Evaluation of Fortress Mussel Control Systems Copper Ion Generator for Effects on Adult Mussels and Evaluation of the Performance of the Copper Ion Generator Under Field Conditions

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February 2021

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Introduction:

Dreissenid mussels, the zebra and quagga mussel, arrived in the United States from Europe in the 1980s and quickly spread to many eastern North American waterways, rivers, and lakes. These mussels are extremely prolific and can produce costly impacts by attaching to and clogging water intakes, trash racks, pipes, fire control systems, cooling water systems, fish screens, and virtually all types of underwater infrastructure.

Since 2007, dreissenid mussels have been present in the lower Colorado River. The mussel populations have proliferated and mussels are now adversely affecting the Hoover, Davis, and Parker Dams. In early 2008, larval zebra mussels were found to be present at Pueblo Reservoir in Colorado, and adult zebra mussels were found at San Justo Reservoir in California. More recently, both zebra and quagga larvae have been detected in several other reservoirs affiliated with Reclamation facilities. In addition to Arizona, California, Colorado and Nevada, mussels are present in Kansas, Nebraska, Texas, and Oklahoma and have been detected in New Mexico, South Dakota and Utah. Flow restriction is the foremost concern because it threatens water delivery and hydroelectric power reliability.

While there are many chemical compounds which will control mussels, most are non-specific and have undesirable side effects on the receiving environment. The toxicity of copper to marine life has been recognized for a long time. Mollusks are particularly sensitive to the presence of copper in the environment. Elevated levels of copper can result in such diverse effects as decreased growth rate, reproductive impairment, enzyme inhibition, reductions or alterations in protein synthesis, and disruptions of ATP synthesis and Ca^{2+} homeostasis (Clayton et al. 2000).

The toxicity of copper in freshwater systems is greatly influenced by the total hardness of water. It would seem reasonable to anticipate that different bodies of water would require different levels of copper ion generation to control dreissenids. The only publication available to-date on the effect of ionic copper is from the Great Lakes (Blume et al. 1994). This study documents a maximum decrease of 58% in settlement of zebra mussel veligers in a flow through system using Lake Michigan water and no effect on adults is documented. In laboratory studies, Kraak et al. (1992) documented that 16 ppb exposure to copper ions did not affect the filtration rate or survival of zebra mussel adults. Further, the authors concluded that at the 16 ppb exposure level there was no increase in the level of copper in the tissue of zebra mussels during the long exposure. This led them to conclude that adult zebra mussels were capable of regulating the body concentration of copper when exposed to the low 16 ppb level. No equivalent studies on quagga mussel adults have been done.

The use of copper ion generator technology for the control of invasive mussels has been promoted for over twenty years. Blume et al. (1994) conducted a series of experiments to determine if the copper ion technology could be used against dreissenid mussels. Working on the Great Lakes they concluded that a continuous dose of 10 ppb of copper ion would decrease veliger settlement in the system to be protected. They speculated that the mussel which settled, or adults already present in the system, would be eliminated by the long-term exposure to low levels of copper ions. The technology was commercialized under the trademark of MacroTech

Copper Ion generator. A small MacroTech Copper ion generator was tested under field conditions at the Lower Colorado River for the US Bureau of Reclamation in 2014/2015 field season (Claudi and Prescott 2015) on quagga mussels. This study found that levels of approximately 20 ppb prevented new infestation from developing and eliminated adult mussels over the period of two to three weeks depending on the ambient temperature. The study also noted numerous performance problems with the MacroTech unit such as scaling of the electrodes, uneven release of copper ions and lack of feedback loop to monitor generator performance.

Fortress Mussel Control Systems, an industrial grade Copper Ion Generator developed by ONG Consulting, LLC has addressed some of the deficiencies observed in the MacroTech unit. This is a report regarding the field test performed to verify these improvements while testing the effect of the different concentrations of copper ions on adult quagga mussels and the settlement of veligers. A brief description of how Fortress Mussel Control Systems copper ion generator performed is included.

Methods:

The evaluation of the Fortress Mussel Control System was conducted at Willow Beach National Fish Hatchery, Arizona from October to December 2020. Water from the end of the bank of raceways was pumped to the Fortress Mussel Control System copper ion generator and to 4 bioboxes (see appendix for schematics and additional details). The outflow from the copper ion generator was collected in a 32-gallon head tank and then pumped through a manifold system to three bioboxes to create three different testing concentrations of copper ions. Target concentrations were 60, 30, and 15 ppb copper in the bioboxes. The fourth biobox only received raw water and acted as the control. Raw water and copper concentrate flows were manually adjusted so as to achieve target concentrations.

In each biobox, 3 settlement plates (10x10 cm gray pvc) placed in a stand were added to the end of each biobox, perpendicular to the flow. Adult quagga mussels were collected from the fish hatcheries head boxes of several raceways. The adults were separated and assessed to ensure broken and dead mussels were not included. Approximately 33 mussels were placed into a mesh container. Each biobox received 3 containers for a total of 100 mussels per biobox.

Once the system was operating, monitoring of water quality and adult mussel mortality occurred every 3 to 4 days. Temperature, dissolved oxygen, pH, and conductivity were measured using Hach HQ40d with a LDO101, CDC401, and PHC201 probes (Hach, Loveland, CO). Copper concentrations of each biobox was measured by the porphyrin method with the DR900 (Hach, Loveland, CO). Copper concentrations coming from the copper ion generator, head tank and the concentrate into the last biobox were measured by the bicinchoninate method with the DR900 (Hach, Loveland, CO). Alkalinity (Hach kit AL-TA then Hach kit AL-AP) and hardness (calcium and magnesium hardness measured by the calmagite colorimetric method) were noted weekly. On each visit, every mesh container was pulled out of the biobox and the adult mussels were assessed for mortality. The adults that showed mortality (failure to close with decaying flesh or empty shells) were removed from the containers and the shell length was measured. Live mussels were returned to the container and replaced back into the biobox. Settlement plates were examined monthly and pictures were taken.

When adult mussels reached 100% mortality in a biobox, another set of mussels was placed into the biobox for up to 3 replicates. The concentration with 60 ppb had short time to death, allowing for three replicates to be achieved. The 30 ppb treatment achieved 2 replicates. Adult mortality in the control and 15 ppb treatments were slow and only one replicate was achieved.

At the end of the two-month testing period, the Fortress Mussel Control System copper ion generator was drained, packed into the original shipping boxes and returned to the client. The equipment was not taken apart by KASF Consulting to determine the condition of the anodes. Copper levels over time were compared and correlations between copper concentration and other variables were calculated using Microsoft Excel’s statistical functions.

Proportional mortality was used to determine success of the treatments. Standard deviations were calculated using Excel pivot table functions. Box plots were constructed in R (Version 3.6.2) with the standard package (R Core Team 2019). T-tests were conducted to determine the significance of the measurement of mussel size across treatment with package lme4 (Bates et al. 2015). Tukey’s HSD was used to determine which treatments were different from each other using package multcomp (Hothorn et al. 2008) and multcompView (Graves et al. 2019).

Results:

Settlement of Quagga Mussels

During the testing period the number of quagga mussel veligers was low in all of the system. At the end of the two-month testing period, 7 settled mussels that were less than 1 mm in length were found on the control settlement plates (Figure 1). No settled mussels were found on the settlement plates in the treated bioboxes (Figure 2). There were no settled mussels visible at the one month testing period.

Algae and biofilm growth decreased with increased copper concentration on the settlement plates as well as in the bioboxes. The control biobox had long filamentous algae and lots of biofilm present, which increased considerably over the two months (Figures 3 &4). In the 15, 30, and 60 ppb bioboxes there was no filamentous algae and minimal growth of other algae and biofilm. Snail densities and macroinvertebrate densities also decreased with increased copper concentrations (Figures 2, 3, &4).

Figure 1. Example of a settled mussel on the control settlement plate.

