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Evaluating Low pH for Control of Zebra Mussels

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Executive Summary

Dreissenid mussels are an environmental and economic nuisance across North America. When 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 is one of chemical control, as it has often proven to be convenient and effective. The major advantage of 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 such as trihalomethanes (THM's) especially in water with high organic loads. Proprietary compounds used for mussel control, with one exception, have to be detoxified by bentonite clay. Both chlorine and proprietary products are non-selective and therefore toxic to all forms of aquatic life.

Dreissenid mussels have a relatively narrow range of pH tolerance, 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 dreissenid mussels in raw water systems by decreasing the pH level. A proof of principle experiment to verify this hypothesis was done on Lake Ontario with quagga mussels. Settlement was virtually prevented at pH 7.1 and 40% mortality of captive adult mussels was recorded at pH of 6.9 after 10 weeks.

The calcium level at the Lake Ontario experimental location is in the range of 40mg/L. California Department of Water Resources (DWR) wished to determine the response of zebra mussels to low pH levels when the ambient water calcium levels were in the range of 20 mg/L. A field experiment was carried out using a custom built flow-through laboratory using water from San Justo Reservoir in San Benito County, California. The experiment tested the ability of zebra mussel pediveligers to settle under conditions of depressed pH and the long-term survival of adult zebra mussels under the same conditions. The potential impact of depressed pH on the materials of construction was evaluated using corrosion coupons of mild steel, stainless steel and copper.

The settlement of dreissenid pediveligers was inhibited with decreasing pH. At the minimum pH tested of 7.0, there was approximately 90% reduction in the maximum settlement compared to the control. No significant mortality of adults was observed at any of the experimental pH levels. The analysis of length to dry shell weight relationship revealed that only at pH of 7 was there a significant loss of weight at any given shell length of captive zebra mussels when compared to the control.

In the second portion of the field experiment, we tested the response of adult zebra mussels to very low pH levels (i.e. pH 2, 3, and 4). At pH 3, there was a 100% mortality of adult mussels after 96 hours, whereas pH 2 and pH 4 had 69.9% and 52.4%, respectively. We conclude that pH depression could be used both as a preventative treatment to minimize settlement by zebra

mussels and as an end of season treatment to eliminate adults, provided the materials of construction are compatible with short term exposure to pH 3.

The results of the low pH experiment from San Justo Reservoir are somewhat different compared to the results obtained in the experiment on Lake Ontario using quagga mussels. This could be due to a difference in susceptibility of zebra and quagga mussels to low pH. Additionally, the conductivity of San Justo Reservoir water is extremely high compared to Lake Ontario. Depending on location and season, the conductivity in Lake Ontario oscillates between 300 and 400 µS/cm. During the experiment at San Justo Reservoir, the conductivity was greater than 600 µS/cm. In order to determine if the response to low pH is species or location specific, the adult exposure portion of the experiment should be repeated using captive quagga and zebra mussels together in the same exposure chamber.

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1 Introduction

When dreissenid mussels invade a new system, calcium and pH are two of the most important environmental variables which will determine the success or failure of the invasion. Without sufficient calcium (>15mg/L), dreissenids are not able to build their shells. How pH limits dreissenid success is less fully understood. Most adult mollusks have an upper and lower lethal pH threshold; the lower pH is generally near 6.5 and the upper pH is near 9 (Harman 1974). Larval stages are assumed to have lower tolerance to pH extremes than adults.

Limited information is available in the literature on the lower pH limit for dreissenid survival. Most authors place the lower pH limit for long-term zebra mussel survival at 7.3. This is based on studies by Ramcharan *et al.* (1992a; 1992b) where European data from 76 lakes pointed to an absence of zebra mussels when the pH fell below 7.3. Another frequently cited study states that veligers only develop to the settlement stage when pH ranges from 7.4 to 9.4, with an optimum of pH 8.4 at temperatures of 18-20˚C (Sprung 1993). 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 (Cohen and Weinsten 1998; Cohen 2008; Hayward and Estevez 1997) and Europe (Trichkova *et al.* 2007).

Only two studies were found linking calcium levels and pH to zebra mussel survival. Hincks and Mackie (1997) tested adult survival, juvenile growth rates and veliger production against different concentrations of calcium, alkalinity, total hardness, chlorophyll, and pH by rearing adults and newly settled juveniles collected from Lake St. Clair in water from 16 Ontario Lakes. Six of these lakes had mean calcium levels below 8.5 mg/L and a mean pH of 8.4 or less. In these low calcium waters all adults died within 35 days, juvenile growth rates were near zero or negative, and no veligers were produced. Studies by Nierzwicki-Bauer *et al.* (2000) documented that adult zebra mussels were able to survive in Lake George water (Ca=12 mg/L, pH=7.15), but the development of veligers failed unless both calcium and pH levels were raised. Lake George has had a minimal level of dreissenid infestation for the last ten years. The reason dreissenid mussels survive at all in Lake George is hypothesized to be due to limestone outcroppings in various parts of the lake which provide microzones with higher calcium levels.

A recent study by Claudi *et al.* (2012a) documented that under flow-through field conditions, adult quagga mussels suffered mortality of almost 40% at pH of 6.9 after 10 weeks of exposure. New settlement was essentially prevented at a pH of 7.1 regardless of the high level of calcium (approximately 40 mg/L) in the source water. This study examines the response of zebra mussels to low pH levels when the ambient water calcium levels are in the range of 20 mg/L. The experiment was carried out using a custom built flow-through laboratory at San Justo Reservoir in San Benito County, California.

2 Methodology

Raw water containing zebra mussel veligers was drawn from San Justo Reservoir into the flow-through laboratory. The water was then split into four streams. Each stream was directed into one of four 160 L mixing tanks. Three tanks had pH individually depressed by the addition of food grade phosphoric acid, the fourth tank had no pH adjustment and remained at the background pH. On exiting the mixing tanks, each water stream was further subdivided into three streams which flowed into individual settling tanks (Figure 1).

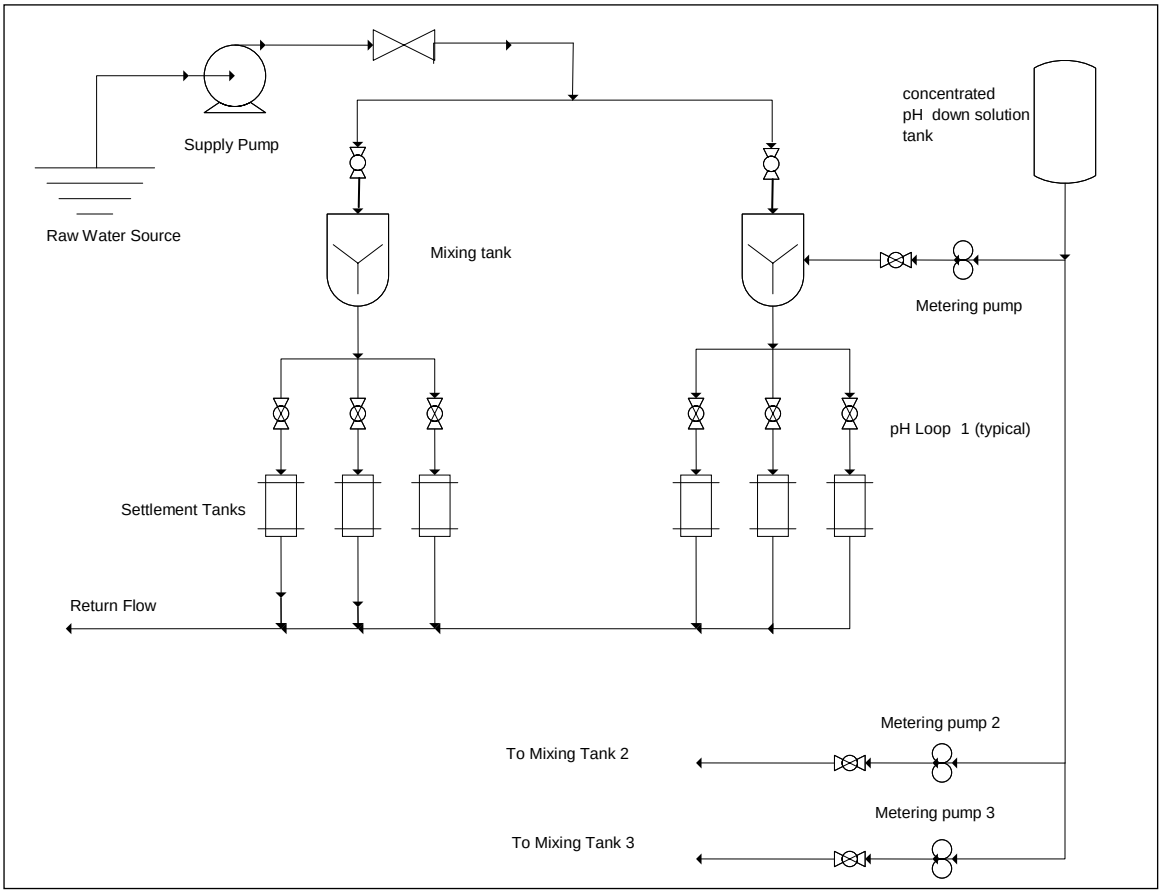

Figure 1. Schematic of the experimental layout showing two out of the four experimental loops in detail.

