Investigation of the KRIA Industrial Ionizer on the Mortality of the Invasive Quagga Mussel

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TABLE OF CONTENTS

EXECUTIVE SUMMARY

The KRIA Ionizing Water Treatment System (KRIA) electrochemically generates negatively charged oxygen (O_2^-, or the superoxide radical) from atmospheric air, removes the atmospheric nitrogen and stores the products in a pressurized reservoir tank. From the reservoir tank the superoxide radical is then injected into the water stream at the discharge nozzle. Discharges of the superoxide radical have been shown by Premier Materials and EcoUSA to increase oxygen content, reduce dissolved nutrients and organic pollutants. The superoxide radical is a reactive oxygen species which is recognized as a strong oxidant, which can cause oxidative stress in organisms. When reactive oxygen species exceed an organism's ability to counteract the effects with superoxide dismutase and other enzymatic reactions, cell damage can occur which can lead to the destruction of overall homeostasis and eventual death. Organisms have different levels of enzymatic processes to overcome exposure to reactive oxygen species. It is hypothesized that mollusk species, especially the invasive dreissenid mussels (quagga and zebra mussels) have low amount of superoxide dismutase enzyme and are more susceptible to reactive oxygen species.

This study investigated a single and multiple pass of water testing system, referred to as flow-through and recirculating test system, with the KRIA technology to determine the best test system to cause mortality of quagga mussel adults and veligers. Studies were conducted in waters from the Lower Colorado River at Willow Beach National Fish Hatchery, Willow Beach, Arizona. Common water quality methods and measurement devices were used to quantify the reactive oxygen species produced by the KRIA technology. The flow-through exposure system was run for 7 days starting September 2015 followed by the recirculating test system run for 17 days.

At 17 days of exposure a total of 74% mortality was achieved in a recirculating configuration with the KRIA technology, with control mortality at 9%. After removed from the exposure, latent mortality occurred in exposed groups of adult mussels. Increased exposure time did increase mortality of adult quagga mussels. The measured dose of superoxide radical was quantified using dissolved oxygen, which reached an average of 204% or 16.81 mg/L at 23.9 C in recirculating tests. Veligers were also exposed and the treatment killed 18.6% more veligers than the control system at 48 hours.

In a flow-through system, where the water went through the KRIA machine only once; the KRIA technology did not produce high enough levels of dissolved oxygen or detectable superoxide radical by-products to cause mortality in quagga mussels at either life stage tested. Levels of dissolved oxygen in the flow-through system in the treated KRIA water was 21% above background and control, which was 102% or 9.3 mg/L at 1 gpm. At higher velocities, 6 gpm, the observed dissolved oxygen was 119% or 10.9 mg/L, which also did not produce mortality in adult or veliger life stages of the quagga mussels.

INTRODUCTION

Premier Materials Technologies, Inc. (Premier Materials, Minneapolis, MN) is marketing the KRIA Ionizing Water Treatment System (KRIA) with EcoUSA (EcoUSA, Denver, CO) as a novel water and wastewater treatment unit. The KRIA electrochemically generates negatively charged oxygen (O_2^-, or the superoxide radical) from atmospheric air while removing the nitrogen and is stored in a pressurized reservoir tank, which is then injected into the water stream at the nozzle discharging pumped water. The patent describes this system as a two part process to ionize the water. The first process is to pull in the polluted water into the device and bring the polluted water into contact with ceramic balls, generating bubbles, and at the same time, inducing negative ions into those bubbles. The second process is to subject the bubbles to both sides of multiple window splines which are fixed, having spaces in between, on high-speed rotating shaft by bar magnets and ceramic rods alternately to the circulating water channel. This method of ionization is described as "ionization by collision", and involves reaction in a magnetic field as the air is drawn through a bed of ceramic balls with reactive minerals (Kunio et al. 1999). The superoxide created is charged into water that is pumped into a treatment unit and then discharged. This discharge can be released into a water body of interest, such as a pond or lake, river or stream, or tank. Discharge of the superoxide radical has been shown by Premier Materials and EcoUSA to increase oxygen content, reduce nutrients and organic pollutants in several field application trials. Studies in conjunction with US Army Corps of Engineers found that the KRIA increased dissolved oxygen content, decreased diesel concentrations in the water, removed PCBs from the water, and reduced the number of algae and cyanobacteria in the water (Medina 2014). The objective of this study was to determine if the KRIA technology system could be successfully applied to kill invasive zebra (*Dreissena polymorpha*) or quagga (*D. rostriformis*) mussels.

Zebra and quagga mussels are an invasive aquatic freshwater species that has infested many waterways of the United States and continue to spread to new areas causing much ecological and infrastructural problems. Current invasive mussel control strategies include physical (e.g. biofiltration, high-pressure spray) and chemical (e.g. sodium hypochlorite) methods, which remove adults from substrates and tend to be reactive in nature (Meesters et al. 2003; Daniels and Shelby 2007; Matthews et al. 2012). Developing cost-effective solutions to mitigate the effects of invasive mussel infestations is a high priority for many facility operators and waterbody managers and additional research is required to identify and develop the most effective solutions.

The superoxide radical is a reactive oxygen species recognized as a strong oxidant. The superoxide radical is a component of other reactive oxygen species and therefore can easily be transformed into metal bound radicals in the presence of certain metals (manganese, iron or copper) or can produce other reactive oxygen species in the presence of organic compounds, such as hydroxyl, hydroperoxyl and alcoxyl radicals (Blough and Zepp 1995). By-products of these reactions include hydrogen peroxide, ozone, and hydroxide (Livingstone et al. 1990, Manduzio et al. 2005, Valavanidis et al. 2006). Surface waters also have naturally occurring reactive oxygen species where levels and types depend on the availability of metal and organic

compounds, and are driven by abiotic photochemical reactions (Blough and Zepp 1995). Biological organisms, including mollusks, produce many of these radicals through biological processes or have evolved adequate enzymatic and non-enzymatic antioxidant mechanisms, including the enzyme superoxide dismutase (SOD) that mediate the effect of the radicals on the organism (Livingstone et al. 1990; Manduzio et al. 2005; Valavanidis et al. 2006). There are several forms of superoxide dismutase in species and each form has a different level of effectiveness against oxidants dependent on metal availability, pH, or seasonality. Mussels, especially the *Mytillus edulis*, a cousin of *D. polymorpha*, has a copper zinc complex with superoxide dismutase (Cu/Zn-SOD)form expressed in tissues, rather than the manganese Mn-SOD form which has higher effect against superoxide radicals (Manduzio et al. 2005).

Using superoxide radicals for a treatment to kill dreissenid species is not well studied. The efficacy of ozone and hydrogen peroxide on zebra mussels has been studied with limited results. Hydrogen peroxide was not found effective to kill veligers due to the need for doses >9 mg/L for 14 days to kill 96% (Van Benschoten et al. 1993). Ozone was more effective in removing veligers from the water column (97% reduction with 5 hr at 0.5 mg/L), but short term exposures did not prove effective (Van Benschoten et al. 1993). Chemical oxidants, such as chlorine, have been studied on zebra and quagga mussel species with high acceptance by facility managers due to the affordability of chlorine and ease of application with high effectiveness (100% reduction of veligers with 20 hrs at 1 mg/L) (Boelman et al. 1997; Van Benschoten et al. 1993). However, there can be unwanted by-products from use of chlorine and toxicity on non-target species. Some studies have demonstrated that mussels exhibit avoidance behaviors, and adult mussels will close to avoid a chemical toxicant for up to 2 weeks (Boelman et al. 1997).

Zebra and quagga mussels are major biofouling organisms and rapidly colonize hard surfaces in flow through facilities or in open waters, clogging water intake structures, such as pipes and screens, reducing pumping capabilities for power and water treatment plants ties (Benson et al. 2015). Managers are searching for cost effective tools that can keep smaller diameter piping clear of infestations. The KRIA system could provide the technology to keep water system intake structures clear, and may hold promise in use within open systems in lakes and rivers.

The KRIA Ionizing Water Treatment System has the potential to cause high mortality on different life stages of zebra and quagga mussels as it produces many types of radicals, increases the dissolved oxygen to very high levels (Mike Mangham, personal communication, April 13, 2015), and as the patent describes has a physical method of destroying the veligers as they pass through the KRIA. Increased exposure to a high level of radicals should outcompete the natural defense mechanisms of the organism and create much damage at the cellular level to induce mortality (Mike Mangham, personal communication, April 13, 2015; Manduzio 2005). Supersaturation of the water with dissolved oxygen as a treatment strategy on dreissenid mussels is novel and at this time, no studies have been published to determine the toxicity of such treatments.

This study will determine a flow-through (single pass) and recirculating (multiple pass) testing system design that will test the KRIA technology to determine if mortality of quagga mussel adult and veligers from the Lower Colorado River will occur. Common water quality methods

and measurement devices will be used to quantify the reactive oxygen species produced by the KRIA technology and recommend the best one for use with this technology. It will also investigate the physical ability of the machine to destroy veligers as they pass through the machine.

METHODS

Study Location

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

Experimental Set-Up

The KRIA technology arrived at WBNFH early July 2015; an electrician was hired to wire the 220V 3 phase 0.75 kw into the hatchery breaker box. The machine provided for testing contained a self-suction pump producing 10 gallons per minute (gpm) discharge. Water was pumped through a ceramics filter on the inlet side (Figure 1A), through the pump in the KRIA housing (Figure 1B) and discharged (Figure 1C) through a 1-1/2" diameter nozzle that was reduced to 1" and then to ½" before the water was discharged (Figure 1D). Atmospheric air flow was filtered through two air filters and then through an oxygen-nitrogen separator. The oxygen was routed to the negative ion generator at about 3 L/min before stored in an air tank and the nitrogen was off-gassed back into the atmosphere. The air leaving the tank was piped into the ½" section of the nozzle (Figure 1D). The KRIA system released off gasses at about 10 second intervals. The manufacturers recommended monitoring the increase of dissolved oxygen (DO) level in a recirculating system. During operations, dissolved oxygen levels after 2 hours of recirculating flow were 4.61 mg/L (53% DO) higher than background dissolved oxygen levels.