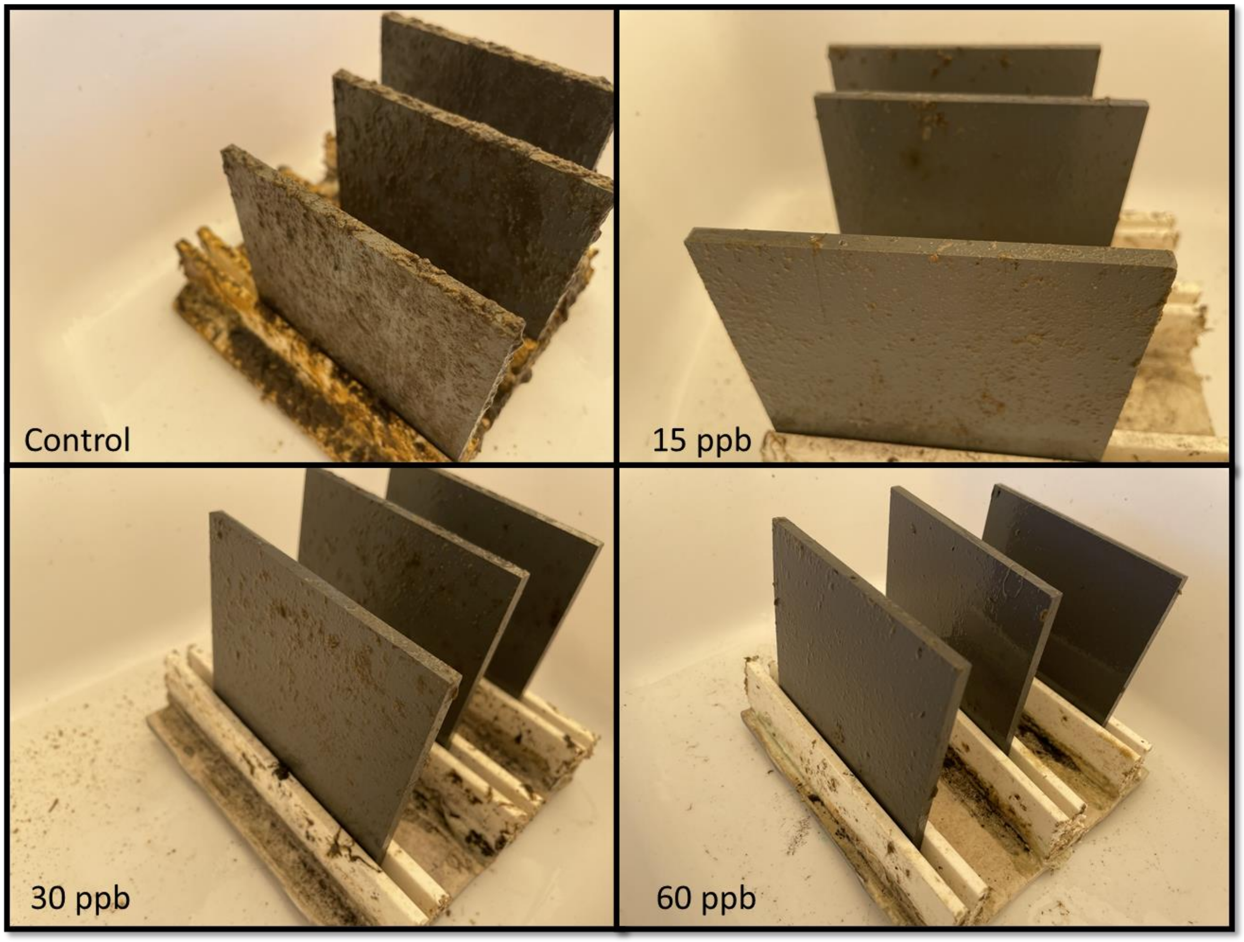

Figure 2. Settlement plates from all treatments after two months of treatment duration.

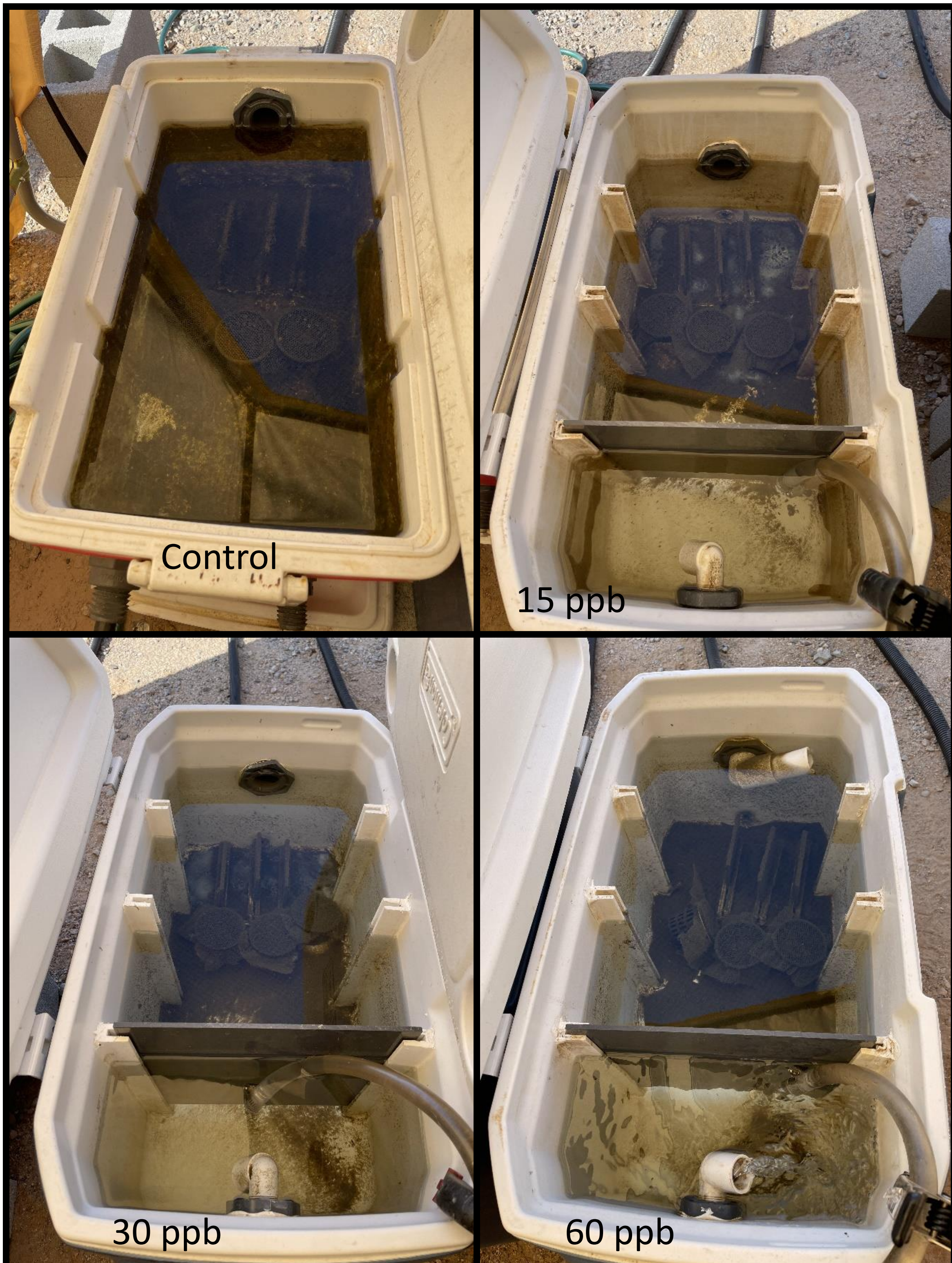

Figure 3. Condition of the bioboxes at the one-month check point.