The settling tanks were insulated coolers with a capacity of 45 L. The coolers had been filled with lake water for approximately 10 weeks prior to this experiment; this pre-soak was performed to remove any potential contamination from the manufacturing process. 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 x 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 was also placed in each cooler (Figure 2). Two coupons each of C1010 carbon steel, 304 stainless steel, and grade CDA110 copper (all supplied by Metal Samples Co. Inc.) were arranged in such a manner as to avoid galvanic coupling between coupons. The coupons were placed downstream of the bag containing captive adults to avoid any potential effects of metal corrosion products. All mixing tanks were monitored continuously for pH and temperature using electronic probes connected to a programmable logic controller. The controller adjusted the pH by adding diluted phosphoric acid via the metering pumps. Prominent Beta-4 pumps were used for the addition of the acid to three of the four mixing tanks. All four tanks were continuously mixed using stainless steel propeller-style paddles. Water exited the bottom of each tank through a housing containing a flow sensor, temperature probe, and pH probe supplied by Prominent Controls. These probes, together with the control module (Dulco Marin 2), monitored and recorded all pH and temperature values, and sent an adjustment signal to the acid addition pumps to add more solution and lower the pH if necessary (Figure 3). Each cooler was tested twice per day with a hand-held pH meter. The readings were compared to the pH readings for the mixing tank displayed on the control module and recorded in a log book.

Figure 2. Settlement chamber with settlement plates, corrosion coupons, and mesh bag containing live adult mussels.

Figure 3. Flow-through laboratory in operation.

2.1 Settlement prevention and long-term impact on zebra mussel adults due to depressed pH

This experiment was carried out at San Justo Reservoir located in San Benito County, California. The water from the reservoir was brought to the laboratory through a 2-inch potable water polyethylene pipe using a 1.5 hp submersible Champion pump. The distance from the pump intake to the laboratory fluctuated somewhat due to fluctuating levels of the reservoir. The maximum distance from the pump intake to the laboratory was approximately 400ft.

The experiment began on August 5, 2011. The ambient water temperature was between 17 ºC and 24ºC, and the number of veligers in the water was very low (Table 1).

Table 1. Veliger density in San Justo Reservoir plankton samples (courtesy of DWR).

Date	Station-Tow	D-Shaped			Umbonal			Pediveliger		
		Intact	Empty	Total	Intact	Empty	Total	Intact	Empty	Total
07/23/11	Int-Hori	1.2	0.2	1.4	0.1	0.4	0.5	0.1	0.0	0.1
08/01/11	Int-Hori	3.0	2.2	5.1	0.0	0.0	0.0	0.0	0.0	0.0
08/01/11	Int-Vert	4.3	0.5	4.7	0.5	0.0	0.5	0.1	0.0	0.1
08/01/11	Int-Oblique	8.9	2.8	11.7	0.0	0.0	0.0	0.0	0.0	0.0

units = number per L

The nominal (target) pH adjustment for the long-term tests was set as follows:

System A: pH 6.9

System B: pH 7.1

System C: pH 7.3

System D: no adjustment (control: pH 7.9 to 8.6)

Temperature and pH data were recorded in a daily log (Appendix 1) both for the mixing tanks and for the individual settlement chambers. To aid mussel settlement within settlement tanks, additional plankton was collected daily and added to the settlement chambers, thereby providing additional veligers. Water from the reservoir was delivered to an area adjacent to the laboratory at a rate of approximately 500 L/hr and emptied into a plankton net (1 m mouth diameter, 53 micron net and bucket mesh) positioned within a large tank (volume of approximately 200 L) filled with water (Figure 4). This arrangement minimized 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 emptied from the net collection bucket into a separate vessel. The collected volume was diluted with reservoir 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 flow through each tank was calibrated each week to verify that the flow through each cooler was approximately 2 L/ min. Adjustments to flow were necessary as the flow had a tendency to diminish in some coolers.

Settlement was detected in the control tanks at the end of September 2011. On October 9, 2011, the corrosion coupons were removed and sent for analysis, and the settling plates and bags containing adults were examined for new settlement and adult mortalities. On October 10, 2011, the flow was stopped, the system was drained, and all experimental vessels were examined for settlement.

Figure 4. Close-up of plankton collection set-up.

2.1.1 Short term exposure of adult zebra mussels to very low pH

Following the settlement experiment described in the previous section, the acute effect of very low pH on captive adult zebra mussels was tested. On October 11, 2011, clumps of live adult mussels were placed in mesh bags and introduced into one cooler in each system. The number of mussels per bag varied as clumps were left intact to preserve the integrity of the adult shells. .

The nominal (target) pH adjustment for the short-term tests was set as follows:

System A: pH 2

System B: pH 3

System C: pH 4

System D: no adjustment (control: pH 8.8 to 8.9)

Each bag of adults was checked for mortality every 24 hours to a maximum of 96 hours. Two bags of mussels at pH 3 were also observed for mortality after 12 hours.

3 Results

3.1 Settlement prevention

The system performed flawlessly until September 9, 2011 when flow to the laboratory was interrupted by blockage of the intake line with fish parts. Flow was restored by September 12, 2011. During this period, the settlement tanks stayed filled and were manually refreshed with lake water to maintain adequate levels of dissolved oxygen. An interruption in acid delivery occurred on October 1, 2011 when the acid supply tank was depleted overnight. The appropriate pH was restored within 12 hours.

Figure 5 to Figure 7 document the pH levels in the individual systems. Figure 8 shows the pH recorded in the head tank supplying the control coolers. The calculated average pH for each settlement tank is shown in Table 2. During the experiment, the background pH in the control tanks fluctuated between 7.87 and 8.6, with an average of 8.27.

Table 2. Average pH recorded in each settlement tank.

Settlement tank	Average pH
Average all A	*7.03*
A1	7.03
A2	7.03
A3	7.04
Average all B	*7.18*
B1	7.18
B2	7.18
B3	7.19
Average all C	*7.37*
C1	7.37
C2	7.37
C3	7.38

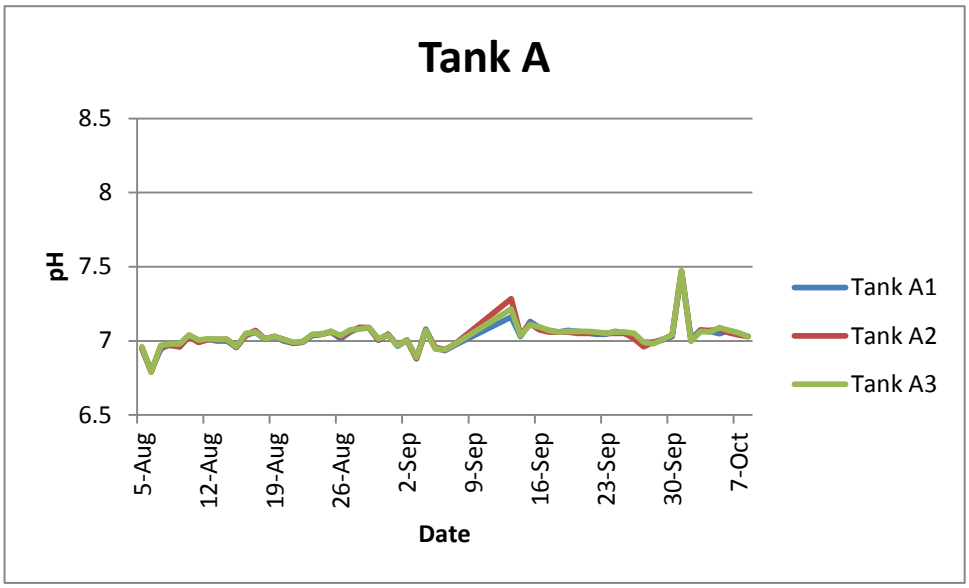

Figure 5. Average pH in test tanks of system A during the long-term experiment.

