Evaluation of citric acid, a common food additive and calcium descaler, for use in adult zebra mussel decontamination

Joseph Perry,¹ Matthew Albright,² and Daniel Stich³

INTRODUCTION

Zebra mussels are costly in both economic and environmental terms. As of 2005, the economic cost of zebra mussel proliferation is estimated to be as high as one billion dollars in the United States annually (Pimentel et al. 2005) and is wide-ranging, affecting many economic sectors including power, water, agriculture, fishing, and recreation (Connelly et al. 2007; Elliott et al. 2005; MacIssac 1996). The billion-dollar figure accounts for tangible costs such as property value depreciation, biofouling of intake pipes in the power, municipal water, and industrial sectors, and resources allocated by nonprofits and local, state, and federal governments to manage their spread. Zebra mussels consume microscopic phytoplankton indiscriminately, competing with other planktivores including smaller zooplankton and indigenous freshwater mussels (MacIssac et al. 1995 and Ricardi et al. 1998) among others. Because zebra mussels are efficient filter feeders, their presence has contributed to increased clarity in many water bodies, causing mass macrophyte growth and die-offs. These factors may be contributing to the benthification of freshwater food webs by redirecting energy and resources from pelagic to benthic zones.

Once zebra mussels have been introduced in a water body, populations tend to rise quickly due to mass spawning events. Such events in mature populations have been reported to yield up to 500,000 veliger larvae per square meter of the water column (Ludyanskiy et al. 1993). To date, little if anything can be done to eradicate populations once established. Like many other aquatic invasive species, zebra mussels are primarily introduced by the overland transport of boats and equipment from infested waters. Mussels can survive overland trips attached to boat hulls, motors, chains, pipes, buoys, and livewells (Davis 2016).

While overland transport of zebra mussels is generally attributed to recreational boating, lake-to-lake movements by those involved in lake research or management activities may themselves serve as vectors of a variety of aquatic invasive species. Particularly, absorbent material which can remain damp for extended periods of time, such as fishing nets, can harbor living zebra mussels and their veligers for weeks. Similar conditions are afforded by anchor and mooring lines, foul weather gears such as waders, sampling gear, etc. As part of a larger set of procedures intended to minimize the spread of exotic species, effective protocols for disinfecting such materials need to be adopted. Here, citric acid was evaluated as such a disinfection agent. To evaluate this, zebra mussel adults were collected from Otsego Lake and were exposed to citric acid solutions of varying concentrations and periods of time. Resulting mortality was measured.

While there are many commercially available treatments proven to disinfect materials moving between water bodies, many are less than ideal in terms of cost, safety, ease of

¹ SUNY Oneonta Biology Department Intern, summer 2016.
² Assistant to the Director, SUNY Oneonta Biological Field Station.
³ Assistant Professor of Biology, SUNY Oneonta.
application in remote locations, environmental impact, and material compatibility (NOAA Fisheries Service 2017). Citric acid is an inexpensive biodegradable food additive that may show promise in application as a targeted disinfectant for invasive mussel species on its own or in conjunction with other compounds. In addition to its use as a food additive, citric acid is commonly used as a commercial descaler for limescale (calcium carbonate) deposits in pipes, boilers, kettles, and espresso machines. As a triprotic species, citric acid undergoes ionization in aqueous environments in a stepwise fashion shown in equations 1-4 below (Al-Khaldi, et al. 2007):

\[
\begin{align*}
H_3AOH & \leftrightarrow H_2AOH^{\text{(aq)}} + H^+ \text{(aq)} & \text{pKa (25°C): 3.13} & (1) \\
H_2AOH^{\text{(aq)}} & \leftrightarrow HAOH^2\text{\text{(aq)}} + H^+ \text{(aq)} & \text{pKa (25°C): 4.76} & (2) \\
HAOH^2\text{\text{(aq)}} & \leftrightarrow AOH^3\text{\text{(aq)}} + H^+ \text{(aq)} & \text{pKa (25°C): 6.40} & (3) \\
AOH^3\text{\text{(aq)}} & \leftrightarrow AO^{4\text{\text{(aq)}}} + H^+ \text{(aq)} & \text{pKa (25°C): 11.6} & (4) \\
\end{align*}
\]

