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Short Communication

Distilled white vinegar (5% acetic acid) as a potential decontamination method for adult zebra mussels

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Abstract

The spread of zebra mussels into new bodies of water is of great concern in the United States due to their economic and ecological costs. Some government agencies suggest the use of vinegar as a decontamination option but do not provide data to explain this decision. This study examined the toxicity of distilled white vinegar on adult zebra mussels at varying concentrations and exposure times. All tested concentrations (25, 50, 75, and 100%) caused complete mortality within four hours. These results indicate that distilled white vinegar can be used for the decontamination of adult zebra mussels.

Key words: zebra mussels, decontamination, vinegar, acute toxicity

Introduction

The introduction of non-native species has become one of the leading causes of native organism diversity declines (Pysek and Richardson 2010). Aquatic invasive species (AIS) are among some of the more commonly known examples. However, AIS are not only a cause of ecosystem alterations but can inflict significant economic damages as well (United States Army Corps of Engineers 2002). In the United States, AIS cost more than $7 billion annually (Pimentel et al. 2005). One AIS of great concern is the zebra mussel, *Dreissena polymorpha* (Pallas, 1771). Zebra mussels alone cost an estimated $1 billion in the United States (Pimentel et al. 2005). In the New York State Canal and Hudson River Systems, zebra mussels cost an estimated $12.5 million in control and management (Pimentel 2005). The ability of zebra mussels to colonize hard substrates using their byssal threads is what makes them such a problem. Biofouling occurs in water intake systems, such as drinking water and power-producing facilities, as mussels attach to the interior of pipes and other infrastructure. In response to a survey, facility managers reported a total cost of over $267 million due to zebra mussels from 1989–2004 (Connelly et al. 2007). The spread of zebra mussels across much of the northeast and upper Midwest of the United States from the Great Lakes, where they were first discovered in the North America, is most likely from overland transport by trailered watercraft (Johnson et al. 2001). In the western United States, many agencies have begun watercraft interception programs to keep dreissenid mussels from spreading to uninfected water bodies (Zook and Phillips 2012). These programs involve watercraft inspection and decontamination. The United States Bureau of Reclamation has approved numerous forms of decontamination (DiVittorio et al. 2012). They include hot water/high pressure washing, heat, freezing, physical removal, desiccation, and chemical treatment (DiVittorio et al. 2012). Chemical treatments can be one of the

more difficult methods to use because some chemicals can be toxic to non-target organisms or they can produce harmful byproducts (Watters 2011). It also may require a well developed plan to handle the chemicals after treating watercraft (DiVittorio et al. 2012). However, chemical treatments may provide an option for remote, low use boat launches if the chemical used is in small quantities and can be easily contained for proper disposal. A low cost chemical that is effective in small doses would allow for maximum decontamination for minimal cost. The use of undiluted white vinegar is one chemical treatment recommended by the United States Bureau of Reclamation for the treatment of veligers, the larval life stage of zebra mussels, but they do not indicate any studies that were used to come to this conclusion (DiVittorio et al. 2012). White vinegar is a common chemical found in many households and can be obtained fairly easily at a relatively low price. Our hypothesis was that distilled white vinegar is effective in killing adult zebra mussels. The goal of this study was to investigate the lethality of distilled white vinegar to adult mussels. The data generated by this study will be used to design a test of distilled white vinegar toxicity to mussel veligers during the upcoming spawning season.

Methods

Adult collection and preparation

Rocks colonized by adult mussels were collected at the State University of New York College at Oneonta Biological Field Station (BFS) Thayer Boathouse on Otsego Lake, New York in water around 1m in depth using a variety of clam rakes. The rocks were brought back to the lab in trays and coolers. Mussels were removed from the rocks using a paint scraper (similar to Costa et al. 2008) and were placed into a small tray with a constant flow of fresh Otsego Lake water. Mussels bunched together via byssal threads were pulled apart and placed into the tray. Once all the mussels were in the tray, eleven mussels were selected at random and placed into a 800-micron mesh bag. Those with any physical damage were discarded. One hundred and fifty bags were filled with mussels. All extra individuals were placed into a separate bag. The bags were placed into a large aquarium (~50L) with a slow constant flow of lake water for at least 72 hours for the mussels acclimate to the bags and to determine any mortality due to handling and/or stress (similar to Comeau et al. 2011). The aquarium was lightly aerated with compressed air. After the 72-hour holding period, the mesh bags were removed one at a time and the mussels inside were examined for mortality. Any dead individuals were removed from the bags. If no mortality occurred, one mussel was selected at random, removed from the bag, and discarded. All bags had 10 individuals so that there were 30 individuals for every concentration-exposure period combination. The number of mortalities during the acclimation period was less than ten.

