Journal of Aquatic Animal Health 30:3–12, 2018

ISSN: 0899-7659 print / 1548-8667 online
DOI: 10.1002/aah.10013

ARTICLE

Toxicity of Potassium Chloride Compared to Sodium Chloride for Zebra Mussel Decontamination

Eric A. Davis
Department of Biology, State University of New York College at Oneonta, 108 Ravine Parkway, Oneonta, New York 13820, USA

Wai Hing Wong*
Department of Biology, State University of New York College at Oneonta, 108 Ravine Parkway, Oneonta, New York 13820, USA; Biological Field Station, State University of New York College at Oneonta, 5838 State Highway 80, Cooperstown, New York 13326, USA; and Massachusetts Department of Environmental Protection, Watershed Planning Program, 8 New Bond Street, Worcester, Massachusetts 01606, USA

Willard N. Harman
Department of Biology, State University of New York College at Oneonta, 108 Ravine Parkway, Oneonta, New York 13820, USA; and Biological Field Station, State University of New York College at Oneonta, 5838 State Highway 80, Cooperstown, New York 13326, USA

Abstract

The use of chemicals to decontaminate watercraft and/or equipment after exposure to zebra mussels *Dreissena polymorpha* is one method of decontamination that has been recommended by multiple government agencies in the United States. The ideal chemical to be used for decontamination would be inexpensive and easily obtained, would have no or limited effect on nontarget species, and would be relatively environmentally friendly. Two chemicals that have been tested are potassium chloride (KCl) and sodium chloride (NaCl). The toxicity of each chemical to both adult zebra mussels and veliger larvae was examined. Sodium chloride was less effective at causing mortality than KCl within the exposure periods tested. Adult mussels required a 4× longer exposure period to exhibit complete mortality when exposed to NaCl at 30,000 mg/L (24 h) compared to KCl (6 h). At 10,000 mg/L, NaCl took 8× longer (96 h) than KCl (12 h) to cause 100% mortality of adult mussels. Veligers that were exposed to KCl at 1,250 mg/L required a 12-h exposure to attain complete mortality, while those exposed to NaCl at 10,000 mg/L required an 18-h exposure to exhibit the same result. To determine whether KCl is more advantageous as a decontamination chemical, the cost and chemical availability must be researched.

Controlling the ongoing spread of aquatic invasive species (AIS) has become a very high priority for numerous natural resource agencies throughout North America (Zook and Phillips 2015). One species of concern is the zebra mussel *Dreissena polymorpha*. The rapid spread of this bivalve throughout the Great Lakes region and the northeastern United States after its discovery in the 1980s, combined with its ability to completely alter the ecosystems it invades, has caused many U.S. agencies to develop watercraft interception programs (Zook and Phillips 2015). These programs are designed to prevent AIS from spreading via trailered watercraft, seaplanes, and other equipment and thus to preserve natural resources (DiVittorio 2015; Zook and Phillips 2015). One major aspect of the

*Corresponding author: david.w.wong@state.ma.us
Received May 25, 2017; accepted October 1, 2017

watercraft interception programs is the physical inspection of those watercraft and the determination of what to do if the boat is found to be a possible source of AIS spread. Trailered watercraft are believed to be the greatest vector for the spread of AIS (Johnson et al. 2001). Once a vessel has been deemed a "threat" with the potential to spread zebra mussels, there are a few recommended courses of action for decontamination. They include chemical treatment, heat, hot-water/high-pressure washing, freezing, physical removal, and desiccation (DiVittorio et al. 2012). Many of these treatment options require specialized training or equipment, making it difficult for watercraft owners to effectively decontaminate their own equipment.

Desiccation is the easiest and least expensive form of decontamination against aquatic organisms but can require greater lengths of time than boat owners are willing to wait (i.e., up to 40 d for attached adult mussels) before reusing their boats (DiVittorio et al. 2012). Physical removal of attached mussels does not require any specialized equipment but is only applicable to the mussel life stages that are visible to the naked eye. Furthermore, it can be a very labor-intensive process to remove attached mussels that have colonized large areas or hard-to-reach locations (DiVittorio et al. 2012). Freezing is another easy decontamination method when ambient temperatures are low enough; otherwise, one would need dry ice crystals or a similar product to spray over the mussels, causing them to freeze. When multiple layers of mussels are present, greater lengths of time may be required to freeze the innermost layer (McMahon et al. 1993). Hot water/high-pressure treatment is probably the most common decontamination method for watercraft. This method uses heated (60°C) water that is sprayed through a pressure washer to heat the mussels and remove them at the same time (Comeau et al. 2011). The water must be sprayed over the affected area for 5–10 s to heat the mussels to lethal temperatures (Comeau et al. 2011). Although extremely effective, hot water wash stations have some drawbacks. Each station relies on electricity and plumbing to operate properly. The electricity is needed to heat the water and run the pressure washers, while plumbing is needed to catch the water leaving the watercrafts to be sure that it does not enter any waterway. There can be substantial costs associated with these amenities—for example, up to $30,000 to build a wash station (D. A. Jensen, presentation at the 16th Annual Conference on Aquatic Invasive Species, 2009; http://www.seagrant.umn.edu/downloads/jensen_boatwash.pdf). In a state with hundreds of boat launch locations, building wash stations at many locations would easily incur a total cost in the millions of dollars. Additionally, due to the temperature and high pressure of the sprayer, it would be necessary for a trained professional to operate each wash station to ensure that watercraft are properly decontaminated and that the sprayer is operated safely.

