

**Research Article** 

# Evaluating high pH for control of dreissenid mussels

Renata Claudi<sup>1</sup>\*, Thomas H. Prescott<sup>1</sup>, Katherine L. Prescott<sup>1</sup>, Sergey E. Mastitsky<sup>1</sup>, Dave Evans<sup>2</sup> and Ana Carolina Taraborelli<sup>1</sup>

RNT Consulting Inc., 823 County Road 35, R.R. #2 Picton, ON K0K 2T0, Canada
 Ontario Power Generation, 889 Brock Road, Location P82-5A, Pickering, ON L1W 3J2, Canada
 E-mail: rnt@kos.net (RC), tprescott@kos.net (THP), klprescott@gmail.com (KLP), aliensinbelarus@gmail.com (SEM), david.evans@opg.com (DE), actaraborelli@gmail.com (ACT)

\*Corresponding author

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#### Abstract

Two field experiments were carried out using a custom built flow-through laboratory to test the effect of elevated pH on dreissenid mussels as a potential control method. Both experiments tested the ability of dreissenid pediveligers to settle under conditions of elevated pH and the long-term survival of adult dreissenids under the same conditions. The two experimental sites had different water quality and different species of dreissenids present. The settlement of quagga mussel pediveligers at the lower Colorado River was inhibited with increasing pH. At the maximum achieved pH of 9.1, there was approximately 90% reduction compared to the maximum settlement observed in the controls. Since the settlement was almost as low in pH 8.9 as at pH 9.1, the inhibition in settlement may have been due to the presence of a precipitate formed under high pH conditions rather than the increase in background pH. No mortality of quagga mussel adults was observed in the experimental pH levels at the lower Colorado River. At San Justo Reservoir, zebra mussel settlement decreased with increasing pH. New settlement was almost entirely absent at the highest pH tested (pH 9.6). The observed mortality of adult zebra mussels was low, but did tend to increase with increasing pH. We also tested the response of adult zebra mussels to short-term exposure to very high pH levels (i.e. pH 10, 11, and 12). Adult mussels in poor physical condition experienced 90% mortality after 12 hours at pH 10 and pH 11. From this study, we conclude that pH elevation could be used both as a preventative treatment to eliminate settlement by dreissenid mussels and as an end of season treatment to eliminate adults. The high pH treatment would have to be tailored to the site water quality to prevent formation of precipitate during treatment and to minimize corrosive action on materials of construction.

Key words: control; elevated pH; field experiment; mortality; proof of principle; quagga mussel; zebra mussel

#### Introduction

When dreissenid mussels (zebra mussel, Dreissena polymorpha Pallas, 1771, and quagga mussel, Dreissena rostriformis bugensis Andrusov, 1897) are present in the source of raw cooling water, they become a serious problem for industrial facilities using this water unless defensive steps are taken. The treatment of choice for most facilities tends to be one of chemical control, as it is convenient and effective. The major advantage offered by chemical treatments is that they can be engineered to protect most of the facility, from intake to discharge. A wide variety of chemical treatment strategies is available for controlling mussel populations; however,

minimizing local environmental impact is frequently difficult. Chlorine, widely used for dreissenid control, creates undesirable by-products. Proprietary compounds used for mussel control generally have to be detoxified by bentonite clay. Both chlorine and proprietary products tend to be nonselective and therefore may be toxic to all forms of aquatic life.

As dreissenid mussels have a relatively narrow range of pH tolerance, with the optimum range being pH 7.5 to 9.3, it was hypothesized that by manipulating this environmental variable it may be possible to control the growth, settlement, and survival of dreissenids in raw water systems. How pH limits dreissenid success, however, is little understood. It is assumed that veligers have lower tolerance to pH extremes than adults. Claudi et al. (2012) examined the impact of low pH on dreissenid mussels and found that the majority of settlement was prevented at pH 7.1. Furthermore, the adult mussels were found to experience significant loss of calcium at pH 7.1. When the pH was reduced to 6.9, calcium loss was accelerated and 40% mortality was observed after 11 weeks. Overall, Claudi et al. (2012) concluded that decreased pH could be a credible mitigation strategy for dreissenid mussels.

