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CONTROLLING THE SPREAD OF NEW ZEALAND MUD SNAILS ON WADING GEAR

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SUMMARY

New Zealand mud snails were first reported in Europe during the 1800s and in North America (Idaho) in 1987. Mud snails quickly colonize habitable waters, and they were first discovered in the Owens River in Eastern California in late 1999 and have since spread to the Mokelumne, Calaveras, and Napa rivers, as well as Rush, Hot and Putah creeks. This invasive species will likely have impacts on native species, fisheries, and aquatic ecosystems of the Sacramento-San Joaquin watershed. Unintentional transport on fishing gear and equipment, notably wading gear, is likely one of the primary vectors spreading mud snails among water bodies. In this study, a phased approach identified several chemicals and cleaning methods that could easily be used in the field, and were efficacious in removing snails from wading gear with minimal corrosiveness to the gear.

New Zealand mud snails were exposed in laboratory tests to solutions of benzethonium chloride, chlorine bleach, Formula 409® Disinfectant, Pine-Sol®, ammonia, grapefruit seed extract, isopropyl alcohol, potassium permanganate, and copper sulfate. With the exception of grapefruit seed extract, potassium permanganate and isopropyl alcohol, these materials all killed mud snails within five minutes. Wading gear was repeatedly exposed to bleach, copper sulfate, Pine-Sol®, benzethonium chloride, and Formula 409® Disinfectant for prolonged periods. Bleach and Pine-Sol®, at concentrations efficacious in killing snails, did structural damage to the wading gear. Solutions of copper sulfate (252 mg/L Cu), 1,940 mg/L benzethonium chloride, and 50% Formula 409® Disinfectant killed New Zealand mud snails within five minutes and had minimal effects on wading gear integrity. Wading gear was completely submersed or put in a dry-sack with the cleaning solutions and shaken in field trials, and copper sulfate solution was sprayed on fishing gear in a separate trial. Copper sulfate (252 mg/L Cu), benzethonium chloride (1,940 mg/L) and Formula 409® Disinfectant (50% dilution) solutions under field conditions can prevent the spread of New Zealand mud snails on wading gear.

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INTRODUCTION

The New Zealand mud snail (NZMS), *Potamopyrgus antipodarum,* is one of many non-native species invading California waters. The NZMS is a cosmopolitan species that was spread to Europe and Australia, during the 19th century (Gangloff 1998). NZMS were first discovered in North America in the Snake River, Idaho in 1987 (Bowler 1990) and have since been reported in all fully western states except New Mexico (Montana State University, New Zealand mud snail website, http://www.esg.montana.edu/aim/mollusca/nzms/). A genetically distinct population of NZMS was also found in the state of New York, in Lake Ontario, in 1991. It is a new invasive species in California waters, only recently discovered in the Owens River in 1999 (Dawne Becker, personal communication). It has since spread by 2005 to the Calaveras, Mokelumne and Napa rivers as well as Hot, Rush and Putah creeks spanning both sides of the Sierra Nevada Mountains.

NZMS is a member of the Gastropod order prosobranchia (lungless or gilled snails). Species in this order have a calcified operculum that fits tightly over the shell opening. The long-range dispersal of NZMS is restricted to transport in water or damp media. The operculum forms a tight seal, and NZMS have been reported to survive out of water for several hours (Gangloff 1998). The survival of NZMS increases if kept in damp media such as a wading boot; Winterbourn (1970) reported 50% survival after 25 days in damp media. It is likely that their spread within California and from Idaho to Montana and Wyoming were the result of unintentionally being transported on damp media such as wading gear.

NZMS can reproduce by parthenogenesis, where generally a female produces offspring without fertilization. The young snails are fully functional versions of the adult, complete with immature larvae developing in their ovaries (Gangloff 1998). The populations in western North America have been found to be predominately females and are believed to be clones originating from one original source population (Gangloff 1998). Several researchers have reported NZMS densities near and in excess of 50,000/m^2 (Hylleberg and Siegismund 1987; Schreiber et al. 1997; Noda 2003); numbers in excess of 100,000/m^2 have been observed in Putah Creek (Ken Davis, personal communication) and rivers in Yellowstone National Park (Riley 2002).

Impacts of NZMS can fall into three categories: (1) out competing native gastropods (Richards 2003); (2) exclusion of other grazing aquatic organisms through high density (Cada 2003); and (3) competing with other macro-invertebrates for periphyton (Gangloff 1998, Cada 2004). It is also possible that very dense snail populations may have a significant adverse impact on available nutrients in streams. Mud snails are capable of passing through the digestive canal of most fish, alive and intact (Bondesen and Kaiser 1949; Haynes et al. 1985). In addition, energetic studies have indicated that NZMS, even when digested is a trophic dead end with fish receiving little, if any nutrition from feeding on them (Vinson 2004, Ryan 1982). This will ultimately have a significant adverse impact on the fish populations through reductions in nutritious benthic invertebrate fauna to the benefit of low-nutritional value mud snails.

Initially in California, NZMS were only found in a portion of the Owens River on the east side of the Sierra Nevada Mountains. Snails have now been found along a significant stretch of the Owens River and some tributaries, as well as in streams and rivers on the west side of the Sierras (Bergendorf 2004). It is believed that the snails were inadvertently transported from one stream location to another by hitchhiking on waders, wading boots and other gear used in infested streams. NZMS are very small, typically < 7 mm in length. The ability of NZMS to survive for days in damp environments, combined with their parthenogenic nature, makes wading boots and equipment that contact infested waters a high risk vector for spreading populations (Chapman 2003). An immediate threat exists of NZMS invasion of suitable habitats within the Sacramento-San Joaquin River Delta.

Little research has been conducted on fishing gear to determine the sensitivity of NZMS to potential cleaning compounds. Studies among various researchers show somewhat inconsistent results, likely due to the lack of a consistent testing method. NZMS in a Petri dish were killed by immersion in a 5% bleach solution for 1 hour (Medhurst and Herbst 2003). Dwyer (2001) however, was unable to kill NZMS with chlorine at any concentration within one minute, but did have some success with copper sulfate. For any cleaning method to gain widespread acceptance for routine use in reducing the spread of NZMS, it should be easy to perform and not damage gear. Medhurst (2003) noted that fishing gear placed in water at a temperature of 54° C for five minutes completely killed NZMS. Medhurst (2003) also noted that freezing fishing gear for four to six hours should also kill NZMS. However, using hot water or freezing to remove NZMS from wading gear in the field is impractical and there is some concern that such methods may damage gear. Little research has been conducted on the efficacy of cleaning compounds to kill NZMS on wading gear or on the potential corrosive effects such treatments would have on gear. This uncertainty limits wide-spread efforts by the public to help prevent the spread of NZMS.

In an effort to prevent the unintentional spread of NZMS to uncontaminated waterways in California, the Department of Fish and Game (DFG) investigated methods of cleaning wading gear between uses. In this study, a phased approach identified several cleaning solutions that were efficacious in killing mud snails, minimally corrosive on wading gear, and could easily and effectively be applied in the field. In the first phase of this study, NZMS were exposed to readily available compounds to determine what concentrations elicited 100% mortality in five minutes. The second phase of the study examined the corrosiveness of the compounds on wading gear under prolonged exposures. Finally, the efficacious compounds that had minimal effects on the integrity of the wading gear were tested under field conditions.

