Davis Dam
2011 Zequanox Facility Treatments Report

Conducted on Behalf of

Bureau of Reclamation

Location
Davis Dam

Work Completed
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Executive Summary

In 2011, Marrone Bio Innovations, Inc. (MBI) in collaboration with the Bureau of Reclamation (Reclamation) conducted a series of quagga mussel (*Dreissena bugensis*) control treatments at Davis Dam. These treatments used Zequanox®, a Marrone Bio Innovations, Inc. (MBI) biological product for controlling invasive freshwater mussel (zebra and quagga mussel) infestations. The objective of the project was to evaluate the efficacy of Zequanox in cooling water systems. Run and operated by Reclamation, Davis Dam is a hydroelectric generation and water storage facility along the Colorado River between Arizona and Nevada and was the first United States facility to be treated with Zequanox for an infestation of quagga mussels.

Under an EPA Section 18 emergency use exemption permit, MBI successfully completed three treatments of Zequanox on a cooling water subsystem at Davis Dam. The treatments occurred in June, September, and November of 2011. These treatments represented the first time that Reclamation treated the cooling water subsystems at Davis Dam for either micro or macro fouling.

The results of these treatments demonstrated that Zequanox can deliver high mussel mortality when used as directed and can play a key role in a mussel management strategy for a cooling water system. Additional key findings include the following:

- High mussel mortality can be achieved with a single application of Zequanox.
- Repeated treatments of Zequanox result in cumulative effects, enabling a regimen of regular treatments to be employed to achieve population control throughout a season.
- Even with an extreme and unusual algae bloom event, Zequanox provided significant mussel control.
- Zequanox caused no impact on Colorado River water quality.
- Treatment concentrations can be adjusted to limit and/or maximize mussel mortality and can be tailored to meet a facility's unique requirements.

Treatment Results

June Treatment

During the month following the June treatment, 43% of the treated mussels died, which is within the expected range for the treatment concentration that was achieved. The concentration was approximately 3 times less than designed due to the dilution that resulted from a significantly higher-than-anticipated flow rate of 5,000 gallons per minute, versus the described rate of 1,300–1,700 gallons per minute.

Monitoring of the mussels placed for the June treatment continued in order to evaluate the cumulative effect of successive treatments. The “June” mussels were subjected to the June and September cooling water subsystem treatments, as well as two lower concentration “preventative” treatments applied in July and August, for a total of four treatments. Final mortality of the June mussels after the four treatments reached 75.6%.

September Treatment

A mortality rate of 77% was measured during the month following the September treatment. This mortality rate demonstrated the efficacy of Zequanox for controlling adult invasive mussel populations.

November Treatment

During the month following the November treatment, MBI field scientists observed a mortality of 44% in the treated mussels. During this treatment, an unusually extensive algae bloom of *Microcystis* was occurring throughout the Lower Colorado River. It is believed that the algae bloom resulted in reduced mussel mortality during this treatment. The complete impact of the algae on Zequanox and on mussel filtration is still unknown and is under study at MBI, but even amidst this rare natural occurrence, Zequanox provided effective control of the mussel population. The November treatment provided evidence that significant invasive mussel control could be gained with Zequanox despite significant algae blooms on the river that would significantly limit the efficacy or control of other chemical and mechanical control alternatives.

Impact on Water Quality

Water quality was monitored throughout the three treatments by testing water samples that were collected both upstream from the injection location and downstream of where the Zequanox-treated water had been returned to the Colorado River. No detectable differences for measured water quality parameters (including turbidity, environmental *Pseudomonas* species concentration, and total organic carbon) were observed between locations upstream of the treatment and downstream of the treatment.

Related Assessment of the Preventative Treatment Program

In addition to monitoring the adult mussel mortality after Zequanox treatments, MBI assessed the use of Zequanox as a tool for control of newly settled juvenile mussel populations. This pilot demonstration involved monitoring juvenile mussel populations that grew on settlement plates within treated and untreated bioboxes at Davis Dam. The experimental plan included Zequanox treatments approximately once a month. The findings were very encouraging and showed that Zequanox can be used on a "preventative" basis to minimize control mussel biomass accumulation by treating the water system at regular intervals throughout a season. The results of this pilot demonstration are available in the *Davis Dam 2011 Zequanox Preventative Treatments Report* (Link, 2012).

1.0 Introduction

Davis Dam, a hydroelectric generation and water storage facility along the Colorado River between Arizona and Nevada, has been a product development site for Marrone Bio Innovations, Inc. (MBI) since early 2009. Access to the facility and collaboration with the Bureau of Reclamation (Reclamation) staff was coordinated through a cooperative research and development agreement between MBI and Reclamation.

In 2011, under an EPA Section 18 emergency use exemption permit, Davis Dam became the first United States facility to be treated with MBI's brand name Zequanox for an infestation of quagga mussels (*Dreissena bugensis*). The goal of the 2011 facility treatments was to demonstrate the efficacy of Zequanox, a naturally derived product for control of invasive freshwater mussels.

MBI successfully completed three Zequanox treatments on a cooling water subsystem at Davis Dam in June, September, and November (Table 1). Because these treatments represented the first time that Reclamation treated the cooling water subsystems at Davis Dam for either micro or macro fouling, average treatment concentration varied during the treatments as better knowledge of flow rates in the cooling water subsystem was obtained. This report describes the methods (Section 2) and results (Section 3), and presents conclusions regarding the efficacy of these treatments (Section 4).

2.0 Methods

MBI applied Zequanox to the infested cooling water system and monitored mussel mortality in the system after the treatment, to determine efficacy. This section describes the treatment and efficacy monitoring methods as follows:

- Section 2.1 describes how Zequanox application concentrations were determined and how system flow rates were monitored and adjusted in the course of treatment monitoring.
- Section 2.2 describes the use of bioboxes to monitor efficacy.
- Section 2.3 describes the monitoring of water quality in the Colorado River, which is the system's source water and discharge location.

2.1 Treatment Monitoring

To monitor the concentration of Zequanox in the water during a treatment, MBI relies on a linear correlation between turbidity and Zequanox concentration. MBI field technician determined a site-specific target turbidity for Zequanox, measured as the product's active ingredient - dry cell weight - in milligrams per liter (mg a.i./L) and turbidity in nephelometric turbidity units (NTU) using water from the cooling water subsystem at Davis Dam. During facility treatments, MBI monitored the application in accordance with MBI Standard Operating Procedure (SOP)#: MBI-G-20.15.0, *Turbidity and MOI-401 Active Ingredient Correlation and Application Monitoring*.

Prior to the June treatment, MBI and Reclamation engineers designed an injection system and determined a Zequanox application rate according to Reclamation's estimated flow rate of 1,300–1,700 gallons per minute (gpm) in the treated subsystem (Unit 3 Cooling Water System). MBI calibrated the injection pumps for the treatment concentration; however, observed Zequanox concentrations were significantly lower than anticipated during the initial June 2011 treatment. This discrepancy between anticipated and observed application concentration was the result of higher-than-anticipated flow rates in the treated system. After the June 2011 treatment, Davis Dam staff indicated that it might be possible to reduce the flow through the treated system by restricting flow to the coolers supplied by the system. Because regulations prohibited the use of other means, such as tracer dyes, for flow rate monitoring, MBI proposed using the Zequanox concentration as a tracer for identifying the impact of flow restrictions on the total system flow. During the September treatment, Davis Dam staff and MBI field technicians manipulated the flow and used the Zequanox concentration to determine flow rates and identify the effects of system restrictions on the flow rates.

