Demonstration of Biweekly Zequanox Treatments to Control Invasive Mussel Populations at Hoover Dam

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1.0 Executive Summary

Marrone Bio Innovations, Inc. (MBI) conducted a Zequanox® demonstration in a cooling water system at Hoover Dam, Nevada, USA during October 2013–January 2014. The series of biweekly (every 2 weeks) Zequanox treatments targeted incoming settling mussels in the system and successfully controlled the mussel settlement, preventing more than 85% of the incoming mussels from settling and growing in the system. A bonus of the treatment, in addition to effectively controlling growth of new mussels in the system, more than one third (36%) of the adult mussels already living within the system were eliminated during the 4 months of treatment.

The biweekly Zequanox treatment strategy over the course of one year uses approximately one half of the product required for a single annual treatment in a flowing system, and therefore costs significantly less for customers who require flowing treatments (as opposed to static treatments). In addition, the biweekly Zequanox treatment strategy requires little space and limited effort to complete, and requires no changes to the cooling water system.

2.0 Introduction

Freshwater quagga and zebra mussels (*Dreissenia bugensis* and *Dreissenia rostiformus*) are invasive species native to Eastern Europe. The species spread throughout Europe and into North America, first infesting the Great Lakes, then the Mississippi River. Since 2007, dreissenids have spread into the Western United States, infesting the lower sections of the Colorado River. These prolific organisms travel through infested waters as planktonic larvae, and then attach and grow to adulthood on any hard surface, including the interior of plumbing systems. Once the mussels attach and grow in these systems, flow is inhibited, causing impacts on system functionality.

Along the Lower Colorado River, a number of dams were built during the 1900s and are now managed by the U.S. Bureau of Reclamation (Reclamation). These dams manage and control usage of the waters in the Colorado River and produce hydroelectric power. Many systems within these dams, such as cooling water systems and fire suppression systems, use raw water for various services. Controlling the populations of mussels that grow and occlude these systems is vital to the systems' continued function, and Reclamation has cooperated with various companies and consultants to conduct trials evaluating the effectiveness of various methods for controlling the mussel populations within its facilities.

Cooling water systems at Hoover Dam combine high-pressure water from the penstocks with low-pressure water from the tailwaters downstream of the dam. Unit AZ1, which was used for the Zequanox demonstration, is configured differently, with water supplied only from the tailwaters with a centrifugal pump. Reclamation made this configuration change in 2010 to reduce the flow rate and water pressure through the cooling water system so that various equipment for testing of mussel control methods could be installed and evaluated. After reconfiguration, it was found that drawing cooling water from only the tailwaters had additional benefits:

- It decreased the entrainment of penstock mussel debris (shells from mussels growing on the walls of the penstocks) in the cooling water system (the largest problem in the cooling water systems at Hoover Dam).
- It reduced the use of headwaters from the lake for cooling water (an important cost benefit given decreasing stored water in the lake due to drought and increased use).
- The high pressure water eductors created high decibel levels that could cause hearing damage. By using the tailwater pump, the noise level was significantly reduced providing protection to the workers hearing.

The benefits of this configuration change are significant enough that Hoover Dam management has planned to change all cooling water system configurations to match that of unit AZ1 in the coming years. Reclamation also has interest in testing Zequanox for use as the backup or secondary control tool if the reconfiguration does not adequately control the mussel macrofouling issues at Hoover Dam.

Zequanox is a biopesticide that controls quagga and zebra mussels with demonstrated specificity to the target organisms. Zequanox does not have the same toxicity to non-target organisms (including human applicators) that alternative chemical mussel control options have, thus Zequanox mitigates the environmental and human health risks associated with chemical options. Zequanox is composed of dead cells of a naturally occurring microbe (*Pseudomonas fluorescens)*, and is perceived as a nonthreatening food source. Zebra and quagga mussels readily consume the product along with their normal phytoplankton diet. Once ingested, Zequanox deteriorates the mussel's digestive lining, causing death.

In this demonstration, Zequanox (EPA Reg. No.: 84059-15), was applied biweekly for 4 months, from October 2013 through January 2014. While past demonstrations at Davis Dam, a nearby Reclamation facility, have demonstrated the efficacy of Zequanox at controlling adult mussel populations with single, high-concentration treatments annually (Link 2012), the objective of this treatment series was to demonstrate Zequanox efficacy at controlling and reducing the population of mussels settling within a cooling water system using low-concentration Zequanox treatments every 2 weeks. The biweekly treatment program offers different benefits than the Zequanox annual adult treatments.

