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Tolerance of the Invasive New Zealand Mud Snail To Various Decontamination Procedures

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I Hereby Reaffirm the Lawrence University Honor Code

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Abstract

In an attempt to stop the spread of invasive species, state governments have established decontamination procedures for use on contaminated equipment. However, different species can tolerate various procedures depending on their morphology and physiology. The New Zealand mud snail (*Potamopyrgus antipodarum*) is invasive to the United States and may alter the food web of streams due to the snail's high reproductive ability, causing potential problems for native trout populations and local economies. We collected mud snails from the recently invaded Black Earth Creek, WI and tested their tolerance to decontamination protocols being considered by the Wisconsin Department of Natural Resources. Treatments included immersion in bleach (200 & 400 ppm), salt (35 ppt), full strength Formula 409, and the standard disinfectant Virkon (2.0%). We also tested effectiveness of spraying vs. immersion of Formula 409 and interference of mud with the cleaning procedure. Snails remained viable after immersion for up to 30 minutes in bleach and salt baths but exposure in Formula 409 baths killed all snails after 10 minutes. The effectiveness of spraying was more variable than immersion. However the percentage mortality in both techniques was significantly decreased by the presence of mud. These results provide a scientific basis for future invasive species management decisions.

Introduction

Aquatic Invasive Species and Impacts

Aquatic invasive species are defined by the US Fish & Wildlife Service as "aquatic organisms that invade ecosystems beyond their natural, historic range" (US Fish and Wildlife Service 2014b). These species have the potential to affect the biodiversity and ecosystem of the invaded area. These species may also affect human commercial, agricultural, or recreational activities in the invaded ecosystem.

Invasive species impact economies as well as ecosystems. Estimates have been calculated that the United States economy loses about $120 billion annually to invasions in terms of invasion control and management costs (Simberloff 2013). The zebra mussel (*Dreissena polymorpha*) is an example of an invasive species that costs the economy an estimated $1 billion annually, with a majority of this cost coming from the cleaning of water intake pipes clogged by the mussel. Impacts on the ecosystem can vary from minimal to immense. The zebra mussel colonizes habitat quickly, out-competes other mollusks, and filters water very quickly. Increased water filtering rates by zebra mussels often changes water quality in the habitat, in turn causing decreased phytoplankton populations. Such changes in the lower food web then lead to decreases in zooplankton and fish populations (Simberloff 2013). Invasive species, such as the zebra mussel, can cause changes to the ecosystem that have large impacts on native flora and fauna.

The New Zealand mud snail (*Potamopyrgus antipodarum*) is another aquatic invasive species in the United States that has the potential to have significant impacts on freshwater ecosystems and the economy. The effect of the snail is not well understood, and there are

conflicting conclusions from published studies. Strzelec (2005) found a negative correlation between the mud snail and species richness as well as abundance of native invertebrates, while Schreiber *et al.* (2002) found a positive correlation. However, due to its life history and reported population densities of up to 300,000 snails/m^2, the mud snail has the potential to displace and compete with native invertebrates (Benson *et al.* 2015, Hall *et al.* 2003). Native fish feed on native invertebrates and increased abundance of mud snails could negatively affect native invertebrates resulting in decreased fish populations and changes in the trophic levels and food web structure of infested aquatic ecosystems. With decreases in fish populations, local and state economies that are at least in part supported by the fishing industry may be negatively impacted. As a result, there is a need to study the New Zealand mud snail related to both its effect on aquatic ecosystems as well as how to limit the inadvertent transportation of the snail between bodies of water. The focus of our study was to address factors related to the spread of the New Zealand mud snail by testing its tolerance to decontamination procedures that might be used by the public.

Morphology

The length of *P. antipodarum* is usually between 4-6 mm in invasive populations, but has been measured up to 12 mm in New Zealand populations (Levri *et al.* 2007, Zaranko *et al.* 1997). The shell is dextral with a right hand coil pattern and elongated with 6-8 whorls separated by grooves (Fig. 1a). Some morphs have a keel in the middle of each whorl while others are smooth. It is believed that this keel exists as an anti-predator trait (Holomuzki and Biggs 2000, Levri *et al*. 2007, Zaranko *et al.* 1997). The aperture of the shell is oval in shape and

is covered by the operculum when the snail retracts into the shell (Fig. 1b). Shell color varies between gray, dark brown, and light brown.

a. b.

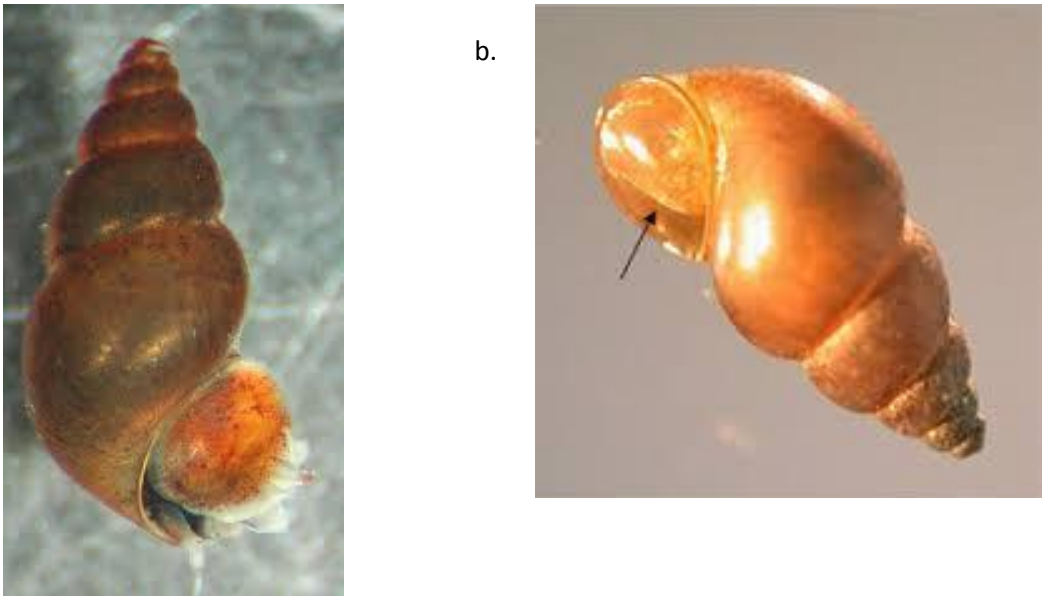


Figure 1: New Zealand mud snail. A) Identification factors include a right hand coil pattern and 6-8 whorls separated by grooves (Photo from Gustafson (2009)). B) Black arrow indicates the operculum (Photo from New Zealand mud snail (2012)).

Preferred Habitats

The New Zealand mud snail is endemic to the islands of New Zealand and adjacent islands (Gangloff 1998). The species primarily lives in freshwater but is euryhaline and can tolerate salinities of 30-35‰, preferring salinities between 0-15‰ (Gerard *et al.* 2003, Jacobsen and Forbes 1997, Zaranko *et al.* 1997). While not thriving in high salinity, tolerance of these conditions allows the New Zealand mud snail to have an increased range of dispersal.

The snail thrives in areas of high nutrient flow such as streams and the littoral zone of lakes. These areas support a higher growth rate of green algae, a significant source of the snail's diet. The snail feeds nocturnally on epiphytic and periphytic algae, diatoms, and detritus from

plants and animals. It can retain residence in areas of high flow by burrowing into the sediment and can also tolerate siltation (Collier *et al*. 1998, Holomuzki and Biggs 1999, Holomuzki and Biggs 2000, Negovetic and Jokela 2000, Weatherhead and James 2001, Zaranko *et al.* 1997). *Potamopyrgus antipodarum* have been reported to occur at depths of 4-45 m, indicating that depth is not a significant limitation on the snail's habitat use (Levri *et al.* 2007, Zaranko *et al*. 1997). The ecology of this animal provides a foundation for the current global invasion. Its ability to thrive in both high flow areas as well as in low flow, littoral zones of lakes provides the snail with multiple invasion strategies and gives it a high invasion success rate when introduced to new habitats.

Physiology

As would be expected from a successful aquatic invasive species, New Zealand mud snails are very tolerant of a range of environmental conditions. However, there is an optimal range of particular conditions for snail growth and reproduction. A summary of the physiological range of mud snails was adapted by Therriault *et al*. (2010; Table 1). New Zealand mud snails thrive in high levels of calcium, alkalinity, and water hardness. Environments that are more basic tend to be more suitable for the snail as well. Optimal temperature ranges are estimated to be between 20-26°C but snails can tolerate temperatures between 0° and 35°C. Conductivity and TDS (total dissolved solids) are not limiting factors for survival of this snail species. The physiology of the mud snail is very adaptable, indicating the high potential for successful future invasions into new ecosystems.

Table 1: Physiological Range of New Zealand mud snails. Adapted from Therriault *et al.* (2010).

Parameter	No Potential for Adult Survival	Little Potential for Egg Development	Moderate Potential for Nuisance Infestation	High Potential for Massive Infestation
Calcium (mg Ca/L)	<2	2-4	4-7	7-?
pH	<6.0?	6.0-6.8	6.9-7.5	7.5-8.5?
Alkalinity (mg CaCO/L)	<10	10-50	50-100	100-350
Hardness (mg $CaCO_3$/L)	<10	10-50	50-100	100-350
Temperature (°C)	<0 and >35	0-15 and 30-35	15-20 and 26-30	20-26
Conductivity (µS/cm)	<25 and >46,000	25-200 and 25,000-46,000	200-1,200 and 9,000-25,000	1,200-9,000
TDS (mg/L)	<17 and >30,800	17-130 and 16,800-30,800	130-800 and 6,000-16,800	800-6,000
Salinity (‰)	<0.01 and >30	0.01-0.1 and 15.0-30.0	0.1-2.0 and 5-15	2.0-5.0

Population Control

One of the key characteristics that aids the New Zealand mud snail in its successful invasion of the United States is its rapid rate of colonization. This ability to rapidly colonize new locations is due to the reproductive biology of the snail. The species is ovoviviparous with young developing inside the female and then being born as fully functioning adults. Female snails can reproduce both sexually and asexually. In the United States, populations are almost entirely clonal due to asexual reproduction, with all individuals genetically identical to the founding mothers that initially invaded the location. Consequently, this results in nearly 100% female populations (Zaranko *et al.* 1997). During asexual reproduction, females produce offspring by parthenogenesis where eggs do not require fertilization to develop (Wallace 1992, Dybdahl and Lively 1995). This reproductive strategy results in the ability for a single individual to establish a

new population. Each individual can produce 230 offspring per year, resulting in population densities as high as 500,000 individuals per m^2 (Hall *et al.* 2003).

Predators of mud snails differ between New Zealand and the United States. In New Zealand, gut analyses documented the presence of mud snails in the diets of short- and long-finned eels (*Anguilla australis*, *A. dieffehbachii*), brown trout (*Salmo trutta)*, and bullies (*Gobiomorphus cotidianus*). However, it was unclear whether the snails had been selectively ingested or ingested accidently along with other prey (McDowall 1990, Levri 1998). In the United States, several species of fish eat the mud snail. However, some fish have been observed to avoid them. In a 2004 study by Cada, only one mud snail was found in the gut content analysis of 29 brown trout and 17 sculpin (*Cottoidei cottoidea*). However, there are anecdotal reports of mountain whitefish (*Prosopium williamsoni*) stomachs containing many mud snails (Proctor *et al.* 2007). Bersine *et al.* (2007) also noted that mud snails were in the diet of juvenile Chinook salmon (*Oncorhynchus tshawytscha*).

While New Zealand mud snails clearly have natural predators in New Zealand, there is no significant evidence that these populations are controlled by predators (Nyström and McIntosh 2003). Parasites do have the potential to be biological control agents of mud snails. In New Zealand, there are 14 known trematode parasites that inhibit host reproduction by sterilizing the host. Experimental studies testing the trematode as a biological control agent of New Zealand mud snails have yielded positive results (Dybdahl *et al*. 2005). One such trematode is *Microphallus sp.* and observations indicate that this species is highly specific (Lively and Dybdahl 2000). However, there are other issues of concern related to introducing

another invasive species as a biological control agent, including potential side effects of the parasite on the rest of the ecosystem.

