RNT CONSULTING INC

Invasive Mussel Research

Evaluation of Copper Ion Generator for Effects on Settlement of Veligers and Impact on Adult Quagga Mussels

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1 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, trashracks, 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 reaches of the Colorado River. The mussel populations have proliferated and mussels are now adversely affecting Hoover, Davis, and Parker Dams. 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 and Nevada, mussels are present in Kansas, Nebraska, Oklahoma and Texas.

The toxicity of copper to marine life has been recognized for centuries. Ocean going sailing vessel hulls were frequently covered by sheets of copper to minimize hull fouling by marine organisms. 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 Ca2+ homeostasis (Clayton et al. 2000).

The use of copper ion generator technology for the control of invasive mussels has been promoted for over 20 years. Blume et al. (1994) conducted a series of experiments to determine if the copper ion technology could be used against zebra mussels. 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 adults already present in the system treated 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.

Copper ion generators have recently been promoted and purchased as a relatively inexpensive dreissenid control technology for several different applications. Given limited available literature on efficacy of copper ion generators there was a need for an independent evaluation of the technology. In this study we tested the effects of various levels of copper generated by the copper ion generator on the settlement of larval quagga mussels (veligers) and the effects of the tested copper level on captive adult quagga mussels. We also evaluated the performance of the copper ion generator itself such as, ease of use and reliability. The evaluation was carried out in a flow through system using veliger rich water from the Colorado River and adult mussels collected from Lake Mohave.

2 METHODOLOGY

2.1 Equipment and Test Loop Set-Up

Upon award of the contract, an order was placed for the smallest available copper ion generator unit from MacroTech Inc. The ZM01 generator was delivered to Davis Dam in mid-July, 2013.

The copper ion generator was installed on a side stream from the cooling water supply to Unit 3 of Davis Dam as indicated in Figure 1 below.

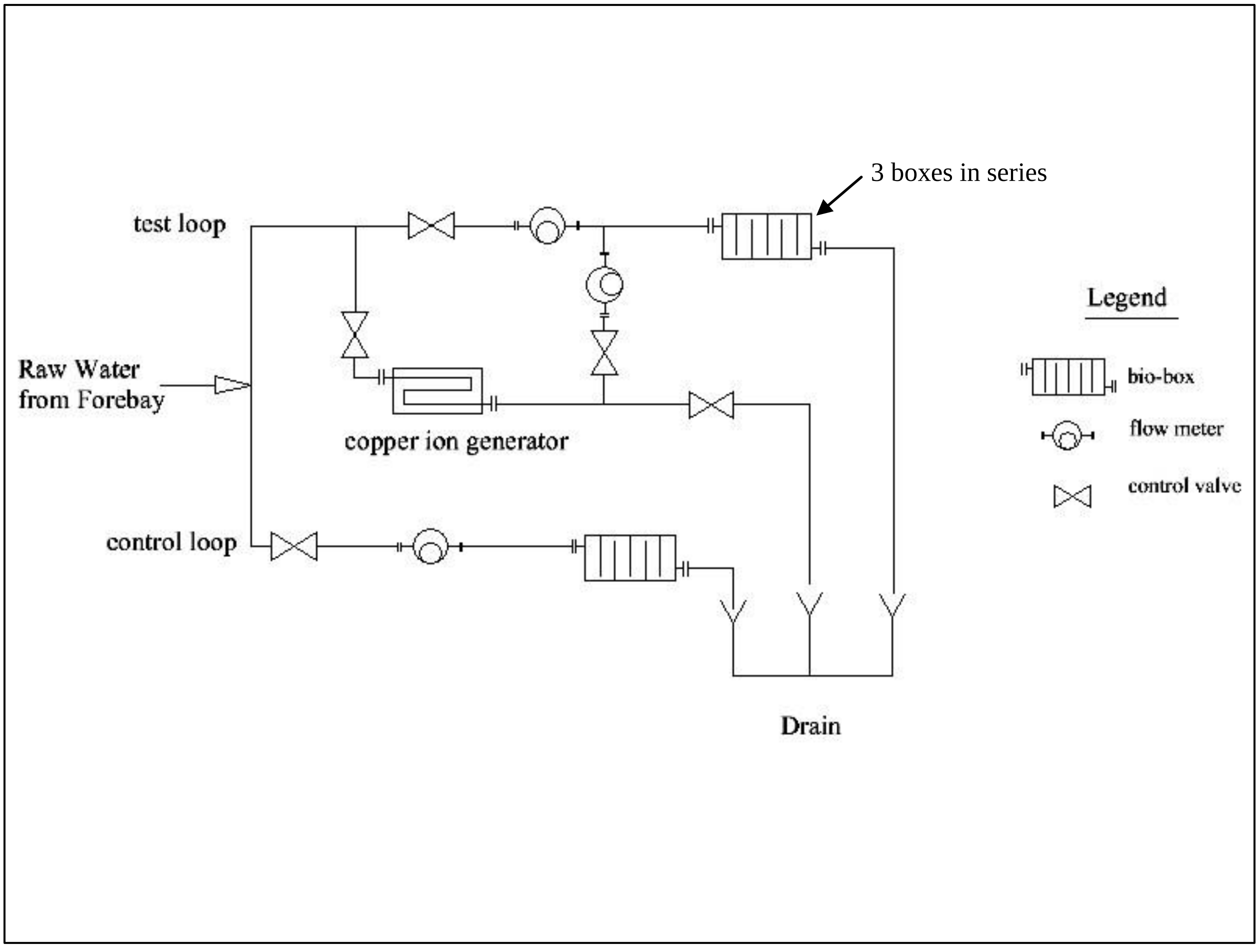

Figure 1 Initial test loop configuration

Two parallel loops were used to evaluate the settlement of incoming quagga mussel veligers and the impact of the copper ions on captive adult mussels.

One loop received input from the copper ion generator, while the second loop received untreated raw water and acted as a control.

The control stream flowed through a single biobox equipped with settlement plates and a mesh bag containing approximately 100 adult mussels. The treated stream flowed through three bioboxes in series to mimic the 20 minute retention time in the service water (Fig.2a). The first and the last treated biobox were equipped with settlement plates and mesh bags containing approximately 100 adults (Fig.2b).

The first treated biobox (referred to as biobox 1) received the copper injection in the incoming water. The water then flowed through the bioboxes to the last treated biobox (referred to as biobox 2). The treated water arrived in biobox 2 approximately 20 minutes after injection.

The flow into each loop was measured with a totalizer which allowed verification that both streams were delivering the same amount of water; approximately 4gpm. The flow rate was chosen as to maximize the number of veligers reaching the settlement plates in each biobox while maintaining speed of flow well below 4ft/s to allow settlement to occur.

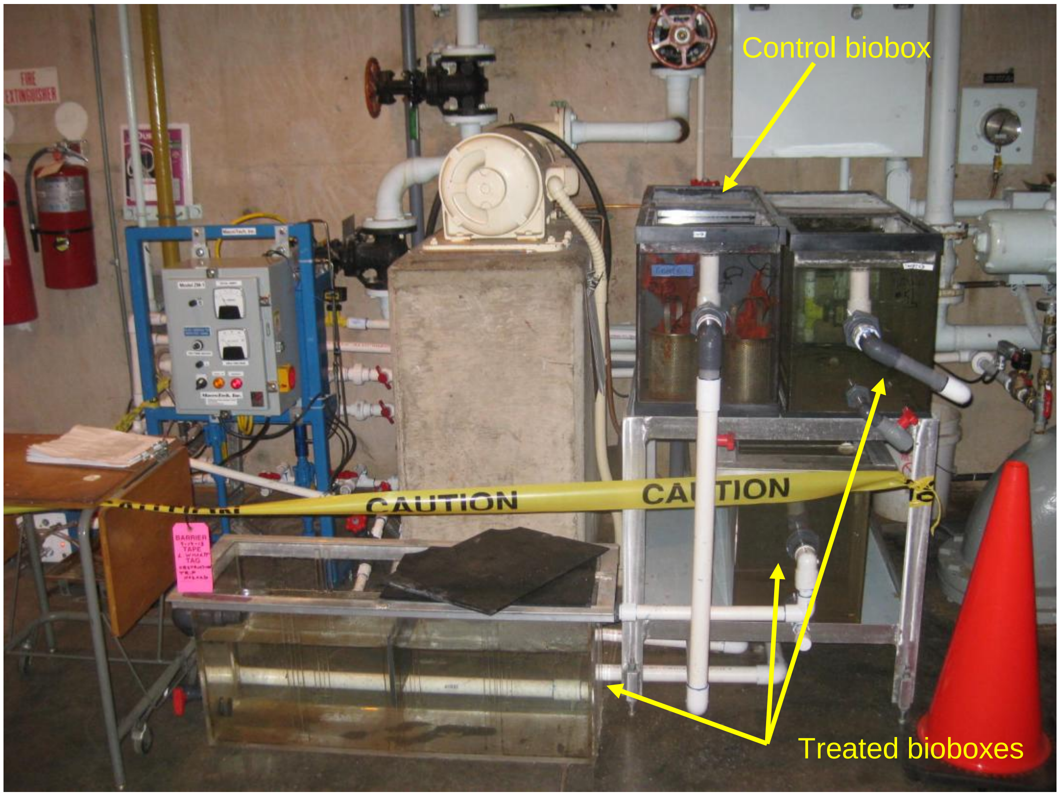

Figure 2a Photograph of test installation

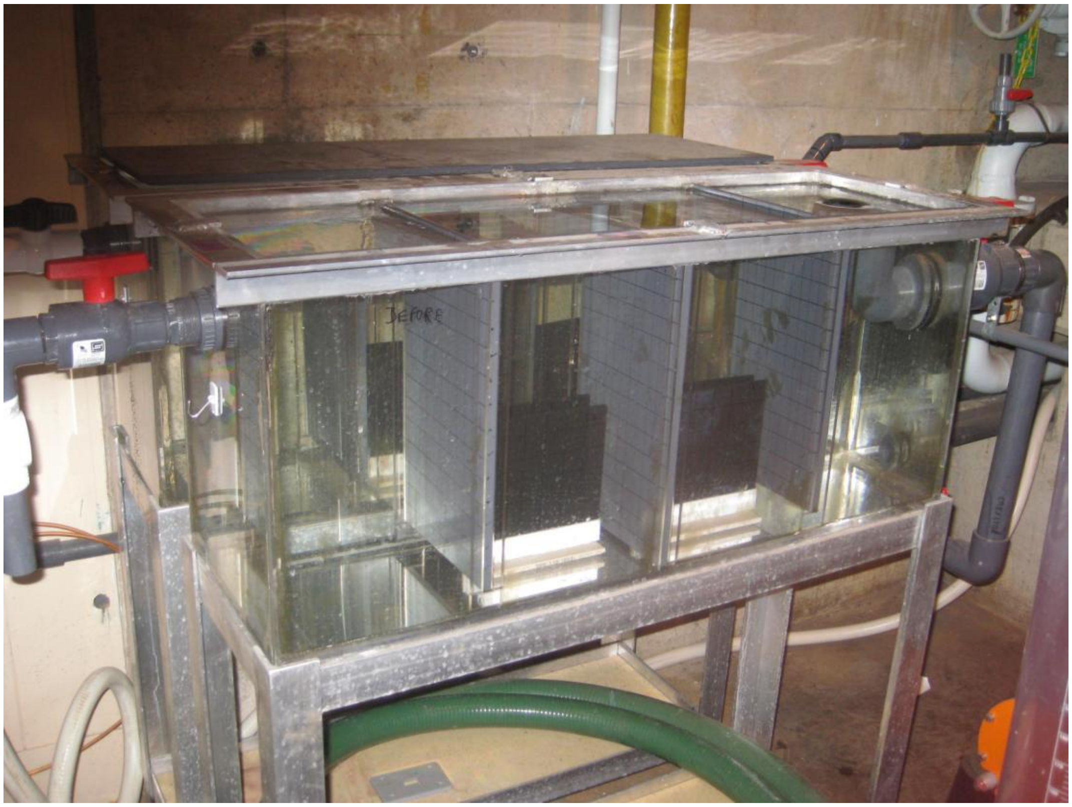

Figure 3b General layout of all bioboxes showing three large plates perpendicular to the flow and two sets of small plates parallel to the flow.

A study by Blume et al. in 1994 suggested that a dose of 10 ppb above background prevented zebra mussel veliger settlement. The Blume study was assumed to be the basis upon which the copper ion generator – ZM01 was designed. The operating manual for the ZM01 indicates that mussel control is achieved for a flow of 2000 gpm at an imposed electric current of 1 ampere. The flow through the generator was set at 13.5 gpm. This means that an injection flow of concentrated free copper ions of 13.5 gpm would treat 2000 gpm. The amount of concentrate removed from the ion generator flow to treat the test bio-box flow of approximately 4 gpm would therefore be 0.027 gpm (0.1 lpm).