The biweekly program minimizes the size of equipment and amount of product used for each treatment, which in turn minimizes the overall impact on facility operations. Most significantly, the biweekly treatments reduce the total amount of product needed annually by approximately one half when compared to the single annual adult treatments, which drastically reduces the overall annual cost to treat and protect a system from mussel problems. For the treatment at Hoover Dam discussed in this report, biweekly treatments (conducted year-round) would reduce the cost to $30,000 a year from $55,000 (for a single annual treatment). If Hoover Dam did not have year round mussel settlement, such as is found in the Great Lakes region, the treatments could be reduced to just the months when settlement occurs, reducing the cost further to approximately $15,000 a year for biweekly treatments. This report describes the results of these demonstration biweekly treatments, evaluating the ability of Zequanox to reduce the population of mussels settling and growing within the system.

3.0 Methods

The general method for these treatments was to apply Zequanox throughout a cooling water system, monitor the treatments, and determine their effectiveness by comparing mussel density and mortality at both treated and untreated points within the system. MBI field scientists injected the cooling water system with Zequanox at 15 milligrams active ingredient per liter (mg a.i./L) for approximately 3.5 hours at 14-day intervals. The number of planned treatments (10) was reduced due to system maintenance; 8 treatments were conducted. During the Zequanox demonstration, the treated cooling water system maintained an approximate flow rate of 1,100 gallons per minute (gpm).

Scientists monitored the density of newly settling mussels and the mortality of adult mussels in tanks (bioboxes) receiving water from the cooling water system. For comparison, the setup (Figure 1) included bioboxes that received water from upstream of the treatment application point (untreated control) and bioboxes downstream of the treatment application point (treated). Effects observed in the bioboxes were a surrogate for those occurring within the cooling water system (See Section 3.3). Comparison of the mussel densities and mortality between the two biobox locations was used to determine the percent reduction in populations of newly settling and established adults, respectively, caused by the Zequanox treatments.

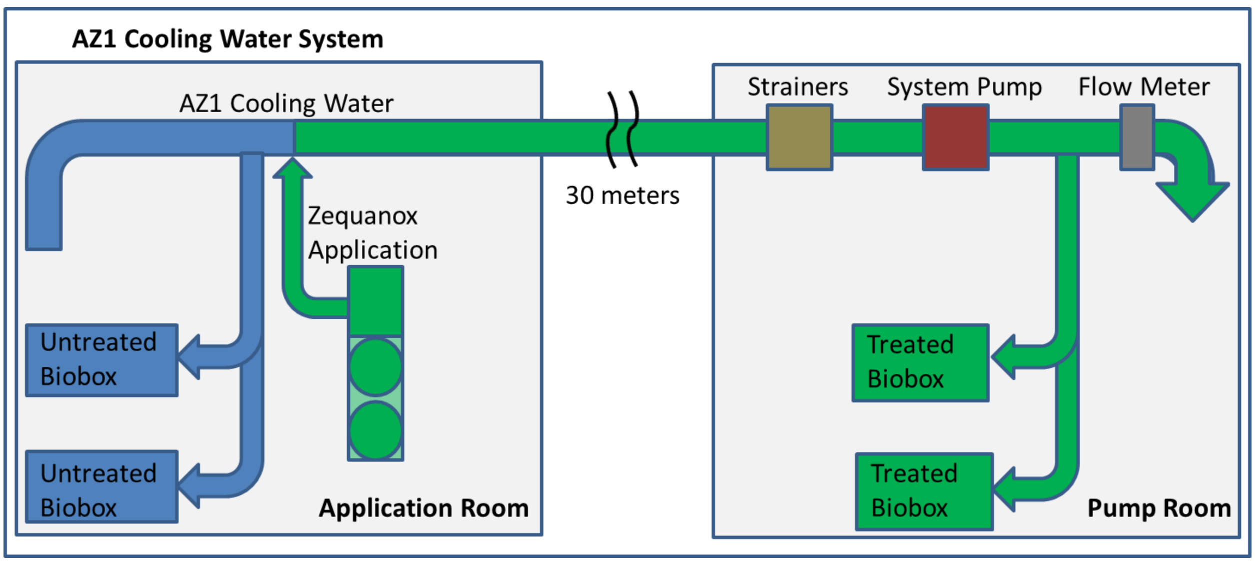


Figure 1. Diagram of AZ1 Cooling Water System at Hoover Dam as Configured during the Zequanox Demonstration

3.1 Equipment Setup

As is noted above, equipment for the Zequanox demonstration included bioboxes upstream (untreated) and downstream (treated) of the Zequanox injection site (Figure 1). The Zequanox application system consisted of two 50-gallon mixing tanks and a small skid with a peristaltic pump and calibration equipment. The pump was connected by a flexible tube to the cooling water system at an existing port with valve (Figure 2). The application system footprint was approximately 3 x 10 feet and was designed to minimize the impact on operations within the facility, requiring only that nearby equipment storage shelves be relocated temporarily.