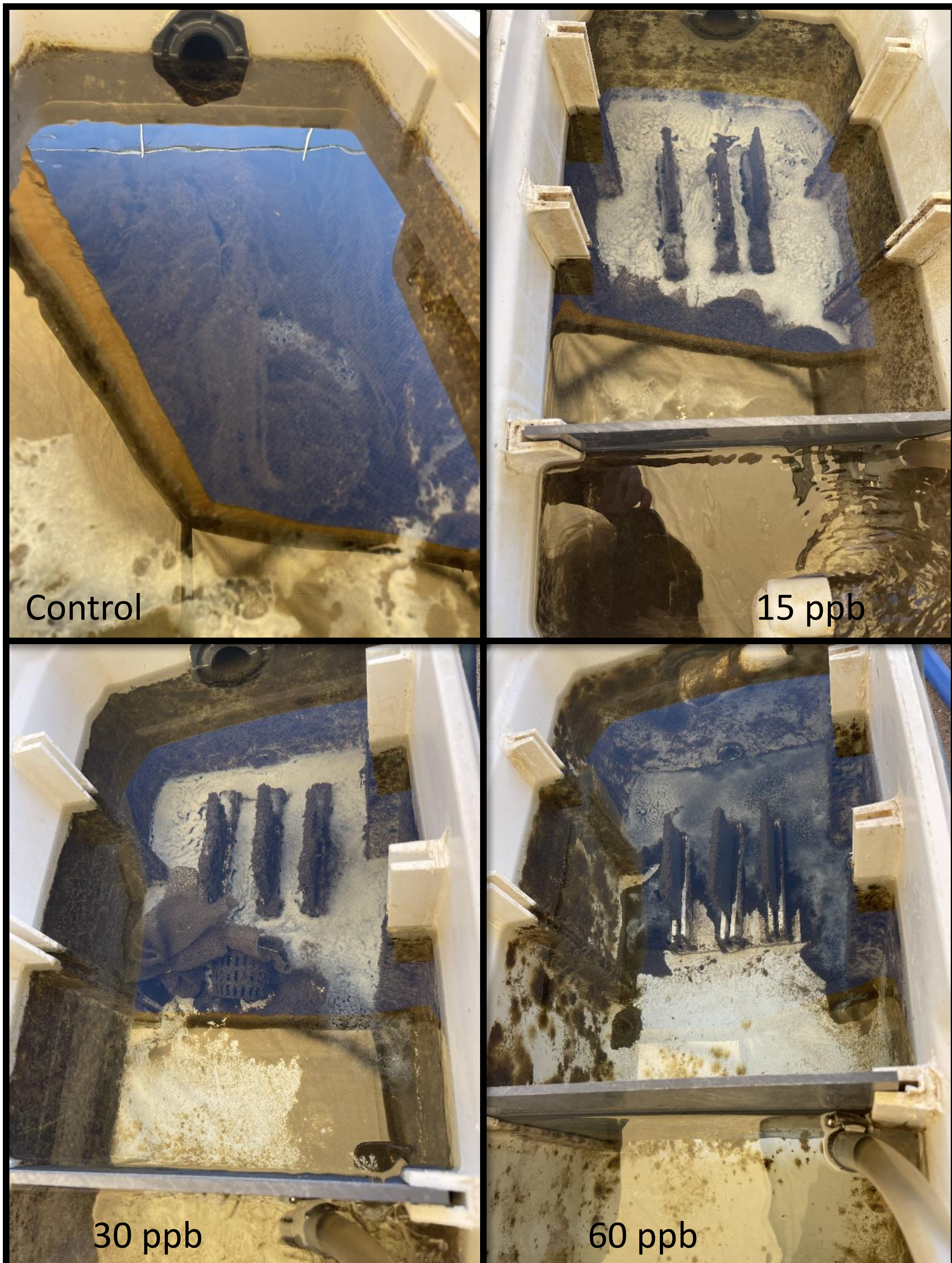

Figure 4. Condition of the bioboxes at the two-month check point.

Adult Mortality

There was mortality in the adult quagga mussels over time in all the bioboxes (Figure 5). Control mortality increased with time and final mortality at two months was 32%. Treatments with copper ions added had higher mortality of adult quagga mussels than the control mortality. The two-month adult mortality for the 15 ppb treatment was 80%. There were two rounds of adults started in the 30 ppb treatment; the first round resulted in 99% mortality after two month of exposure and after 25 days for exposure to 30 ppb the second round showed 94% mortality (Figure 5). The three rounds of adults tested in the 60 ppb treatment had 100% mortality in 11 days with an average water temperature of 16°C (Figure 5).

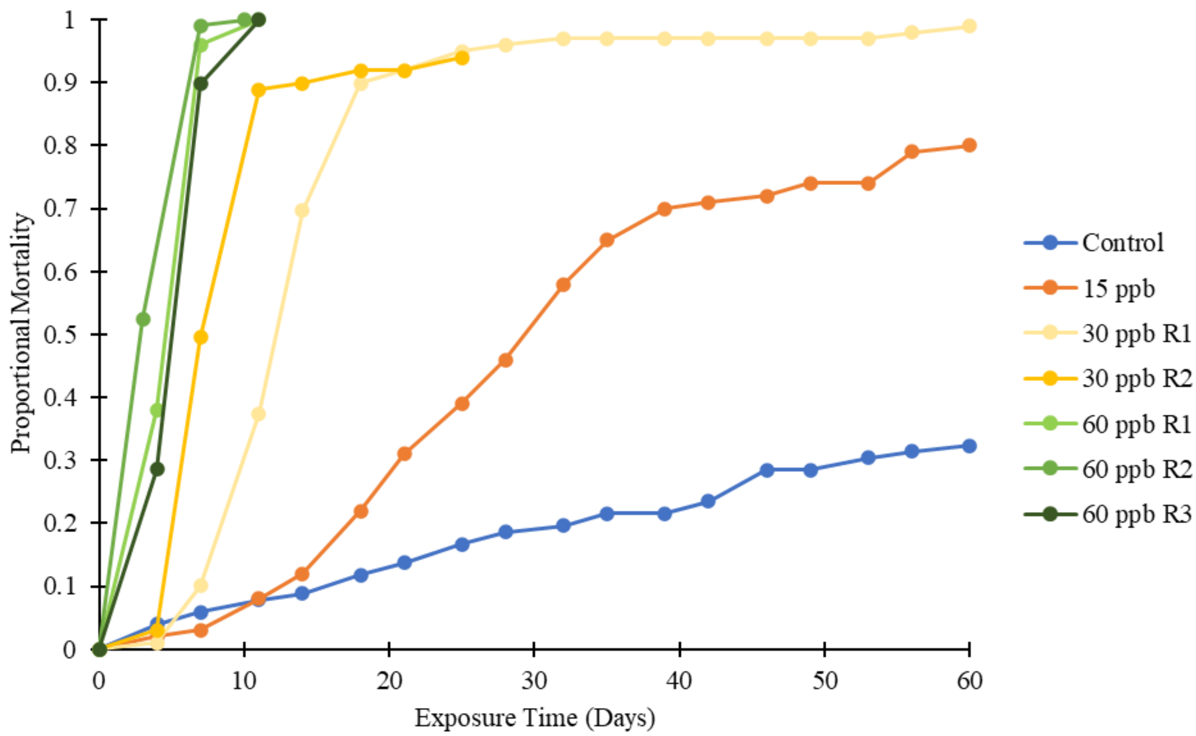

Figure 5. Adult quagga mussel mortality of each treatment with each round separated out to show each mortality over exposure time.

The mortality curves of each round were very similar to each other (Figure 5). The 30 ppb treatment had the greatest variation of when the adult mussels died over time, but final mortality results were very similar. In the 60 ppb treatment, the adult mussels died quickly, within 11 days with very little variation (Figure 5). Mussel shell length was measured for each round at death of the animal or termination of the study (Figure 6). The second round of mussels picked were slightly smaller than the first round; and the third round of mussels picked were slightly bigger than the original mussels. Mussel size did not affect time to mortality at 60 and 30 ppb (Figure 5). Despite these small differences there was not much variation and no significant difference (F_3=1.609, p-value=0.186) in mussel size across the treatments.

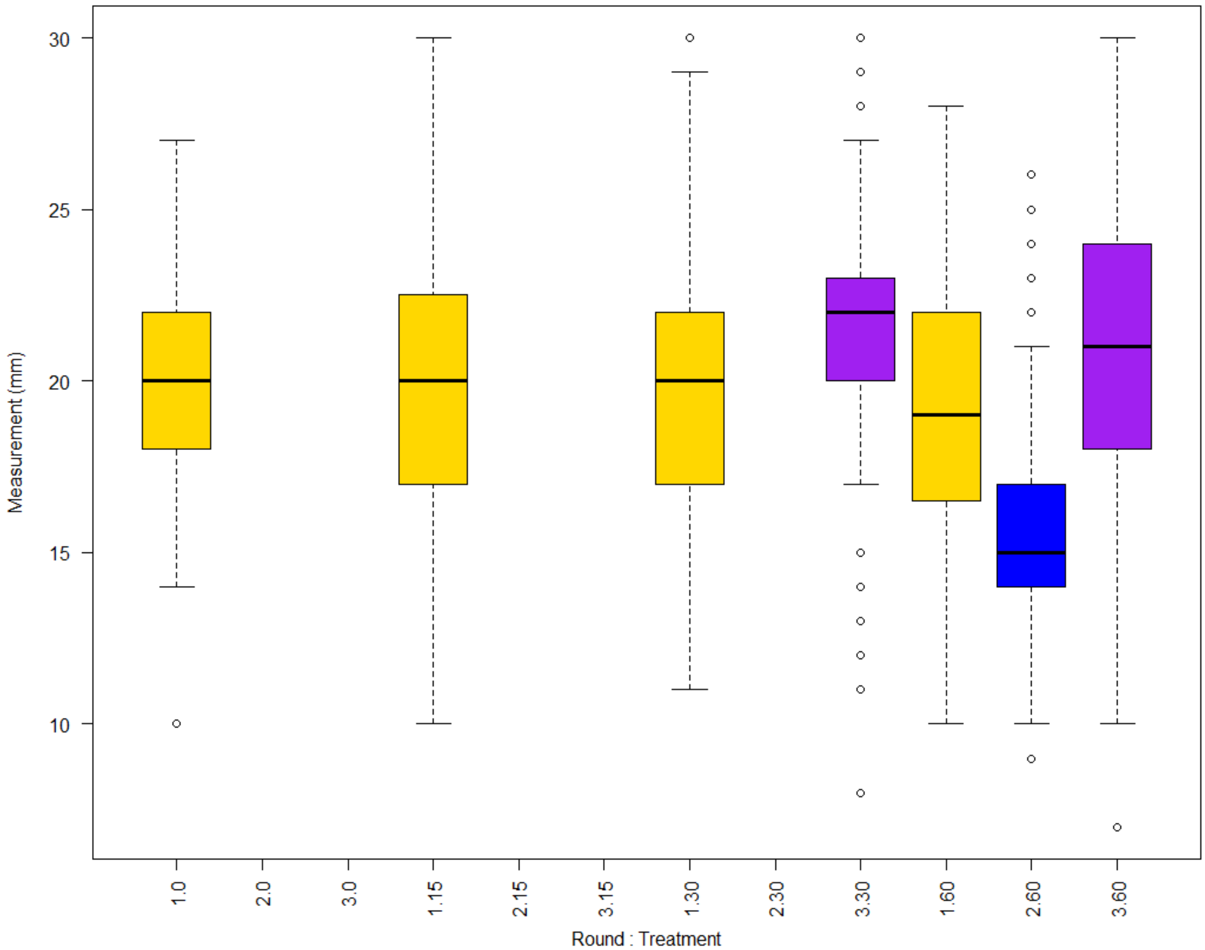

Figure 6. Box plot of the length measured for each adult quagga mussel used in each round of testing. Yellow corresponds to mussels picked at the beginning of the study, blue corresponds to the mussels picked two weeks into the study and purple corresponds to the mussels picked at one month.