Where \( A = C_6H_4O_6 \)

Because citric acid typically yields an acidic environment in solution without the addition of a strong base such as sodium hydroxide, equation 4 is unlikely to apply to the conditions of this study. Once ionized, citrate may participate in a reaction with calcium carbonate, the primary mineral constituent of zebra mussel valves (Pathy and Mackie 1992). The primary stepwise reaction of citric acid and calcium carbonate under acidic conditions is shown in equations 5-7 below (Al-Khaldi et al. 2007):

\[
\begin{align*}
2H^+ \text{(aq)} + CaCO_3 & \leftrightarrow Ca^{2+} \text{(aq)} + H_2O + CO_2 & \text{pH: 1.8 – 4} & (5) \\
H_2AOH^+ \text{(aq)} + Ca^{2+} \text{(aq)} & \leftrightarrow CaH_2AOH^+ \text{(aq)} & \text{pH: 1.8 – 4} & (6) \\
Ca^{2+} \text{(aq)} + 2(CaH_2AOH^+) \text{(aq)} & \leftrightarrow Ca_3(AOH)_2(s) & \text{pH: 6} & (7) \\
\end{align*}
\]

Where \( A = C_6H_4O_6 \)

In addition to reacting with protein components in the soft tissues, we hypothesize that citric acid will also directly react with the mineral components of zebra mussel valves, specifically calcium carbonate crystals which predominate the valve structure. Attacking the structure of the valves may be of some importance since zebra mussels are known to close tightly on exposure to noxious chemicals, potentially protecting soft tissues for long periods of time (Rajagopal et al. 2002).
MATERIALS AND METHODS

Mussel collection and setup

Adult zebra mussels were harvested from rocks collected from the source of the Susquehanna River (N 42° 41’ 58.9” W 74° 55’ 13.2”’) in water 1 – 2 meters deep. Mussels were removed from rocks with a paint scraper (Davis 2016) and transported to the lab for placement into mesh bags (Doc Foster CE-22541) in sets of ten live mussels each. A target range of 15-30 mm valve length was sought during selection and bagging in order to exclude mussels in the juvenile life stage. Bags were suspended by dowels in one of two 50-L aquaria with slow, constant flows of aerated lake water for at least 20 hours to acclimate mussels to ambient laboratory conditions.

After the acclimation period, fifteen lightly aerated 20-L aquarium tanks were filled with filtered lake water and dosed with food grade anhydrous citric acid (Duda Energy) to yield three replicates of the following concentrations in the low-dose trial: 1.00, 0.50, 0.25, and 0.10 %. Nine 20-L tanks filled with filtered lake water were dosed with citric acid to yield concentrations of 4.00, 2.00, and 0.500 % in the high-dose trial. Additionally, three negative control tanks filled only with filtered lake water were used in each trial.

Treatment

Ten bags of mussels were suspended by dowels in each 20-L aquarium. One bag was removed from each tank at ten predetermined time points (5 and 30 minutes; 1, 2, 4, 6, 8, 12, 24, and 72 hours) before rinsing in a 500-L aquaculture tank filled with lake water to remove any residual treatment. Bags were then moved to one of two 50-L aquaria with slow, constant flows of aerated lake water for 48-72 hours post-treatment. This recovery period was intended to allow those mussels that might have appeared dead to show signs of recovery, as mussels treated with disinfectants sometimes falsely appear dead immediately after chemical exposure (Pucherelli et al. 2014).

Mortality and measurement

Mussels were individually evaluated for mortality after holding in post-treatment aquaria according to the criteria listed above. Mussel lengths were obtained using a digital caliper and recorded along with mortality.

Water quality assessment

A calibrated multi-parameter water quality sonde (YSI Incorporated, Model Number 6820V2-M) was used to monitor physical and chemical parameters of water quality in each experimental, holding, and recovery tank. Data on conductivity, pH, dissolved oxygen, and temperature were obtained at 8:00 each morning. Calcium concentrations were determined on of each high-dose and control tanks approximately one week after the study was completed using the EDTA titrimetric method (APHA 2012).