Figure 2. Zequanox Application System. Product injection occurs where flexible tube connects to port on blue pipe at center right in the photo.

3.2 Application Methods and Monitoring

Prior to application, Zequanox concentration targets were calculated based on a dry cell weight of the active ingredient using standard laboratory techniques. Zequanox (a wettable powder) was mixed with system water in the mixing tanks (Figure 2) to create a concentrated liquid solution for application through the peristaltic pump.

The treatment parameters were as follows:

- <u>Flow Rate</u> - Flow through the cooling water system was approximately 1,100 gpm. Prior to each treatment, the flow rate was checked on the Siemens flow meter in the pump room. (Figure 1)
- <u>Application</u> - Product was applied constantly to reach treatment concentrations. At the injection point (Figure 2) Zequanox was metered into the system using a peristaltic injection pump. Field scientists checked the injection rates using a calibration column contained on the skid and a timer.
- <u>Treatment Monitoring</u> - To monitor the concentration of Zequanox in the water during a treatment, MBI relies on the correlation (a linear relationship) between turbidity and Zequanox concentration (Appendix 7.1). MBI field scientists determined a site-specific target turbidity for Zequanox measured in mg a.i./L, and turbidity measured in nephelometric turbidity units using water from the bioboxes at Hoover Dam. During treatments, MBI monitored the application by sampling water in the treated bioboxes and measuring the turbidity in accordance with MBI Standard Operating Procedure (SOP)#: MBI-RD-4008-SOP Turbidity and MOI-401 Active Ingredient Correlation and Application and Monitoring Procedure.

Zequanox label usage rates for control of juvenile mussels (mussels settling and growing in the pipes) allow for treatments of up to 50 mg a.i./L for 8 hours every 2 weeks. For many facilities, effective control can be obtained at lower concentrations and with shorter treatment times. For this demonstration at Hoover Dam the application target concentration was 15 mg a.i./L with an acceptable range between 12 and 18 mg a.i./L, and the target treatment time was 3.5

hours, with an acceptable range between 3 and 4 hours. All product used was labeled Zequanox (EPA Reg. No.: 84059-15) product (i.e., no experimental formulations were used) and all treatments were conducted under a permit issued by the state of Nevada Department of Agriculture.

3.3 Efficacy Monitoring

The cooling water subsystem was not accessible for direct observation of the mussels within it. The efficacy of Zequanox applications was therefore determined by monitoring the settlement of mussels on sample plates and the mortality of a sample population of mussels held in bioboxes within the facility. Bioboxes (Figure 3) are modified, flow-through tanks that are plumbed to receive a small, continuous stream of the water in a treated system. Because the treated mussels in a biobox are exposed to water with the same properties as the water in the treated system, observed mussel settlement and mortality in the bioboxes is considered the closest estimate of the mussel behavior inside the treated system.

For this demonstration, four bioboxes were used: two upstream of the application site (control untreated) and two downstream of the application site (treated). All bioboxes received a constant flow rate of 3 gpm, checked and adjusted for each treatment. The bioboxes contained settlement plates for assessment of settlement (juvenile mussels) and containment tubes for mortality monitoring of adult mussels (Figure 3).

Figure 3. Monitoring Biobox. Visible in the biobox are three trays of settlement sample plates (one in the field scientist's hand) and two adult mussel containment tubes (of the three used in each biobox).

3.3.1 Assessment of Settlement

Field scientists performed assessment of the mussel densities in the untreated portion of the system (just upstream of application/injection) and the treated portion of the system 2 weeks after each treatment. Assessment included observation and measurement of mussel densities on settlement plates, which are 10 x 10 centimeter (cm) pieces of ABS (thermoplastic) spaced 1 cm apart in an acrylic tray, with up to eight plates held in each tray.

The equipment included 48 settlement plates upstream and 48 plates downstream, split evenly into two bioboxes (24 in each). The plates were monitored for the full length of the demonstration. More plates were monitored initially—when densities were low—than later in the demonstration, due to the increased time required to check each plate as densities increased, as described below.

3.3.1.1 Visual Analysis of Settlement

At the beginning of the demonstration, mussels growing on each plate were counted by visually inspecting the back of each settlement plate and recording the number of observed mussels (Figure 4). Each plate was removed from the biobox and visually inspected within a 2-minute time limit before it was placed back into the biobox. The time limit was used both for consistency and to prevent the plate from drying out.

Figure 4. MBI Field Scientist Catherine Bagley holds a settlement plate by the corners to minimize damage to mussels during analysis.