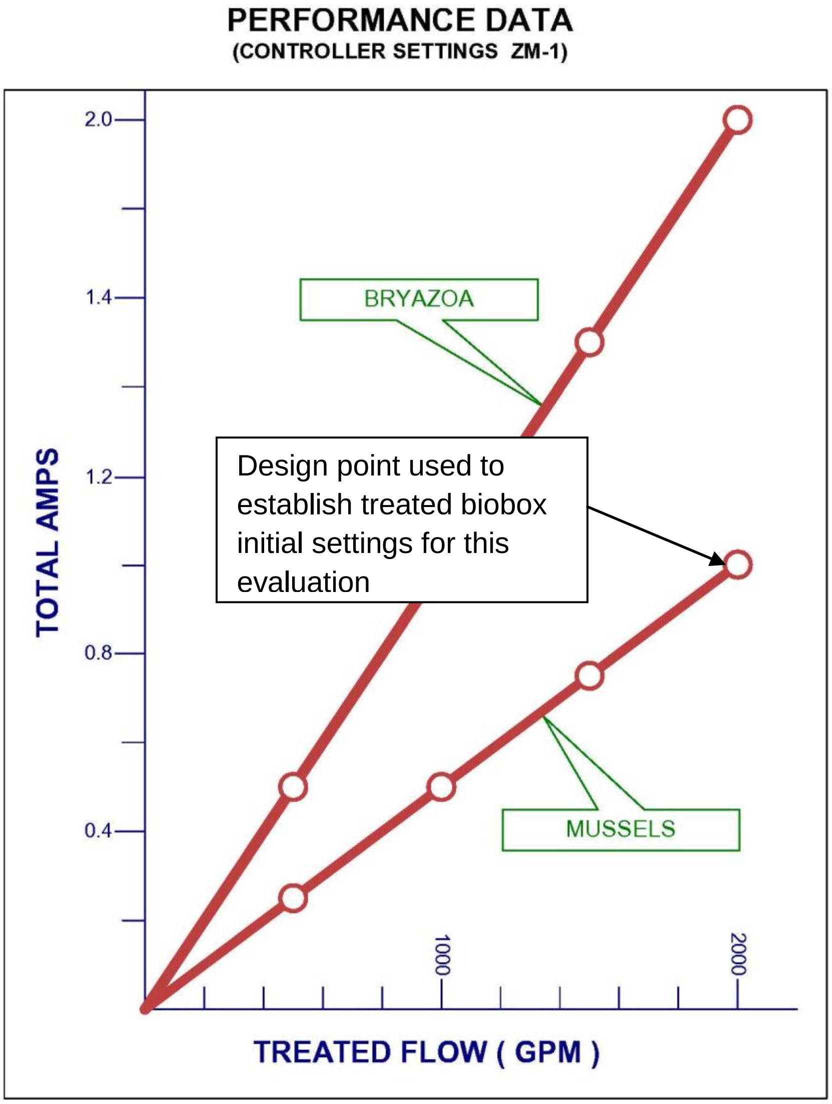

Figure 4 Performance chart from ZM01 Copper Ion Generator Manual

The copper ion generator is an industrial size unit and its minimum flow is greater than required for the test loop. Therefore the piping was set up so that the flow from the ion generator was split into 2 streams. One stream was directed to the mixing chamber preceding the treated bio-boxes and the other stream directed the remaining copper ion concentrate to waste. The amount of injection into the mixing chamber was adjusted to achieve the desired residual copper level in bio-box 1 by changing the back pressure of the generator discharge line that went to waste drain. This method proved to be unsteady and it was not possible to maintain a constant copper residual with the copper amount drifting significantly above or below the target level.

To address the above problems, the copper ion delivery system was modified to use a peristaltic pump to extract a constant quantity of copper ion solution from the generator discharge. The pump then injected the known quantity into the test biobox 1. Except for daily checks to adjust the drift in the ion generator itself and minor changes in the background copper levels, this change resulted in improved control of the copper concentration in the test biobox as defined by reproducible measurements of copper ion in biobox1 over a period of several hours. The revised test configuration is shown below. At a current of 1.1 ampere and an injection rate of 1.0 lpm a free copper concentration of just slightly above background was achieved. According to our instrumentation the background copper level fluctuated from 0 to 10ppb. The two outside laboratories had results of below 3ppb for the background level. 3ppb was their detection limit. Given the observed fluctuation we assumed a background of 3 to 5ppb for most of our experimental set-up.

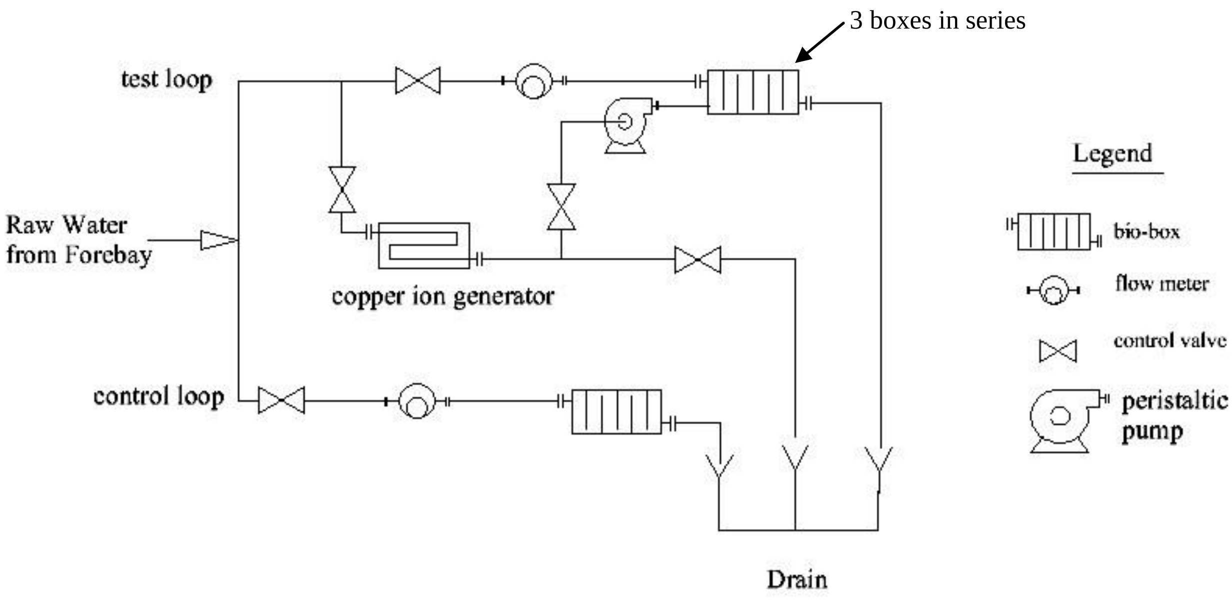

Figure 5 Final test loop configuration

2.2 Testing of Environmental Parameters

Water parameters such as temperature and pH were measured weekly using Hach HQ40d Multiprobe handheld instrument.

Figure 6 Instrumentation used to measure environmental parameters

2.3 Testing of Copper Ions Present

Copper concentrations were measured in both control and treated streams at start up and thereafter on weekly basis using Hach DR900 colorimeter. The collected water sample from each location was used to create three replicate 10 ml samples to be measured by the Hach meter using the porphyrin method for free copper. The porphyrin method is very sensitive to trace amounts of free copper. Due to the sensitivity of the method, a masking agent is used to prepare a “blank” for each sample. The masking agent removes any interference from other metals which might give false positive readings. The method does not require any sample extraction or concentration before analysis. The porphyrin indicator forms an intense, yellow-colored complex with any free copper present in the sample. The measurement wavelength is 425 nm for spectrophotometers or 420 nm for colorimeters. The instrument range was between 4 and 200ppb of copper with accuracy of +/- 6ppb. Thus a concentration of 13 ppb could be shown as a value anywhere between 7 ppb and 19 ppb on our instrument. To minimize the potential instrument error, all copper concentration readings were done in triplicate and the three values obtained were averaged.

The water sample measured using the Hach instrument was also periodically subdivided and a portion of the water sample was sent to Mohave Environmental Laboratories or to Accutest Laboratories (sometimes both) to verify the Hach instrument readings. The use of two independent laboratories to verify copper readings allowed for some comparison of the experimental error in the copper readings obtained.

Figure 7 Hach Colorimeter used to measure copper ion concentration

2.4 Monitoring Settlement and Adult Mortality

Each of the bioboxes monitored, control and test biobox 1 and test biobox 2 were equipped with three large settlement plates oriented perpendicularly to the flow and two sets of small settlement plates oriented in parallel to the flow through the biobox (Fig.2a). In addition a mesh bag containing approximately 100 adult mussels was introduced into each biobox at the beginning of each test cycle.

During the weekly visits the plates were visually evaluated for settlement and the bags containing adults were opened and all dead individuals were counted and removed, one bag at a time. Remaining adults were replaced in the mesh bag and re-introduced into the original biobox.

Veliger levels in the incoming raw water were not monitored. Veliger densities tend to vary in space and time and a weekly evaluation of a plankton sample would not offer an assurance of equal veliger densities reaching control and biobox1 at all times. We therefore relied on flow measurements which verified that control and biobox 1 received approximately the same amount of raw water and therefore approximately the same number of veligers.

2.5 Test Cycles

The objective of the experiment was to establish at what level of total copper ion present in raw water we would suppress the settlement of pediveligers. Secondary objective was to observe what happened to captive adult mussels during the long term exposure to the target copper ion level. Our initial target was the 10ppb level above background as suggested by the manufacturer. This target level was difficult to achieve given the variability of +/- 6ppb reading of the instrument and the apparent variability of the background level of copper ion in the lower Colorado River. After the initial two test cycles the copper ion level was increased till we obtained high adult mortality and commensurate reduction in settlement.

The first test cycle was initiated on August 16, 2013 with test objective of 13 to 15ppb copper concentration in the biobox1. On September 20, 2013 the water and power to the system was turned off during electrical outlet maintenance. Both test loops were stagnant for at least 32 hours. The test cycle was discontinued as we found very high copper level in the test biobox (55ppb), all the captive adults were dead and there was no settlement on the test plates.

A new test cycle (C1) was initiated on September 20th. Sufficient flow from the copper ion generator was added to obtain a reading of 13-15 ppb in the test Biobox 1 (first biobox immediately after copper addition). On October 1st we received a work stop order due to US Government shutdown. The shutdown continued until October 17th, 2013. On October 29th we were able to re-enter the facility and evaluate the results.

Test cycle (C2) commenced on November 6th, 2013 and continued until December 3rd, 2013. Once again the objective was to obtain a reading of 13 -15 ppb in the test Biobox 1. Due to the difficulties experienced with maintaining the target copper level in the test bioboxes during the past two cycles, it was decided to modify the test loop set up using the peristaltic pump. This configuration was used for all subsequent cycles. In concert with our BOR technical contact we designed a process whereby the copper ion concentration from the generator was measured as previously described and the initial amount of injection from the peristaltic pump was proportioned according to the biobox flow. This approach reduced the amount of flow adjustments needed to achieve the target copper ion concentration in the test biobox. The peristaltic pump was ordered and the system was re-configured in mid –December 2013.

Test cycle (C3) began on January 3rd, 2014. The copper addition level was such as to have a reading of 13 to 15 ppb in the first treated biobox – biobox 1 with the assumption that the level in biobox 2 may be lower given the fate of copper in raw water. On February 3rd the generation unit which supplied water to this experiment went out of service. Partial data was collected for this cycle.

Following the return of the generating unit to service, three additional independent test cycles, C4, C5 and C6 were carried out between February 28th, 2014 and June 9th, 2014.

During the test cycle C4 (February 28th to April 15th, 2014) the objective was to test copper levels at 20 and 30 ppb due to settlement being observed at the lower levels tested. This level is well within the acceptable limits for drinking water (1ppm).

Test cycle C5 **started** on April 16th, 2014 and ended on May 13th, 2014. The copper level in the first treated biobox was set to be between 15 and 20 ppb.

The last test cycle, C6, started on May 14th and terminated on June 9th. The copper level in the first treated biobox was to be between 10 and 15 ppb). The return to the low copper test levels was done in an attempt to establish a threshold for long term survival of the adult mussels.

3 RESULTS

3.1 Veliger Settlement and impact on Captive Adult Mussels

3.1.1 Test Cycle 1

This cycle was initiated on September 20th. Sufficient flow from the copper ion generator was added to the first test biobox to obtain a reading of 13 - 15ppb, with background level measured between 0 and 3ppb. On October 1st we received a work stop order due to US Government shutdown. The shutdown continued until October 17, 2013. On October 29th we were able to re-enter the facility and terminate the experiment. The test biobox was at the same level as the control, background copper level of 7ppb. During the shutdown we were not able to collect data or adjust the copper levels in the bioboxes. The ambient temperature was between 21.7 and 20ºC.