Untreated WBNFH water was released into a raceway at about 20 gpm to provide a recovery system for adult quagga mussels after testing. The water quality parameters were the same as the other untreated water flowing through the hatchery. Raceways were cleaned on a weekly basis to remove settled debris off of the bottom of the raceways.

To test the hypothesis that there would be mortality of veligers by being pumped through the KRIA technology, veligers were collected two times with a 35 µm mesh plankton net at the inlet into the vertical tank for three hours at 6 gallons per minute (1080 L filtered) of both the treated system and control system. The collection was analyzed with the fast green protocol described in this study.

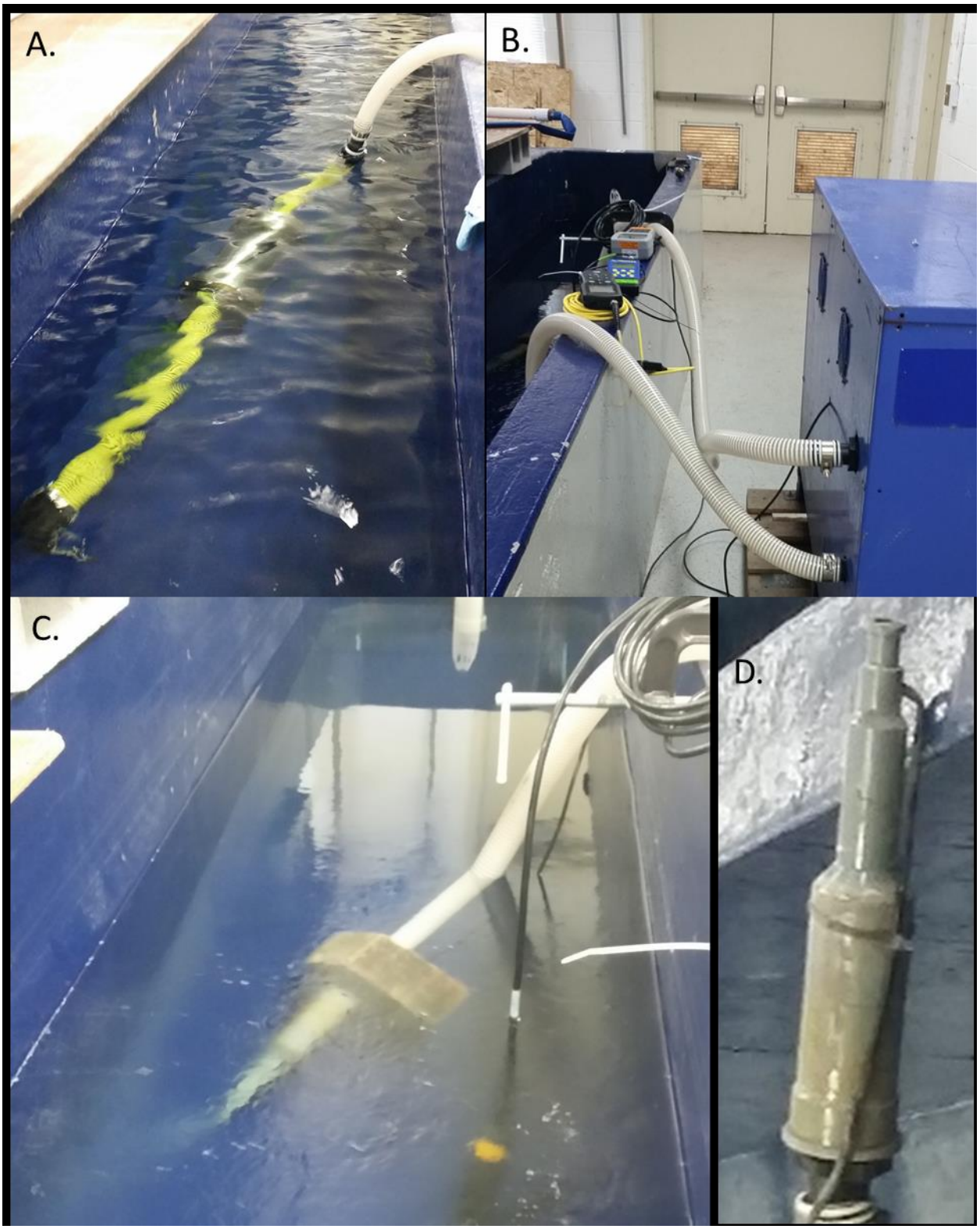

Figure 1. Water discharge system of the KRIA technology, including A) the inlet side where water traveled through the ceramic filter and then B) into the pump housed within the KRIA machine and C) discharged into the water through the D) nozzle.

Flow-Through Testing Conditions

The tests of the flow-through, single pass system began 9 September 2015 with the KRIA pumping untreated water from a 240 x 30 inch concrete raceway inside the WBNFH building and the discharge being plumbed with flexible hosing into PVC pipe. A portion of the treated flow was diverted with a series of ball valves through a flow meter and into a 25-gallon testing tank (cooler) and a 50 gallon vertical testing tank with excess water being drained out to the hatchery discharge pipe (Figure 2 and Figure 3). Water flowed through the 25-gallon testing tank and was gravity fed through a ¾" tube into another 25-gallon testing tank. From here the water was again gravity fed through a 3/4" tube into a 240 x 30 x 16 inch concrete lined raceway before exiting the hatchery discharge pipe (Figure 3). A control system was set up that mirrored the treated system with a submersible sump pump (Little Giant 506171 6-CIA-RFS 115 Volt 2760 Watt Cast-Iron Sump Pump) to pump water through the system.

Figure 2. Actual configuration of flow-through testing system design.

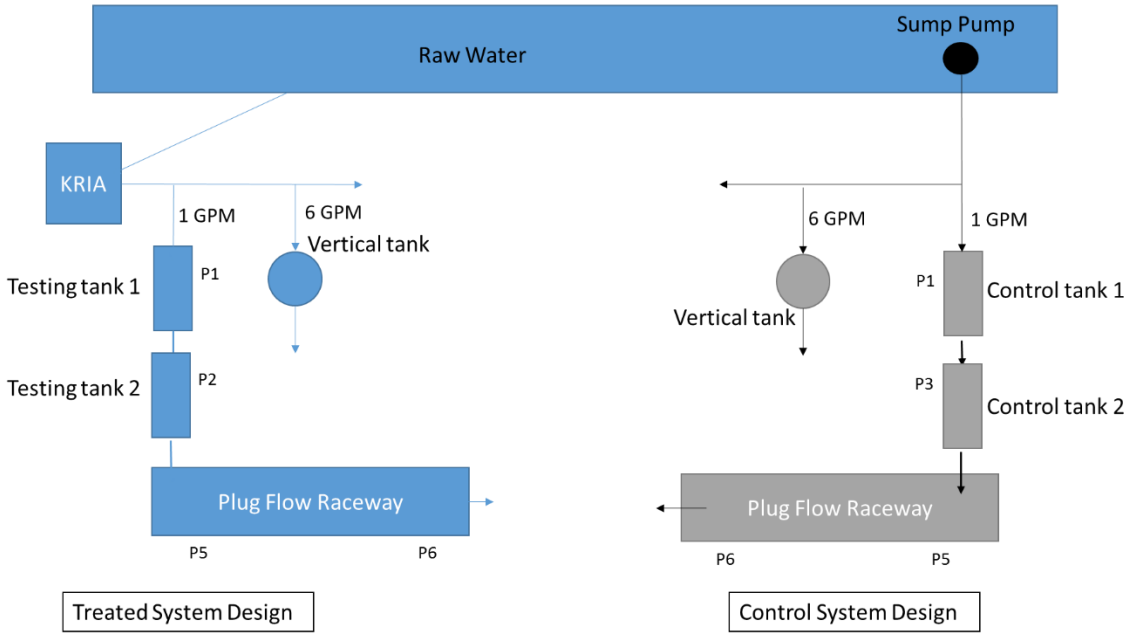

Figure 3. Schematic of the flow-through system with water quality and mussel testing positions.

Flow into the control and testing tanks were set at 1 gallon per minute (gpm) and flow into the vertical tanks was 6 gpm. Water quality measurements were taken and quagga mussels were tested at the four positions labeled throughout the system (P1, P3, P5 and P6) and at the top and bottom of the vertical tank (Figure 3). Distance that the water traveled from the nozzle at each testing location was P1: 5 ft, P3: 10 ft, P5: 25 ft, P6: 40 ft, and top of vertical tank: 10 ft and bottom of vertical tank: 13 ft. The KRIA was operating throughout the seven days of testing and adult mussels were exposed to treatment for seven days before put into recovery system. Quagga mussel veligers were exposed for 4 and 24 hours.

Recirculating Testing Conditions

To establish a recirculating system, a 240 x 30 x 18 inch plug-flow concrete lined raceway was filled with river water on 18 September 2015 with a drain plug in the discharge pipe. The inlet pipe of the KRIA technology was positioned to draw from the bottom of the raceway and the discharge end was positioned at the top of the raceway (Figure 4 and Figure 5). The nozzle on the discharge end was submersed under the surface of the water at least 3 inches and clamped in place to the raceway wall. Flow going through the KRIA was at 10 gpm and was held constantly on through the 17 days of testing.

