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Research Article

Impact of pH on survival and settlement of dreissenid mussels

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Editor's note:
This special issue of *Aquatic Invasions* includes papers from the 17th International Conference on Aquatic Invasive Species held in San Diego, California, USA, on August 29 to September 2, 2010. This conference has provided a venue for the exchange of information on various aspects of aquatic invasive species since its inception in 1990. The conference continues to provide an opportunity for dialog between academia, industry and environmental regulators within North America and from abroad.

Abstract

A field experiment was conducted in 2009 using Lake Ontario water in a continuous flow through system to determine the impact of low pH on dreissenid mussel (zebra mussel, *Dreissena polymorpha* Pallas, 1771, and quagga mussel, *Dreissena rostriformis bugensis* Andrusov, 1897) settlement and survival in calcium rich waters. Raw water containing veligers was pumped to the field laboratory where the incoming water was divided into four streams. Three of the streams had the pH adjusted using phosphoric acid to pH 7.3, 7.1 and 6.9. The fourth stream was used as a control. Three replicates of each pH resulted in 9 treatment tanks and three control tanks. Three bags of caged adults were placed in each tank. Adult mortality of almost 40% was recorded at a pH of 6.9 after 10 weeks of exposure. Analysis of the weight/length relationship of adult mussels confirmed that for any given length the mussels were significantly lighter at all three pH levels when compared to the controls. Visually, erosion and perforation of the shells was noted, leading to the conclusion that the loss in weight was primarily due to loss of calcium from the shells of the adults. The visual loss of calcium was the greatest at a pH of 6.9. New settlement was essentially prevented at a pH of 7.1. Based on these results, downward adjustment of pH in calcium rich waters may be a viable treatment for prevention of dreissenid fouling in industrial cooling water systems and raw water conveyances.

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

Introduction

Dreissenid mussels are aggressive bio-foulers. These non-native invasive 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 tends to be one of chemical control, as it has often proven to be 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. Additionally, under hot sunny conditions, chlorine dissipates quickly in open channel applications, such as aqueducts. In such situations, multiple points of chlorine addition are required in order to maintain adequate treatment levels throughout the system. 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.

When dreissenid mussels invade a new system, calcium and pH are the two most important environmental variables which will determine the success or failure of the invasion. As summarized by Cohen and Weinstein (2001), a number of authors have examined the calcium and pH limits of dreissenid mussels. Of particular note is the study by Nierzwicki-Bauer et al. (2000) documenting some adult mussels survival in Lake George water (calcium 12 mg/L, pH 7.15) but failure of veligers unless both calcium and pH levels were raised. How low pH at high calcium levels limits dreissenid success has not been explored. The only exception is the report by Smythe et al. (1998), who found that at pH of 5, even with adequate calcium levels, adult zebra mussels experienced significant mortalities after one month of exposure. What happens to veligers in water with sufficient calcium but low pH has not been studied.

As dreissenid mussels have a relatively narrow range of pH tolerance, with the optimum range being 7.5 to 9.3, we 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 with a single point addition of acid. A proof of principle experiment was required to verify this hypothesis and to collect data that would allow for the comparison of the cost of lowering pH with that of more conventional methods of biofouling control.

Materials and methods

Study area

The experiment drew water from a relatively shallow, sheltered bay in the eastern part of Lake Ontario. This area has had a well established population of dreissenid mussels for more than 15 years. At present, a mixed population of quagga and zebra mussels is colonizing this area, with quagga mussels now becoming more populous (pers. observation by authors). All industrial facilities in the area are, therefore, dealing with mixed populations of dreissenid mussels. The pH of the water in the bay oscillates between 7.8 and 8.5 and the recorded alkalinity is over 103 mg/L as calcium carbonate. The alkalinity reading was obtained from the local water treatment plant and a calcium level was calculated using the following relationship:

$$Ca + CO_3 = CaCO_3 \qquad (1)$$

This relationship (1) assumes that all alkalinity is due to calcium carbonate. The amount of calcium predicted by this calculation may be higher than the actual level as there will be some magnesium (as magnesium carbonate) present. This, however, has been ignored in the calculation. At an alkalinity of 103 mg/L, the calcium level at the research site was estimated to be approximately 41 mg/L (40 mg/L of calcium is the value assumed for Lake Ontario by majority of publications). This level of calcium is generally steady throughout the year due to the large volume of Lake Ontario and its long retention time. Having confirmed that the area has plentiful calcium to support massive dreissenid population, this variable was no longer monitored, and the focus of the study was on the effect of pH.