Introduction to the United States

New Zealand mud snails were first discovered in the United States in 1987 but have spread quickly across the country (Fig. 2). There are three known genotype clones of the New Zealand mud snail. The western clone was first found in the Snake River in Idaho before reaching the Madison River in Montana in 1995. The next year, the snail was found in Yellowstone National Park. In 1997, populations were found in Oregon in the Columbia River and in the Owens River in California. Since then, the species has become established in the Green River in Utah (2001), the Colorado River in Arizona (2002), and in Boulder Creek in Colorado (2004; Proctor *et al*. 2007).

The eastern clone was first discovered in the Great Lakes region in Lake Ontario (1991; Zaranko *et al*. 1997). Since then, it has been found in Lake Superior (2001; Grigorovich *et al*. 2003), Lake Erie (2005; Levri *et al.* 2007), and Lake Michigan (2006; Benson *et al*. 2015). The only currently known population in the state of Wisconsin is in Black Earth Creek in Dane County and is the eastern clone. The eastern and western clones are morphologically nearly identical (Proctor *et al*. 2007).

A third clone was discovered in a short section of the Snake River in Idaho. Genetic testing has verified that it is a separate genotype; no match has been found to any other known mud snail genotype (Proctor *et al*. 2007).

New Zealand mud snails are believed to have been introduced to the Great Lakes in ballast water from European cargo ships originating where there are nonindigenous populations (Leppäkoski & Olenin 2000, Levri *et al.* 2007, Zaranko *et al.* 1997). Another theory suggests that the snails were introduced in the western rivers of the United States through water containing live game fish imported from infested waters containing the snail (Proctor *et al*. 2007).

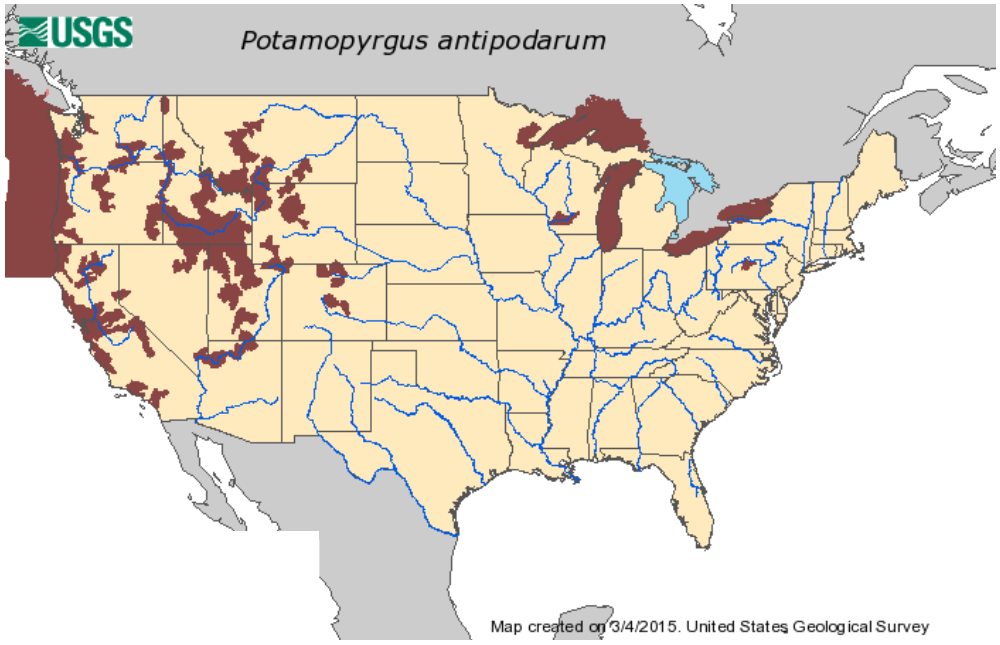


Figure 2: Distribution map of the New Zealand mud snail in the continental United States. Dark brown coloration indicates water bodies with confirmed populations. Map from Benson *et al.* (2015).

Transportation Vectors

Anthropogenic activities are one of the primary means facilitating the invasion success of the New Zealand mud snail (Dwyer 2003, Richards 2002, Richards *et al.* 2004). Snails have been documented to be trapped on boots and stuck in nets following their use in an

environment containing the mud snail. As the snail can survive out of water for up to 25 days if moist (Winterbourn 1970), gear not efficiently decontaminated and then used in a new water system can result in introduction and potential invasion success of the snail. As previously discussed, the snail can survive in ballast water of ships and thus invade new countries and continents.

In addition to anthropogenic vectors, the New Zealand mud snail can be transported through other means. It has the ability to survive passage through fish and bird digestive tracts and remain reproductively viable (Bersine *et al.* 2008, Bruce 2006, Haynes *et al.* 1985). Since the snail can reproduce parthenogenetically, only one snail needs to survive the passage through the gut of a fish or bird and be introduced into a new area to establish a new population. The snail has been documented attaching to fallen leaves in the aquatic environment, floating by itself, or attaching to mats of *Cladophora* (green algae) and subsequently being transported to new areas for potential colonization (Zaranko *et al*. 1997).

Management and Control

The management of New Zealand mud snails falls under the jurisdiction of each state. Several states (including Wisconsin, Minnesota, Colorado, Utah, Washington, and Wyoming) list the mud snail as "prohibited" which is defined as "illegal to possess, transport, or import without special license" (Proctor *et al.* 2007). Other states do not have specific regulations on the New Zealand mud snail but general laws prohibit the transportation and possession of aquatic invasive species. All states put the emphasis on reducing the spread of the mud snail from one body of water to another because any means of total eradication from the ecosystem

using physical or chemical methods would be time consuming and/or have negative impacts on the ecosystem.

In terms of decontaminating equipment, control of *P. antipodarum* is also not under federal regulation and states maintain their own policies. In the report prepared for the Aquatic Nuisance Species Task Force, Proctor *et al.* (2007) recommend "cleaning all mud and debris that might harbor NZ mud snails from boots, waders and gear with a stiff brush" and then using freezing and desiccation techniques. Freezing is described as putting gear in a freezer for 6-8 hours which will result in the mortality of all mud snails. Putting gear in water maintained at 48.9°C was also reported to kill all mud snails (Richards *et al.* 2004). It is also recommended that drying gear at 28.9-30°C for at least 24 hours or at 40°F for at least two hours results in snail mortality (Richards *et al.* 2004). These control measures are sufficient if 1) scientific, commercial, and recreational users have facilities large enough for these recommendations and 2) if users will consent to use these procedures on their own equipment.

Due to the difficulty of implementing these recommendations for all users, there has been limited research into effective field decontamination techniques. The California Department of Fish and Game conducted laboratory tests on mud snails to determine effectiveness of chemical procedures. They concluded the most effective chemicals to be copper sulfate (252 mg/L Cu), benzethonium chloride (1,940 mg/L) and 50% Formula 409 solution for 5 minutes (Hosea and Finlayson 2005). Schisler *et al.* (2008) contradicted these findings with results indicating the 5 minute 50% Formula 409 solution was not sufficient in achieving 100% snail mortality. They recommended a 10-min exposure in 100% Formula 409 or 10-min exposure to a 3.1% Sparquat solution. Due to these contradictory findings, our research

focused on further testing chemical means to effectively decontaminate materials potentially transporting New Zealand mud snails.

Research Questions

Since the mud snail is invasive in the United States and its effect on ecosystems is not well known, state legislatures have emphasized management activities focused on reducing the spread of this species. However, there is little scientific research on the best decontamination practices for commercial and recreational equipment to stop the species from being transported by humans. Our research tested the tolerance of the New Zealand mud snail to decontamination procedures applied to various materials. We focused on three categories of questions that guided our research: application, material, and chemical. These categories were tested for effectiveness of reaching 100% snail mortality. We also wanted our procedures to be practical in real life scenarios that could potentially be utilized by the public, researchers, and management agencies, such as the Wisconsin Department of Natural Resources staff.

This research focused on five main research questions; Application: 1) Does the application of the chemical in spray or immersion techniques have an effect on mud snail survivorship? 2) Does the presence of mud decrease the effectiveness of the spray and immersion techniques? Material: 3) What effect does the material have on the effectiveness of decontamination techniques? Chemical: 4) Are the currently recommended Virkon and bleach decontamination procedures effective? and 5) Is salt an effective decontamination chemical for New Zealand mud snails?

Methods

Animal Collection

Snails were collected from Black Earth Creek, WI (43.1250°N, -89.7102°W) on 7/23/2014 and 8/6/2014 next to South Valley Road, Black Earth, WI. On 7/23/2014, the weather conditions were sunny with few clouds. Air temperature was 24°C and water temperature was 19°C. Snails were collected at 11:00 am. Dip nettings and hand collection of rocks with attached snails were the primary methods of collection. Snails were very abundant in the creek and approximately 10,000 were collected. On 8/6/2014, the weather conditions were similar as it was sunny with an air temperature of 23°C. Collection time was 12:00 pm. An estimated 3,000 snails were collected on this second date.

Once collected, snails were placed in Rubbermaid tubs filled with water taken from Black Earth Creek. Snails were transported back to Lawrence University in these holding bins in accordance with WI Administrative Code NR 40 and all applicable permitting requirements under the Lawrence University Scientific Collector's Permit. In the laboratory, bins were placed in a controlled environment aquarium room at a constant temperature of 20°C.

To clean collection equipment and waders, a Virkon Aquatic 2.0% solution was prepared as recommended by the Wisconsin Department of Natural Resources. Collection equipment and waders were submerged for 20 minutes and then rinsed with tap water.

Snail Length and Operculum Measurements

The length of the shell and size of the operculum opening were determined for 310 snails used in the experiments. Shells of previously frozen snails were aligned vertically in the

imaging frame of a Sony Handycam Model HDR-HC9 camcorder attached to a dissecting microscope. Images were taken of the shells and then calibrated using a stage micrometer. Shell length and operculum opening size were measured using the program Image J (ver. 1.48). Shell length was calculated from the tip of the whorls of the shell to the bottom of the shell below the operculum opening. The operculum opening was measured vertically to permit a comparison to shell length.

Experimental Setup

Throughout the testing, a range of immersion and spray times were employed for different tests and materials. However, each test shared common practices and followed a general procedure with conditioning, testing, and recovery phases (Fig. 3). Each test included 5 replicates for each time tested (Fig. 4). Each replicate consisted of 15 snails placed on the material in a glass finger bowl. For each test, 3 replicate control containers with 15 snails in each bowl were also tested using collected river water. Snails were selected from those attached to the side of the holding bin to ensure test animals were healthy at the beginning of the experiment. Snails were placed in their assigned replicate bowl on the tested material with approximately 100 mL of water. Following a conditioning period of one hour during which the replicates were kept in the aquarium room, the snails were brought into the laboratory. The material, along with the snails, was moved to an empty glass finger bowl in which the chosen decontamination procedure took place.

Snails were tested using one of two techniques. A spray technique was tested in which each replicate was sprayed with a light mist consisting of the chosen chemical solution 5 times

from a spray bottle bought from a local hardware store. The volume of chemical delivered by this procedure was estimated by choosing the desired force of spray from the nozzle and then spraying 5 times into a graduated cylinder. The collected liquid from this spray technique averaged 5.1 mL (SD= 0.211, n=10). The spray covered the material and the bottom of the bowl so all surfaces were coated evenly. An immersion technique was also employed in which the replicate was fully submerged in the chemical solution. The solution bath volume varied depending on the depth of the test material. However, both the snails and the material were completely immersed for the duration of the test. Following the selected spray or immersion technique and time, the material and snails were moved to another finger bowl for a 20 minute recovery bath with 40 mL of 0.45-µm filtered (Millipore brand membrane filters) Black Earth Creek water at 22°C. This was intended to simulate the transfer of snails into a new body of water. Following 20 minutes, each snail was tested to determine the total number surviving. We decided that the snail was alive if there was movement of any kind, including retraction further into the shell, when touched with a blunt metal probe, as utilized by Stockton & Moffitt (2013).