Chemical treatments can be recommended to watercraft owners but entail some caveats. One drawback to chemical treatments is that the chemicals often require a plan for post-treatment disposal (DiVittorio et al. 2012). Another potential problem is that some chemicals (e.g., Zequanox, Virkon Aquatic, or EarthTec) with toxicity to adult mussels or veligers are registered pesticides that require special storage, licensing, or handling. Other chemicals (e.g., chlorine bleach) can be effective, but their use results in harmful byproducts (Watters et al. 2013). However, some chemicals that have been recommended for use can be easily obtained by the general public, are relatively inexpensive, and are safe to handle; potassium chloride (KCl) and sodium chloride (NaCl) are two chemicals that fit this description. These two chemicals may require an extended exposure period to effectively decontaminate watercraft or gear. To apply the appropriate contact time, a watercraft and/or trailer would have to be left in a treatment pond or bath for upwards of 24 h (Davis et al. 2015a). Therefore, decontamination with these chemicals may be more practical for use with gear and equipment.

Potassium chloride and/or NaCl have been tested as chemical treatments on adult zebra mussels, veliger larvae, or both (Fisher et al. 1994; Waller et al. 1996; Lewis et al. 1997; Wildridge et al. 1998; Edwards et al. 2000, 2002; Sykes 2009; Fernald and Watson 2013; Pucherelli et al. 2014; Davis et al. 2015a). Fisher et al. (1991) found that potassium was toxic to zebra mussels at elevated concentrations. Those authors reported vacuolization of the gill epithelial cells in mussels exposed to elevated potassium levels, and they suggested that the pathology was likely related to a loss of fluid and/or electrolyte balance in the cells due to functional or structural changes in the plasma membrane. Fisher et al. (1991) also reported that the median lethal dose (LD50) of KCl was 138 mg/L for a 24-h exposure. Waller et al. (1996) found that a 2,500-mg/L concentration of KCl caused 100% mortality in veligers; furthermore, they observed that NaCl at 10,000 mg/L caused 100% mortality with a 24-h exposure. Ellis and MacIssac (2009) found that NaCl at 30,000 mg/L did not cause complete mortality in adult zebra mussels. These previous studies did not provide a direct comparison of KCl and NaCl toxicity to adult zebra mussels and veliger larvae. Thus, we sought to compare the toxicity of KCl and NaCl and to compare our results with previous findings. It was hypothesized that both chemicals would cause mortality to adult zebra mussels and veliger larvae but that KCl would cause mortality (1) sooner at the same concentration and (2) at lower concentrations for similar exposure periods.

METHODS

Adult collection and acclimation.— Adult zebra mussels were collected via the same procedure used by Davis et al.

(2015a, 2015b). Adult mussel-colonized rocks in 1 m of water were collected from Otsego Lake, New York, during fall 2014. Otsego Lake is a meso-oligotrophic, dimictic lake that was formed from glacial over-deepening in the Susquehanna River valley (Harman 1997). In the laboratory, mussels were physically removed from the rocks with a paint scraper (Costa et al. 2008). After removal from the rocks, mussels were put into a small tray, and a flow of lake water was supplied to the tray. Any mussels with byssal threads that were intertwined were separated by gently pulling them apart by hand to reduce any physical damage. After the tray had all of the mussels in it, 11 mussels were randomly removed and put into a mesh bag. Mussels with physical damage from the paint scraper were discarded. Mussels were placed into 150 mesh bags. All remaining mussels in the tray were gathered and placed into one separate mesh bag. A large tank (~150 L) was filled with lake water and aerated before all 150 bags of mussels were placed into it. A flow of lake water (~15°C) was left running in the tank. Mussels (in bags) were kept in the tank for at least 72 h to allow acclimation to the mesh bags. After the acclimation period, the mesh bags were removed from the tank one at a time, and the mussels within each bag were examined for mortality. Dead individuals were removed from the bags. If more than one dead mussel was found, live mussels were taken from the bag of leftover mussels at random and were added to the mesh bag to obtain 10 live mussels. If no mortality occurred, one mussel was selected at random, removed from the mesh bag, and discarded. There were 30 mussels for each concentration × exposure period combination. Mortality was less than 1% throughout the acclimation period.

Chemical treatment.— Lake water was used to rinse 15 glass tanks. The tanks were scoured with a wet sponge; they were then emptied, rinsed, and emptied again. Twenty liters of water (~15°C) taken from Otsego Lake were added to each tank. During the study, the water in experimental tanks warmed to about 19°C by the 24-h mark and stayed at this temperature for the remainder of the study. An electronic scale was used to measure the correct amount of KCl (Amresco, Solon, Ohio). We tested KCl concentrations of 500, 3,000, 10,000, and 30,000 mg/L, which allowed for direct comparison of our results with those of Davis et al. (2015a). After KCl was added to a tank, a wooden dowel was used to stir the water until the chemical dissolved. There were three replicate tanks at each concentration as well as three control (chemical-free) tanks. Each tank was aerated to ensure constant mixing of the water.

After the tanks were prepared, 10 bags of mussels—one bag for each of the 10 exposure periods (0, 1, 2, 4, 6, 12, 24, 48, 72, and 96 h)—were placed in each tank. After mesh bags were placed in all tanks, one bag was removed from each tank to represent the 0-h exposure. Upon removal from a treatment tank, each mesh bag was labeled with its exposure time and was hung from a wooden dowel in one of two large holding tanks. Bags of mussels were transported from the experimental tanks to the holding tanks in a large plastic tray. The holding tanks were supplied with continuously flowing lake water (~15°C), allowing any chemical left on the mussels or on the mesh bags to be slowly diluted and removed from the tanks. Over the course of the study, the water temperature in the holding tanks averaged about 16°C due to their location within a greenhouse. Mortality was assessed at 48 and 72 h postexposure. It has been shown that mussels can recover from some chemicals after being placed in chemical-free water (Wildridge et al. 1998; Pucherelli et al. 2014). Mussels were removed from the mesh bag and were examined for gaping valves, the factor used to determine mortality. Any mussel with a slight gape was probed between the valves by using a blunt probe; if there was no response to this stimulus, the mussel was considered dead. Probing was only conducted for mussels with a slight valve gape or when more than half of the mussels in the sample group were gaping. The overall shell length was taken with a digital caliper (Model CD-6″CX; Mitutoyo Corp., Kawasaki, Japan). The shell length and mortality status of the mussel were recorded.