Research set-up

Water was withdrawn continuously from the bay at a depth of 4.5 m located 60.6 m from the shoreline. The water was pumped up through a plastic foot valve into a 2.54 cm potable water pipe which brought the water to the laboratory. The rate of flow was approximately 6 L/min. Once in the laboratory, the water was split into four streams and directed to four separate mixing tanks (Figure 1).

Each mixing tank had a volume of 175 L (Figure 2). The water was introduced into the top of each mixing tank. There was an overflow at the top of each tank to maintain a constant level. The flow was adjusted so that some overflow was always present. In three of the tanks, diluted phosphoric acid was added at a predetermined rate to obtain the desired pH. Prominent brand Beta-4 pumps were used for the addition of the acid. The fourth tank was the control and contained lake water only. 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. These probes, together with the control module (Dulco Marin 2), monitored and recorded all pH and temperature values and, if necessary, sent an adjustment signal to the phosphoric acid addition pumps.

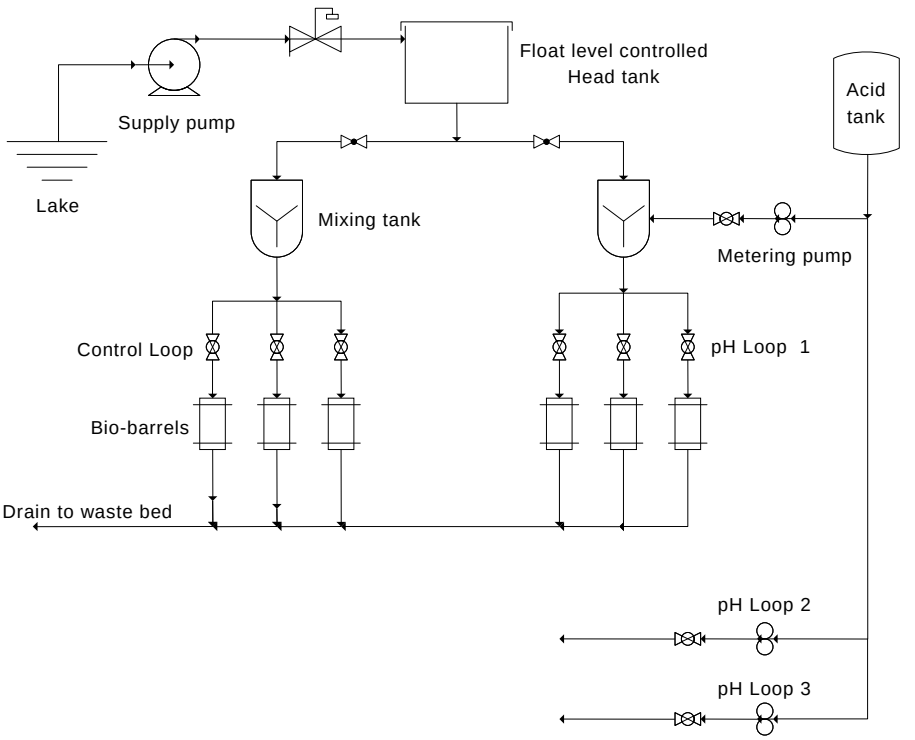

Figure 1. Schematic representation of laboratory set-up showing two of the four streams in detail.

Water exiting each mixing tank was split into three streams. Each stream was directed into a 175 L bio-barrel which contained settlement substrates and mesh bags containing adult mussels. In each bio-barrel, the water entered at the top of the barrel and exited at the bottom. This arrangement resulted in one control and three treatments. Each treatment and the control had three replicates.

Experimental protocol

The experiment was initiated on June 15, 2009. Three pH treatment levels were chosen: 7.5, 7.3, and 7.1. Each bio-barrel was equipped with a string of five 10×10 cm settling tiles made of unglazed fired clay. The string was suspended in the middle of the barrel from a metal crosspiece. On July 16, 2009, clumps of adult mussels were introduced into each of the bio-barrels. Adult mussels were present at the study site; however, no divers were available to collect mussels from this area. As such, adult mussels were collected by divers in Lake Huron during an underwater cleaning operation and shipped to site in a chilled container by courier. The adult mussels received were a mixture of zebra and quagga mussels. Lake Huron has average calcium levels of 23 mg/L (Mackie 2004) and pH of 8.1 to 8.5.