3.3.1.2 Digital Microscope Analysis of Settlement

Once densities on the untreated plates reached around 300 mussels per plate, the density could no longer be accurately inspected within 2 minutes and it was difficult to distinguish between mussels. A microscope was used thereafter. A Dinolite digital microscope and a laptop computer with DinoCapture 2.0 software provided the ability to quickly take magnified photos of sample sections of the plates. The photographs were analyzed later to determine the mussel density in each image. To locate consistent sample sections on each plate, a grid was placed over each plate before taking the picture. For each assessment, the photos included eight sections of the back of each plate: four at the top of the plate and four mid-way down the plate (Figure 5). When the assessment methods were switched, both methods were used to analyze the plates on the same day. The resulting correlation in densities was approximately direct (Appendix 7.2); therefore, no data transformation was necessary for comparison of densities determined by the two methods.

Figure 5. An MBI field scientist (at left) uses the digital microscope method to collect photos of settlement plates (example at right).

3.3.2 Adult Mussel Mortality Assessment

Adult mussel population monitoring was conducted by placing adult mussels (approximately 15 mm in length) in tube enclosures (Figure 6) within the untreated and treated bioboxes, and monitoring mortality of these mussels throughout the demonstration. Fifty mussels were placed in each tube, and the tube was capped with mesh on both ends to allow for water to travel through the tube. Three tube enclosures were placed in each biobox, for a total of 300 untreated adult mussels and 300 treated adult mussels. Two weeks after each treatment, the adult mussels were evaluated for viability. Gaping mussels that did not respond to stimuli with a finger were identified as dead. Dead mussels were counted and removed; live mussels were counted and returned to the tube enclosure and appropriate biobox.

Figure 6. Adult Quagga Mussels in the Foreground and a Containment Tube in the Background.

3.3.3 Visual/Photographic Monitoring

Visual monitoring of the waters downstream was conducted during and after each treatment. The objective of the visual monitoring of the waters was to identify any visual indications/changes associated with the treatment. Representative photographs are presented in the results (Figures 10–13 in Section 4.2).

To provide additional qualitative information, pictures (taken by Reclamation staff) of a strainer basket on the treated unit are included in the results. The strainer location is such that it captures mussel debris from within the cooling water system that had been treated with Zequanox. MBI took additional pictures after clean-out of the same strainer on a different day to show mussel debris in more detail. Figures 14–15 in Section 4.2 show the mussel debris.

4.0 Results

Below are the results of treatment monitoring, assessment of treatment impact on the population of settling mussels and adult mussels already resident in the system, and visual monitoring of the downstream water quality and the cooling water strainer system.

4.1 Treatment Monitoring

Target treatment concentrations and treatment durations were met satisfactorily throughout the demonstration (Table 1). All treatments were at target turbidity concentrations of approximately 15 mg a.i./L, and lasted approximately 3.5 hours. Water temperature data for the treatment days was recorded to provide a reference temperature range for the treatment efficacy data.

Table 1. Hoover Dam Zequanox Treatment Parameter Measurements

Date of Treatment	Temperature (°C)	Average Treatment Concentration (mg a.i./L)	Treatment Duration (hours)
10/10/2013	17	16.6	3.8
10/25/2013	17	13.2	3.8
11/8/2013	16	14.4	3.5
11/22/2013	16	12.6	3.4
12/5/2013	14	14.7	3.5
12/20/2013	12	17.0	4.0
1/3/2014	11	13.7	4.0
1/17/2014	11	13.5	4.0
Average	**14**	**14.5**	**3.8**

mg a.i./L = milligrams active ingredient per liter

4.2 Assessment of Settling Mussel Population

The average densities of settled mussels on the untreated sample plates indicate that two significant waves of mussel settlement occurred during the treatment series (Figure 7). Ongoing mussel settlement occurred from commencement of monitoring in early October through mid-December, then appears to have decreased for approximately one month, and then began increasing again from mid-January to the end of January when the demonstration was completed. Veliger (larvae) densities in the water were not monitored during the demonstration, but ongoing settlement year round is observed in the Lower Colorado River.