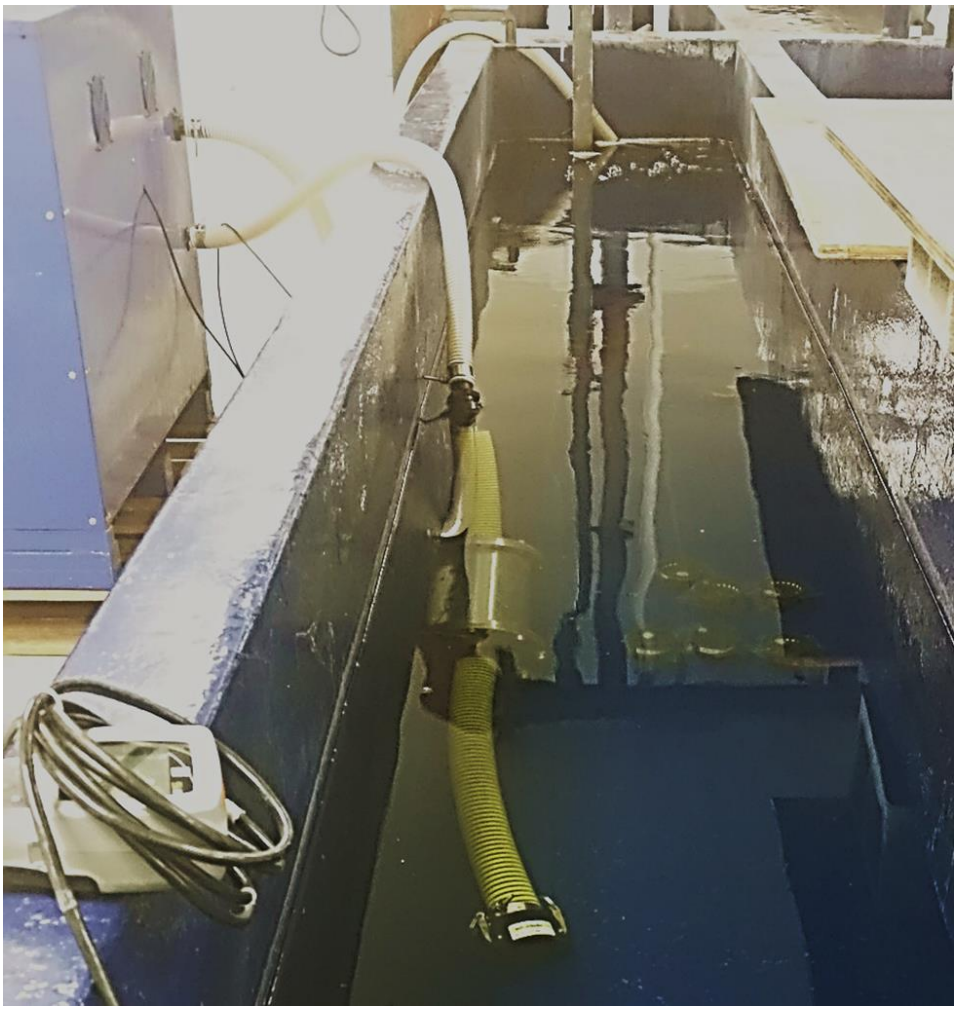

Figure 4. Configuration of the KRIA treated water in the recirculating testing design.

A control system was kept with minimally flowing water and 20 gpm was recirculated back into the raceway (Figure 5). The submersible sump pump discharged at 40 gpm, the extra flow was diverted to the flow-through control system at 2 gpm and 6 gpm for the vertical tank. To keep the recirculating control system full of water, an extra 20 gpm of WBNFH river water was added.

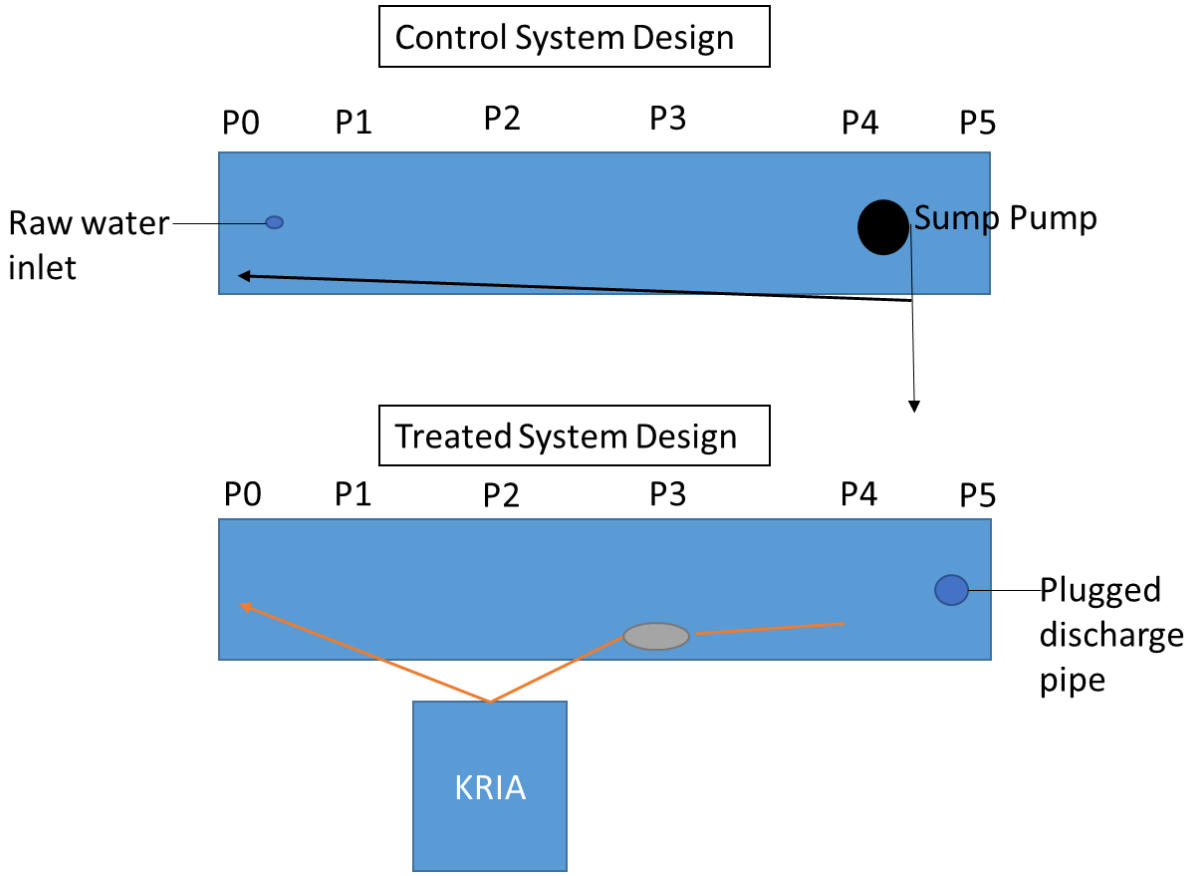

Figure 5. Schematic of the recirculating testing and control systems with water quality and quagga mussel testing positions.

Water quality measurements were taken and quagga mussels were tested at the 4 positions labeled throughout the system (P1, P2, P3 and P4) (Figure 5). Additional water quality measurements were taken at P0 and P5. The distance from the nozzle of these positions were P0: <1 ft, P1: 4 ft, P2: 8 ft, P3: 12 ft, P4: 17 ft, and P5: 19 ft. Adult mussels were pulled from treatment, assessed, and placed into the recovery system at 3, 5, 7, 11, and 17 days. Adult mussels were placed into the treated and control raceway before the startup of the system. All mussels were assessed at 3 days of exposure and one set was pulled out and put into recovery follow-through water. This protocol continued for day 5, 7 and 11. The 17 day mussels were evaluated on day 14 for mortality and were put back into the treatment conditions until day 17. Adult mussels in recovery were assessed on day 7, 14 and 21. Quagga mussel veligers were exposed for 24 and 48 hours.

Water Quality Analysis

Water quality parameters were measured with a variety of probes and test kits to determine the best instrumentation for monitoring. The instruments were cross calibrated with each other. An YSI 556 multi-probe (YSI Yellow Springs, OH 45387) was used to measure temperature, dissolved oxygen (Clark Electrode: membrane method), pH, conductivity, salinity, and total dissolved solids (TDS) and oxidation-reduction potential (ORP). Background water quality measurements for the incoming untreated water were consistent through the testing period (Table 1).

Table 1. Water quality parameters of the untreated water measured with the YSI 556 for the period of testing at Willow Beach National Fish Hatchery.

Date	Temp (C)	Sp. Cond (mS/cm)	Cond. (mS/cm)	pH	TDS (mg/L)	Salinity (ppt)	DO (mg/L)	DO %	ORP (mV)
9/12	18.7	1.02	0.90	7.8	0.67	0.51	7.3	78.3	-90
9/14	17.7	1.02	0.87	7.9	0.66	0.51	7.0	73.3	-153
9/16	17.8	1.00	0.86	7.7	0.65	0.50	6.9	72.7	-92
9/18	18.0	1.02	0.88	7.7	0.66	0.51	6.5	67.5	-95
9/21	19.0	1.02	0.91	7.7	0.67	0.51	5.7	61.2	-179
9/25	19.0	1.02	0.91	7.8	0.67	0.51	6.0	65.3	-192
10/2	18.4	1.01	0.88	8.0	0.66	0.50	7.7	82.2	-212

To measure dissolved oxygen with a luminescent/optical dissolved oxygen probe a Hach HQd LDO probe (Hach, Loveland, CO) was used that also measured temperature. A Point Four Tracker total dissolved gas pressure meter (Pentair Aquatic Eco-Systems, Sanford, NC 27330) was used to measure total dissolved gas pressure, barometric pressure, and temperature. Hach hydrogen peroxide test kit, model HYP-1, was used to determine the amount of hydrogen peroxide in the water and a Hach ozone test kit, model OZ-2, was used to determine the amount of ozone in the water. Two other chemical titration methods were investigated, D2180: Standard Test Method for Active Oxygen in Bleaching Compounds and reduction of superoxide radical by superoxide dismutase by presence of hydrogen peroxide content, but they were not applicable and were unreliable to testing results of the superoxide content. The approach to detect superoxide radical with the reaction to superoxide dismutase to form elevated levels of hydrogen peroxide were not sensitive enough to determine the difference between background hydrogen peroxide levels and amount of superoxide radicals.

