North American Journal of Fisheries Management

ISSN: 0275-5947 (Print) 1548-8675 (Online) Journal homepage: http://www.tandfonline.com/loi/ujfm20

Efficacy of Commercially Available Quaternary Ammonium Compounds for Controlling New Zealand Mudsnails Potamopyrgus antipodarum

J. Benjamin Stout, Brian W. Avila & Eric R. Fetherman

To cite this article: J. Benjamin Stout, Brian W. Avila & Eric R. Fetherman (2016) Efficacy of Commercially Available Quaternary Ammonium Compounds for Controlling New Zealand Mudsnails Potamopyrgus antipodarum, North American Journal of Fisheries Management, 36:2, 277-284, DOI: 10.1080/02755947.2015.1120830

To link to this article: http://dx.doi.org/10.1080/02755947.2015.1120830

Published online: 22 Mar 2016.

North American Journal of Fisheries Management 36:277–284, 2016

ISSN: 0275-5947 print / 1548-8675 online
DOI: 10.1080/02755947.2015.1120830

MANAGEMENT BRIEF

Efficacy of Commercially Available Quaternary Ammonium Compounds for Controlling New Zealand Mudsnails *Potamopyrgus antipodarum*

J. Benjamin Stout
Colorado Parks and Wildlife, 317 West Prospect Road, Fort Collins, Colorado 80526, USA

Brian W. Avila
Department of Fish, Wildlife and Conservation Biology, Colorado State University, Fort Collins, Colorado 80523, USA

Eric R. Fetherman*
Colorado Parks and Wildlife, 317 West Prospect Road, Fort Collins, Colorado 80526, USA

Abstract

The New Zealand mudsnail *Potamopyrgus antipodarum* is an invasive species that can be transported to and established in new bodies of water on gear used by aquatic professionals, anglers, and aquatic recreationists. Sparquat 256, a standard disinfectant for controlling the spread of mudsnails, was recently discontinued by the manufacturer. Our objective was to find an industrial-strength, commercially available quaternary ammonium compound (QAC) that could replace Sparquat 256 for disinfection purposes. The efficacy of three products—Quat 4, Green Solutions High Dilutions Disinfectant 256 (GS 256), and Super HDQ Neutral (Super HDQ)—were tested using bath disinfection at multiple concentrations and exposure durations. For bath disinfection purposes, GS 256 and Super HDQ were the most effective. Super HDQ caused higher mortality rates at 48 h postexposure and was therefore tested and found to be highly effective for spray disinfection to prevent transporting mudsnails on field equipment. Regardless of the QAC chosen, we recommend a bath disinfection rate of 0.4% and a spray disinfection rate of 0.8% QACs in solution with an exposure duration of 10 min. These concentrations meet or exceed minimum effective disinfection requirements for quagga mussels *Dreissena rostriformis bugensis*, zebra mussels *Dreissena polymorpha*, whirling disease *Myxobolus cerebralis*, and chytrid fungus *Batrachochytrium dendrobatidis*.

Aquatic nuisance species (ANS) are a growing concern in the Intermountain West and around the world. They are typically difficult if not impossible to remove or control after introduction. The disruptive and damaging effects of these species on native aquatic ecosystems are well known. Aquatic nuisance species can displace or outcompete native species, introduce disease, or even physically alter habitats (see Ellender et al. 2014). New Zealand mudsnails *Potamopyrgus antipodarum*, a highly fecund, nonnative species (Winterbourn 1970), were first found in the western United States in 1987 in the Snake River in Idaho (Bowler 1991). Since then, their spread throughout the country has been rapid, and they are now found in Arizona, California, Colorado, Idaho, Montana, Nevada, Oregon, Utah, Washington, and Wyoming (Benson et al. 2014). The New Zealand mudsnails' ability to reproduce by parthenogenesis allows entire populations to be started by one individual (Dybdahl and Lively 1995). Densities of 500,000 mudsnails per square meter have been reported, and their ability to reproduce asexually combined with high densities can cause negative ecological impacts in affected streams (Richards et al. 2001). Mudsnails also have the ability to dominate the consumption of primary production (Hall et al. 2003) while providing little nutritional content for fish (Vinson and Baker 2008). Unfortunately, there is currently no effective method to remove New Zealand mudsnails once they are established in a body of water.

The development and use of disinfection protocols to prevent the spread of ANS is a priority in most places. Therefore, numerous disinfection methods exist to prevent the spread of aquatic invaders, including New Zealand mudsnails. Disinfecting gear

