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Efficacy of Copper Based Algaecides for Control of Quagga and Zebra Mussels

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1.0 Introduction

Dreissenid mussels, the zebra and quagga mussels, arrived in the United States from Europe in the 1980s and quickly spread to many Eastern waterways, rivers, and lakes. These mussels are extremely prolific and can produce costly impacts by attaching to and clogging water intakes, trashracks, pipes, fire control systems, cooling water systems, fish screens, and virtually all types of underwater infrastructure.

Since 2007, dreissenid mussels have been present in the lower Colorado River. The mussel populations have proliferated and mussels are now adversely affecting the Hoover, Davis, and Parker Dams. More recently, dreissenid were found in California, Kansas, Nebraska, and Oklahoma and have been detected in New Mexico, and Utah.

Various aquatic herbicide formulations have been reported as having a negative effect on dreissenid mussels during aquatic weed and algae control treatments. The objective of this study was to determine the efficacy of some frequently used algaecides on mussel mortality. Selected for this trial was Green Clean Sodium Carbonate Peroxyhydrate Powder (27.6% Hydrogen Dioxide by weight), Green Clean Liquid (27% Hydrogen Dioxide), SePRO Corporation Natrix™ and Captain™, non-proprietary copper sulfate crystal and EarthTec® algaecide/bactericide.

Copper products (copper sulfate and copper carbonates or chelates) can be used to control mollusks in open water systems, but require a Special Local Need Label (also known as a Section 24-c) issued by the USEPA.

2.0 Scope

The purpose of the experiment described in this report was to evaluate the efficacy of algaecide formulations to control dreissenid mussels at concentrations normally used for the control of aquatic weeds and algae. The experiment was done in the following steps;

1. Determine dose/exposure response curves of adult quagga mussels to selected algaecides under temperature regimes which may be encountered during weed/algae control applications. The source water for the tests was the Lower Colorado River Water (Arizona).
2. Determine dose/exposure response curves of adult zebra mussels to selected algaecides under temperature regimes which may be encountered during weed/algae control applications. The source water for the tests was San Justo Reservoir water (San Benito County, California).
3. As most algaecides are not applied for a period of 96 hours in the open environment, additional tests were done to determine if a short exposure to any product which caused at least 60% mortality in the 96 hour test product will result in delayed post exposure mortality of adults. Exposure of eight and twelve hours was followed by a recovery period of 36 hours. Mussel mortality was assessed every 12 hours.

3.0 Methodology

The experiments were carried out in mobile flow-through laboratories situated next to the two sources of water. One laboratory was at Davis Dam (Figure 1) on the Lower Colorado River to test the effects on quagga mussels, and the second laboratory was situated at San Justo Reservoir to test the response of zebra mussels. The same research protocol was followed at both locations, with ambient water temperatures between 16.5 and 19.5 ºC.

Five different algaecides were tested:

- GreenClean Sodium Carbonate Peroxyhydrate Powder (27.6% Hydrogen Dioxide by weight) and Green Clean Liquid (27% Hydrogen Dioxide). Both products were tested at the maximum strength approved for use in open water. This translates to the equivalent treatment of 90 lbs/ acre-foot for the powder and 30 gal/acre-foot for the liquid. This product dissipates within 12 hours in the environment. On the advice of the manufacturer we limited the experiment to a 12 hour exposure of adult mussels.

- Copper sulfate is a chemical compound with the chemical formula $CuSO_4$. This salt exists as a series of compounds that differ in their degree of hydration. The anhydrous form is a pale green or gray-white powder, whereas the pentahydrate ($CuSO_4 \cdot 5H_2O$), the most commonly encountered salt, is bright blue. This form is commonly used as an aquatic herbicide. The chemical data sheets suggest a maximum of 2 ppm of product for algae treatment, and guarantee a minimum of 25% copper ions in the product. Therefore the maximum recommended application is 0.5 mg/L copper equivalent.

- Natrix™ (SePro Corporation) is a copper carbonate ethanolamine complex originally listed as an algaecide. It now has an EPA special use label in some states for control of invasive mollusks. For the mollusk control applications the recommended application rates are higher than for algae control. As our purpose was to test collateral damage during treatment for algae the maximum recommended dose recommended for algae control was chosen as the upper limit of application (1 mg/L copper equivalent).

- Captain™ (SePro Corporation) is a double chelated copper compound containing 9% copper by weight. It is primarily used for the control of planktonic and filamentous algae. There are no drinking water or fish consumption restrictions with Captain-treated waters. The maximum application rate is 1 mg/L of copper.