MATERIALS AND METHODS

Toxicity of Potential Cleaning Solutions

Several cleaning solutions were selected for testing on NZMS. A protocol was developed for determining the lowest concentrations of these products/compounds that killed 100% of the NZMS within 5 minutes of exposure (Appendix 1).

Compounds investigated for killing NZMS were:

- Grapefruit seed extract (GSE), (Nutribiotic "The Original GSE®", 33% Citricidal)
- Benzathonium chloride (BZCl), (Alfa Aesar, 97% benzethonium chloride)
- Household bleach, (Clorox®, 6% sodium hypochlorite)
- Formula 409® Disinfectant
- Copper sulfate pentahydrate, (Malinckrodt, 99.1% cupric sulfate granular U.S.P.)
- Potassium permanganate, (J.T. Baker, 0.1N volumetric solution)
- Isopropyl alcohol, (generic rubbing alcohol 70% isopropyl alcohol by volume.)
- Pine-Sol®, (5% pine oil)
- Household non-sudzing, unscented ammonia, (generic ammonia, 4% as NH_3)

NZMS used for toxicity testing were collected from Putah Creek upstream of Lake Solano in Yolo County, California. Toxicity tests were conducted at temperatures of 5°C and 15°C. Large colonies of collected snails were maintained at the Aquatic Toxicology Laboratory (ATL) in Elk Grove in 2,000-ml glass beakers in constant temperature incubators set at temperatures of 5°C or 15°C. NZMS were maintained on a diet of deciduous leaves and algae, *Selenastrum* sp. in excess. NZMS used in the tests had a carapace length > 2 mm.

All toxicity tests employed laboratory controls using well water from ATL. Well water was also used for dilution of the nine compounds. Quality of ATL well water was 68 mg/L $CaCO_3$ hardness, 84 mg/L $CaCO_3$ alkalinity, 8.2 pH, and 255 µmho/cm conductivity. Concurrent toxicity tests were conducted using NZMS with opercula open or closed at two water temperatures (5°C and 15°C). Forty NZMS were exposed per treatment, with four chambers, 100-ml borosilicate glass beakers, per each treatment (10 NZMS per chamber). Test temperatures were maintained at 5 ± 1°C and 15 ± 1°C using constant temperature incubators. Prior to testing, NZMS were confirmed to be alive by observation of movement by the individual snails. For tests with opercula open groups, NZMS were placed in the individual test chambers with 10 ml of laboratory water ensuring the snails were active with their opercula open at the time of exposure. For tests with opercula closed, NZMS were gently disturbed with a blunt probe to close their opercula prior to placement in the test chambers with the test solutions. Test solutions for the opercula open tests were prepared at a concentration 11% higher than needed to account for the dilution of the 10 ml of laboratory water in the test chambers at the beginning of the test. This was not possible for the materials that were tested as original strength or "undiluted".

NZMS were exposed to the test solutions for 5 minutes (Figure 1), after which the test solutions were decanted and NZMS rinsed twice each with 50 ml of ATL well water. The test chambers were then refilled with 100 ml of ATL well water, covered, and returned to the incubators for 48 hours. NZMS were not fed during this period. At the end of 48 hours NZMS were observed under 10X magnification for survival. The criteria for death were

opercula open and no movement for a minimum of 5-minutes; occasionally the body of the snail was observed to be separated from the shell. At the end of the tests all NZMS were killed by immersion in undiluted bleach; NZMS were killed within five to ten minutes.

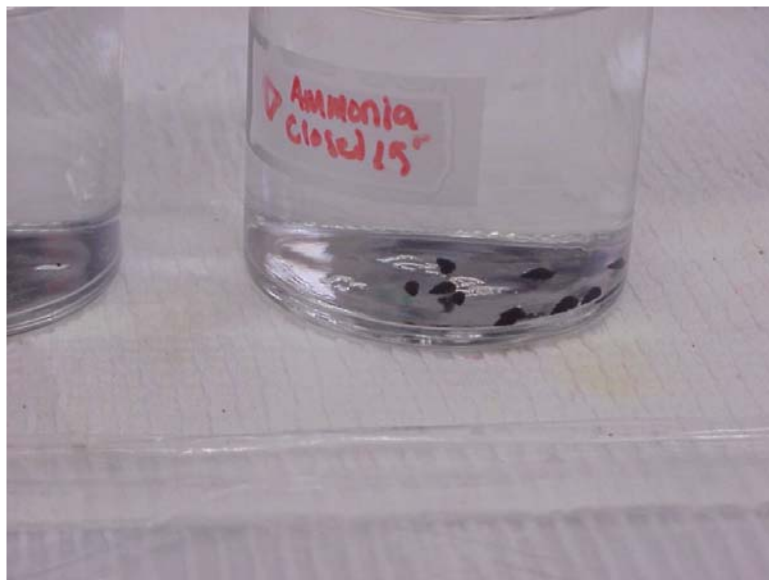

Figure 1. NZMS in test chamber with ammonia test solution

Preliminary results indicated that the solutions were more toxic to NZMS at lower temperatures with their opercula open. To mimic worst-case conditions, dilutions of the test solutions found to be most effective during the initial tests were retested on NZMS at a temperature of 15°C with their opercula closed. In the next phase of the study, solutions that were efficacious at killing NZMS were tested for corrosiveness on wading gear. The solutions that were efficacious were undiluted bleach, 50% Pine-Sol®, 50% Formula 409® Disinfectant, 1,940 mg/L benzethonium chloride, 991 mg/L copper sulfate (252 mg/L Cu), and undiluted non-sudzing household ammonia.

Corrosiveness of Cleaning Solutions

The second phase of the study tested the corrosiveness of the efficacious cleaning solutions on wading gear. The testing was conducted using the protocol in Appendix 1. Waders and wading boots were donated by three manufacturers (Orvis Outfitters, Simms, and Patagonia).

Each cleaning solution was tested on waders and boots from each manufacturer. One mate from each pair of gear was used as a control (ATL well water) and the other mate was exposed to the cleaning solution. Exposure consisted of immersing the gear in a five-gallon plastic container containing a cleaning solution or control water for 30 minutes, followed by drying in direct sunlight at ambient temperatures for a period of not less than one hour or until the gear were dry to touch, which ever was longer. Gear exposed to the cleaning solutions were not rinsed with control water following exposure. Gear were exposed on alternating days for a period of two weeks; a total of seven exposures for each set of gear. Test solutions were not renewed during the course of the testing.

At the end of the seven exposures, the gear were examined and physically tested to identify any adverse effects from exposure to the test compounds. Examination included checking for changes in: fabric flexibility, fabric color, texture of fabric, water repellency, seam integrity (stitching and glue), the condition of neoprene as well as the presence of surface and/or structural cracking on rubber parts, corrosion on metal parts, surface residues, and odors. The efficacious cleaning solutions that did not significantly damage the gear were tested under field conditions for removing NZMS from wading gear. The cleaning solutions that were efficacious against NZMS and were not corrosive on wading gear were 991mg/L copper sulfate (252 mg/L Cu), 1,940 mg/L benzethonium chloride, and 50% Formula 409® Disinfectant.