The different rounds of adult mussel testing can be combined to show that copper dosage influences mussel mortality (Figure 7). As concentration of copper ions from the copper ion generator increased the mortality of adult quagga mussels increases. The time to 100% mortality decreased with increasing copper ion concentration.

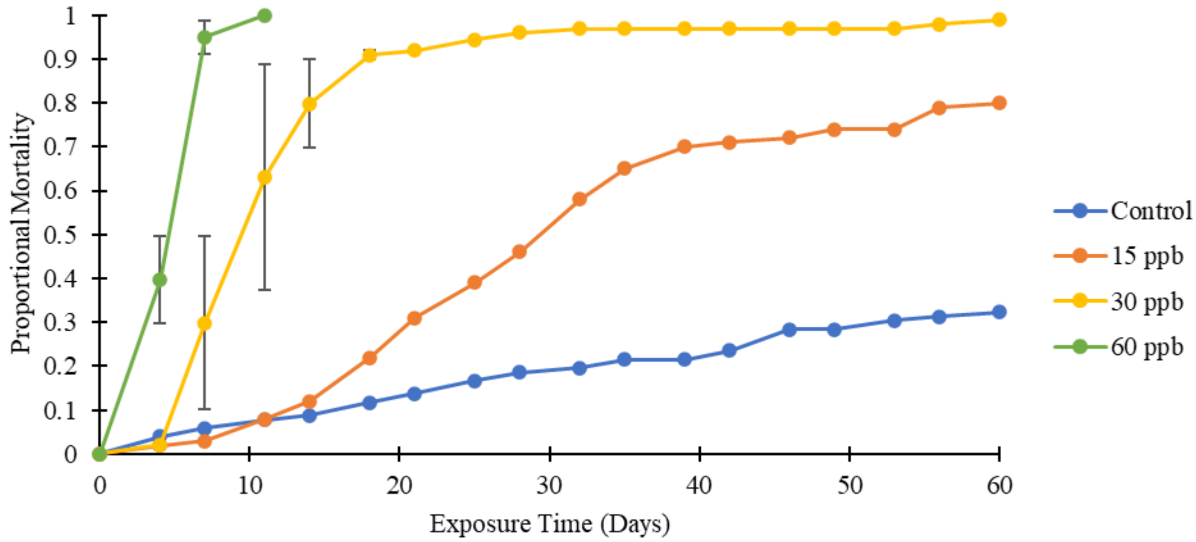

Figure 7. Average mortality of adult quagga mussels with standard deviation bars on the doses that had multiple rounds of testing.

With increased copper concentration, there was less clumping and attachment of the adult quagga mussels to the containers and each other. The control adult quagga mussels were tightly clumped together and difficult to separate (Figure 8). At 15 ppb, clumping was less and easier to pull apart and the mussels did not stay in the containers when dumped out (Figure 8). Both the 30 and 60 ppb mussels had no byssal attachment, therefore, were easy to separate and remove from the containers.

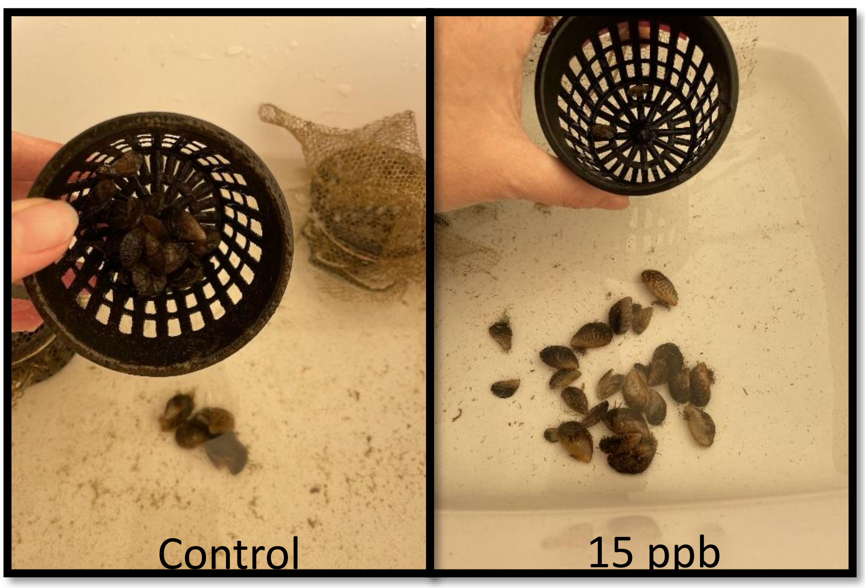

Figure 8. Adult quagga mussels in containers from the control and 15 ppb treatments showing degree of attachment.

Water Quality

Temperature over the two-month study period ranged from 17.8 to 14.4 °C (Table 1). This was a 3°C decrease over time, average temperature was 16.1°C. Temperature was not different between the different treatment bioboxes (F_3=0.022; p-value=0.99). Since the water source was at the tail end of the bank of 4 large raceways, the dissolved oxygen was low (Table 1), but adequate enough for mussel survival. The average dissolved oxygen for the study period was 6.43 mg/L. The dissolved oxygen in the control was lower than the dissolved oxygen in the treatment bioboxes (F_3=7.094; p-value=0.0003). This might help explain the mortality in the control biobox combined with the large amount of sediment and algae (Figure 3 &4).

Table 1. Water chemistry results taken at 3-to-4-day intervals during the study period as an average of all bioboxes.

Treatment Day	Temp °C	DO (mg/L)	pH	Cond. (µS/cm)	Alkalinity (mg/L)
4	17.8±0.0	5.16±0.08	7.83±0.10	912±1	<385
7	16.8±0.1	6.84±0.44	7.22±0.08	914±2	<385
11	17.7±0.0	5.68±0.16	7.70±0.02	917±0	
14	17.5±0.2	6.75±0.69	7.18±0.08	909±5	<385
18	17.5±0.2	6.31±1.13	7.09±0.10	913±3	
21	16.2±0.0	5.85±0.40	7.11±0.07	914±0	<385
25	16.0±0.3	6.42±0.43	7.08±0.10	916±1	
28	16.1±0.0	6.28±0.76	7.05±0.11	912±1	<385
32	16.6±0.4	6.33±1.64	7.03±0.28	915±7	
35	15.9±0.0	6.28±0.15	7.03±0.11	913±1	175±9
39	16.2±0.1	7.17±0.62	6.96±0.10	918±1	
42	15.8±0.0	7.44±0.36	7.17±0.05	916±1	180±0
46	15.2±0.0	6.90±0.06	7.71±1.27	918±1	
49	14.9±0.1	6.82±0.23	7.28±0.02	915±0	270±17
53	15.2±0.1	6.39±0.2	7.23±0.06	920±1	
56	14.8±0.0	5.94±0.05	7.17±0.16	924±1	260±14
60	14.4±0.1	6.71±0.16	7.03±0.13	917±1	

Average pH was 7.23 (Table 1), and there were no significant differences in pH reading across the bioboxes (F_3=0.562, p-value=0.642). Conductivity was also consistent over time with an average of 915 µS/cm and the bioboxes did not have significantly different conductivity readings (F_3=1.222, p-value=0.309). Alkalinity of the water did increase over time but did not significantly differ between the bioboxes even with the more precise test kit used in the second month (Table 1).

Hardness was measured in all of the bioboxes starting in the second month (Table 2). The total hardness was not significantly different between the bioboxes (F_3=1.668, p-value=0.211). Hardness as magnesium (F_3=1.739, p-value=0.197) and as calcium (F_3=2.789, p-value=0.072) were not significantly different among the different treatments. Hardness did not significantly vary over day either (F_8=1.584, p-value=0.228).

Table 2. Hardness of water used in testing over the study period.