Chemical treatment

Fifteen glass tanks were rinsed with lake water and scrubbed with an abrasive sponge soaked in lake water. The tanks were then emptied, rinsed, and emptied again. Lake water was added to each tank in the volume needed to result in a total of 20L once the vinegar was added. The four concentrations of vinegar used were 25, 50, 75, and 100% vinegar. There were three duplicates of each concentration as well as three control tanks with no chemicals. The distilled white vinegar used was a readily available commercial product (5% acetic acid, Great Value, Distributed by Wal-Mart Stores, Inc., Bentonville, AR). The vinegar was kept in a greenhouse at the BFS main lab overnight before testing. Airstones were added to each tank and they were lightly aerated with compressed air to ensure continued mixing of the treatment water. Once all tanks had been filled, ten bags of mussels were hung from a wooden dowel in each aquarium. Each bag represented an exposure time period (0, 1, 2, 4, 6, 12, 24, 48, 72, and 96 h). After the bags in all tanks were immersed, the time 0 bags were then removed from each tank. Once bags were removed from the treatment tanks, they were labelled with their exposure time and hung from wooden dowels in two large holding tanks with continuously flowing water from Otsego Lake. This created an environment that was very close to the natural conditions. Mussels removed from the 0 and 25% vinegar treatment mussels were placed into one tank and the mussels in the 50, 75, and 100% vinegar solutions were placed into the other tank. One dowel held all the bags from an individual tank. Mussels were left in this tank for at least 48 hours for recovery. Mussels were examined for mortality between 48 and 72 hours after being removed from their chemical treatments. This was done because it has been shown that mussels that appear dead at the end of a chemical treatment can recover after being placed in clean water free of chemicals (Pucherelli et al. 2014).

Mortality was determined by the mussel having gaping valves when removed from the mesh bag and placed on a paper towel. Mussels with a slight gap had a blunt probe placed into the valve gap and if the mussel closed it was considered alive. A mussel with no movement of the valves after probing was considered dead. Only mussels with a slight valve gap were probed, or when more than half of a sample group was fully gaping showing mortality. After mortality assessment, the shell length of each mussel was measured using a Mitutoyo Absolute digital caliper (Model Number: CD-6"CX, Mitutoyo Corporation, Kawasaki, Japan) and recorded along with the mortality status.

Water quality

An YSI (Model Number: 6820V2-M, YSI Incorporated, Yellow Springs, Ohio) multiparameter water quality sonde was used in each chemical treatment tank at times 0, 24, 48, 72, and 96 h. Prior to each measurement, the YSI was calibrated following standard operation protocols to ensure probe accuracy. All tanks of the same concentration were measured starting from the control tanks and increasing in concentration. The probe was rinsed with lake water between concentration levels. The water in the recovery/holding tanks was measured at 0, 24, 48, 72, and 96 h. The parameters measured were temperature, specific conductivity, pH, and dissolved oxygen as percentage and as mg/L. Measurements were taken from the chemical treatment tanks after mussels had been removed for the same exposure time. Measurements in the recovery tank were also taken after mussels of the same exposure time were added to the tank. This was to measure the maximum amount of chemicals being added at a time in the holding tank, as the constant flow of fresh lake water would replace the water in the tank over time diluting the chemical.