Adult Quagga Mussel Testing

Live quagga mussels were collected from dock structures at WBNFH. Following collection and sorting, 35 live mussels were placed into 3” round plastic net pots, with an l mm nylon mesh screen over the top held in place by a rubber band (Figure 6A). The mussels were placed into the test system (three replicates for test location and control). Adult mussels were assessed for live

and dead condition (Figure 6B and 6C), where dead mussels were either empty shells or contained rotting flesh and did not respond to touch stimulus. Dead mussels were counted and removed from the test system at least weekly. After treatment duration, mussels were put into the recovery system. Mussel mortality was monitored until day 25 in flow-through treatment and until day 21 for the recirculating treatment.

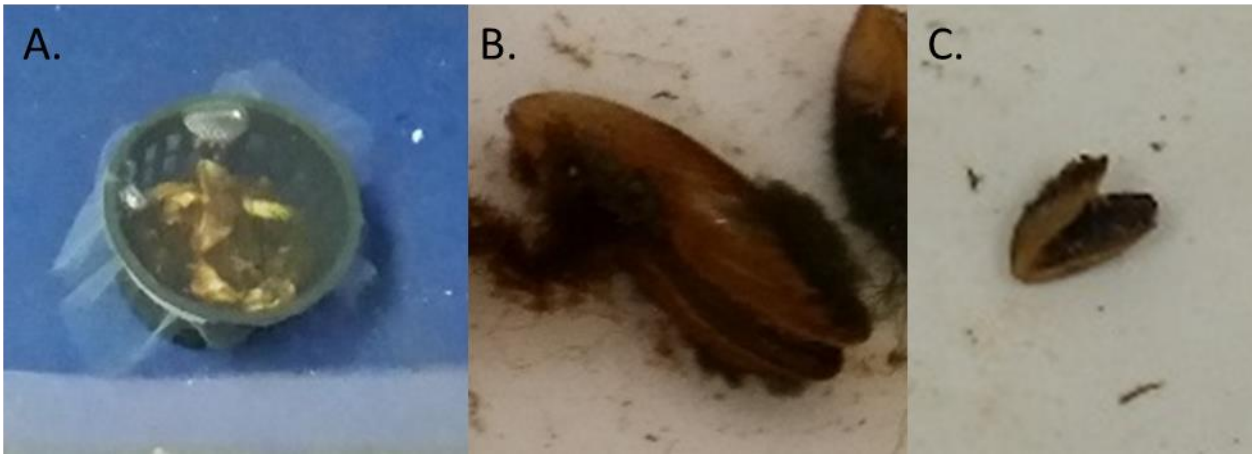

Figure 6. Adult quagga mussels A) in plastic net pots. B) Alive adult quagga mussel. C) A dead adult quagga mussel.

Quagga Mussel Veliger Testing

Quagga mussel veligers were collected from river water with 35 µm mesh plankton tow nets attached to 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. Densities were greater than 100 veligers per 1 mL of sample counted.

Trials were conducted with 3 replicate containers per interval and water type. To conduct a trial, 3 to 4 mL of concentrated veligers from the stock collection was put into the testing containers. The testing containers were created with ¾” diameter electrical conduit couplers glued together with PVC and a 35 µm nylon mesh filter was positioned between the male and female couplers (Figure 7). Testing containers were placed into the bottom of the test system for the duration of the treatment with water flow directed through the test containers.

Figure 7. Veliger test systems.

At the end of the test interval, the testing containers were pulled up through the water column to concentrate plankton to the bottom. The top end was rinsed with 10 µm filtered water in a squirt bottle and removed. The sides of the testing container were rinsed down and all of the test water was drained through the bottom filter. The sample was then placed into a solution of 0.4% fast green dye for 20 min, then rinsed to remove the dye from the samples and were retained in fresh filtered water until microscopic evaluation with a gridded Sedgewick-Rafter counting cell. For each treatment, a minimum of 100 individual veligers were scored as live, dead, or as empty shells (Figure 8). The condition of veliger was assessed with the aid of 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.

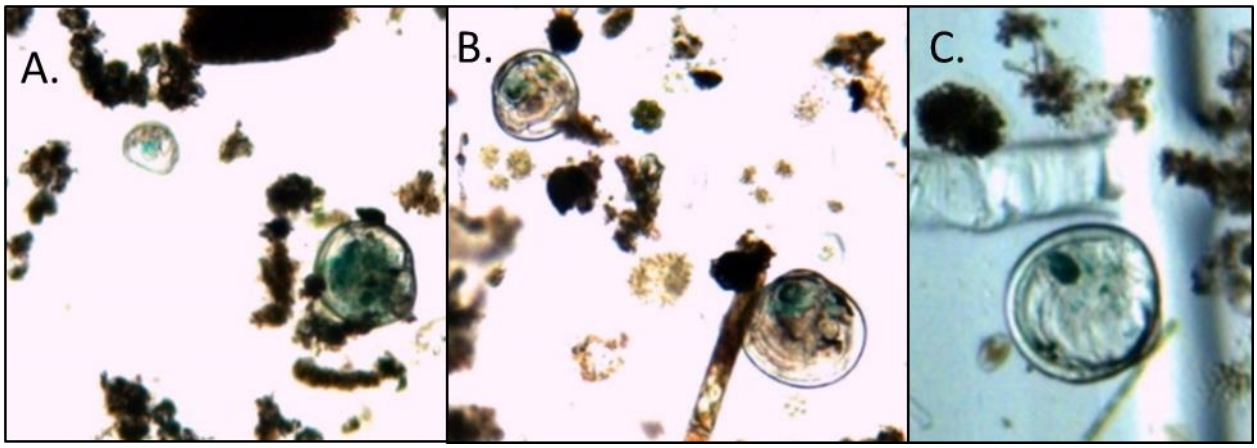

Figure 8. Examples of A) Dead, B) Live, and C) Empty shell of quagga mussel veligers after being stained with fast green dye.

Statistical Analysis

Mussel mortality was calculated by dividing number of dead by total number of live and dead counted and multiplying by 100%. Averages and standard deviations were calculated with the formulas in Microsoft Excel (2013). Statistical analysis was conducted in R (R Development Core Team, 2015) with package *multcompview* (Graves et al. 2012). An ANOVA was used to determine significant differences between variables with α=0.05, and Tukey's HSD tests were analyzed to determine which variables were significantly different from each other. T-tests were also used to determine differences between the mortality of control and treated results, which were calculated with the formulas in Microsoft Excel.

For veliger analysis the percentage of empty shells was calculated by dividing the number of empty shells counted by the total number of veligers counted. Further analysis was conducted to determine if the empty shells could be included as dead mussels in the longer treatment durations (greater than 24 hours) with *Proc Freq* using SAS version 9.2 (SAS Institute, Cary, NC). Using the chi-square analysis in this procedure to determine if there was a significant difference between treatment and control given location by duration as an interval.

RESULTS

Analysis of Veligers Survival through the KRIA System

Collected organisms from the discharge of the control and treated systems were observed to be swimming and behaving normally; there was no difference in the condition of veligers passing through the KRIA system compared to the control (Table 2). Mortality of the quagga mussel veligers were slightly lower in the treated discharge than the control discharge. The amount of veligers collected in the treated discharge was higher, which was not a treatment effect, but related to the position of the inlet in the flowing raceway between the two test systems. If there was a treatment effect there would have been a higher percentage of dead or empty shells in the treated samples compared to the control samples.

Table 2. Analysis of the quagga mussel veligers through the test and control system during a 3 hour collection at 6 gpm.

Treatment	Rep.	# Live	# Dead	Empty	Total	% Mortality w/o empty	% Empty
Control	1	92	4	32	128	4.2%	25.0%
	2	66	3	22	91	4.3%	24.2%
Treated	1	130	4	52	186	3.0%	28.0%
	2	123	3	19	145	2.4%	13.1%

Mortality of Quagga Mussels in a Flow-Through Application of the KRIA System

Post Exposure Adult Mortality

After 7 days of continuous application, the mortality of adult quagga mussels was assessed. There were no significant differences between the treated and control mortality (Figure 9). The adults were placed into recovery for an additional 18 days and checked regularly for additional mortality. At the last check, Day 25, there was still no significant differences in mortality between treated and control adult mussel mortalities (Figure 9). The quagga mussels retained in the vertical tanks held at 6 gpm had significantly higher mortality than adults held at 1 gpm, but the mortality of both control and treated were not significantly different (Figure 10). Because of this effect, the treatment was not considered effective.

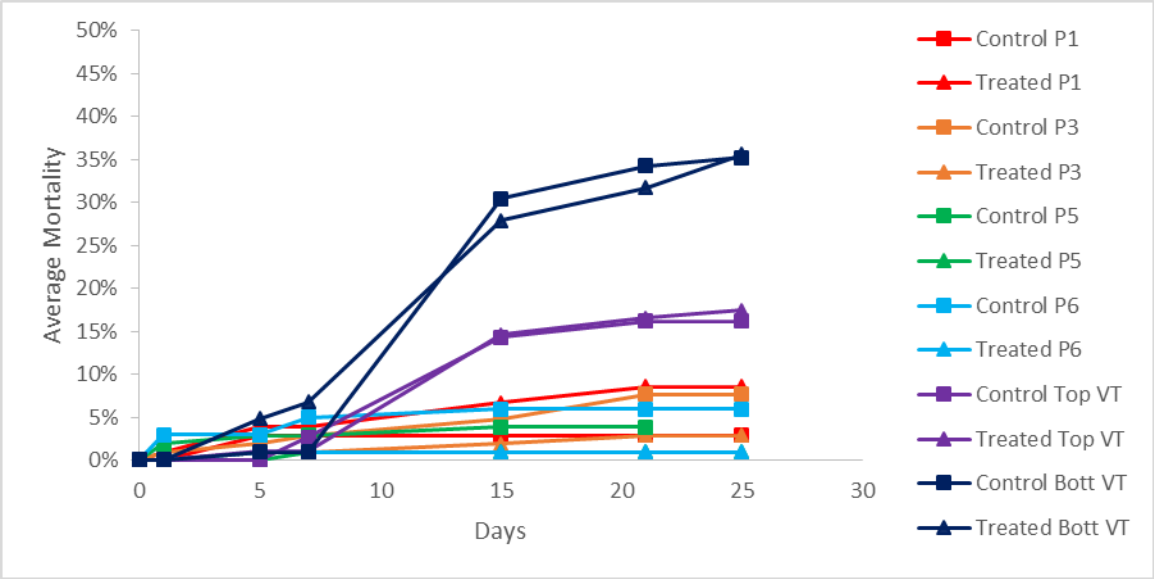

Figure 9. Average mortality of adult mussels exposed to 7 days of flow-through KRIA treated water and its associated control in each position tested. At day 7, treated mussel containers were moved in with control containers at the corresponding locations and monitored for mortality until day 25.