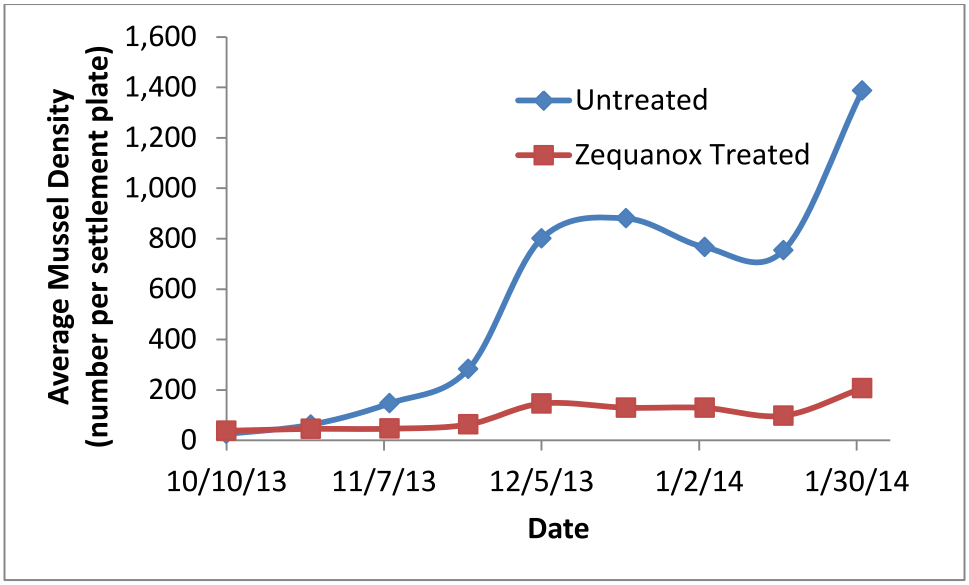


Figure 7. Average Mussel Densities on Treated and Untreated Settlement Plates during the Zequanox Demonstration.

The level of control, indicated by the percent reduction (calculated as [untreated density - treated density]/untreated density) in the settled mussel population, increased during the first four treatments, and then maintained a level of control of approximately 85% for the remaining treatments (Table 2 and Figure 8).

Table 2. Percent Reduction in Mussel Settlement by Treatment

Number of Zequanox Treatments	Percent Reduction in Mussel Settlement
0	-51.5
1	26.8
2	68.6
3	77.7
4	81.8
5	85.3
6	83.2
7	87.0
8	85.1
Average after Treatment 4	**85.1**

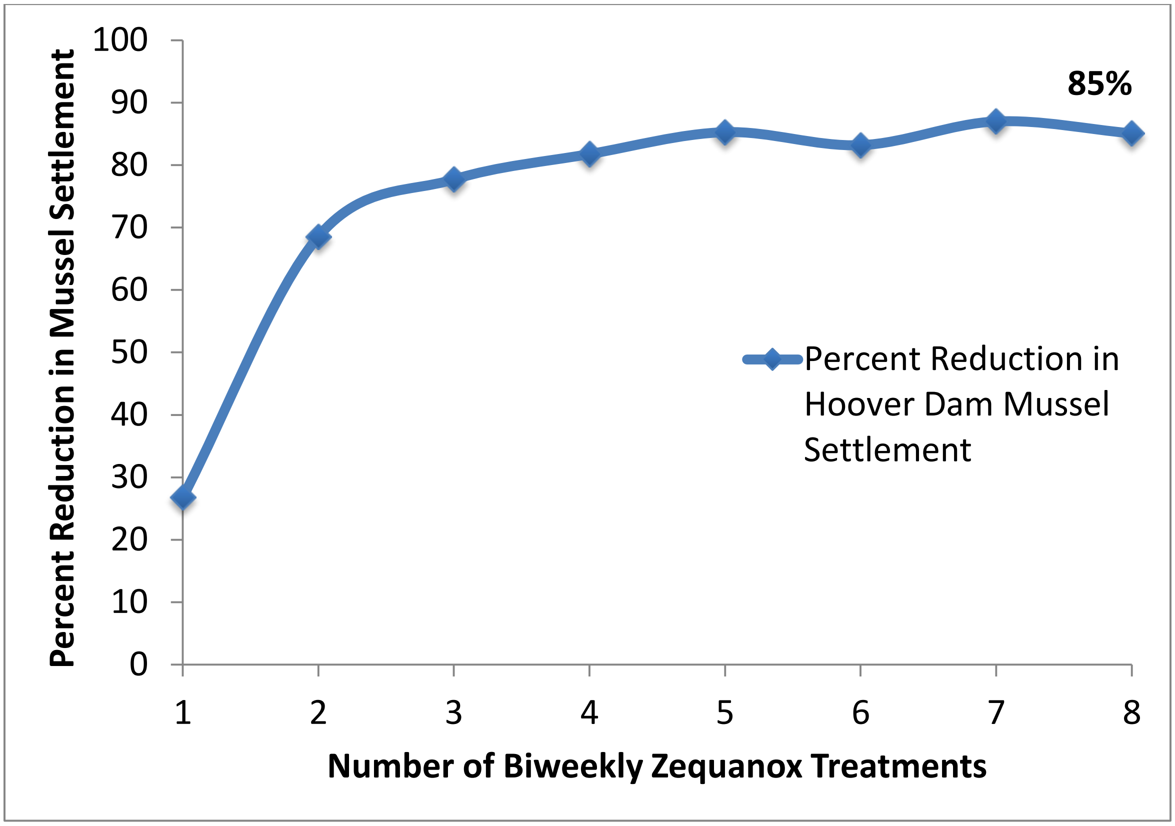


Figure 8. Percent Reduction in Mussel Settlement in Hoover Dam Cooling System Unit during Biweekly Zequanox Treatment