	Magnesium as $CaCO_3$(mg/L)				Calcium as $CaCO_3$ (mg/L)				Total as $CaCO_3$ (mg/L)			
Day	**0 ppb**	**15 ppb**	**30 ppb**	**60 ppb**	**0 ppb**	**15 ppb**	**30 ppb**	**60 ppb**	**0 ppb**	**15 ppb**	**30 ppb**	**60 ppb**
4	0.46				1.24				1.70			
7	1.42				1.41				2.83			
14	1.14				1.55				2.69			
21	1.34				1.71				3.05			
28	1.78				1.22				3.00			
35	1.18	1.20	1.34	1.49	1.60	0.55	0.94	1.15	2.78	1.75	2.28	2.64
42	1.25	0.88	1.30	1.58	1.17	1.16	1.07	1.16	2.42	2.04	2.37	2.74
49	1.55	1.99	1.48	2.02	0.77	1.02	1.06	0.84	2.32	3.01	2.54	2.86
56	1.51	1.49	1.54	1.96	0.81	0.69	0.76	0.98	2.32	2.18	2.30	2.94

Copper Concentration and Mussel Mortality

The copper concentrations were set on the check days, which was every 3 to 4 days, to ±10 ppb of test concentration, usually preferring a higher value. On the next check day, the copper concentration was usually much lower than initially set (Table 3). Usually, the tubing was plugged with organic matter, sediment, and small amounts of copper pieces. Flows were reset, tubing was unplugged and concentrations were remeasured. There was no measurable copper detected in the control biobox throughout the study period.

Table 3. Copper concentrations (ppb) measured at initial arrival and after flushing and flows were reset in each treatment biobox.

	15 ppb		30 ppb		60 ppb	
Day	**Initial Cu**	**Post Cu**	**Initial Cu**	**Post Cu**	**Initial Cu**	**Post Cu**
4	0	17.0	6.3	44.4	78.2	82.0
7	5.1	15.1	23.0	31.6	64.8	75.9
11	10.3	20.7	19.8	33.8	70.1	65.4
14	16.8	21.2	18.7	31.5	50.0	57.0
18	10.9	18.2	2.7	28.9	128.5	72.3
21	3.5	18.6	0.0	29.7	24.7	68.2
25	3.6	17.5	0.0	31.8	69.2	72.4
28	15.2	21.8	0.0	39.2	60.5	59.5
32	29.4	14.6	40.7	34.6	116.7	65.9
35	2.9	15.9	26.9	33.3	41.7	57.2
39	0.0	33.0	22.4	49.4	58.8	88.0
42	14.7	14.7	27.5	27.5	63.7	63.7
46	7.6	15.5	10.3	38.3	53.7	67.2
49	3.5	19.3	25.5	37.7	67.8	63.0
53	15.1	16.1	0.0	43.7	1.3	72.6
56	0.0	17.4	21.9	37.2	24.4	53.4
60	4.4		30.0		37.0	

In the 15 ppb biobox, mussel mortality occurred over the two-month period and did not seem to be impacted by higher copper concentrations. When the copper was above 15 ppb, there was no large increase in mussel mortality following the increased copper concentration (Figure 9). Low copper concentrations might have slowed the mortality of the adult quagga mussels especially in the second month of testing. Since these were one-time readings over time and not continuous readings, it is not known how long the copper concentrations were at the measured levels. Therefore, direct correlations between spikes and troughs of copper concentrations as they relate to mussel mortality was not predictable.

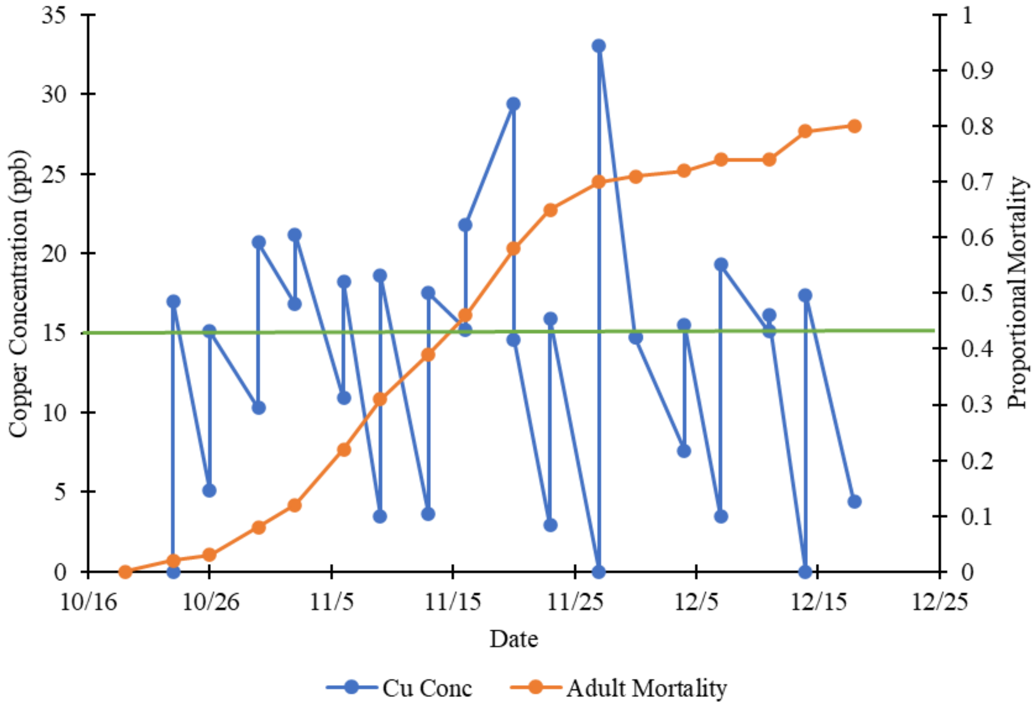

Figure 9. Copper concentrations of the 15 ppb biobox as potentially correlated to the adult mortality of the quagga mussels.

The 30 ppb biobox, two rounds of adult mussels were put into the biobox in the two-month testing period. The adult mussel mortality curves were similar, however the increased set point on day 39 (11/27/2020) may have increased the mortality rate of the second round of mussel testing (Figure 10, Table 3). This most likely explains the high standard deviation observed in the adult mussel mortality in Figure 7. However, the increased copper concentration did not affect the few mussels surviving the copper treatment in round 1 (Figure 10). With additional testing time and controlled copper concentrations, the second round of testing would have resulted in approximately 99% mortality.

Within the 60 ppb biobox, three rounds of adult mussels were tested in the two-month period. All mussels were dead within 10 to 11 days and copper concentrations did not seem to impact the speed of mortality (Figure 11). The dose of 60 ppb was enough to cause harm to the adult quagga mussels and doses higher than 60 ppb did not seem to accelerate the mortality of the adults.

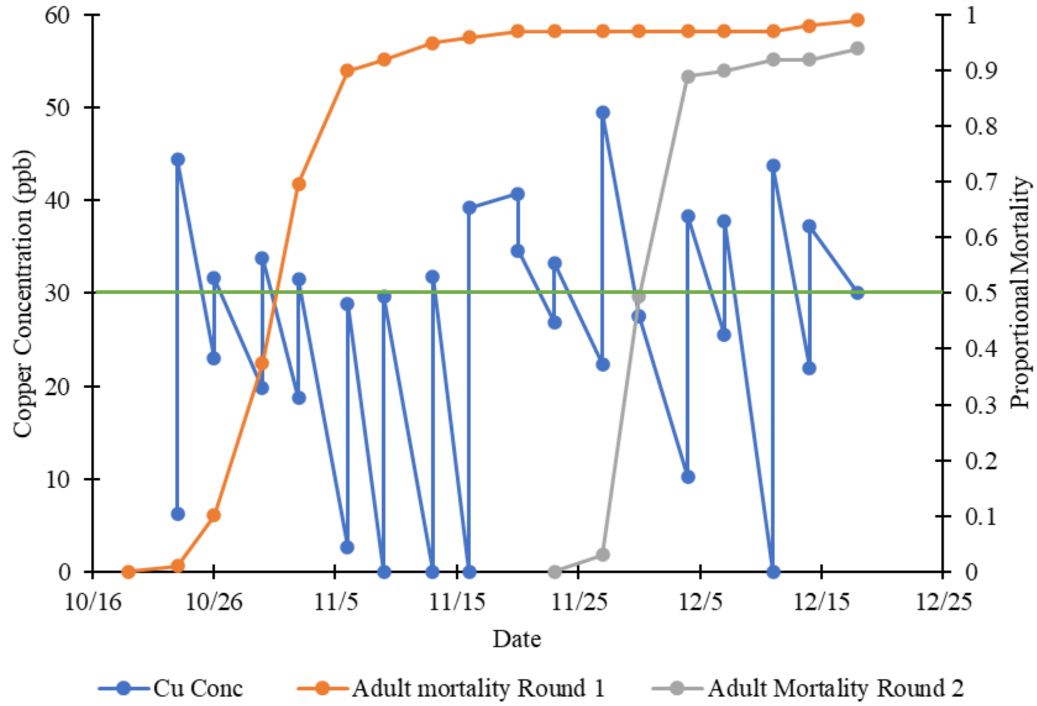

Figure 10. Copper concentrations of the 30 ppb biobox as potentially correlated to the adult mortality of the quagga mussels.