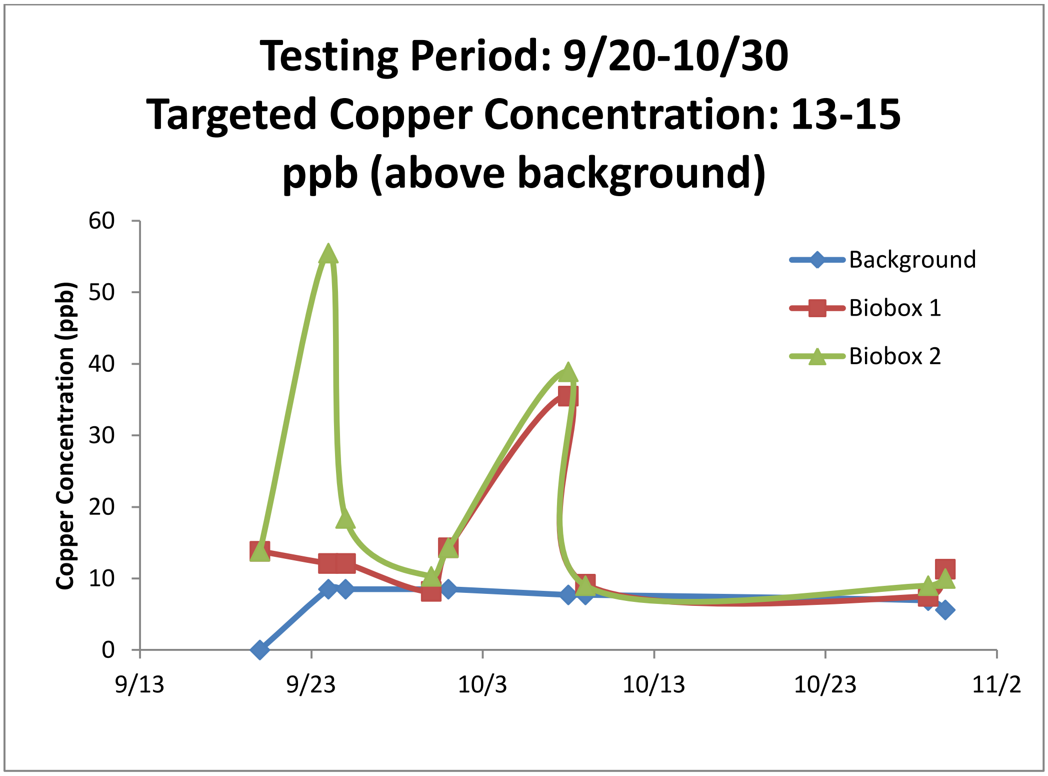

Figure 8 Cycle 1 Copper Concentration Levels.

There was settlement in the treated loop. The difference in settlement densities between control and the first treated biobox (biobox 1) were significantly different (p-value 0.01), with the treated biobox having a much lower settlement density than the control biobox. The last treated biobox (biobox 2) also had significantly lower densities compared to control and the first treated biobox densities. The third treated biobox was not examined. The total amount of water flowing into the control biobox was similar to the total amount flowing into test loop where the three treated bioboxes were in series. Therefore the settlement in the control biobox is most directly comparable to the settlement in treated biobox1.

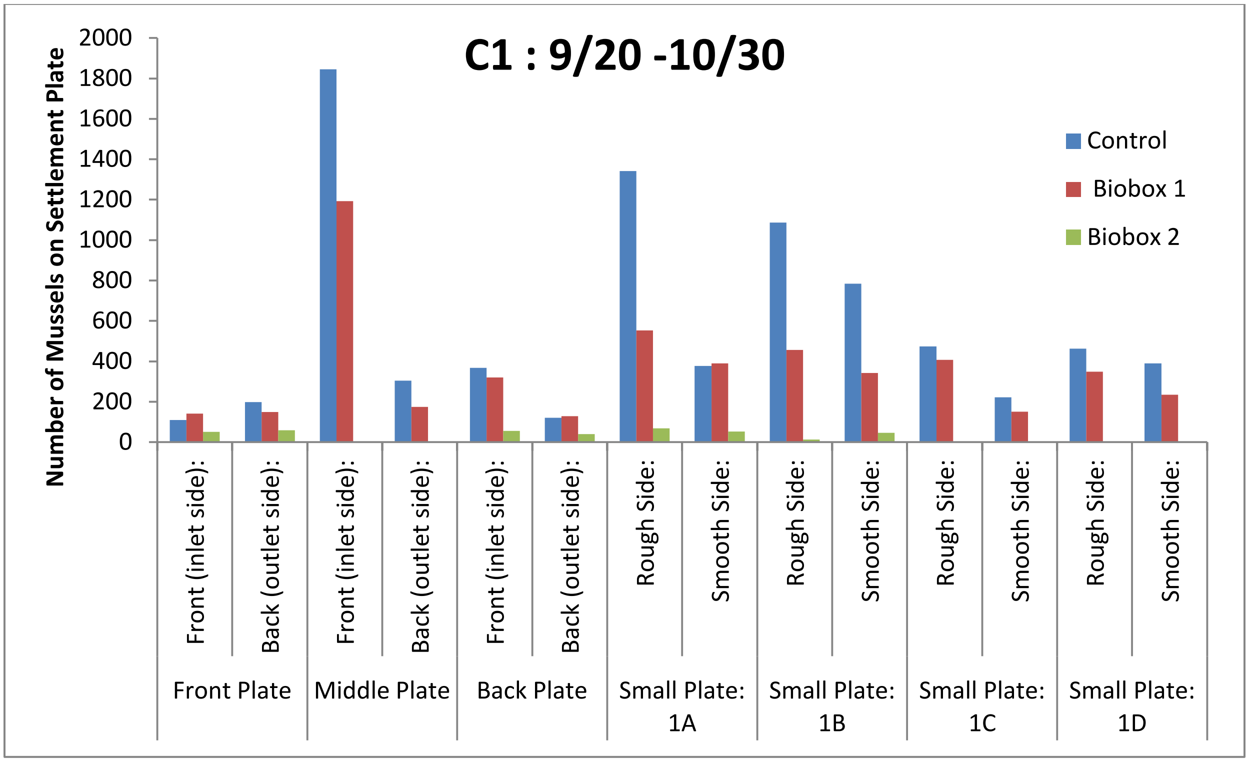

Figure 9 Cycle 1 Settlement Results

The settlement in both the control and treated bioboxes was not evenly distributed as seen in Figure 8. Settlement densities varied both with the position of the settlement plate within the biobox and the orientation of the surface with respect to the direction of flow.

Ninety-six percent of the caged adults in biobox 1 were dead. In the second test biobox, biobox 2, 88% were dead. In the control there was only 4% mortality of adults. The high mortality of adults in the treated bioboxes is likely due to the two high copper levels early on in the cycle as shown in *Figure 8* . The observed settlement could have occurred after the copper levels decreased to the level observed on October 29th.

3.1.2 Test Cycle 2

The cycle commenced on November 6, 2013 and continued until December 3, 2013. Once again the objective was to obtain a reading of 13 - 15ppb in the test biobox 1. The actual copper levels achieved were below this target. The ambient temperature during the experiment was between 16.9 ºC and 12 ºC.

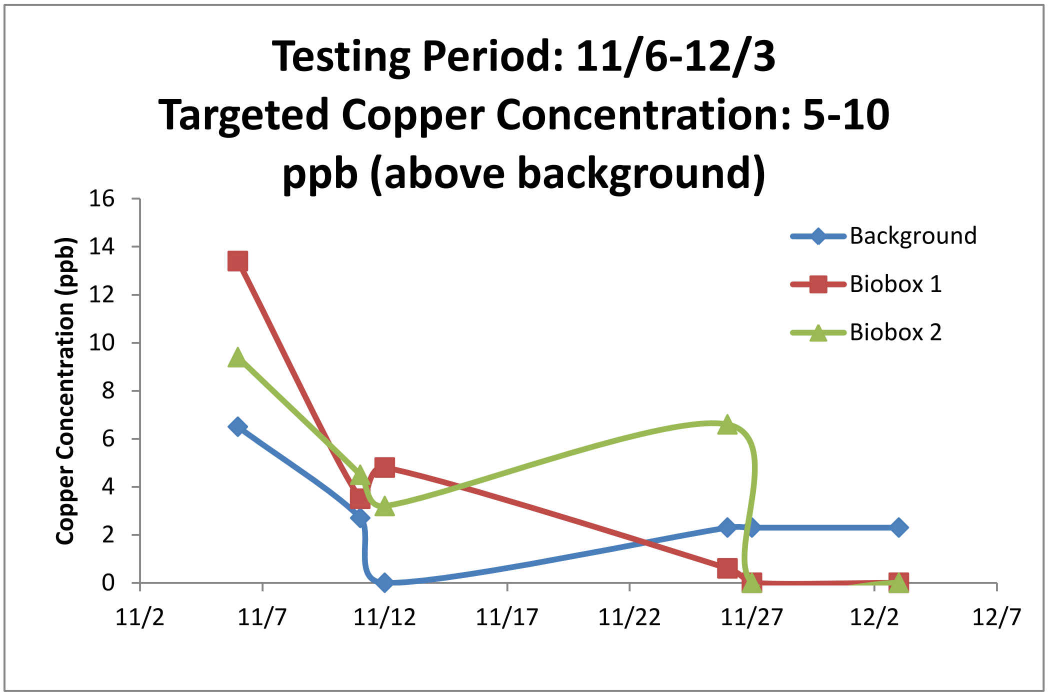

Figure 10 Cycle 2 Copper Concentration Levels

There were no significant differences between the settlement in control and the treated loop (p-value 0.64); Average densities in the treated biobox 1 were higher than the control average densities. Settlement densities in biobox 2 were significantly lower than the densities in biobox1 and control biobox (p-value= <0.001).There was settlement in both the treated and control systems.

The copper value recorded in biobox 2 on 27th of November may have been an artifact given the low levels of copper present.

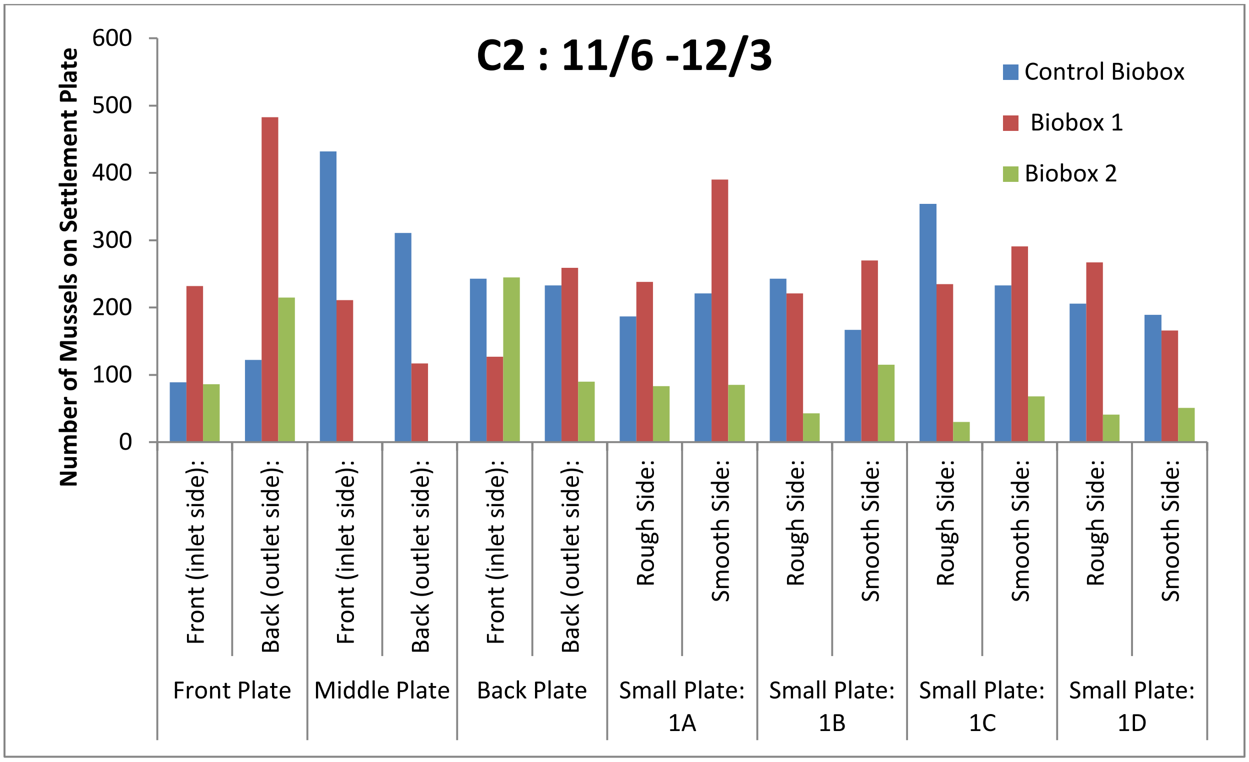

Figure 11 Cycle 2 Settlement Results

There was slightly higher adult mortality in the treated bioboxes compared to control at the end of the test. Fourteen percent of the caged adults in biobox 1 were dead. In biobox 2, 12% were dead. In the control there was 9% mortality of adults.