Few studies have been done on the upper pH limit for dreissenid survival. Most authors place the upper pH limit for long term dreissenid survival between 9.3 and 9.5. This assumption appears to be based on very sparse data. Most authors quote the paper by Sprung (1993), who states that veligers develop to settlement stage when pH ranges from 7.4 to 9.4 with an optimal pH of 8.4 at temperatures of 18–20°C. These values are used by a number of authors when constructing invasion models (Naddafi et al. 2011) or assessing invasion risk for dreissenids in North America (Hayward and Estevez 1997; Cohen and Weinstein 1998; Cohen 2008) and in Europe (Trichkova et al. 2007).

The only other pertinent study found on upper pH tolerance of adult dreissenids was done by Bowman and Bailey (1998). Their laboratory experiment used a very small sample size and very small treatment containers, and the final pH levels were uncertain. The authors concluded that the upper pH limit for zebra mussels was 9.3-9.6. This conclusion was based on the results obtained after 30 days of exposure where 100% of adults were alive in the low NaOH treatment (final pH = 9.3) and control, 60% were alive in the medium NaOH treatment (final pH = 9.50), and 10% were alive in the high NaOH treatment (final pH = 9.55).

The purpose of our study was to carry out a "proof of principle" experiment to examine the effects of elevated pH on growth, settlement, and survival of dreissenid mussels at adequate calcium levels. We also examined the suitability of highly elevated pH as a control strategy for dreissenid fouling.

### Material and methods

The experiment was carried out in two parts. In the first part, a mobile laboratory was set up at Lake Havasu, Arizona. Water containing quagga mussel veligers was drawn from Lake Havasu on the lower Colorado River. Settlement and adult mortalities were monitored for eight weeks. The experiment was terminated when settlement was observed in control tanks. Upon completion of the Lake Havasu experiment, the mobile laboratory was disassembled, disinfected, and moved to San Justo Reservoir, California where the entire experiment was repeated on zebra mussel veligers.

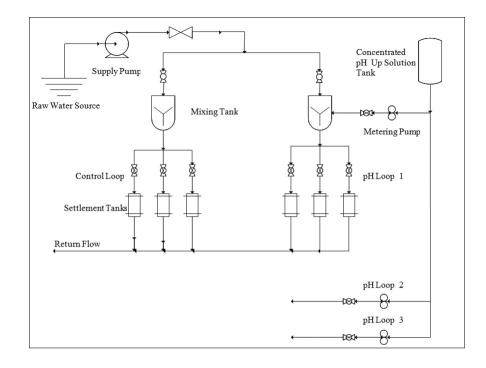
### Experimental set-up

On location, raw water containing dreissenid veligers was drawn into the mobile field laboratory. The water was then split into four streams which entered 160 L mixing tanks. Three tanks had pH individually increased by the addition of sodium hydroxide solution and the fourth stream acted as control at background pH. On exiting the mixing tanks, each water stream was further subdivided into three streams which flowed into individual settling tanks (Figure 1).

The settling tanks were insulated coolers with capacity of 45 L. The coolers had been filled with lake water for approximately one week prior to the experiment to condition them and remove any possible contaminants. Immediately prior to the start of the experiment, the coolers were emptied and dried with paper towels. Once each cooler was filled with lake water and adjusted to the proper pH, a 20 cm  $\times$  10 cm mesh bag containing live adult dreissenid mussels, collected on location, was placed in each cooler. A rack containing four settlement plates and six corrosion coupons (two each of C1010 carbon steel, 304 stainless steel, and grade CDA 110 copper) was also placed in each cooler (Figure 2).

The complete laboratory set-up showing mixing tanks, settlement tanks, and monitoring devices is shown in Figure 3. All tanks were monitored continuously for pH and temperature electronic probes connected to using а programmable logic controller. The controller adjusted the pH by adding a concentrated solution of sodium hydroxide via the metering pumps. Prominent Beta-4 pumps were used for the addition of the concentrated sodium hydroxide solution to three of the four mixing tanks. All four tanks were continuously mixed using stainless steel propeller style paddles. Water exited each tank on the bottom, through a housing containing a flow sensor, temperature probe, and pH probe acquired from Prominent Controls. These probes, together with the control module (Dulco Marin 2), monitored and recorded all pH and temperature values and, if necessary,

Figure 1. Schematic of the experimental layout showing the control loop and one of three pH loops in detail.