Field Testing of Cleaning Solutions and Methods

The last phase of the study tested the efficacy of the cleaning solutions under field conditions using the cleaning protocol in Appendix 1. Three cleaning methods used during the field trials were: (1) Full immersion of gear in a solution (approximately 20 to 30 liters of cleaning solution); (2) Shaking gear in a dry sack with a solution (approximately 8 to 10 liters of cleaning solution); and (3) Spraying gear with a solution to the point of saturation/runoff (only done using copper sulfate solution (252 mg/L Cu)). Following the 5-minute cleaning procedure, the gear was rinsed in control water. The cleaning solutions were not renewed during the course of the field trials.

Over fifty volunteers were recruited from local fishing clubs (Figure 2). The volunteers, wearing their own wading gear, followed a marked trail along approximately 1,300 feet of the stream bed in Putah Creek in Yolo County, California. This section of the creek had been previously identified as supporting high densities of NZMS (>100,000 snails/m^2). After wading the trail, gear were removed and randomly assigned to one of seven treatment groups (Table 1). Field trials continued over a period of two days until a minimum of seven sets of gear had been processed using each of the cleaning solution/method combinations.

Table 1. Distribution of wading gear sets by combination of cleaning solution and method.

Treatment	Cleaning Solution	Cleaning Method	Sets of Wading Gear
1	Copper sulfate (252 mg/L Cu)	Immersion	7
2	Benzethonium chloride (1,940 mg/L)	Immersion	7
3	Formula 409® Disinfectant (50% dilution)	Immersion	8
4	Copper sulfate (252 mg/L Cu)	Shaken in dry sack	8
5	Benzethonium chloride (1,940 mg/L)	Shaken in dry sack	7
6	Formula 409® Disinfectant (50% dilution)	Shaken in dry sack	8
7	Copper sulfate (252 mg/L Cu)	Sprayed	7

Figure 2. Volunteers participating in the field trials

All wading gear were examined after cleaning to detect the presence of living or dead NZMS. If substrate material (mud, gravel, etc) that could harbor NZMS was present, the gear was rinsed again in fresh water and examined. If substrate material remained, the wading gear was scrubbed using a stiff, nylon-bristled brush until all foreign material was removed. The cleaning solutions and rinse water were filtered through a 500-µm mesh nylon net following each cleaning (Figure 3). The filter and filtrate for each pair of wading gear were placed into individually labeled, clean containers with fresh water. The containers were placed on wet ice and returned to the laboratory to identify the numbers of living and dead NZMS. Live NZMS were collected from Putah Creek during the field trials and held in clean containers with fresh water served as process controls.

The filtrate samples were examined in the laboratory under 10x magnification. Survival was determined for each sample. Because size may have affected survival, all NZMS were counted and assigned to the following size classes based on the length of the carapace: (1) $\leq$ 1 mm; (2) 2 mm; (3) 3 mm; (4) 4 mm; (5) 5 mm; and (6) $\geq$ 6 mm. Data from the examination of the samples were tabulated to determine the efficacy for each cleaning solution/method combination.

Figure 3. Filtering cleaning solution after removing wading gear.

RESULTS

Toxicity of Cleaning Solutions

Benzethonium chloride (1,940 mg/L), copper sulfate (504 mg/L Cu), undiluted Formula 409® Disinfectant, undiluted household ammonia, and a 50% dilution of Pine-Sol® were effective at killing NZMS in a 5-minute exposure at a temperature of 5°C (Table 2). A 5% dilution of bleach (3,000 mg/L sodium hypochlorite), potassium permanganate (200 mg/L), undiluted isopropanol (700,000 ml/L) and grapefruit seed extract (700 ml/L) were ineffective at killing NZMS at temperatures of either 5°C or 15°C.

The survival rate for NZMS was notably higher for isopropyl alcohol and potassium permanganate at the higher temperature (15°C) (Table 2). Similarly, the survival rate was higher when the NZMS opercula were closed when exposed to isopropanol, potassium permanganate, and bleach. Hence, additional tests were conducted only at a temperature of 15°C and with the NZMS opercula closed.

Additional toxicity tests were performed using dilutions of those materials that were efficacious on NZMS to determine the minimal effective dose. Copper sulfate solution at 252 mg/L Cu and the 50% dilution of Formula 409® Disinfectant continued to exhibit 100% control (Table 2). Dilutions of Pine-Sol® below 50% and of benzethonium chloride below 1,940 mg/L were not effective. Grapefruit Seed Extract (GSE) was retested at a higher concentration (2,000 ml/L) but remained ineffective in killing NZMS (Table 2).

Laboratory toxicity tests demonstrated that copper sulfate pentahydrate (252 mg/L Cu), Formula 409® Disinfectant (50% dilution), Pine-Sol® (50% dilution), benzethonium chloride (1,940 mg/L), and undiluted household ammonia (4% NH_3) were effective at killing NZMS over a range of temperatures (5°C - 15°C). Dilutions of bleach appeared to be effective at controlling NZMS when their opercula were open (Table 2).

Table 2. Survival of New Zealand mud snails at temperatures of 5°C and 15°C following 5-minute exposure to cleaning solutions and a 48-hour recovery in fresh water

Compound	5° C		15° C	
	Opercula Open	Opercula Closed	Opercula Open	Opercula Closed
ATL Well Water (Control)	97.5	99.5	99.5	100
Grapefruit seed extract (700 ml/L)	100	97.5	100	100
Grapefruit seed extract (2,000 ml/L)	-	-	-	62.5
Isopropanol (undiluted, 700,000 ml/L)	47.5	75	85	72.5
Potassium permanganate (200 mg/L)	10	25	22.5	52.5
Bleach (5% dilution; 3,000 mg/L HOCl)	0	30	0	80
Bleach (17% dilution; 10,000 mg/L HOCl)	-	-	-	97.5
Benzethonium chloride (1,940 mg/L)	0	0	0	0
Benzethonium chloride (970 mg/L)	-	-	-	67.5
Benzethonium chloride (485 mg/L)	-	-	-	87.5
Formula 409® Disinfectant (undiluted)	0	0	0	2.5
Formula 409® Disinfectant (50% dilution)	-	-	-	0
Pine-Sol® (undiluted; 50,000 ml/L pine oil)	0	0	0	0
Pine-Sol® (50% dilution)	0	0	0	0
Pine-Sol® (25% dilution)	-	-	-	62.5
Pine-Sol® (10% dilution)	-	-	-	100
Ammonia (undiluted; 40,000 ml/L)	0	0	0	0
Ammonia (50% dilution)	-	-	-	30
Ammonia (25% dilution)	-	-	-	47.5
Copper sulfate (504 mg/L Cu)	0	0	0	0
Copper sulfate (252 mg/L Cu)	-	-	-	0

Corrosiveness of Solutions

Examination revealed the most noticeable changes were to those waders and boots exposed to bleach (undiluted) and Pine-Sol® (50% dilution) (Table 3).

Bleach (undiluted) – Bleach caused a noticeable color change in the fabric of waders and wader boots. Cracking of neoprene, overall loss of flexibility (stiffening) of the fabric, failure of stitching, and tearing of fabric on the boots were all observed (Figure 4 and 5). The waders exposed to bleach leaked through the neoprene booties. However, water continued to bead up on the fabric portion of the Simms® waders exposed to bleach.