At site, any crushed or damaged mussels were removed, but clumped mussels were not separated in order to have as robust a population of adults as possible. Separating the mussel would require cutting of the byssal threads and might result in specimens more susceptible to the pH treatment than they would be in real life situations. Approximately 225 g of adult dreissenid mussels were placed in a bag made of 1.5×1.5 mm mesh (Figure 3). Three mesh bags were tied to a string and suspended from the same crosspiece as the settling tiles in each bio-barrel.

The flow through each barrel was approximately 0.5 L/min. The experiment was monitored daily. Temperature was verified in each bio-barrel with a handheld thermometer and compared to the values in the data logger. The pH was verified in each barrel with a handheld portable microprocessor-controlled pH meter and probe with accuracy of ±0.01 supplied by Prominent Controls. The measurements were taken in the top 10 cm of each bio-barrel. The probe was calibrated weekly against a known standard. Weekly, plankton samples were taken from the bio-barrels to verify that live veligers were present and settlement tiles were visually inspected for fresh settlement.

Figure 2. Mixing tanks and bio-barrels. Photo by RNT Consulting Inc.

Figure 3. Mesh bags with adult mussels at the end of the experiment. Photo by RNT Consulting Inc.

On August 11, 2009, a mid-point evaluation of the experiment was conducted. There was no settlement on the tiles and no visible settlement on the sides of the bio-barrel. However, there was evidence of settlement on the adult shells in the screen bags held at pH of 7.5 and in the control. As settlement was taking place at pH of 7.5 the decision was made to lower the pH of 7.5 to pH of 6.9. Before this step was taken, all barrels were emptied and pressure washed. Clay tiles were also washed and dried. Caged adults were placed in separate buckets filled with lake water during this process. On August 12, 2009, each mesh bag containing adult mussels was opened and placed in a separate white metal tray. Newly settled mussels (i.e. individuals less than 4 mm) were removed from the surface of adult shells. Any new settlers found on the outside of the bags, on the strings connecting the bags, and on the bio-barrels were included in the counts. Once the tanks were refilled and stabilized at the desired pH, the clumps of adult mussels were replaced in the mesh bags and returned to the bio-barrel from which they were removed. All bags appeared to contain adults which were in good condition, no gaping shells were observed and there was no de-clumping. Adults that were in the pH 7.5 treatment were now in the treatment kept at pH of 6.9. An additional string of settlement substrate, this time small clay flower pots, 3 flowerpots to a string, was placed in each bio-barrel. This was done to maximize the settlement substrate available. In addition, several strings of settlement tiles (5 tiles to a string) were placed on the lake bottom in the vicinity of the intake to the experiment. The experiment was re-started on August 13, 2009 and ran continuously until November 15, 2009.

The bio-barrels were tested weekly for larval presence and level of settlement. Numerous live veligers were present in the plankton but no visible settlement was observed. In an effort to boost settlement, oblique plankton tows, using a 50 micron mesh plankton net, were collected from the bay. The individual plankton tow samples were washed down into four separate buckets and each mixing tank received the contents of one bucket. This procedure was repeated at least once a week, weather permitting, beginning on August 23, 2009. Confounding the plankton collection and re-introduction was a heavy bloom of blue-green algae in the bay. At the height of the bloom, in late August, the blue green algae plugged the plankton nets and the small diameter water delivery piping in the flow laboratory had to be cleaned weekly to maintain proper flow. The plankton collection continued until November 7, 2009 when microscopic examination of plankton determined that the incoming veligers had dropped to very low levels.

On November 1, 2009 all bags containing adults were removed from the bio-barrels. Each bag was emptied into an individual tray. The mussels were then divided into dead and alive individuals. From the group of live adult dreissenid mussels, 30 individuals with non-perforated shells were selected for further analysis. These 30 individuals were placed in an aluminum tray and dried for 3 hours at 177°C.

Once dry, the mussels were measured (shell length) using electronic calipers and were weighed to the nearest milligram using an electronic scale (GemPro-500).

On November 15, 2009 the experiment was terminated.