**Figure 2.** Inside of a settlement chamber showing a rack with four settlement plates and a mesh bag containing live adult mussels. Photograph by Renata Claudi.



**Figure 3.** Complete laboratory set-up showing four mixing tanks (blue barrels), twelve settlement tanks (red coolers), and associated monitoring devices. Photograph by Renata Claudi.

sent an adjustment signal to the sodium hydroxide solution addition pumps to add more solution to raise the pH. Each cooler was tested twice per day with a handheld pH meter. The readings were compared to the pH readings for the mixing tank displayed on the control module and recorded on a log sheet. All tanks were monitored weekly for settlement.

#### Experimental protocol

The mobile laboratory was put into full operation at Lake Havasu on March 7, 2011. The ambient water temperature was 13°C and the number of veligers in the water was very low, approximately 2 veligers/L. No ready-to-settle veligers were observed in the incoming stream and no new settlement was observed on the adults collected in the forebay and placed in the mesh bags. The initial pH treatments for the experiment were set at 9.1, 9.3, and 9.5.

Within 48 hours of the start of the experiment, a precipitate formed in the treatment tanks at pH 9.5, coating all upper surfaces in the settling tanks and instrument probes. On March 9, 2011, the portion of the system running at a pH of 9.5 was shut down and drained. Bags of adult mussels were rinsed and the adults were checked for mortality and placed in separate containers with ambient water from the river. The system was de-scaled using Safety Acid solution (an inhibited hydrochloric acid formulation marketed as Magic Acid). On March 11, 2012, the system was returned to service with modified pH treatment levels (Table 1). Temperature and pH data were collected in a daily log both for the mixing tanks and for the individual settlement chambers starting March 12, 2011.

To increase mussel settlement within settlement tanks, additional plankton was collected daily from the forebay. A sump pump was used to lift water from the forebay through a garden hose at a rate of approximately 1,000 L/hr. The garden hose emptied into a plankton net (1 m mouth diameter, 53-micron net and bucket mesh) positioned within a large tank (approximately 200 L) filled with water. This arrangement was observed to minimize the trauma to the plankton collected. The collection of plankton through the net was done continuously. Twice each day (9:00 am and 5:00 pm) the collected plankton was removed from the net collection bucket into a separate vessel. The collected volume was diluted with river water to 4 L, mixed with a glass rod, and quickly divided into four parts. Each mixing tank received 1 L of the concentrated plankton. The incoming veliger count increased throughout the experiment, peaking at approximately 150 veligers/L.

At the end of April 2011, after the end of the experiment at Lake Havasu, the mobile field laboratory was sterilized and re-located to San Justo Reservoir in San Benito County, California. At this time, the reservoir was in the process of being re-filled with a mix of water from San Luis Reservoir and the Sacramento Delta. It appeared that there had been a significant kill of zebra mussels in the reservoir during the winter. Only a few live adults were found on several buoys in the reservoir; the water was too high to locate any mussels on the shoreline. The water from the reservoir was brought to the laboratory through a 5 cm potable water polyethylene pipe using a 1.5 hp submersible Champion pump. The distance from the pump intake to the laboratory was approximately 120 m.

The mobile laboratory was put into operation at San Justo Reservoir during the first week in May 2011. The first test carried out was to determine the effect of short-term exposure to very high pH on adult zebra mussels. Live adult mussels were placed in mesh bags and introduced into each of the coolers in the lab. The number of mussels per bag varied between 10 and 27 individuals. Mussels in the bags were in clusters; the clusters of mussels were randomly selected from a small pool of adults available. Any adults with perforated shells were excluded. The pH levels of each treatment system are shown in Table 1. Each bag of adults was checked for mortality after 12, 24, 36, and 48 hours.

As a result of potentially harsh winter conditions in the San Justo Reservoir, the adult zebra mussels collected in May 2011 may have been under physiological stress leading to quick mortalities during the high pH experiment. The experiment was repeated in October 2011 to verify that the mortalities observed in the May experiment were a result of elevated pH conditions alone and not due to a combination of high pH and the impact of stressful winter

 Table 1. pH levels for the long-term and short-term exposure experiments for quagga mussels at Lake Havasu, Arizona and zebra mussels at San Justo Reservoir, California.