*Corresponding author: eric.fetherman@state.co.us
Received January 8, 2015; accepted November 8, 2015

before use can prevent the dispersal of this species between bodies of water, and is considered to be one of the most effective tools for slowing their expansion into novel systems. Methods of disinfection include freezing, desiccation, heating, and exposure to chemicals such as bleach, potassium permanganate, isopropyl alcohol, generic ammonia, benzethonium chloride, copper sulfate pentahydrate, and quaternary ammonium (Dwyer et al. 2003; Richards et al. 2004; Hosea and Finlayson 2005; Schisler et al. 2008). Quaternary ammonium compounds (QACs) are some of the most effective chemicals for disinfection that work by interfering with gill membrane function in invertebrates (Schisler et al. 2008) while minimally affecting wading gear (Hosea and Finlayson 2005). A wide variety of ANS can be treated with quaternary ammonium, including quagga mussels *Dreissena rostriformis bugensis* (Britton and Dingman 2011), zebra mussels *Dreissena polymorpha* (Waller et al. 1993), whirling disease *Myxobolus cerebralis* (Hedrick et al. 2008), and chytrid fungus *Batrachochytrium dendrobatidis* (Johnson et al. 2003). Quaternary ammonium is the active ingredient in household cleaning products such as Formula 409 and industrial-strength disinfectants such as Sparquat 256, both of which have been recommended for mudsnail disinfection (Hosea and Finlayson 2005; Schisler et al. 2008).

Sparquat 256 has been shown to help prevent the spread of New Zealand mudsnails and was therefore used by Colorado Parks and Wildlife as a chemical disinfectant for sampling gear (Schisler et al. 2008). Unfortunately, Sparquat 256 was recently discontinued by the manufacturer (Spartan Chemical). The objective of this study was to find an industrial-strength, commercially available QAC to replace Sparquat 256 for use in disinfecting waders and other gear that had come in contact with New Zealand mudsnail-infested waters. The efficacy of three products—Quat 4, Green Solutions High Dilutions Disinfectant 256 (GS 256), and Super HDQ Neutral (Super HDQ)—were tested using bath disinfection at multiple concentrations and exposure durations. The product producing the highest mortality rates at 48 h postexposure during bath disinfection was also tested for spray disinfection efficacy to prevent transporting mudsnails among bodies of water on field equipment when bath disinfection was not an available option.

METHODS

Bath disinfection.—Adult New Zealand mudsnails (N = 2,000; mean length = 4.12 mm; SE = 0.06 mm) were collected in July 2013 from South Delany Lake, Jackson County, Colorado, at an elevation of 2,469 m. Mudsnails were removed from loose vegetation that had washed up along the shoreline, and 25 individuals were randomly allocated to eighty 473-mL plastic beakers filled with reservoir water.

The experimental design was a 4 × 3 × 2 factorial (three chemical treatments and one control treatment × three concentrations × two exposure durations) with four replicate beakers per treatment. Treatments included QAC concentrations (active ingredient in solution, diluted with distilled water [DW]) equivalent to the manufacturer's label recommendation as well as 0.5 and 1.5 times the recommended concentration, for Quat 4 (Choice Manufacturing; 0.39, 0.19, and 0.59%, respectively), GS 256 (Spartan Chemical; 0.85, 0.41, and 1.27%, respectively), and Super HDQ (Spartan Chemical; 0.66, 0.33, and 1.0%, respectively). All of the products have different types and combinations of QACs: Quat 4 contains a total of 10% QACs, GS 256 contains a total of 21.7% QACs, and Super HDQ contains a total of 16.9% QACs. Rather than standardize on QAC concentration, we standardized on manufacturer's label recommendations as those using the products are likely to follow label instructions when creating disinfection baths. Distilled water served as a control treatment.

For exposures, mudsnails were transferred to a 15-mL tube containing the chemical treatment, and tubes were turned upside-down four times to mix mudsnails in the disinfectant. Mudsnails were exposed to the chemical and control treatments for a duration of 5 or 10 min. After exposure, mudsnails and the disinfectant were poured through a sieve and mudsnails were rinsed with reservoir water for 5 s to remove any chemical residue. Rinsed mudsnails were returned to their respective 473-mL beakers and placed in a cooler for transport and holding to prevent inadvertent escape. Water in the beakers was not changed and mudsnails were not fed during the postexposure observation period. Mudsnail condition was assessed at 48 h, 1 week, 1 month, and 55 d postexposure. Mudsnails were classified into three categories: alive, compromised (lethargic snails protruding from their shells and displaying weak movement), or dead (per Schisler et al. 2008). Condition was assessed at 55 d to determine the potential for compromised snails to recover, reducing ambiguity about mudsnail condition. Evidence of reproduction (i.e., the presence of juvenile mudsnails) was also recorded at 48 h, 1 week, 1 month, and 55 d postexposure.

Spray disinfection.—Adult New Zealand mudsnails (N = 800; mean length = 4.02 mm; SE = 0.45 mm) were collected in September 2014 from Dry Creek east of Baseline Reservoir (elevation of 1,615 m) in Boulder County, Colorado. Mudsnails were collected by overturning rocks located in the creek and scraping them from the rocks into a bucket filled with creek water using plastic spoons.

Experimental design was a 4 × 2 factorial (three concentrations of Super HDQ and one DW control × two exposure durations) with four replicate beakers per treatment. Super HDQ was the only QAC used in this experiment because it produced the higher rates of mortality across concentrations at 48 h postexposure during the bath disinfection experiment (see below). All spray disinfection experiments were conducted in a plastic tray to prevent potential mudsnail escape as well as to prevent chemicals from contacting the surrounding environment. A piece of breathable wader material mounted on multiple sponges within the tray served as the field equipment being disinfected. Wader material was sprayed with DW prior to the experiments to simulate equipment exiting an infested stream.