Statistical analysis:

Binomial logistic regression models were used to analyze the effects of citric acid concentration and exposure time on the mortality of zebra mussels. A Bayesian hierarchical approach was used to model variation in mortality (p) due to the interactive effect of citric acid
concentration and exposure time (\textit{TIME}) for each trial \((i)\). To incorporate potentially different responses to exposure time between the different doses, we modeled dose as a random effect on the slope \((\beta_{\text{time},j})\) of the linearized relationship between mortality and time:

\[
\logit(p_{i,j}) = \beta_0 + \beta_{\text{time},j} \times \text{TIME}_i
\]

The approach assumed that the number of dead mussels in each \(i\)th trial \((D_i)\) was drawn from a binomial distribution defined by the probability of mortality in each \(j\)th citric acid concentration \((p_{i,j})\) and the number of zebra mussels in each trial \((N_i)\):

\[
D_i \sim \text{Binomial}(p_{i,j}, N_i)
\]

We used uninformative prior distributions for model parameters to allow data to guide conclusions about the process of interest. We assumed a shared intercept \((\beta_0)\) among all doses because all trials started at time zero, with zero mortalities. We used a diffuse normal prior distribution with a mean of zero and a variance of 10 on the intercept.

\[
\beta_0 \sim \text{Normal}(0, 10)
\]

We hypothesized that increased exposure time would have a positive effect on the number of dead zebra mussels in each trial across all doses, but that the effect of exposure time \((\beta_{\text{time},j})\) would vary between doses. In order to incorporate variability in the intensity of this effect (i.e. shape of the dose-response curve), we assumed that the effect of exposure time was drawn from a global population of possible effects represented by a normal distribution with hyperparameters \(\mu\) and \(\sigma^2\).

\[
\beta_{\text{time},j} \sim \text{Normal}(\mu, \sigma^2)
\]

The mean of the global distribution for \(\beta_{\text{time},j}\), \(\mu\), was assigned a diffuse normal prior with a mean of zero and a variance of ten, and we used a uniform prior distribution on \(\sigma^2\) that ranged from zero to ten. This approach allowed us to share information across all trials to estimate hyperparameters for the global distribution of \(\beta_{\text{time}}\) to improve parameter estimation while allowing the effect of time to vary between citric acid concentrations.

We used Markov chain Monte Carlo (MCMC) methods to estimate model parameters in JAGS using the ‘R2jags’ package (Su and Yajima 2015) in R (R Development Core Team 2017). We used a burn-in of 3,000 runs, and simulated an additional 30,000 samples from each posterior distribution, saving every 30th sample to reduce autocorrelation between samples and increase the number of independent samples from the posterior distribution (Kruschke 2010). We ran a total of three Markov chains for each parameter, resulting in a total of 2,700 samples from which to construct posterior distributions. We assessed convergence among the three chains for each parameter using the Gelman-Rubin convergence diagnostic (Gelman & Rubin, 1992), and visually inspected plots of Markov chains to ensure adequate mixing (Kruschke 2010).
RESULTS AND DISCUSSION

Low-dose trial

A total of 1,500 mussels were assessed over three replicate trials, ten time points, and five concentrations of citric acid treatments (including negative controls). The overall average mussel length was 21.98 mm with a standard deviation of 3.107. Average lengths of dead and live mussels in this trial were 21.88 mm and 22.00 mm respectively. Although no concentration in the low-dose trial yielded 100% mortality at any time point, mortality was above 90% in the 0.500% and 1.00% treatments after 72 hours. In the negative control group, 3 of 300 mussels were dead with one mortality at two, four, and seventy-two hours (Figure 1).
Figure 1. Individual posterior predictive dose-response curves for zebra mussels exposed to low doses of citric acid. Outer lines indicate the 95% credible interval while percentages indicate dose.
High-dose trial

In the high-dose trial, the overall average mussel length was 21.50 mm with a standard deviation of 9.061. Living mussels averaged 21.89 mm and dead mussels averaged 20.83 mm. Because of major valve degeneration in mussels at higher time and doses, a total of 77 mussels were not able to be measured. Treatment concentrations of 2.00 and 4.00% each yielded greater than 50% mortality after 2 hours, while the treatment of 0.500% yielded greater than 50% mortality after 48 hours. Complete mortality was achieved in 0.500% citric acid after 72 hours, 2.00% citric acid after 24 hours, and 4.00% citric acid after 24 hours. All 300 mussels in the negative control group survived (Figure 2).