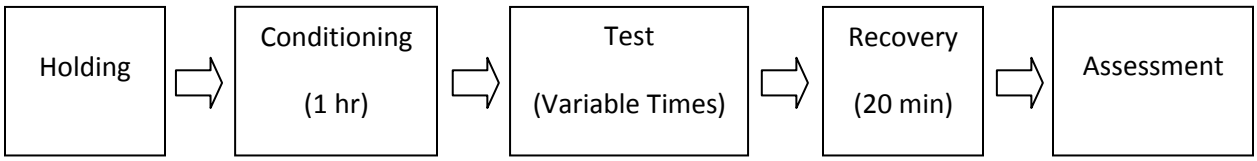


Figure 3: General .Procedure for Test Methods.

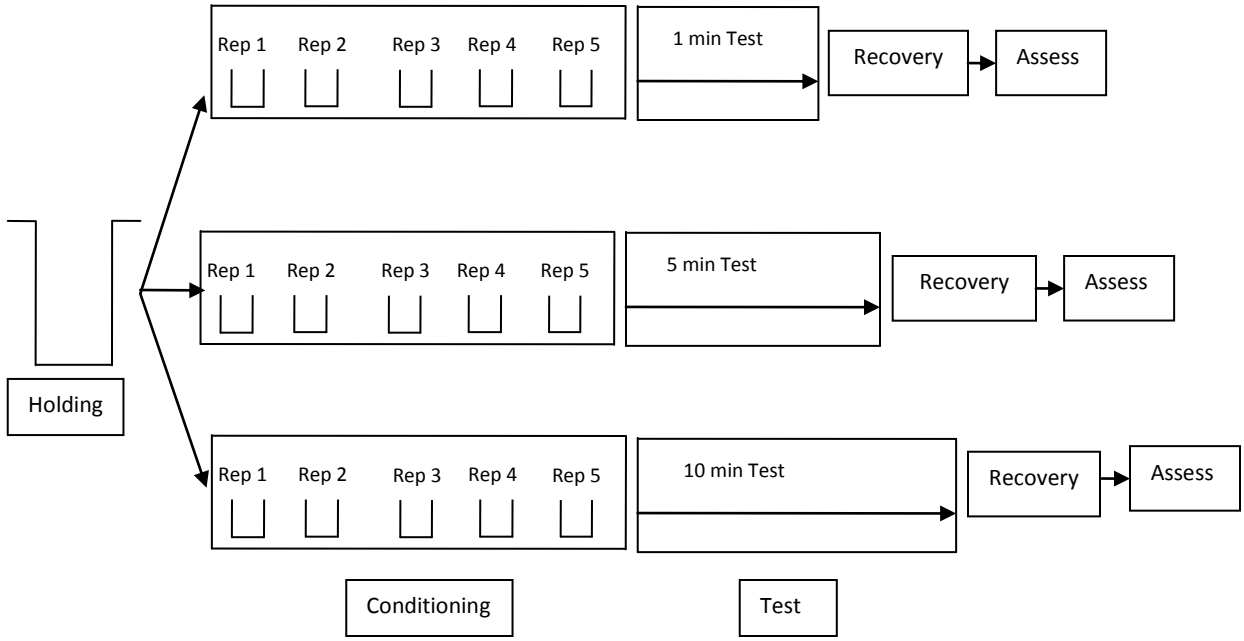


Figure 4: Diagram of Standard Test Method.

Test Materials

Rubber

One material tested for decontamination effectiveness was rubber from waders. This material was taken from the sole of Hodgeman brand waders (size 10). Wader boot soles/treads were cut into approximately 1”x1” (2.5 cm x 2.5 cm) squares using a table saw and scissors. Each piece of rubber was placed such that the treads were facing upward and the snails were placed in and on these treads in an attempt to mimic a realistic situation with snails being transported in the treads of rubber boots/waders.

Felt

Felt was also tested for decontamination effectiveness. Felt from felt soled waders (Caddis Systems Replacement Felt Sole kit, La Pine, OR) was cut into approximately 1”x1” (2.5

cm x 2.5 cm) squares using a retractable box cutter. Snails were placed directly onto the felt surface to mimic situations in which they might be transported in real-world scenarios.

Canvas

Canvas was cut into approximately 1”x1” (2.5 cm x 2.5 cm) squares using scissors. Snails were placed on one side of the square. This material was chosen to mimic transport and effectiveness of decontamination procedures on both scientific (e.g. ecological dip nets) and recreational equipment (e.g. waders, fishermen bags, etc).

Decontamination Solutions

2.0% Virkon Aquatic

Virkon Aquatic (2.0%) is recommended by the Wisconsin Department of Natural Resources (WI DNR) as the standard decontamination agent for New Zealand mud snails (Wisconsin Department of Natural Resources 2013). We conducted several preliminary tests to determine the effectiveness of this solution. The solution was made by mixing 75.6 g of Virkon Aquatic with 1 gallon of aged tap water. This solution was employed only for the immersion techniques.

100% Formula 409

Several studies have cited Formula 409 as effective when attempting to decontaminate materials from New Zealand mud snail infested waters. However, the effectiveness across different materials has not been documented. Formula 409 solution was obtained at local retail stores. This solution was used in both spray and immersion techniques on the tested materials.

50% Formula 409

Some research has indicated that a 50% Formula 409 solution was just as effective as a 100% solution at decontaminating New Zealand mud snails (Hosea and Finlayson 2005). Our research tested the effectiveness of 50% Formula 409 in a limited number of experiments, but testing of this solution was halted due to time constraints.

Bleach

Due to its observed ability to kill many organisms such as bacteria and other microbes, bleach has been recommended by some states as a means to stop the spread of New Zealand mud snails. We tested 200 ppm and 400 ppm solutions of bleach. Bleach solutions were created according to WI DNR recommendations by adding one or two tablespoons of Clorox Bleach per gallon of aged tap water for the 200 and 400 ppm solutions, respectively.

Salt

Some theories suggest that because New Zealand mud snails are native to freshwater, saltwater might be an effective decontamination solution. We tested this hypothesis by creating a 3.5% (35 ppt) salt water solution, effectively the same as naturally occurring sea water. This solution was made by adding ½ cup (118.29 cm^3) of NaCl (i.e. Morton table salt) to one gallon (3.79 L) of aged tap water.

Treatment Combinations

Due to the large number of possible combinations of techniques, materials and solutions, as well as time constraints, we prioritized some tests over others (Table 2). However, we put priority on tests that would be useful under realistic situations. As a result, we shifted our later efforts onto the effectiveness of Formula 409 when mud was present on the

materials. We created mud that was a mixture of 100 g of dirt (Green-Gro potting soil) and 60 mL of aged-tap water. For mud treatments, one tablespoon of mud was added on top of the snails following the conditioning period of one hour. Any snails that had moved off of the test material during the conditioning period were manually placed into the mud. Following the experimental time, the material containing the mud and snails was placed in the recovery bath with the mud side facing downward in the bowl.

Table 2: Summary of the solutions, materials, and techniques tested.

		Rubber		Felt		Canvas	
		No Mud	Mud	No Mud	Mud	No Mud	Mud
Virkon	Spray						
	Immersion	X		X		X	
409	Spray	X	X	X	X	X	X
	Immersion	X	X	X	X	X	X
200 ppm Bleach	Spray						
	Immersion	X		X		X	
400 ppm Bleach	Spray						
	Immersion					X	
Salt	Spray						
	Immersion					X	

Time of Experiments

Previous research on decontamination of materials containing New Zealand mud snails indicated that 10 minutes would be sufficient to result in 100% snail mortality in full strength Formula 409 (Hosea and Finlayson 2005, Schisler *et al*. 2008). Using this knowledge, we wanted to test whether this same time frame would be effective for all solutions and materials. As a result, we decided to begin testing with 1, 5, and 10 minute contact times. For some combinations we tested times for longer than ten minutes because snails were still surviving at

high rates after ten minutes. We extended exposure times for up to 30 minutes given that the target user for these decontamination procedures was the general public. This was based on the idea that if users moved between water systems, 30 minutes would be a reasonable time between leaving one water system and arriving at another. In addition, tests were ceased once 100% mortality was found across all five replicates. For these reasons, maximum testing time varied for different combinations of solutions and materials (Table 3).

Table 3: Summary of the times tested (minutes) for the combinations of various solutions, materials, and techniques.

		Rubber		Felt		Canvas	
		No Mud	Mud	No Mud	Mud	No Mud	Mud
Virkon	Spray						
	Immersion	1,5,10		1,5,10		1,5,10	
409	Spray	1,5,10	5,10,15,20,25,30	1,5,10	5,10,15,20,25,30	1,5,10	5,10,15,20,25,30
	Immersion	1,5,10	5,10,15	1,5,10	5,10,15,20,25,30	1,5,10	5,10,15
200 ppm Bleach	Spray						
	Immersion	1,5,10		5,10,15,20,25,30		1,5,10,15	
400 ppm Bleach	Spray						
	Immersion					5,10,15,20	
Salt	Spray						
	Immersion					5,10,15,20,25,30	

Confidence Intervals

In order to compare the overall effectiveness of the various techniques, mean and 95% confidence intervals were calculated for each combination of chemical, method of application, and material. This comparison was based on snail survivorship at the maximum time employed for each combination.

Calculations

Statistical tests and graph construction were created using the Paleontological Statistics software package (PAST; Hammer *et al*. 2001) and Microsoft Excel (Office 2007). Data were examined for normality and homogeneity of variance prior to statistical analysis and transformed to meet assumptions of tests. We employed the arcsine transformation which is recommended for percentage data (McDonald 2009). Two-way analysis of variance (ANOVA) with replication was used to compare percent survivorship across treatment conditions and times. P-values were considered significant when equal to or less than 0.05.

Results

Controls

In all experiments, snail survivorship in the controls was significantly greater than snail survivorship in the treatments. Snail survivorship of New Zealand mud snails in the laboratory setting was at or near 100% across all tests with a minimum survival percentage of 93.3% (Table 4). This percentage indicates the death of one snail out of the fifteen in a given replicate.

Table 4: Summary table of number of replicates with different percent survivorship values for each control treatment. N=number of replicates.

Control	% Survival		N
	100	93.3	
Water	243	0	243
Water+Material	232	14	246
Water+Material+Mud	90	0	90

Shell Length and Operculum Opening

Median shell length of the tested New Zealand mud snails was 4.39 mm and lengths ranged from 2.45 to 5.5 mm (Fig. 5). Lengths exhibited a slightly negative skew (skewness= -0.81) and were leptokurtic (kurtosis=2.16). Operculum opening had a median value of 1.28 mm with a range of 0.66 mm to 1.75 mm. Operculum measurements were essentially normally distributed (skewness=-0.04; kurtosis=0.05). Operculum opening was relatively consistent, and was not significantly correlated with length of the shell (p=0.7; Fig. 6).

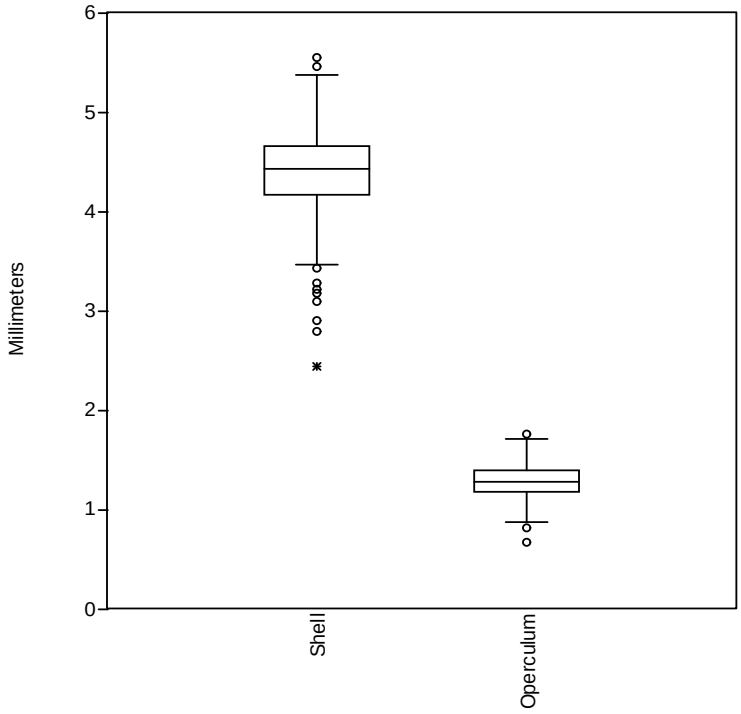


Fig. 5: Shell and operculum opening lengths. Open circles indicate outliers.