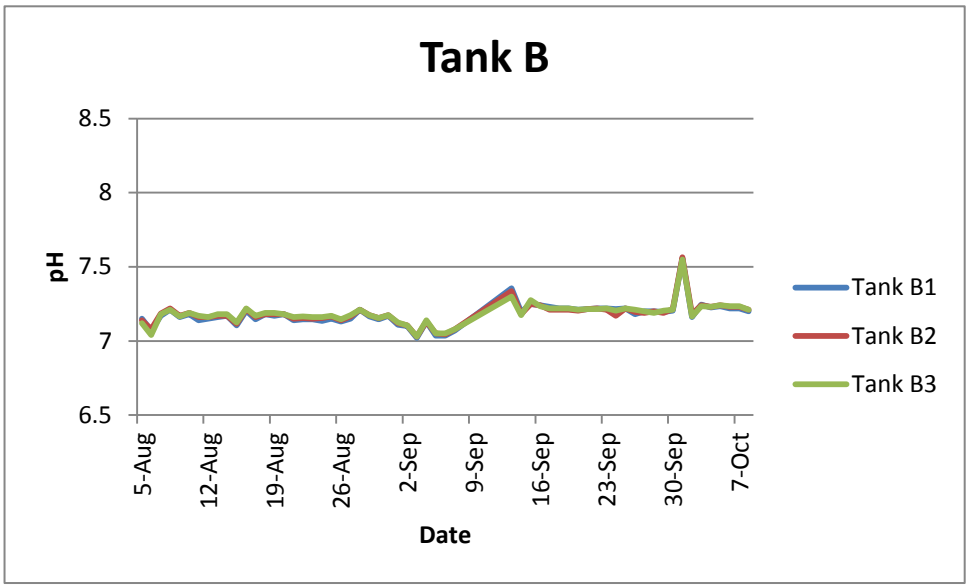

Figure 6. Average pH in test tanks of system B during the experiment.

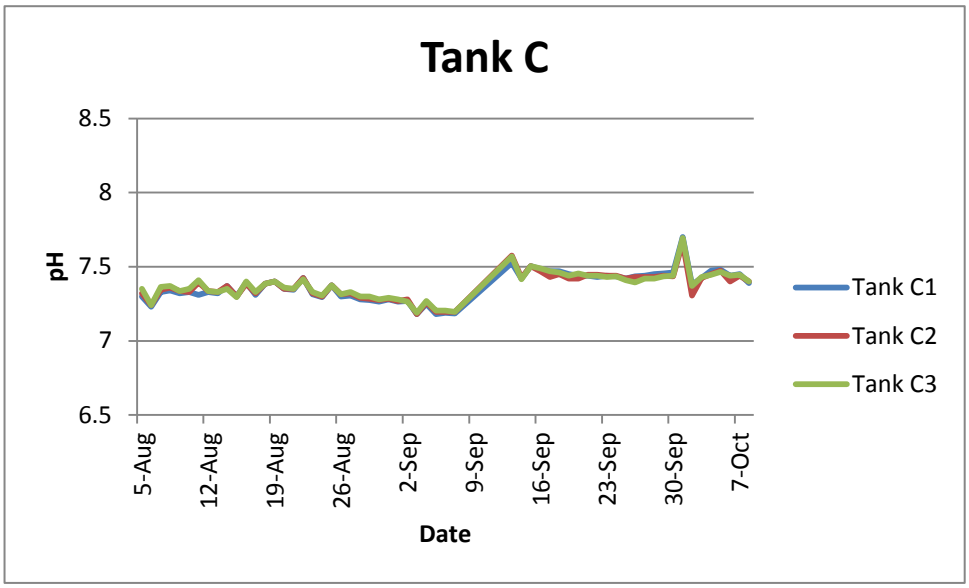

Figure 7. Average pH in test tanks of system C during the experiment.

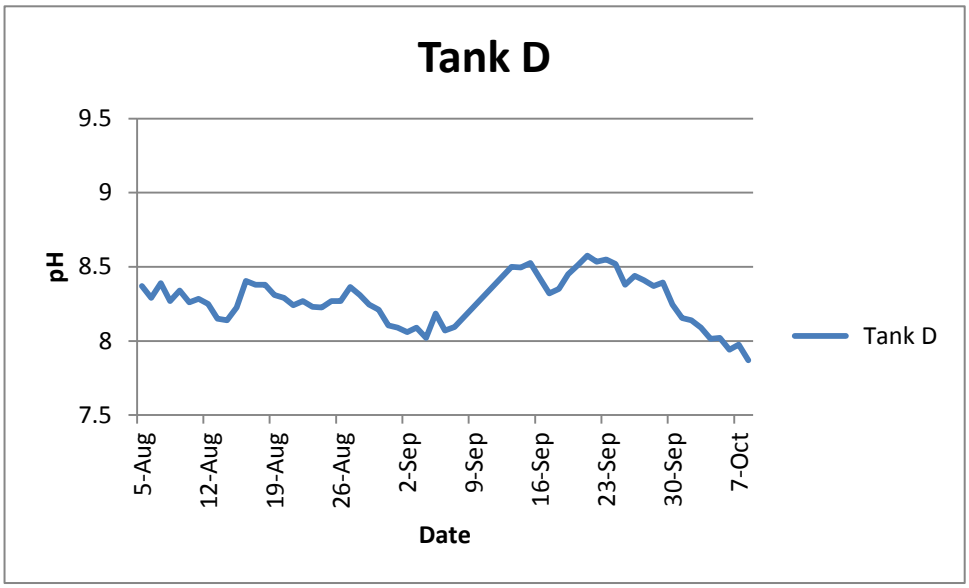

Figure 8. Average pH in the Control Head tank during the experiment.

Table 3 summarizes the settlement data we observed at the end of the experiment. Statistical analysis on the data in Table 3 is difficult due to the variation between individual coolers in each treatment, particularly in the control system (Figure 9).

Table 3. Settlement of zebra mussels in test tanks.

System	On Coupons	On Rack	On Bags of Adults	On Settlement Plates	In Cooler	Total Number
A1	0	1	2	3	8	14
A2	0	6	1	14	22	43
A3	0	0	0	0	5	5
B1	0	8	4	18	45	75
B2	0	3	1	17	29	50
B3	0	3	5	14	28	50
C1	0	6	10	48	65	129
C2	0	9	0	43	46	98
C3	2	3	0	11	28	44
D1	2	9	35	132	107	285
D2	1	1	9	48	23	82
D3	0	0	4	11	16	31

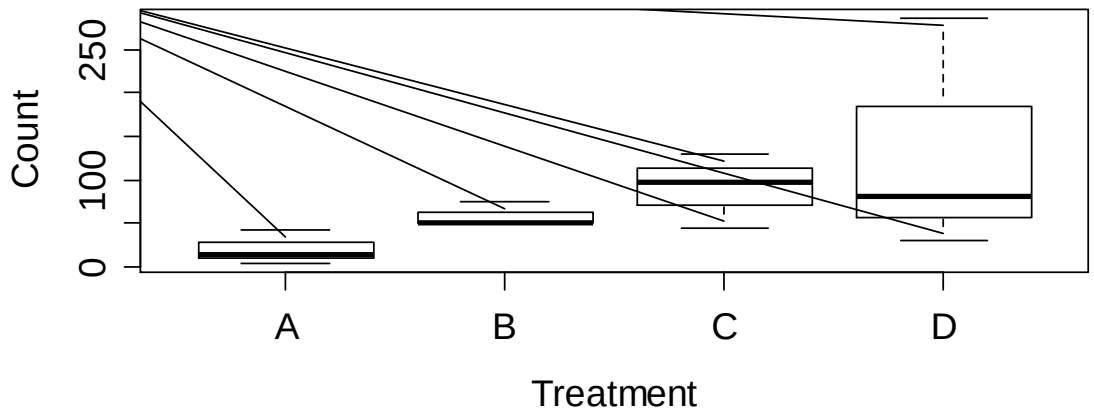

Figure 9. Boxplot of the settler counts for the four experimental pH conditions. A-pH 7.0; B-pH 7.2; C-pH 7.4; D-control. Horizontal lines within the boxes represent median values. The height of the box corresponds to the interquartile range (IQR). Whiskers are 1.5xIQR.