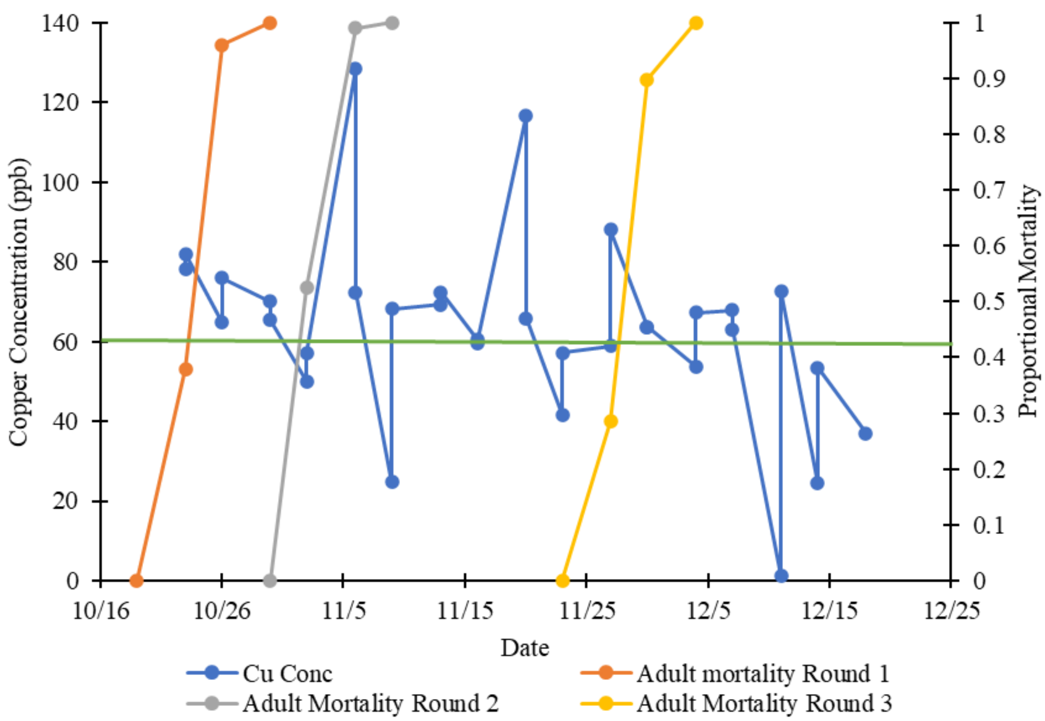

Figure 11. Copper concentrations of the 60 ppb biobox as potentially correlated to the adult mortality of the quagga mussels.

Performance of Fortress Mussel Control Systems Copper Ion Generator

After two months of running Fortress Mussel Control Systems copper ion generator, the copper ion level seemed to maintain consistency (Figure 12). Flow through the copper ion generator did not influence the copper output from the generator sample port; and Pearson's correlation showed flow was not correlated to copper concentration (r=-0.384; p-value= 0.31).

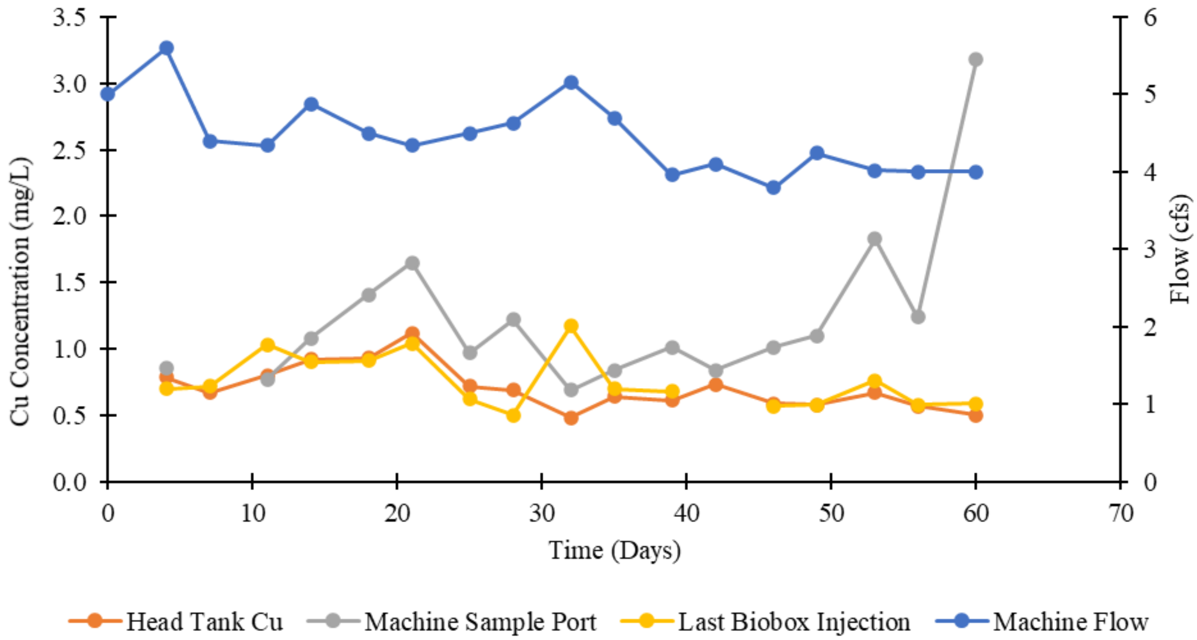

Figure 12. Concentration of copper ions from the Fortress Mussel Control System copper ion generator over time measured at the generator sample port, head tank, and injection tubing at last biobox compared with flow supplied to the generator.

Fortress Mussel Control Systems copper ion generator was cleaned per client's direction with a weak solution of muriatic acid on day 21 (11/9/2020) and day 39 (11/27/2020). The tubing delivering the copper concentrate were also cleaned out with the weak solution of muriatic acid on these days. There were no noticeable impacts on copper concentration or flow, but the next check day involved very little adjustments and flushing. Maintenance with of the copper ion generator was easy and control of copper concentration, while minimal, only involved adjusting flow rate. Variability in copper concentrations observed while testing (Figures 9, 10, and 11) were due to the plugging of copper delivery tubing to the bioboxes (see appendix for additional details) and not to generator variability.

Discussion:

The Fortress Mussel Control System copper ion generator performed well during the field test. It was easy to operate and took little maintenance during testing. A consistent amount of copper ions was generated to supply the head tank and then the bioboxes for testing (average copper concentration in the head tank was 0.71±0.16 mg/L; Figure 12). Biobox system configuration could be improved in following rounds to make control of copper concentration more uniform, but there is additional the cost to implementing these improvements. (see Appendix for additional details).

Copper ions generated by the Fortress Mussel Control System were effective in causing adult mortality. At the highest concentration tested (60 ppb) 100% mussel mortality occurred within 11 days at 16°C. With lower concentrations mortality was lower and achieved more slowly, but was significantly higher than control mortality. In this study control mortality was higher than desired, but the control biobox consistently had lower dissolved oxygen, lower flow, and more algae and debris compared to the treatment bioboxes. These factors could have easily contributed to the higher adult mortality in the control biobox. The 15 ppb copper treatment had 80% mortality in the two month treatment time, but a more consistent concentration could have increased mortality. However, this study results were similar to Claudi and Prescott's (2015) results which obtained 50% mortality at 15 to 20 ppb copper ions in 4 weeks at 19°C.

Settlement was controlled by the addition of copper. There were no settled mussels present in the 15, 30 or 60 ppb treatment. Even though settler sized veliger numbers were low, there was some growth on the control plates. More striking was the control of algae and biofilm the copper ions from the Fortress Mussel Control System copper ion generator exhibited. The control biobox was full of filamentous algae at the end of the treatment period and the treated bioboxes did not have any.

Copper ions have been shown to affect the gills of bivalves and disrupt metabolic processes (Yen Le et al. 2021). However, copper is an essential element involved in many organism's metabolism (Kraak et al. 1992, Le et al. 2021). Kraak et al. (1992) found that the no effects concentration was 16 µg/L for copper in zebra mussels in 48 h. However, we showed that there was an effect with chronic exposure at this concentration. Le et al. (2021) found that pH (8.3, 7.3 & 6.5) and possibly sodium concentration could affect the toxicity of copper on zebra mussels. As pH decreased, the accumulation of copper in the gills decreased, showing a potential interaction between H^+ and Cu^+ at uptake sites. Mussels have been shown to regulate the amount of copper in their cells, but there was a tipping point of "no return" where the mussels could not regulate enough of the copper ion to ensure survival and the osmotic balance was disrupted (Jorge et al. 2013; Le et al. 2021). This study showed that at low levels of copper ions (15 ppb) byssal thread production was minimal and death eventually occurred; and impact increased with increased copper concentration. Kobak et al. (2002) showed that zebra mussels exposed to copper plates had lower attachment strength to surfaces.