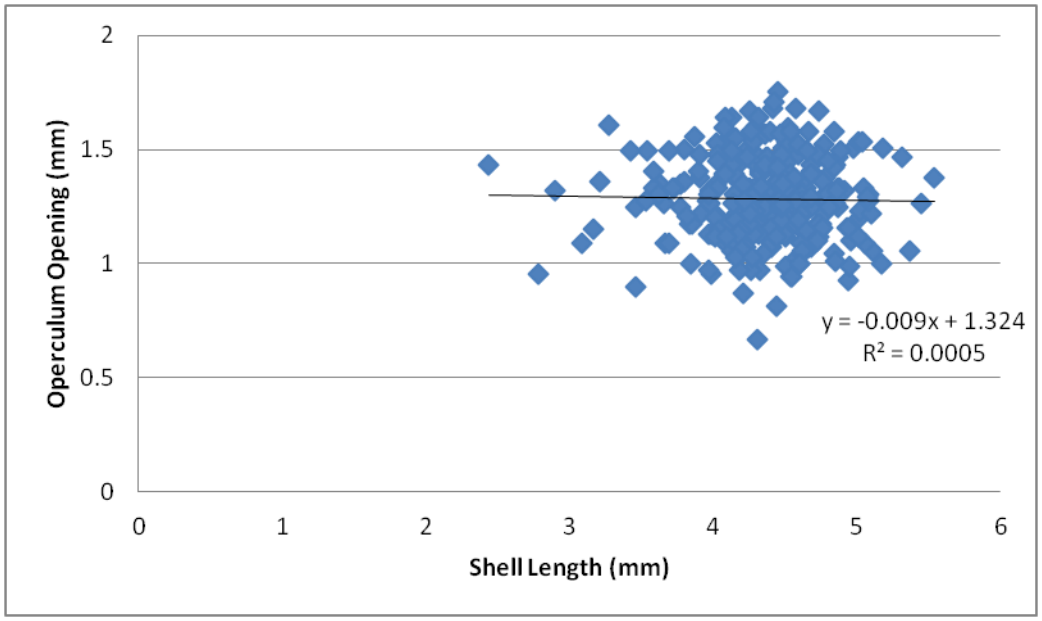


Fig. 6: Relationship between shell length and operculum opening. Linear relationship was slightly negative. Shell length and operculum opening were not correlated (p=0.7).

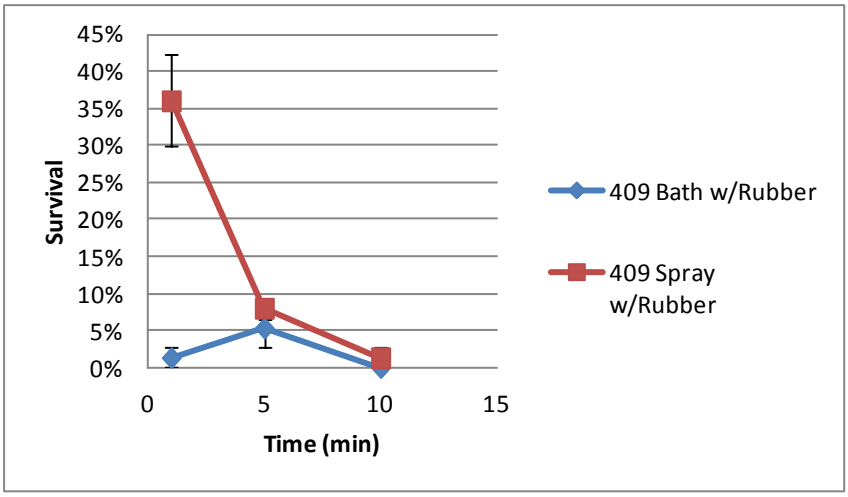
Spray vs. Immersion Technique Effectiveness

To test the effectiveness between the spray and immersion technique, we compared Formula 409 spray and immersion techniques for each material without mud (Fig. 7). On rubber, the survival percentage at one minute differed dramatically between the spray and immersion technique (Fig. 7a). However, at both the five and ten minute intervals, both techniques had similar survival rates. For rubber, there was a significant difference between the spray and immersion techniques as well as a significant effect of time on snail survivorship (Table 5; $p<0.01$ and $p<0.01$, respectively).

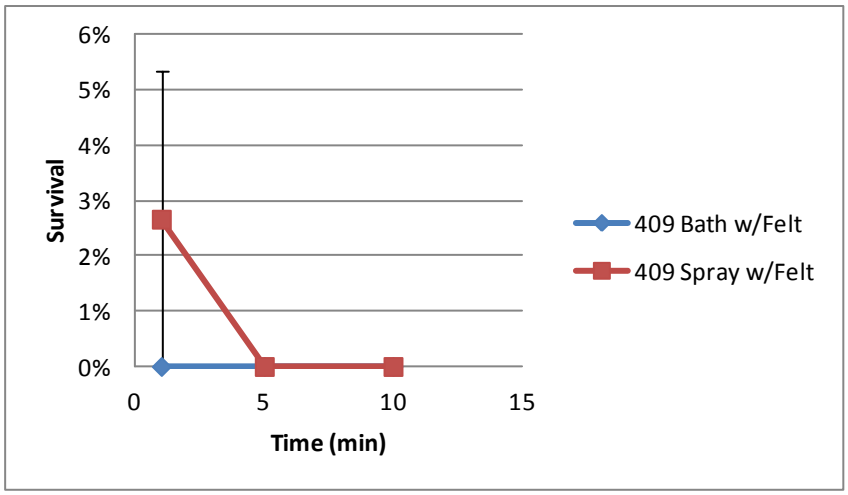
On felt, the percent survival at all three time intervals tested was very low as only the one minute test of Formula 409 spray did not result in 0% survivorship (Fig. 7b). Spray resulted in higher snail survivorship than immersion on felt and there was also a significant effect of time (Table 5; $p=0.03$ and $p=0.01$, respectively). The effect of the immersion and spray techniques resulted in different trends across time as snail survivorship decreased after spraying and was consistently 0% at all immersion times tested ($p=0.01$).

On canvas, spraying with Formula 409 resulted in decreasing survivorship as exposure time increased (Fig. 7c). Interestingly, there was no significant overall difference between the spray and immersion techniques (Table 5; $p=0.10$). However, snail survivorship was higher using the spray technique across time compared to the immersion technique ($p<0.01$). This pattern was due to the effect of time as the interaction of technique and time was not significant ($p=0.74$). Comparing the spray and immersion techniques overall, the effect of time was significant on snail survivorship across all three materials (Table 5).

a.



b.



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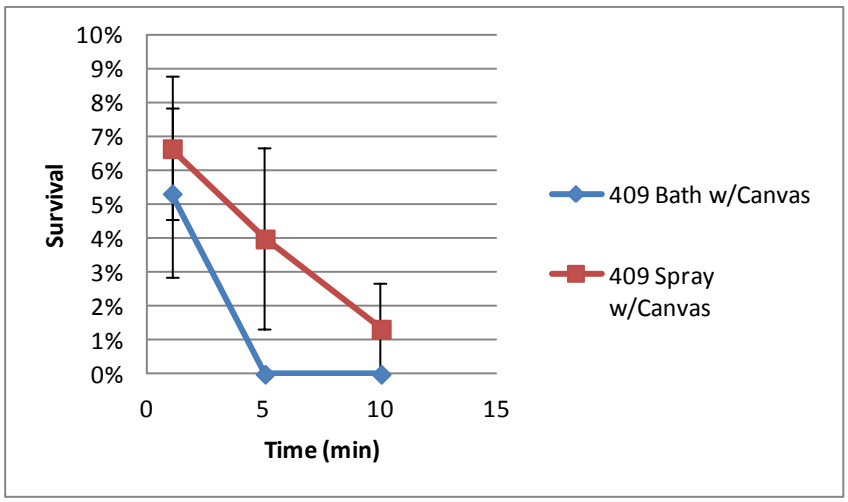


Fig. 7: Survivorship of snails exposed to Formula 409 using bath and spray techniques on three different materials: (a) rubber, (b) felt, (c) canvas. Y-axis denotes % survival of the snails at the given time interval. Error bars are ± 1 SE.

Table 5: P-values from two-way ANOVA for the effect of immersion and spray techniques on snail survivorship. Degrees of freedom indicated by "df": Tr=Treatment, Ti=Time, I=Interaction. Red highlight indicates significant values (alpha value equal to 0.05).

Treatments	Treatment	Time	Interaction	df (Tr, Ti, I)
409 Bath vs Spray_Rubber	4.71E-06	8.98E-06	3.486E-05	1,2,2
409 Bath vs Spray_Felt	0.027	0.010	0.010	1,2,2
409 Bath vs Spray_Canvas	0.103	0.009	0.744	1,2,2

Effect of Mud

After completing tests employing both immersion and spray techniques using Formula 409, the same tests were repeated with a coating of mud over the mud snails. Mud generally increased survivorship for all treatments tested (Fig. 8, Table 6) and had similar effects in all combinations, as evidenced by non-significant interaction effects (Table 6). The effect of time was not significant for nearly all tests with the exception of the comparison between the spray technique on rubber with and without mud. Mud therefore inhibited the effectiveness of Formula 409 across time (Fig. 8b and 8c).

On rubber, at all times tested in common, snail survival was higher in the mud tests than the non-mud test when the spray technique was utilized (Fig. 8a). The effectiveness of Formula 409 was significantly decreased when mud was present in the spray experiment (Table 6; $p<0.01$). Interestingly, there was also a significant difference in snail survivorship across time when the spray technique was utilized on mud ($p=0.03$). The overall decreasing trend in snail survivorship is evident in both spray technique experiments. For the immersion technique, the 5 and 10 minute times were again compared and a similar pattern was seen between the tests with and without mud. However, Formula 409 was found to be equally effective when mud was

present (p=0.42). Percent survival was found to be 0% following a 15 minute immersion with mud compared to 0% survival following a 10 minute immersion when mud was absent. The effect of time was not significant in the immersion technique tests on rubber (p=0.42).

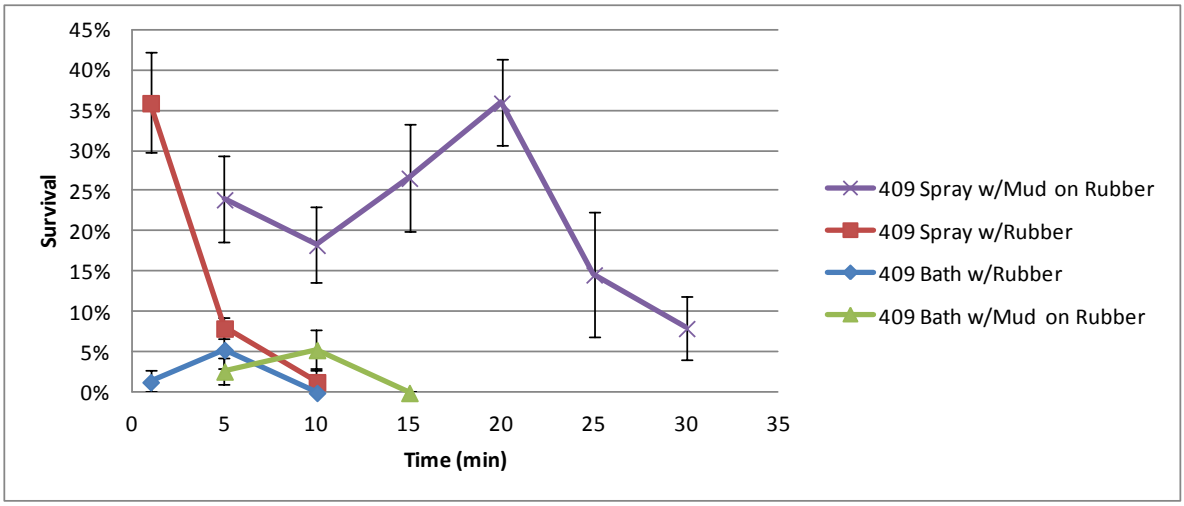
On felt, there is a clear distinction between the spray technique tests with mud and without mud (Fig. 8b). The effectiveness of Formula 409 on snail survival decreased when mud was present (p<0.01). Time did not have a significant effect on snail survivorship and there was not a significant interaction between time and mud effect (p=0.48, p=0.48 respectively). The flat trend across time for the spray test involving mud indicates that a longer contact time with the Formula 409 spray was not effective for eliminating snails. The immersion technique test results with and without mud display a similar relationship to the spray technique tests with and without mud. Overall, percent survival was higher when mud was present for the immersion technique. As a result, the effectiveness of Formula 409 was decreased when mud was present (p=0.01). The effect of time was not significant when mud was present (p=0.41).