To account for the high variation in counts, a statistical model with a quasi-Poisson error distribution was applied (Zuur *et al.* 2002). The model was fitted with the help of *nlme* package for the R v2.13 statistical computing environment (R Development Core Team 2011). Despite a slight tendency for increase of the settlers' counts with pH (Figure 9), there was no statistically significant effect of pH on the mussel settlement (P = 0.086, chi-square test of the GLM deviance; treatment d.f. = 3, residual d.f. = 8).

This result may be due to variations between the test coolers which have not been accounted for by the model. In particular, the flow into each cooler had a tendency to vary in-between weekly calibrations. Lower flow will generally result in lower settlement as fewer individual pediveligers are brought into the cooler. In future experiments, the flow-through individual coolers will be monitored with flowmeters. The second variable was the presence of large snail populations in some coolers while they were absent in others. The feeding by snails, which is accomplished by the scraping of surfaces with rasp-like radula, may interfere with the settlement process of the mussels and may even result in their mortality. Although snails were observed in much greater numbers in some coolers compared to others, no notes were kept on their numbers and distribution. This variable will be recorded in future experiments.

Due to the variations in the coolers, the more indicative result of the experiment may be the comparison of maximum settlement achieved in each treatment (Figure 10). This comparison of settlement appears to clearly demonstrate the effect of decreasing pH. At pH 7 we see only 15% of the settlement recorded in the controls.

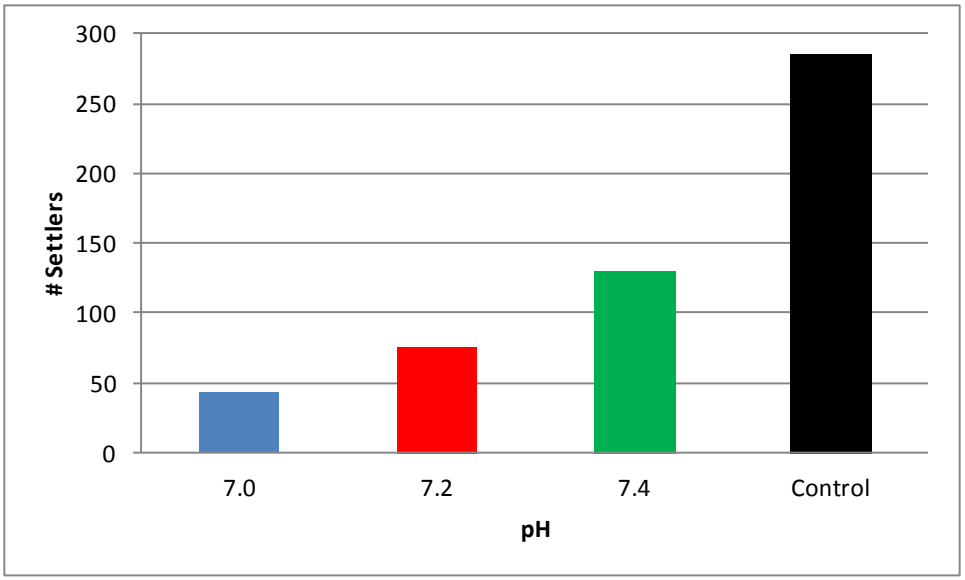

Figure 10. Maximum settlement recorded in each treatment.

3.2 Long-term survival of adult zebra mussels at depressed pH

As seen from Table 4, there was very low mortality of adult zebra mussels in any of the test coolers. In all treatments, the adults remained firmly clumped and had to be separated for counting. There were very few mussel shells which had any perforations even at a pH of 7. Three of the most affected mussels are shown in Figure 11.

Figure 11. Example of worst shell condition observed at the end of the experiment.

Table 4. Adult survival in the pH experiments.

Date	System	Cooler	Number of Adults	
			Alive	Dead
7-Oct-11	A	1	101	1
7-Oct-11	A	2	90	2
7-Oct-11	A	3	89	3
7-Oct-11	B	1	88	2
7-Oct-11	B	2	86	2
7-Oct-11	B	3	114	2
7-Oct-11	C	1	96	0
7-Oct-11	C	2	87	0
7-Oct-11	C	3	113	0
7-Oct-11	D	1	98	0
7-Oct-11	D	2	89	2
7-Oct-11	D	3	104	3

3.2.1 Changes in shell weight to length ratio in adult zebra mussels

The relationship between the size and the 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 length-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/weight relationship between treatment groups can indicate that a particular treatment is causing stress to the test animals, as indicated by different line elevations between treatment groups, even though mortality does not result. In dreissenids, the shell weight accounts for 90% to 95% of total dry weight recorded.

The live mussels from each of the mesh bags were placed in individual aluminum pans and dried for 3 hours at 350ºF. Subsequently, each mussel shell was measured using electronic calipers (Powerfist) and weighed to the nearest milligram using an electronic scale (GemPro-500). An exploratory analysis revealed four outliers which were removed from the subsequent analysis.

The relationship between length and weight of zebra mussels follows a power function and therefore, for purposes of line fitting and significance testing, the data were log-transformed. As the condition of zebra mussels might have randomly varied between individual replicate coolers, a linear mixed-effects model was fitted to assess the effect of pH on the length/weight relationship:

$$\log Weight_{ij} = \beta_0 + \beta_1 \times \log Length_{ij} + \beta_2 \times Treatment_{ij} + \beta_3 \times \log Length_{ij} : Treatment_{ij} + a_i + \varepsilon_{ij} \quad (1)$$

$LogWeight_{ij}$ is the log-transformed dry weight of mussels *j* from cooler *i*; β_0 is the overall mean log-transformed weight of mussels in control group; β_1 is the effect of shell length; β_2 is the effect of pH treatment; β_3 is the effect of interaction between shell length and pH treatment. The term a_i is a random intercept associated with the effect of cooler; it is assumed to be normally distributed with mean zero and variance σ^2_a. The residuals ε_{ij} are similarly assumed to have a normal distribution with mean zero and variance σ^2_i. The index *i* attached to the residual variance indicates that this variance was allowed to be different among experimental groups (see (Zuur *et al.* 2002) for details on this type of model parameterization). The analysis was conducted using the functionality of the *nlme* v3.1-100 package for R (Pinheiro *et al.* 2011).

All of the main effects in Model (1) appeared to be statistically significant (Table 5). The dry weight of zebra mussels was found to be strongly associated both with their shell length and the pH. Also, there was a statistically significant interaction between the pH treatment and shell length, suggesting that the character of relationship between the shell length and weight varied under different pH regimes.

In addition to the main effects, there was a low yet statistically significant random effect of the replicate coolers (σ^2_a = 0.038; lower 95% confidence limit = 0.021, upper 95% limit = 0.070).

This means that the average weight of mussels slightly yet significantly varied among the replicate coolers.

Table 5. Analysis of variance to test the effects of shell length and pH level on the dry weight of zebra mussels (as fitted by Model (1)).

Effect	Degrees of freedom for the F-statistic (nominator, denominator)	F-statistic	P-value of the F-test
Length	1, 1127	23155.625	< 0.001
Treatment	3, 8	7.502	0.010
Length:Treatment	3, 1127	4.217	0.007

In the control group, the relationship between shell length and weight was as follows:

$$\log Weight = -9.989 + 2.977 \times \log Length \quad \text{(Control group)}$$

A closer examination of the individual differences between the control and other treatments revealed the following patterns. In treatment A (pH ~ 7.0), there was a significant decrease (P < 0.00, t-test) of the effect of shell length and an insignificant (P = 0.125, t-test) increase of the regression intercept:

$$\log Weight = -9.706 + 2.834 \times \log Length \quad \text{(Treatment A)}$$

Treatment B (pH ~ 7.2) did not differ significantly from the control in the effect of shell length (P = 0.420, t-test) or regression intercept (P = 0.150, test):

$$\log Weight = -10.251 + 3.021 \times \log Length \quad \text{(Treatment B)}$$

Treatment C (pH ~ 7.4) similarly did not differ from the control (effect of the shell length: P = 0.509, test; intercept: P = 0.272, t-test):

$$\log Weight = -10.179 + 3.013 \times \log Length \quad \text{(Treatment C)}$$

All four regression lines (i.e. control and treatments A to C) are plotted in Figure 12. Thus, the overall significant effect of pH shown in Table 5 was only determined by the difference between control and treatment A in the effect of shell length. This analysis shows that only at pH of 7 is there a significant loss of weight at any given shell length when compared to control.