For exposures, 25 mudsnails were randomly chosen from the bucket, placed on the wet wader material, and allowed to acclimate to the material for 3 min to ensure that mudsnails were out of their shells and moving upon exposure to a treatment. After the 3-min acclimation period, mudsnails were sprayed with either DW (control) or one of three concentrations of Super HDQ (0.4, 0.8, and 1.2% QAC concentration in solution); these concentrations represent one, two, and three times our final recommendation for bath disinfection using Super HDQ (see below). Spray application lasted 5 s, which was enough time to thoroughly coat the wader material with the chemical solution. Following this 5-s application, mudsnails were exposed to the treatment for a duration of either 5 or 10 min.

Following treatment, mudsnails were collected from the wader material, rinsed with stream water for 5 s to remove any chemical residue, and placed in 473-mL beakers filled with stream water for later observation. Beakers were transported to the laboratory in a cooler, where the mudsnails remained for the duration of the observation period. Holding conditions were similar to those in the bath disinfection experiment, where the water in the beakers was not changed and mudsnails were not fed during the postexposure observation period. Similar to the bath disinfection experiment, mudsnail condition—classified as alive, compromised, or dead—was assessed at 48 h, 1 week, 1 month, and 55 d postexposure.

Statistical analyses.—Percent mortality was calculated for each chemical, concentration (as a function of manufacturer's label recommendation), and exposure duration for the bath disinfection experiment at 48 h, 1 week, 1 month, and 55 d postexposure. For the bath disinfection experiment, an incomplete factorial ANOVA was conducted to evaluate the effect of chemical, concentration, exposure duration, and their interactions on mortality of mudsnails at 55 d postexposure (PROC GLM; SAS Institute 2014). An interaction between chemical and concentration was not included in the analysis because the two were correlated, i.e., concentration was determined by the manufacturer's label recommendation for a given chemical. Results were based on type III sum of squares to account for the incomplete design in some factors (e.g., chemical; $n = 8$ for water controls and $n = 24$ each for Quat 4, GS 256, and Super HDQ). Juvenile mudsnails were found in several experimental beakers in the bath disinfection experiment; therefore, a factorial ANOVA was conducted to evaluate the effect of concentration, exposure duration, and their interaction on mudsnail reproduction at 55 d postexposure. Percent mortality was calculated for each concentration and exposure duration for the spray disinfection experiment at 48 h, 1 week, 1 month, and 55 d postexposure. A factorial ANOVA was also conducted to evaluate the effects of concentration, exposure duration, and their interaction on mortality of mudsnails at 55 d postexposure using data from the spray disinfection experiment. Results for mudsnail reproduction and the spray disinfection experiment were based on type I sum of squares. If significant effects were identified ($P < 0.05$), the least-squares means method with a Tukey's adjustment for multiple comparisons was used to quantify differences between treatments and exposure durations.

RESULTS

Bath Disinfection

At 48 h postexposure, only three treatments resulted in 100% mortality (1.0 times the manufacturer's label recommendation for Super HDQ with a 5-min exposure duration, and 1.5 times the label recommendation for Super HDQ with a 5- and 10-min exposure duration; Table 1). Mortality was high (≥84%) as a result of exposure to Super HDQ and GS 256, and relatively low (≤80%) as a result of exposure to Quat 4 at 48 h postexposure. In addition, compromised mudsnails were found in all treatments that did not result in 100% mortality at 48 h postexposure. Fewer compromised mudsnails were observed following exposure to Super HDQ than to GS 256 or Quat 4, and the highest numbers of compromised mudsnails were observed in experimental beakers containing mudsnails exposed to Quat 4. Between 48 h and 1 week, compromised mudsnails recovered or died. In the beakers containing mudsnails exposed to Super HDQ and GS 256, all compromised mudsnails were classified as dead (with the exception of one) one week postexposure. However, in the beakers containing mudsnails exposed to Quat 4, 40 of the 97 compromised mudsnails were classified as dead, whereas the other 57 recovered and were classified as alive at 1 week postexposure. In comparison to the QAC treatments, only 12 snails were dead in the beakers containing mudsnails exposed to the DW control at 48 h postexposure, and an additional 13 died between 48 h and 1 week; no mudsnails were classified as compromised in the control beakers during any postexposure assessments. Very few changes in classification occurred between 1 week postexposure and 55 d postexposure; therefore, results for 1 month postexposure are not shown. At 55 d postexposure, exposure to all but two concentrations of GS 256 and Super HDQ resulted in 100% mudsnail mortality. In contrast, exposure to Quat 4 did not result in 100% mortality, although higher mortality was observed using higher concentrations of Quat 4 with longer exposure durations (Table 1).