Sustam	Lake Havasu	San Justo	Reservoir
System	Long-Term Exposure pH	Short-Term Exposure pH	Long-Term Exposure pH
А	8.9	10	9.2
В	9.0	11	9.4
С	9.1	12	9.6
D (control)	8.2 - 8.6	8.8 - 8.9	8.2 - 8.6

conditions. Larger numbers of adults were exposed in each pH tested in October 2011 (70 - 168individuals/cooler), and mortality was recorded after 24, 48, and 72 hours.

To examine the impact of high pH on mussel weight and shell length, live mussels from each of the mesh bags were placed in individual aluminum pans and dried for 3 hours at 350°F. Subsequently, each mussel shell was measured using electronic calipers (Powerfist) and weighed to the nearest milligram using an electronic scale (GemPro-500).

Following the May 2011 short-term exposure test, the pH in all settlement tanks was stabilized and the long-term experiment commenced on May 22, 2011. There was no precipitation in the test tanks at San Justo Reservoir and we were able to adjust the test pH to the desired upper level (Table 1). The veliger numbers in the plankton were very low throughout the experiment (average density 53 veligers/L). Settlement was detected in the control tanks in mid-July. At that time, the veliger numbers were declining rapidly in the plankton. Although settlement was observed in the control tanks, the experiment was terminated because further settlement was not expected due to declining veliger counts in the plankton samples. On July 30, 2011, the bags containing adults were examined and a detailed examination of the settling plates was performed. On July 31, 2011, the flow was stopped, the system was drained, and all experimental vessels were examined for settlement.

### Statistical analysis

We employed a number of statistical models to test hypotheses about the data obtained in this study. For the experiment with quagga mussels from Lake Havasu, a one-way analysis of variance was used to test if the total numbers of settled molluscs counted at the end of the experiment differed among the pH treatments. Before this analysis, the counts were log(x + 1)transformed to achieve homogeneity of variance and normality. As there was no settlement in the system B coolers (see Results), that series was excluded from the analysis. The analysis was conducted using statistical computing environment R v2.14.0 (R Development Core Team 2011).

As there were multiple measurements originating from the same experimental coolers, we fitted a linear mixed-effects model (LMEM) to test whether the weight of quagga mussels differed among the pH treatment groups, with shell length of the molluscs considered as a covariate. The variation among experimental coolers was fitted in the form of a random intercept of the LMEM. In addition, an inter-action between the shell length and pH level was added to the model to test whether the regression slopes differed among the pH treatments. Insignificant terms were stepwise backward eliminated from the initially fitted model based on the log-likelihood ratio test (Zuur et al. 2009). This analysis was conducted using the *nlme* package for R (Pinheiro et al. 2012).

For the experiment with the zebra mussels at San Justo Reservoir, a generalized linear model (GLM) with a quasi-Poisson error structure was employed to test whether there was a difference in settler counts among the pH treatments. To test statistically the effect of pH on zebra mussel mortality, a GLM with a binomial error structure was fitted (Zuur et al. 2009). Both models were fitted using the base functionalities of R (R Development Core Team 2011).

Similar to the experiment with guagga mussels from Lake Havasu, we fitted a LMEM to examine if the dry weight of zebra mussels from the San Justo Reservoir differed among the pH levels, with shell length treated as a covariate. Analysis of the results produced with this model revealed no significant variation among the experimental coolers, and thus a simpler, generalized least squares (GLS) model with only fixed effects of pH and shell length was fitted instead. In this GLS model, the variance of the dry weight measurements was allowed to vary among coolers (see Zuur et al. (2009) for details on this type of parameterization). Insignificant terms were stepwise backward eliminated from the initially fitted model based on the loglikelihood ratio test. The model was fitted using the nlme package for R (Pinheiro et al. 2012).

Data from the October 2011 experiment on short-term exposure of zebra mussels to elevated pH were used to fit a binomial log-logistic doseresponse model of the following functional form:

$$p = \frac{d}{1 + \exp(b \times (\log(Time - e)))},$$

where p is the proportion of mussels found dead at a certain time of exposure, d the maximum proportion of dead mussels reached, b the scale parameter that determined the shape of the doseresponse curve, and e the exposure time resulting in 50% of the maximum effect observed in a treatment group. All parameters of this model were allowed to vary among the pH treatment groups, and were estimated using the *drc* package for R (Ritz and Streibig 2005).