Water quality.— Temperature, specific conductivity, pH, and dissolved oxygen concentration were measured in each test tank and in the two holding tanks by using a YSI sonde (Model 6820V2-M; YSI, Inc., Yellow Springs, Ohio) at 0, 24, 48, 72, and 96 h. The sonde was calibrated prior to each measurement. Measurements were first taken from the three control tanks, then the three tanks with the lowest chemical concentration, and were repeated for each successive concentration level. The probe was rinsed with lake water between concentration levels and before use in sampling the holding tanks. Water quality variables were measured in the treatment tanks after the removal of mussels at the same exposure period. The holding tanks were sampled after the bags of mussels had been added; this was done to measure the maximum amount of chemical that was added to the holding tank via chemical solution soaked into the mesh bags or left on or in adult mussels.

Veliger collection.— Veliger collection and enumeration followed the methods used by Davis et al. (2016). A 63-μm plankton net was used to collect veligers from the top 3 m of Otsego Lake in summer 2015 by using approximately 750-m horizontal tows. The contents of the net were placed into an opaque, 1-L bottle. This was repeated until the opaque bottle was 75% full. The bottle was then brought to the main laboratory of the Biological Field Station (Cooperstown, New York; State University of

New York College at Oneonta). At the laboratory, a portion of the bottle's contents was placed into a 500-mL beaker and was concentrated using a cup with a bottom consisting of 63-μm net material. This was repeated until all of the bottle's contents were concentrated to about 100 mL of liquid. A gridded Sedgewick–Rafter counting cell was examined by cross-polarized light (CPL) microscopy to determine the number of veligers contained in 1 mL of the concentrated sample. Two repetitions were performed, and the three concentrations were averaged. A concentration of 50–100 veligers per milliliter of sample was desired; we further concentrated the sample or diluted it with filtered lake water until this goal was met. A sample of the veligers was preserved in ethanol for analysis of the distribution of veliger stages and sizes.

Veliger chemical treatment.— In a 50-mL beaker, 24 mL of chemical solution were prepared by combining lake water filtered through 63-μm net material with the corresponding amount of chemical needed to make 25 mL of solution for each tested concentration level (0, 1,250, 2,500, and 5,000 mg/L for KCl; 0, 5,000, 10,000, and 15,000 mg/L for NaCl). Waller et al. (1996) suggested that a 2,500-mg/L concentration of KCl was effective with a 24-h exposure for veligers taken from Lake Erie; in that same study, a 10,000-mg/L concentration of NaCl was found to cause 100% mortality at an exposure period of 24 h when the water temperature was 12°C. We selected a series of concentrations that were lower and higher than both of those suggested concentrations, and we included one similar concentration to allow for comparison. Three beakers were used for each concentration × exposure period combination (exposure durations = 18 and 24 h for NaCl; 12, 18, and 24 h for KCl) during each test. After chemical solution was added to the beakers, 1 mL of concentrated veliger sample water was added to each beaker by using a pipette with the tip cut to prevent damage to the veligers. At the end of the exposure period, each beaker was poured into a veliger holding device (VHD), rinsed with filtered lake water, and poured into the VHD again. Each VHD was labeled with the chemical name, concentration, and exposure time and was placed into a recovery tank of filtered lake water that was lightly aerated with compressed air. See Davis et al. (2016) for a detailed description of the VHDs' design and construction. The VHDs allowed the veligers to be exposed to chemical-free water while also allowing easy re-collection for recovery investigations. Some chemicals have been shown to cause veligers to appear dead immediately after exposure, but after being placed into water without the chemicals, the veligers can recover (Pucherelli et al. 2014). Upon removal from the chemical solution and placement into the recovery tank, veligers were observed for mortality by using the Sedgewick–Rafter counting cell and CPL microscopy. A 1-mL sample from each VHD was observed, and the numbers of dead and live veligers were counted. Samples for each concentration × exposure period combination were observed consecutively: for example, replicate 1 was observed first, replicate 2 was observed second, and replicate 3 was observed last for the 0-mg/L NaCl treatment with an 18-h exposure. The total number of veligers for the three replicates was determined, and if the total did not exceed 30 veligers, another 1-mL sample was observed from each replicate. If samples contained more than 100 veligers, only the first 100 veligers observed were used for the mortality assessment. After the mortality observation was complete, the contents of the slide were rinsed back into the VHD with filtered lake water. This process was repeated every 24 h until the control groups reached greater than 50% mortality or 72 h, whichever occurred first. Veligers were considered dead if they met any of the following criteria: (1) no internal organs were visible within the shell, (2) internal organs were leaking out of the shell, (3) there was no movement of internal organs, and (4) there was no movement of cilia (Watters et al. 2013).

Statistical analysis.— The mean and SD were determined for adult zebra mussel mortality. Adult mortality data were used to create the LD50 and LD99 with 95% confidence intervals (CIs) for times 4, 6, 12, and 24 h by using a probit regression. For each exposure period, the LD50 was the chemical concentration that caused mortality of 50% of the mussels, and the LD99 was the concentration that caused mortality of 99% of the mussels (Davis et al. 2015a, 2015b). We determined that a 24-h period was the greatest exposure time for watercraft disinfection. The results of the KCl test were compared to the results of Davis et al. (2015a). The effectiveness of KCl was compared to that of NaCl by using ANCOVA, with the concentration as the covariate for each exposure interval (Zar 1996). An ANOVA was performed to compare the average shell length between the dead and live adult mussels; a *t*-test was performed to compare the mean shell length of adult mussels from the KCl and NaCl tests. The average observed mortality (%) from the veliger mortality assessments was calculated for each concentration × exposure period combination. The mortality data were arcsine transformed due to a nonnormal distribution and the low replicate number. To compare the effectiveness of the KCl and NaCl treatments, an ANCOVA was performed for mortality at the 5,000-mg/L concentration (the only concentration used for both tests), with exposure period as the covariate. The level of significance α was set at 0.05 for all tests. Statistical analyses were performed with SAS version 9.3 (SAS Institute, Inc., Cary, North Carolina).