Table 3. Effects of NZMS cleaning solutions on wading gear.

Compound and Concentration	Color Change	Seam Integrity	Leaks[A]	Comments
ATL control well water	None	No observed impact	No	No observed changes
Copper sulfate (252 mg/L Cu)	None	No observed impact	No	No observed changes
Benzethonium chloride (1,940 mg/L)	None	No observed impact	No	Rubber of boot toe guard developed small cracks, waders lost surface water repellency
Formula 409® Disinfectant(50% dilution)	None	No observed impact	No	Rubber of boot toe guard developed small cracks, waders lost surface water repellency
Pine-Sol® (50% dilution)	Some dark staining inside and outside	Glue dissolved on neoprene seams	Yes in the seams	Fabric retained odor of Pine-Sol®, developed a greasy feeling, rubber of boot toe guard developed small cracks, waders lost surface water repellency
Bleach (undiluted)	yellowing, fading	Stitching began to dissolve on boots	Yes	Fabric became brittle, neoprene developed cracks, fabric fractured and tore along boot seams

[A] All waders were tested for leaks prior to initiation of Phase 2 testing.

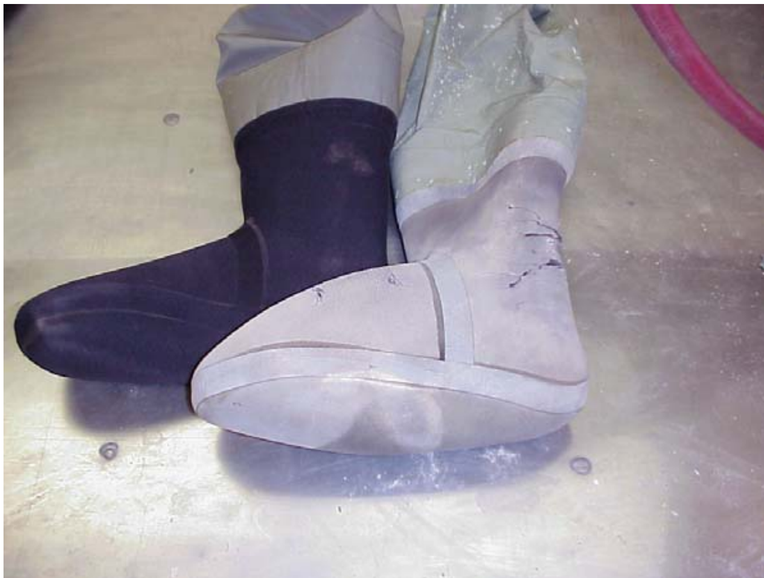

Figure 4. Neoprene wader bootie exposed to bleach (right) compared to bootie exposed to ATL control well water. The fabric of the neoprene bootie exposed to bleach has cracked and lost color.

Figure 5. Wader boot exposed to bleach had changed color of boot liner and failure of material where tongue had been sewn.

Pine-Sol® (50% dilution) – Wading gear exposed to Pine-Sol® (50% dilution) continued to emit an odor characteristically associated with the compound several weeks after the tests ceased. The seams of the neoprene booties were weakened and in one case actually failed. The glue used in the seams appears to have been dissolved (Figure 6). Surface cracking of the rubber toe portion was observed for boots. The inside of the leg of the Simms® brand waders exposed to the Pine-Sol® solution exhibited some staining but did not leak.

Figure 6. Neoprene bootie exposed to Pine-Sol® had seam failure. The glue appears to have dissolved.

Formula 409® Disinfectant (50% dilution) – Cracking of the rubber toe portion of the boots was observed. The cracking was similar to that observed for the boots exposed to the Pine-Sol® solution. This cracking appeared to be surficial and did not appear to have compromised the integrity of the boots. Water did not continue to bead up on the exterior of the waders. However, they did not develop any leaks.

Benzethonium Chloride (1,940 mg/L) – The only observed changes in the wading gear exposed to benzethonium chloride were minor, surficial cracking of the rubber toe guard of the boots similar to that observed with Formula 409® Disinfectant and Pine-Sol® exposure (Figure 7), and a loss of surface water repellency (beading of water) on the legs of the waders similar to that observed for Formula 409® Disinfectant. Waders exposed to benzethonium chloride did not develop any leaks. The fabric in the waders or the boots did not appear to lose any flexibility.

Figure 7. Wader boot exposed to benzethonium chloride had cracking of rubber toe.

Copper Sulfate (252 mg/L Cu) – There were no observed changes in wading gear exposed to the copper sulfate solution (252 mg/L Cu). The fabric of the waders and the boots did not appear to have become stiff or brittle. Cracks were not observed in any of the rubber on the wader boots. There were no observable stains present on the outside or the inside of the waders. The waders did not develop any detectable leaks. Water continued to bead on the surface of the waders unlike those exposed to the benzethonium chloride, Pine-Sol® or Formula 409® Disinfectant solutions.

ATL Well Water – There were no observed changes to any of the waders or boots exposed to the ATL well water (controls).

Field Testing of Cleaning Solutions and Methods

Fifty-two sets of wading gear were tested during the field trials. At least seven sets of wading gear were tested for each cleaning solution and method. NZMS were separated from each filtrate sample and divided into 1-mm size classes based on length of the carapace (Table 4 and Appendix 2). All NZMS collected as control samples survived confinement in the containers, transportation and storage at the laboratory. All cleaning solutions and methods were 100% efficacious in removing NZMS from wading gear. No NZMS were recovered alive from wading gear, or from the filtrate samples.

Table 4. Numbers of NZMS isolated from individual filtrate samples for each treatment group. Refer to Table 1 for treatment specifics.

Treatment	NZMS Size Class						Total NZMS
	≥6 mm	5 mm	4 mm	3 mm	2 mm	≤1 mm	
1	0	3	25	24	40	116	208
2	5	53	64	27	66	309	524
3	0	17	30	29	51	150	277
4	3	18	47	52	53	185	358
5	0	16	11	17	21	81	146
6	0	2	1	9	18	36	66
7	0	7	15	10	39	80	151

The majority of NZMS recovered were associated with wading boots. NZMS were observed on the tongue area of wading boots, associated with the laces or the area of the tongue that was tucked beneath the lacing eyelets. Large numbers of small NZMS were present inside of the boots, having worked down between the boot and the neoprene bootie of the wader. If the boots contained padded insole inserts, NZMS were also found underneath the inserts, associated with sand grains. NZMS were recovered from every treated set of wading gear. Numbers of NZMS per sample (Figure 8) ranged from 1 to 227 with a mean of 33 (Appendix 2). Over 50% of NZMS recovered were ≤ 1 mm in size (Table 4).

Figure 8. NZMS recovered from one set of wading gear cleaned during the field trials.

DISCUSSION

Prior to this study, the primary methods of eliminating NZMS from wading gear were primarily physical in nature, either freezing equipment for over 6 hours at temperatures of -10°C (Bergendorf 2004) or immersing in heated water at a temperature of 45°C for at least 1 minute (Gangloff et al. 1998). Desiccation has also been reported to be effective at removing NZMS from equipment. However, all three physical methods have significant limitations (lack of a freezer or heating device) for making them feasible for cleaning gear in the field. Complete desiccation of NZMS could take several days. Several studies report greater than 50% survival of NZMS for 25 days on "damp media" (Bergendorf 2004). "Damp media" may consist of small void spaces under seams on waders and gravel guards or under insoles in wading boots that could retain small amounts of moisture for days. During this drying period the gear would not be available for use in other waterways.