#### Results

#### Quagga mussels at Lake Havasu, Arizona

The long-term exposure experiment carried out on the lower Colorado River at Lake Havasu was plagued by precipitation of calcium carbonate when pH was increased from the ambient level. The laboratory water systems required extensive labour to continue to function. The pH fluctuated in all treatments on a daily basis due to the ongoing fouling of the pH probes. On April 2, 2011, system B, which was supposed to maintain a pH of 9.0, accidentally overdosed to a pH of 12. As the overdose happened during the night, the high pH persisted for more than 10 hours in the settlement chambers. Subsequent to this overdose, we found all adults in the mesh bags were dead and no settlement was recorded in system B. At the same time, there was virtually no mortality of adult guagga mussels in test coolers A (<0.5%), C (0%) and D (<1.5%).

Average numbers of settled quagga mussels were found to significantly differ among the treatments (P = 0.007, one-way ANOVA). Posthoc Tukey tests were performed to find which particular groups differed from each other. This analysis showed no differences between treatments A and C (P = 0.99), while both A and C significantly differed from D (P = 0.011 and P = 0.012, respectively), which was the control.

Dry weight of the quagga mussels was found to be strongly related both to their shell length and pH level (P < 0.01, *F*-tests, for all parameters in the mixed-effects model fitted as described in Material and Methods). However, there was no significant interaction between shell length and pH, indicating that the regression lines for all pH levels had the same slope. In addition, variance of the random intercept in the fitted model significantly differed from zero ( $\sigma_b^2 = 0.043$ ; 95% confidence interval: 0.022–0.082), indicative of a significant inter-cooler variation of the mussels' weight within each of the pH groups.

According to the conducted analysis, the relationships between the dry weight and shell length of quagga mussel under different pH treatments were as follows:

$$\log(Weight) = -9.715 + 2.972 \times \log(Length)$$

Group C:

 $\log(Weight) = -9.744 + 2.972 \times \log(Length)$ 

Group D:

 $\log(Weight) = -9.905 + 2.972 \times \log(Length)$ 

On the original (non-log) scale, dry weight of quagga mussels in group A was on average 20.9% higher than in group D. In group C, it was 17.5% higher than in group D, whereas the difference between groups A and C was as low as 2.9%.

#### Zebra mussels at San Justo Reservoir, California

During the May 2011 experiment with very high pH levels, the highest mortality occurred in system C (pH 12) where after 12 hours 90% mortality was recorded in all three test coolers. The remaining mussels, except for a few in one of the test coolers with pH of 12 (99% mortality), died in the next 24 hours. Mortalities were low for the other two pH treatments, i.e. 5% at 12 hours and an additional 2% mortality after 36 hours in system A (pH 10), and only 1.5% mortality at 36 hours in system B (pH 11). No mortality was found in system D (control).

When the experiment was repeated in October 2011, lower mortalities were observed in all treatments at any given time (Figure 4). A mortality of approximately 90% at pH 12 was only reached after 120 hours. Also after 120 hours, significant mortality was observed at both pH 10 and pH 11, though not as high as at pH 12. According to the fitted log-logistic dose response model (Table 2), the exposure times resulting in 50% of the maximum effect recorded in each treatment were 102.2 h at pH 10 (95% confidence interval: 75.4 - 128.9 h), 88.7 h at pH 11 (95% confidence interval: 80.3 - 97.1 h), and as low as 14.5 h at pH 12 (95% confidence interval: 10.9 – 18.1 h). During both experiments (May and October 2011), mortality appeared to be accompanied by the swelling and rapid disintegration of body tissues (Figure 5).

The settlement prevention at high pH experiment ran from May 22 to July 30, 2011. Settlement in all coolers was relatively low compared to settlement recorded on the lower Colorado River at Lake Havasu. The low settlement was due to the low remaining population of zebra mussels in the San Justo reservoir in

Figure 4. Cumulative percent mortality of zebra mussels in the October 2011 short-term exposure experiment, with the fitted dose-response curves indicated.