Alkalinity and hardness should be in the same magnitude. The measurements in this study showed that hardness was around 1 mg/L while alkalinity was around 200 mg/L. Data from the USGS site located on the Colorado River above Willow Beach, Arizona on December 9, 2020 measured an alkalinity level of 140 mg/L. The measured hardness was 170.5 mg/L; measured as 170 mg/L (as bicarbonate) and 0.5 mg/L (as carbonate) (USGS 2021). Moffit et al. (2016) reported that calcium ion concentrations were at 79 mg/L for Willow Beach National Fish Hatchery. Both these sources indicate that there was something wrong with the hardness measurements in this study and should not be used. Copper toxicity decreases with increasing hardness but over a large range (Ebrahimpour et al. 2010), small variations on a daily basis should not completely change the toxicity.

Recommendations:

Use of the Fortress Mussel Control System copper ion generator is recommended to control settlement of quagga mussels and kill adult mussels. The system was easy to operate and was effective at low doses of copper ion concentrate. Amount of control needed by client could dictate the concentration of copper used in the system. Water quality parameters must be taken into account to ensure the effective dose of copper is maintained through treatment period.

Additional studies might include work with different levels of sodium in the water. Copper may compete with sodium for sites of ion transport on the plasma membrane and disrupt osmoregulation (Nogueira et al. 2013). Kraak et al. (1994) found that copper and cadmium had an additive toxicity effect when used together to decrease filtration rate and a larger affect than just cadmium or copper alone. However, the concentrations used in Kraak et al. (1994) exceeds the aquatic life ambient water quality criteria which is 1.8 µg/L (EPA 2016) compared to treatment concentrations of 194 µg/L. Zinc and copper were less toxic than copper alone (Kraak et al. 1994).

If additional studies are warranted by the client at Willow Beach National Fish Hatchery, modifications to the test system set up should be considered to address the issues referenced in the appendix. Some of those modifications include lifting the sump pump off the floor of the raceway to decrease the settled solids and try to find a location that is above the raceways closer to the inflow. This study could also benefit from using inline flow mixers and peristaltic pumps to deliver the ion concentrate to the bioboxes. With these modifications and additional testing copper concentrations in each biobox would be better controlled and more uniform through the testing period.

Acknowledgements:

We would like to thank the staff at Willow Beach National Fish Hatchery, Willow Beach, Arizona for their support on this project. They provided electricity, water, space, quagga mussels and checked on the system during KASF Consulting’s absence from the site. Staff was also instrumental in receiving and shipping equipment.

Works Cited:

Bates D, M Maechler, B Bolker, and S Walker. 2015. Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software, 67(1), 1-48.

Blume WJ, PC Fraleigh, and WR van Cott. 1994. Evaluation of copper ion and aluminum floc for preventing settlement of zebra mussels. In: Proceedings of the Fourth International Zebra Mussel Conference, Madison, USA.

Claudi R and T Prescott. 2015. Evaluation of Copper Ion Generator for Effects on Settlement of Veligers and Impact on Adult Quagga Mussels. Prepared for Bureau of Reclamation, Task Order Number: R13PD80252, IDIQ Contract: R10PC80264.

Clayton ME, R Steinmann, and K Fent. 2000. Different expression patterns of heat shock proteins hsp 60 and hap 70 in zebra mussels (*Dreissena polymorpha*) exposed to copper and tributyltin. Aquatic Toxicology, 47: 213-226.

Ebrahimpour M, H Alipour, S Rakhshah. 2010. Influence of water hardness on acute toxicity of copper and zinc on fish. Toxicol Ind Health 26:361-365.

EPA (US Environmental Protection Agency). 2016. Aquatic Life Ambient Water Quality Criteria Update for Cadmium-2016. https://www.epa.gov/sites/production/files/2016-03/documents/cadmium-final-factsheet.pdf Accessed: 15 February 2021.

Graves S, HP Piepho, L Selzer, and S Dorai-Raj. 2019. multcompView: Visualizations of Paired Comparisons. R package version 0.1-8. https://CRAN.R-project.org/package= multcompView

Hothorn T, F Bretz and P Westfall. 2008. Simultaneous Inference in General Parametric Models. Biometrical Journal 50: 346-363.

Jorge MB, VL Loro, A Bianchini, CM Wood, and PL Gillis. 2013. Mortality, bioaccumulation and physiological responses in juvenile freshwater mussels (*Lampsilis siliquoidea*) chronically exposed to copper. Aquatic Toxicology 126:137-147.

Kobak J, E Klosowska-Mikulan, R Wisniewski. 2002. Impact of copper substrate on survival, mobility and attachment strength of adult *Dreissena polymorpha* (Pall.). Folia Malacologica 10: 91-97.

Kraak MHS, D Lavy, WHM Peeters, et al. 1992. Chronic ecotoxicity of copper and cadmium to the zebra mussel *Dreissena polymorpha*. Arch. Environ. Contam. Toxicol. 23: 363–369.

Kraak, MHS, D Lavy, H Schoon, M Toussaint, WHM Peeters, and NM van Straalen. 1994. Exotoxicity of mixtures of metals to the zebra mussel *Dreissena polymorpha*. Environmental Toxicology and Chemistry 13: 109-114.

Le TTY, D Grabner, M Nachev, WJGM Peijnenburg, AJ Hendriks, and B Sures. 2021. Modelling copper toxicokinetics in the zebra mussel, *Dreissena polymorpha*, under chronic exposures at various pH and sodium concentrations. Chemosphere 267: 12978.

Moffitt, CM, KA Stockton-Fiti, R Claudi. 2016. Toxicity of potassium chloride to veliger and byssal stage dreissenid mussels related to water quality. Management of Biological Invasions 7: 257-268.

Nogueira, LS, CM Wood, PL Gillis, A Bianchini. 2013. Isolation and fractionation of gill cells from freshwater (*Lasmigona costata*) and seawater (*Mesodesma mactroides*) bivalves for use in toxicological studies with copper. Cytotechnology 65: 773-783.

R Core Team. 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

USGS (US Geological Survey). 2021. USGS 355241114401601 Colorado River above Willow Beach, Arizona. Water Quality Samples for USA: Sample Data. URL: https://nwis.waterdata.usgs.gov/nwis/qwdata? Accessed: 15 February 2021.

Appendix: System Configuration and Performance

Biobox System Configuration

A sump pump was placed at the tail end of a bank of four raceways to supply the water to the biobox system. All of the raceways were growing rainbow trout at various sizes and densities. Water leaving the raceway was discharged to the Colorado River. The sump pump supplied water and pressure to feed the copper ion generator which sat on a table and the four treatment bioboxes (Figure A1). Water discharged from the four treatment bioboxes and the overflow for the mixing/head tank were discharged back into the raceway discharge.