3.1.3 Test Cycle 3

Cycle 3 began on January 3, 2014. The targeted copper level was 13 to 15 ppb absolute in biobox 1. On February 3, the generation unit which supplied raw water to the test loops went out of service. The readings in biobox 1 were almost 50ppb on February 3. The average ambient water temperature was 11.5º C.

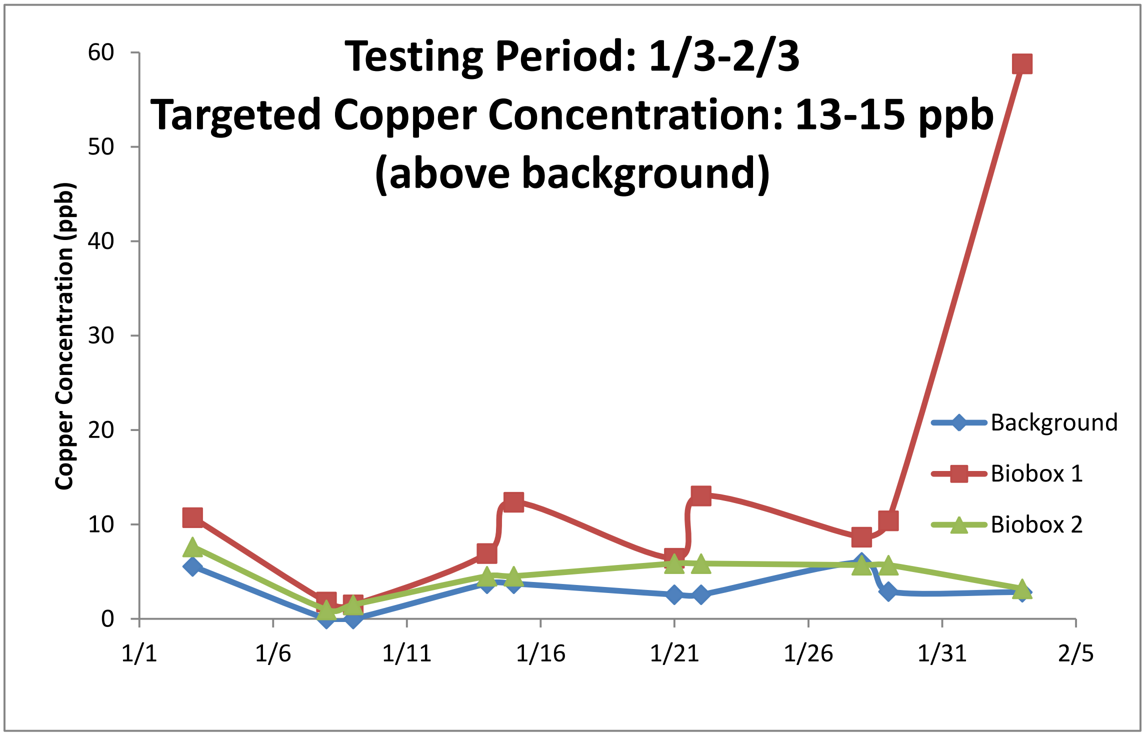

Figure 12 Cycle 3 Copper Concentration Levels

There was settlement in the control and treated systems. The difference in settlement densities between control and biobox 1 were not significantly different (p-value 0.056), Biobox 2 had significantly higher densities compared to the control and biobox 1 densities (p-value 0.04). Average densities per plate in the last biobox were 175.13 ± 21.15.

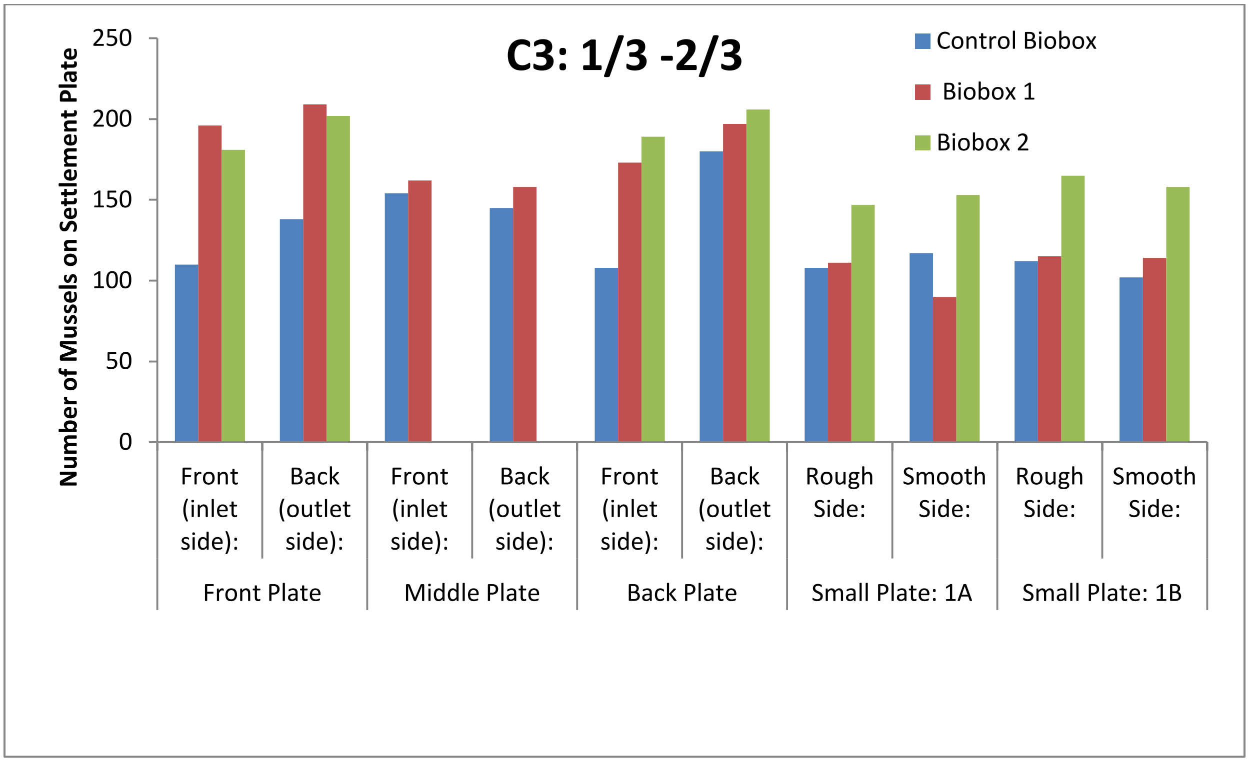

Figure 13 Cycle 3 Settlement Results

There was no significant difference in adult mortality between control and the two treated bioboxes. Fourty percent of the caged adults in biobox 1 were dead. In biobox 2, 36% were dead. In the control there was 40% mortality of adults.

3.1.4 Test Cycle 4

Cycle 4 was initiated on February 28th when the generating unit was returned to service and water flow resumed. The cycle continued until April 15th. The target for the copper level in the first treated biobox was to be between 20 and 30ppb. However the actual levels measured in the biobox were between 25 and 65ppb. The average ambient temperature was 15º C.

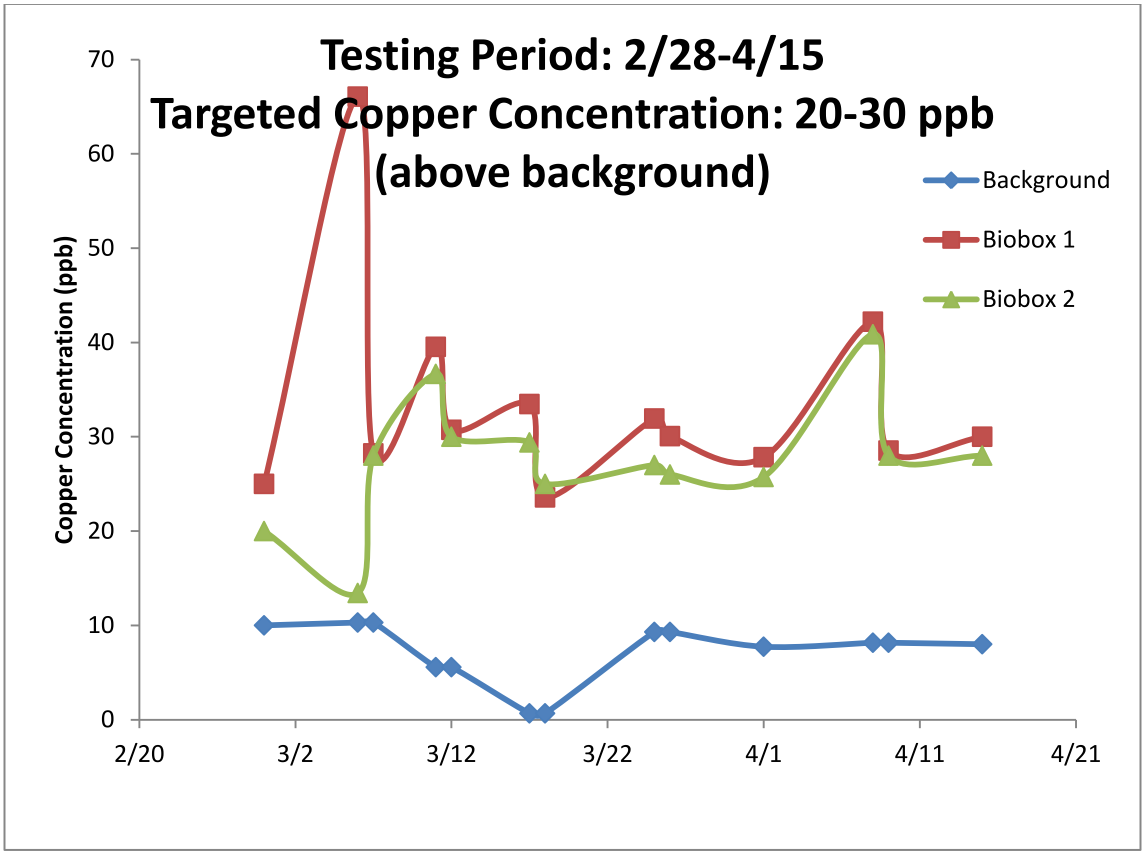

Figure 14 Cycle 4 Copper Concentration Levels

Settlement was present in the treated bioboxes but no settler was larger than 0.5mm. In the control biobox settlers ranged from 0.5mm to 5mm. In terms of absolute settlement numbers there was not a significant difference between the treated system and control (p-value is 0.11). This was due to the large variation in settlement on the plates in the control biobox. In the control biobox, the average settlement density per plate was 127.5 with a standard deviation of 166.6. Biobox 1 had a much smaller average settlement density per plate; 29.6 with a standard deviation of 21.8. Densities in biobox 2 were significantly lower than the control and first biobox 1.

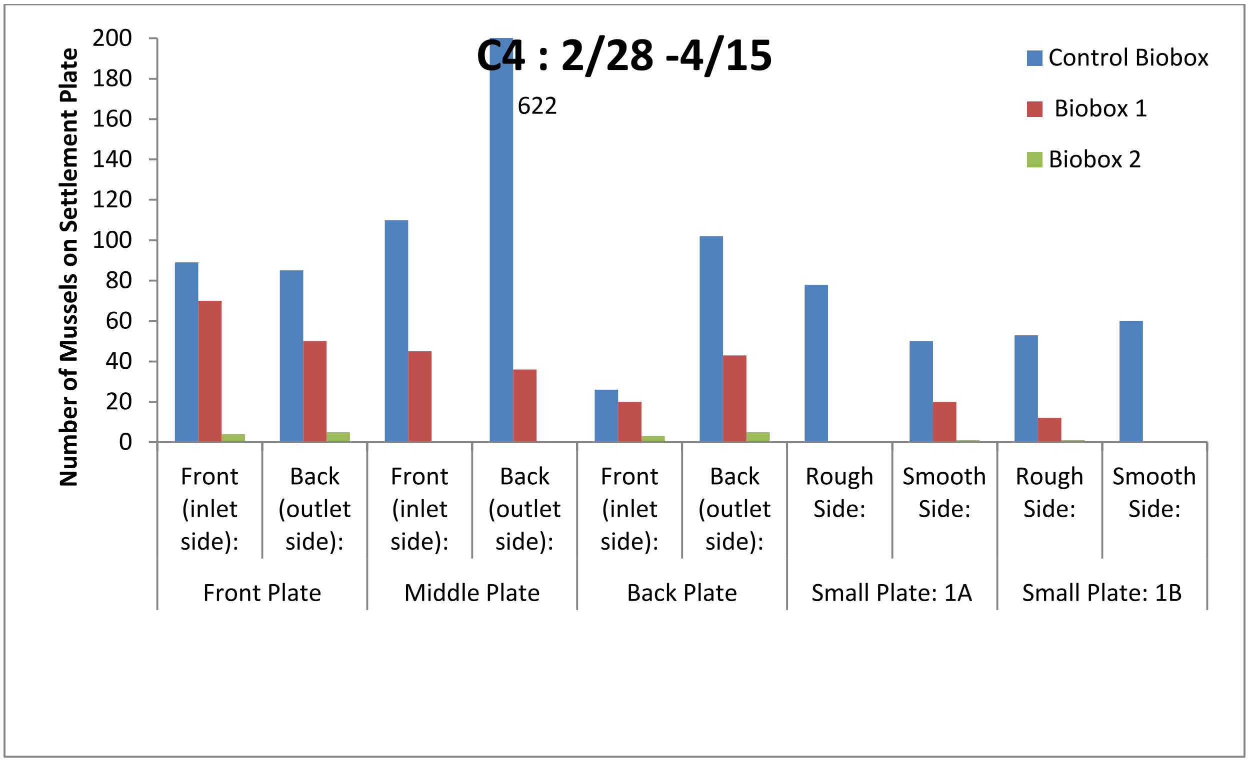

Figure 15 Cycle 4 Settlement Results

During this cycle we observed adult mortality beginning after the first week of treatment. By the end of the cycle test biobox 1 had almost 90% mortality of adults, biobox 2 had over 80% mortality, while control had just over 20% mortality of adults.