Statistical analysis

All data was entered into a Microsoft Excel spreadsheet. Descriptive statistics were run on the shell length of all mussels. It was also used to determine the average mortality for each concentration at each time interval and to create a graph showing mortality at each interval. This was used to create the LD_{50} and LD_{99} with 95% confidence intervals for times 1 and 2 h with SAS® (Version 9.3 SAS Institute Inc., Cary, NC). The LD_{50} and LD_{99} values are the concentrations of vinegar that are required to produce 50% and 99% mussel mortality. A t-Test was performed to compare the mean length of dead and alive mussels. A one-way ANOVA was performed for the average values for each water quality parameter grouped by concentration over time. A t-Test was performed to compare the mean values of each group whenever the ANOVA was significant. The water quality parameters of the two holding tanks were compared using a t-Test. The level of significance was set at $\alpha = 0.05$.

Results

The mean temperatures of the experimental tanks did not differ by concentration for the duration of the experiment (p-value 0.876). The mean dissolved oxygen did not differ between concentrations as either a percentage or in mg/L (p-values 0.354 and 0.807). Mean specific conductivity was significantly different between concentration levels (p-value <0.001). The mean square of variation between groups was 1.34 and was 0.01 for within groups. The mean pH was also different between concentration levels (p-value <0.001). Mean square of variation between groups was 26.35 while the mean square for within groups was 0.07. Each concentration was different from all other concentrations for specific conductivity and pH when compared with t-Tests (all p-values <0.001). The temperature of each holding tank was significantly different over the duration of the study (p-value 0.045). The specific conductivity of each tank was not different (p-value 0.115). Dissolved oxygen concentration as % was not different between tanks (p-value 0.116) and neither was dissolved oxygen as mg/L (p-value 0.106). The pH values were different between holding tanks (p-value 0.026).

Mean shell length for the mussels was 13.29 mm with a standard deviation of 2.94 mm. This number was calculated using the lengths of all mussels in exposure periods of 48 hours and shorter. Mussels exposed to any vinegar treatment for longer than 48 hours were not able to be measured due to the vinegar's effects to the mussels (Figure 1). There was a significant difference between the length of dead and alive mussels (p-value <0.0001). The mean length of dead mussels was 12.99 mm and the mean length of live mussels was 13.92 mm. All concentrations of vinegar led to complete mortality of mussels in less than 24 hours (Figure 2). The concentration to cause 100% mortality the fastest was the full strength vinegar, with a one hour exposure period. The 75% vinegar

Table 1. Average water quality parameters measured from recovery tanks and chemical solution tanks at various times during testing.

Tank/Concentration (% vinegar)	Time (h)	Temperature (ºC)	Specific Conductivity (µS/cm)	pH	Dissolved Oxygen (%)	Dissolved Oxygen (mg/L)
Recovery Tank 1	0	15.25	0.300	6.55	92.9	9.30
	24	14.51	0.303	7.16	93.9	9.56
	48	14.97	0.307	7.06	97.8	9.87
	72	15.84	0.312	6.64	94.0	9.29
	96	15.14	0.306	6.85	94.7	9.51
Recovery Tank 2	0	15.25	0.300	6.55	92.9	9.30
	24	14.59	0.305	7.10	94.2	9.59
	48	15.04	0.303	6.77	97.2	9.79
	72	15.84	0.306	6.41	90.3	8.92
	96	15.18	0.304	6.71	93.7	9.40
0%	0	17.97	0.309	8.54	94.7	8.97
	24	17.27	0.306	7.25	87.4	8.38
	48	17.83	0.307	7.19	84.0	7.96
	72	18.94	0.308	7.21	89.3	8.29
	96	18.00	0.308	7.55	88.9	8.40
25%	0	15.17	0.839	2.63	91.4	9.15
	24	16.93	0.834	2.66	81.3	7.86
	48	17.66	0.879	2.70	63.8	6.94
	72	18.94	1.119	2.78	87.8	8.12
	96	18.99	1.205	2.69	81.1	8.02
50%	0	12.85	1.163	2.32	83.2	8.77
	24	16.51	1.139	2.42	78.8	7.68
	48	17.68	1.326	2.54	77.7	7.36
	72	18.97	1.378	2.54	91.6	8.37
	96	19.02	1.392	2.46	82.8	8.05
75%	0	10.94	1.397	2.15	83.6	9.19
	24	16.41	1.322	2.31	84.4	8.25
	48	17.72	1.492	2.44	86.5	8.19
	72	18.95	1.550	2.44	82.1	7.55
	96	19.05	1.655	2.33	84.1	8.29
100%	0	8.92	1.589	2.03	82.5	8.83
	24	16.63	1.475	2.24	82.2	7.97
	48	17.90	1.593	2.33	77.5	7.33
	72	19.10	1.668	2.34	90.7	8.34
	96	19.10	1.715	2.23	83.2	8.12