On canvas, a distinction between percent survival was observed between the spray technique tests with and without mud (Fig. 8c). Again, Formula 409 effectiveness was decreased in the tests with mud compared to the test without mud (p=0.02). An unexplained spike in survivorship occurred during the 20-minute exposure in the spray technique test with mud. Snail survival was not significantly decreased by longer contact time with Formula 409 following the spray technique (p=0.34). For the immersion technique, the observed findings are similar to those of the spray technique. The effectiveness of Formula 409 in decontamination of mud snails was decreased when mud was present using the immersion technique on canvas

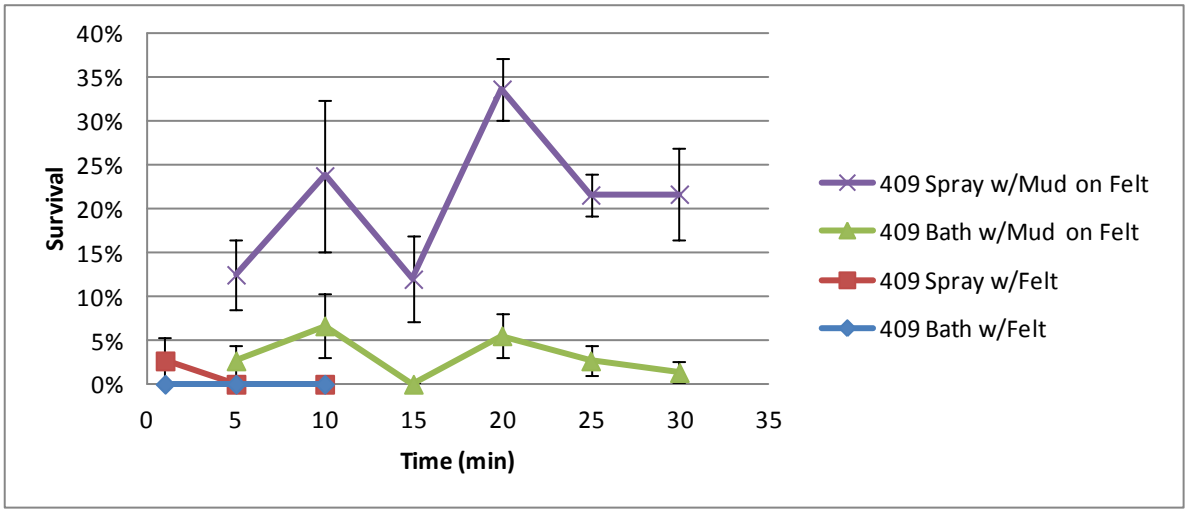
($p<0.01$). Similar to the spray technique, time was not a significant factor in determining snail survivorship ($p=0.11$).

For the spray technique tests involving mud, only the test on rubber displayed a decreasing snail survivorship trend across time. On felt and canvas, snail survivorship either increased or did not change with increased Formula 409 contact time. None of the spray tests with mud successfully killed all of the snails, even after 30 minutes of exposure (Fig. 8).

a.



b.



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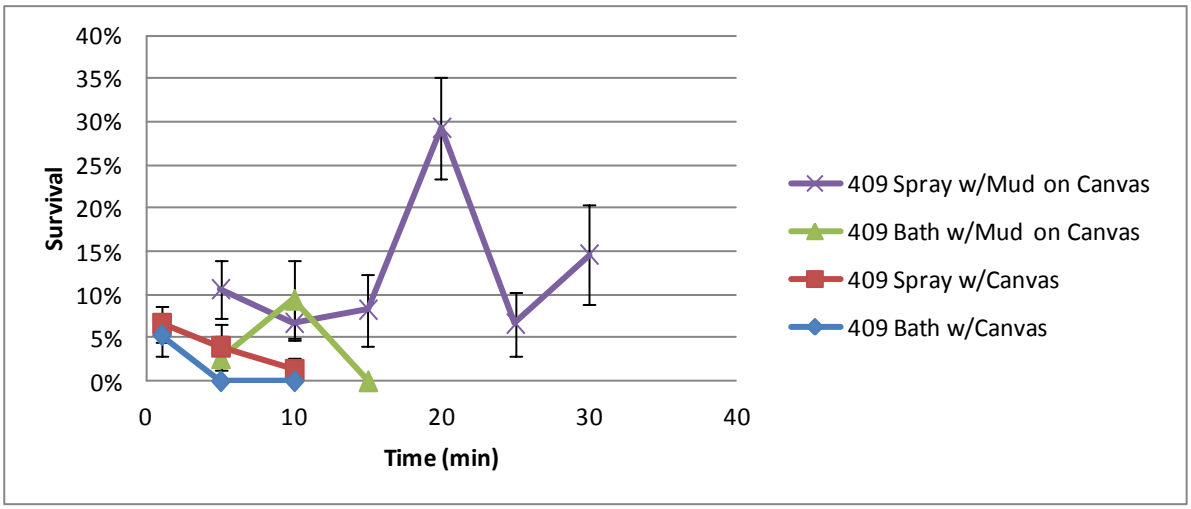


Fig. 8: Efficiency of 409 bath and spray techniques with and without mud on three different materials: (a) rubber, (b) felt, (c) canvas. Y-axis denotes % survival of the snails at the given time interval. Error bars are ± 1 SE.

Table 6: P-values from two-way ANOVA for the effect of mud on snail survivorship after 5 and 10 minute exposure times. Degrees of freedom indicated by "df": M=Mud, T=Time, I=Interaction. Red highlight indicates significant values (alpha value equal to 0.05).

Treatments	Mud	Time	Interaction	df (M, T, I)
409 Spray vs Spray Mud_Rubber	0.001	0.026	0.346	1,1,1
409 Bath vs Bath Mud_Rubber	0.416	0.416	0.059	1,1,1
409 Spray vs Spray Mud_Felt	4.59E-05	0.479	0.479	1,1,1
409 Bath vs Bath Mud_Felt	0.014	0.409	0.409	1,1,1
409 Spray vs Spray Mud_Canvas	0.024	0.344	0.923	1,1,1
409 Bath vs Bath Mud_Canvas	0.008	0.110	0.110	1,1,1

Effect on Different Materials

To test the effect of decontamination procedures on different materials, tests involving the same chemical on these different materials were compared (Fig. 9). The effects of the immersion technique in Formula 409 resulted in low snail survival percentages across all materials (Fig. 9a). The effectiveness of Formula 409 on rubber was lower than on felt (Table 7; p=0.02). When the snails were on rubber, 0% survivorship was not achieved until 10 minutes. In contrast, there was 0% survivorship on felt at 1, 5, and 10 minutes. However, the time of the immersion did not have a significant effect on snail survivorship between rubber and felt (p=0.07). The overall patterns of survivorship over time were higher when snails were on rubber compared to on felt, but this pattern was not significant (p=0.07).

In comparing the survival percentages between rubber and canvas with Formula 409 immersion, the two materials had no differing effects on snail mortality (Fig. 9a, Table 7; p=0.67). As a result, the time of immersion on these two materials did not result in significant differences in survivorship (p=0.06). However, the materials did have contrasting trends on snail mortality across time. While there was higher snail survivorship on the canvas after one

minute, this trend was reversed as more snails survived the 5 minute bath on the rubber ($p=0.02$). The effects of canvas and felt were significantly different as a higher snail survivorship occurred on canvas than felt following the 1 minute test ($p=0.03$). The effect of the time of immersion also differed between these materials as indicated by the decrease in survivorship from the 1 to 5 minute immersion test on canvas ($p=0.01$). Felt exhibited 0% snail survivorship while canvas had a decreasing trend across time ($p=0.01$). There was no significant difference due to material or time of immersion for the Formula 409 immersion tests ($p=0.057$, $p=0.057$ respectively). However, there were differences in the trends of each material across time as evidenced by the differing patterns of snail survivorship on rubber and canvas ($p=0.005$). Variations in the significant effects of material, time, and interaction between material and time indicate a complicated array of effects leading to snail mortality (Table 7).

For the Formula 409 spray technique, decreasing trends of survivability were observed as time increases on all three materials (Fig. 9b). Between rubber and felt, snail survivorship was lower on felt following all three immersion times indicating that there was a difference in snail survivorship between the materials and between immersion times ($p<0.01$ and $p<0.01$ respectively). While snail survivorship decreased across time for both rubber and felt, there was a more drastic decrease in survivorship on rubber than on felt ($p<0.01$). Similar results were observed between rubber and canvas. Rubber resulted in higher survivorship of snails than canvas and this pattern was consistent at each time interval ($p<0.01$ and $p<0.01$ respectively). In addition, the higher snail survivorship on rubber was consistently higher across time compared to canvas ($p<0.01$). In comparing the materials of felt and canvas, the effect of the material on snail survivability with the Formula 409 spray technique was not as differentiated.

No significant difference in survivorship was found between the materials ($p=0.10$). However, the immersion time had a significant effect on snail survivorship as survivorship was higher on canvas than felt ($p<0.01$). Across all immersion times, canvas and felt had similar decreasing trends of snail survivorship ($p=0.74$). For the Formula 409 spray tests, significant effects of material, time, and the interaction of material and time were observed ($p<0.01$, $p<0.01$, $p=0.003$ respectively).

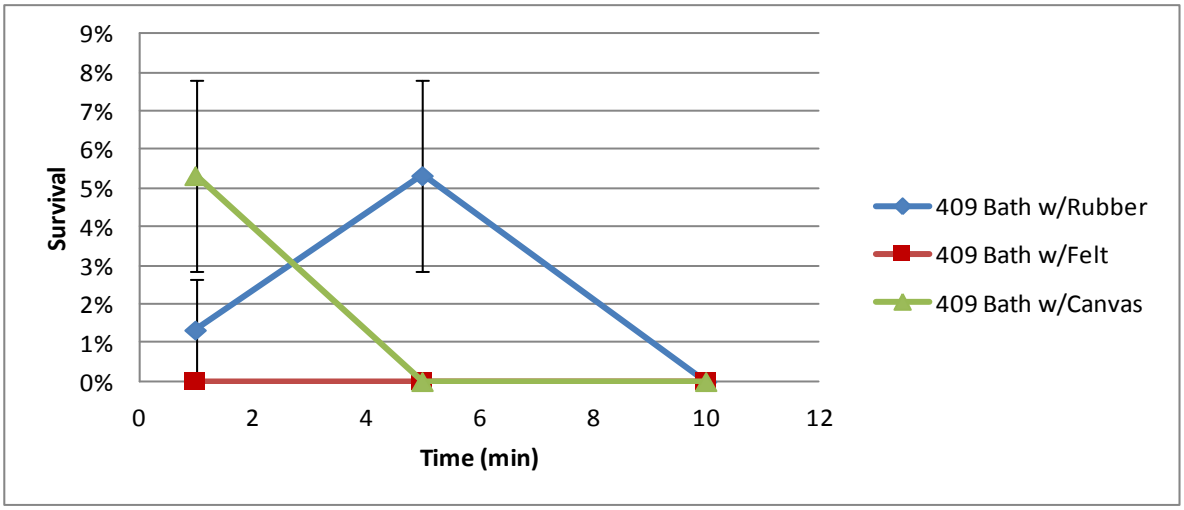
Due to the differences in testing techniques, the three bleach tests had different time intervals and immersion times. This limited the times at which the different materials could be evaluated as only times that were the same between the tests could be compared.

