Toxicity of EcoClean to Quagga Mussel Veligers

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December 2015

INTRODUCTION

Quagga mussels (*Dreissena rostriformis bugensis*) were introduced into the Great Lakes region of North America in the 1980s and have since been transported to other regions via recreational boaters (Johnson et al. 2006). These mussels are considered an invasive species because they cause much ecological and economic damage in areas where they establish and proliferate into the environment (Wong and Gerstenberger 2011). Quagga mussels attach to hard surfaces, grow by filtering out nutrients from the water, and spawn to release millions of eggs, up to three times per year. The eggs are fertilized and within a few weeks the viable larval stage of the mussel grows into a planktonic organism, a veliger. The veliger stage feeds and is maintained in the water column until it reaches a size where it can settle on a hard surface and grow into a juvenile and then into an adult, restarting the cycle. Veligers are considered a hearty but vulnerable life stage, which can be easily transported to other areas and start an infestation of a new water body (Choi et al. 2013).

There is a growing need for options and tools to use to disinfect and eradicate the quagga mussel from transport vessels and from water bodies. However, many of the available options are either too expensive, environmentally unsafe, or limited to certain uses (Moffitt et al. 2015). A possible option for disinfection of equipment contaminated with quagga mussel veligers that has not been investigated is EcoClean.

EcoClean, distributed by EcoUSA, is formulated from 100% ionized water; it is a very stable $H_3O_2^-$ (Mike Mangham, personal communication, September 28, 2015; EcoUSA 2013a). The website of this product describes many benefits of using this product including that it is transparent, odorless and non-flammable; it is harmless to the environment, gentle on skin and clothing, and easily removes carbon, nicotine, oil and fat from surfaces (EcoUSA 2013a). In the FAQ section, the website describes the product as safe for the environment because it does not contain active surface agents, phosphates or plasticizers (EcoUSA 2013b). It is produced with specially designed equipment that discharges negatively charged ions creating hydroxyl ions in the water. Due to this equipment, EcoClean has a longer shelf life and more cleaning strength than other ionized water product commercially available (EcoUSA 2013b).

The objective of this study is to understand the toxicity of the EcoClean solution on quagga mussel veligers. EcoUSA provided 1 L of EcoClean product to conduct experiments with on the veligers. Static laboratory trials were conducted as this was the prescribed use of the product.

METHODS

This study was conducted at Willow Beach National Fish Hatchery (WBNFH), Willow Beach, AZ from 29 September through 1 October 2015. WBNFH has year round access to adult mussels and there were at least two annual spawning events for this population that produced all veliger life stages from May through January. The hatchery provided access to raw river water, electricity, laboratory space, and security of equipment.

Quagga mussel veligers were collected from river water with 35 µm mesh plankton tow nets attached a raceway in the hatchery at flows of 80 L/min. Collections of veligers lasted approximately 1 to 2 h (4,800 to 9,600 L filtered). Water and filtrate from the plankton net cod jar was filtered again with a 150 µm nylon mesh net and then transferred into Nalgene sample bottles and brought into the lab. Samples were transferred to beakers and retained at room temperature (~20ºC) creating a stock collection for treatment. An aliquot of 1-2 mL was removed with a plastic serological pipette to evaluate the density of live veligers with a gridded Sedgewick-Rafter counting cell and compound microscope. D-shaped and umbonal sized veligers were used for testing and were mixed in with other planktonic organisms in the treatment sample.

Trials were conducted with 3 replicate containers per interval and water type. To conduct a trial, 19 mL of EcoClean solution, composed of 3% active ingredient and 97% water, was added to 1 mL of concentrated veligers from the stock collection into a 150 mL glass beaker for a 95% concentration of EcoClean, 0.0285% active ingredient, $H_3O_2^-$. For controls, 19 mL of untreated 10 µm filtered water and 1 mL of concentrated veligers were put into the beakers. At the end of a test interval, the beaker contents were drained over a 10 µm nylon mesh filter, then the filter and contents suspended in a small Petri dish. The sample was immersed into a solution of 0.4% fast green dye for 20 min. The samples were rinsed and retained in fresh filtered untreated water until microscopic evaluation with a gridded Sedgewick-Rafter counting cell and compound microscope. For each treatment, a minimum of 50 individuals were scored as live or dead (Figure 1). The condition of veligers were assessed as live or dead using the fast green dye that stains dead tissue a greenish blue, while leaving living tissue clear. Veligers were considered dead if they were completely green with no visual cilia, internal organ, or body movement observed for 5 seconds.