- EarthTec® algaecide/bactericide, manufactured by Earth Sciences Laboratories Inc., contains 20% copper pentahydrate in liquid form from which 5% metallic copper content is derived. The product has a low pH. It is used for the control of planktonic, filamentous and chara algae in lakes, ponds, fish farms, fountains and other water systems. EarthTec® is registered by the EPA in all 50 states and certified to ANSI/NSF Standard 60 for addition to drinking water. The maximum allowed application rate is 1 mg/L of copper.

Most of the copper based algaecides have a maximum suggested application of 1 mg/L of copper (Cu^{2+}). Therefore, two copper levels were tested in this experiment: a high level of 1 mg/L of copper and a low level of 0.5 mg/L of copper. The notable exception is copper sulfate, which has a recommended application rate of 2 mg/L of product, which is 0.5 mg/L of copper equivalent.

This was the value used for the high concentration while the low concentration was 0.25 mg/L of copper. In this report, mg/L is always used to refer to the mg/L of copper equivalent in order to be able to directly compare effects of different products.

The copper levels to be used in the experiment were calculated in the same manner as would be done when carrying out an algaecide control campaign. In order to obtain the highest, manufacturer recommended level of copper equivalent, we calculated the weight or volume of each product to be added to 50 gallons of raw water in each drum using the manufacturer provided product label sheets. Appendix 2 shows the worksheets used to calculate the amounts of product to be used. The second treatment with each product was carried out at 50% of the maximum recommended level.

For copper sulfate in granular form, 0.37 mg was dissolved in 50 ml of raw water and added directly to the drum for the high copper concentration, and the low concentration was achieved using 0.18 mg of dissolved copper sulfate. For the other algaecides, 10 mL of product was first dissolved in 100 mL of filtered lake water to minimize experimental error on product addition as the amounts of product needed at full concentration were very small. A precision pipette was used to measure the diluted product into drums containing 50 gallons of raw water. The amount of diluted product and the resultant copper concentration is shown in Table 1.

Table 1. Amount of product used (mg of product or mL of diluted product solution in 50 gallons of raw water) for each algaecide and the resulting copper concentration.

	Copper Sulfate		**Natrix™**		**Captain™**		**EarthTec®**	
Treatment Level	**amount of product**	**copper conc.**	**amount of product**	**copper conc.**	**amount of product**	**copper conc.**	**amount of product**	**copper conc.**
Low	0.18 mg	0.26 mg/L	8.7 mL	0.5 mg/L	8.7 mL	0.5 mg/L	8.3 mL	0.5 mg/L
High	0.37 mg	0.52 mg/L	17.4 mL	1.0 mg/L	17.4 mL	1.0 mg/L	16.7 mL	1.0 mg/L

Please see Appendix 2 for the calculation tables used to determine algaecide amounts. After the algaecide was added, a drill mounted paint mixer was used for thorough mixing (Figure 2).

Figure 1. Field Laboratory at Davis Dam

Figure 2. Mixer

3.1 Methodology at Davis Dam for dose response curves of quagga mussels

Adult mussels were collected from the docks at Katherines Landing National Park. The alkalinity at this location was measured as 130 mg/$CaCO_3$, the calcium concentration was approximately 80 mg Ca/L, and pH varies from 7.9 to 8.6. The mussels were sorted immediately, discarding empty shells and crushed individuals. Groups of 100 (±10) adults were placed into individual mesh bags. The bags containing adults were placed in aerated lake water to acclimate to laboratory conditions over 48 hours. Natural clumps of mussels were kept as intact as possible to minimize the stress on the adults and to evaluate if algaecides would cause de-clumping. De-clumping in dreissenids has been observed as a sub-lethal response to noxious environments.

Five 50 gallon drums in the laboratory were filled with raw water from the Colorado River and allowed to stabilize over 24 hours. The temperature was maintained by maintaining the ambient air temperature in the laboratory to match the desired temperature of the water in the drums (Figure 3). Then the algaecides were added (see section 3.0).

Figure 3. Drums and associated coolers for Algaecide Experiment.

Following the mixing process, the drip valves installed on the bottom of each drum were opened and the solution from each drum started flowed into individual coolers (Figure 4). When coolers were filled to approximately 50%, 3 mesh bags containing 100 (± 10) adults each were placed in

each cooler. Each bag contained a waterproof label to identify the individual bags in each cooler as 1, 2 or 3 (Figure 5). On initial exposure, clumps of quagga mussel were observed filtering and individuals were moving in the bag.