With the high number of compromised mudsnails observed at 48 h, statistical analyses were conducted only with data from 55 d postexposure. Chemical had a significant effect on mudsnail mortality ($F_{3,\ 60} = 46.39$, $P < 0.001$), as did concentration ($F_{6,\ 60} = 7.18$, $P < 0.001$), and exposure duration ($F_{1,\ 60} = 4.30$, $P = 0.043$). However, neither of the interactions between chemical and exposure duration ($F_{3,\ 60} = 1.94$, $P = 0.113$) nor between concentration and exposure duration ($F_{6,\ 60} = 0.22$, $P = 0.968$) were significant. Efficacy discrepancies at all three concentrations of Quat 4 contributed to significantly lower mortality overall within the 10-min versus 5-min exposure durations. Mortality

TABLE 1. Summary of chemical (chem) and concentration, as a function of the manufacturer's label recommendation (label), bath disinfection treatments applied to New Zealand mudsnails exposed for a duration of 5 or 10 min (four beakers per treatment; 25 mudsnails per beaker), and the resulting number of mudsnails that were alive, compromised (comp), or dead at 48 h, 1 week, and 55 d postexposure. Treatments included a DW control (0% QACs in solution), Quat 4, GS 256, and Super HDQ at three concentrations each: 0.5, 1.0, and 1.5 times the manufacturer's label recommendation.

			48 h			1 week			55 d		
Chem	Label	Time	Alive	Comp	Dead	Alive	Comp	Dead	Alive	Comp	Dead
DW	0	5	95	0	5	84	0	16	78	0	22
		10	93	0	7	91	0	9	88	0	12
Quat 4	0.5	5	60	21	19	81	0	19	49	0	51
		10	84	5	10	89	0	10	77	0	22
Quat 4	1.0	5	11	30	59	22	0	78	14	0	84
		10	32	13	55	42	0	58	40	0	60
Quat 4	1.5	5	1	20	79	7	0	93	5	0	95
		10	12	8	80	16	0	84	15	0	85
GS 256	0.5	5	5	11	84	3	0	97	2	0	98
		10	1	7	92	0	0	100	0	0	100
GS 256	1.0	5	1	8	91	0	0	100	0	0	100
		10	0	5	95	0	0	100	0	0	100
GS 256	1.5	5	0	3	97	0	0	100	0	0	100
		10	0	1	99	0	0	100	0	0	100
Super HDQ	0.5	5	1	1	98	0	1	99	0	0	100
		10	4	10	86	4	0	96	3	0	97
Super HDQ	1.0	5	0	0	100	0	0	100	0	0	100
		10	0	3	97	0	0	100	0	0	100
Super HDQ	1.5	5	0	0	100	0	0	100	0	0	100
		10	0	0	100	0	0	100	0	0	100

was lowest following exposure to DW and 0.5 times the label recommendation for Quat 4, and these treatments did not differ significantly from each other ($P = 0.353$; Figure 1). Exposure to 1.0 and 1.5 times the label recommendation for Quat 4 resulted in significantly higher mortality than did exposure to DW and 0.5 times the label recommendation for Quat 4 ($P \leq 0.002$), but the two did not differ significantly from each other ($P = 0.575$). Mortality did not differ significantly between any pair of treatments in which mudsnails were exposed to GS 256 and Super HDQ ($P \geq 0.974$). Additionally, mortality did not differ significantly between any pair of treatments in which mudsnails were exposed to GS 256 or Super HDQ and 1.0 or 1.5 times the label recommendation for Quat 4 ($P \geq 0.061$ and $P \geq 0.974$, respectively). Exposure to GS 256 and Super HDQ resulted in significantly higher mortality than did exposure to DW and 0.5 times the label recommendation for Quat 4 ($P \leq 0.002$; Figure 1). Overall, exposure to DW caused significantly lower mortality than did exposure to a QAC ($P < 0.001$). Significantly lower mortality rates were observed following exposure to Quat 4 than to GS 256 or Super HDQ ($P < 0.001$), whereas mortality rates did not differ between GS 256 and Super HDQ ($P = 1.000$).

Exposure to QACs also affected mudsnail reproduction. Offspring were found in all control beakers and were observed as early as 1 week postexposure, whereas offspring were not observed in a QAC beaker (with the exception of the beakers containing mudsnails exposed to 0.5 times the label recommendation for Quat 4) until 1 month postexposure (Figure 2). Concentration had a significant effect on reproduction ($F_{9, 60} = 24.33$, $P < 0.001$), whereas exposure duration ($F_{1, 60} = 2.08$, $P = 0.155$) and the interaction between concentration and exposure duration ($F_{9, 60} = 0.54$, $P = 0.841$) did not have a significant effect on reproduction. A least-squares means procedure showed that juvenile mudsnails were observed in significantly fewer beakers containing mudsnails exposed to 1.5 times the label recommendation for Quat 4 compared with 0.5 and 1.0 times the label recommendation for Quat 4 and DW ($P \leq 0.002$ among comparisons). The number of beakers in which offspring were observed did not differ significantly between 0.5 times the label recommendation for Quat 4 and DW ($P = 0.985$). In the other two QAC products, offspring were only observed in beakers containing mudsnails exposed to 0.5 times the label recommendation for GS 256 and Super HDQ for 5 min, and significantly fewer beakers within these treatments contained offspring as compared with 0.5 and 1.0 times the label recommendation for Quat 4 or DW ($P \leq 0.001$), but not 1.5 times the label recommendation for Quat 4 ($P \geq 0.985$). Interestingly, the number of beakers in which