In reviewing potential threats to California habitat and fisheries posed by NZMS, Becker (2001) noted that control measures for the snails appeared to be more effective at cold temperatures. Our data corroborate these findings as survival of NZMS was notably lower at a temperature of 5°C compared to a temperature of 15°C for bleach, potassium permanganate, and isopropanol. We believe this is related to the increased time for the NZMS to close the opercula at lower temperatures, allowing more cleaning solution inside the shell.

Our results indicate that copper sulfate (252 mg/L Cu), Formula 409® Disinfectant (50% dilution) and benzethonium chloride (1,940 mg/L) are effective at removing NZMS from wading gear in a feasible amount of time. These materials were equally effective if the snails' opercula were open or closed and had no apparent effect on gear integrity. Observed impacts to the gear appeared to be cosmetic rather than structural, and the exposure was far more rigorous than the necessary exposure period indicated by our lab trials (5-minutes). All three solutions were effective at removing NZMS from wading gear under field conditions. We conclude that wading gear properly cleaned using any one of the three methods are free of live NZMS that could be transported to another water body. It also appears that exposure to materials causes NZMS to release from the substrate they're in contact with, which facilitates their removal. The data further support the conclusion that solutions of these materials can remain efficacious for cleaning several sets of wading gear without renewal.

CONCLUSIONS

We believe that the use of copper sulfate, benzethonium chloride or Formula 409® Disinfectant immersion baths or in dry sacks provides an acceptable alternative to the current physical methods of removing NZMS from wading gear. Copper sulfate was also effective when sprayed on the gear. These have the advantage of requiring less than 30 minutes to complete versus freezing (4 to 6 hours) or desiccation (possibly days) and cleaning can be done in the field. However, it may be necessary to carry a container to place the gear in during cleaning. After cleaning, the gear should not be rinsed with site water as this may place NZMS back on the gear. Care must also be taken to ensure that the cleaning solutions not enter surface water. We propose that a possible cleaning protocol based on the results of this study could be distributed through an outreach program to various fishing groups, consultants and researchers that may visit NZMS infested waters (Appendix 3).

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APPENDICES

Appendix 1:

Standard Operating Procedure (SOP) For Testing The Effects Of Cleaning Solutions On New Zealand Mud Snail, *POTAMOPYRGUS ANTIPODARUM* And Wading Gear

STANDARD OPERATING PROCEDURE (SOP) TESTING THE EFFECTS OF CLEANING SOLUTIONS ON
NEW ZEALAND MUD SNAIL, *POTAMOPYRGUS ANTIPODARUM*
AND WADING GEAR

Prepared by: ______________________________ Date:
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1.0 Scope and Application

1.1 The purpose of this protocol is to screen for short-term lethal effects of select cleaning solutions on the New Zealand mud snail (NZMS) *Potamopyrgus antipodarum* with their opercula open and with their opercula closed. Previous studies have suggested a considerable difference in toxicity between the two opercula states. The compounds tested are being considered for use to clean waders, wading boots and water contact fishing equipment containing NZMS in an attempt to prevent its spread to uncontaminated waters. The compounds currently being considered include (1) Grapefruit seed extract; (2) Benzethonium chloride (a quaternary ammonium compound); (3) Household bleach (6% sodium hypochlorite); (4) Formula 409® Disinfectant brand all purpose cleaner; (5) Copper sulfate; (6) Potassium permanganate; (7) 70% Isopropyl alcohol; (8) Pine Sol®; and (9) Household use non-sudzing ammonia.

2.0 Equipment

2.1 100-ml borosilicate clear glass beaker test chambers (four replicates per test solution and four control replicates).

2.2 NZMS of a size large enough to be seen with the unaided human eye, approximately 2-mm carapace length or larger (ten snails per replicate, including controls).

2.3 1-L chemically clean amber glass bottles for mixing and holding test solutions.

2.4 Temperature control incubator to hold test chambers.

2.5 Disposable glass pipettes.

2.6 Glass graduated cylinders.

3.0 Preparation of Test Equipment

3.1 Tests are conducted at the Aquatic Toxicology Laboratory (ATL) of the Department of Fish and Game (DFG), Elk Grove. Tests are run at two temperatures, 5°C and 15°C (40° and 60° F) using constant temperature incubators.

4.0 Preparation of Test Solutions for Opercula Open Tests[1]

Test materials are diluted using ATL control water (1:1 ATL well water : RO water).

4.1 0.20% (2,000 ppm) grapefruit seed extract (GSE), 33% Citricidal in glycerin: 200 drops (165 ml) of GSE diluted with control water to a total volume of 900 ml.

4.2 0.194% (1,940 ppm) benzethonium chloride (QAC): 2 g crystalline benzethonium chloride (97% purity) in control water to a total volume of 900 ml.

4.3 5% (3,000 ppm) household bleach (6% sodium hypochlorite): 50 ml bleach diluted with control water to a total volume of 900 ml

4.4 100 % Formula 409® Disinfectant (undiluted):

4.5 0.198% (1,980 ppm) copper sulfate pentahydrate (504 ppm CU): 2 g copper sulfate pentahydrate (99.1% purity) in control water to a total volume of 900 ml

4.6 0.02% (200 ppm) potassium permanganate ($KMnO_4$): 63.7 ml of 3.171 mg/ml potassium permanganate standard solution diluted with control water to 900 ml total volume

4.7 100% household rubbing alcohol (70%, isopropyl alcohol):

4.8 100% Pine Sol® cleaner (5% pine oil):

4.9 100% household non-sudzing ammonia (4% as NH_3):

5.0 Preparation of Test Solutions for Opercula Closed Tests

Test materials are diluted using ATL control water (1:1 ATL well water : RO water).

5.1 0.20% (2,000 ppm) grapefruit seed extract (GSE), 33% Citricidal in glycerin: 200 drops of GSE (165 ml) diluted with control water to a total volume of 1,000 ml.

5.2 0.194% (1,940 ppm) benzethonium chloride (QAC):

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[1] The diluted materials are 11% stronger than indicated to account for dilution by 10 ml of control water in the test chambers

2 g crystalline benzethonium chloride (97% purity) in control water to a total volume of 1,000 ml.

5.3 5% (3,000 ppm) household bleach (6% sodium hypochlorite):
50 ml bleach diluted with control water to a total volume of 1,000 ml

5.4 100% Formula 409® Disinfectant (undiluted):

5.5 0.198% (1,980 ppm) copper sulfate pentahydrate (504 ppm Cu):
2 g copper sulfate pentahydrate (99.1% purity) in control water to a total volume of 1,000 ml

5.6 0.02% (200 ppm) potassium permanganate ($KMnO_4$):
63.7 ml of 3.171 mg/ml potassium permanganate standard solution diluted with control water to 1,000 ml total volume

5.7 100% household rubbing alcohol (70%, isopropyl alcohol):

5.8 100% Pine Sol® cleaner (5% pine oil):

5.9 100% household non-sudzing ammonia (4% as NH_3):

6.0 Preparation of Dilutions of Efficacious Solutions for Opercula Closed Tests

6.1 (10,000 ppm) Bleach: 167 ml Bleach (60,000 ppm) diluted to 1,000 ml with control water.