Following SOP#: MBI-G-20.15.0, the day before the September Zequanox application, the field technicians determined a site-specific correlation between Zequanox and turbidity. On the day of the treatment, they measured turbidity in water samples to monitor concentrations of Zequanox during treatment. At 45 minutes into the treatment and at various points thereafter, Davis Dam staff manipulated the flow rate through the treated system using system valves. MBI staff evaluated the concentration of Zequanox in the treated waters approximately 40 minutes after each valve restriction—the estimated time for the impact of the restriction to be observed in the treated waters coming through the monitoring points. The flow rate of the cooling system was then calculated under the various restrictions with the following equation:

$$C_1F_1 = C_2F_2$$

- C_1 = concentration of stock Zequanox (100 grams per liter)
- F_1 = injection rate
- F_2 = flow in cooling water system
- C_2 = observed Zequanox concentration based on turbidity measurements

In November, MBI used the same method in conjunction with direct readings from the flow meter (newly installed by Reclamation) to compare the flow rates determined by the two different methods.

2.2 Efficacy Monitoring

Typical of hydropower cooling water systems, the treated cooling water subsystem at Davis Dam is not accessible for direct observation of the mussels within it, as it operates with high pressure and volume, and is required to be in continual operation. The efficacy of Zequanox applications was therefore determined by monitoring the mortality of a sample population of mussels held in bioboxes within the treated facility. Bioboxes (Figure 1) are modified, flow-through aquaria 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 mortality in the bioboxes is considered the closest estimate of the mussel mortality inside the treated system. In the northeastern part of North America, similar biobox setups have been used for the same purpose for more than 20 years to monitor the efficacy of other invasive mussel control technologies.

Mortality of the treated mussels is compared with that of the untreated mussels (in a control biobox) to confirm that mortality in the treated system is the result of the treatment, and not the result of handling, old age, or other reasons unrelated to the efficacy of Zequanox. Multiple control bioboxes were placed in in two locations (See Section 2.2.1 and Figures 1 through 5):

- Upstream of the Zequanox injection location, so that they received the same water as the treated system, but without the Zequanox.
- In a separate, identical system, in the same locations within the system as the treated bioboxes.

Water enters the biobox via the supply pipe on the left side of the photo in Figure 1. The water then travels through the bioboxes with serpentine flow over or below the baffles (dark grey plates within the biobox) before exiting through a standpipe on the right end of the biobox in this photo. During treatment and monitoring, there is constant flow of water through the biobox.

Figure 1 - Photo of a biobox.

2.2.1 Biobox Locations

Davis Dam facility staff installed the bioboxes as follows (See the schematic in Figure 2).

- Two bioboxes near the inlets (Figure 3) of both the control and treated systems
- One biobox at the midpoint of each system (Figure 4)
- One biobox at the outlet of each cooling water subsystem (Figure 5), just before the subsystem's water is released back into the Colorado River

The only bioboxes supplied with treated water were the bioboxes at the midpoint and outlet of the treated system. All other bioboxes served as monitoring locations of untreated water and mussels.

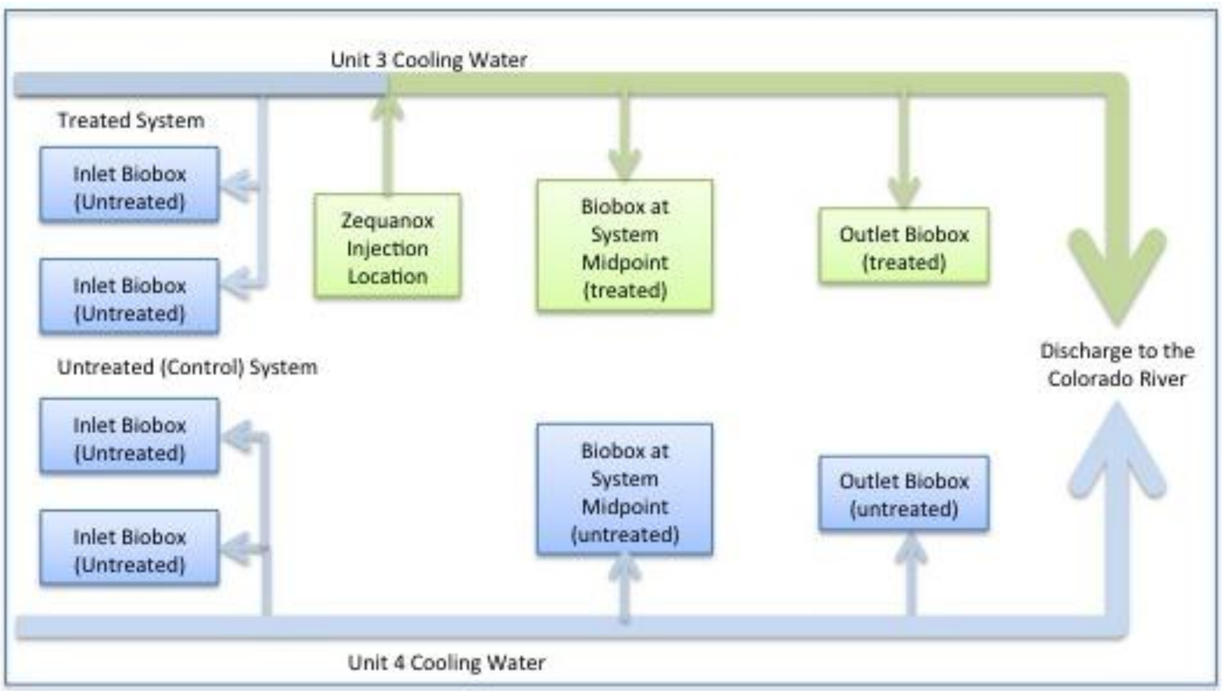

Figure 2 - Schematic of Biobox Placement at Davis Dam

Flow is from left front to right rear in this photo. These inlet bioboxes (circled in red) receive system water just prior to the treatment injection location (boxed in blue in the rear/ right of the photo).

Figure 3 - Bioboxes (untreated) at the Inlet of the Treated System.

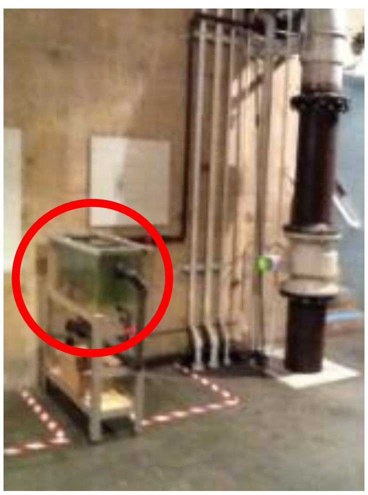

Figure 4. Biobox (treated) at the Midpoint of the Treated System.

Figure 5. Biobox (treated) at the Outlet of the Treated System.

2.2.2 Mussel Selection for Efficacy Monitoring

All mussels used for monitoring the treatments were collected by MBI 2 kilometers (km) upstream of Davis Dam at Katherine's Landing, Arizona. Field technicians harvested mussels as needed for all treatments at least 3 days prior to treatments. During monitoring after the September treatment, seasonal senescence (aging after maturity) increased the observed mortality in untreated mussels, so mussel collection for the November treatment was rescheduled to 10 days prior to treatment, to facilitate extended population monitoring for health. A diver performed the collection manually, by removing the mussels from the smooth, bottom side of floating dock structures with a scraper. Scraping, as opposed to pulling, ensured that the mussels were removed by release of the byssal threads from the structure and not by tearing the threads from the mussels.