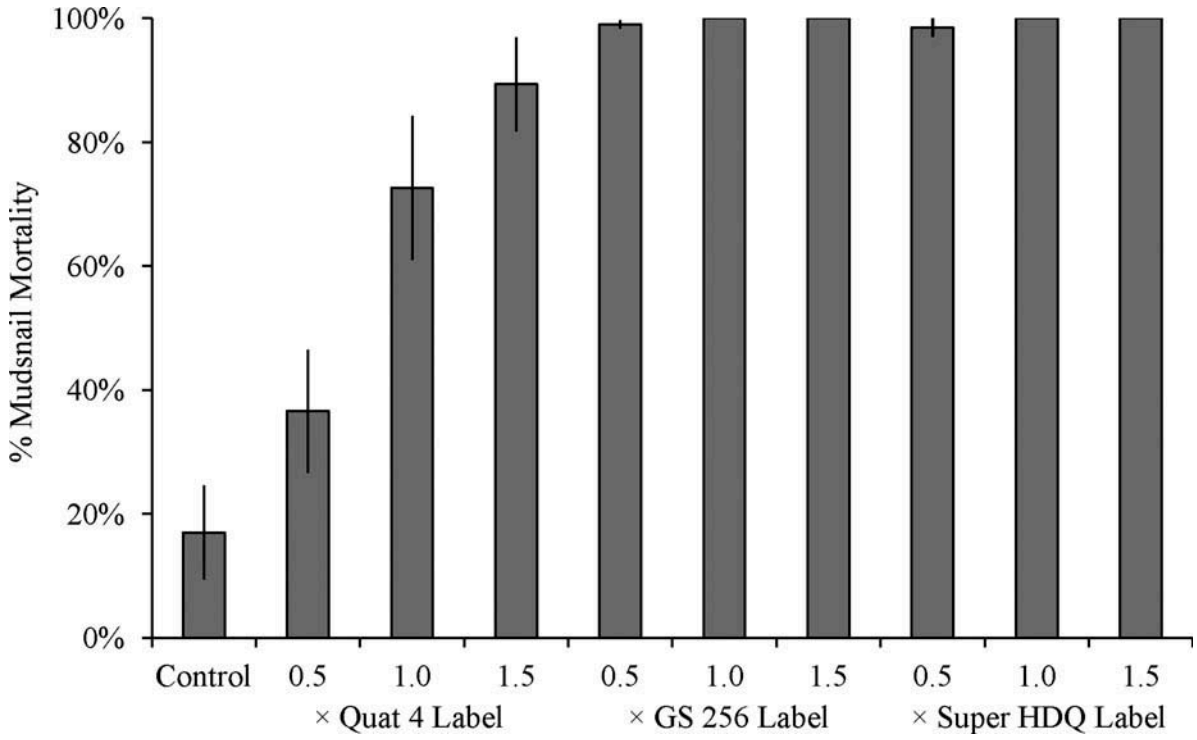

FIGURE 1. Mean percent mortality (±SE) of New Zealand mudsnails exposed to DW (control) and 0.5, 1.0, and 1.5 times the manufacturer's label recommendation for Quat 4, GS 256, or Super HDQ at 55 d postexposure. Absence of error bars indicates 100% mortality across all experimental beakers (eight beakers per concentration; 25 mudsnails per beaker).

offspring were observed was reduced between 1 month and 55 d postexposure in the beakers containing mudsnails exposed to 1.5 times the label recommendation for Quat 4, and 0.5 times the label recommendation for GS 256 and Super HDQ (Figure 2), suggesting that exposure to Super HDQ, GS 256, or higher concentrations of Quat 4 reduced offspring viability.

Spray Disinfection

Due to the higher mortality rates produced across all concentrations at 48 h postexposure in the bath disinfection experiment, Super HDQ was the only chemical evaluated in the spray disinfection experiment. At 48 h and 55 d postexposure, all mudsnails in the Super HDQ treatments were classified as dead. In the DW controls, one mudsnail was dead and three others were compromised at 48 h postexposure, and two of the compromised mudsnails recovered and were classified as alive by 55 d postexposure (Table 2). Very few changes in classification occurred between 1 week postexposure and 55 d postexposure; therefore, results for 1 month postexposure are not shown. Concentration had a significant effect on mudsnail mortality ($F_{3, 24} = 29{,}403$, $P < 0.001$). Exposure duration did not have a significant effect on mudsnail mortality ($F_{1, 24} = 3.0$, $P = 0.096$), and the interaction between concentration and exposure duration was marginally insignificant ($F_{3, 24} = 3.0$, $P = 0.051$); both effects were driven entirely by mortality occurring in the water control treatments (two mudsnails died with a 5-min exposure duration whereas zero mudsnails died with a 10-min exposure duration). Exposure to Super HDQ at any concentration or duration resulted in 100% mortality, and was significantly higher than that of the controls ($P \leq 0.001$).