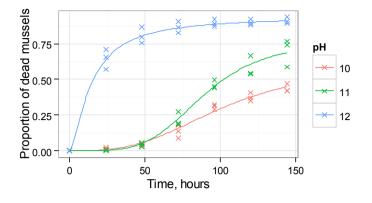


 Table 2. Parameters of the log-logistic dose-response model fitted to data from the October 2011 short-term exposure experiment (see "Material and methods" section for definition of the model parameters).

Parameter	Estimate	Standard error of the estimate	t-statistic	P-value
b [pH 10]	-3.00	0.40	-7.49	< 0.001
b [pH 11]	-4.28	0.42	-10.17	< 0.001
b [pH12]	-1.48	0.35	-4.13	< 0.001
d [pH 10]	0.61	0.10	5.92	< 0.001
d [pH 11]	0.77	0.05	15.22	< 0.001
d [pH12]	0.94	0.03	32.98	< 0.001
e [pH 10]	102.2	13.6	7.50	< 0.001
e [pH 11]	88.7	4.2	20.76	< 0.001
e [pH12]	14.5	1.8	7.87	< 0.001

the spring of 2011 following winter kill of adults. The remaining low population resulted in low veliger counts.

Overall, there was a statistically significant effect of pH on the mussel settlement (P = 0.0017, Chi-squared test of the GLM deviance). Pairwise comparisons of the pH treatments revealed a marginally significant difference between the control coolers and those in group A (P = 0.053, t-test), as well as in the control and group C coolers (P = 0.053, t-test), whereas no difference was observed between the control and system B (P = 0.225, t-test). Also, there was a marginally significant difference between system A and system B (P = 0.0498, t-test), but no difference between A and C (P = 1.00, t-test).

Overall, there was a significant effect of the pH on the mortality of adult zebra mussels (P < 0.001, Chi-squared test of deviance). The particular differences were revealed between the control and group A coolers (P = 0.025, z-test), and between the control and group C coolers (P

= 0.005, z-test). At the same time, there was no difference between the control and group B coolers (P = 0.147, z-test).

The dry weight of zebra mussels from San Justo Reservoir was found to be associated only with shell length, whereas no effect of pH was detected:

$$\log(Weight) = -10.643 + 3.250 \times \log(Length)$$

Overall, the fitted regression model was highly significant statistically (P < 0.001, F-test) and explained 92.8% of the variance in data (Figure 6). Accordingly, both of its coefficients were also highly significant (P < 0.001, t-tests).

#### Corrosion coupon tests

The pH treatment had little effect on the corrosion rates for carbon steel and copper, compared to the control in the Lake Havasu experiment. However, the pH adjustment was found to significantly increase the corrosion



**Figure 5.** Dead mussel removed from the pH 12 treatment after 24 hours. Although the mode of death was not determined, the bloated appearance of the tissue suggests failure of osmoregulation. Photograph by Renata Claudi.

penetration for stainless steel compared to control, although in all treatments the corrosion rates for 304 stainless steel were quite low in absolute terms (i.e., ca. 7.6 to  $10.2 \,\mu$ m/yr). The 304 stainless steel corrosion took the form of a uniform light etch, while the C1010 carbon steel showed evidence of some limited localized attack, in addition to general corrosion. In system A, grade CDA110 copper corrosion was generally a uniform light etch, whereas systems B, C, and D showed spotty etching.

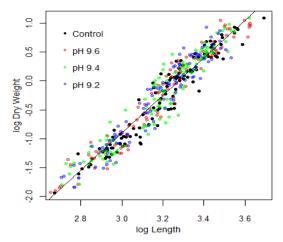
The San Justo Reservoir long-term exposure results for the various corrosion coupon materials indicate that system A (pH 9.2) resulted in some decrease in corrosion rates compared to the control, for all three alloys. Further increases in pH (i.e., systems B and C) showed little additional benefit. The 304 stainless steel and grade CDA110 copper corrosion took the form of a uniform etch, while the C1010 carbon steel showed evidence of some limited localized attack, in addition to general corrosion.

#### Discussion

# Preventing settlement of dreissenid mussels using elevated pH

Results for the settlement experiment at Lake Havasu showed that settlement was considerably lower at pH of 9.1 than in the control tank and at a pH of 8.9. No settlement data were available





**Figure 6.** Relationship between the shell length and total dry weight of zebra mussels at San Justo Reservoir.

for the pH 9.0 treatment due to an overdose of sodium hydroxide causing pH to rise to 12 for approximately 10 hours. Regardless of the failure of the pH 9.0 tanks, the Lake Havasu results suggest that elevated pH may hinder settlement of quagga mussels.