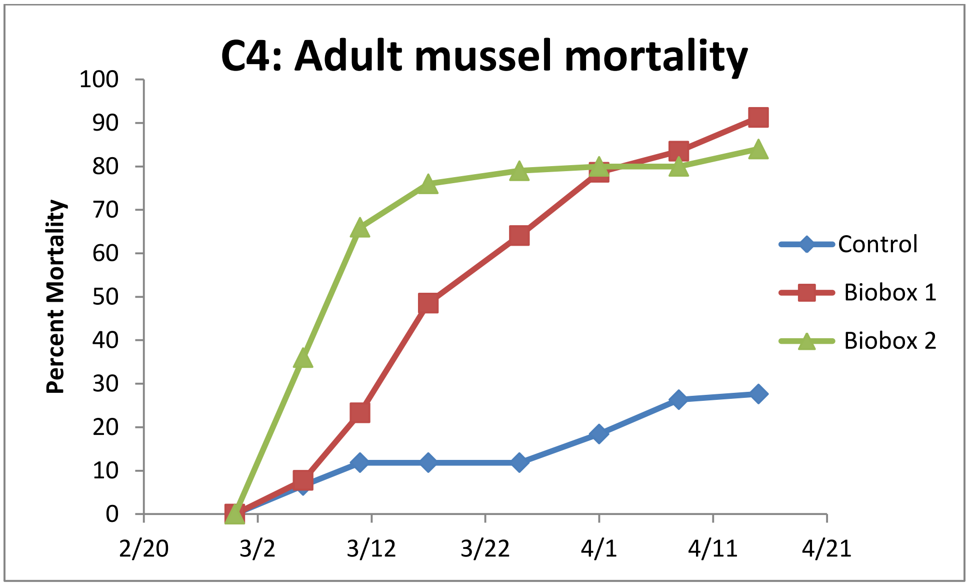

Figure 16 Cycle 4 Adult Mortality Results

3.1.5 Test Cycle 5

This cycle started on April 16th, 2014 and ended on May 13th, 2014. The copper level in the first treated biobox was targeted between 15 and 20ppb. The ambient water temperature was 17ºC.

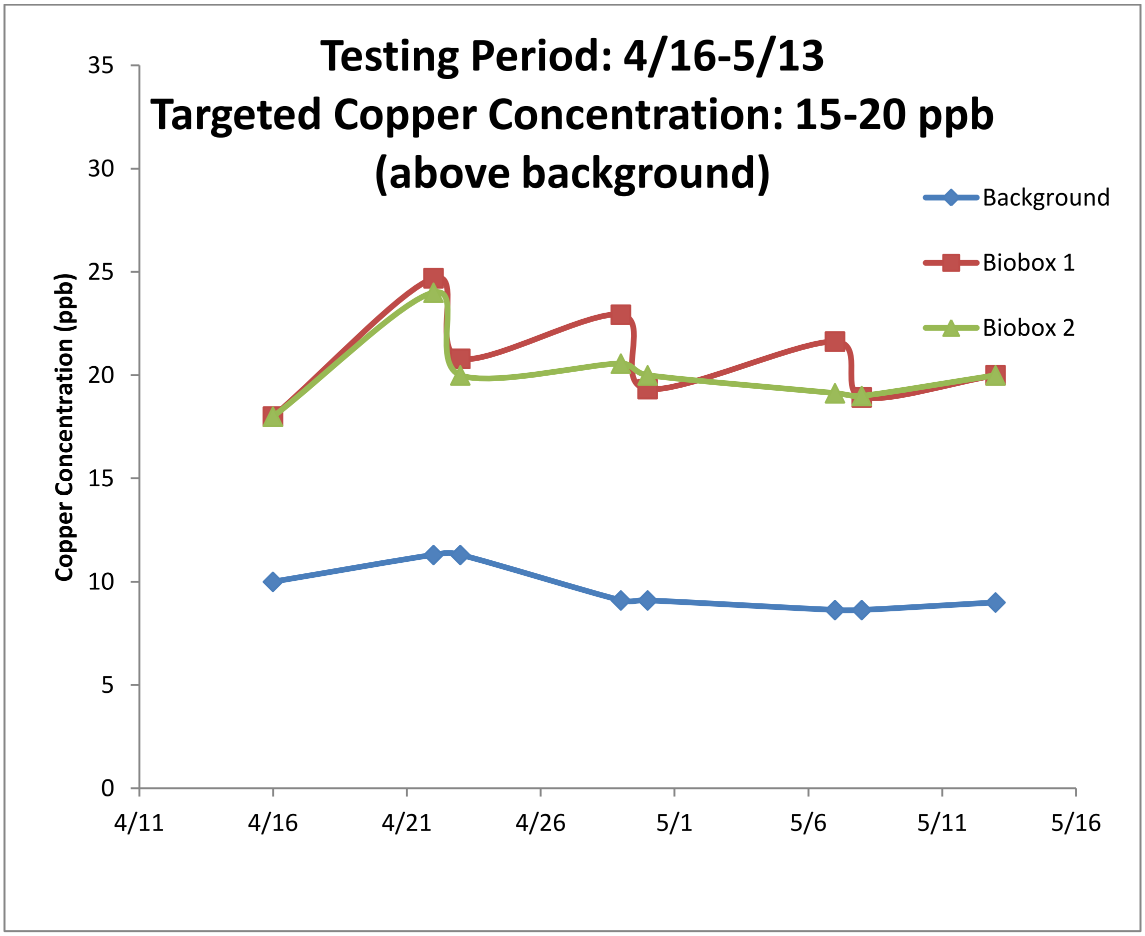

Figure 17 Cycle 5 Copper Concentration Levels

Settlement was present in the treated bioboxes but no settler was larger than 0.5mm while the settlers in the control bioboxes ranged from 0.5mm to 5mm. In terms of absolute settlement numbers there was a significant difference between the treated system and control (p-value 0.03). The average density per plate in biobox 1 was 19.3 with a standard deviation of 13.5. Average densities per plate of the control were 74.3 with a standard deviation of 62.6.

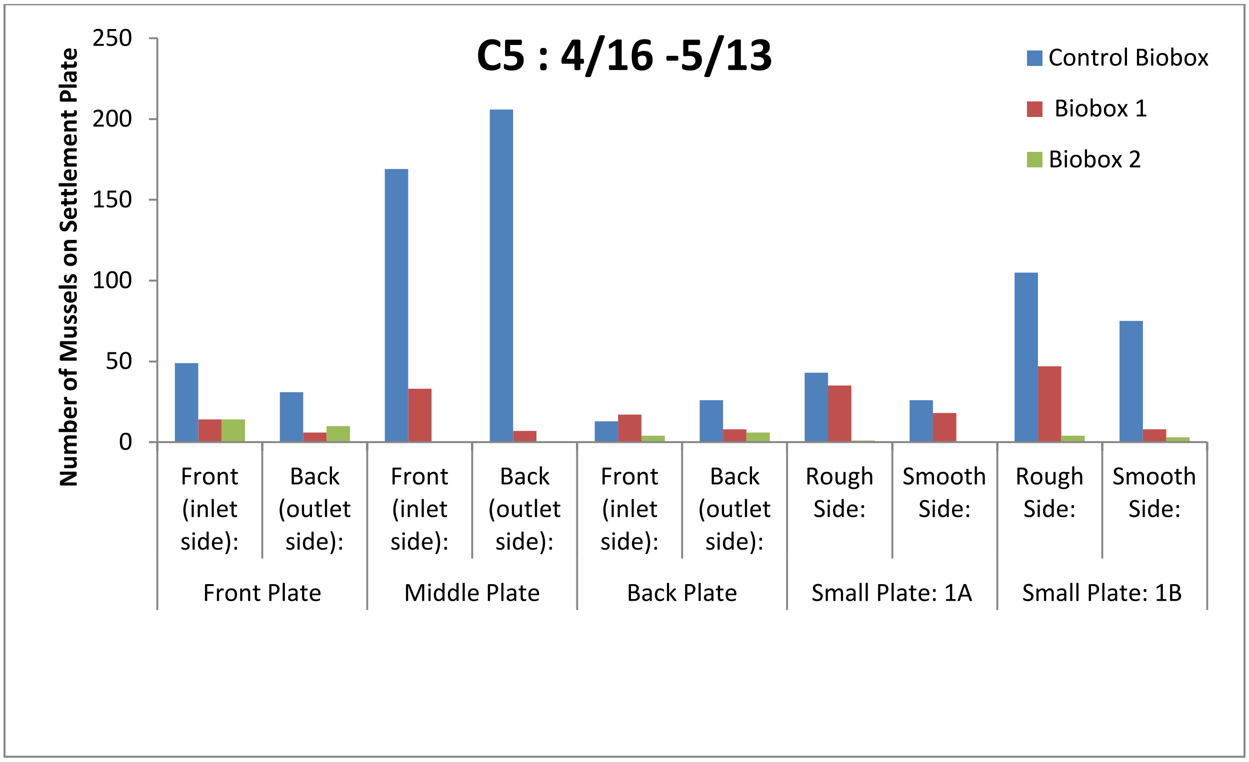

Figure 18 Cycle 5 Settlement Results

During this cycle we observed adult mortality beginning after the third week of treatment. By the end of the cycle biobox 1 had just over 40% mortality of adults, while biobox 2 and control had just under 20% mortality of adults.

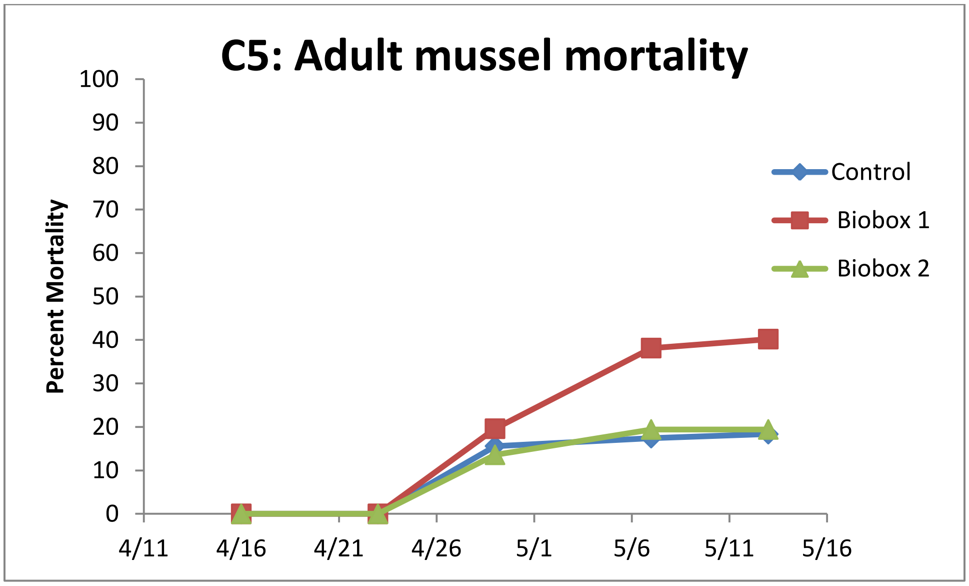

Figure 19 Cycle 5 Adult Mortality Results

3.1.6 Test Cycle 6

This cycle started on May 14th and terminated unexpectedly on June 9th. An electrical spike appeared to have destroyed the fuse in the copper ion generator and this shut down the copper addition. The copper level in the first treated biobox was targeted to be between 10 and 15ppb but has moved between 15 and 20ppb. The ambient water temperature was 19.3º C.