Table 2. Estimated LD50 and LD99 of distilled white vinegar concentrations (% vinegar) for adult zebra mussels vinegar with 95% confidence intervals for exposure durations of 1 and 2 hours and the sample dose(s) that caused 100% mortality during the experiment.

Exposure Time (h)	LD_{50} (95% CI)	LD_{99} (95% CI)	SD_{100}
1	37.78[a]	220.89[a]	100
2	24.65 (17.49-29.86)	83.30 (62.04-160.40)	75, 100

Note: [a] indicates that no 95% confidence interval was generated.

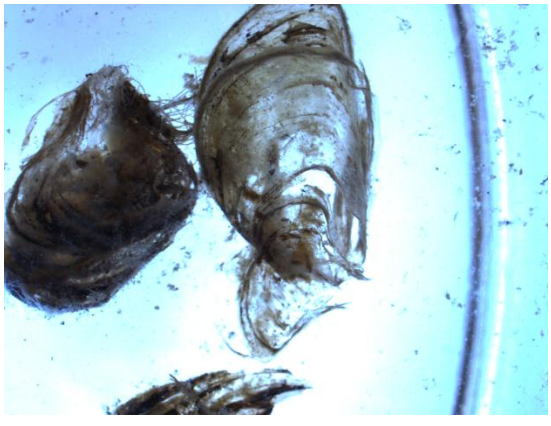

Figure 1. Adult zebra mussels after exposure to 50% distilled white vinegar for 72 hours. Photograph by Eric Davis.

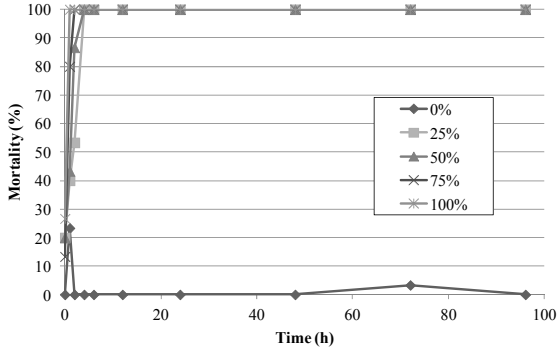

Figure 2. Average mortality (%) of adult zebra mussels (N=3) from Otsego Lake after exposure to distilled white vinegar of varying concentrations (% vinegar) in Fall 2014.

treatment led to complete mortality with a two hour exposure, and the 50% and 25% vinegar treatments caused complete mortality with a four hour exposure. All exposure periods longer than four hours also caused complete mortality. The control group had 8 total mortalities. Seven mortalities occurred at the 1 hour exposure (4, 3, and 0 in the three replicates, respectively) and one mortlity at the 72 hour exposure. The LD_{50} and LD_{99} values were calculated in % vinegar in the treatment and were 37.78% and 220.89% for the one hour exposure period and 24.65% (17.49–29.86 [95% CI]) and 83.30% (62.04–160.40 [95% CI]) for the two hour exposure period (Table 2).