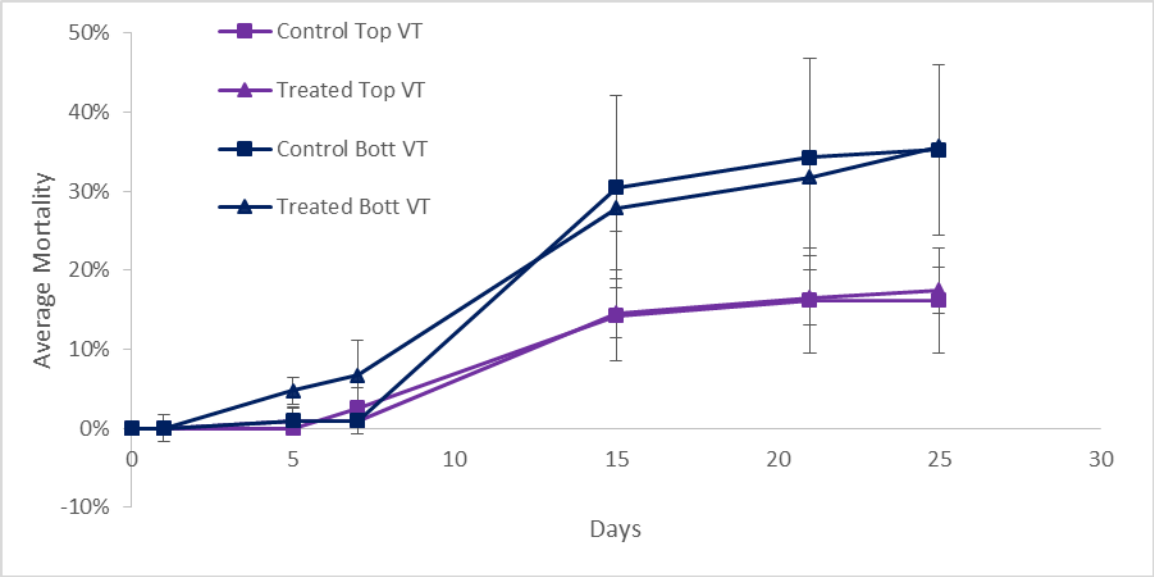

Figure 10. Average mortality with standard error bars of adult mussels exposed to 7 days of flow-through KRIA treated water in the vertical tanks and its associated control in each position tested. At day 7, treated mussel containers were moved in with control containers at the corresponding locations and monitored for mortality until day 25.

Veliger Mortality after Contact Time

In the flow-through experimental test system, collected veligers were exposed to the test system in the holding chambers and then removed and assessed at 4 and 24 hours at P1: 5 ft, P3: 10 ft, P6: 40 ft and in the vertical tank (VT) 10 ft. Mortality of the veligers was very low, an average of 4.5% with a range of 1 to 8.1% mortality (Table 3). The ANOVA calculated no significant differences in the mortality of veligers based on treatment, position or duration of treatment and t-tests also showed no significant differences between the treatments of each combination of position and duration.

The frequency of empty shell counts was assessed to identify the possibility of the test system immediately causing death causing erosion of veligers from the shells and only leaving empty shells behind. There were no significant differences found by the ANOVA between control and treated amounts of empty shells in the veliger samples (Table 3). The KRIA treatment did not have an effect on veliger mortality in a flow-through condition at either 1 gpm or 6 gpm.

Table 3. Average mortality of veligers exposed for 4 and 24 hours in the test system at the different locations with standard deviation and associated percentage of the amount of empty veliger shells in the treatment.

Treatment	% Mortality	% Empty
P1: 5 ft		
4 hrs: Control	5.5 (±1.8)	31.5 (±7.6)
Treated	6.3 (±4.6)	20.7 (±2.2)
24 hrs: Control	3.5 (±1.8)	15.4 (±4.0)
Treated	1.9 (±0.8)	16.6 (±8.4)
P3: 10 ft		
4 hrs: Control	5.5 (±3.2)	41.7 (±3.0)
Treated	5.2 (±4.8)	46.0 (±0.6)
24 hrs: Control	7.9 (±5.3)	30.4 (±0.4)
Treated	4.6 (±1.8)	26.5 (±1.9)
P6: 40 ft		
4 hrs: Control	3.2 (±0.6)	26.4 (±4.4)
Treated	4.5 (±1.8)	21.5 (±0.4)
24 hrs: Control	6.5 (±5.5)	21.3 (±5.4)
Treated	8.1 (±3.0)	15.6 (±0.5)
Vertical Tank: 10 ft		
4 hrs: Control	1.1 (±0.9)	14.0 (±4.0)
Treated	1.4 (±0.4)	15.1 (±0.7)
24 hrs: Control	5.2 (±5.9)	16.5 (±4.0)
Treated	1.5 (±0.2)	17.5 (±1.4)

Water Quality during Treatment

Water quality measurements were taken in the flow-through system and compared against the water quality measurements of the untreated water. It was observed that the test system did change some of the parameters measured including temperature and dissolved oxygen. There were slight changes in conductivity, pH, ORP, TDS and salinity, but not outside of accuracy readings of the equipment so there were no significant differences and therefore no effect of treatment was observed for these parameters. A supersaturation probe was also used and the total gas pressure was 100% for treated and control test systems and did not change through the treatment duration. Hydrogen peroxide and ozone kits were also used and there were no differences from control in these measurements for the flow through test system.

The temperature of the untreated water ranged from 18.0 to 18.9 C with an average of 18.45 C for the 7 days of testing. Transport of water through the KRIA increased the water temp by 0.2 C but not through the control pump, as the difference was 0.0 C. The water warmed up when in the raceway at P5 and P6 by an additional 0.05 C at P5 and an additional 0.1 C at P6 in the treated, whereas in the control the increase at P5 was 0.2 C and an additional 0.05 C at P6, though the

accuracy of the equipment was ±0.15 C. There was no change in temperature within the vertical tanks. This increase in temperatures was not significant in the survival of the mussels.

Dissolved oxygen was measured in mg/L and as a percent saturation. Percent dissolved oxygen was mostly analyzed as it accounted for temperature changes. The starting percent dissolved oxygen in the untreated water was 75% (±6.4%). As the water moved through the test system in the control side the water experienced an increase in DO% (Table 4). At control P1 and P2 there was only a slight increase in DO% which was within the equipment's accuracy, but the DO% increased by 9% at control P5 and P6. In the treated test system, the DO% decreased as the KRIA treatment water moved through the test system. Initially, at treated P1, the DO% increased by 21% over background DO% levels, there was no significant change at P3, but at P5 and P6 the DO% was at +24%. If the system was to increase the DO% by 9% as observed in the control system, the treated water only increased 3%, which indicated that some of the dissolved oxygen created by the KRIA was lost through the system. In the vertical tanks, the DO% increased 37% in the treated and there was no measured increase in the controls.

Table 4. The measurements of dissolved oxygen made with the Hach LDO probe of the control and KRIA treated water through the test system in flow-through conditions. Measurements listed as amount of dissolved oxygen measured above the untreated water readings and the standard deviation of the percent dissolved oxygen and the amount of increased dissolved oxygen in mg/L.

Treatment	Untreated water	P1	P3	P5	P6	Vertical Tank Top	Vertical Tank Bottom
Control							
DO%	73.48	+0.27	+2.0	+9.23	+9.73	+0.13	+0.03
SD(DO%)*	(±2.1)	(±1.02)	(±0.94)	(±2.24)	(±2.44)	(±0.32)	(±0.58)
DO (mg/L)	6.73	+0.02	+0.18	+0.81	+0.85	+0.01	+0.01
Treated							
DO%	77.43	+21.73	+21.88	+24.15	+24.3	+37.4	+37.0
SD(DO%)*	(±9.0)	(±1.71)	(±1.69)	(±6.43)	(±7.32)	(±0)	(±0.34)
DO (mg/L)	7.07	+1.93	+2.01	+2.16	+2.16	+3.39	+3.37

*Accuracy of the DO probe is ±2% and 0.2 mg/L.

The difference in measured dissolved oxygen between treated and control test systems was significant. The KRIA did increase the measured dissolved oxygen of the water and in a flow-through system at 1 gpm the percent dissolved oxygen increased by over 20%. When the flow rate was higher, 6 gpm, the dissolved oxygen levels increased by 37% as seen in the vertical tanks (Table 2). There was not an increase in measured hydrogen peroxide, ozone or change in ORP. In the vertical tanks the ORP did decrease slightly from the untreated water, 8 (±7.5) mV at the top and 13 (±10.4) mV at the bottom.

Mortality of Quagga Mussels in a Recirculating Application of the KRIA System

The treated recirculating system pulled water out at 16 ft from the head of the raceway and discharged into the front of the raceway at 10 gpm. The control recirculating system pulled water out at 18 ft from the head of the raceway and discharge into the front at 20 gpm. Within 24 hrs the dissolved oxygen level in the treated had maxed out at about 206% or 17.7 mg/L at 21.6 C, there was no change in the control system.

Treatment and Post Exposure Adult Mortality

Mortality in test systems was higher than the control mortality in treatments that had greater than 7 days of exposure (Figure 11). The highest mortality achieved was 74% after 17 days of exposure; at day 17 the adult mussel mortality was 57 (±7)% and after 4 days in the recovery system there was an additional mortality of 17% for at cumulative mortality of 74 (±4)%. Mortality decreased as exposure time decreased (Figure 11), as an 11 day exposure achieved 36 (±9)% mortality on check day 21. Control mortality was 9 (±4)% of all of the treatments combined, as there were no significant outliers during the different removal days (Figure 12).