RESULTS

Adult Mussel Exposure to Potassium Chloride

The average shell length of adult zebra mussels in the study was 13.57 mm (SD = 3.70 mm). There was no

significant difference in mean shell length between live and dead adult mussels or in the shell lengths used when comparing KCl concentrations (ANCOVA: $F = 0.51$, $P = 0.77$).

As expected, higher concentrations of KCl caused mortality at a faster rate than lower concentrations (Figure 1). Exposures up to 4 h did not cause complete mortality at any KCl concentration. Complete mortality was achieved at 6-, 12-, and 24-h exposures with a KCl concentration of 30,000 mg/L. The 10,000-mg/L concentration also caused 100% mortality for the 12- and 24-h exposures. No other KCl concentration produced complete mortality in less than 48 h. The control samples had 100% survival of adults. All test concentrations resulted in some degree of mortality (0–93% mortality) at exposures less than 24 h.

A wide range of LD50 values was found based on the exposure period (Table 1). The 4-h LD50 for KCl was 12,916 mg/L, and the 24-h LD50 was 278.9 mg/L. The calculated values for the LD99 also spanned a wide range; the 4-h LD99 was 45,865 mg/L, while the 24-h LD99 was 25,084 mg/L. The average temperature of the experimental tanks began at approximately 15°C, as they were filled with fresh lake water right before the experiment began (Table 2). The temperature then rose to about 18.5°C in the first 24 h and then remained at that level, which was the room temperature of the laboratory. The pH of the holding tanks averaged 8.39–9.18 from the start to the end of the experiment. Average pH never dropped below 7.70 (range = 7.70–8.60 for all concentrations) in any of the tanks. Dissolved oxygen concentration was high in all tanks throughout the experiment (range = 7.54–10.22 mg/L). Average specific conductivity in the control tanks was 0.298, 0.315, 0.291, 0.291, and 0.292 mS/cm during the experiment; these values were similar to those of the recovery tanks (0.312, 0.368, 0.288, 0.299, and 0.309 mS/cm). Specific conductivity ranges were 1.210–1.303 mS/cm at the KCl concentration of 500 mg/L; 5.419–5.850 mS/cm at 3,000 mg/L; 16.197–17.477 mS/cm at 10,000 mg/L; and 43.723–47.263 mS/cm at 30,000 mg/L.

Chemical Comparison for Adult Mussels

The mean shell lengths of adult zebra mussels in the KCl and NaCl tests were significantly different ($N = 3{,}000$; $t = 3.30$, $P < 0.001$). The mean lengths differed by 0.4 mm. The tested chemical and the concentration were significant factors for mortality in every exposure period other than 0 h. The chemical × concentration interaction was a significant factor for mortality in exposure periods other than 0, 12, and 24 h.

Veliger Exposure to Potassium Chloride

Veligers in the experimental groups exposed to KCl appeared to exhibit complete mortality for the first 48 h after removal from the chemical treatments (Table 3). During the 72-h postremoval mortality assessment, veligers that were exposed to KCl at 500 mg/L showed some recovery. The average mortality rate was 98.48% for the 12-h exposure period, 99.19% for the 18-h exposure, and 98.55% for the 24-h exposure. Average mortality rates at the postremoval assessments (0-, 24-, 48-, and 72-h assessments) were 38.15, 33.98, 37.76, and 68.98% for the 12-h exposure control group; 16.61, 23.80, 32.72, and 68.03% for the 18-h exposure control group; and 16.80, 24.16, 27.73, and 54.85% for the 24-h exposure control group.

Veliger Exposure to Sodium Chloride

Average mortality percentages for veligers in the 18-h NaCl exposure control group were 22.47, 30.38, and

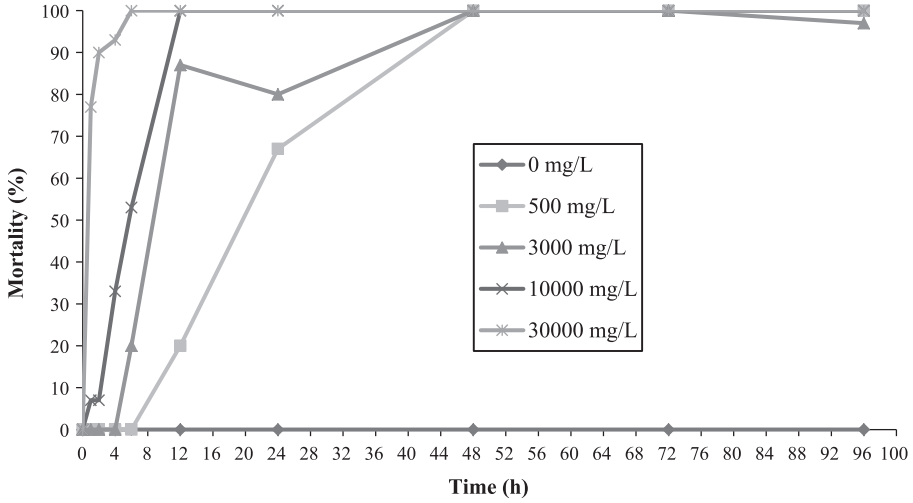

FIGURE 1. Mortality (%) of adult zebra mussels ($N = 3$ groups, each with 10 mussels) after exposure to potassium chloride at varying concentrations in fall 2014. Zebra mussels were collected from Otsego Lake, New York.