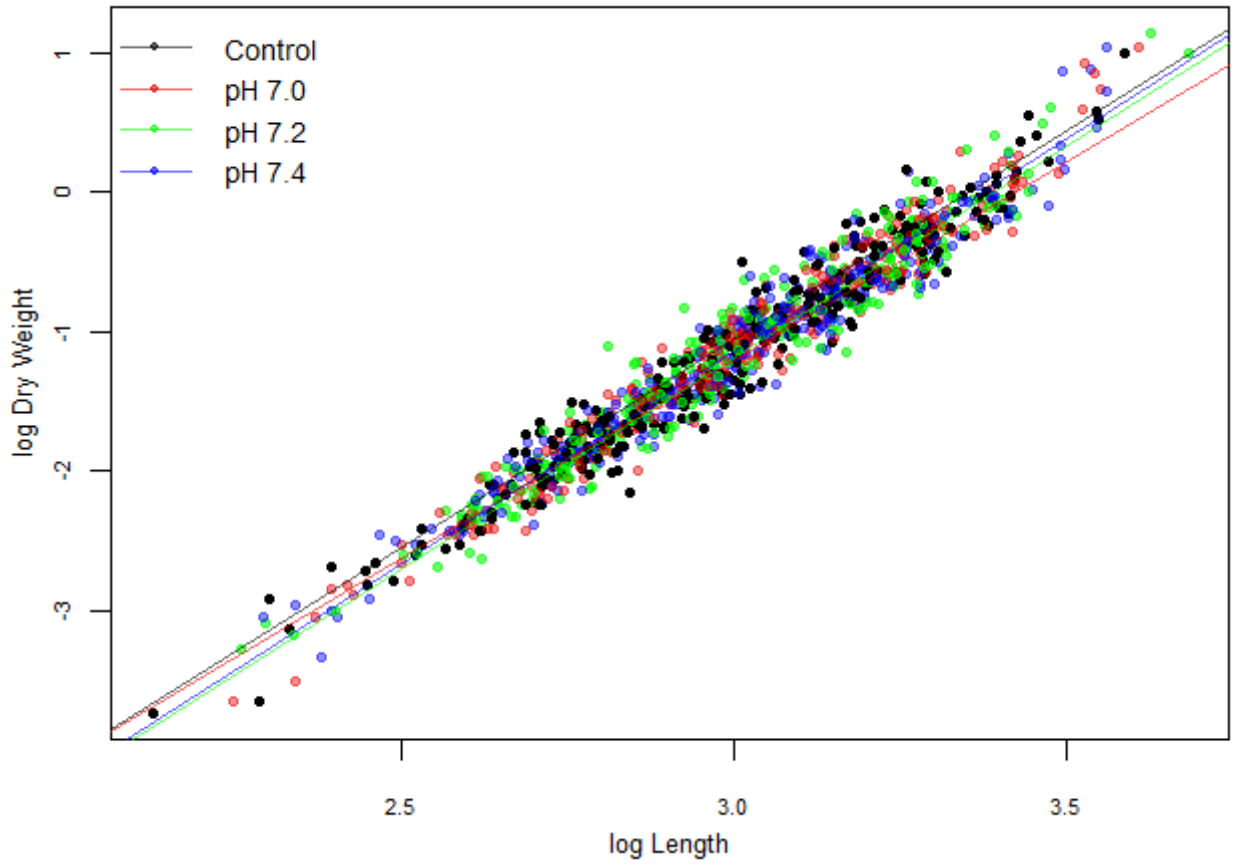

Figure 12. Relationships between the (log-transformed) shell length and dry weight of zebra mussels from San Justo Reservoir at four different pH regimes.

3.3 Corrosion Coupon Results

The corrosion coupons in Treatments A, B, C and D were exposed to the following pH conditions in the long-term tests:

System A: pH 7.03 (average)

System B: pH 7.18 (average)

System C: pH 7.37 (average)

System D: no pH adjustment (control: pH 7.9 to 8.6)

In all cases, the corrosion coupons were supplied and analyzed by Metal Samples Company, Inc. Average corrosion penetration rates were determined gravimetrically and all coupons were visually inspected to assess the nature of the attack (i.e. general etching versus localized attack). The long-term (1512 hours) exposure results for the various materials at each

treatment level are summarized in Table 6 and further details are given in Appendix 2. Table 6 shows that the pH reduction treatments had negligible effect on the rates of stainless steel and copper corrosion compared to the control.

Table 6. Average corrosion rates (mpy) for different coupon materials in the long-term pH treatments for the San Justo Reservoir low pH tests.‡

pH Treatment	Material		
	304 Stainless Steel	C1010 Carbon Steel	Grade CDA110 Copper
A	0.0414 ± 0.0072	1.902 ± 0.049	0.507 ± 0.036
B	0.0398 ± 0.0083	2.093 ± 0.203	0.527 ± 0.039
C	0.0392 ± 0.0074	2.689 ± 0.473	0.443 ± 0.032
D Control	0.0380 ± 0.0063	6.937 ± 0.588	0.508 ± 0.040

‡ 1512 hr nominal exposure time; mpy = mils per year;
to convert to μm/year multiply mpy values by 25.4

By way of comparison, the rates of stainless steel corrosion found in the reduced pH tests were somewhat higher than those found in the previously reported high pH tests using San Justo Reservoir water (Claudi *et al.* 2012b), where the stainless steel corrosion rates ranged from about 0.02 to 0.03 mpy (1680 to 1656 hr test duration). However, the stainless steel corrosion rates in the low pH tests were low in absolute terms and comparable to the corrosion rates in the untreated control.

Similarly, the corrosion rates for the grade CDA110 copper were slightly higher in these current low pH tests than in the previous elevated pH tests at San Justo Reservoir, where the copper corrosion rates ranged from about 0.31 to 0.42 mpy. That is not surprising since the minimum corrosion rate for copper is known to lie on the alkaline side of neutral in the absence of specific complexing agents (such as ammonia). However, the copper corrosion rates in the low pH tests are low in absolute terms and comparable to the corrosion rates in the untreated control.

More complex behaviour was observed for the C1010 grade carbon steel, where the corrosion rate was found to decrease with decreasing pH over the range tested (8.6 to 7.03). The corrosion rate for the control ("D") in the present tests (6.94 mpy) is in good agreement with the control corrosion rate found in the previous elevated pH tests at San Justo Reservoir (6.45 mpy). A standard representation of the effect of pH on carbon steel corrosion rates is shown in Figure 13. This suggests that the corrosion rate is relatively unaffected by pH over the range pH 10 to pH 3 at 22°C (72°F) or pH 8.5 to pH 4 at 40°C (104°F). The observed carbon steel corrosion rate for the pH 7.9 to 8.6 controls (6.95 mpy or 176 μm/year) is consistent with the generic corrosion rates in aerated water (Figure 13).

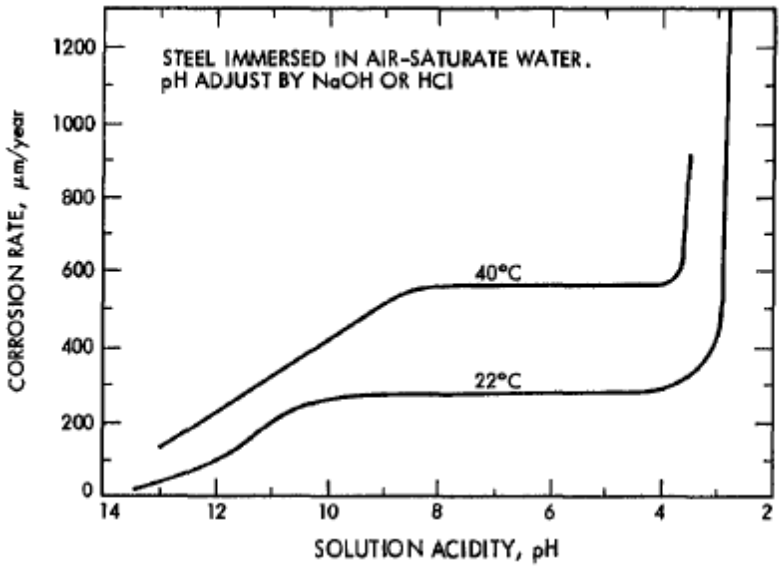

Figure 13. The effect of pH on carbon steel corrosion rates.