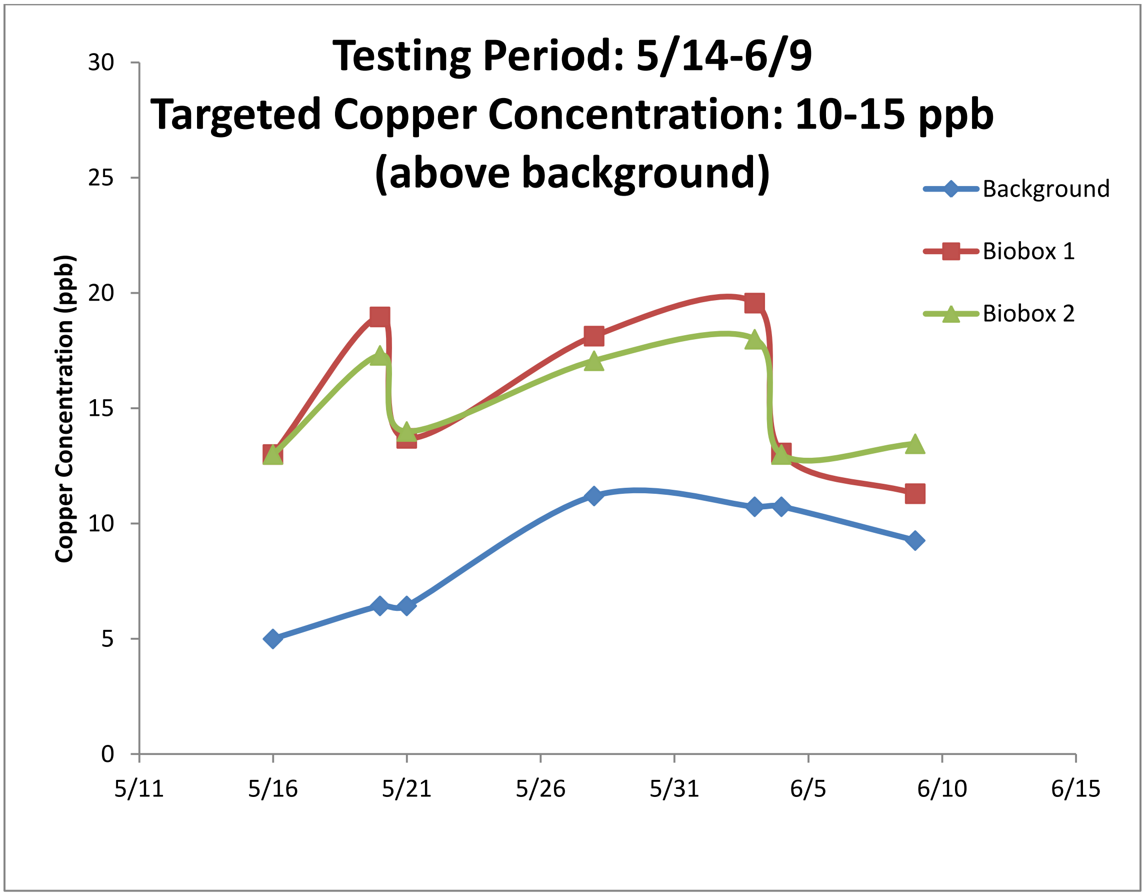

Figure 20 Cycle 6 Copper Concentration Levels

Settlement was present in the treated bioboxes but no settler was larger than 0.5mm while the settlers in the control bioboxes ranged from 0.5mm to 5mm. In terms of absolute settlement numbers there was a significant difference between the treated system and control (p- value of 0.01), with lower settlement in the treated biobox1. Densities of biobox 2 were significantly lower than the control and the treated biobox. The hypothesis for this is that settlement of ready to settle pediveligers occurs on the first available surface, i.e. biobox1. The same phenomenon is observed in pipelines where the settlement is heaviest near the intake and tapers off with distance.

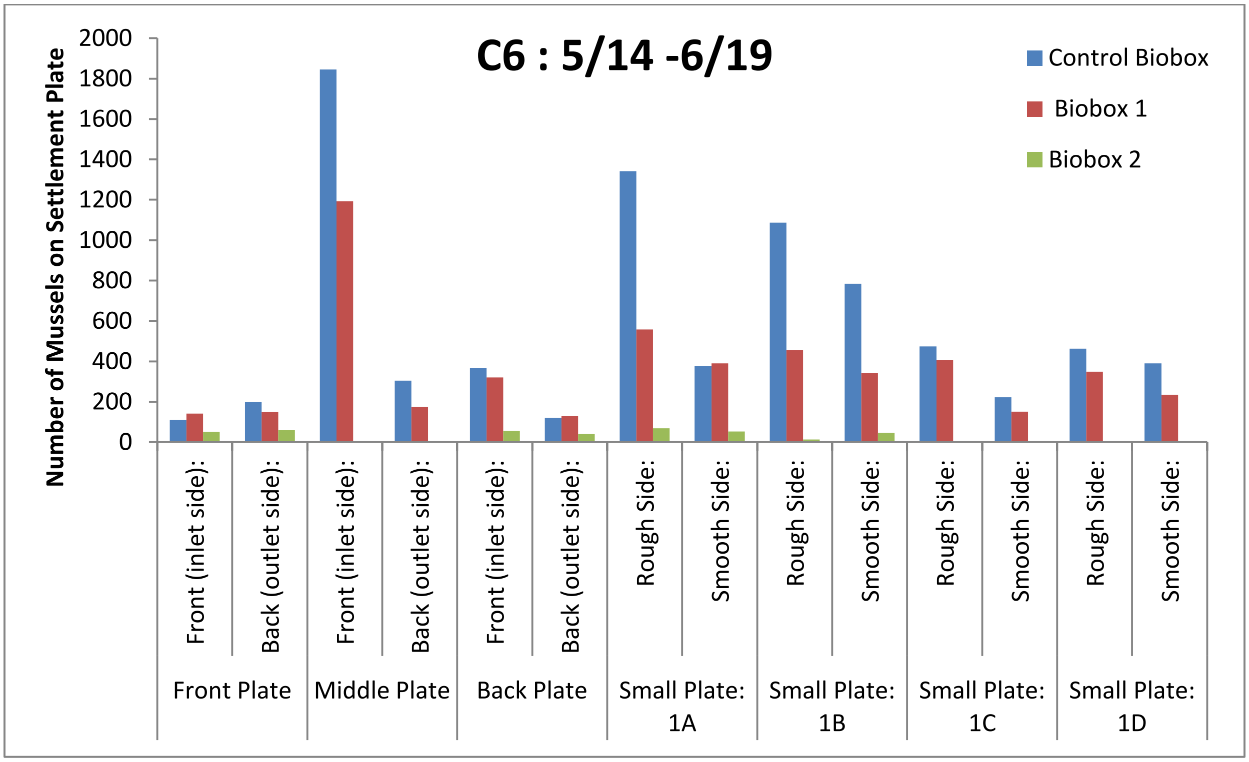

Figure 21 Cycle 6 Settlement Results

During this cycle we observed adult mortality beginning after the first week of treatment. By the end of the cycle both treated bioboxes had over 50% mortality of adults, while the control had just under 5% mortality of adults.

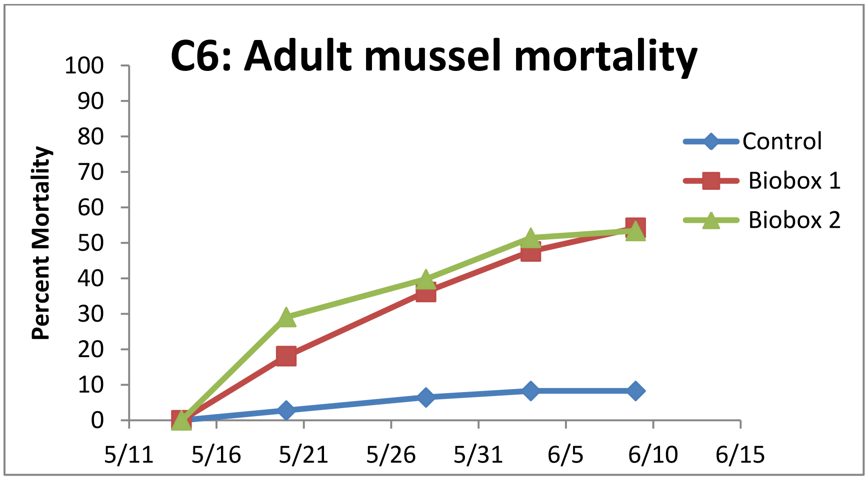

Figure 22 Cycle 6 Adult Mortality Results

4 DISCUSSION

4.1 Performance of the Copper Ion Generator

4.1.1 Initial Setup and Operation

The manufacturer's representative attended at site to assist in installation and startup of the ion generator. The installation and setup process was simple and straight forward requiring less than half a day to complete. Once operation began, the only attention required was occasional adjustment of the voltage control knob to keep the current at the target setting. There is no auto-compensation present in this device.

The raw water flow through the generator was set to 13 gpm and the current was adjusted to 1 ampere. Throughout the duration of the evaluation the flow and current were monitored once per day and adjusted as necessary. The flow was in the range of 11 -13.5 gpm which was within the manufacturer's recommended range of 10 - 15 gpm. The current variation during the evaluation was in the range of 0.9 to 1.3 amps.

4.1.2 Operating Experience

During test cycles1 and 2 attempts to achieve the target copper ion concentration in the test bioboxes were performed by adjusting the valves in the test loop. With this method it was difficult to maintain a stable concentration of copper ion in biobox 1. As the amount of concentrate from the generator injected into the test biobox was very small, it was postulated that the small piping line and valve were sensitive to any particulate in the water stream. For cycles 3-6 a positive displacement pump was used so that the amount of fluid injected could be set. This method improved the stability of copper concentration flow rate, but some drifting still occurred.

The porphyrin method to determine copper concentration is time consuming. To reduce the number of adjustments to the pump to achieve the target concentration, the concentration of the discharge from the copper ion generator was measured and the amount of injection required was predicted based on flow ratios. The pump could then be set at an initial flow which reduced the number of iterations to arrive at the target flow and copper concentration.

At the start of Test Cycle 3, the discharge water stream from the copper ion generator typically had copper ion concentration of 145 ppb but there were occasional excursions between 100 - 195 ppb. Based on the concentration of the discharge from the ion generator and a desired concentration of 10 ppb above background, the ZM01 unit would be capable of treating a total flow stream of no more than 200 gpm. This is significantly lower than what we would expect based on the manufacturers manual, which suggests that 1 ampere of impressed current the ZM01 unit would be capable of treating 2000 gpm. Although the flow through the generator and the impressed current were at all times within the manufacturer's recommended settings, there was no diagnostic indication on the equipment that the performance of the generator was not producing the required amount of copper ions. As the condition of all the external equipment and associated values appeared normal, the electrodes were suspected of being consumed or damaged. An attempt was made to open the generator to examine the electrodes but the lid of the ion chamber was seized shut and would not release. Rather than risk damaging the unit with excessive force it was decided to continue the evaluation until completion and then transport the unit to the shop where the lid could be removed with heavy duty tools. By the time Test Cycle 6 was carried out, the discharge from the ion generator had deteriorated to 80-90ppb while the raw water flow and current were still being maintained within the manufacturer's recommended values.

4.2 Ion Chamber

At end of Test Cycle 6 the copper ion generator was moved to the plant maintenance shop and the lid was removed. Approximately 2/3 of the surface of the aluminum electrode was coated with a cream colored and light brown colored deposit. The copper electrodes were coated with deposits which were mostly light blue in color. The bottom of the chamber was covered with loose scale suggesting the scale was being shed from the electrodes. There were patches of scale that were different thickness and shape consistent with shedding, exposing different areas of the electrodes. This may provide a possible explanation of why the copper ion concentration measurements were frequently erratic.

The generator had been operating but at a reduced rate of copper ion production which would suggest that electrons or ions were participating in unwanted side reactions resulting in different products being formed. The light blue color of the scale on the copper electrodes is typical of copper carbonate and may be a result of the high alkalinity (130mg/l in $CaCO_3$ units) of the Colorado River water. Based on water quality monitoring done by Southern Nevada River Authority and Central Arizona Project, alkalinity levels remain fairly constant in the Colorado River water in this area.