Between rubber and felt treatments with bleach, there was a significant difference between the materials as snail survivorship was higher on rubber (Fig. 9c; Table 7; $p<0.01$). However, this pattern was not significant for all three compared immersion times of 5, 10, and 15 minutes ($p=0.54$). In addition, the effect of time was different for rubber and felt as snail survivorship on rubber increased from the 5 minute test to the 10 minute test and then decreased in the 15 minute test. On felt, an opposite pattern was observed as snail survivorship decreased from the 5 to 10 minute tests and then increased in the 15 minute immersion test (interaction effect, $p=0.12$).

For the rubber and canvas tests, there was no significant difference between the materials or time as snail survivorship was similar in the compared immersion tests of 5 and 10 minutes ($p=0.25$, $p=0.16$ respectively). The trends across time also differed as survivorship increased on rubber and decreased on felt between the 5 and 10 minute tests.

In comparing the results of felt and canvas, a significant difference was found between the materials as snail mortality was much lower on felt (Table 7; $p<0.01$). However, time did not cause differences in survivorship for both felt and canvas and the interactions across materials and the compared times of 5 and 10 minutes were also similar ($p=0.41$, 0.39 respectively). Across all materials, the material did have an effect on snail survivorship ($p<0.01$). However, both time and the interaction of material and time were not significant ($p=0.36$, $p=0.34$ respectively).

a.



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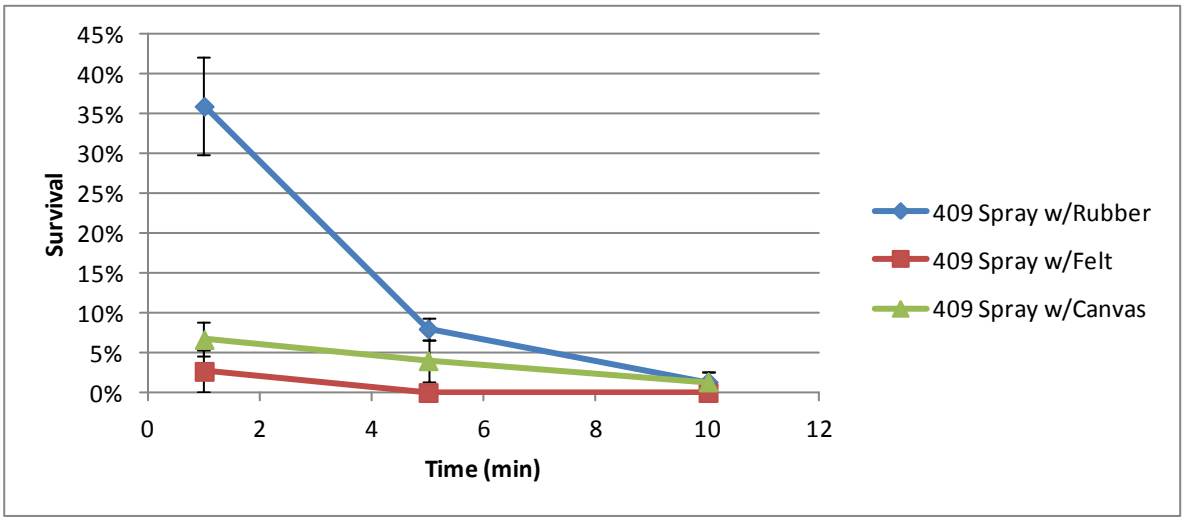

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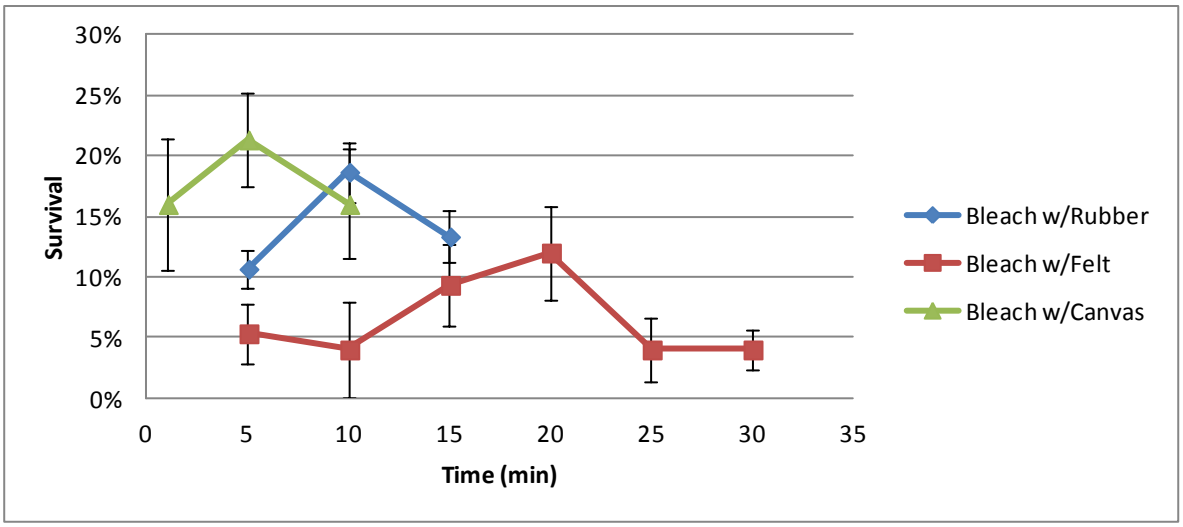


Fig. 9: Comparison across the three test materials using chemical treatments: (a) 409 bath, (b) 409 spray, (c) bleach. Y-axis denotes % survival of the snails at the given time interval. Error bars are ± 1 SE.

Table 7: P-values from two-way ANOVA for the effect of material on snail survivorship. Degrees of freedom indicated by "df": M=Material, T=Time, I=Interaction. Red highlight indicates significant values (alpha value equal to 0.05).

Treatments	Material	Time	Interaction	df (M, T, I)
409 Bath_All Materials	0.057	0.057	0.005	2,2,4
409 Bath_Rubber vs Bath_Felt	0.019	0.069	0.069	1,2,2
409 Bath_Rubber vs Bath_Canvas	0.666	0.064	0.016	1,2,2
409 Bath_Felt vs Bath_Canvas	0.027	0.010	0.010	1,2,2
409 Spray_All Materials	2.17E-06	8.1E-08	0.003	2,2,4
409 Spray_Rubber vs Spray_Felt	3.93E-07	8.12E-08	0.001	1,2,2
409 Spray_Rubber vs Spray_Canvas	0.001	4.02E-06	0.007	1,2,2
409 Spray_Felt vs Spray_Canvas	0.103	0.009	0.744	1,2,2
Bleach_All Materials	9.77E-05	0.362	0.337	2,2,4
Bleach_Rubber vs Bleach_Felt	0.001	0.538	0.121	1,2,2
Bleach_Rubber vs Bleach_Canvas	0.250	0.163	0.809	1,2,2
Bleach_Felt vs Bleach_Canvas	0.001	0.414	0.391	1,2,2

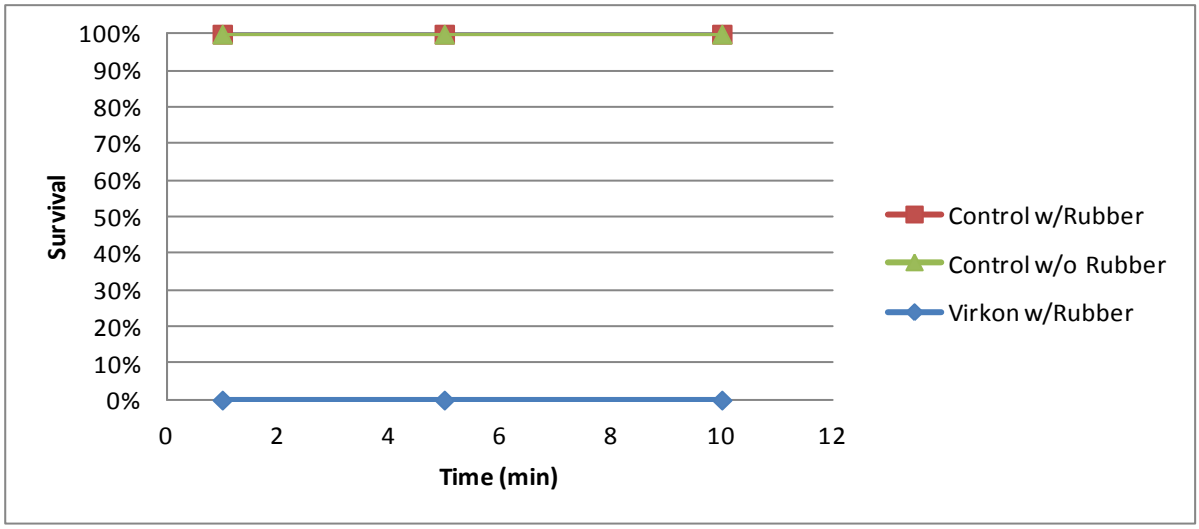
Virkon and Bleach Recommendations

Virkon Aquatic, the currently recommended chemical treatment by the Wisconsin DNR, resulted in 0% snail survivorship across all test times (Fig. 10). This indicates that this chemical is effective for disinfecting gear exposed to the New Zealand mud snail. As snail survivorship in the controls was 100% across all tests, the 0% snail survivorship was attributed to the effect of Virkon Aquatic.

Bleach did not cause 0% snail survivorship on any of the three tested materials (Fig. 9c). However, the material did have an effect on snail survivorship ($p<0.01$). Recall that time was not significant in determining snail survivorship in the bleach tests (Table 6). Bleach is not effective at achieving 100% mortality, considering multiple snails survived the procedure at 200 ppm. However, snail survivorship decreased to 0% at the 5, 10, and 20 minute exposure times when bleach concentrations were increased to 400 ppm (Fig. 11). This was a significantly lower survivorship in bleach than observed in the controls ($p<0.01$). Both time and interaction of

bleach and time were not significant, indicating the low survivorship across time (Table 8). This result gives promise to the effectiveness of bleach at higher concentrations on the decontamination of adult New Zealand mud snails.

a.