The lower carbon steel corrosion rates seen in the reduced pH treatments might seem counter-intuitive, but the pH reduction agent used in this study, phosphoric acid, can form an iron phosphate coating (known as a “conversion coating”) on carbon steel. Such conversion coatings confer increased corrosion resistance and form the basis for the wide-spread use of phosphate conversion coatings in the automotive sector. It should be noted that the corrosion performance resulting from the use of other acids for the pH adjustment could be significantly different than the corrosion rates obtained with phosphoric acid. For example, hydrochloric acid (HCl) would be expected to produce substantially higher carbon steel corrosion rates than those found for phosphoric acid, since the resulting reaction product, ferric chloride, is known to be an aggressive corrosion agent for mild steels.

The 304 SS and copper 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. Further details are given in Appendix 2.

3.4 Short term exposure of adult zebra mussels to very low pH

When adult mussels were placed in the individual treatments, approximately 100 ml of water was withdrawn from each test cooler into a glass beaker. At the designated time/exposure interval, five mussels were placed in each beaker and their behaviour was observed and mortality assessed. In beakers with water at pH 2 and 3, the mussels did not re-open, crawl, or re-attach during the entire duration of the experiment. Their mortality was not assessed as the

behaviour was the primary focus for the observation. At pH 4, the mussels did move within the beaker and were seen filtering within 3 hours of exposure.

Table 7 shows the cumulative mortality observed in the test coolers at each interval of the experiment. Mussels experienced the highest mortality at pH 3, with 47% mortality after 24 hours, 84.8% mortality after 48 hours, and 100% mortality after 96 hours. At pH 2, 56.3% mortality occurred after 72 hours. Nearly 30% of the mussels survived after 96 hours of exposure. At pH 4, only 52.4% mortality was achieved after 96 hours. The raw data are included in

Appendix 3. Data in Table 7 are plotted for graphical representation in Figure 14.

Table 7. Cumulative percent mortality of adult zebra mussels in low pH treatments.

Time (hr)	pH		
	2	3	4
12	-	33.5	-
24	16.8	47.0	1.1
48	34.9	84.8	8.8
72	56.3	96.3	40.9
96	69.9	100.0	52.4

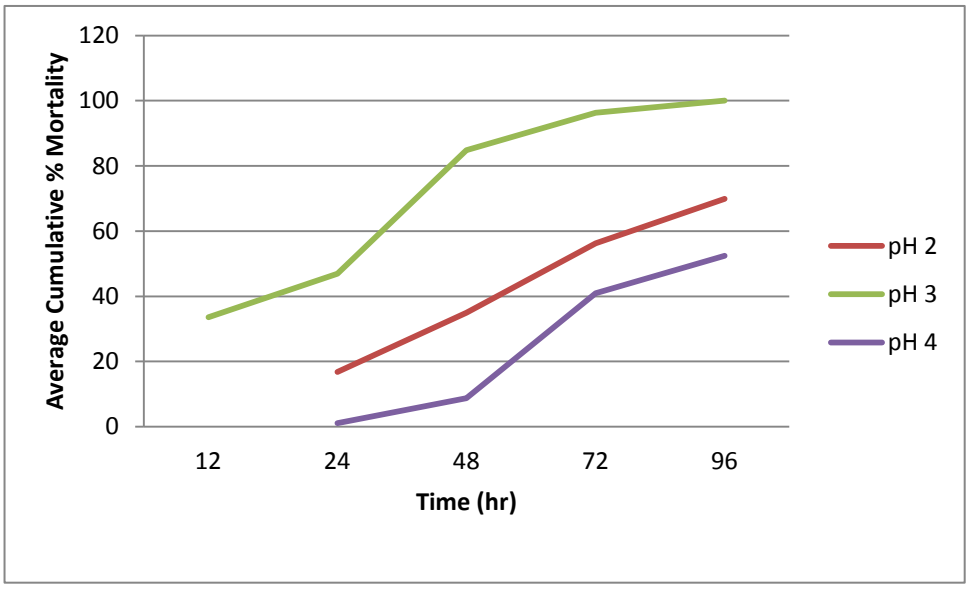

Figure 14. Mortality curves for adult zebra mussels in different treatments.

4 Discussion

The experiments described in this report confirm that chronic depression of pH can serve as a preventative treatment strategy for the control of zebra mussels (i.e., prevention of mussel settlement). Furthermore, extremely low pH (i.e., pH of 3 for 48 hours) could be used as an end of season treatment to eliminate settled adults. In both types of treatment, attention must be given to the materials of construction to insure their compatibility with pH depression.

When comparing the results obtained at San Justo Reservoir to those recorded for Lake Ontario, significant differences were observed both in veliger settlement and adult mortality under virtually the same pH conditions. In the Lake Ontario experiment, there was no primary settlement in treatments with pH of 7.1 and adults exposed to this pH suffered significant mortality after 10 weeks. At San Justo Reservoir, some settlement occurred at a pH of 7 and there was virtually no mortality of adults at this pH level after 8 weeks.

In Lake Ontario, the primary dreissenid species present is the quagga mussel, *Dreissena bugensis*. At San Justo Reservoir, the species present is the zebra mussel, *Dreissena polymorpha*. There may be some species-specific difference in response to low pH.

The minimum average pH in the lowest pH treatment was recorded as 6.9 on Lake Ontario and as 7.0 at San Justo Reservoir. This difference is within the industry accepted error for pH electrodes of Offset: 7.00 +/- 0.2 pH (+/- 12mV) (Ross 2004) and is therefore considered very small. The average pH in the lowest pH treatment in the San Justo experiment appeared to increase from the initial set-point of 6.9 to the final end point of 7.0. Field observation at both locations has shown that the pH in the mixing tank is slightly lower than the pH observed in the coolers at any given pH level. This is probably due to the relatively long retention time in the coolers. During this time, the natural buffering in the water will raise the pH slightly. In the San Justo experimental set-up, we controlled the pH based on real-time readings in the mixing tank. This resulted in slightly lower pH levels in the coolers than was desired. A better strategy would have been to set the mixing tanks at a slightly higher level and thus achieve the desired pH in the settlement coolers. The slight difference in pH may have contributed to the difference in results.

Another profound difference between the two experiments is the conductivity of the source water. During the experiment, the conductivity of San Justo Reservoir ranged from 613 µS/cm to 624 µS/cm. During the 2009 experiment in Lake Ontario, conductivity was not measured. However, Howell (2010) noted the conductivity of Lake Ontario varied from 300 µS/cm to 360 µS/cm in 2008 at a site near the experimental location.

It is possible that the high concentration of ions in San Justo Reservoir water is mitigating the impact of low pH on the zebra mussels in the experiment. Equally, the high conductivity, high alkalinity and relatively high pH may also be responsible for the large population of zebra mussels present in the reservoir despite what would normally be considered marginal calcium availability. Water bodies with calcium and pH levels similar to San Justo Reservoir, but with much lower conductivity (100-150 µS/cm) and alkalinity tend to have very low dreissenid populations (personal observation). This suggests that calcium, pH, alkalinity and conductivity may have to be considered together when assessing the vulnerability of a water body to mussel invasion, particularly in areas with relatively low calcium levels.

It would be beneficial to DWR to determine if quagga mussels have a different low pH tolerance than zebra mussels. For this, a side by side experiment using adults of the two species is suggested. At the same time, it would be possible to determine if high alkalinity and high conductivity will partially mitigate the impact of low pH. This could be done by running one set

of experiments in San Justo Reservoir water and a second set of experiments using water from Check 13 (225 µS/cm) or Sacramento River at Hood (140 µS/cm).