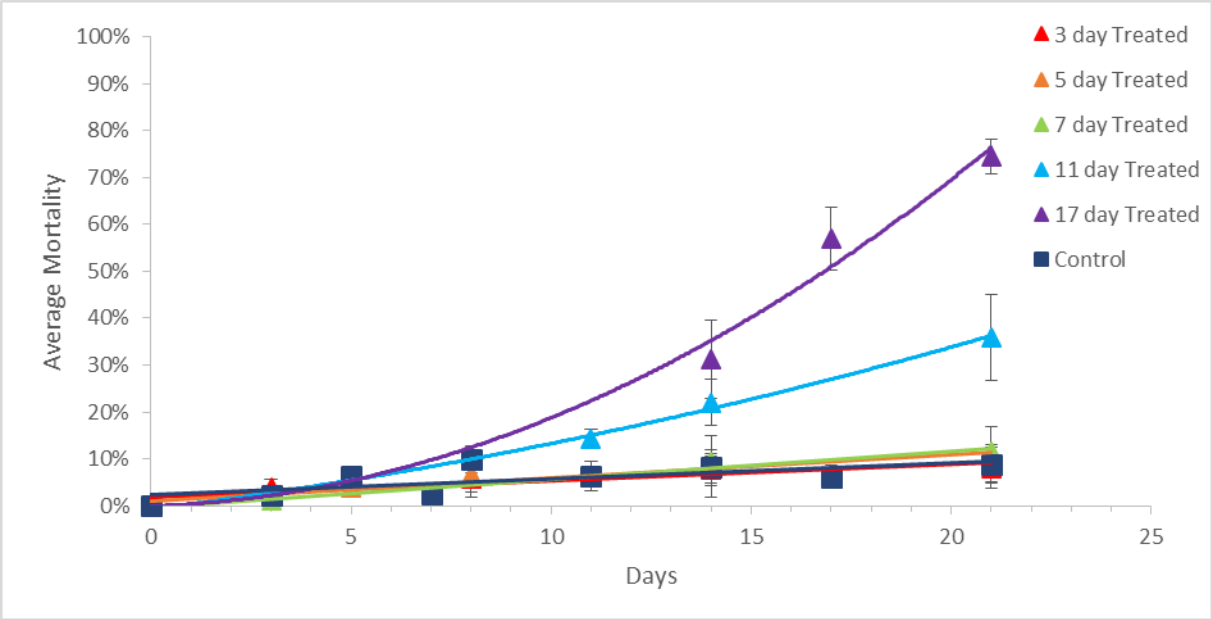

Figure 11. Average mortality with standard error bars and trend lines of adult mussels exposed to 3, 5, 7, 11 and 17 days of recirculated KRIA treated water and the control with each position tested averaged together. At the end of each exposure duration, treated mussel containers were moved in with control containers at the corresponding locations and monitored for mortality until day 21.

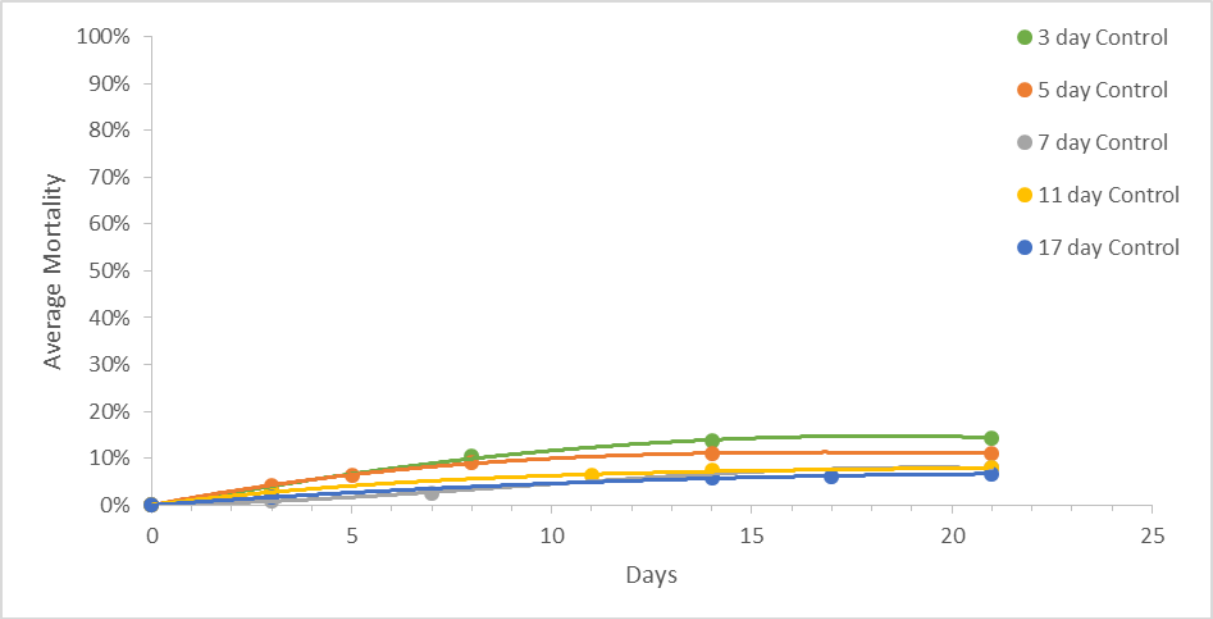

Figure 12. Average mortality of the control mussels removed at 3, 5, 7, 11 and 17 days of recirculated water with each position tested averaged together.

Exposure Veliger Mortality

There were no significant differences between control and treatment responses when positions and durations were combined using percent mortality with live and dead. However, when the variables were separated and run through an ANOVA, there was a significant difference in the treatment response. T-tests were used to compare the individual treatment, position and duration combinations to determine a treatment effect (Table 5). P3 48 hours was the only treatment that had significantly higher mortality in the treatment compared to the control. The other significant differences were because the control had higher mortality than the treated samples.

Table 5. Average mortality of veligers exposed for 24 and 48 hours in the test system at the different locations with standard deviation and associated percentage of the amount of empty veliger shells in the treatment and with percent mortality with all counts combined. (*) was placed on the highest value when there was a significant difference from t-test analysis.

Treatment	% Mortality	% Empty	% Mortality w/ Empty
P1: 4 ft			
24 hrs: Control	35.9 (±10.6)% (*)	35.5 (±1.5)%	58.5 (±9.4)%
Treated	1.8 (±0.5)%	36.1 (7.2)%	37.2 (±9.0)%
48 hrs: Control	6.4 (±4.6)%	30.9 (±0.5)%	35.4 (±3.5)%
Treated	16.1 (±9.7)%	53.0 (±5.1)% (*)	60.2 (±9.9)% (*)
P2: 8 ft			
24 hrs: Control	2.7 (±2.1)%	22.6 (±5.0)%	24.6 (±7.8)%
Treated	2.2 (±0.8)%	22.2 (±4.5)%	24.0 (±5.0)%
48 hrs: Control	6.7 (±3.8)%	32.7 (7.4)%	37.3 (8.0)%
Treated	2.8 (±0.7)%	42.7 (±3.6)%	44.4 (±4.3)%
P3:12 ft			
24 hrs: Control	0.9 (±0.0)%	10.8 (±7.4)%	11.6 (±10.4)%
Treated	4.0 (±2.3)%	23.4 (±2.8)%	26.4 (±5.3)% (*)
48 hrs: Control	22.8 (±13.1)%	29.7 (±3.6)%	46.1 (±8.9)%
Treated	65.6 (±6.3)% (*)	43.7 (1.6)% (*)	80.6 (±4.9)%
120 hrs: Control	29.8 (±14.3)%	77.6 (13.6)%	83.6 (±14.7)%
Treated	26.7 (±37.7)%	96.6 (2.2)%	98.3 (±0.7)%
P4: 17 ft			
24 hrs: Control	28.6 (±10.5)% (*)	45.9 (±3.8)%	61.2 (±8.9)% (*)
Treated	5.5 (±1.4)%	35.5 (11.1)%	39.2 (±12.3)%
48 hrs: Control	54.4 (10.5)%	55.0 (±11.7)%	78.4 (±12.4)%
Treated	63.3 (±9.5)%	63.3 (1.8)%	86.7 (±3.4)%

The next step was to look at the amount of empty veliger shells in the sample as the treatment and the long duration may be causing faster death or ripping the veligers out of the shells and only leaving empty shells behind. There were also no significant differences when position and duration were combined between control and treated with the amount of empty shells present. T-tests were used to compare the individual treatment, position and duration combinations to determine a treatment effect (Table 5). There were two instances that there were more empty shells in the treated than in the control, P1 48 hrs and P3 48 hrs. The other two treated position, P2 and P4, had about 10% higher percent empty shells in the treated samples compared to the paired control samples at 48 hours. This trend did not occur in the 24 hour samples.

Chi-square analysis of the live to dead and empty combined frequencies determined that there was a treatment effect in the 48 hour exposure samples (Table 6) and confirmed the trend seen in Table 6. In all of the treatments that were exposed greater than 24 hours there was a higher frequency of dead and empty veligers in the treated samples and lower number of live veligers

compared to the control samples. The higher amount of empty shells relative to the control at 48 hours was indicative that mortality occurred in the treatment system after 24 hours and the veliger flesh became detached from the shell before the 48 hour observation point. Therefore, the treatment killed 18.6% more veligers than the control system at 48 hours (Table 5).

Table 6. Frequency counts and percentage of response with the chi-square analysis of the test conducted for greater than 24 hours to determine effect of treatment compared to control.