After collection, MBI staff sorted the mussels by size. Adult mussels (15–25mm) were selected for use in the treatments described in this report. The field technicians then placed the selected mussels into sorting trays that contained sufficient water to cover the mussels. The mussels were allowed to sit, undisturbed, until siphoning behavior was observed. The mussels were then gently prodded by an observer, and if a mussel quickly retracted its siphon and closed, the mussel was selected for use in monitoring. The selected mussels were placed in enclosures in groups of 50. The enclosures consisted of containment tubes with mesh caps, which allow water to flow through them.

2.2.3 Mortality Observations

The biobox at each monitoring location held a minimum of three tubes of mussels. Before a treatment, field technicians checked the mussels daily and removed and replaced any expired or damaged mussels. After each Zequanox treatment, mussel mortality was recorded at least weekly by an MBI scientist, who counted the mussels that were alive (closed valves) and those that were expired (gaping and unresponsive to prodding). The scientist then removed the expired mussels and did not replace them, so that only live mussels that had been in the tube since the treatment remained. The number of living mussels in each tube was compared with the original number of live mussels (50) and the percent mortality (the percent of expired mussels) was calculated. The percent mortality from each tube of mussels was then averaged with the other tubes associated with the same treatment, to calculate a mean mortality and standard deviation for the treatment.

Field technicians completed the final evaluation once mortality appeared to be stable, at approximately 30 days after each treatment. Thereafter, all mussels were disposed of (in accordance with MBI's Hazardous Analysis and Critical Control Point Plan) and the tubes were cleaned for reuse. One exception to the 30-day monitoring period is associated with the June treatment, after which the mussels remained in the bioboxes with continued monitoring for 105 days, to evaluate the impact of multiple treatments on mussel mortality (See discussion in Section 3.1.3).

2.3 Water Quality Monitoring

This effort included water quality monitoring to determine whether the treatments at Davis Dam had any effect on Colorado River water quality. Calculations completed for the NEPA Environmental Assessment (DOI 2011) indicated the dilution downstream of the dam would result in Zequanox concentrations of 0.06–0.29 mg/L. These low levels of Zequanox were considered unlikely to have a negative impact on water quality, and monitoring was completed to identify any unexpected increases in measurements. During treatments, the field technicians monitored turbidity with onsite equipment, and collected samples for biological oxygen demand (BOD) and total organic carbon (TOC), which were both analyzed by Mohave Environmental Laboratory. Upstream sampling was performed within the dam, from a sample tap on the treated cooling water subsystem, approximately 1 meter upstream from the point of Zequanox injection. Downstream sampling occurred approximately 1 km downstream of Davis Dam at the Laughlin Bridge, the closest accessible midstream sampling location. All water sampling activities were completed by MBI in compliance with SOP# MBI-G_20.17.1, *MOI-401 EP Discharge Monitoring for Turbidity and Pseudomonas fluorescens*. During this effort, the requirements of SOP MBI-G_20.17.1 were amended by MBI and the Environmental Protection Agency to include fewer sample times; therefore, more sample times are listed for the June treatment than for the September and November treatments. Field technicians also collected water samples to monitor the environmental levels of *Pseudomonas* per MBI SOP# M 24-210, *Enumeration of Pseudomonas fluorescens in Water by the Membrane Filter Method.*

3.0 Results

Below are results from each of the three cooling water subsystem treatments, with treatment and mussel monitoring data first, organized by month. The water quality monitoring section follows the treatment and mussel monitoring, and is also discussed by month.

3.1 June Treatment Results

3.1.1 June Zequanox Concentration Monitoring

Reclamation and MBI staff planned the injection system calibration to have a Zequanox concentration of 175–185 mg active ingredient per liter of water (mg a.i./L) in the treated system for the June treatment. Actual average application concentration over the duration of treatment (370 minutes) equaled 55 mg a.i./L. Figure 6 shows the initial escalation of treatment concentration, followed by a plateau at approximately 50 mg a.i./L, the point at which previous calculations had predicted 175 mg a.i./L would be observed. The next increase, beginning at about 120 minutes, corresponds to the period during which the Zequanox injection rate was increased to the maximum rate possible while still having enough material to treat for the desired 6 hours.

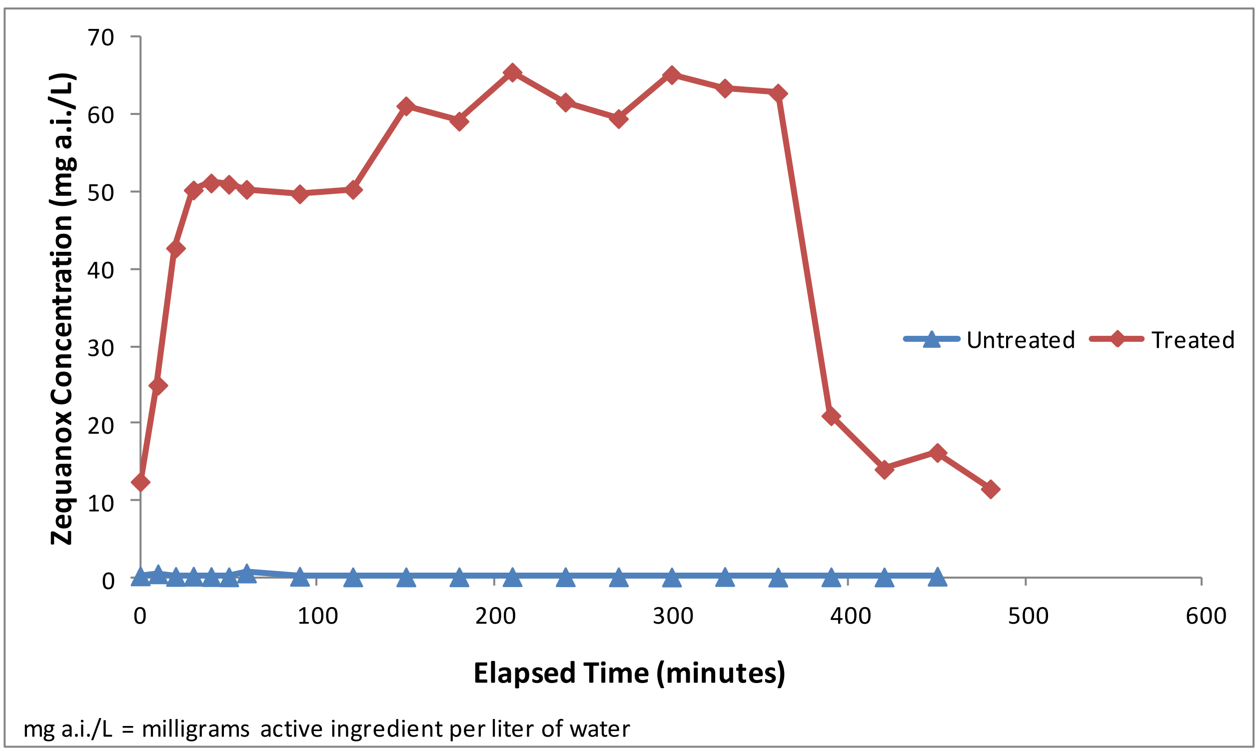

Figure 6. Zequanox Concentration Observed during Treatment in June 2011.