DISCUSSION

Using the manufacturer's label recommendations for disinfection, differences in the efficacy for controlling New Zealand mudsnails were apparent among the three QACs tested. Our results showed that 100% mudsnail mortality was not achieved with any concentration or exposure duration using Quat 4 for bath disinfection. Additionally, some mudsnails classified as compromised subsequently recovered after exposure to Quat 4, and reproduction occurred in many Quat 4 treatments. This presents an issue when using Quat 4 for disinfection as mudsnails can reproduce by parthenogenesis and one mudsnail can start a new population (Dybdahl and Lively 1995). The fact that holding conditions were harsher than those experienced in a natural setting (no food and no water exchange) and the mudsnails were still able to survive and reproduce emphasizes the need to use a product that results in complete mortality following disinfection. Although GS 256 performed well, more mudsnails were

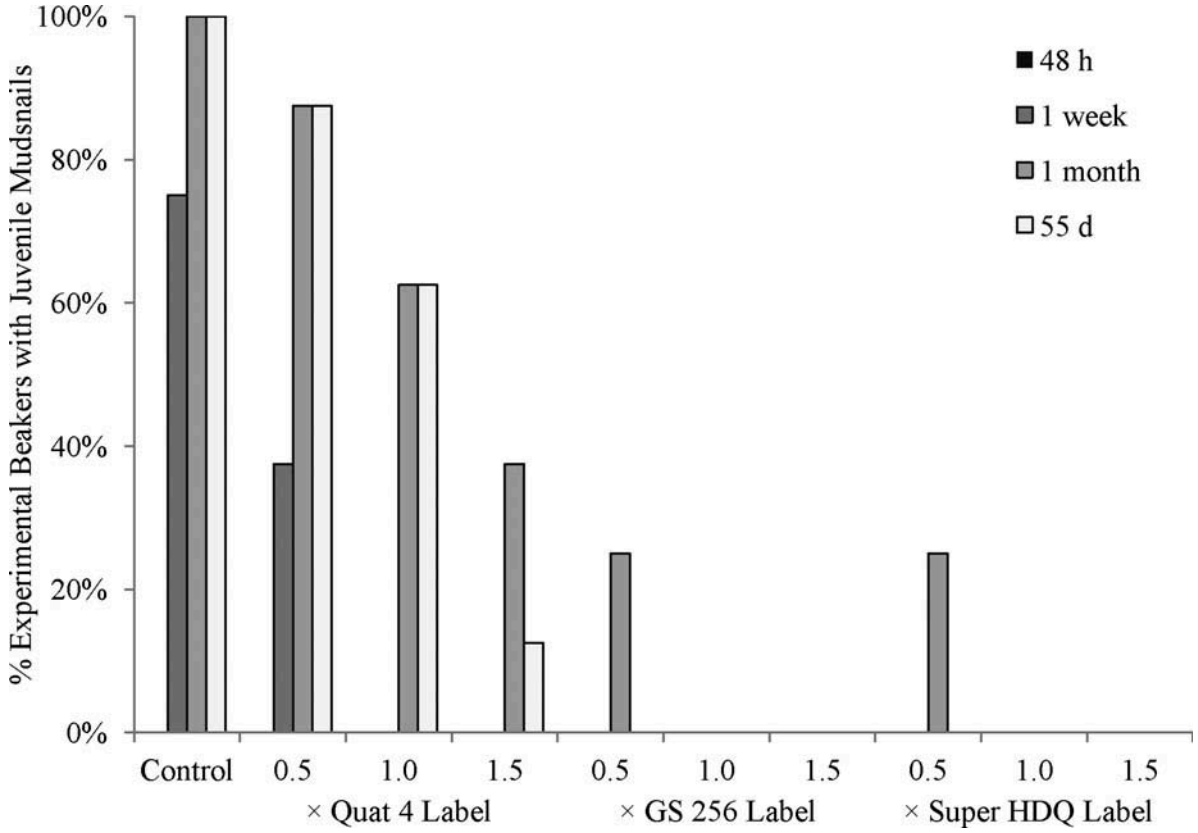

FIGURE 2. Percent of experimental beakers containing juvenile mudsnails at 48 h, 1 week, 1 month, and 55 d postexposure to DW (control), Quat 4, GS 256, or Super HDQ.

classified as compromised initially, and lower initial mortality rates were observed compared with Super HDQ. Super HDQ produced the highest mortality rates at shorter exposure durations. Super HDQ was used in the spray disinfection experiment because exposure to Super HDQ resulted in higher rates of mortality at 48 h postexposure in the bath disinfection experiment. Our spray disinfection results showed that Super HDQ achieved 100% mudsnail mortality at all concentrations and exposure durations.