Discussion

The low mean square of variation values given for the pH and the specific conductivity within treatment groups indicates that the values did not vary greatly throughout the experiment. The source of variation was much greater between groups, as it was expected. The ratio of vinegar and water in the tanks differed by concentration, leading to differences in the amount of dissolved ions in the water when measured as specific conductivity. The difference in the amount of ions within each concentration also materialized in the pH readings. The greater the concentration of vinegar there was, the lower the pH value. The temperature at the beginning of the experiment was lower in the vinegar solutions compared to the recovery tanks, but the largest temperature difference was less than seven degrees. Adult mussels have been shown to handle temperature increases up to 10 degrees and decreases of up to 15 degrees with no mortality (Nichols 1992). The difference in the temperature of the holding tanks was found to be significant; however the difference between their means was 0.0375°C. The small sample size likely played a role in the difference being significant. The water coming from the lake was well oxygenated and had a consistent amount of dissolved ions in it. Neither tank came close to having anoxic conditions that would contribute to addition mortality not caused by the chemical treatments. The pH was different between the two recovery tanks and can be explained by how they were used. The first tank only received the mussels from the control and 25% vinegar treatments. The second tank received mussels from the 50, 75, and 100% vinegar treatments. When the mussels were added to the recovery tank, they usually had some amount of their treatment solution left in the mesh bag or on the surface of the mussels. So by adding three bags that came from tanks with significantly lower pH values, the second recovery tank pH value should decrease more than the first recovery tank. Also, the timing of measurement taking could have played a role. Water quality was measured directly after the bags of mussels were added to the recovery tanks. This would mean the lowest pH would be when the measurements were taken, but they would get diluted out as fresh lake water was flowing into the tank.

It has been suggested that the pH tolerance range of adult zebra mussels is 6.5 (McCauley and Kott 1993) to 9.3 (Bowman and Bailey 1998). Claudi et al. (2012) found that a pH value of 6.9 caused about 40% mortality to adult mussels after an exposure of 10 weeks. The pH values of all vinegar concentrations in this study were well below 6.5, so mortality was expected. However, the speed of mortality caused by vinegar was not known and was of the greatest interest. The short exposure time causing complete mortality is a very positive indication for the use of distilled white vinegar as a decontamination method. It is recommended elsewhere that contaminated equipment be exposed to undiluted white vinegar for 20 minutes (DiVittorio et al. 2012). However, DiVittorio et al. (2012) do not indicate where they came up with this recommendation. This exposure period is intended for the treatment of veligers, not adults. It could be assumed that the time needed to kill veligers is shorter than needed to kill adults based on previous findings (Fisher et al. 1994). DiVittorio et al. (2012) do not recommend chemical treatments for adults because they claim adults can close their valves for up to 10 days when exposed to chemicals. The current study indicates that adult mussels can be treated with chemicals to induce complete mortality in under 4 days. With all concentrations causing complete mortality in 4 hours, it can be assumed that an even lower concentration could be used as the minimum concentration to cause complete mortality with a 24 hour exposure. The four hour exposure with 25% vinegar solution does allow for a fast turn-around time without using as much vinegar. The use of such a strong acid solution may be problematic in certain applications where the materials that need to be disinfected could be harmed by the chemical treatment. A test under field conditions would also be beneficial to investigate the effects it may have on equipment. Further testing to examine lower vinegar concentration solutions could be

beneficial so that there is a lower potential for damage. The likelihood of using the chemical treatment to disinfect the exterior of a watercraft is low because of the exposure time that is needed to induce complete mussel mortality. However, the use of a vinegar solution to disinfect equipment that is submersible such as lines, chains, and anchors is very feasible. The use of a vinegar solution to disinfect areas of a watercraft that can hold volumes of liquid such as livewells, bilges, and anchor boxes is also feasible because the chemical solution can be held in those places for the needed exposure period. Examining the exposure periods needed to cause complete mortality of veligers is of great value because they can be easily transported by trailered watercraft while not being visible to anyone inspecting the watercraft for AIS.

The difference in the shell length of live and dead mussels was found to be significantly different. However, this difference should not be considered conclusive due to the fact that all shell lengths of mussels in exposure times of 72 and 96 hours were not included in the calculations. The addition of these shell lengths may have caused the mean lengths of dead and live mussels to be similar. All mussels used in this study were greater than 8mm in length, so they can be considered adults regardless of their mean lengths. Also, all mussels were killed regardless of length during exposures greater than 4 hours in all vinegar solutions.

This study suggests that distilled white vinegar can be used at varying concentrations for the decontamination of adult zebra mussels. Vinegar is a common household chemical that is relatively inexpensive ($2.49 per gallon for this test) so it may have a positive impact on the decontamination effort put forth by the lay watercraft owner. An investigation into the impact vinegar solution have on equipment would be very informative to help determine its practical use as a decontamination chemical.

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