3.1.2 June Mussel Mortality

During the month following the June treatment, 43.3% of the treated mussels died (Table 2 and Figure 7); this rate is within the expected range for the 55 mg a.i./L treatment the system received.

Table 2. Total Mussel Mortality in the Treated and Untreated Cooling Water Systems at Davis Dam after the June 2011 Treatment.

Treatment	Applied Concentration (mg a.i./L)	Time (hours)	Check (days post treatment)	Mortality (%)	Std. dev. (+/-%)
Untreated	0		27	1.3	0.8
Treated	55	6	27	43.3	6.1

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

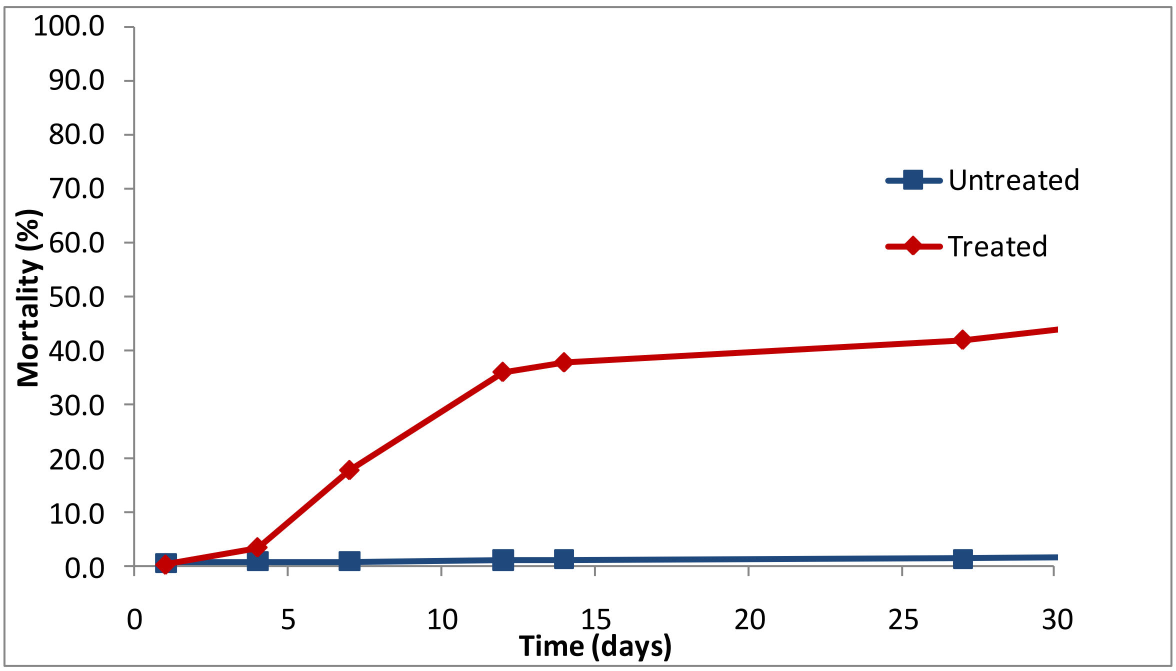

Figure 7. Mortality over time of mussels treated with Zequanox in June 2011 at Davis Dam.

After the June treatment, most of the observed mortality had occurred by day 14, with only a slight increase in mortality between day 14 and day 27 post treatment.

3.1.3 Cumulative Treatment Results

Monitoring of the mussels placed for the June treatment continued after the first 27 days to evaluate the cumulative effect of successive treatments. The “June” mussels were subjected to the June and September cooling water subsystem treatments, as well as two 25 mg a.i./L “preventative” treatments that were applied to the treated bioboxes in July and August as part of a preventative treatment demonstration (see below), for a total of four treatments.

Monitoring of the mussels continued 22 days after the September facility treatment (the final cumulative treatment of four), with final mortality evaluation occurring 105 days after the initial June treatment. The 105-day mortality observation thus represents the combined effects of four treatments at approximately 1-month intervals. Table 3 and Figure 8 show the mortality results associated with these treatments. Final mortality of the June mussels after the four treatments reached 75.6%. During the same 105-day monitoring period, the untreated mussels had a final mortality of 18.5%. As discussed in Section 3.2.2 regarding the September treatment, some of the mortality exhibited by the untreated mussels was likely due to annual senescence in September.

Preventative Treatment Program Demonstration

In addition to monitoring the adult mussel mortality after Zequanox treatments, MBI assessed the use of Zequanox as a tool for control of newly settled juvenile mussel populations and the prevention of biomass accumulation. This assessment involved monitoring juvenile mussel populations growing on settlement plates within the treated and untreated bioboxes at Davis Dam. The demonstration study plan included Zequanox treatments approximately once a month, with treatment concentrations of at least 25 mg a.i./L. As there were no cooling water subsystem treatments in July or August, Zequanox was applied once in each month at 25 mg a.i./L directly into the treated bioboxes for 6 hours. The results of this assessment are available in the *Davis Dam 2011 Zequanox Preventative Treatments Report (Link 2012)*.

Table 3. Total Mussel Mortality in the Treated and Untreated Cooling Water Systems at Davis Dam of the June 2011 Monitoring Mussels, Cumulative over 105 Days, Inclusive of Four Zequanox Treatments

Treatment	Applied Zequanox Concentration (mg a.i./L)	Treatment Length (hours)	Check (days after initial treatment)	Cumulative Mortality (% of initial mussels)	Standard Deviation (+/-%)
Untreated Mussels/Control					
June 23	0	N/A	27	1.3	0.8
July 21	0		47	5.7	3.3
August 10	0		82	5.8	5.0
September 14	0		105	18.6	9.3
Treated Mussels					
June 23	55	6	27	43.3	6.1
July 21	25	6	47	50.9	2.7
August 10	25	6	82	60.8	2.2
September 14	85	6	105	75.6	3.0

Notes:
mg a.i./L = milligrams active ingredient per liter of water
Treated mussels were subjected to 4 treatments, with cumulative effects. Expired mussels were removed and not replaced.