All of the products used had different combinations of QACs. Quat 4 contains 5% alkyl dimethyl benzyl ammonium (ADBAC) and 5% alkyl dimethyl ethyl benzyl ammonium

TABLE 2. Summary of DW and Super HDQ concentrations (conc) sprayed on wader material containing New Zealand mudsnails, exposed for a duration of 5 or 10 min (four beakers per treatment; 25 mudsnails per beaker), and the resulting number of mudsnails that were alive, compromised (comp), or dead at 48 h, 1 week, and 55 d postexposure. The three concentrations of Super HDQ represent one, two, and three times the recommended concentration for bath disinfection.

			48 h			1 week			55 d		
Chem	Conc (%)	Time	Alive	Comp	Dead	Alive	Comp	Dead	Alive	Comp	Dead
DW	0	5	97	2	1	99	0	1	98	0	2
		10	99	1	0	100	0	0	100	0	0
Super HDQ	0.4	5	0	0	100	0	0	100	0	0	100
		10	0	0	100	0	0	100	0	0	100
Super HDQ	0.8	5	0	0	100	0	0	100	0	0	100
		10	0	0	100	0	0	100	0	0	100
Super HDQ	1.2	5	0	0	100	0	0	100	0	0	100
		10	0	0	100	0	0	100	0	0	100

chloride (ADEAC), for a total QAC of 10%; GS 256 contains 6.51% octyl decyl dimethyl ammonium chloride (ODDAC), 3.255% dioctyl DAC (DioDAC), 3.255% didecyl DAC (DidDAC), and 8.68% ADBAC, for a total QAC of 21.7%; and Super HDQ contains 10.14% DidDAC and 6.76% ADBAC, for a total QAC of 16.9%. The different types and concentrations of the compounds that make up the different products could have had an influence on product performance in our experiments, especially when standardizing on label recommendations versus QAC concentration in solution. For comparison, Sparquat 256, the chemical discontinued by the manufacturer, which prompted this study, contains 5% ADBAC, 3.75% ODDAC, 1.875% DioDAC, and 1.875% DidDAC, for a total QAC of 12.5%, with similar label recommendations as the other QACs tested. Therefore, if following the label recommendations, it is likely that Sparquat 256 would have performed more similarly to Quat 4 rather than GS 256 or Super HDQ based on total QAC concentration. However, little is known about how the variety and percent composition of the various compounds that account for total QAC concentration affect disinfection. Examining these effects could help with the development of a QAC made exclusively for ANS disinfection purposes.

The mudsnail's physiological response to disturbance, or lack thereof, also likely resulted in differences in observed mortality during bath and spray disinfection. The operculum of the New Zealand mudsnail is thought to help limit exposure to chemicals while closed. Studies have shown that mudsnails with open opercula were killed at higher rates than mudsnails with closed opercula when exposed to isopropanol, potassium permanganate, and bleach (Hosea and Finlayson 2005). In the bath disinfection experiment, mudsnails were moved from a beaker to a tube containing a treatment, likely causing at least some of the mudsnails to close their opercula due to disturbance. This could have had an effect on the efficacy of the chemicals used for the bath disinfections. However, results from Hosea and Finlayson (2005) suggest that an open or closed operculum did not change the effectiveness of a treatment when using QACs. In the spray disinfection experiment, all mudsnails were out of their shell at the time of exposure, ensuring contact with the chemical. Therefore, mortality rates observed in this study may be higher than what would be expected under normal conditions. For example, simulating the removal of waders upon exiting an infested body of water prior to spray application may have resulted in some mudsnails closing their opercula, which may have reduced the effectiveness of Super HDQ. Due to this potential physiological response, we suggest using higher concentrations of QACs during spray disinfection, especially if equipment is moved or disturbed immediately prior to application.

Because New Zealand mudsnails cannot be removed once they have been established in a body of water, it is extremely important to limit their spread as much as possible (Schisler et al. 2008). Aquatic professionals, anglers, and aquatic recreationists are the most likely modes of transportation due to their use of multiple waters (Gates et al. 2009). Disinfection of gear is the most important part of preventing the spread of ANS. For both bath and spray disinfection, all debris, mud, and vegetation should be removed prior to disinfection. Muddy disinfection solution can lose its effectiveness and therefore its ability to kill ANS (Colorado Division of Wildlife 2005). Gear should be visually inspected prior to disinfection, and any ANS observed should be removed. Regardless of product chosen, bath disinfection with a minimum of 0.4% QACs in solution for a duration of 10 min is recommended to ensure a 100% mortality rate. This concentration is achieved using the label recommendations for GS 256 and Super HDQ, but not for Quat 4. Our recommendation is similar to that of Schisler et al. (2008), who recommended a 0.4% Sparquat solution with a 10-min exposure duration. Despite our results showing a 100% mortality rate with Super HDQ, a 0.8% QAC solution with a 10-min exposure duration is suggested for spray disinfection to avoid issues with uneven application, shorter application times (than the 5-s application used here), or disturbance causing mudsnails to close their opercula prior to application. A 10-min exposure duration is long enough to kill mudsnails but short enough to prevent prolonged disinfection times in the field if performing work in multiple bodies of water in the same day. Anyone using spray application is cautioned to be diligent in ensuring that all crevices are exposed to the chemical because mudsnails have been found in all possible locations on wading boots, including underneath the insole (Hosea and Finlayson 2005).