Statistical analysis

As there were replicate counts of dead mussels originating from the same experimental bio-barrels (i.e. counts of dead mussels in the three mesh bags in each barrel), a mixed-effects logistic regression was fitted to test whether the mortality differed among treatments:

$$\text{logit } p_{ij} = \beta_0 + \beta_1 \times Treatment_{ij} + a_i \quad (2)$$

where logit $p_{ij} = \log[p_{ij} /(1 - p_{ij})]$ is the log odds of finding dead adults in bag j from bio-barrel i; β_0 is the overall proportion of dead molluscs in control group (on the logit scale); β_1 is the effect of pH treatment, and a_i is the random intercept that is assumed to be normally distributed with mean zero and variance σ_a^2 (see Zuur et al. 2009 for details). The analysis was carried out using the *MASS* package (Venables and Ripley 2002) for the R v2.12 statistical computing environment (R Development Core Team 2011).

As there were multiple individuals measured from the same bio-barrels, we fitted the following linear mixed-effects model to account for the possible barrel effect when assessing the weight/shell length relationship:

$$\log Weight_{ij} = \beta_0 + \beta_1 \times \log Length_{ij} + \beta_2 \times Treatment_{ij} + \beta_3 \times \log Length_{ij} : Treatment_{ij} + a_i + \varepsilon_{ij} \quad (3)$$

$\log Weight_{ij}$ is the log-transformed dry weight of mollusc j from bio-barrel i; β_0 is the overall mean weight of molluscs in control group (on the log scale); β_1 is the effect of shell length; β_2 is the effect of pH treatment, which is a nominal variable with four levels; 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 barrel; it is assumed to be normally distributed with mean zero and variance σ_a^2. The residuals ε_{ij} are similarly assumed to have a normal distribution with mean zero and variance σ_i^2. The residual variance was allowed to vary among experimental groups (see Zuur et al. 2009 for details on this type of model parameterization). Since the model included a continuous predictor variable (length), a nominal predictor variable (treatment group), and their interaction, this model was analogous to the analysis of covariance (ANCOVA) with mixed effects. The analysis was conducted using the functionality of the nlme v3.1-100 package for R (Pinheiro et al. 2011).

Results

New settlement on caged adults from July 16, 2009 to August 12, 2009

The settlement observed during this period was used to select the pH levels to be tested for the remainder of the experiment. Adult mussels were introduced into the bio-barrels on July 16, 2009. The growth rate for new settlers is assumed to be approximately 1 mm/week (Claudi and Mackie 1994). In just over 4 weeks, individuals smaller than 4 mm represented new settlement which occurred after the adult mussels were introduced into the bio-barrels.

Figure 4 shows the size distribution of newly settled mussels from July 16, 2009 to August 11, 2009 for each of the treatments and the control. Based on the number of settlers found, it was determined that a pH of 7.5 or 7.3 may not sufficiently affect mussel settlement and survival. As a result of these findings, the treatment using a pH of 7.5 was lowered to a pH of 6.9.

Adult dreissenid mortality in the mesh bags

In the pH 6.9 and pH 7.1 treatments, many of the mussels had perforated shells and the shells appeared almost white (Figure 5). Although most of the mussels with perforated shells were dead, some were still alive. The average mortality of adults (±standard deviation) varied on from 3.0 ± 2.5% in control group to 37.8 ± 7.4% in the pH 6.9 treatment (Figure 6).

The variance of the random intercept in the mixed-effects logistic regression (Equation 2) initially fitted to the mortality data appeared to be close to zero ($\sigma_a^2 = 4.7 \times 10^{-5}$). This suggested that the barrel effect was negligible, and thus the model was refitted without this random effect. The resultant logistic regression was highly significant (null deviance 477.4 (d.f. = 35) vs. model residual deviance 84.5 (d.f. = 32), $P << 0.001$, χ^2-test), which, in addition to Figure 6, provides a strong statistical evidence of the effect of pH on survival of adult dreissenids.

The mortality in each of the pH treatments was found to significantly differ from that in the control treatment ($P < 0.02$ in all cases, z-tests).

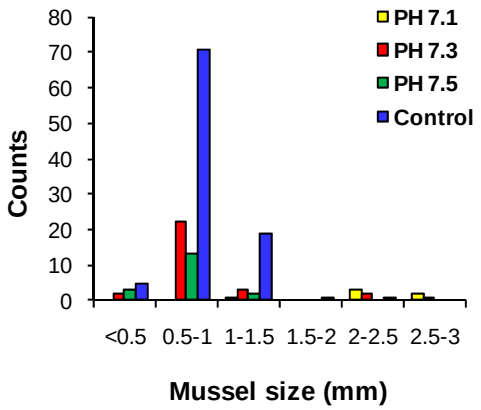

Figure 4. Size frequency distribution of the newly settled dreissenid, July 16, 2009 to August 12, 2009.