TABLE 1. Estimated doses that were lethal to 50% and 99% of adult zebra mussels (LD50 and LD99; 95% confidence intervals in parentheses) during exposure to potassium chloride for 4, 6, 12, or 24 h; and the sample dose(s) that caused 100% mortality for a given exposure period during the experiment. Zebra mussels were collected from Otsego Lake, New York.

Exposure duration (h)	LD50 (mg/L)	LD99 (mg/L)	Sample dose (mg/L) causing 100% mortality
4	12,916 (10,285–16,091)	45,865 (31,429–98,153)	None
6	7,217 (5,410–9,549)	50,750 (29,859–137,900)	30,000
12	1,067 (737.3–1,492)	8,213 (4,703–23,130)	10,000, 30,000
24	278.9 (40.65–610.2)	25,084 (8,656–463,332)	10,000, 30,000

TABLE 2. Average water quality characteristics of the recovery tanks and experimental tanks during trials investigating the toxicity of potassium chloride (KCl) to adult zebra mussels.

KCl concentration (mg/L)	Time (h)	Temperature (°C)	Specific conductivity (mS/cm)	pH	Dissolved oxygen (mg/L)
Recovery	0	16.40	0.312	8.39	9.10
	24	16.30	0.368	8.59	9.99
	48	17.05	0.288	9.11	8.92
	72	16.86	0.299	9.15	8.95
	96	16.67	0.309	9.18	8.97
0	0	15.05	0.298	8.40	9.63
	24	18.27	0.315	8.14	10.19
	48	18.75	0.291	8.43	9.09
	72	18.81	0.291	8.51	9.06
	96	18.88	0.292	8.60	9.03
500	0	15.19	1.253	8.09	9.52
	24	18.16	1.303	8.14	10.22
	48	18.55	1.210	8.22	9.06
	72	18.67	1.214	8.27	9.04
	96	18.79	1.219	8.33	9.02
3,000	0	15.18	5.628	8.08	9.46
	24	18.20	5.850	8.18	10.04
	48	18.70	5.419	8.16	8.90
	72	18.79	5.427	8.19	8.88
	96	18.89	5.435	8.22	8.86
10,000	0	15.18	16.813	7.91	9.20
	24	18.26	17.477	8.07	9.63
	48	18.81	16.197	8.08	8.51
	72	18.89	16.230	8.09	8.45
	96	18.98	16.263	8.11	8.38
30,000	0	14.88	45.257	7.70	8.47
	24	18.41	47.263	7.97	8.53
	48	18.91	43.723	7.99	7.60
	72	18.97	43.815	7.98	7.57
	96	19.02	43.907	7.97	7.54

37.84% at the 0-, 24-, and 48-h postremoval assessments, respectively (Table 4). The 24-h control group had an average mortality of 23.05, 34.14, and 85.45% at the 0-, 24-, and 48-h assessments, respectively. During the 48-h postremoval assessment, recovery was observed for veligers that were exposed to NaCl at 5,000 mg/L. The average observed mortality was 97.84% for the 18-h exposure and 99.56% for the 24-h exposure. The 10,000- and 15,000-mg/L concentrations produced 100% mortality, with no veliger recovery.

TABLE 3. Average mortality (%) of zebra mussel veligers after removal from treatments involving a 12-, 18-, or 24-h exposure to potassium chloride (KCl) at varying concentrations. Mortality was observed at 0, 24, 48, and 72 h after removal from the KCl treatment (postremoval observation period). Observed recovery rate for veligers at 72 h is also presented. Veligers were collected from Otsego Lake, New York.

Exposure duration (h)	KCl concentration (mg/L)	Postremoval observation period				Observed recovery (%) at 72 h postremoval
		0 h	24 h	48 h	72 h	
12	0	38.15	33.98	37.76	68.28	N/A
	500	100.00	100.00	100.00	98.48	1.52
	1,250	100.00	100.00	100.00	100.00	0.00
	2,500	100.00	100.00	100.00	100.00	0.00
	5,000	100.00	100.00	100.00	100.00	0.00
18	0	16.61	23.80	32.72	68.03	N/A
	500	100.00	100.00	100.00	99.19	0.81
	1,250	100.00	100.00	100.00	100.00	0.00
	2,500	100.00	100.00	100.00	100.00	0.00
	5,000	100.00	100.00	100.00	100.00	0.00
24	0	16.80	24.16	27.73	54.85	N/A
	500	100.00	100.00	100.00	98.55	1.45
	1,250	100.00	100.00	100.00	100.00	0.00
	2,500	100.00	100.00	100.00	100.00	0.00
	5,000	100.00	100.00	100.00	100.00	0.00

Chemical Comparison for Veligers

The ANCOVA indicated that chemical treatment and exposure period were significant factors for veliger mortality ($F = 30.28$, $P < 0.0001$) and that KCl was more lethal than NaCl ($P = 0.0476$). The treatment × exposure period interaction was not significant ($P = 0.2340$).

The majority (80%) of veligers used during the tests were D-stage (Figure 2). Umbonal-stage veligers constituted 16% of the sample, whereas pediveligers comprised only 4%.