4.3 Assessment of Adult Mussel Population

The adult mussel population within the Hoover cooling water system experienced 36% mortality during the course of the demonstration (Figure 9). This mortality occurred gradually during the series of treatments, and was still increasing with each treatment at the end of the demonstration. Gradual mortality of the existing adult mussels within a system is ideal for facilities, as it immediately begins improving the flow of water through a system, and thereby its function. At the same time, the debris associated with the mussels as they detach from inside the pipes is gradual enough to be managed by facility staff. This result is in contrast to methods of mussel treatment that do not affect the resident adult mussel population at all, and methods that affect a large percentage of the population at once, creating large debris slugs, which can cause severe blockages and shutdowns.

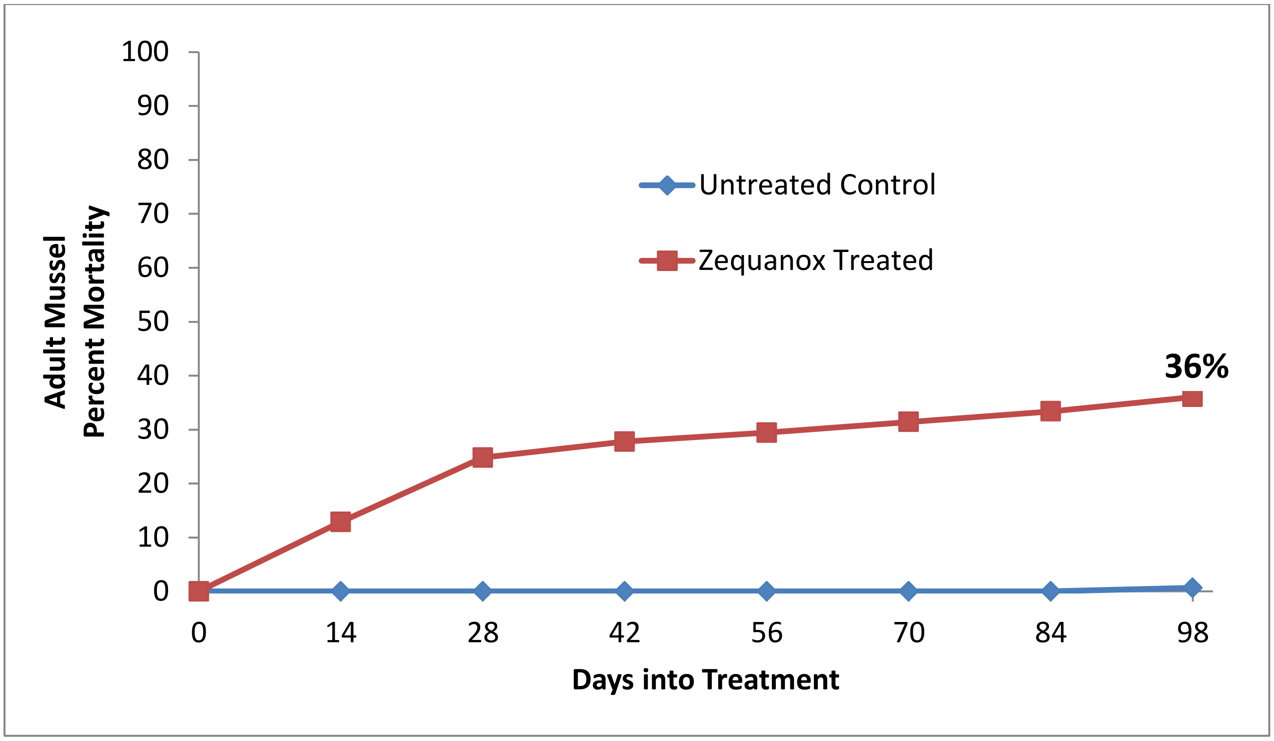


Figure 9. Adult Mussel Mortality in Hoover Dam Cooling System Unit during Biweekly Zequanox Treatments

4.4 Visual/Photographic Monitoring

During each Zequanox treatment, water quality was checked visually at the Hoover Dam tailrace, where the water from the treated cooling system discharges into the tailwaters exiting the dam. While other untreated systems have white bubbles associated with their discharge into the tailrace (Figure 10), some white foam was visible adjacent to the discharge location of the Zequanox-treated system during each treatment (Figure 11). This foam always dissipated before the water left the tailrace area (Figure 12), and did not remain after treatment (Figure 13). Foam residue did not persist in waters exiting the Dam property, nor persist after the treatments. MBI does offer environmentally safe products that can be mixed with Zequanox to reduce foaming. Verbal communication with the Hoover Dam staff indicated that approximately 1.5 weeks after the first treatment, during a system shutdown and restart, additional white material was observed in the tailrace adjacent to the treated system discharge; however, no explanation of how Zequanox could have remained in the system, or confirmation that the material was Zequanox, could be made. Later shutdowns and restarts of the treated system occurred during the demonstration, but no additional reports of changes in downstream water quality were reported.