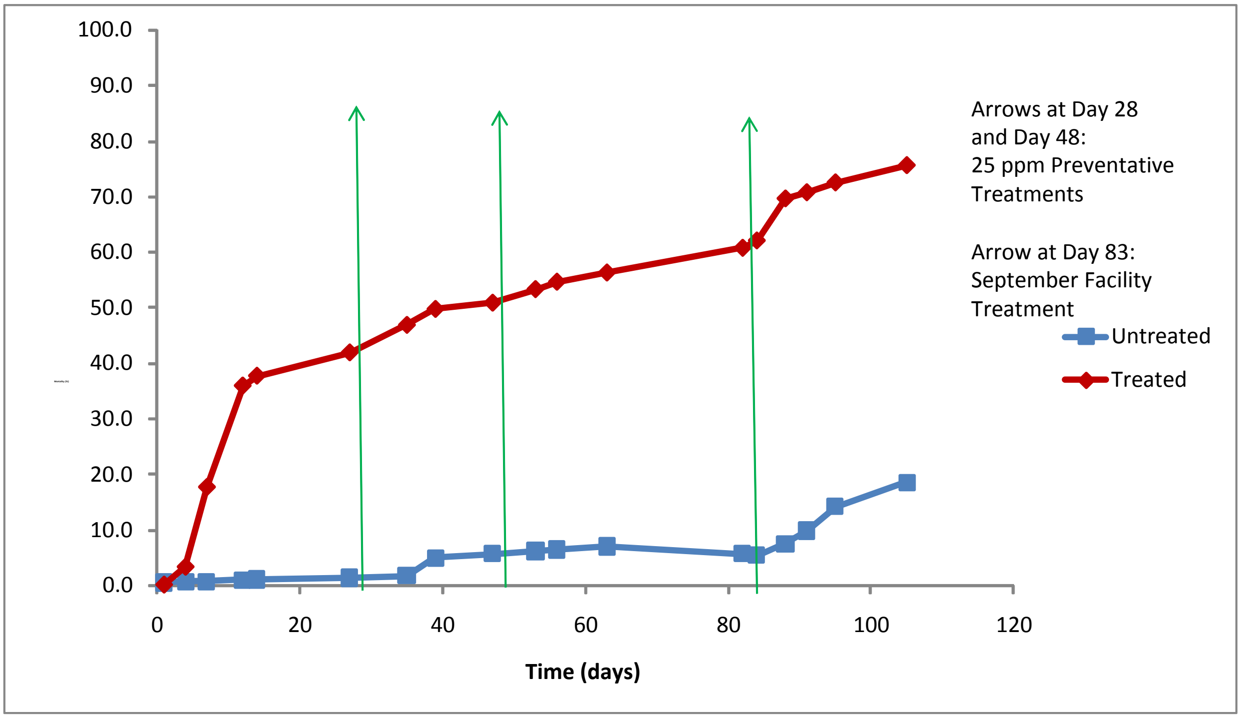

Figure 8. Cumulative Mortality of Mussels Treated 4 Times with Zequanox: in June, July, August, and September 2011 at Davis Dam.

3.2 September Results

3.2.1 September Tracer Study

As described in Section 2.1, MBI and Reclamation conducted a tracer study in September to determine potential abilities to adjust flow through the cooling water systems at Davis Dam. During the September treatment, Davis Dam staff made five flow-restricting actions. The final restriction caused an unexpected 6-times drop in flow rate, causing the observed concentration of Zequanox to significantly spike (Figure 9). Immediately upon detection, Davis Dam staff reversed the flow restrictions, to return application concentrations to within the permitted range.

In summary, the tracer study found that flow rates through the treated cooling water subsystem can be decreased significantly through restriction of water supplied to the main coolers. A 90% closure of valves for the main coolers resulted in a 60% reduction in flow through the subsystem (Table 4). Further reduction in flow could be obtained, but loss of flow through some equipment might occur as a result.

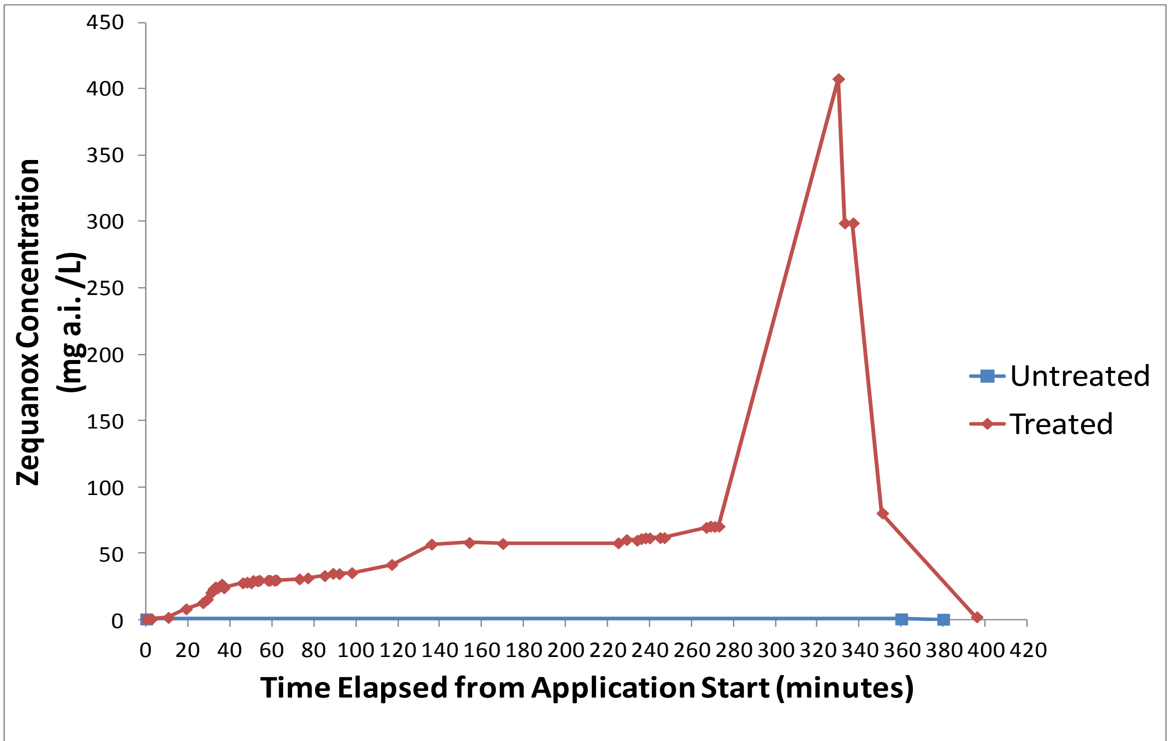

Figure 9. Calculated Concentration of Zequanox through the Davis Dam Treated Cooling Water System Based on Observed Turbidity during the September 2011 Treatment.

Table 4. Calculated Flow Rates through the Treated Cooling Water System with Corresponding Flow Restricting Actions during the September 2011 Treatment.

Time from Start of Application (minutes)	Flow Setting	Observed Zequanox Concentration 40 Minutes after Flow Adjustment (mg a.i./L)	Calculated Flow Rate (gpm)
0	Full flow: 7.5 turns open on coolers	26.7	5,091
40	Cooler restriction 40%: 3.0 turrns closed	33.1	4,103
70	Cooler restriction 80%: 6.0 turns closed	41.5	3,275
100	Cooler restricted 90%: 7.0 turns closed	61.5	2,147
240	Plug valve closed 50%	70.1	1,882
275	Plug valve closed 75%	407–299	324–442

Notes:
mg a.i./L = milligrams active ingredient per liter of water
gpm = gallons per minute

3.2.2 September Mussel Mortality

During the month following the September treatment, 77.0% of the treated mussels died (Table 5 and Figure 10), demonstrating the efficacy of Zequanox for controlling adult mussel populations. Mortality observed by MBI in the control populations was higher than that observed in the control populations for the June and November treatments (1.3% and 2.7%, respectively). This was likely due to physiologic weakening of the population, which is often observed at the end of summer (Wong et al. 2012). Monitoring after the September treatment was limited to 19 days because the cooling water system was taken offline and dewatered for the addition of a flow meter; however, based on the trending mortality (Figure 10), additional mortality was not anticipated. The 85 mg a.i./L Zequanox application concentration during the September treatment is the average applied concentration over the 6-hour treatment.

Table 5. Total Mussel Mortality in the Treated and Untreated Cooling Water Systems at Davis Dam after the September 2011 Treatment.