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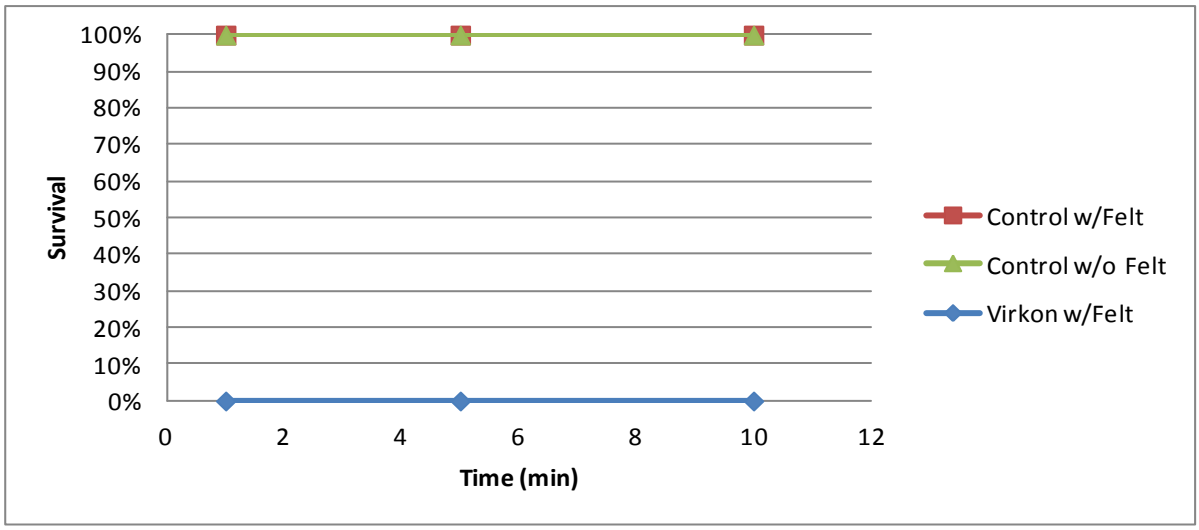

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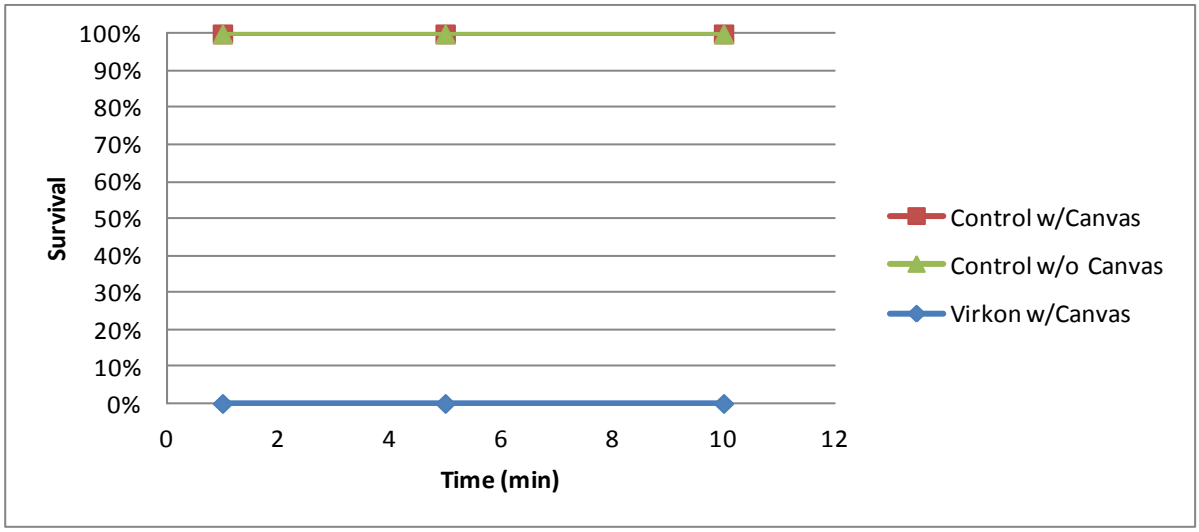

Fig. 10: Comparison across the three test materials using Virkon: (a) rubber, (b) felt, (c) canvas. All tests were done using the immersion technique. Y-axis denotes % survival of the snails at the given time interval. Error bars are ± 1 SE.

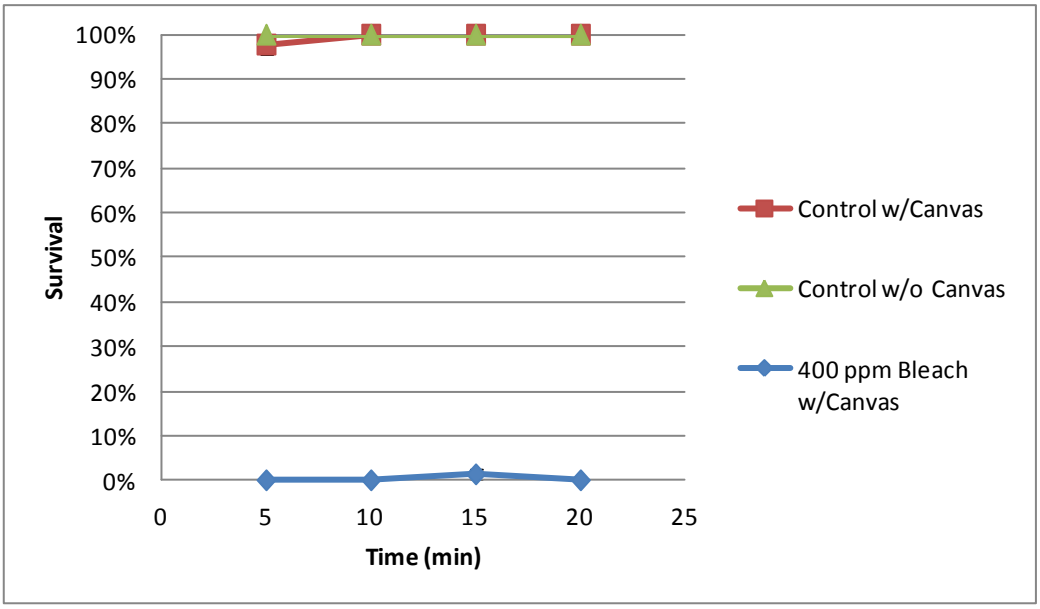

Fig. 11: Effect of 400 ppm concentration bleach on snail survivorship. Y-axis denotes % survival of the snails at the given time interval. Error bars are ± 1 SE.

Table 8: P-values from two-way ANOVA for the effect of 400 ppm bleach on snail survivorship. Degrees of freedom indicated by "df": B=Bleach, T=Time, I=Interaction. Red highlight indicates significant values (alpha value equal to 0.05).

Treatments	Bleach	Time	Interaction	df (B,T,I)
Bleach_Canvas vs Control	7.53E-61	0.058	0.128	2,3,6

Salt as Decontamination Chemical

Snail immersion in the salt solution never reached the desired 0% survival but did decrease sharply between the 15 to 20 minute contact times (Fig. 12). This decrease occurred only between these test times and displayed a flat pattern between 5, 10, 15 and then 20, 25, and 30 minutes. The salt solution, time, and the interaction of salt and time were all significant indicating salt decreases survivorship (Table 9). As 0% survivorship was not reached, salt is not effective as a decontamination chemical.

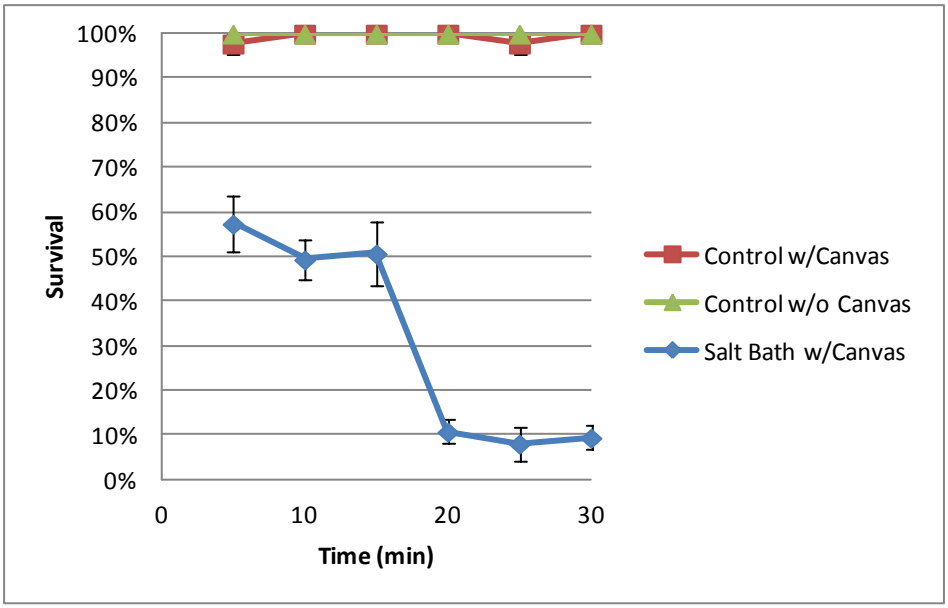

Fig. 12: Effect of salt as a decontamination chemical. Y-axis denotes % survival of the snails at the given time interval. Error bars are ± 1 SE.

Table 9: P-values from two-way ANOVA for the effect of salt on snail survivorship. Degrees of freedom indicated by "df": S=Salt, T=Time, I=Interaction. Red highlight indicates significant values (alpha value equal to 0.05).

Treatments	Salt	Time	Interaction	df (S, T, I)
Salt_Canvas vs Control	4.35E-57	8.12E-12	3.42479E-16	2,5,10

Overall Effectiveness

In the immersion technique, neither 200 ppm bleach nor 3.5% salt solutions reached 0% survivorship (Fig. 13). When Formula 409 was tested on felt with mud added, the confidence interval overlapped with 0% survivorship. For all other tests using the immersion technique, snail mortality was 100% at the maximum time tested.

When Formula 409 was sprayed on snails without mud on rubber and canvas, the 95% confidence interval crossed 0% snail survivorship (Fig. 14). Using the spray technique, average

snail mortality reached 100% only on felt at the maximum time tested. The spray technique with the presence of mud was not effective in reaching 0% snail survivorship on any of the three materials tested.

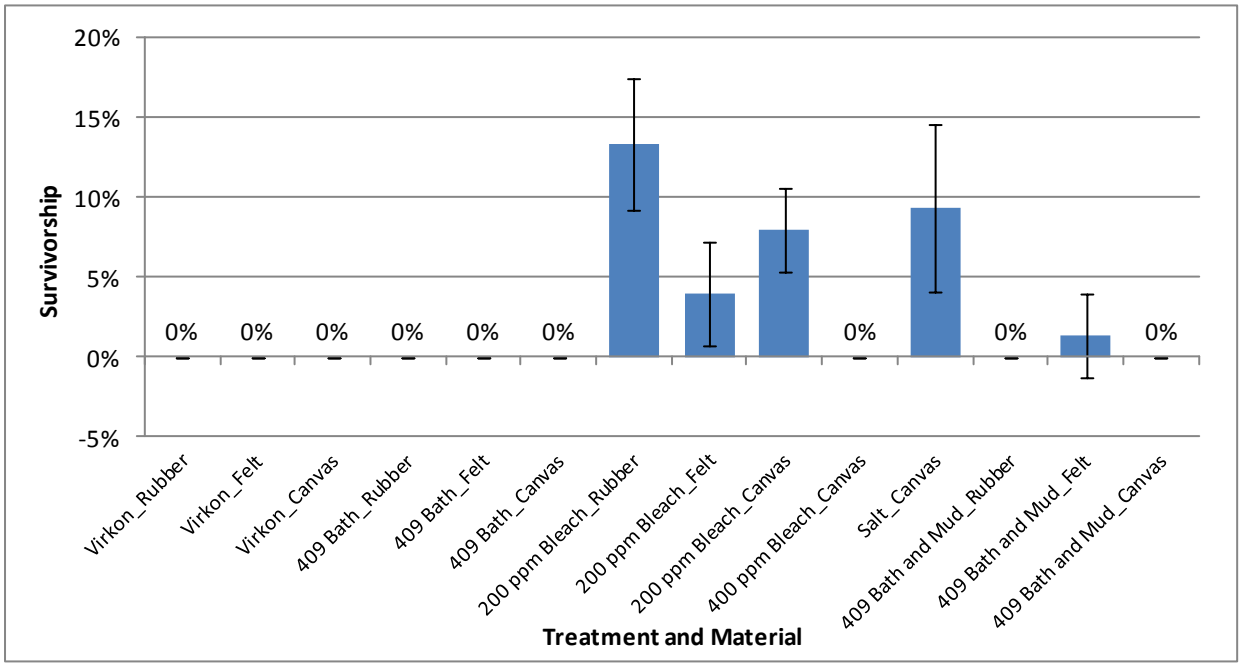

Fig. 13: Snail survival (mean and 95 % confidence interval) after the maximum time of exposure to Formula 409 in immersion technique tests. Y-axis denotes % survival of the snails at the given time interval.