Figure 5. Bleached and eroded shells of live individuals from the pH of 7.1 treatment. Photo by RNT Consulting Inc.

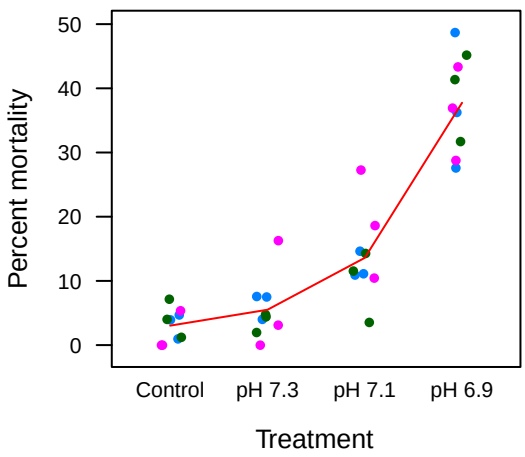

Figure 6. Mortality of adults exposed to different pH regimes from July 16, 2009 to October 31, 2009. The red curve describes the change of average mortality across treatments. Within a treatment, dots of the same color represent replicate counts of dead mussels from the same bio-barrel.

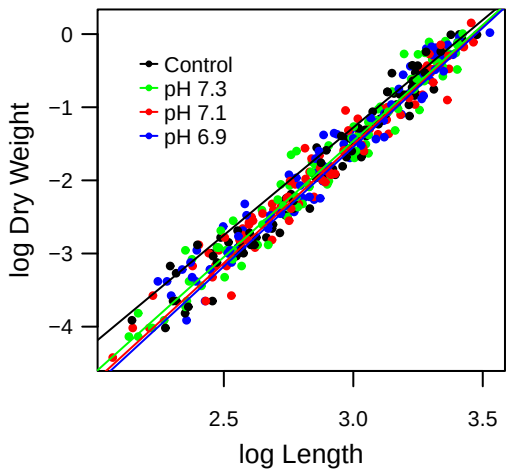

Figure 7. Log transformed data showing the length to weight relationship of adult dreissenids exposed to different pH regimes.

Length to weight relationship of adult dreissenids exposed to different pH regimes

The length to weight relationship for each measured mussel is shown in Figure 7. On the original scale, the relationship between length and weight follows a power function and, therefore, for purposes of line fitting and significance testing, the data were log-transformed. The mixed-effects linear model initially fitted to this data (Equation 3) appeared to have a random intercept with negligible variance ($\sigma_a^2 = 7.11 \times 10^{-6}$), suggesting that the random bio-barrel effect could be ignored. All terms in the model refitted without the random intercept were highly significant (Table 1).

Table 1. Results of ANCOVA to test the effects of shell length and pH regime on the dry weight of adult dreissenids (as fitted by Equation 3, but with no random intercept included). Residual d.f. = 353.

Factor	d.f.	F-value	P-value
Length	1	15062	<< 0.001
Treatment	3	74.2	< 0.001
Length:*Treatment*	3	10.6	< 0.001

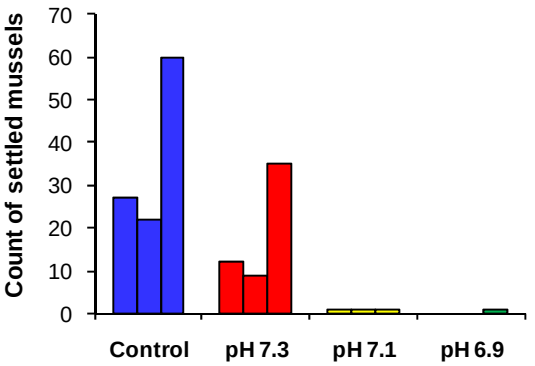

Figure 8. Dreissenid settlement under different pH regimes in bio-barrels between August 12, 2009 and November 15, 2009. Values from three replicate barrels are shown under each treatment.