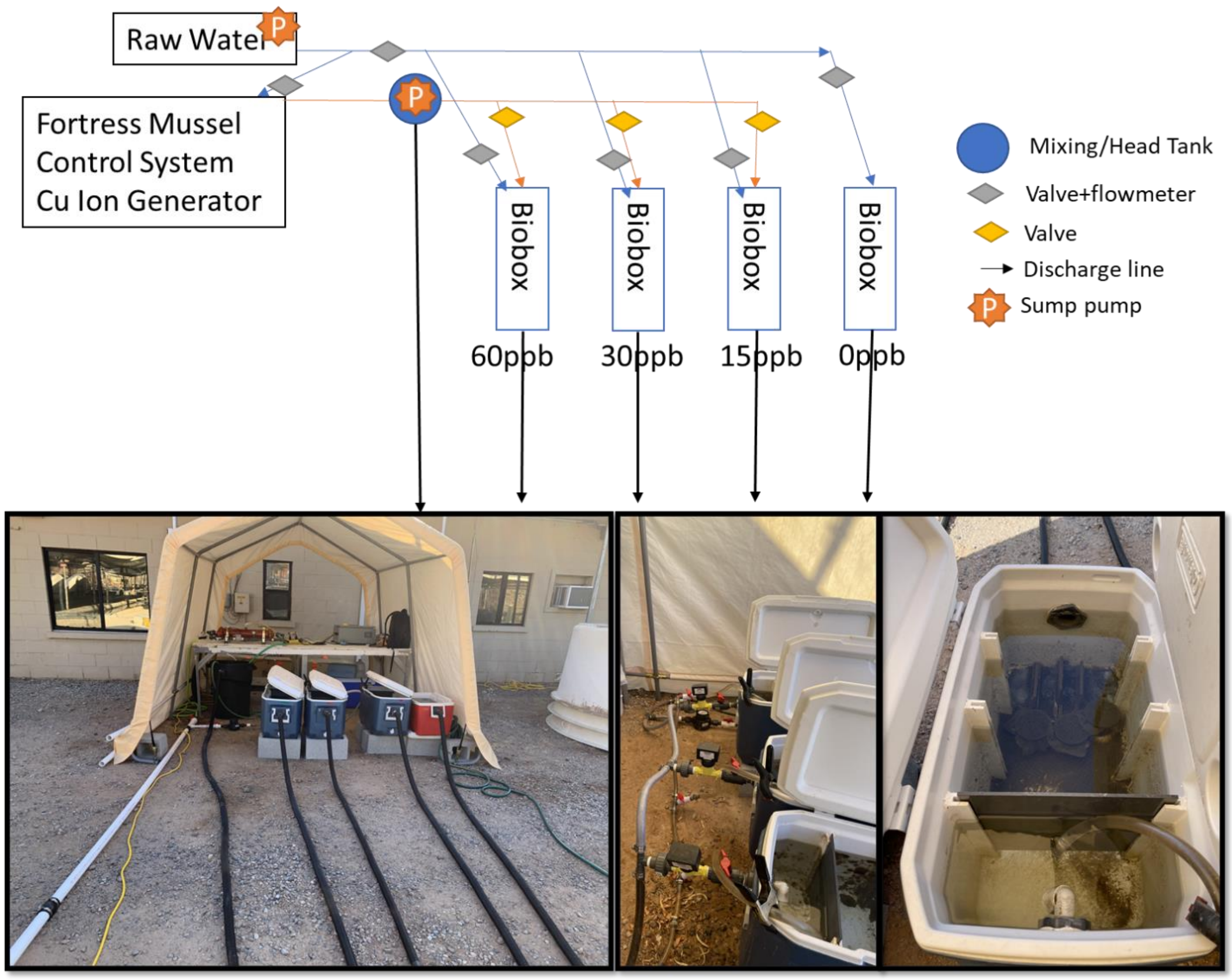

Figure A1. Schematic of biobox system set up with pictures of the field site showing how the actual configuration.

The copper ion generator had a flow meter with valve to control flow and the electrical box was configured to control amperage and volts supplied to the anodes of the copper ion generator. Only a 120 V outlet was needed for this copper ion generator. Once the generator was set to a certain amperage and voltage, no adjustments were made; flow through the generator was the only thing that was adjusted. The copper ion generator had a sample port installed to take small amounts of water from the discharge for copper concentration measurement. Flow from the copper ion generator was discharged into a 32 gallon mixing head tank.

A sump pump was located in the bottom of the mixing head tank to supply the pressure for feeding the copper ion concentrate. A manifold of tubing and ball valves were used for the copper ion delivery to each biobox, except the control biobox. At the end of the tubing in each biobox a plug was placed in the tubing with 5 to 6 holes drilled into the end of the plug to make a mixing sparger. These ends were placed at the beginning of the biobox by the raw water inflow before the baffle (Figure A1).

Flow from the sump pump in the raceway supplied water for the bioboxes. A valve and flow meter were installed prior to all bioboxes. The water was then diverted from the main tubing line to each of the four bioboxes. Each biobox had a flow meter and ball valve to control flow to the bioboxes. Each treatment biobox had a baffle in the front position to encourage mixing. All water quality measurements were taken in the middle of the bioboxes.

Performance of the Biobox Configuration

Overall, the system performed well to deliver copper ions to three bioboxes to determine adult quagga mortality and the prevention of settlement. There was much organic debris, sediment and algae that accumulated in the biobox (Figure 4) from the raceways. To fix this in the future, the sump pump needs to be placed on a stand above the settling solids or located at the head end of the bank of raceways. A stand may not work because of high flows through the raceway; however, this flow was not high enough to wash out the sediment and organic debris.

Some of the organic debris and sediment, and potentially water hardness, had impacts on the copper concentrations to need more copper ions than expected in the bioboxes (Figure A2). The amount of flow needed over expected was not consistent and became more variable with time. This made adjusting the copper concentrations in each biobox time consuming and frustrating. The other thing that made control of the copper concentrations more difficult was using ball valves to change the flow rate, these provided less precision than was optimal. Flow of the copper ions to all three bioboxes were pressurized with one sump pump, so changing flow into one biobox effected the other two bioboxes. To improve these problems, it is recommended to utilize flow with less organic debris and sediment, such as at the entrance to the hatchery or from the first bank of raceways. Another option would be to install a filter system after the water supply sump pump to remove much of the organic debris and sediment. Installation of metering pumps to deliver the copper solute to each biobox would eliminate the ball valves and impact of flow changes on the other bioboxes.

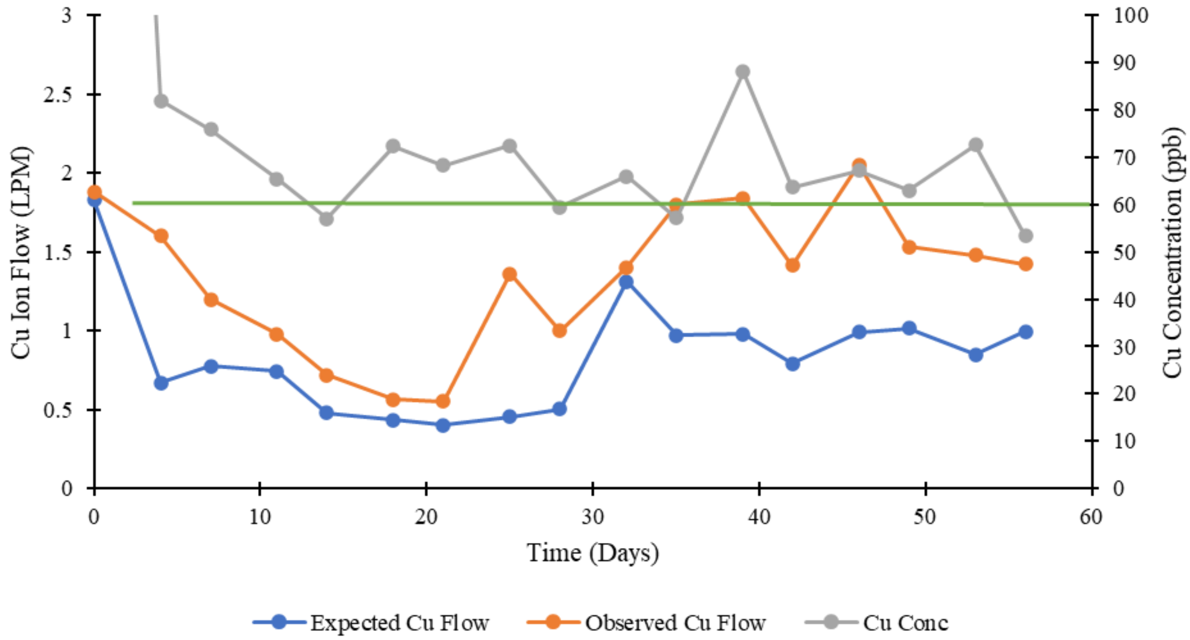

Figure A2. Calculated copper ion concentrate flow versus observed copper flow into the 60 ppb biobox with actual copper concentration in the biobox as an example of the difficulty experienced of keeping the bioboxes at desired concentrations.

The mixing sparger at the end of the copper ion tubing would become plugged with organic debris, sediment, and even solid copper pieces. This clogging would cause copper concentrations in the bioboxes to decrease. On every check day, the tubing would need to be flushed, back washed, or cleaned with muriatic acid. Future design could include bigger holes in the tubing to create a mixing sparger that does not plug, or if utilizing independent peristaltic pumps an inline flow mixer would mix solute with raw water prior to entry into the biobox.

Flow meters were very useful in this system set up. The flow meters to the bioboxes were digital paddlewheel flow meter (Blue-White Industries) that worked well and the ball valves were easy to manipulate to change flow by increments of 0.1 gpm. There was one time a snail had become lodged in the paddlewheel housing and stopped the flow meter, but it was easy to take apart the flow meter and dislodge the snail. The flow meters placed at the head of all the bioboxes and on the control biobox were analog positive displacement mechanical flowmeters (Badger). These flow meters plugged with algae and snails. Once these flow meters were plugged, they had to be taken out of the system, cleaned with hot water and Virkon disinfectant for at least 24 h and rinsed before being clean enough to be replaced. The flow meter prior to the bioboxes was removed completely on 20 November 2020 and the flow meter for the control was switched out with a clean flow meter every two weeks after 20 November 2020. It is not advised to use the Badger type flow meters in the presence of snails or high organic matter.

Plugging of the flow meter prior to the bioboxes and the general pressure of the biobox system put a lot of back pressure on the sump pump in the raceway. The sump pump had to be replaced one month into the study because it was not supplying enough water to the system. Upon replacement, an additional relief discharge was installed to bleed off extra pressure and decrease the strain on the sump pump. The extra water was diverted back to the raceways. A more consistent flow was achieved and the health of the pump was sustained. This modification is highly recommended for future configurations and a pressure valve that reads small pressure (<10 psi) will be helpful to increase pump life and stability of the raw water flow. An overflow or pressure release system should be installed with every sump pump used in the study configuration.

This was a low cost study and we used what we had available to keep costs low. This was also the first time doing a flow through study by KASF Consulting with the water supply from the outside raceways of Willow Beach National Fish Hatchery. Additional testing at this location as warranted should include many of these recommendations to make the study more precise, less time consuming, but will be more costly.