6.2 Pine Sol® 25% Solution: 250 ml of Pine Sol® diluted to 1,000 ml with control water.

6.3 Pine Sol® 10% Solution: 100 ml of Pine Sol® diluted to 1,000 ml with control water.

6.4 Household ammonia 50% Solution: 500 ml of ammonia diluted to 1,000 ml with control water.

6.5 Household ammonia 25% Solution: 250 ml of ammonia diluted to 1,000 ml with control water.

6.6 0.097% (970 ppm) benzethonium chloride (QAC):
1 g crystalline benzethonium chloride (97% purity) in control water to a total volume of 1,000 ml.

6.7 0.049% (485 ppm) benzethonium chloride (QAC):

0.5 g crystalline benzethonium chloride (97% purity) in control water to a total volume of 1,000 ml.

6.8 0.099% (991 ppm) copper sulfate pentahydrate (252 ppm Cu):
1 g copper sulfate pentahydrate (99.1% purity) in control water to a total volume of 1,000 ml.

6.9 Formula 409® Disinfectant 50% Solution: 500 ml of Formula 409® Disinfectant diluted to 1,000 ml with control water.

7.0 Collection of Test Organisms

7.1 A minimum of 440 NZMS (2 mm or larger) are needed for the tests. Each replicate will require ten (10) snails..

7.2 There will be one trial with four replicates each for a control and each of the nine (9)) test solutions. A five minute exposure will be conducted for each material. One set of 40 snails will be retained as a control group. The total is ten (10) test and control groups.

8.0 Preparing the Test Chambers

8.1 Ten snails will be held in each test chamber and covered by ten (10) ml of control water (1:1 ATL well water:RO water) at the appropriate test temperature for the opercula open tests.

8.2 Snails will be held in the 2,000-ml glass beaker with control water at the appropriate test temperature until ready for use in the opercula closed tests.

9.0 Loading the Organisms

9.1 Snails will be maintained in the laboratory in 2,000-ml beakers containing control water at either 5°C or 15°C. The snails will be fed deciduous leaves and algae, *Selenastrum* sp. to excess as a maintenance diet. Snails will be randomly assigned to each test chamber. Snails will not be fed for one day before test solutions are administered.

10.0 Daily Tasks

10.1 Opercula Open Tests – Day 0: Place ten snails (at least 2 mm in size) in each test chamber (four replicates per test solution and control), add 10 ml of control water to each chamber and bring to a temperature of 5°C or 15°C. Allow snails to acclimate for 1 hour prior to exposure. This ensures that the

snails are active with open opercula during exposure. Add ninety (90) ml of test solution to each test chamber to make a final solution of 100 ml. Test solutions take into account the additional 10 ml in the test chamber. Exposure times in each test chamber are five minutes. Remove all test solution, and rinse snails in each test chamber with 50 ml of control water (1:1 ATL well water : RO water), twice. Drain second rinse and fill test chamber with clean control water. Replace test chamber in rack. At completion of all tests, randomize placement of test chambers in rack and return test chambers to incubator for 48 hours. Snails will not be fed during the test.

Day 1: Do not disturb snails.

Day 2: At the end of 48 hours remove test chambers from incubator and record numbers of live snails (active snails) in each test chamber. All snails are viewed under magnification to confirm survival or mortality.

10.2 Opercula Closed Tests – Day 0: Place 100 ml of test solutions into 4 chambers and bring temperature to either 5°C or 15°C. Add 100 ml of control water to four test chambers and bring temperature to either 5°C or 15°C. Disturb snails with blunt probe prior to transfer to test chamber to ensure opercula are closed. Transfer the snails, making sure that the opercula are closed prior to immersion into test chambers. Exposure times in each test chamber are five minutes. Remove all test solution, and rinse snails in each test chamber with 50 ml of control water (1:1 ATL well water : RO water), twice. Drain second rinse and fill test chamber with clean control water. Replace test chamber in rack. At completion of all tests, randomize placement of test chambers in rack and return test chambers to incubator for 48 hours. Snails will not be fed during the test.

Day 1: Do not disturb snails.

Day 2: At the end of 48 hours remove test chambers from incubator and record numbers of live snails (active snails) in each test chamber. All snails are viewed under magnification to confirm survival or mortality.

10.3 Opercula Closed Tests – Day 0: Place 100 ml of each test solution into each of 4 chambers and bring temperature to 15°C. Add 100 ml of control water to four test chambers and bring temperature to 15°C. Disturb snails with blunt probe prior to transfer to ensure opercula are closed. Transfer snails, making sure that the opercula are closed prior to immersion into test chambers. Exposure times in each test chamber are five minutes. Remove all test solution, and rinse snails in each test chamber with 50 ml of control water (1:1 ATL well water:RO water), twice. Drain second rinse and fill test chamber with clean control water. Replace test chamber in rack. At completion of all tests, randomize placement of test chambers in rack and return test chambers to incubator for 48 hours. Snails will not be fed during the test.

Day 1: Do not disturb snails.

Day 2: At the end of 48 hours remove test chambers from incubator and record numbers of live snails (active snails) in each test chamber. All snails are viewed under magnification to confirm survival or mortality.

11.0 Ending the Test

11.1 Count the number of snails surviving in each of the test chambers.

11.2 Write up test summary.

11.3 At the completion of the tests all contents of test chambers will be disposed of in a concentrated solution of bleach. All test chambers and equipment contacting snail containing water will be rinsed in a concentrated bleach solution to preclude inadvertently introducing snails to any uncontaminated waterways.

12.0 Corrosiveness Testing

12.1 Samples of various waders and wader boots are identified and obtained.

12.2 Specific compounds and dilutions from Phase I toxicity tests on NZMS are identified.

12.3 Samples of each of the waders and wader boots are completely immersed in solutions of the identified Phase I compounds under the following conditions on alternate days for a period of two weeks:

12.3.1 Immersion in the designated concentration of the compound for a period of thirty (30) minutes. The waders and wader boots are then placed in direct sunlight until dry or not less than one hour which ever is greater.

12.4 Following completion of the exposure regimen, all waders and wader boots are examined for evidence of adverse impacts due to exposure to the specified compounds. Adverse impacts include:

12.4.1 Discoloration of waders or wader boots

12.4.2 Cracking or evidence of brittleness of wader material or wader boots.

12.4.3 Separation of layers of materials in waders or wader boots.

12.4.4 Failure of seams in waders.

12.4.5 Loss of water repellency

12.4.6 Presence of odors

12.4.7 Presence of surface residues

12.5 The compounds identified from Phase I to be tested for adverse effects to wader material include:

12.5.1 Formula 409® Disinfectant (50% dilution)

12.5.2 Copper sulfate (252 mg/L Cu)

12.5.3 Benzethonium chloride (1,940 mg/L)

12.5.4 Pine-Sol (50% dilution)

12.5.5 If time and resources permit, ammonia (4% NH_3, undiluted) and bleach (6% sodium hypochlorite, undiluted) will also be tested.

13.0 Field Testing

13.1 Different solutions and methods for decontaminating waders and wader boots (equipment) contaminated with immature and mature NZMS are tested.