The San Justo Reservoir experiment also showed low settlement with significant differences between the control tanks and those at pH 9.2 and between the control tanks and those at pH 9.6. There was no significant difference between the control tanks and the system held at pH 9.4. The low settlement at San Justo Reservoir may have been a result of low veliger counts in the reservoir and, as such, results for this experiment should be interpreted with caution due to the small data set.

Although the Lake Havasu results showed reduced settlement, the experiment was hindered by the production of a precipitate as pH was increased. The pH was originally increased through the addition of sodium carbonate  $(Na_2CO_3)$ . Since the calcium levels of the lower Colorado River are high, with an average of 80 mg/L, it is believed that the precipitate was calcium carbonate. Even after the exchange of sodium hydroxide (NaOH) for the sodium carbonate, a precipitate formed in the test tanks. This suggests that the lower Colorado River is naturally high in carbonates and, in conjunction with the high calcium carbonate saturation index (SI).

The saturation index is used as an indicator of the scaling potential for water. A saturation index of zero represents a system at equilibrium. A saturation index below zero indicates the system is under-saturated and therefore unlikely to precipitate  $CaCO_3$ , and a saturation index above zero represents a system that is oversaturated and considered likely to precipitate CaCO<sub>3</sub>. Calcium carbonate exists as three different polymorphs (i.e., calcite, aragonite and vaterite). The most common form in freshwater systems is calcite (American Public Health Association - APHA 2005), and thus the saturation index for calcium carbonate is typically based on solubility coefficients for that polymorph. The saturation index for calcite (SI<sub>calcite</sub>) can be calculated from calcium concentration (mg/L), temperature (°C). alkalinity (mg CaCO<sub>3</sub>/L), conductivity ( $\mu$ S/cm), and pH (American Public Health Association -APHA 2005). Based on site specific average values for the aforementioned parameters, the SI<sub>calcite</sub> at the lower Colorado River site is 0.88, which indicates that the lower Colorado River is oversaturated with calcite. As pH increases, the SI<sub>calcite</sub> also increases. With all other variables held constant, increasing pH from 8.3 to 8.9 for the lower Colorado River site increases the SI<sub>calcite</sub> to 1.48. In comparison, the experiment at San Justo Reservoir did not result in the formation of a precipitate. Average parameter values for San Justo Reservoir result in a SIcalcite of 0.07. At the highest pH tested in the San Justo Reservoir experiment (i.e., pH 9.6), the SI<sub>calcite</sub> increased to 1.32. Although this value suggests a system with the potential to form CaCO<sub>3</sub> at elevated pH, other factors may be influencing the formation of a precipitate (Howard et al. 1984; Meyer 1984; House 1987; House et al. 1989; Neal et al. 2002). Saturation indices do provide some indication of water's potential to form a scale; however, they are limited in that they do not indicate that a scale will definitely form. As such, the SI<sub>calcite</sub> can be used as a preliminary indicator of the potential for the system to form a scale, but it should be followed by site specific testing at elevated pH to determine if the system will definitely form a precipitate.

# Impact of elevated pH on adult dreissenid mussels

During the elevated pH experiments, we examined potential effects on adult dreissenid mussels. Specifically, we looked for impacts on adult mortality and on the mussel shell lengthtotal dry weight relationship. The relationship between the shell length and total dry weight of a dreissenid mussel has been frequently used as an index of condition; at any given size, a heavier individual is normally considered to be in better condition. Having a population of organisms of various sizes, a shell length-total dry weight plot can similarly be used to assess their condition. The elevation of the fitted line provides an index of condition, with better condition being indicated by higher elevation of the line. The differences in shell length-total dry weight relationship between treatments can provide an indication that, although not causing mortality, a particular treatment is causing stress to the test animals.

The statistical analyses on results for quagga mussels in Lake Havasu at the lower Colorado River found that there was a considerable increase in mussel weight with both increase in shell length and pH. It is likely that the precipitate in the test tanks may have settled on the shells contributing to increased shell weight despite best efforts of rinsing the shells prior to drying them. At the San Justo Reservoir, pH had no statistically significant effect on shell length to total dry weight relationship of adult zebra mussels. This results further supports the likelihood of precipitate being responsible for shell weight increase at the lower Colorado site.