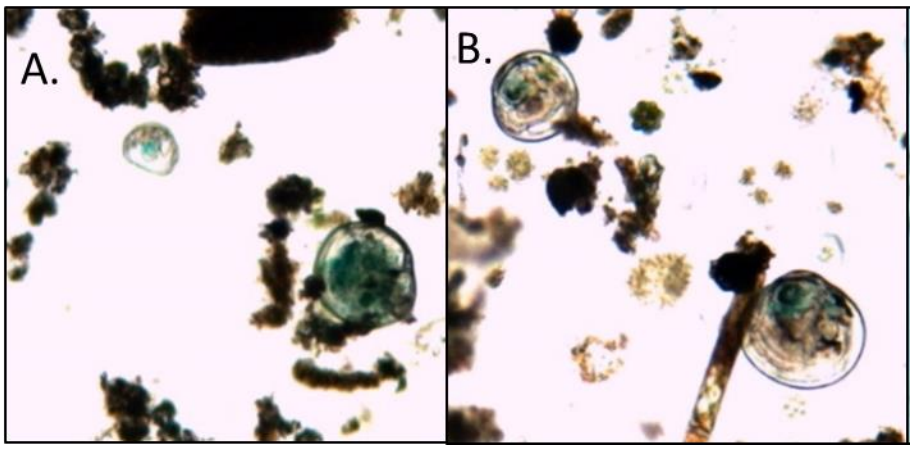

Figure 1. Examples of A) Dead and B) Live quagga mussel veligers after being stained with fast green dye.

Measurements of conductivity, specific conductivity, pH, salinity, TDS, and ORP with a YSI 556 multi-probe (YSI Yellow Springs, OH 45387) of each test duration at the end of tests. The EcoClean solution was analyzed to determine the temperature, specific conductivity, conductivity, pH, salinity, TDS, ORP, dissolved oxygen, ozone and hydrogen peroxide levels.

The veliger mortality results were analyzed by calculating the proportions of live and dead by each replicate treatment and interval and averaging together the replicates for final results and to obtain a standard deviation.

RESULTS

The EcoClean solution was tested from the bottle for its water quality characteristics prior to testing of the veligers (Table 1). Water chemistry readings of the WBNFH water used was also measured (Table 1). The EcoClean solution had a very high, basic, pH at 12.18 and also had a high amount of hydrogen peroxide at 1.2 mg/L. Specific conductivity, conductivity, total dissolved solids and salinity were also very high.

Table 1. Water chemistry readings of the EcoClean solution and of the WBNFH water used in testing.

	Temp C	pH	DO (mg/L)	DO%	ORP (mV)	H_2O_2 (mg/L)
EcoClean	20.9	12.18	7.46	85	-145.9	1.2
WBNFH water	19	7.8	6	65.3	-192	0

	Ozone (mg/L)	Sp. Cond. (mS/cm)	Cond. (mS/cm)	TDS (mg/L)	Salinity (ppt)
EcoClean	<0.05	5.593	5.155	3.635	3.03
WBNFH water	0	1.02	0.91	0.666	0.51

EcoClean was effective in killing quagga mussel veligers in 30 minutes with a 95% concentration of solution (Table 2). Lower exposure times were effective with a 10 minute exposure killing 91%.

Table 2. Mortality results of the quagga mussel veligers at the tested exposure periods.

Treatment	# Replicates	# Live	# Dead	Percent Mortality (± Standard Deviation)
Control	9	1167	48	4.1 (±2.8)
EcoClean				
10 min	6	68	674	91.0 (±4.2)
20 min	3	2	396	99.6 (±0.7)
30 min	6	0	508	100 (±0.0)

Water quality of the testing solutions was performed at the end of the testing duration. The three replicate samples were combined to obtain enough solution for a reading. Readings were averaged for repeated exposures and a standard deviation was obtained (Table 3). Dissolved oxygen, ozone and hydrogen peroxide measurements were not taken because these reading are

changed when the sample passes through the filter, which was used to collect the veligers prior to water chemistry testing. Water chemistry values did not change significantly with exposure duration, which indicated that the sample loading and organic content was low enough to have no effect on the chemical solution.