Other ANS of concern include whirling disease, chytrid fungus, quagga mussels, and zebra mussels. Quaternary ammonium compounds have been shown to be highly effective for controlling the spread of these species. A 0.3% QAC in solution treatment for 10 min eliminated any evidence of infectivity for whirling disease myxospores (Hedrick et al. 2008). Low concentrations (0.012% or 0.008%) of QACs in solution for durations as short as 30 s resulted in a 100% mortality rate for chytrid fungus (Johnson et al. 2003). A 10-min exposure to 0.39% QACs in solution was sufficient to achieve 100% mortality of quagga mussel veligers (Britton and Dingman 2011). Zebra mussel veligers have exhibited a 100% mortality rate at a concentration of 0.0625% QACs in solution for 1 min (Wong 2012). Our recommendations for the disinfection of New Zealand mudsnails (0.4% and 0.8% QACs in solution for an exposure duration of 10 min) are higher than for these other ANS of concern, and therefore should be sufficiently effective for preventing the spread of these species as well.

ACKNOWLEDGMENTS

This work was sponsored in part by the Colorado Parks and Wildlife Aquatic Nuisance Species Program. The authors would like to thank G. Schisler for his assistance with New Zealand

mudsnail collection and consultation on experimental design, E. Brown for her assistance in obtaining chemicals and equipment, and W. Keeley and City of Boulder Open Space and Mountain Parks for allowing us to collect mudsnails in and conduct the spray disinfection experiment at Dry Creek.

REFERENCES

Benson, A. J., R. M. Kipp, J. Larson, and A. Fusaro. 2014. *Potamopyrgus antipodarum* (New Zealand mudsnail) mollusks–gastropods, exotic. Fact sheet. NAS (Nonindigenous Aquatic Species) [online database]. U.S. Geological Survey, Gainesville, Florida. Available: http://nas.er.usgs.gov/queries/factsheet.aspx?SpeciesID=1008. (December 2014).

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

Britton, D. K., and S. Dingman. 2011. Use of quaternary ammonium to control the spread of aquatic invasive species by wildland fire equipment. Aquatic Invasions 6:169–173.

Colorado Division of Wildlife. 2005. New Zealand mudsnail management plan: current status and suggested management actions. Colorado Division of Wildlife, Denver.

Dwyer, W. P., B. L. Kerans, and M. M. Gangloff. 2003. Effect of acute exposure to chlorine, copper sulfate, and heat on survival of New Zealand mudsnails. Intermountain Journal of Sciences 9:53–58.

Dybdahl, M. F., and C. M. Lively. 1995. Diverse, endemic and polyphyletic clones in mixed populations of a freshwater snail (*Potamopyrgus antipodarum*). Journal of Evolutionary Biology 8:385–398.

Ellender, B. R., D. J. Woodford, O. L. F. Weyl, and I. G. Cowx. 2014. Managing conflicts arising from fisheries enhancements based on non-native fishes in southern Africa. Journal of Fish Biology 86:1890–1906.

Gates, K. K., C. S. Guy, A. V. Zale, and T. B. Horton. 2009. Angler awareness of aquatic nuisance species and potential transport mechanisms. Fisheries Management and Ecology 16:448–456.

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

Hedrick, R. P., T. S. McDowell, K. Mukkatira, E. MacConnell, and B. Petri. 2008. Effects of freezing, drying, ultraviolet irradiation, chlorine, and quaternary ammonium treatments on the infectivity of myxospores of *Myxobulus cerebralis* for *Tubifex tubifex*. Journal of Aquatic Animal Health 20:116–125.

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

Johnson, M. L., L. Berger, L. Philips, and R. Speare. 2003. Fungicidal effects of chemical disinfectants, UV light, desiccation and heat on the amphibian chytrid *Batrachochytrium dendrobatidis*. Diseases of Aquatic Organisms 57:255–260.

Richards, D. C., L. D. Cazier, and G. T. Lester. 2001. Spatial distribution of three snail species, including the invader *Potamopyrgus antipodarum*, in a freshwater spring. Western North American Naturalist 61:375–380.

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

SAS Institute. 2014. SAS system software, release 9.3. SAS Institute, Cary, North Carolina.

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

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

Waller, D. L., J. J. Rach, W. G. Cope, L. L. Marking, S. W. Fisher, and H. Dabrowska. 1993. Toxicity of candidate molluscicides to zebra mussels (*Dreissena polymorpha*) and selected nontarget organisms. Journal of Great Lakes Research 19:695–702.

Winterbourn, M. 1970. The New Zealand species of *Potamopyrgus* (Gastropoda: Hydrobiidae). Malacologia 10:283–321.

Wong, W. H. 2012. Develop effective decontamination protocols for wildland firefighting equipment exposed to quagga/zebra mussels: testing the efficacy of quaternary ammonium compounds on killing dreissenid veligers and adults. Report to the U.S. Fish and Wildlife Service, Las Vegas, Nevada.