	Frequency (%)		**Chi-Square**		
Treatment	**Live**	**Dead+Empty**	**DF**	**Value**	**Prob.**
P1: 48 hrs					
Control	167 (32.12)	93 (17.88)	1	31.55	<0.0001
Treated	103 (19.81)	157 (30.19)			
P2: 48 hrs					
Control	236 (35.17)	131 (19.52)	1	4.89	0.027
Treated	170 (25.34)	134 (19.97)			
P3: 48 hrs					
Control	215 (23.22)	188 (20.30)	1	118.81	<0.0001
Treated	100 (10.80)	423 (45.68)			
P4: 48 hrs					
Control	54 (11.89)	190 (41.85)	1	5.90	0.0151
Treated	28 (6.17)	182 (40.09)			
P3: 120 hrs					
Control	35 (7.97)	166 (37.81)	1	33.32	<0.0001
Treated	4 (0.91)	234 (53.20)			

Longer durations in the treated KRIA system did increase the mortality of veligers. In the one 120 hour duration conducted at P3, there was a very high amount of empty shells in the treated replicates, 96.6%, but the control had high counts as well, 77.6%, with actual mortality quite low (Table 5). The frequency and chi-square tests showed that the higher amount of empty shells was significant and that there was a treatment effect (Table 6). It should be noted though that this high amount of mortality in the controls indicated that the veligers were not surviving in the containers very well for long periods of time.

Treatment Water Quality Analysis

Water quality measurements were taken of the recirculating system and compared against the water quality measurements of the untreated water. There were only small, non-significant changes in parameters as the measurement locations became further from the discharge end of the machine or inlet of the control. In the treated system there was evaporation of the water out of the test system which raised the specific conductivity, pH, total dissolved solids (TDS), and salinity over the testing period (Table 7). This rise in these parameters were not significant enough to effect the final mortality results. The ORP values were lower in the KRIA treated

system by about 10 to 20 mV than in the control system. As the duration of the experiment continued the ORP in both the control and treatment became more negative and oxidative.

Table 7. Average readings of the water quality measurements taken at each position over the treatment duration for treated and control test systems for specific conductivity, pH, total dissolved solids and salinity, showing that increase in parameters was from evaporation out of the test system.

	Sp. Cond. (mS/cm)		pH		TDS (g/L)		Salinity (ppt)		ORP (mV)	
Day	Treat.	Cont.	Treat.	Cont.	Treat.	Cont.	Treat.	Cont.	Treat.	Cont.
0	1.018	1.018	7.75	7.75	0.662	0.662	0.51	0.51	-91.8	-98.8
3	1.032	1.023	8.28	7.83	0.671	0.665	0.51	0.51	-183	-182
7	1.045	1.024	8.34	7.83	0.679	0.666	0.52	0.51	-212	-197
14	1.067	1.011	8.61	7.91	0.694	0.657	0.53	0.50	-224	-203

The temperature of the hatchery water ranged from 17.5 to 19.6 C throughout the testing period. Temperature of the control water was 0.1 (±0.24) C higher than the hatchery water. The treated temperature water was 5 (±0.96) C, with the average measured treated water temperature at 23.9 (±0.81) (range: 21.5-25.2) C. There was no additional water being added to lower the temperature and the air temperature was over 30 C in the WBNFH facility. The temperatures were consistent throughout the raceways in both the control and treated test systems. Increased temperature of the treated system was due to the KRIA system heating up the water. When the machine was turned on the water temperature increased 1 C in three hours. Overnight when temperatures decrease, the KRIA treatment system had an increased temperature. The average increase in temperature over 19 hours of initial temperature logging was an increase of 1 C per 4 hours. The temperature of the treated KRIA test system equilibrated at a temperature 5 C higher than the control.

As noted in the above section, the recirculating treated raceway stabilized at 206% dissolved oxygen which was 17.7 mg/L at 21.6 C. The treated dissolved oxygen stayed above 200%; the average was 204% or 16.81 mg/L at 23.9 C. There were no differences in the distribution of dissolved oxygen in the treated system between the two edges of the raceway or at different depths (bottom, middle or top) at each position. The KRIA system increased the amount of dissolved oxygen from the background untreated water by 136.6 (±4.65)% or an increase of 10.67 (±0.59) mg/L. In the control system the recirculation of water increased by 10 (±1.44)% dissolved oxygen or 0.96 (0.15) mg/L.

The supersaturation probe was also used and the total gas pressure was 103% for treated and 97% in control test systems and did not change through the treatment duration. There was an increase in the amount of hydrogen peroxide observed in the treated system after 14 days, 0.9 mg/L. The control was also low at 0.25 mg/L. The ozone levels in the KRIA treated water were 0.05 mg/L after 14 days at P1 and P2 only; 0.0 mg/L was recorded in the control.

Evaluation of the Water Quality Equipment

The best tool that was currently on the market, reasonably priced, and worked well to detect the KRIA technology produced changes in the treated water was an optical sensor dissolved oxygen meter. The KRIA produced superoxide radicals that reacted very quickly with the environmental compounds and resulted in elevated dissolved oxygen levels. Dissolved oxygen sensors were very convenient to use to determine the difference between KRIA treated water and untreated water. Other oxygen species, such as hydrogen peroxide, ozone, and other testing methods for increases in dissolved oxygen were also evaluated.

There are generally two types of dissolved oxygen sensors used in the field, an electrochemical sensor and an optical sensor. An electrochemical sensor has a membrane that the oxygen diffuses through and reacts with KCl and sends changes the electrical gradient in the KCl solution which sends an electrical signal to the machine (YSI Incorporated 2009). This type of sensor uses up the dissolved oxygen in the immediate vicinity of the probe and must be constantly moved or experience a high flow rate to obtain accurate readings. Optical sensors have a paint layer to the sensing layer that the oxygen diffuses through that changes the luminescence of the sensing layer (YSI Incorporated 2009).

The optical sensor does not use up any oxygen when reading and therefore does not have to be moved to obtain an accurate reading. Due to the high amounts of oxygen and other forms of oxygen species in the KRIA treated water, the YSI 556 probe with the electrochemical sensor read high in the recirculating system, especially when recording levels on a continuous basis overnight (Figure 13). When the probe was moved at the velocity recommended by YSI protocol the readings dropped and were consistent to the readings of the Hach optical sensor probe that was also used. Therefore the flow rate of the KRIA treated system was not high enough to get accurate readings if the probe was left stationary in the recirculating system. Thus the optical sensor dissolved oxygen probe was easier to use and just as accurate as the electrochemical probe, but did not have the function of recording or logging over a period of time.

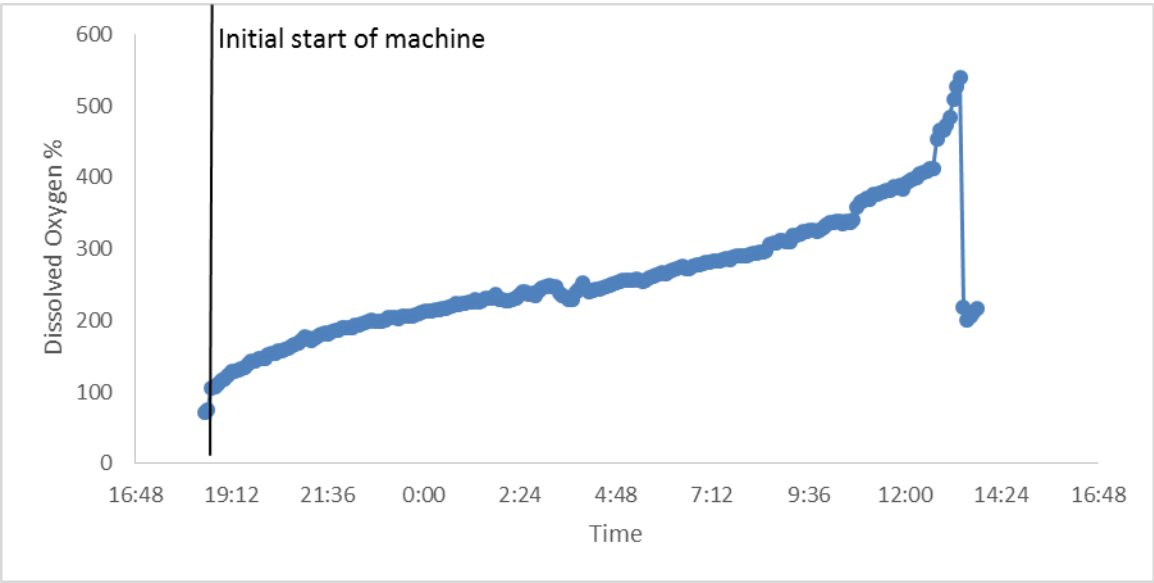

Figure 13. Percent dissolved oxygen reading logged with the YSI 556 electrochemical sensor from the initial startup of the KRIA to the next afternoon. The drop in readings at the end or the monitoring periods was due to user moving probe at YSI specified reading velocity for accurate readings.

Hydrogen peroxide and ozone chemical test kits were also used to test the KRIA treated water. In flow-through conditions there was no hydrogen peroxide or ozone found in either the treated or control water. In the recirculating system conditions there were some positive samples in both the treated and control water that had small amounts of hydrogen peroxide and ozone in the test samples. The ozone levels in the KRIA treated water were 0.05 mg/L after 14 days at P1 and P2 only in the recirculating system, but 0.0 mg/L was recorded in the control. Hydrogen peroxide levels were also elevated slightly in the KRIA treated recirculating system over the control, but was not at levels different enough to be considered significant as the standard deviations were high (Table 8).

Table 8. Average of hydrogen peroxide and ozone readings for the recirculating system of KRIA treated water and associated control with standard deviation.

	H_2O_2 (mg/L)		**Ozone (mg/L)**	
Day Tested	**Treated**	**Control**	**Treated**	**Control**
Day 3	0.25 (±0.19)	0.00	0.0	0.0
Day 7	0.45 (±.38)	0.20 (±0.28)	0.0	0.0
Day 14	0.90 (±0.20)	0.25 (±0.10)	0.025 (±0.029)	0.0

A supersaturation meter was used to determine if the KRIA treated water was gas supersaturated and to determine the amount of total gas pressure in the treated water. In the flow-through system, the total gas pressure was 100% in the treated and control system. During the recirculating test system the treated total gas pressure was 103% and the control was 98%. The total gas pressure did not change with duration or location. Nitrogen was removed from the

atmospheric air that the KRIA machine intakes and therefore was not released as part of the superoxide generation mechanism.