DISCUSSION

Potassium chloride caused complete mortality of adult zebra mussels at every concentration in less than 24 h. A 6-h exposure to 30,000 mg/L resulted in complete mortality, with the estimated LD99 at 50,750 mg/L; the CI was as low as 29,859 mg/L. This concentration would be useful for people who want to be able to deploy their watercraft or equipment in a different water body than the one they originally used. At the 12-h exposure, both the 10,000- and 30,000-mg/L concentrations of KCl caused 100% mortality. The LD50 was 278.9 mg/L (95% CI = 40.65–610.2) for the 24-h exposure; this was slightly higher than the 138-mg/L (123–161 mg/L) LD50 identified by Fisher et al. (1991). However, the two results are not significantly different, as the 95% CIs overlap. Another study (Lewis et al. 1997) examined adult mussel exposure to KCl at 100 mg/L under a variety of water temperatures to determine the time required to cause 95% mortality based on the temperature used; 95% mortality was achieved in 56 h at room temperature (~20°C) and in 165 h at 12–14°C. Areas that are sensitive to corrosion due to their material construction can be treated with a lower concentration for a longer exposure period. Decreased concentrations with longer exposure periods may also be useful for electrical components, which may have problems when exposed to solutions with high ionic concentrations. For example, we found that a 24-h exposure to KCl at 10,000 mg/L was completely lethal to adult mussels; the estimated LD99 was 25,084 mg/L, with the CI including concentrations as low as 8,656 mg/L. This still allows for complete mortality of adult mussels within a reasonable time frame before resuming use of the gear and equipment. This would also allow for a decreased cost of decontamination.

In our previous study on three forms of NaCl (Davis et al. 2015a), decontamination of adult zebra mussels with NaCl was suggested as feasible. The salinity tolerance of zebra mussels is related to the salinity of the water body they are inhabiting (McMahon 1996). Therefore, conducting NaCl toxicity experiments using mussels collected from waters representing diverse saline levels may be necessary to provide more accurate recommendations for decontamination protocols.

When comparing the effectiveness of both chemicals for adult mussel decontamination, NaCl was less effective than KCl for every exposure period other than 0 h. At the 30,000-mg/L concentration, KCl caused complete mortality in one-fourth the time compared to NaCl (6 h for KCl versus 24 h for NaCl). Concentration was also a significant cause of mortality, which can be expected because lower concentrations typically will cause less mortality

TABLE 4. Average observed mortality (%) of zebra mussel veligers after removal from treatments involving an 18- or 24-h exposure to sodium chloride (NaCl) at varying concentrations. Mortality was observed at 0, 24, and 48 h after removal from the NaCl treatment (postremoval observation period). Observed recovery rate for veligers at 48 h is also presented. Veligers were collected from Otsego Lake, New York.

Exposure duration (h)	NaCl concentration (mg/L)	Postremoval observation period			Observed recovery (%) at 48 h postremoval
		0 h	24 h	48 h	
18	0	22.47	30.38	37.84	N/A
	5,000	98.67	100.00	97.84	2.16
	10,000	100.00	100.00	100.00	0.0
	15,000	100.00	100.00	100.00	0.0
24	0	23.05	34.14	85.45	N/A
	5,000	100.00	100.00	99.56	0.44
	10,000	100.00	100.00	100.00	0.0
	15,000	100.00	100.00	100.00	0.0

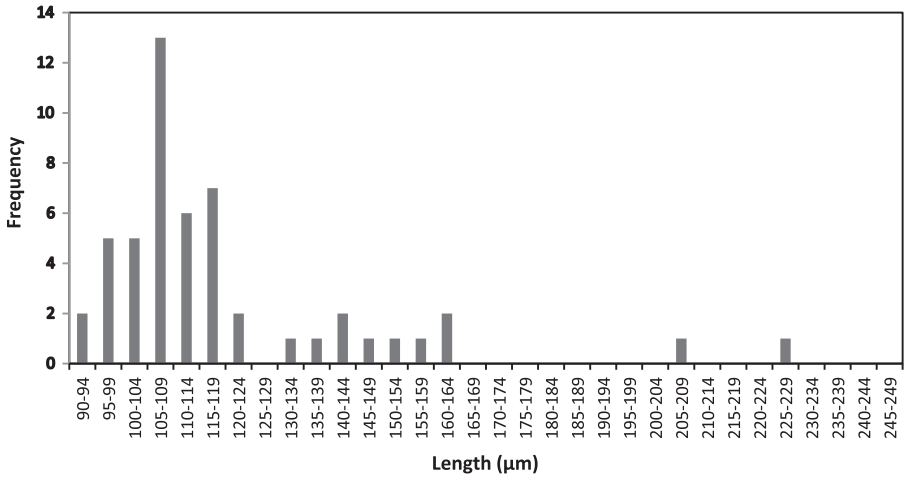

FIGURE 2. Length (µm) distribution of zebra mussel veligers ($N = 51$) sampled when toxicity testing was performed. Veligers were collected from Otsego Lake, New York.

than higher concentrations for the same exposure period, regardless of the chemical being used. The size difference between the adult mussels used in the two experiments was minimal (0.4 mm) but significant. The large sample size likely caused the significant difference in shell length ($N = 3{,}000$). However, all mussels tested were adults (>8 mm) and were collected from the same location within a few weeks of each other. Therefore, the influence of mussel size on testing results is likely minimal.

For veligers of zebra mussels, KCl caused 100% mortality without any veliger recovery at 1,250 mg/L, a lower concentration than has been reported by others to be effective. Potassium chloride at 2,500 mg/L caused complete mortality by 24 h for zebra mussel veligers taken from Lake Erie (Waller et al. 1996). Pucherelli et al. (2014) reported that a KCl exposure of 2,500 mg/L for 24 h, with or without a formalin secondary treatment, was effective for causing mortality in veligers of the quagga mussel *D. rostriformis bugensis*; those authors also found that KCl at 50 mg/L with a secondary treatment of formalin was effective for quagga mussel veligers. Edwards et al. (2000, 2002) reported that a 750-mg/L treatment of KCl for 1 h plus a formalin secondary treatment was effective against zebra mussel veligers, but those studies did not include a recovery period. The exclusion of a recovery period raises concerns over the possible recovery of veligers after placement into chemical-free water, similar to what was observed in the current study. Other studies have found KCl to be ineffective at levels similar to those tested here. Sykes (2009) found that KCl at 4,250 mg/L with a secondary treatment of formalin was not effective for causing mortality of quagga mussel veligers; the lack of

effectiveness was suggested to be due to (1) the elevated hardness of the water at the veliger collection site and (2) the water used during the chemical treatments.