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Appendix 1

Raw data low pH. n/a = no data available.

				pH														
Date	Time	Lab Amb. Temp (°C)	Water Temp (°C)	Tank A mon.	Tank A hand	A1	A2	A3	Tank B mon.	Tank B hand	B1	B2	B3	Tank C mon	Tank C hand	C1	C2	C3
5-Aug	15:30	24.5	24.0	6.89	6.87	6.95	6.95	6.96	7.11	7.11	7.15	7.14	7.12	7.30	7.28	7.30	7.32	7.35
6-Aug	18:00	18.0	18.0	6.90	6.77	6.80	6.79	6.79	7.10	7.08	7.07	7.08	7.04	7.29	7.23	7.23	7.24	7.24
7-Aug	10:30	18.8	22.8	6.91	6.94	6.95	6.99	7.00	7.11	7.15	7.17	7.19	7.19	7.31	7.32	7.33	7.36	7.38
7-Aug	19:15	24.5	23.3	6.92	6.94	6.94	6.94	6.94	7.11	7.19	7.17	7.18	7.17	7.31	7.31	7.33	7.33	7.35
8-Aug	9:25	17.4	22.7	6.92	6.96	6.98	6.97	6.98	7.11	7.21	7.21	7.22	7.21	7.30	7.33	7.34	7.36	7.37
9-Aug	10:40	19.8	23.1	n/a	n/a	n/a	n/a	n/a	7.09	7.22	7.21	7.21	7.20	7.28	7.37	7.37	7.38	7.37
9-Aug	16:15	24.0	23.5	6.92	6.92	6.96	6.96	6.98	7.10	7.09	7.11	7.13	7.13	7.29	7.25	7.27	7.29	7.30
10-Aug	9:00	16.4	22.4	6.90	7.02	7.03	7.03	7.04	7.10	7.16	7.18	7.19	7.19	7.29	7.31	7.33	7.33	7.35
11-Aug	14:00	21.9	22.9	6.92	6.98	7.00	7.01	7.02	7.10	7.15	7.14	7.16	7.17	7.28	7.26	7.31	7.47	7.48
11-Aug	17:30	21.2	22.9	6.92	6.96	6.98	6.97	6.99	7.10	7.14	7.14	7.16	7.17	7.31	7.30	7.31	7.32	7.34
12-Aug	10:00	17.1	22.5	6.91	7.01	7.02	7.03	7.04	7.11	7.16	7.16	7.17	7.17	7.31	7.30	7.33	7.34	7.34
12-Aug	16:35	23.7	23.6	6.90	6.98	7.00	6.99	6.99	7.10	7.13	7.14	7.15	7.15	7.32	7.29	7.33	7.33	7.33
13-Aug	9:45	18.5	22.6	6.90	6.98	7.00	7.01	7.01	7.11	7.13	7.16	7.17	7.18	7.31	7.29	7.32	7.33	7.33
14-Aug	8:50	19.3	22.6	6.91	6.99	7.00	7.01	7.01	7.11	7.15	7.17	7.17	7.18	7.31	7.35	7.37	7.37	7.35
15-Aug	9:05	21.5	23.0	6.91	6.95	6.96	6.97	6.98	7.12	7.11	7.13	7.14	7.15	7.32	7.27	7.29	7.30	7.30
15-Aug	14:40	28.0	24.4	6.91	6.92	6.95	6.94	6.94	7.10	7.06	7.08	7.09	7.10	7.32	7.31	7.31	7.30	7.29
16-Aug	9:30	17.8	22.9	6.90	7.06	7.07	7.08	7.09	7.11	7.21	7.23	7.24	7.24	7.31	7.37	7.41	7.41	7.42
16-Aug	14:20	26.7	22.9	6.91	6.97	7.00	7.00	7.01	7.10	7.15	7.18	7.19	7.20	7.32	7.36	7.38	7.36	7.38
17-Aug	11:00	22.5	23.5	6.92	7.02	7.08	7.09	7.07	7.11	7.18	7.11	7.14	7.15	7.31	7.40	7.27	7.29	7.31
17-Aug	16:25	26.5	24.1	6.91	7.04	7.04	7.05	7.05	7.11	7.15	7.18	7.18	7.19	7.32	7.33	7.35	7.35	7.35
18-Aug	9:20	17.4	22.9	6.91	6.97	7.03	7.04	7.03	7.11	7.12	7.16	7.17	7.19	7.31	7.37	7.39	7.39	7.39
18-Aug	15:55	23.0	20.6	6.90	6.96	6.99	6.99	7.00	7.09	7.17	7.20	7.19	7.19	7.31	7.35	7.38	7.38	7.38
19-Aug	12:40	22.4	23.4	6.90	7.00	7.03	7.03	7.03	7.09	7.14	7.17	7.18	7.19	7.33	7.39	7.40	7.40	7.40
20-Aug	11:20	20.2	22.8	6.92	6.97	7.00	7.01	7.01	7.12	7.16	7.18	7.18	7.18	7.31	7.32	7.35	7.35	7.36
21-Aug	11:05	18.7	22.8	6.91	6.95	6.99	6.99	7.00	7.11	7.10	7.14	7.15	7.16	7.32	7.33	7.35	7.36	7.36
21-Aug	16:30	24.2	23.3	6.91	6.95	6.98	6.98	6.98	7.11	7.13	7.14	7.15	7.16	7.30	7.31	7.34	7.34	7.34
22-Aug	11:10	19.4	22.8	6.91	6.98	6.99	7.00	7.00	7.10	7.11	7.15	7.16	7.17	7.31	7.43	7.44	7.44	7.43
22-Aug	15:55	26.4	23.6	6.91	6.98	6.99	6.99	6.99	7.10	7.11	7.14	7.15	7.16	7.31	7.41	7.41	7.41	7.40
23-Aug	11:00	24.5	23.1	6.91	7.08	7.08	7.08	7.09	7.10	7.15	7.17	7.18	7.19	7.31	7.29	7.33	7.34	7.35
23-Aug	16:10	31.0	22.0	6.91	6.98	6.99	7.00	7.00	7.10	7.10	7.12	7.12	7.13	7.31	7.27	7.30	7.30	7.31
24-Aug	8:55	20.2	22.8	6.90	7.04	7.06	7.06	7.06	7.11	7.11	7.14	7.16	7.16	7.31	7.26	7.29	7.30	7.30
24-Aug	14:25	28.9	24.1	6.91	7.00	7.03	7.03	7.03	7.11	7.10	7.13	7.15	7.16	7.31	7.27	7.30	7.30	7.31
25-Aug	9:15	20.1	22.9	6.91	7.05	7.06	7.07	7.07	7.11	7.11	7.14	7.16	7.17	7.31	7.37	7.38	7.38	7.38
25-Aug	14:05	27.1	24.2	6.92	7.05	7.06	7.06	7.06	7.12	7.12	7.16	7.17	7.17	7.31	7.36	7.37	7.37	7.37
26-Aug	9:10	18.3	22.6	6.91	6.91	6.95	6.97	6.99	7.11	7.05	7.10	7.11	7.12	7.33	7.30	7.32	7.33	7.33
26-Aug	15:45	26.8	24.2	6.91	7.07	7.08	7.08	7.08	7.10	7.13	7.16	7.17	7.17	7.31	7.25	7.28	7.30	7.30
27-Aug	9:55	19.9	22.8	6.91	7.04	7.06	7.06	7.08	7.10	7.12	7.15	7.17	7.18	7.30	7.29	7.33	7.34	7.35
27-Aug	14:40	26.5	24.2	6.90	7.04	7.06	7.07	7.07	7.11	7.11	7.15	7.16	7.17	7.29	7.24	7.28	7.30	7.31
28-Aug	10:40	19.4	22.8	6.91	7.08	7.09	7.09	7.08	7.12	7.19	7.21	7.21	7.21	7.28	7.25	7.28	7.29	7.30
29-Aug	8:55	16.1	22.5	3.90	7.10	7.11	7.12	7.12	7.10	7.19	7.21	7.21	7.21	7.31	7.25	7.28	7.29	7.30
29-Aug	14:10	24.8	23.9	6.90	7.05	7.06	7.06	7.06	7.11	7.09	7.12	7.14	7.14	7.31	7.24	7.27	7.28	7.30
30-Aug	10:15	17.6	22.7	6.91	6.91	6.94	6.94	6.95	7.11	7.11	7.13	7.14	7.14	7.31	7.23	7.26	7.27	7.27
30-Aug	15:05	23.6	22.9	6.91	7.06	7.07	7.07	7.08	7.12	7.14	7.16	7.17	7.17	7.32	7.23	7.27	7.28	7.29
31-Aug	9:55	17.5	22.4	6.92	7.05	7.07	7.07	7.06	7.11	7.13	7.15	7.16	7.16	7.31	7.25	7.28	7.28	7.29
31-Aug	14:20	21.7	23.3	6.92	6.99	7.02	7.02	7.02	7.12	7.16	7.19	7.19	7.19	7.32	7.26	7.28	7.29	7.29
1-Sep	8:40	18.1	22.2	6.92	6.94	6.96	6.96	6.96	7.12	7.10	7.11	7.12	7.13	7.31	7.23	7.25	7.26	7.27
1-Sep	15:20	24.2	23.3	6.92	6.94	6.97	6.98	6.98	7.12	7.09	7.11	7.12	7.12	7.32	7.26	7.28	7.28	7.29
2-Sep	10:00	21.2	22.4	6.92	6.99	7.01	7.01	7.01	7.12	7.09	7.11	7.11	7.11	7.32	7.26	7.28	7.29	7.29
2-Sep	15:00	25.9	23.6	6.92	6.98	7.00	7.00	7.00	7.12	7.08	7.09	7.10	7.10	7.31	7.24	7.26	7.27	7.24
3-Sep	12:00	22.9	22.9	6.91	6.87	6.88	6.88	6.89	7.10	7.00	7.02	7.03	7.03	7.31	7.16	7.18	7.18	7.19
4-Sep	11:05	19.5	22.4	6.91	7.07	7.08	7.07	7.07	7.11	7.12	7.13	7.13	7.14	7.31	7.23	7.25	7.26	7.27
5-Sep	8:45	17.2	21.7	6.91	6.94	6.95	6.96	6.94	7.10	7.02	7.04	7.05	7.05	7.30	7.16	7.18	7.20	7.20
5-Sep	15:00	26.7	23.4	6.91	6.93	6.95	6.95	6.95	7.10	7.01	7.03	7.05	7.05	7.31	7.16	7.18	7.19	7.21
6-Sep	9:05	17.7	22.2	6.91	6.96	6.98	6.98	6.98	7.10	7.03	7.05	7.07	7.07	7.31	7.17	7.19	7.20	7.21
6-Sep	15:45	32.4	23.7	6.90	6.88	6.89	6.90	6.90	7.12	7.00	7.02	7.02	7.03	7.32	7.18	7.19	7.19	7.20
7-Sep	9:00	21.2	22.6	6.91	7.01	7.01	7.01	7.01	7.11	7.08	7.10	7.11	7.11	7.30	7.16	7.19	7.20	7.20
7-Sep	15:00	32.5	24.4	6.91	6.92	6.93	6.94	6.94	7.11	7.02	7.04	7.05	7.05	7.30	7.16	7.18	7.19	7.19
8-Sep	7:15	13.3	20.9	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a