Along with pH, this probe also measures oxidation-reduction potential (ORP), which is the measurement of the amount of oxidizing or reducing agents that is in the water. Oxygen radicals are part of the equation and are considered oxidizing agents. More positive ORP values are an indication of large amounts of reducers in the water and large negative values are an indication of oxidizers in the water; more oxygen radicals present will give a large negative ORP reading. ORP probe readings are dependent on temperature but are not temperature adjusted and are not very accurate, probe reading accuracy is ±20 mV.

During testing in both the flow-through and recirculating system designs the ORP readings were within the ±20 mV range between control and treated and untreated water readings. The ORP for the KRIA treated water was slightly more negative (-3.2 mV) than the untreated water and the control was only (-0.5 mV) different from the untreated water as an average of all of the readings. In the recirculating system, the ORP values of the treated KRIA system were about -2.2 mV different from the untreated water and the control system was very similar to the untreated water system with an average difference of 0.01 mV.

DISCUSSION

This study determined that the KRIA technology can be used to kill quagga mussel adults. After 17 days of exposure a total of 74% mortality was achieved in a recirculating configuration. The mortality at the end of the exposure was 57%. Exposed adult mussels continued to die as there was an additional 17% mortality 4 days after exposure in recovery. When the adults were exposed for fewer days, 11, the 21 day mortality was 36%. Longer exposure times would achieve higher mortality of adult mussels. The measured dose was high dissolved oxygen, an average of 204% or 16.81 mg/L at 23.9 C. Control mortality was low, 9%, in the recirculating configuration, which was expected given the time the experiment was conducted, as adult mussels experience a natural senescence at this time of year in the Lower Colorado River.

In the recirculating system, veligers were also exposed and there was an increase in the proportion of empty shells present in the treated exposures than in the control exposures at 48 hours. This increase was indicative that mortality occurred in the treatment system after 24 hours and the veliger flesh became detached from the shell before the 48 hour observation point. The treatment killed 18.6% more veligers than the control system at 48 hours.

The KRIA technology system used in this study was able to increase the dissolved oxygen, but it also increased the temperature of the water in the recirculating system by about 5 C, mostly from the heat off of the pump. It has been observed that there was no increased temperature of the water going through the KRIA technology system in an open area where the machine had good air flow and the air temperatures were below 30 C. There was no supersaturation by nitrogen or other compressed gasses in the water. Low levels of hydrogen peroxide were measured, but not

much higher than levels in the control recirculated water. There were no increases in detectable ozone in the water.

The KRIA produced superoxide radicals, which were only measureable as dissolved oxygen in the treatment systems. Superoxide radicals reacted with organic constituents present in freshwater very quickly and formed other oxygen species such as hydrogen peroxide, ozone, and metal complexes (Blough and Zepp 1995). Hydrogen peroxide and ozone was not highly detectable with measurement tools used. Lastly, ORP could be used to determine the levels of oxidative or reductive ions in the water, but in the KRIA treated water in this test system the difference between treated and control were less than the accuracy of the probe. Therefore, the only detectable superoxide radical by-product was dissolved oxygen. The best tool that was currently on the market, reasonably priced, and worked well to detect the KRIA technology produced changes in the treated water was an optical sensor dissolved oxygen meter. This meter distinguished between when the KRIA was working and not working, read accurately and easily, and accounted for changes in temperature to the parameter.

The KRIA technology in a flow-through (single-pass) system did not provide high enough levels of dissolved oxygen or detectable superoxide radical by-products to cause mortality in quagga mussels at either life stage tested in the amount of exposure time tested. Levels of dissolved oxygen in the flow-through system in the treated KRIA water was 21% above background and control, which was 102% or 9.3 mg/L at 1 gpm. This was not enough to kill mussels with a 7 day exposure. At higher velocities, 6 gpm, the observed dissolved oxygen was 119% or 10.9 mg/L, which also did not produce mortality in adult mussels. Veligers that were exposed for 4 and 24 hours also had no difference in mortality from control treatments. The KRIA unit tested in these trials did not have the ability to pulverize veligers as they passed through the KRIA unit.

Recommendations for Future Work

Results from this study can direct future work regarding the KRIA technology and its use on dreissenid mussels. In this study, utilizing the KRIA technology configured in a recirculating system, the mussels started to die after at least 11 days of exposure. Further studies would include testing the KRIA technology in a recirculating configuration with longer exposure periods to determine if 100% mortality is achievable in adult quagga mussels at dissolved oxygen levels at 204% or 16.81 mg/L at 23.9 C. The next step would be to determine a dose-response relationship with different levels or days of treatment to determine the mortality of quagga mussels. The dissolved oxygen levels produced by the KRIA technology can be controlled by setting the timer to operate for a period of time to obtain desired DO levels to find a dose-response curve. Elevated levels of dissolved oxygen produced by the KRIA technology have been observed up to ½ mile away from the discharge nozzle. This study only looked at short distances from the discharge nozzle and it would be recommended to include further distances when determining a dose-response curve for quagga mussels.

The next step would be to determine the ability to produce higher and lower dissolved oxygen levels based on temperature and quantity of organic content in the water to determine how these

variables effect the dose-response curve. Lower temperatures will increase the amount of dissolved oxygen the water will retain and hypothetically increase the levels produced by the KRIA technology. Different amounts and species of organic matter will also change the reaction time and successive complexes that are formed from the superoxide radicals. Pure water should increase the amount of superoxide radicals present, lower the amount of successive complexes and produce higher mortality of the quagga mussels. Determination of how temperature and organic content influence the superoxide radical could be necessary to fine-tune recommendation for how long to run the KRIA machine to get the desired effect on the quagga mussel. Since the levels of superoxide dismutase enzymes and other antioxidant molecules vary over the season (Manduzio et al. 2005), seasonality of the effect of the KRIA technology should also be investigated. The antioxidant molecules are also inhibited by different pH and presence of metals (Manduzio et al. 2005), water chemistry influences may become an important variable when applying the technology as a treatment.

This study found that the KRIA technology did not produce high enough levels of toxic substance in a single pass through the machine within 7 days, therefore, testing a single pass flow-through system for longer periods of time based on results from the dose-response curve calculated in a recirculating system would also be valuable before marketing the KRIA technology to facility operators. Settlement of veligers is also a problem for facility operators, further studies should include determining if the KRIA technology would prevent the settlement of quagga mussels in infrastructure vulnerable to mussel colonization. The veliger mortality results from the recirculating system configuration showed that there was a treatment effect. In other types of treatment studies, lower doses of substance can limit the colonization of mussels on to infrastructure, i.e. UV, Zequanox. These results can be applicable to zebra mussels as they are closely related species to the quagga mussel, as other control tools developed to date produce similar outcomes on both dreissenid species, though a confirmation test could prove insightful, if treatment results are found to be different.

Another opportunity to consider is testing the effects of the KRIA technology and the very high levels of dissolved oxygen on non-target species within treatment systems. In open-water systems, where the KRIA technology could be used in a recirculating set-up, other invertebrate and fish species need to be tested to determine the effect of such high levels of dissolved oxygen for the long periods of time that it takes to cause mortality in dreissenid mussels. Levels of dissolved oxygen can be controlled by the operator of the KRIA technology to stay below the toxic levels of other desired species to limit the impact. There have been a number of studies conducted on fish species at different life stages to different levels of hyperoxia (above 100% saturated oxygen levels) for short exposure periods that have resulted in different outcomes. Physiologically stressed or sensitive life stages, such as Atlantic salmon (*Salmo salar*) and Coho salmon (*Oncorhynchus kisutch*) smolts, incurred mortality after being exposed to hyperoxic conditions constantly for 96 hours to 1 week (Brauner 1999, Brauner et al. 2000). The mode of action for toxicity was the decrease in the ability for cells to osmoregulate ions across its membranes and increased impaired gill function, which led to an elevation in blood and tissue CO_2 levels (Brauner 1999). This matched results found by Liepelt et al. (1995), which exposed rainbow trout (*Oncorhynchus mykiss*) to hyperoxia (200% atmospheric O_2 levels) for 6 h; and

they also found that there was a significant increase in DNA single strand breaks indicative of gill cell damage.

However, non-sensitive life stages such as one-year old rainbow trout showed no mortality and found that after 14 days of exposure to hyperoxic conditions, where DO% was a 173±24%, there was lowered oxygen transport ability of the blood, but once the trout were put into recovery at normal DO levels, they recovered (Ritola et al. 2002). This matched results found by Edsall and Smith (1990), which showed that in a 125-day experiment with rainbow trout in hyperoxic (180% oxygen) conditions a decrease in the number of erythrocytes was observed, but observed no mortality and conditions returned to normal after being replaced into normal conditions. Hyperoxic conditions has been shown to produce better growth rates and feed conversion rates in fish species such as cod and sturgeon (Olsvik et al. 2006; Bagherzadeh Lakani et al. 2012). Another study with juvenile Central Valley Fall-run Chinook salmon (*Oncorhynchus tshawytscha*) and Central Valley Steelhead (*Oncorhynchus mykiss irideus*) found no effect to hyperoxic conditions (200%) after a 5 day exposure period (Jackson et al. 2010).

There are a few studies with invertebrate species, which showed an effect on the organisms. At hyperoxia levels for a 72 hour exposure, the crayfish excreted positive ions, such as Ca^{2+}, Na^{+}, H^{+}, K^{+}, and regulated the excretion with uptake of negative ions, such as Cl^{-} ions, but once in recovery the crayfish recovered to normal levels (Wheatly 1989). Longer exposure levels to hyperoxia conditions could completely change the internal balance of ions within the cells of the crayfish and lead to death. In snails, Remon Koopman et al. (2015) showed that gastropods, had a varied response to hyperoxic conditions within a few hours of exposure.

Changes and advancements in KRIA technology may change the outputs of the machine and the concentration of superoxide radicals produced and therefore the amount of dissolved oxygen saturation achievable. Engineering changes that effect the amount of superoxide radicals produced should also be investigated to ensure that similar or better results are obtained.

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