Treatment	Applied Concentration (mg a.i. /L)	Time (hours)	Check (days post treatment)	Mortality (%)	Std. dev. (+/-%)
Untreated	0		19	19.1	8.1
Treated	85	6	19	77.0	3.3

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

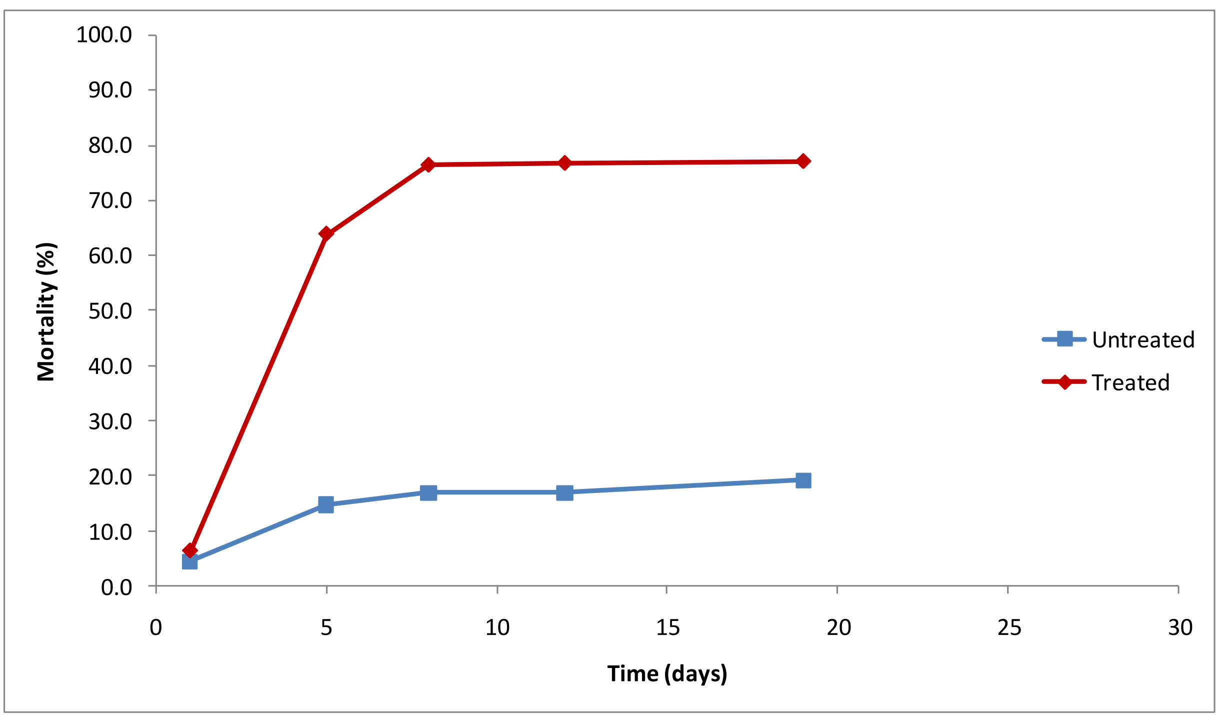

Figure 10. Mortality over Time of Mussels Treated with Zequanox in September 2011 at Davis Dam.

3.3 November Results

3.3.1 November Zequanox Concentration and System Flow Monitoring

At the start of the November treatment, Davis Dam facility staff and Reclamation regional staff manipulated the flow rate in the coolers to approximately 2,000 gpm, as measured by the newly installed flow meter.[1] This flow rate did not correlate to the flow rate determined by calculations from the observed concentration of Zequanox (Table 6), and the staff made additional restrictions during the course of the treatment to provide the desired flow rate for the 7.5-hour Zequanox treatment. Some variability between the September and November flow rates estimated with the observed Zequanox concentration is to be expected because cooler flow restriction settings are rough estimates. As the turbidity correlation to Zequanox concentration is direct, and the same calculations are regularly used by MBI at other facilities with calculated flow rates close to flow meter readings (within 5%), investigation of alternate explanations for this difference may be warranted. While sources of treated system dilution were avoided by closing all visible points of connection between the treated system and nearby systems, the valve integrity can be difficult to confirm, and some sections of the treated system are neither accessible nor visible.

Table 6. Observed and Calculated Flow Rates in the Treated System during November 2011 Treatment for Comparison.

Flow Setting	September Calculated Flow Rate (gpm)	November Calculated Flow Rate (gpm)	Flow Meter Flow Rate (gpm)
Cooler restricted: 7.0 turns closed	2,147	2,500	1,000
Cooler restricted: 6.75 turns closed		2,800	1,400
Cooler restricted: 6.5 turns closed		3,400	2,000

gpm = gallons per minute

[1] *The installed flow meter is a Siemens SITRANS F M MAGFLO MAG 6000, which was calibrated by the manufacturer prior to purchase and installation. It was installed by Davis Dam facility staff between the September and November Zequanox applications, at approximately the midpoint of the treated system. The flow meter appears in Figure 4, where its greenish yellow display screen is visible to the right of the biobox.*

At the start of the November treatment, one of the two Zequanox injection pumps had a fuse failure, and was not repaired until approximately 3 hours into the treatment. To adjust in response to this situation, MBI technicians increased the Zequanox application as much as possible by using an increased Zequanox stock concentration, and Davis Dam staff manipulated flow in the system. These combined actions resulted in an application concentration ranging from 60 to 80 mg a.i./L during the first 3 hours (Figure 11). When the second injection pump became available after repair, the settings were readjusted for the remainder of the treatment, and the final average treatment concentration for the November 2011 treatment was 100 mg a.i./L.