Adult guagga mussels in the Lake Havasu experiment experienced very low mortality (e.g. <2%) in all tanks except those in system B. An unexpected overdose of sodium hydroxide occurred in system B causing pH to increase to 12 resulting in complete mortality of adults within 12 hours. The short term exposure to very high pH experiment at San Justo Reservoir in May 2011 resulted in 90% mortality after 12 hours and 99% mortality after 24 hours. Coolers at a pH of 10 or 11 also experienced some adult mussel mortality, however it was very low (i.e.  $\leq$ 7% after 36 hours). This experiment was repeated at San Justo Reservoir in October 2011 at which time lower mortalities resulted and a greater length of time was required to achieve similar mortality rates as those seen in May 2011. For example, at a pH of 12, 120 hours were required to reach 90% mortality in October 2011, whereas only 12 hours were required to reach 90% mortality in the May 2011 experiment. Due to harsh conditions during winter 2010-2011, it is assumed that the mussels in San Justo

Reservoir were under stress at the time when the May 2011 experiment was completed. This may have contributed to the higher mortalities observed. During the October 2011 experiment, the mussels would have had several months to recover from the stressful winter and may have been more able to withstand the elevated pH conditions. Although mortalities in October 2011 were lower than those seen in the May 201, significant mortalities were observed. In fact, 50% of the maximum mortality reached in each treatment occurred at 102.2 h at pH 10, 88.7 h at pH 11, and as little as 14.5 h at pH 12. The very rapid mortality of quagga mussel adults at pH of 12 (system B overdose) suggests that quagga mussels may be more susceptible to high pH treatments than zebra mussels. The greater susceptibility of adult guagga mussels has been observed by the authors when working with other chemical control strategies. It would be desirable to test the effect of very high pH on quagga mussels using water from San Justo Reservoir to establish the differences between the species in response to very high pH.

In all very high pH experiments the mussels exhibited swelling and disintegration of body tissues indicating that possibly their osmoregulating system was adversely affected.

# Impact of elevated pH on materials of construction

When examining elevated pH as a possible control measure for dreissenid mussels, it is important to determine the impact that different pH levels may have on different materials commonly used for systems in contact with water. The water of the lower Colorado River is known to be very aggressive on a variety of metals under normal conditions as observed by the authors. In the test tanks for the lower Colorado River, elevated pH was found to have little effect on the corrosion rates for carbon steel and copper, compared to the control. However, the pH adjustment was found to significantly increase the corrosion penetration for stainless steel although in absolute terms the increase was small. At San Justo Reservoir, a decrease in corrosion rates was noted on all materials. Carbon steel and copper corrosion rates were lower than the control at all test pH levels. For stainless steel, corrosion rates were the lowest at a pH of 9.2 and they were comparable to the control at pH 9.4 and pH 9.6.

## Elevated pH as a dreissenid mussel control

Elevated pH was shown to inhibit settlement of dreissenid veligers and to cause tissue damage and increased mortality in adult mussels. The quick mortality observed when adult dreissenids were exposed to very high pH (i.e. pH 12) is a new finding, which offers the possibility of a novel end of season treatment for dreissenid mussel control. It is possible that quagga mussels may be more susceptible to high pH treatment than zebra mussels.

The observed impact the chemistry of raw water had at elevated pH levels was profound. Under certain conditions, elevated pH may result in the formation of calcium carbonate precipitate that may hinder the use of high pH as a control measure for mussels. Determination of the calcium carbonate saturation index may help identify systems that are more likely to be affected by calcium carbonate precipitate. Site specific testing is recommended to determine to which pH the system can be elevated without the formation of a precipitate. High pH waters may affect corrosion rates for materials of construction. The results from corrosion tests in this study suggest that water systems designed with carbon steel or copper pipes may be better able to withstand elevated pH treatments, whereas stainless steel systems may be at greater risk to corrosion. The variation in corrosion results at Lake Havasu and San Justo Reservoir indicate that it may be important to carry out site specific testing to more carefully determine the impact of elevated pH on materials of construction.

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