13.2 Three specific solutions have been identified (see 13.6) for 100 % control of NZMS (Phase I) and subsequently, no adverse structural changes to equipment (Phase II).

13.2.1 The solutions to be tested during Phase III include:

13.2.1.1 Copper Sulfate (252 mg/L Cu)

13.2.1.2 Benzethonium Chloride (1,940 mg/L)

13.2.1.3 Formula 409® Disinfectant (50% dilution)

13.3 Equipment tested in Phase III is either donated to the program or belongs to fishermen fishing in Putah Creek who are actively solicited by DFG staff to participate in the decontamination trials. The focus of the trials is on those configurations of equipment that are most prone to becoming contaminated with NZMS.

13.4 Equipment is worn in Putah Creek in an area known to support high concentrations of NZMS. The equipment is examined upon exiting Putah Creek to confirm the presence of NZMS. Equipment contaminated with NZMS is subjected to one of three decontamination protocols using one of the three test solutions (seven test groups). Each treatment group will be used for at least 7 sets of waders and wader boots for a total of at least 49 sets of waders and wader boots (Table 1).

Table 1. Combinations of decontamination methods and solutions to be investigated (49 sets of waders and boots)

Solution	Tub	Dry Sack	Spray Bottle
Copper sulfate (1,000 ppm)	7 Sets	7 Sets	7 Sets
Benzethonium chloride (2,000 ppm)	7 Sets	7 Sets	N/A[1]
Formula 409® Disinfectant (50% solution)	7 Sets	7 Sets	N/A

[1] Combination not planned for initial field trials

13.5 The following three protocols are used to investigate the decontamination of waders and wader boots. Sufficient sets of contaminated waders and wader boots are used for each decontamination method/test solution combination to obtain a statistically valid result (minimum 7). The waders and wader boot sets are assigned to a specific combination of test solution and decontamination method by means of a random number table. Each set of waders and wader boots is assigned to one of seven treatment test groups using a table established prior to testing based on a random number table.

13.5.1 The waders and wader boots are placed in a large (approximately 40 liter capacity) plastic tub containing a sufficient quantity of the decontamination solution for complete immersion (25-30 Liters). The waders and wader boots are immersed in the decontamination solution for five minutes.

13.5.1.1 At the end of the five minute exposure period the decontamination solution is poured through a mesh filter into a holding container. All filtered material is placed in a clean Ziploc bag and sealed for later examination to determine the presence, sizes and numbers of NZMS, and to determine if any snails are alive.

13.5.1.2 The waders and wader boots are removed from the container and rinsed with ATL control water. The rinseate is poured through a mesh filter. All filtered material is placed in a clean Ziploc bag and sealed for later examination to determine the presence, sizes and numbers of NZMS, and to determine if any snails are alive.

13.5.1.3 The waders and wader boots are examined using appropriate magnification equipment to determine if NZMS remain and if they are alive or dead. If NZMS are present and determined to be alive the numbers and estimated sizes of snails are recorded. Locations where the snails are found on the waders and wader boots is recorded and the waders and wader boots are actively scrubbed with a brush and rinsed using ATL control water. They are reexamined and returned to the fishermen once it is determined that all NZMS have been removed.

13.5.2 Waders and wader boots are placed in a 65 liter dry sack and sufficient decontamination solution added to the sack to ensure all surfaces will be exposed to the decontamination solution. The sack is sealed and shaken for 30 seconds. The waders and wader boots then remain in the sack for five minutes. Prior to removing the waders and wader boots they are shaken again for 30 seconds.

13.5.2.1 The liquid solution is poured out of the dry sack through a mesh filter and returned to the tub container. All material filtered from the decontamination solution is placed in a clean container and sealed for later examination to determine the presence, sizes and numbers of NZMS, and to determine if any snails are alive.

13.5.2.2 The waders and wader boots are rinsed with ATL control water and the rinseate is poured through a mesh

filter. All material filtered from the rinse water is placed in a clean container and sealed for later examination to determine the presence, sizes and numbers of NZMS, and to determine if any snails are alive.

13.5.2.3 The waders and wader boots are examined using appropriate magnification equipment to determine if NZMS remain and if they are alive or dead. If NZMS are present and determined to be alive the numbers and estimated sizes of snails are recorded. Locations where the snails are found on the waders and wader boots is recorded and the waders and wader boots are actively scrubbed with a brush and rinsed using ATL control water. They are reexamined and returned to the fishermen once it is determined that all NZMS have been removed.

13.5.3 The waders and wader boots are placed in an empty plastic tub. Using a hand operated kitchen type spray bottle containing the copper sulfate test solution, the waders and wader boots are thoroughly sprayed by hand to the point of runoff of decontamination solution. The waders and boots are manipulated by hand as necessary to ensure all surfaces are sprayed with the decontamination solution. The waders and wader boots are then left to sit for 5 minutes.

13.5.3.1 The waders and boots are removed from the tub and placed into a second tub. Any decontamination solution and any snails that have fallen off of the equipment are rinsed into a mesh filter. All material filtered from the decontamination solution is placed in a clean container and sealed for later examination to determine the presence, sizes and numbers of NZMS, and to determine if any snails are alive.

13.5.3.2 The waders and wader boots are then rinsed using ATL water. The rinseate is poured through a mesh filter and any filtered material is placed in a clean container and sealed for later examination to determine the presence, sizes and numbers of NZMS, and to determine if any snails are alive.

13.5.3.3 The waders and wader boots are examined using appropriate magnification equipment to determine if NZMS remain and if they are alive or dead. If NZMS

are present and determined to be alive the numbers and estimated sizes of snails are recorded. Locations where the snails are found on the waders and wader boots is recorded and the waders and wader boots are actively scrubbed with a brush and rinsed using ATL control water. They are reexamined and returned to the fishermen once it is determined that all NZMS have been removed.

13.6 The effectiveness of immersion decontamination method is to be investigated and compared to the dry sack method and the spray bottle method to determine if the test solutions are effective using any of the three decontamination methods under field conditions. Following completion of the initial Phase III trials it will be decided if other decontamination techniques are to be investigated further.

Appendix 2:

Distribution Of New Zealand Mud Snails In Filtrate Samples From Field Trials Of Cleaning Solutions And Methods

®Distribution and numbers of NZMS, by size class, for individual sets of wading gear from field trials at Putah Creek, Yolo County California, March 5-6, 2005.