Figure 23 Lid of ion generator removed exposing the scale coated copper and aluminum electrodes

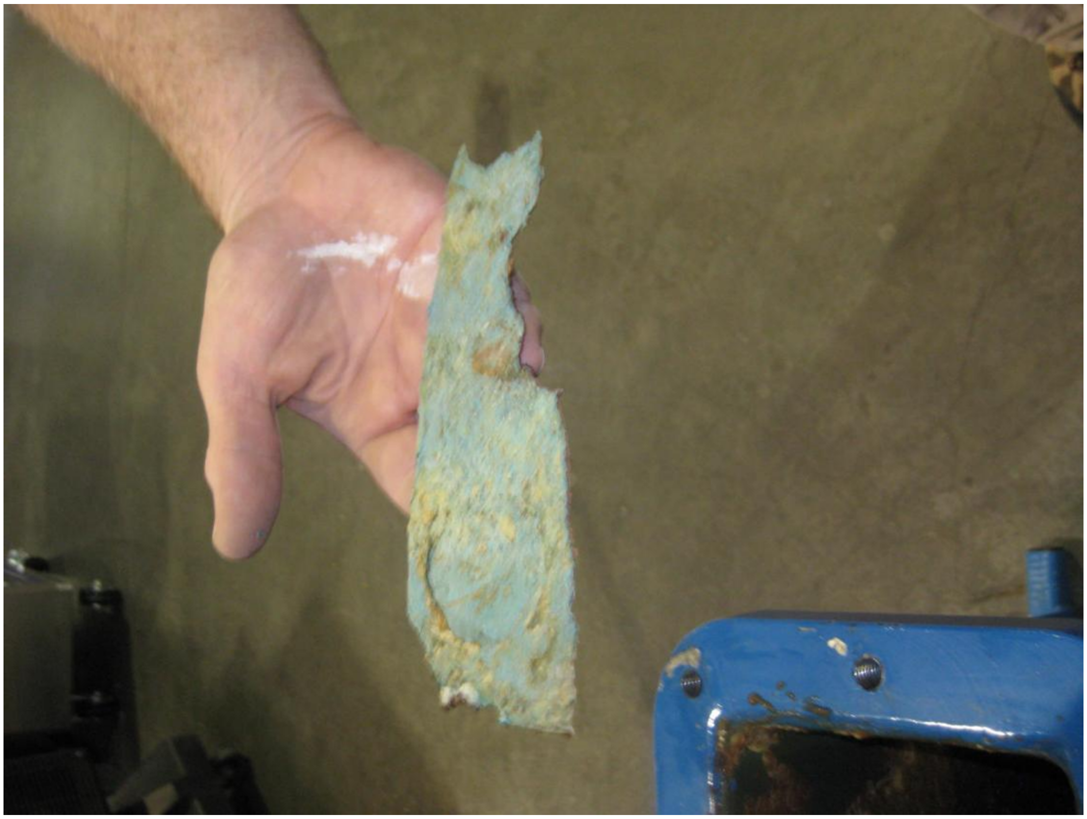

Figure 24 Piece of scale deposit from the copper electrode

4.3 Measuring Copper Ion in Raw Water

Free copper readings in raw and treated water were taken using the Hach DR-900 colorimeter using the porphyrin method. Readings of background copper in the raw water stream were taken, as were readings of the copper contained in the stream from the copper ion generator and from treated bioboxes 1 and 2.

Copper is widely distributed in water since it is a naturally occurring element. According to Moore and Ramamoorthy (1984) copper levels in river waters range from 0.6 to 400 µg/L (ppb), with a median of 10 µg/L (ppb). The EPA (2007) suggests that naturally occurring copper ranges from 0.20 to 30 µg/L (ppb) in freshwater.

The measured background level of copper in the raw water of the control biobox, ranged from 0 to 10ppb. The Hach DR900 colorimeter used has a range between 4 and 200ppb of copper with accuracy of +/- 6ppb. We periodically obtained measurements of 4ppb when measuring background levels. When samples were submitted to the two test laboratories, they generally returned non-detect levels for background of less than 3ppb.

With what would appear to be fluctuating background levels of copper, we assumed an average background level of 3-5 µg/L (ppb) when setting the treatment level of copper ion for our experimental set-up. However, given the fluctuation in the background level, the +/- 6ppb reading error of the instrument we feel that when working with this extremely low level of copper ion we can only speak about the effect of a range of concentrations rather than an absolute number.

We collected water samples from the control, test biobox 1, test biobox 2 and from the outflow of the copper ion generator all at the same time, when possible. The sample from each location was used to create three replicate 10ml samples to be measured by the Hach meter using the porphyrin method for free copper and in some instances the remaining water sample was sent to one or both of the analytical laboratories (Accutest and MEL) for analysis to verify measurements. In some instances there was good agreement between our data and the laboratory data and in some cases there was a difference of up to 10ppb. In some instances there was also a difference in results between the two laboratories (*Figure 25*). Given the detection limit of our instrument and the very low dose of copper ions we were dealing with it is advisable to consider our results within a range of concentration rather than an absolute number.

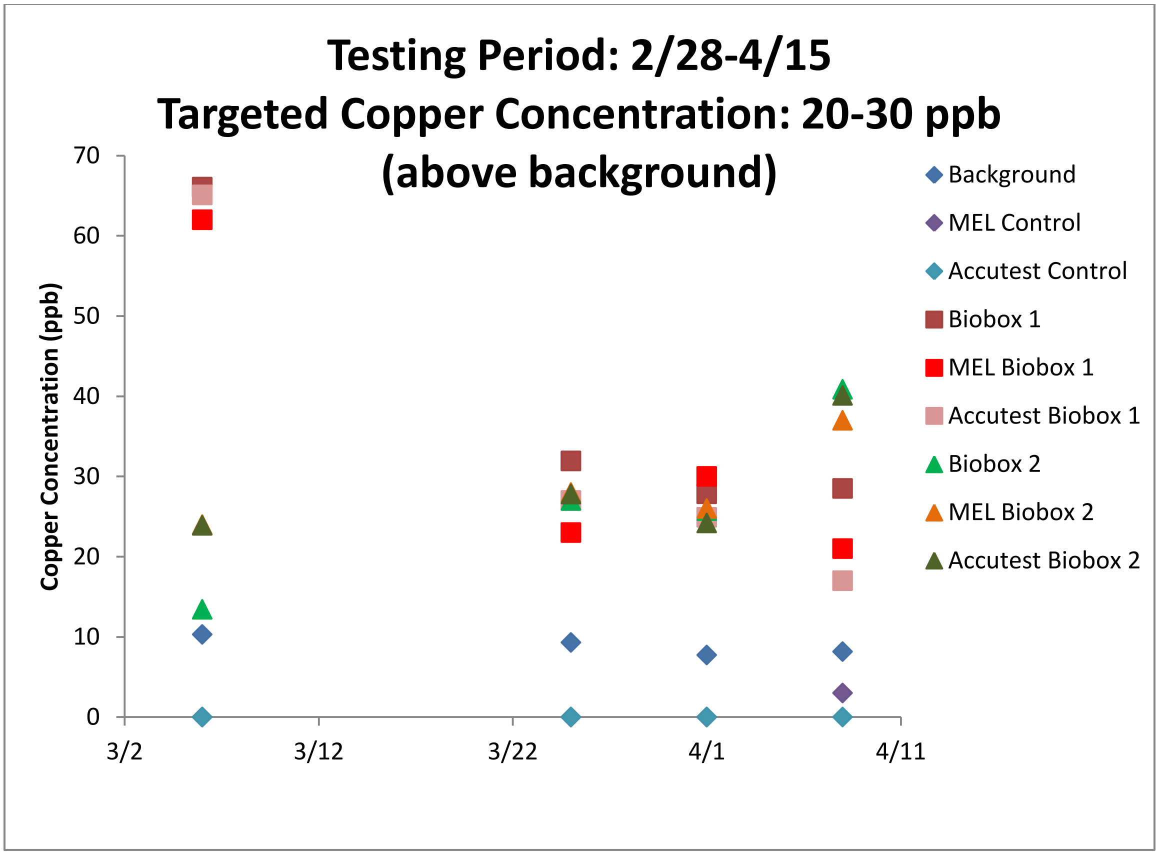

Figure 25 Comparison of Field Copper Measurement with Lab Measurements

4.4 Toxicity of Copper Ion in Raw Water

Copper is an essential micronutrient for plants and animals, but it is toxic at low concentrations to fish and other aquatic life. Cupric ion (Cu+2) is the primary toxic form of copper to algae (McKnight 1981) and to other aquatic life. Unlike other forms of copper, the cupric ion reacts with biological membranes, allowing it to pass into cells. Therefore, the concentration of the free metal ion is what determines the toxicity of copper in water. Water chemistry determines the speciation (i.e., chemical forms) of copper. Copper speciation in freshwater is examined in detail by Leckie and Davis (1979). In freshwater, the solubility of copper salts is decreased under reducing conditions and is further modified by water pH, temperature and hardness; size and density of suspended materials; rates of coagulation and sedimentation of particulates; and concentration of dissolved organics. There is a lack of knowledge on the adsorption characteristics of most cupric ion (Cu+2) complexes which contributes to uncertainties about the behavior of known copper species (Leckie and Davis 1979).

Due to the impact water quality has on copper toxicity to aquatic life, EPA's 2007 revision of the copper standard is based on the Biotic Ligand Model (BLM) and replaces the hardness-based approach to calculating the water quality criterion used previously. The BLM directly estimates the bioavailability of copper depending on the water chemistry of the water body. The model only calculates the acute water quality criterion. The chronic criterion is then calculated from an acute to chronic ratio. BLM requires ten water quality inputs for copper: temperature, pH, alkalinity, dissolved organic carbon (DOC), major cations (Ca, Mg, Na, K), and major anions (Cl, SO4). Copper is measured for use in comparing to the site-specific criteria, but it is not a required input for the BLM. The result is primarily driven by the DOC concentration.

Given the influence water chemistry has on the toxicity of the copper ion, all the results collected as part of this evaluation are primarily applicable to Colorado River water where the testing was conducted. The response of quagga mussels to the same copper ion levels may differ under different water chemistry regimes.

4.5 Prevention of Quagga Settlement by Copper Ions

In all of the six cycles of the evaluation we noted settlement in both the controls and in the treated bioboxes. However, when the copper ion concentration was in the 15 to 20 ppb (absolute) range, the settlers observed in the treated bioboxes were all very small 0.5 to 1mm (*Figure 26*), suggesting there was very little or no growth after settlement. By comparison, in the control biobox settled mussel size ranged from 0.5mm to 5mm (*Figure 27*)

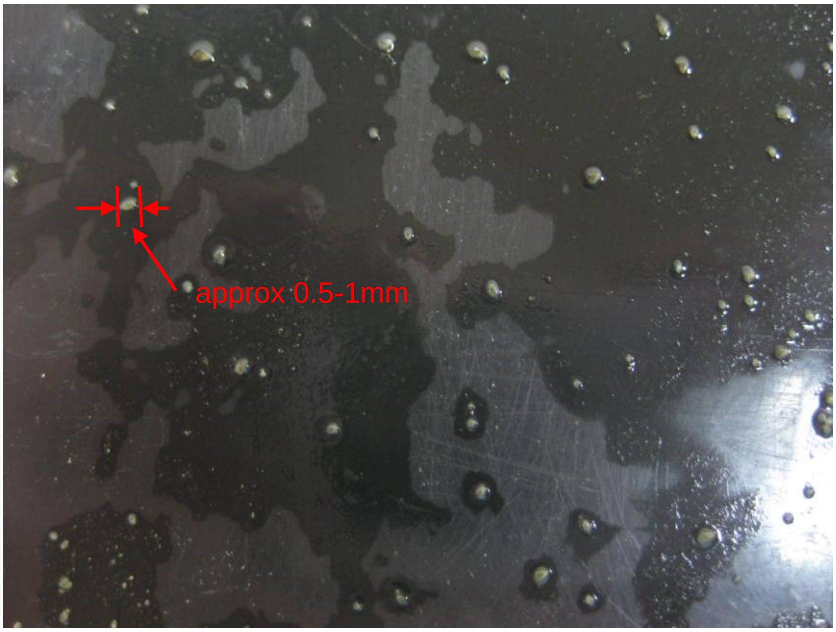

Figure 26 View of a settlement plate from copper ion treated biobox

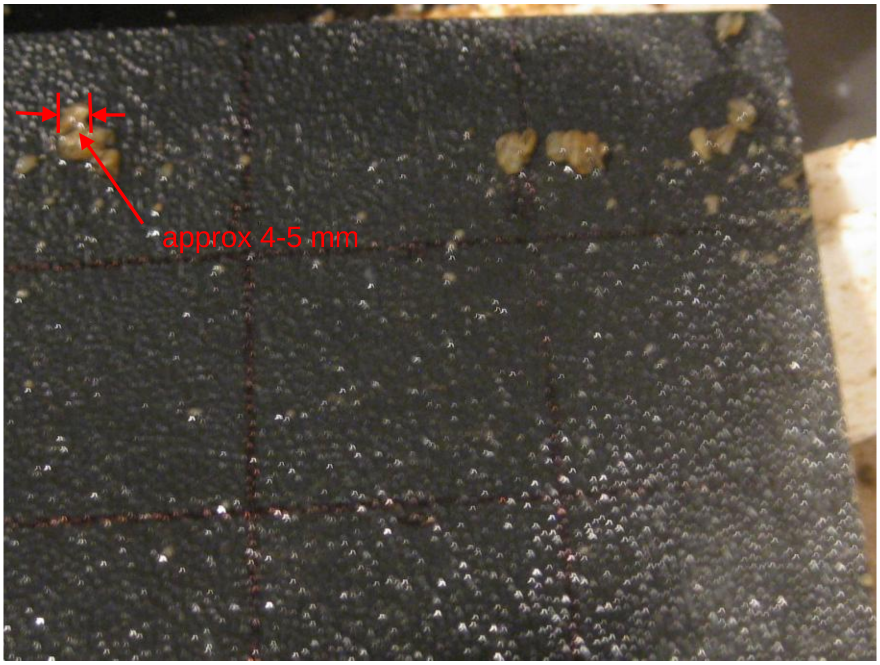

Figure 27 View of a settlement plate from the control biobox

In terms of absolute numbers, when all the settlers in the treated bioboxes were small, there was also less settlement in the treated bioboxes versus the control biobox. There was approximately 75% reduction of settlement in the treated bioboxes with treatments that had greater than 15 ppb absolute copper concentration. Reduction of settlement dropped to 38% when treatment concentrations were lower than 15 ppb absolute.