Appendix 2

Corrosion Test Results from the Long-term pH Reduction Tests at the San Justo Reservoir

Material	Location	Coupon ID #	Penetration Rate	Nature of Attack		Average	Standard Dev
			mpy			mpy	mpy
304	A1	0025	0.0360	uniform etch	A1	0.0414	0.0076
304	A1	0026	0.0467	uniform etch			
304	A2	0027	0.0520	uniform etch	A2	0.0460	0.0085
304	A2	0028	0.0400	uniform etch			
304	A3	0029	0.0414	uniform etch	A3	0.0367	0.0066
304	A3	0030	0.0320	uniform etch			
304	B1	0031	0.0294	uniform etch	All A Coupons	0.0414	0.0072
304	B1	0032	0.0320	uniform etch			
304	B2	0033	0.0360	uniform etch	B1	0.0307	0.0018
304	B2	0034	0.0467	uniform etch			
304	B3	0035	0.0480	uniform etch	B2	0.0414	0.0076
304	B3	0036	0.0467	uniform etch			
304	C1	0037	0.0467	uniform etch	B3	0.0474	0.0009
304	C1	0038	0.0267	uniform etch			
304	C2	0039	0.0374	uniform etch	All B Coupons	0.0398	0.0083
304	C2	0040	0.0467	uniform etch			
304	C3	0041	0.0374	uniform etch	C1	0.0367	0.0141
304	C3	0042	0.0400	uniform etch			
304	D1	0043	0.0427	uniform etch	C2	0.0421	0.0066
304	D1	0044	0.0400	uniform etch			
304	D2	0045	0.0307	uniform etch	C3	0.0387	0.0018
304	D2	0046	0.0294	uniform etch			
304	D3	0047	0.0414	uniform etch	All C Coupons	0.0392	0.0074
304	D3	0048	0.0440	uniform etch			
					D1	0.0414	0.0019
					D2	0.0301	0.0009
					D3	0.0427	0.0018
					All D Coupons	0.0380	0.0063
					All 304 SS Coupons	0.0396	0.0070

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Material	Location	Coupon ID #	Penetration Rate mpy	Nature of Attack		Average mpy	Standard Dev mpy
C1010	A1	B94791	1.8205	general plus localized	A1	1.8718	0.0725
C1010	A1	B94792	1.9231	general plus localized			
C1010	A2	B94793	1.8791	general plus localized	A2	1.9198	0.0575
C1010	A2	B94794	1.9604	general plus localized			
C1010	A3	B94795	1.9297	general plus localized	A3	1.9131	0.0235
C1010	A3	B94796	1.8964	general plus localized			
C1010	B1	B94797	2.4055	general plus localized	All A Coupons	1.9015	0.0486
C1010	B1	B94798	1.9284	general plus localized			
C1010	B2	B94799	2.2762	general plus localized	B1	2.1670	0.3374
C1010	B2	B94800	2.0630	general plus localized			
C1010	B3	B94813	1.9231	general plus localized	B2	2.1696	0.1508
C1010	B3	B94814	1.9604	general plus localized			
C1010	C1	B94815	1.8564	general plus localized	B3	1.9418	0.0264
C1010	C1	B94816	2.7520	general plus localized			
C1010	C2	B94817	3.1158	general plus localized	All B Coupons	2.0928	0.2028
C1010	C2	B94818	2.4402	general plus localized			
C1010	C3	B94819	3.0332	general plus localized	C1	2.3042	0.6333
C1010	C3	B94820	2.9346	general plus localized			
C1010	D1	B94821	7.6430	general plus localized	C2	2.7780	0.4777
C1010	D1	B94822	7.2205	general plus localized			
C1010	D2	B94823	6.0571	general plus localized	C3	2.9839	0.0697
C1010	D2	B94824	6.4409	general plus localized			
C1010	D3	B94825	7.2978	general plus localized	All C Coupons	2.6887	0.4733
C1010	D3	B94826	6.9606	general plus localized			
					D1	7.4318	0.2988
					D2	6.2490	0.2714
					D3	7.1292	0.2384
					All D Coupons	6.9367	0.5882
					All C1010 Coupons	3.4049	2.1354

Material	Location	Coupon ID #	Penetration Rate mpy	Nature of Attack		Average mpy
CDA110	A1	0349	0.5397	uniform etch	A1	0.5185
CDA110	A1	0350	0.4973	uniform etch		
CDA110	A2	0351	0.4737	uniform etch	A2	0.4731
CDA110	A2	0352	0.4725	uniform etch		
CDA110	A3	0353	0.5609	uniform etch	A3	0.5297
CDA110	A3	0354	0.4984	uniform etch		
CDA110	B1	0355	0.5621	uniform etch	All A Coupons	0.5071
CDA110	B1	0356	0.5338	uniform etch		
CDA110	B2	0357	0.5279	uniform etch	B1	0.5480
CDA110	B2	0358	0.5303	uniform etch		
CDA110	B3	0359	0.4537	uniform etch	B2	0.5291
CDA110	B3	0360	0.5550	uniform etch		
CDA110	C1	0361	0.4160	uniform etch	B3	0.5044
CDA110	C1	0362	0.4949	uniform etch		
CDA110	C2	0363	0.4101	uniform etch	All B Coupons	0.5271
CDA110	C2	0364	0.4584	uniform etch		
CDA110	C3	0365	0.4537	uniform etch	C1	0.4555
CDA110	C3	0366	0.4277	uniform etch		
CDA110	D1	0367	0.4808	uniform etch	C2	0.4343
CDA110	D1	0368	0.4961	uniform etch		
CDA110	D2	0369	0.4702	uniform etch	C3	0.4407
CDA110	D2	0370	0.5149	uniform etch		
CDA110	D3	0371	0.5032	uniform etch	All C Coupons	0.4435
CDA110	D3	0372	0.5821	uniform etch		
					D1	0.4885
					D2	0.4926
					D3	0.5427
					All D Coupons	0.5079
					All CDA110 Coupons	0.4964

Appendix 3

Low pH Mortality Data

pH	Bag	Time Elapsed	# Live	# Dead
2	1	24	55	12
2	1	48	38	17
2	1	72	20	18
2	1	96	15	5
2	2	24	87	17
2	2	48	67	20
2	2	72	45	22
2	2	96	26	19
2	3	24	94	18
2	3	48	83	11
2	3	72	65	18
2	3	96	48	17
3	1	24	61	54
3	1	48	18	43
3	1	72	7	11
3	1	96	0	7
3	2	12	65	52
3	2	48	13	52
3	2	72	6	7
3	2	96	0	6
3	3	12	82	24
3	3	48	20	62
3	3	72	0	20
3	3	96	0	0
4	1	24	91	2
4	1	48	84	7
4	1	72	47	37
4	1	96	32	15
4	2	24	102	0
4	2	48	94	8
4	2	72	69	25
4	2	96	62	7