Figure 10. Discharge Water Exiting Hoover Dam from a Unit Not Treated with Zequanox. Visible bubbles from an untreated unit visible as white area in the water just left of center in the photo.

Figure 11. Discharge Water Exiting Hoover Dam during a Zequanox Treatment. Foam visible at center of image is adjacent to discharge of cooling water system treated with Zequanox.

Figure 12. View from Hoover Dam Tailrace towards Downstream during a Zequanox Treatment. Small amount of foam is visible in the bottom left corner; no foam is visible downstream as water exits Hoover Dam tailrace.

Figure 13. Post Treatment Picture of Tailrace Taken from the Top of Hoover Dam. Bubbles are visible adjacent to motoring units (untreated) at center of picture. No foam residue visible from the recently completed Zequanox treatment.

Coarse strainers are cooling water system devices that direct flow through strainer baskets to remove debris before the water enters pumps or other equipment which may be damaged by the debris. The facility does not have a schedule for strainer cleaning; rather, the strainers have associated pressure meters that alert maintenance staff when the strainers may be blocked. In late October, maintenance staff notified the Zequanox treatment coordinator at Hoover Dam that strainer cleanout had been required recently on the Zequanox-treated system, and that mussel debris (and a small fish) had been found in the baskets (Figure 14). While it was not unusual to find this kind of debris in the baskets, the maintenance staff had never before found the mussel debris to be to an extent that suggested notification of others. The image of the basket shows that while the walls of the basket are somewhat occluded by mussel shells, the basket is not full of mussel debris. This manageable amount of mussel debris may be contrasted with the large slugs of debris that would occur with other adult mussel control methods, which could completely block flow through the strainer. MBI staff was not present on the day of the strainer cleanout in late October, but was able to document the debris removed from the strainer (Figure 15) in early December shortly after another strainer cleanout.

Figure 14. Strainer Basket that Required Cleanout on October 23, 2014. The baskets walls are covered in mussel shells in the image, while the small fish sits in the bottom of the cylindrical strainer basket. Credit Kevin Zito, Reclamation.

Figure 15. Mussels Removed from a Strainer Basket during Cleanout at Hoover Dam on December 5, 2013.

5.0 Conclusions

Biweekly Zequanox treatments successfully controlled mussel settlement, preventing more than 85% of the mussels settling in the system from remaining and growing in the system. In addition, more than one third (36%) of the adult mussels already living within the system were eliminated during the 4 months of treatment. Considering these impacts on the resident population and the newly settling population of mussels, the Zequanox treatment series demonstrated that control of invasive mussels within cooling water systems can be obtained with minimal space, no system alterations, and minimal debris management/impact on system function, at half the cost of annual adult treatments in flowing systems.

For this demonstration, MBI conducted all treatments, and coordinated all permits and licensing. This removed the need for Reclamation personnel to conduct the treatments, Reclamation only needed to arrange the security clearance and access to the facility for each treatment. Alternate arrangements can be made with customers based on their unique needs, such as training of their staff on how to use a turn-key self-automated treatment system, requiring only a couple minutes of an operator's time to complete each treatment, allowing a customer to complete treatments easily on their own.

6.0 References

Link, C. 2012. Davis Dam 2011 Facility Treatment Report.

Rackl, S., B. Gruber. 2011. MBI-RD-4008-SOP Turbidity and MOI-401 Active Ingredient Correlation and Application and Monitoring Procedure.