Wader #	Treatment #	Size Classes 6mm	5mm	4mm	3mm	2mm	1mm	Total	Treatment Total	Treatment Mean
3	1	0	0	0	0	0	1	1		
5	1	0	0	1	3	4	10	18		
32	1	0	2	0	2	2	9	15		
38	1	0	0	0	8	11	23	42		
43	1	0	0	24	0	23	48	95		
47	1	0	1	0	11	0	23	35		
49	1	0	0	0	0	0	2	2	208	30
7	2	5	28	49	16	21	98	217		
9	2	0	0	0	0	1	7	8		
13	2	0	0	4	0	6	15	25		
15	2	0	0	3	2	0	10	15		
28	2	0	0	0	0	2	22	24		
39	2	0	25	8	7	36	151	227		
45	2	0	0	0	2	0	6	8	524	75
8	3	0	1	6	4	0	2	13		
12	3	0	1	2	1	1	6	11		
18	3	0	3	7	5	17	45	77		
22	3	0	1	0	0	1	0	2		
30	3	0	0	1	1	3	4	9		
33	3	0	11	14	8	8	48	89		
48	3	0	0	0	8	12	19	39		
51	3	0	0	0	2	9	26	37	277	35
4	4	0	0	2	2	1	6	11		
20	4	3	7	9	6	7	28	60		
26	4	0	7	20	27	30	91	175		
29	4	0	4	2	2	3	6	17		
50	4	0	0	0	3	0	4	7		

41	4	0	0	4	6	7	18	35		
44	4	0	0	10	5	2	28	45		
53	4	0	0	0	1	3	4	8	358	45
1	5	0	1	1	2	0	2	6		
6	5	0	0	1	1	4	37	43		
11	5	0	0	0	10	6	14	30		
19	5	0	0	0	1	1	0	2		
24	5	0	0	0	1	1	4	6		
25	5	0	4	0	2	2	8	16		
27	5	0	11	9	0	7	16	43	146	21
2	6	0	1	1	0	0	2	4		
10	6	0	1	0	0	2	11	14		
17	6	0	0	0	1	2	4	7		
21	6	0	0	0	1	3	6	10		
31	6	0	0	0	1	3	4	8		
35	6	0	0	0	1	0	3	4		
40	6	0	0	0	3	2	2	7		
54	6	0	0	0	2	6	4	12	66	8
14	7	0	0	2	6	3	21	32		
16	7	0	7	6	1	14	16	44		
23	7	0	0	3	2	13	15	33		
34	7	0	0	4	0	2	7	13		
52	7	0	0	0	1	6	10	17		
42	7	0	0	0	0	0	6	6		
46	7	0	0	0	0	1	5	6	151	22
Totals by size class		8	116	193	168	288	957	1730		
Mean # snails by size class		0.1	2	4	3	6	18	33		

Appendix 3.

Proposed Cleaning Procedure for NZMS Infested Wading Gear

The following procedures for cleaning NZMS infested wading gear can be followed upon exiting NZMS infested waters. Wading gear should be cleaned prior to leaving the site. If this is not possible then wading gear should be completely sealed inside of a large plastic bag and cleaned before it is used in any other waters. Three different cleaning protocols have been tested and found to be effective using specific cleaning solutions:

1) Immersion Procedure
    a. Remove wading gear upon exiting NZMS infested waters. **Avoid allowing infested wading gear to come in contact with interior surfaces of vehicles or camping gear such as tents or trailers.** NZMS can be transferred to any surface they come in contact with and they could later be transferred back to cleaned wading gear. Turn waders right side out and remove insoles from wading boots.
    b. Place waders, wading boots, boot insoles and the streambed contact end of a wading stick, if used, in a container of sufficient size to allow the gear to be completely covered by a cleaning solution.
    c. Pour sufficient cleaning solution into the container with the infested wading gear to completely cover the gear. It may be necessary to weight down the gear to ensure that it remains immersed in the cleaning solution.
    d. Allow the wading gear to remain in the cleaning solution for at least 5 minutes.
    e. Remove the wading gear from the cleaning solution one piece at a time and inspect it to make sure that all debris that could harbor NZMS has been removed from the gear as well as any NZMS that could be lodged in cracks or crevices. If necessary, use a stiff plastic bristled brush such as a kitchen brush to remove any remaining debris and mud.
    f. Rinse wading gear in clean water. **DO NOT USE WATER FROM THE NZMS INFESTED SOURCE**. This may reintroduce NZMS to the wading gear.
    g. Return cleaned wading gear to it appropriate storage container.

2) Dry Sack Procedure
    a. Remove wading gear upon exiting NZMS infested waters. **Avoid allowing infested wading gear to come in contact with interior surfaces of vehicles or camping gear such as tents or trailers.** NZMS can be transferred to any surface they come in contact with and they could later be transferred back to cleaned wading gear. Turn waders right side out and remove insoles from wading boots.
    b. Place waders, wading boots, and boot insoles into a dry sack (recommended size: 65 liter). Walking sticks will need to be cleaned separately outside of the dry sack to avoid rupturing the sack.
    c. Add 8 to 10 liters of cleaning solution to dry sack and the seal dry sack.
    d. Pick up the dry sack and shake it back and forth using a rolling motion to ensure that the contents are thoroughly coated with the cleaning solution. Continue shaking for approximately 30 seconds.

e. Let dry sack sit undisturbed for at least 5 minutes. Then repeat the shaking and mixing for another 30 seconds.
f. Open the dry sack and remove the contents one piece at a time and inspect it to make sure that all debris that could harbor NZMS has been removed from the gear as well as any NZMS that could be lodged in cracks or crevices. If necessary, use a stiff plastic bristled brush such as a kitchen brush to remove any remaining debris and mud.
g. Rinse wading gear in clean water. **DO NOT USE WATER FROM THE NZMS INFESTED SOURCE**. This may reintroduce NZMS to the wading gear.
h. Return cleaned wading gear to it appropriate storage container.

3) Spray Bottle Procedure (**Note:** this procedure has only been tested using a copper sulfate cleaning solution).
   a. Remove wading gear upon exiting NZMS infested waters. **Avoid allowing infested wading gear to come in contact with interior surfaces of vehicles or camping gear such as tents or trailers.** NZMS can be transferred to any surface they come in contact with and they could later be transferred back to cleaned wading gear. Turn waders right side out and remove insoles from wading boots.
   b. Place waders, wading boots, boot insoles and the streambed contact end of a wading stick, if used, in a container of sufficient size to allow the gear to be easily accessed.
   c. Using a standard 1 liter squeeze-trigger type spray bottle containing the cleaning solution, spray the wading gear to the point of saturation and runoff with the cleaning solution. Be sure to treat the inside of the wading boots as well as the outside. Use the stream setting to be sure and dislodge any debris from the wading boots. Be sure to treat both top and under side of gravel guards if they are permanently attached to the waders.
   d. Allow the wading gear to set for at least 5 minutes with the cleaning solution on it. Remove the wading gear one piece at a time and inspect it to make sure that all debris that could harbor NZMS has been removed from the gear as well as any NZMS that could be lodged in cracks or crevices. If necessary, use a stiff plastic bristled brush such as a kitchen brush to remove any remaining debris and mud.
   e. Rinse wading gear in clean water. **DO NOT USE WATER FROM THE NZMS INFESTED SOURCE**. This may reintroduce NZMS to the wading gear.
   f. Return cleaned wading gear to it appropriate storage container.

4) Cleaning Solutions.
   a. Copper sulfate: Dissolve 3.785 grams of copper sulfate pentahydrate crystals (99.1% purity) for each gallon of solution you want to make. This will achieve a concentration of 252 mg/L of copper in the cleaning solution.

b. Benzethonium chloride: Dissolve 7.57 grams of benzethonium chloride (97% purity) for each gallon of cleaning solution you want to make. This will achieve a concentration of 1,947 mg/L in the cleaning solution.
c. Formula 409® Disinfectant: Dilute the commercially available solution 1:1 with clean water to achieve the needed concentration for the cleaning solution (i.e. 1 gallon of Formula 409® Disinfectant to 1 gallon of water).