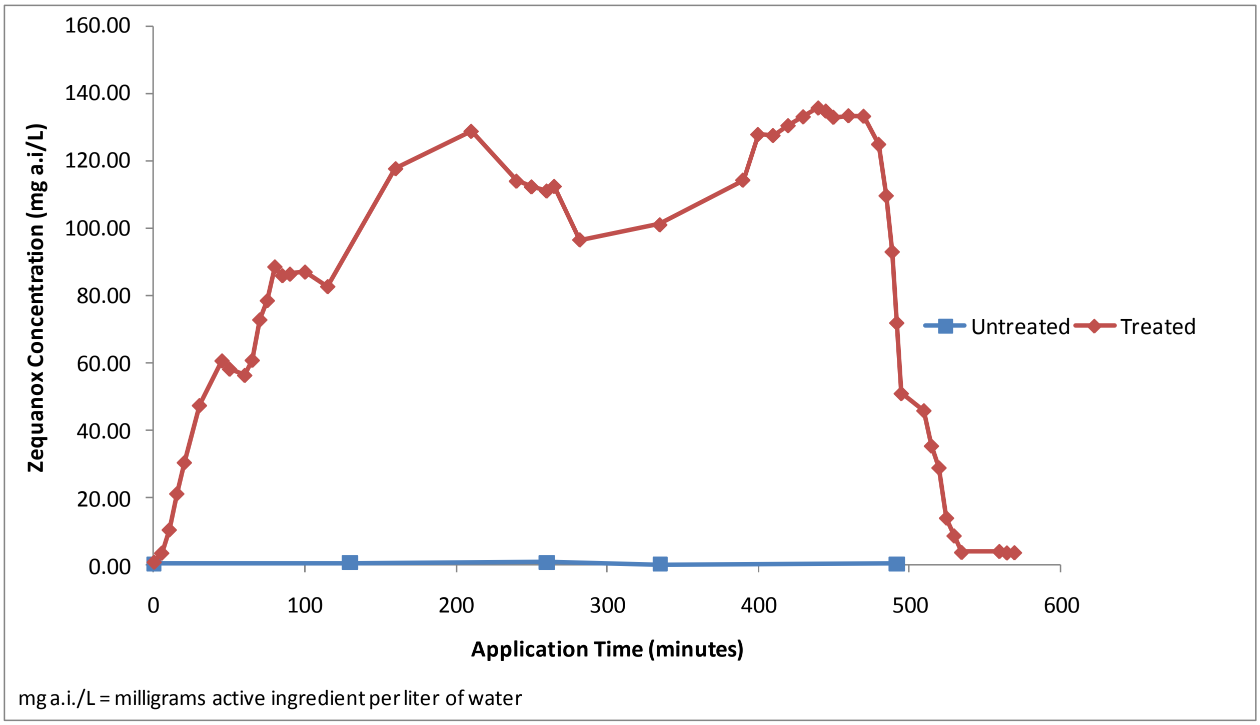

Figure 11. Calculated Concentration of Zequanox through the Treated Cooling Water System based on Observed Turbidity during the November 2011 Treatment.

3.3.2 November Mussel Mortality Monitoring

During the month following the November treatment, MBI field technicians observed a mortality of 43.5% in the treated mussels (Table 7, Figure 11). At the same time, the mortality in the untreated mussels was 2.7%.

During this treatment, a significant algae bloom of *Microcystis* was occurring throughout the Lower Colorado River (Figure 12). The observed amount of algae in the water had not been seen previously by Davis Dam facility staff (Lammers 2011), or by MBI technicians. The potential impacts of the algae bloom on Zequanox when mixed with the Colorado River water prior to injection, and the subsequent product efficacy on mussels, are under study at MBI, but are unknown at this time. The November treatment provided evidence that significant invasive mussel control could be gained despite significant algae blooms on the river that would significantly limit the efficacy or control of other chemical and mechanical control alternatives.

Table 7: November 2011 Treatment Observed Mortalities

Treatment	Applied Concentration (mg a.i. /L)	Time (hours)	Check (days post treatment)	Mortality (%)	Std. dev. (+/-%)
Untreated	0		26	2.7	3.3
Treated	100	7.5	26	43.5	13.4

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

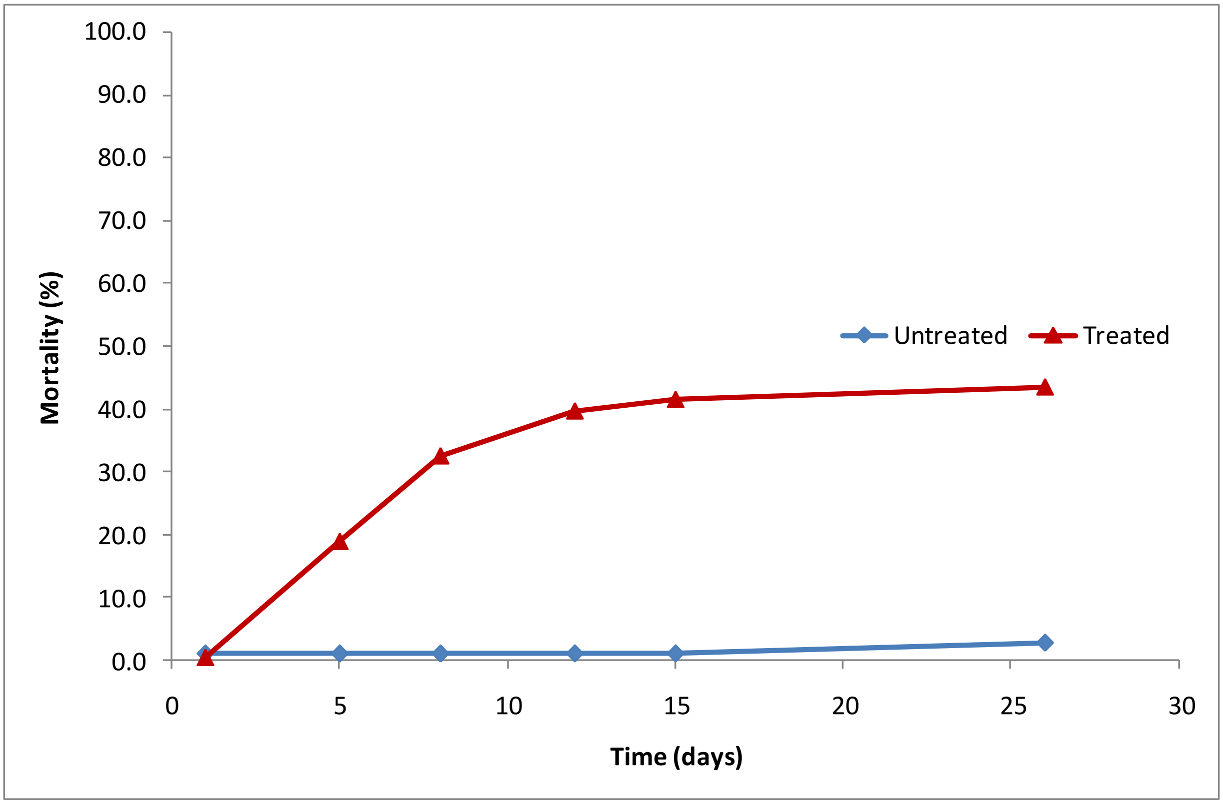

Figure 11. Mortality over Time of Mussels Treated with Zequanox in November 2011 at Davis Dam.

Figure 12. Microcystis algae (green clumps) in concentrated product solution of Zequanox in a bucket during the November 2011 treatment.

3.4 Water Quality Monitoring

These treatments included monitoring to determine whether the treatments at Davis Dam had any effect on Colorado River water quality. The sections below present the water quality monitoring results associated with each treatment. MBI collected both upstream, untreated samples and downstream samples, collected at a location downstream of where the Zequanox-treated water had been returned to the Colorado River. All parameters were monitored for level increases in the downstream samples that were not matched by increased readings in the upstream, untreated samples.

3.4.1 June Water Quality Monitoring

Water quality monitoring data collected by field technicians during the June treatment showed no detectable impact of Zequanox on TOC, turbidity, or environmental *Pseudomonas* concentrations (Table 8). Upstream *Pseudomonas* samples at 1 hour post treatment contained bacteria colonies too numerous to count in all replicates (6 total), likely due to a sample processing error by laboratory staff; however, no increase was observed in the downstream samples. The BOD levels were measurable by laboratory staff in the downstream I hour post treatment sampling; however, all other samples were below limit of detection. No increase in TOC was detected at the 1 hour post treatment sampling, which would be expected if BOD levels elevated. This data suggests that the elevated BOD was likely small particle contamination, sample variability, or collector or laboratory contamination, which could not be determined, as sample replicates were not collected. The temperature of water in the downstream samples increased during the June treatment; this was due to general warming of the surface waters, which were sampled on a diurnal basis in the Colorado River near Laughlin in June.