7.0 Appendices

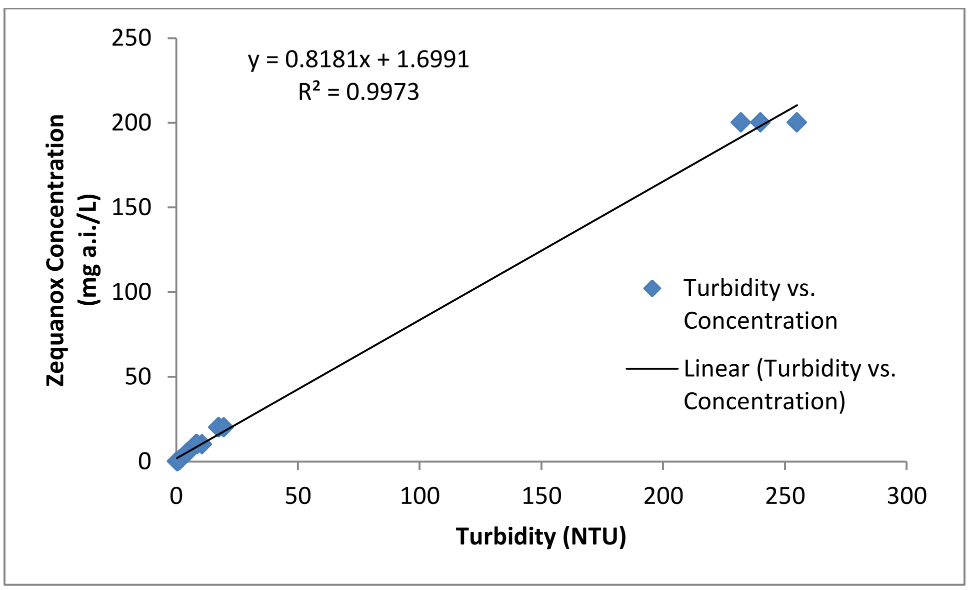
7.1 Turbidity Correlation

Presented here is the correlation completed in accordance with MBI-RD-4008-SOP Turbidity and MOI-401 Active Ingredient Correlation and Application and Monitoring Procedure. The Zequanox concentration and the turbidity of the treated water are strongly correlated (Table 3. and Figure 16). Turbidity is monitored during Zequanox treatments and the concentration is calculated using the equation of the trend line (Figure 16).

Table 3. Raw Data for Correlation between Zequanox Concentration and Turbidity

Zequanox Concentration (mg a.i./L)	Replicate Turbidity (NTU)		
	Replicate 1	Replicate 2	Replicate 3
0	0.47	0.62	0.34
1	1.52	1.39	1.52
5	4.40	4.45	4.41
10	10.6	8.16	8.65
20	17.6	17.3	19.6
200	240	232	255

mg a.i./L = milligrams active ingredient per liter, NTU = nephelometric turbidity units



mg a.i./L = milligrams active ingredient per liter, NTU = nephelometric turbidity units

Figure 16. Zequanox Turbidity and Concentration Correlation in Waters at Hoover Dam.

7.2 Settlement Assessment Method Correlation

Densities of mussels on settlement plates were determined using two methods: the visual counting method (Section 3.3.1.1) and the digital microscope method (Section 3.3.1.2). The densities determined from each method were then compared for each plate and plotted (Figure 17). The trend line equation shows the relationship between the two densities to be approximately a one-to-one ratio.

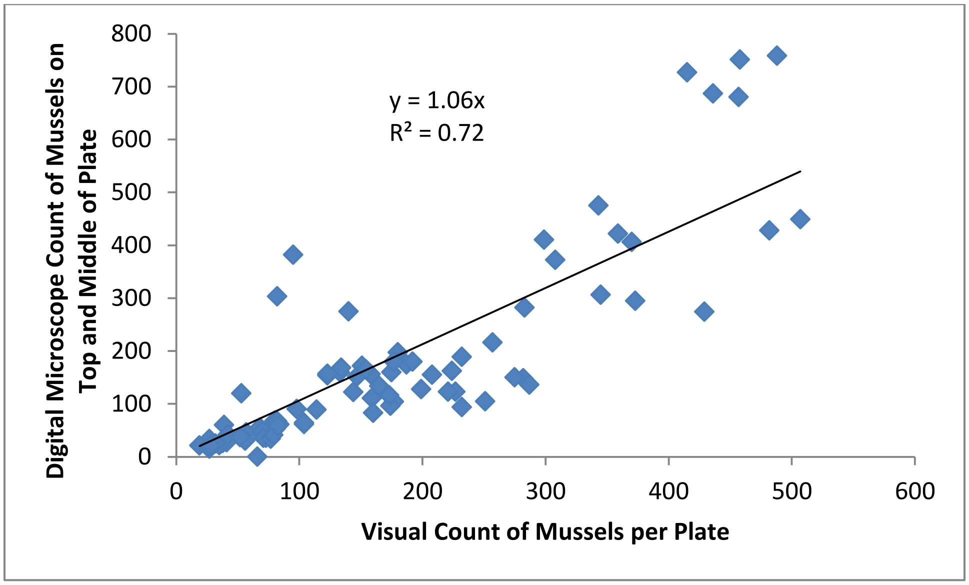


Figure 17. Densities of Mussels on Settlement Plates Compared by Two Analysis Methods