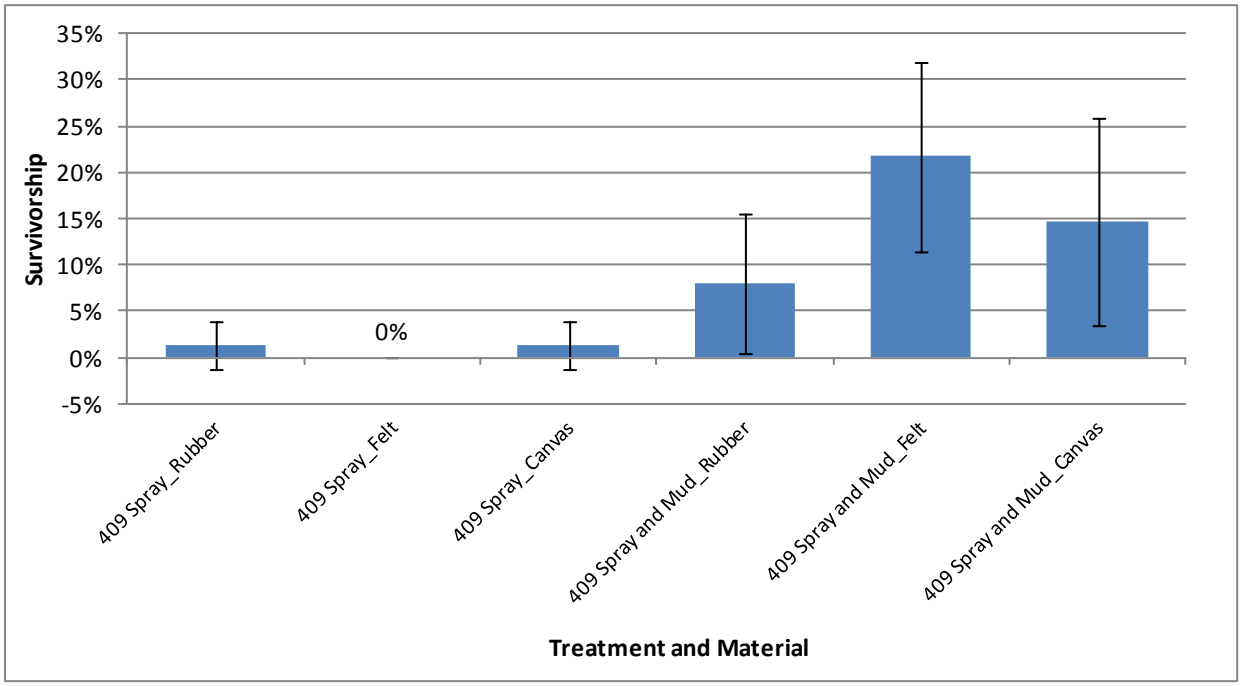

Fig. 14: Snail survival (mean and 95% confidence interval) after the maximum time of exposure to Formula 409 in spray technique tests. Y-axis denotes % survival of the snails at the given time interval.

Discussion

Recommended Treatment

The most effective decontamination procedure for the New Zealand mud snail (NZMS) was the immersion technique using either a 2.0% Virkon Aquatic solution for 15 minutes or a 100% Formula 409 solution for 10 minutes. Both of the chemicals in the immersion technique resulted in 100% snail mortality. Therefore the standard Wisconsin DNR recommended chemical cleaning procedure of 20 minutes in 2.0% Virkon Aquatic can ensure 100% snail mortality. However full strength Formula 409 offers a more economical and accessible option for achieving 100% NZMS mortality on infested gear. In addition, obtaining and using Virkon Aquatic presents more of a challenge and potential health risk to the user than Formula 409. Neither bleach nor salt water disinfection were found to be effective using realistic times of exposure.

Our results agree with previous New Zealand mud snail decontamination studies. Snail mortality of 100% was reached following a 10 minute exposure to a 100% Formula 409 solution (Schisler *et al.* 2008). In their project, Schisler *et al*. observed snail vitality following both a 48 hour and 56 day recovery bath when exposed to lower concentrations of the chemical. In a test studying the effectiveness of a 50% Formula 409 solution, snails appearing to be dead following the 48 hour test were still alive and reproductive following a 56 day recovery. However, their results indicated a 0% survival of snails in both the 48 hour and 56 day recovery baths when a 100% Formula 409 immersion technique was utilized. This further supports our results and recommendation of at least a 10 minute 100% Formula 409 immersion technique.

Stockton & Moffitt (2013) recommended a 2.0% Virkon Aquatic solution exposure for 15-20 minutes. This treatment resulted in 100% mortality of both adult and neonate NZMS on boot surfaces. As a single adult or neonate NZMS could start a new population, an effective treatment needs to achieve 100% mortality for both stages. While our experiment found an effective decontamination following 10 minutes in the 2.0% Virkon Aquatic solution, our test focused only on the adult stage of the NZMS. Following a 15 minute immersion in the 2.0% solution, Stockton & Moffitt reported 0 live neonates. To account for the possible differences in tolerance of adults and neonates, our recommendation for the Virkon Aquatic is a 2.0% solution for at least 15 minutes.

Spray vs. Immersion Technique

The spray technique is not recommended as a decontamination method due to its inconsistent effect. While decontamination effectiveness between the spray and immersion techniques was similar following 10 minutes when no mud was present, the effectiveness was significantly reduced with mud. In addition, effectiveness was lower for the spraying technique compared to immersion (Fig. 7). Again, because asexual reproduction of the snail can result in a new population from a single individual, a consistent 100% mortality is desired in the decontamination procedure. This finding is supported by Stockton & Moffitt (2013) who found that the spray disinfectant did not result in a reliable method for complete mortality of NZMS. Live adult and neonate NZMS were found in all recovery baths following all spray concentrations and exposure times.

Effect of Mud

A layer of organic material, such as mud, greatly increases the survivorship of NZMS in decontamination procedures. While the Wisconsin DNR advises that the user "Scrub equipment with a stiff brush, including crevices, to remove all mud and snails", the effect of organic material has not been quantified. Stockton & Moffitt (2013) tested the effect of organic material on Virkon Aquatic and found that mud deactivated the chemical's effectiveness after 4-24 hours of exposure. However, this length of time is not likely to be a factor in the observed increased snail survivorship in our mud tests. It is more likely that the mud provided a protective layer between the snail and the chemical. However, when the mixture of mud and snails was immersed in the chemical treatment, the mud was likely penetrated more easily by the chemicals than in spray treatments. This probably resulted in the higher observed survival rate of snails using the spray technique compared to the immersion technique (Fig. 8).

The mud in this experiment was made just prior to use in the tests. As a result, it still contained high amounts of water and was very pliable. However, users may clean equipment following an extended period of time out of the water. In such cases, mud may have more time to dry. If dry, it is predicted that the mud would be less penetrable and thus offer an even more protective coating for the NZMS. Future testing is needed in this area.

In addition, the mud in this research was not taken from Black Earth Creek. In a realistic scenario, any mud trapped in the treads of the boot may protect NZMS or other potential invaders that could be transported to another body of water.

Effect of Material

Our results indicate that there are differences in the effectiveness of the treatment on NZMS mortality across different materials. However, there is not a clear pattern in differences between materials or between different techniques. In the tests without mud, longer exposure times resulted in a reduced difference in snail survival between rubber, felt, and canvas. Stockton & Moffitt (2013) reported that there was no difference in material on snail survival following a 2.0% Virkon Aquatic solution immersion. This finding supports the recommendation for Virkon Aquatic. However, the only solution utilized was the 2.0% Virkon Aquatic in that study. Our results for Formula 409 immersion, spray, and bleach immersion indicate that the type of material does matter in terms of decontamination. Snail survivorship on felt was the lowest of the tested materials. Due to the rough and fibrous texture of the felt, the fibers may interfere with the closing and sealing of the operculum as witnessed by fibers being trapped between the shell and operculum. As a result, more chemical comes in contact with the snail resulting in the observed higher snail mortality. However with a longer immersion time, the snail mortality between materials was similar and had a confidence interval overlapping 0% (Fig. 13, 14).

Broader Application

The effectiveness of the Formula 409 as a decontaminant raises questions regarding what makes the chemical so effective. Schisler *et al.* (2008) suggest that the increased levels of snail mortality could be attributed to the toxicity of Quantum Ammonium Compounds (QAC) that are present in Formula 409. In their experiments, they found higher mortality rates in

solutions of 100% Formula 409 and 3.1% and 4.7% Sparquat 256, both of which contain QACs. These QACs interfere with gill membrane function of invertebrates and have been used in molluscicides to reduce nuisance mollusk populations from industrial cooling systems (Dobbs *et al*. 2005). In addition, Schisler *et al.* (2008) found that Formula 409 contains a degreaser which may aid in the effectiveness of the chemical by loosening the snail's operculum seal. This would result in more of the chemical interacting with the snail, allowing for a thorough exposure at a faster rate compared to other decontamination chemicals. The scientific support for the effectiveness of QACs with the addition of the degreaser strengthens our support for the use of Formula 409 as a decontamination chemical for the NZMS. In addition, while this research focused on the invasive NZMS, the results are applicable to a wider range of species. Other mollusk and snail species could be decontaminated using Formula 409 as the effects may be similar to those found in this research. Species that are difficult to decontaminate due to an operculum or other sealing technique such as *Pomacea canaliculata*, *Dreissena polymorpha,* and *Dreissena rostriformis bugensisi* may have higher rates of mortality with the use of Formula 409. However, one of the challenges of invasive species decontamination is the observed species or life stage specificity that can protect certain species or life stages of a species from a decontamination strategy. For example, it is known that the resting eggs of *Bythotrephes longimanus* and zebra mussel veligers can survive Virkon Aquatic (Wisconsin DNR 2014). Further research is needed to determine what species or life stages of those species can tolerate the use of Formula 409.

A significant challenge in limiting the spread of invasive species is the problem of differing decontamination procedures among states and at the national level. Invasive species

can be easily transported across state lines if attached to recreational gear. The standards in place for decontamination procedures at the national and state levels often differ, leading to differing laws and recommendations for the general public to attempt to follow. The National Voluntary Guidelines to Prevent the Introduction and Spread of Aquatic Invasive Species (USFWS 2014a) recommends that scuba diving gear utilized in freshwater dives be soaked in a 3.5% salt solution for 30 minutes to clean the equipment before leaving it to dry for 5 days. However, given the findings of our research, the salt solution would not decontaminate the equipment and the surviving mud snails would still be alive following the 5 day drying period (Fig. 12). Similarly, a general public user in the state of Wisconsin could utilize the Boat and Gear Disinfection Protocol (2014) which recommends disinfection with a 200 ppm chlorine solution for a 10-minute contact time. This procedure would be ineffective on the NZMS given our results (Fig. 9c). While both of these methods are ineffective for NZMS, these differences in decontamination recommendations are one example which suggests that variations among states and at the national level provide a means for the continued spread of invasive species.

The focus of the methods and recommendations of our research was to stop the secondary spread of the NZMS. Limiting the spread of the NZMS will isolate the potential effects of the snail. Given the large number of lakes and streams in Wisconsin, surrounding states, and Canada and the high traffic area of Black Earth Creek by anglers, there is a significant chance that this population of NZMS will be the basis for secondary spread. These inland lakes and streams are invaluable sources of ecological and recreational value (Wilson & Carpenter 1999). Due to the potential effects that this species may have on an ecosystem, the impact of NZMS has the potential to be tremendous if the population spreads.

Risks

One of the considerations that must be taken into account is the environmental and health risks of using chemicals to decontaminate equipment. Careful precautions during the use of the chemicals are necessary to ensure that it does not run into natural water systems. The harsh properties of the chemicals needed to disinfect equipment could have negative effects on the biodiversity of the water system if released in high concentrations. However, the chemical usage has benefits over other methods of disinfection. The use of steam cleaning requires either a 120°F (48.9°C) water or steam cleaner, both of which are difficult to obtain, safely use and are expensive in a realistic situation (USFS 2011). Freezing decontamination requires a freezer large enough for the contaminated equipment and has the disadvantage of requiring an 8-hour period before achieving the desired effect. Drying equipment shares the disadvantage of requiring a long time to be effective, given that at least 24 hours are needed. In addition, drying requires temperatures above 84°F (28.9°C; Richards *et al.* 2004*)*. Chemical disinfection offers quicker and equally efficient means of ensuring NZMS mortality; however the risks of the chemical should be taken into consideration before recommending general use by the public.

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