Sodium chloride was found to be an effective chemical for the treatment of veligers. The exposure periods required for complete mortality with NaCl do not allow for a rapid decontamination but would be sufficient for many other situations. Our results were consistent with previously reported results. A 10,000-mg/L concentration of NaCl was found to cause 100% mortality at an exposure period of 24 h when the water temperature was 12°C (Waller et al. 1996). We observed that NaCl at 10,000 mg/L was effective at both the 18- and 24-h exposures. The water temperature in our study was closer to 20°C; this higher water temperature may have contributed to a higher mortality rate by causing an increase in veliger metabolism, thereby leading to a greater rate of chemical ingestion by the veligers. Waller et al. (1996) reported that multiple species of fish were tolerant of a 10,000-mg/L concentration with an exposure period of 24 h at both 12°C and 17°C; the goal of their study was to find a zebra mussel decontamination method that was safe for use during aquaculture practices. During that same study, a 20,000-mg/L concentration of NaCl caused complete mortality in zebra mussel veligers with a 6-h exposure period at 17°C (Waller et al. 1996). In a study examining the chemical control of quagga mussel veligers, Pucherelli et al. (2014) found that NaCl at 10,000 mg/L was not effective with a 24-h exposure. The difference in results may be due to interspecific differences in the veligers or to differences in the sources of the samples; additionally, the water used by Pucherelli et al. (2014) had a much higher ionic concentration than the water from Otsego Lake.

Potassium chloride was more effective than NaCl for use as a veliger decontamination tool. The type of treatment was a significant indicator of mortality in the ANCOVA, as was the exposure period. The exposure period was likely significant because KCl caused complete mortality at a shorter exposure (12 h), whereas NaCl did not produce complete mortality based on the observations made after veliger removal from the chemical treatments. It is not surprising that KCl was more effective than NaCl, as potassium was shown to cause damage to the epithelial cells of mussel gills (Fisher et al. 1991).

The control groups for both of the veliger experiments experienced high mortality that may detract from the results obtained for the treatment groups. Observing the same group of veligers from the start to the end of the experiment could help reduce the error in estimating mortality. Furthermore, the repeated removal of veligers from the VHDs to the slides and subsequent transfer of veligers back to the VHDs could have led to stress-induced mortality that otherwise might not have occurred. In water bodies where zebra mussels have been established for more than a few years, the natural survival rate of veligers that become settled juveniles is often less than 1%.

Although KCl is often more expensive than NaCl, the overall increase in effectiveness at decontamination compared to NaCl is likely a greater trade-off. Due to the required contact time needed to achieve 100% mortality, the use of KCl or NaCl to disinfect an entire watercraft and/or trailer does not appear to be a practical option. To disinfect a trailered watercraft, a holding pond would have to be built and filled with the chemical treatment, and the trailered watercraft would have to be left in the treatment for the required time. However, these chemicals may be acceptable options for the decontamination of gear that can be placed into a holding receptacle, such as a live well or a bucket. The ability to have gear and equipment ready for use sooner could result in an increased likelihood of decontamination protocols being followed by recreational watercraft owners. This could also lead to an increase in productivity of individuals collecting scientific data. Potassium chloride is sometimes recommended because it is considered to be less corrosive than NaCl; however, this does not mean that KCl is noncorrosive. A KCl solution was created and used for decontamination of gear and equipment during summer 2015 by the intern program at the Biological Field Station. For items that were left in the solution for extended periods of time (i.e., days), surfaces that were susceptible to corrosion were often heavily corroded upon removal from the solution. Iodized table salt was shown to work as effectively as high-grade NaCl, which would allow for decontamination costs to be decreased significantly when using NaCl (Davis et al. 2015a). Iodized table salt cost $1.17 per kilogram in the Davis et al. (2015a) study, much less expensive than the $40-per-kilogram cost of reagent-grade NaCl. One 737-g container of iodized table salt would produce around 18 L of a 30,000-mg/L solution, resulting in an average cost of $0.05 per liter of solution. Preparation of a 10,000-mg/L NaCl solution would have an average cost of $0.02 per liter. A KCl-based table salt alternative was found to be similarly effective as reagent-grade KCl for causing mortality in adult mussels (E. A. Davis, unpublished data). This also can reduce decontamination costs: a 311-g can of KCl-based table salt costs $4.96, or $15.95 per kilogram. Potassium chloride-based water softener salt could offer an even cheaper alternative as a KCl-based chemical treatment ($28 for an 18-kg bag), but testing should be conducted to determine whether it is effective for use with zebra mussels.

Due to the low number of replicates performed during this study, another study that uses more replicates would allow for greater statistical analysis of the results and would strengthen the basis upon which decontamination protocols can be selected. The present results can be used to guide future work that is focused on preventing the spread of zebra mussels.

ACKNOWLEDGMENTS

Funding for the majority of this study was provided by the Environmental Conservation Fund administered by the New York State Department of Environmental Conservation. A Student Research Grant from the State University of New York at Oneonta provided funding for the chemical decontamination testing on veligers. We thank Ed Woltmann, Catherine McGlynn, Matthew Albright, and Holly Waterfield for constructive discussions that aided in project design and execution. The views in this paper are those of the authors and do not necessarily reflect the views or policies of the Massachusetts Department of Environmental Protection. There is no conflict of interest declared in this article.

REFERENCES

Comeau, S., S. Rainville, W. Baldwin, E. Austin, S. H. Gerstenberger, C. Cross, and W. H. Wong. 2011. Susceptibility of quagga mussels (*Dreissena rostriformis bugensis*) to hot-water sprays as a means of watercraft decontamination. Biofouling 27:267–274.