Table 3. Water quality parameters of the testing solutions post exposure.

Treatment	Temp C	Sp. Cond. (mS/cm)	Cond. (mS/cm)	pH	TDS (mg/L)	Sal (ppt)	ORP (mV)
Control	23.60 (±0.64)	1.05 (±0.04)	1.02 (±0.05)	8.52 (±0.18)	0.68 (±0.03)	0.52 (±0.02)	-193.8 (±59)
EcoClean							
10 min	22.49 (±0.38)	4.67 (±0.01)	4.45 (±0.05)	11.96 (±0.06)	3.04 (±0.01)	2.51 (±0.01)	-209.4 (±1)
20 min	23.12	4.63	4.47	12.02	3.01	2.48	-222.4
30 min	22.70 (±0.21)	4.56 (±0.03)	4.37 (±0.03)	11.87 (±0.08)	2.96 (±0.02)	2.44 (±0.02)	-171.2 (±73)

DISCUSSION

This study found that EcoClean can be used to kill 100% of quagga mussel veligers with a 30 minute exposure time. The pH of the solution was very high at 12.0, which was the most likely mode of action for EcoClean. MSDS for EcoClean, also labeled inorganic ion cleaning liquid "KRIA ION" has a chemical composition of water and sodium metasilicate acid (Figure 2) (Fukuda 2001). Elevated pH was the result of the compounds in EcoClean reacting to form hydroxide ions (OH^-), or sodium hydroxide. Bases, such as sodium hydroxide, also disassociate forming hydroxide ions, which make them so toxic.

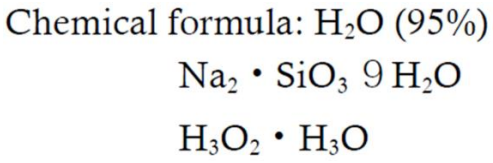

Figure 2. Chemical formula for EcoClean.

Other studies with sodium hydroxide have found 100% veliger mortality at a pH of 12 with exposure periods of 30 minutes (Moffitt et al. 2015). Time to mortality was dependent on temperature; at lower temperature a longer exposure time was necessary to achieve 100% mortality of quagga mussel veligers. Sodium hydroxide killed juvenile and adult mussels, but the exposure time was 3 days and also dependent on temperature (Barenberg et al. 2015). Claudi et al. (2012) determined that sodium hydroxide was effective at controlling quagga and zebra mussels though seasonality was a potential issue, with 99% mortality of adult zebra mussel mortality occurring in 24 h tested in May and only 90% mortality in 120 h of exposure tested in October.

Sodium hydroxide is also lethal to other planktonic organisms, such as the zooplankton *Daphnia magna* (cladoceran) and *Eucyclops* spp. (copepod) over short exposure times (TenEyck et al. 2009). Moffitt et al. (2015) found that elevated pH also killed all freshwater ballast organisms greater than 50 µm. Other studies have shown that elevated pH was 100% effective in 72 hour on environmental and fish pathogenic bacteria (Starliper and Watten 2013). Therefore use of EcoClean should be targeted and waste should be properly disposed of in accordance with the MSDS.

Further studies that might be of interest with EcoClean could be to investigate how the product changes over time at different temperatures or due to other environmental conditions and therefore its effect on the mortality of quagga mussels. Though the manufacturer has found that the active ingredient, $H_3O_2^-$, disassociated at 32 F and cleaning power ends at 120 F and is stable in sunlight and open air (Mike Mangham, personal communication, December 27, 2015).

ACKNOWLEDGEMENTS

I am grateful to Mark Olson, Tom Frew, Stephanie Karpowicz, Giovanni Cappellii, Andrew Flaten, Joseph Saccomanno and Scott Peterson at Willow Beach National Fish Hatchery for their support and assistance. I also am appreciative of Dr. Christine Moffitt Professor and Assistant Unit Leader at the USGS Coop Unit in the Department of Fish and Wildlife Sciences of the University of Idaho, Moscow for her support, assistance, advice, and use of equipment.

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