Figure 2. Individual posterior predictive dose-response curves for zebra mussels exposed to high doses of citric acid. Outer lines indicate the 95% credible interval while percentages indicate dose.
Calcium levels were 70.3 mg/L (SD = 1.00) in the 0.500% citric treatment tank, 114 mg/L (SD = 2.00) in the 2.00% tank, and 172.3 mg/L (SD = 12.02) in the 4.00% tank. There was a perfect linear correlation between the citric acid and calcium concentrations in the tanks ($R^2 = 1$). The negative control tank was found to have a calcium concentration of 23.4 mg/L (SD = 0.200), which is not inconsistent with normal calcium levels in the lake from which water was sourced (Figure 3).

Figure 3. Calcium concentrations of high-dose treatment tanks recorded after the completion of the study.
Like EDTA, citrate also binds calcium to form an insoluble product, calcium citrate (Equation 7 above). Although this may theoretically deflate numbers obtained from the titrimetric method used to quantify calcium in each sample, the concentration of EDTA is almost 50 times higher than the concentration of citrate in the highest dose treatment solution (100 mM vs 2.12 mM, respectively), so any interference is considered to be negligible.

Mussels exposed to higher dose and time conditions had valves that were soft, pitted, and occasionally harbored a white, crystalline substance. While the compound was not tested, it may be reasonable to assume that it could be calcium citrate, the product of equation 7. Mussels exposed to 2.00% and 4.00% citric acid at higher time points were reduced to piles of slime which made the measurement of mussels in these cohorts impossible (Figure 4).

Figure 4. Mussels exposed to 0% (A), 0.500% (B), 2.00% (C), and 4.00% (D) citric acid solutions over 72 hours’ time.
CONCLUSION

Citric acid produced 100% mortality of adult zebra mussels at the higher concentrations tested (2-4%), when the contact time was between 12 and 24 hours (Figure 2). Because of this, further investigation is recommended to assess if mortality may be achieved more quickly when using higher concentrations of citric acid. That said, citric acid may hold some promise as an anti-biofouling agent due to significant calcium leeching and near dissolution of zebra mussel valves when exposed to the concentrations tested. The effect of citric acid on materials to be defouled, the evolution of CO$_2$ gas during the process (equation 5), and the effect of higher concentrations of citric acid on dissolution time should all be considered before application. Additionally, mortality should be evaluated on zebra mussel veligers, as this juvenile life stage is that which is most likely to be accidentally transported in field sampling gear, and is the stage generally most susceptible to chemical decontamination (Perry et al. 2017; Davis 2016; Kennedy et al. 2006).

Additional work that may be performed includes increasing sample size and replicates for both phases of the project to improve statistical power and performing a thorough validation of solution stability over time. Solutions of relatively low concentrations of citric acid are very biodegradable – fungi and bacteria readily grew in uncovered beakers of citric at 2.00% and were observed in high-dose tanks two weeks after the conclusion of this study. Food grade citric acid is also relatively safe to handle and it is not expensive, at $75 US (2016) for 50 lb ($3.30/kg).

According to Diggins (2001) and Costa et al. (2008), adult zebra mussels may alter their filtration rates by season and may vary by a factor of 22 between the summer and winter. The downscaling of filtration by the adult mussels in the fall and winter seasons (when this study was performed) may make the mussels less susceptible to chemical treatments. Because of this, the study should also be performed in the warmer months to determine whether less citric acid may be used for shorter periods of time.

REFERENCES


Su, YS. and Yajima M. 2015. R2jags (R Package version 0.5-7).