Costa, R., D. C. Aldridge, and G. D. Moggridge. 2008. Seasonal variation of zebra mussel susceptibility to molluscicidal agents. Journal of Applied Ecology 45:1712–1721.

Davis, E. A., W. H. Wong, and W. N. Harman. 2015a. Comparison of three sodium chloride chemical treatments for adult zebra mussel decontamination. Journal of Shellfish Research 34:1029–1036.

Davis, E. A., W. H. Wong, and W. N. Harman. 2015b. Distilled white vinegar (5% acetic acid) as a potential decontamination method for adult zebra mussels. Management of Biological Invasions 6:423–428.

Davis, E. A., W. H. Wong, and W. N. Harman. 2016. Livewell flushing to remove zebra mussel (*Dreissena polymorpha*) veligers. Management of Biological Invasions 7:399–403.

DiVittorio, J. 2015. Equipment inspection and cleaning: the first step in an integrated approach to prevent the spread of aquatic invasive species and pests. Pages 226–242 *in* W. H. Wong and S. L. Gerstenberger, editors. Biology and management of invasive quagga and zebra mussels in the western United States. CRC Press, Boca Raton, Florida.

DiVittorio, J., M. Grodowitz, J. Snow, and T. Manross. 2012. Inspection and cleaning manual for equipment and vehicles to prevent the spread of invasive species. U.S. Bureau of Reclamation, Technical Memorandum 86-68220-07-05, Denver.

Edwards, W. J., L. Babcock-Jackson, and D. A. Culver. 2000. Prevention of the spread of zebra mussels during hatchery and aquaculture activities. North American Journal of Aquaculture 62:229–236.

Edwards, W. J., L. Babcock-Jackson, and D. A. Culver. 2002. Field testing of protocols to prevent the spread of zebra mussels *Dreissena polymorpha* during fish hatchery and aquaculture activities. North American Journal of Aquaculture 64:220–223.

Ellis, S., and H. J. MacIssac. 2009. Salinity tolerance of Great Lakes invaders. Freshwater Biology 2009:77–89.

Fernald, R. T., and B. T. Watson. 2013. Eradication of zebra mussels (*Dreissena polymorpha*) from Millbrook Quarry, Virginia: rapid response in the real world. Pages 195–213 *in* T. F. Nalepa and D. W. Schloesser, editors. Quagga and zebra mussels: biology, impacts, and control. CRC Press, Boca Raton, Florida.

Fisher, S. W., H. Dabrowska, D. L. Waller, L. Babcock-Jackson, and X. Zhang. 1994. Sensitivity of zebra mussel (*Dreissena polymorpha*) life stages to candidate molluscicides. Journal of Shellfish Research 13:373–377.

Fisher, S. W., P. Stromberg, K. A. Bruner, and L. D. Boulet. 1991. Molluscicidal activity of potassium to the zebra mussel, *Dreissena polymorpha*, toxicity and mode of action. Aquatic Toxicology 20:219–234.

Harman, W. N. 1997. A comparison of the Otsego Lake macrobenthos communities between 1935 and 1993. State University of New York College at Oneonta, Biological Field Station, Annual Report 30, Cooperstown.

Johnson, L. E., A. Riccardi, and J. T. Carlton. 2001. Overland dispersal of aquatic invasive species: a risk assessment of transient recreational boating. Ecological Applications 11:1789–1799.

Lewis, D. P., J. M. Piontkowski, R. W. Straney, J. J. Knowlton, and E. F. Neuhauser. 1997. Use of potassium for treatment and control of zebra mussel infestation in industrial fire protection water systems. Fire Technology 33:356–371.

McMahon, R. F. 1996. The physiological ecology of the zebra mussel, *Dreissena polymorpha*, in North America and Europe. American Zoology 36:339–363.

McMahon, R. F., T. A. Ussery, and M. Clarke. 1993. Use of emersion as a zebra mussel control method. U.S. Army Corps of Engineers, Waterways Experiment Station, Vicksburg, Mississippi.

Pucherelli, S. F., D. E. Portz, K. Bloom, J. Carmon, S. Brenimer, and D. Hosler. 2014. Quagga mussel contamination of fish haul trucks by fish and development of effective potassium chloride and formalin treatments. Journal of Applied Aquaculture 26:132–148.

Sykes, C. L. 2009. Efficacy of potassium chloride and formalin for removing quagga mussel veligers from transport tanks at Willow Beach National Fish Hatchery. U.S. Bureau of Reclamation, Final Report, Boulder City, Nevada.

Waller, D. L., S. W. Fisher, and H. Dabrowska. 1996. Prevention of zebra mussel infestation and dispersal during aquaculture operations. Progressive Fish-Culturist 58:77–84.

Watters, A., S. L. Gerstenberger, and W. H. Wong. 2013. Effectiveness of EarthTec for killing invasive quagga mussels (*Dreissena rostriformis bugensis*) and preventing their colonization in the western United States. Biofouling 29:21–28.

Wildridge, P. J., R. G. Werner, F. G. Doherty, and E. F. Neuhauser. 1998. Acute toxicity of potassium to the adult zebra mussel *Dreissena polymorpha*. Archives of Environmental Contamination and Toxicology 34:265–270.

Zar, J. H. 1996. Biostatistical analysis, 3rd edition. Prentice Hall, Upper Saddle River, New Jersey.

Zook, B., and S. Phillips. 2015. Uniform minimum protocols and standards for watercraft interception programs for dreissenid mussels in the western United States (UMPS). Pages 175–202 *in* W. H. Wong and S. L. Gerstenberger, editors. Biology and management of invasive quagga and zebra mussels in the western United States. CRC Press, Boca Raton, Florida.