3.4.2 September Water Quality Monitoring

Water Quality monitoring by MBI and Mohave Environmental Laboratories during the September treatment showed no evidence of an impact from Zequanox on the river. September data (Table 8) showed no impact of Zequanox on TOC, BOD, turbidity, temperature, or environmental *Pseudomonas* concentrations. The downstream *Pseudomonas* concentration did increase during the treatment, but not to the same extent as in the upstream, untreated waters.

3.4.3 November Water Quality Monitoring

Water quality monitoring during the November treatment provided no evidence that the treatment had an impact on Colorado River water quality (Table 8). November monitoring by field technicians detected no impact of Zequanox on TOC, BOD, turbidity, temperature, or environmental *Pseudomonas* concentrations. A slight increase in turbidity was detected by MBI in the downstream samples from 2 hours into the treatment to post treatment; however, any readings below 1 NTU are considered to be ambient variability.

Table 8. Colorado River Water Quality during June, September, and November 2011 Zequanox Treatments.

Treatment Month	Location of Collection	Time of collection	TOC (mg/L) (LOD = 1)	BOD (mg/L) (LOD =5)	Turbidity (NTU)	Temp (°C)	*Pseudomonas* Spp. (colonies/mL)
June	Upstream	One hour pre-treatment	2.7	<5.0	0.14	15	4.1
	Upstream	Mid treatment	2.8	<5.0	1.38	15	0.08
	Upstream	One hour post treatment	2.8	<5.0	1.54	15	TNTC
	Downstream	One hour pre-treatment	2.9	<5.0	0.33	15	0.43
	Downstream	Mid treatment	2.8	<5.0	0.32	21	0.21
	Downstream	One hour post treatment	2.9	11	0.37	21	0.25
September	Upstream	2 hours into treatment	2.8	<5.0	0.38	20	0.43
	Upstream	2 hours post treatment	2.7	<5.0	1.25	20	1.84
	Downstream	2 hours into treatment	2.6	<5.0	0.35	21	0.44
	Downstream	2 hours post treatment	2.7	<5.0	0.38	21	1.18
November	Upstream	2 hours into treatment	3.1	<5.0	1.00	19	0.5
	Upstream	2 hours post treatment	2.8	<5.0	0.81	19	1.6
	Downstream	2 hours into treatment	4.3	<5.0	0.79	20	1.4
	Downstream	2 hours post treatment	3	<5.0	0.86	20	0.1

LOD= Limit of Detection of analysis equipment used. TOC = Total Organic Carbon. BOD = Biological Oxygen Demand. NTU = nephelometric Turbidity Unit

4.0 Summary and Conclusions

> ***Conclusions***
>
> - ***Large-scale application can achieve high mussel mortality.***
> - ***Mussel management strategies can use repeated treatments to achieve goals by taking advantage of cumulative effects.***
> - ***Even with an unusually extensive algae bloom event, Zequanox provided significant mussel mortality. The impact of the algae bloom on Zequanox and subsequent mussel mortality is being further investigated.***
> - ***Treatments caused no impact on Colorado River water quality.***

The results of these facility treatments provide evidence regarding large-scale application efficacy and mussel management strategies. First, the mortality results (43.3%, 77.0%, and 43.5% for the June, September, and November treatments, respectively) indicate that large-scale application of Zequanox can achieve high mussel mortality. The June treatment (at 55 mg a.i./L) resulted in 43.3% mortality throughout the system at day 27. Successive treatments on the June mussels in the months following the June treatment, with continued monitoring, demonstrated that significant additional mortality could be gained by applying lower concentration, monthly treatments, with final treated mussel mortality reaching 75.6% at 105 days after initial treatment. These results indicate that mussel population management strategies incorporating repeated treatments do not need to be based on achieving high mortality in the initial treatment, because target mortalities can be reached using multiple treatments with cumulative effects.

The September treatment provided confirmation that a single, large-scale application (e.g., an entire cooling water subsystem) can be completed with high resulting mussel mortality (77.0%, using an 85 mg a.i./L treatment concentration). Monitoring for the September treatment showed 19.1% mortality in the untreated mussels, which is higher than untreated mussel mortality during other Davis Dam treatments, but this higher mortality was understandable due to the timing of the treatment. Annual end-of-summer senescence of the mussel population is common; the weaker physiologic state of mussels is often part of the motivation for all types of end-of-season treatment planning, inclusive of chemical treatments.

The November Zequanox treatment achieved 43.5% mortality within 26 days after treatment, using a 100 mg a.i./L treatment concentration during a high algae event. During this treatment, a unique large-scale algae event was occurring on the Colorado River. At the Interagency Quagga Mussel Meeting in November 2011, Southern Nevada Water Authority and Bureau of Reclamation field staff confirmed and discussed the algae event, its unusualness, and that its cause was unknown. The impact of the algae on Zequanox and on mussel filtration is still unknown. With a mortality rate similar to that of the June treatment, the November Zequanox treatment, if combined into a repeated treatment strategy, would likely have resulted in high cumulative mortality, as was demonstrated in the June cumulative treatment results. In addition, the November treatment provided evidence that significant invasive mussel control could be gained despite significant algae blooms on the river that would significantly limit the efficacy or control of other chemical and mechanical control alternatives.

Monitoring of water quality, conducted during the three treatments in 2011, showed little-to-no impact on the Colorado River. No detectable difference was observed between locations upstream of treatment and downstream of treatment for measured water quality parameters, including turbidity, environmental *Pseudomonas* concentration, and TOC. An increase in BOD was observed in the hours after the June treatment (but not during the treatment) downstream of the Davis Dam; however, the increase was from a concentration below the level of detection to a concentration just above the level of detection, and was not accompanied by an increase in TOC or turbidity, which contradicts any correlation to an increase associated with the Zequanox treatment. In addition, no increase in BOD was observed in subsequent treatments, with all samples in September and November reporting below BOD levels of detection.

5.0 References

5.1 External References

Interagency Quagga Mussel Meeting at the Southern Nevada Water Authority on November 17, 2011.

Lammers, Vince (Davis Dam Operation and Maintenance Supervisor II). 2011. Personal communication. November 2, 2011.

US Department of the Interior, Bureau of Reclamation. 2011. Finding of No Significant Impact and Final Environmental Assessment: Controlling Quagga Mussels in the Cooling Water System at Davis Dam Using Zequanox™ (MOI-401) Laughlin, Nevada and Bullhead City, Arizona. No. LC-11-12. http://www.usbr.gov/lc/region/g2000/envdocs/Davisquagga2011/FONSIFinalEA.pdf accessed on December 1, 2011.

Wong, D., S. Gerstenberger, W. Baldwin, B. Moore. 2012 (in press). Settlement and growth of quagga mussels (*Dreissena rostriformis bugensis* Andrusov, 1897) in Lake Mead, Nevada-Arizona, USA. Aquatic Invasions (2012) Volume 7.

5.1 MBI Standard Operating Procedures

SOP#: MBI-G-20.15.0, *Turbidity and MOI-401 Active Ingredient Correlation and Application Monitoring*

SOP#: MBI-G-20.15.0,*Turbidity and MOI-401 Active Ingredient Correlation and Application Monitoring*

SOP# M 24-210, *Enumeration of Pseudomonas fluorescens in Water by the Membrane Filter Method*

SOP# MBI-G-20.17.1 *MOI-401 EP Discharge Monitoring for Turbidity and Pseudomonas fluorescens*

5.2 MBI Reports

Link, C. 2012. Davis Dam 2011 Zequanox Preventative Treatments Report.