There was a considerable difference between settlement in biobox 1 situated immediately after the addition of the copper ion solution vs. biobox 2 which received treated water approximately 20 minutes after the addition of copper. With one exception, biobox 2 consistently had fewer settlers than either biobox 1 or the control. Settlement densities in biobox 2 were reduced by greater than 80% when treatment concentrations were greater than 15 ppb absolute as occurred in cycles 4, 5 and 6. However, the reduction in settlement in biobox 2 may not have been due to copper concentrations as much as to the position of biobox 2 in the treated system. In the field we have observed fouling in pipelines is always heavier at the upstream ends of the pipeline, tapering off with distance from intake. It appears that ready to settle individuals will do so immediately when encountering appropriate surface which most likely accounts for this phenomenon. With the exception of cycle 3, the observation of lower settlement in biobox 2 is generally consistent with this field experience.

The reason for placing biobox 2 approximately 20 minutes after copper ion addition was to test if copper would act swiftly on ready to settle veliger and prevent any settlement from occurring or conversely if the free copper ions would become bound up by the water chemistry leaving biobox 2 unprotected. Neither scenario was observed in any of the evaluation cycles.

4.6 Impact of Copper Ion on Captive Adult Quagga Mussels

During Cycle 3 we observed a very high mortality in the control group (same mortality as in the test groups) despite several weeks of acclimation of the adults. This may have been due to an end of season post spawning die-off and not due to exposure to the copper ion.

The mortality rate of captive adult mussels appears to have varied with copper ion concentration level, length of exposure and possibly increased temperature of ambient water. Higher copper levels did increase the rate of mortality. At levels between 24 and 40 ppb (Cycle 4) adult mussels experienced almost 90% mortality in seven weeks of exposure at ambient water temperature of 15ºC. At the five week point of this cycle they already exhibited almost 80% mortality, while during Cycle 6 at concentrations between 15 -20 ppb at temperature of 19.3 ºC the mussels reached less than 60% mortality in 5 weeks. However, it is possible that the mortality of adult mussels during Cycle 6 would have been even lower if the temperature was the same as during Cycle 4. Copper toxicity has been shown to increase with increasing temperature in a number of aquatic species; goldfish, channel catfish and trout (Smith and Heath 1979), freshwater snails (Gupta et al. 1981 and Das et al. 2012), amphipods (Levent et al. 2000), cladocerans and copepods (Boeckman and Bidwell 2005). Temperature differences during the various cycles make comparison of dose response curves for adult quagga mussels difficult.

5 CONCLUSIONS

In the lower reaches of the Colorado River the presence of copper ions in raw water between 10 and 40 ppb above background does not prevent settlement of quagga pediveligers. The veligers do not detect the presence of copper and are able to settle. The toxic effect of copper occurs after mussel settlement. The growth of settled mussels is retarded and mortality follows. When copper ions are present in levels between 15 and 20 ppb, mortality of settlers appears to begin within a few days after settlement and may begin sooner at higher copper ion concentrations.

In most instances the levels of copper measured were very similar in treated biobox1 and biobox2 suggesting there was no loss of copper ion due to binding with other elements in the water. Therefore in a typical hydraulic power plant or pumping station where the residence time of the once through cooling water is on the order of 20 minutes or less, all of the cooling water piping downstream of the copper injection point should be protected if the copper ion level is sufficiently high (15-20ppb absolute level).

Captive adults also experience mortality when copper ions are in the 15 – 20 ppb range. Mortality increases with copper level and exposure time. At higher levels of copper ion concentration, 20 to 50 ppb, the mortality of adults is higher for any particular time exposure. At levels between 24 and 40 ppb adult mussels experienced almost 90% mortality in seven weeks of exposure at ambient water temperature of 15ºC.

Given the observed mechanism of action, a continuous treatment at 20 – 25 ppb would prevent infestation from developing and eventually eliminate any adults which may be present. Equally, a periodic treatment at 40 to 50 ppb for four weeks is likely to cause mortality of all juveniles and adults present in the system, particularly during warmer weather.

The copper ion generator performance was significantly degraded from its manufacturer-claimed design operating capacity probably due to accumulation of deposits on the electrodes that could not be detected by the instrumentation on the generator.

The persistent instability of the copper ion stream being added to the treated system, the experimental error in the measurement of copper ion at the extremely low level used and the changing temperature during the experiment precluded the possibility of constructing a dose response curve for the adult quagga mussels.

While copper ion is an effective molluscicide even at very low levels, the copper ion generator, in its present form, does not appear to be a practical means for mussel control in an industrial setting.

6 RECOMMENDATIONS

Based on the results of the evaluation of a copper ion generator, the following recommendations are offered:

1. Repeat this copper toxicity evaluation using an alternative source of copper ions such as the addition of a copper algaecide (e.g., EarthTec or equivalent) to verify the dose findings in this evaluation. We believe that in an industrial setting, it would be easier to add a precise amount of copper to the treated stream using a liquid source of copper ions with stable copper concentration and thereby avoid the instability issues observed with the ion generator method of using copper as a treatment. The predicted costs of a liquid chemical vs. electrolysis approach are similar with a small advantage in favor of the electrolysis approach (EarthTec at $7.20 per Mgal and Copper Ion generation at $6.60 per Mgal both at 30 ppb copper ion concentration).
2. Verify that the response of zebra mussels is identical to the response of quagga mussels under similar environmental conditions using a liquid form of copper ion.
3. Verify the response of quagga mussels in waters with lower calcium level which would increase the toxicity of the copper ions, perhaps achieving control of settlement and adult mortality at lower copper ion levels then found in this report.
4. Any copper ion generator being proposed for commercial or industrial use should include provision for the following:
    a. An automatic on board alarm when electrodes are deteriorated and not able to produce the design concentration. The existing current and voltage meters do not provide an indication of deteriorating performance.
    b. The lid of the ion chamber is difficult to remove. Consider incorporating a threaded jack bolt on the generator housing flange.
    c. Provide a method of cleaning the electrodes of deposits without opening the generator. Consider a clean in place (CIP) circuit, mechanical wipers or soften the water that is used in the ion generator.
    d. The current requires daily attendance to confirm current level and make any adjustments. This is an operational nuisance. Consider an automatic current regulator for this equipment.

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RAW DATA

ADULT MORTALITY RAW DATA											
	Control			Front Biobox			End Biobox				
	Live	Dead		Live	Dead		Live	Dead		Temp	Avg Cu
9/20/2013	100	0		100	0		100	0			
9/24/2013											
9/25/2013											
9/30/2013											
10/1/2013											
10/8/2013											
10/9/2013											
10/29/2013											
10/30/2013	98	2		4	96		12	88			
11/6/2013	102	0		103	0		102	0			
11/11/2013											
11/12/2013											
11/26/2013											
11/27/2013											
12/3/2013	93	9		89	14		90	12			
1/3/2014	103	0		103	0		107	0			
1/8/2014											
1/9/2014											
1/14/2014											
1/15/2014											
1/21/2014											
1/22/2014											
1/28/2014											
1/29/2014											
2/3/2014	63	40		63	40		71	36			
2/28/2014	76	0	0.00	103	0	0.00	100	0	0.00		30
3/6/2014		5	6.58		8	7.77		36	36.00	14.5	30
3/11/2014		4	11.84		16	23.30	34	30	66.00	14.4	30
3/17/2014		0	11.84		26	48.54		10	76.00	14.7	30
3/25/2014		0	11.84		16	64.08		3	79.00	15.5	30

4/1/2014		5	18.42		15	78.64		1	80.00	14.9	30
4/8/2014		6	26.32		5	83.50		0	80.00	16.1	30
4/15/2014		1	27.63		8	91.26		4	84.00	16.1	30
4/15/2014	75	21		9	94		16	72			
4/16/2014	109	0	0.00	97	0	0.00	103	0	0.00		20
4/23/2014			0.00			0.00			0.00	16.3	20
4/29/2014		17	15.60		19	19.59		14	13.59	17.4	20
5/7/2014		2	17.43		18	38.14		6	19.42	17.4	20
5/13/2014		1	18.35		2	40.21		0	19.42	17.5	20
5/13/2014	89	20		58	39		83	20			20
5/14/2014	109	0	0.00	105	0	0.00	103	0	0.00		15
5/20/2014		3	2.75		19	18.10		30	29.13	19.5	15
5/28/2014		4	6.42		19	36.19		11	39.81	19.4	15
6/3/2014		2	8.26		12	47.62		12	51.46	19.5	15
6/9/2014		0	8.26		7	54.29		2	53.40	19.4	15
6/9/2014	100	9		48	57		48	55			15

	MUSSEL SETTLEMENT RAW DATA							
Target Concentration			13-15ppb	5-10ppb	13-15ppb	20-30ppb	15-20ppb	10-15ppb
		Start	9/20/2013	11/6/2013	1/3/2014	2/28/2014	4/16/2014	5/14/2014
		End	10/30/2013	12/3/2013	2/3/2014	4/15/2014	5/13/2014	6/9/2014
			Total	Total	Total	Total	Total	Total
Control Biobox	Front Plate	Front (inlet side):	109	89	110	89	49	109
		Back (outlet side):	199	122	138	85	31	199
	Middle Plate	Front (inlet side):	1845	432	154	110	169	1845
		Back (outlet side):	305	311	145	622	206	305
	Back Plate	Front (inlet side):	368	243	108	26	13	368
		Back (outlet side):	120	233	180	102	26	120
	Small Plate: 1A	Rough Side:	1342	187	108	78	43	1342
		Smooth Side:	378	221	117	50	26	378
	Small Plate: 1B	Rough Side:	1086	243	112	53	105	1086
		Smooth Side:	785	167	102	60	75	785
	Small Plate: 1C	Rough Side:	474	354				474
		Smooth Side:	222	233				222
	Small Plate: 1D	Rough Side:	462	206				462
		Smooth Side:	390	189				390
	Adult mortality		0.02	0.0882353	0.38835	0.21875	0.183486	0.090909
			98l 2d	93l 9d	63L 40D	75L 21D	89L 20D	100L 10D

Biobox 1	Front Plate	Front (inlet side):	142	232	196	70	14	142
		Back (outlet side):	149	483	209	50	6	149
	Middle Plate	Front (inlet side):	1193	211	162	45	33	1193
		Back (outlet side):	174	117	158	36	7	174
	Back Plate	Front (inlet side):	320	127	173	20	17	320
		Back (outlet side):	128	259	197	43	8	128
	Small Plate: 1A	Rough Side:	553	238	111	0	35	558
		Smooth Side:	390	390	90	20	18	390
	Small Plate: 1B	Rough Side:	456	221	115	12	47	456
		Smooth Side:	342	270	114	0	8	342
	Small Plate: 1C	Rough Side:	408	235	92			408
		Smooth Side:	150	291	128			150
	Small Plate: 1D	Rough Side:	348	267	189			348
		Smooth Side:	234	166	148			234
	Adult mortality		0.96	0.1359223	0.38835	0.9370629	0.402062	0.551402
			4 l 96d	89 l 14d	63L 40D	9L 134D	58L 39D	48L 59D
Biobox 2	Front Plate	Front (inlet side):	51	86	181	4	14	51
		Back (outlet side):	59	215	202	5	10	59
	Middle Plate	Front (inlet side):						
		Back (outlet side):						
	Back Plate	Front (inlet side):	56	245	189	3	4	56
		Back (outlet side):	40	90	206	5	6	40
	Small Plate: 1A	Rough Side:	68	83	147	0	1	68
		Smooth Side:	52	85	153	1	0	52
	Small Plate: 1B	Rough Side:	13	43	165	1	4	13
		Smooth Side:	46	115	158	0	3	46
	Small Plate: 1C	Rough Side:		30			18	
		Smooth Side:		68			56	
	Small Plate: 1D	Rough Side:		41			26	
		Smooth Side:		51			8	
	Adult mortality		0.88	0.1176471	0.336449	0.8181818	0.194175	0.54717
			12l 88d	90l 12d	71L 36d	16L 72d	83L 20d	48L 58D
					unit went off line system overdosed 2/3/2014 60ppb in Biobox1	settlement visible on treated not growing presumed dead	settlement visible on treated not growing presumed dead	