The residuals were randomly distributed and demonstrated homogenous variance across the entire range of observations (not shown here), suggesting that the model was valid. Treatment-specific regression lines were estimated with that model as follows:

$$\text{Control: } \log Weigth = -10.106 + 2.942 \times \log Length$$

$$\text{Treatment A (pH 7.3): } \log Weigth = -10.982 + 3.173 \times \log Length$$

$$\text{Treatment B (pH 7.1): } \log Weigth = -11.235 + 3.241 \times \log Length$$

$$\text{Treatment C (pH 6.9): } \log Weigth = -11.331 + 3.261 \times \log Length$$

The intercept in control group was statistically significantly higher than in any of the pH treatments ($P < 0.001$ in all cases, *t*-tests), indicative of a considerably higher average weight per unit shell length in control animals. At the same time, the slope in control group was significantly lower than in any of the pH treatments ($P < 0.001$ in all cases, *t*-test), suggesting that the dry weight in control animals increased faster per unit shell length.

To test differences in elevation of the regression lines in the treatment groups, analysis of covariance was similarly performed on the three treatments alone. The analysis showed no differences in the slopes ($F = 0.659$, d.f. = 2, $P = 0.518$) but the elevations were significantly different between the three treatments ($F = 5.853$, d.f. = 2, $P = 0.003$; Figure 7).

Evaluation of new settlement from August 12, 2009 to November 15, 2009

No settled mussels were found on any of the clay tiles or clay flower pots in any of the bio-barrels. All settlement found was on the walls of the bio-barrels.

Mussel settlement between August 12, 2009 and November 15, 2009 is shown in Figure 8. Although only minimal numbers of settlers were found on the walls of the barrels, the counts did differ significantly between the four experimental groups (Kruskal-Wallis ANOVA by ranks, $P = 0.024$), specifically between the control and the two lowest pH treatments (Tukey HSD test, $P < 0.03$ in both cases).

Settlement tiles placed on the bottom of the lake near the intake to the laboratory were clear of any new settlement when examined in mid-November. The submerged portion of the intake pipeline was also examined and irregular patches of new settlement interspaced with no settlement at all were noted.

Discussion

The object of this study was to verify if pH adjustment of calcium rich water could prevent the settlement of dreissenid veligers and if prolonged exposure to low pH would eliminate adult mussels. The amount of settlement recorded in the bio-barrels was low. It is not clear why there was such a low settlement both in the laboratory and in the bay that provided the source water for this experiment. It is possible that the blue-green algae bloom interfered with

settlement in the latter part of the summer in both locations.

Despite overall low settlement in the experiment, it would appear that a pH of 7.1 will prevent the majority of dreissenid settlement from occurring. Juvenile mussels settling at this pH are not likely to survive to maturity. This conclusion is based on the observed weight/shell length relationships. The analysis shows that adult shells experience significant loss of calcium at a pH of 7.1. At a pH of 6.9 the loss of calcium is further accelerated resulting in almost 40% mortality of adults in 11 weeks.

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/weight relationship between treatments would have been greater had live adults been selected at random for this portion of the study. By selecting only adults without shell perforations, some of the differences between treatments may have been masked.

The adults in the treatment using a pH of 6.9 were only exposed to that pH since August 13, 2009. Prior to this date, these adults were in treatment using pH of 7.5 (July 16, 2009 to August 11, 2009). At the end of that period, no mortality, shell bleaching or perforations were observed in these adults. Therefore, the almost 40% mortality which was recorded in this treatment occurred in just over 11 weeks (August 12, 2009 to October 31, 2009). However, as the pH of 7.5 is below that of the controls and below that observed in Lake Huron, the prior exposure may have contributed to the final mortality.

It is important to note that during the study adult mussels exposed to low pH continued to stay in their original clumps. Normally, when mussels are placed in a “noxious environment” such as water treated with a low level of oxidant, they tend to break their byssal threads in an effort to escape. This reaction has been observed by one of the authors (RC) during numerous industrial treatments of cooling water circuits with sodium hypochlorite. De-clumping is observed well in advance of any mortality. The lack of de-clumping in the bagged adults in this experiment suggests that the low pH was not detected as a noxious.

Although a significant impact of low pH on mussels was observed, the study was carried out with background calcium levels of 41 mg/L. The effect of low pH may be more profound when background calcium levels are lower. This hypothesis should be tested in future experiments.

Overall, the proof of principle study suggests that pH adjustment could be a credible mitigation strategy both for large systems such as aqueducts and for small cooling water systems used by industry.

Acknowledgements

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