Figure 4. Cooler for captive adults

Figure 5. Mesh bag containing captive adults

The drip valves continuously passed solution from the drum into the associated 40 L cooler over a period of 96 hours at a rate of approximately 2 L/hour. The discharge from each cooler was collected into buckets for disposal at the evaporation basin at Davis Dam.

Each bag was examined every 12 hours to determine if adults were dying. The dead mussels were removed from the bag, counted and the bag was returned to the cooler. At that time, the temperature, pH and dissolved oxygen measurements were taken using a Hach HQ40d multiprobe. All mussels remaining in the bags were removed from the test coolers after 96 hour exposure and placed in flow-through recovery coolers. Post-exposure mortality was monitored for up to 102 hours.

At the start of the experiment, a clump of adult mussels was placed in a glass beaker containing water with each concentration of each test product. The mussels were observed for filtering behavior.

The exceptions to the above protocol were the experiments using the GreenClean Sodium Carbonate Peroxyhydrate Powder (27.6% Hydrogen Dioxide by weight) and Green Clean Liquid (27% Hydrogen Dioxide). Both products were tested at the maximum strength approved for use in open water. These products dissipate within 12 hours in the environment. On the advice of the manufacturer we limited the experiment to a 12 hour exposure of adult mussels within the drums used to mix the chemicals. Three bags of approximately100 mussels were placed in each drum for 12 hours, then removed and evaluated for mortality. The experimental temperature was 25ºC.

3.2 Methodology at San Justo Reservoir for dose response curves of zebra mussels

Adult mussels were collected from various settling substrates in the San Justo Reservoir, and kept in a large drum with continuous flow-through water. The protocol from Davis Dam was then followed for the remainder of the experiment. The discharge from the experiment was collected into a soaking pit outside of the trailer. The alkalinity of San Justo Reservoir water was between 80 and 85 mg/$CaCO_3$ and the calcium concentration was between 18 and 20 mg Ca/L, pH varied from 7.9 to 8.6.

3.3 Methodology for a short term exposure and recovery test to determine post-exposure mortality

As most algaecides are not applied for a period of 96 hours in the open environment, we devised the following test to determine if a short exposure to a product will result in delayed post exposure mortality of adults. Only products which after 96 hours had caused mortality above 60% were tested in the short term recovery experiments.

In these experiments, the product was mixed into 50 gallon drums as described in section 3.1. Into each drum, we placed 6 bags containing adult mussels. Each bag consisted of approximately 50 or 100 adult mussels, depending on the experiment. The groups of mussels were contained in labelled mesh bags.

We withdrew 3 bags of mussels from the drum with product being tested after 8hrs, and 12hrs, rinsed them in raw water, examined the adults for mortality, removed any dead individuals and placed the bags in flow through recovery coolers.

We examined each bag every 12 hours until the 36-hour recovery time was reached following the longest exposure (12 hours), a total of 48 hours. We monitored temperature, pH and dissolved oxygen in the drum and in the recovery coolers.

3.4 Methodology for statistical modelling

Statistical modeling was used to understand the effects of our experiments while controlling for covariates such as temperature. While the effects of the algaecides may appear to be evident, there was some variability in temperature both between and within experiments (i.e. each concentration tested was in a separate cooler). While we attempted to control temperature, statistical modeling was used to ensure that the apparent effects were indeed true effects after controlling statistically for temperature.

Statistical modeling was also used to understand which covariates played the biggest role in determining mussel mortality. Our initial models included concentration, month (July or November for some algaecides), and temperature. We reduced our initial models to the final models presented in the results based on which variables were statistically significant. In other words, the modeling enabled us to say that in some cases not all variables were useful in predicting mussel mortality.

Depending on the properties of the mortality data at hand, we used two different approaches for modeling the response of dreissenids exposed to algaecides. If there was no temporal autocorrelation present in the mortality values, we used the generalized linear models (GLMs) with the log link function (a.k.a. "logistic regression"; Venables and Ripley 2002). When the autocorrelation was present, we used the generalized additive models (GAMs) with correlated residuals (Wood 2006). The two approaches are described below in more detail.

GLMs were fitted to the dreissenid mortality data in order to derive equations describing the change of mortality over time, and also to test which of the measured covariates (e.g., concentration, temperature, etc.) influenced that change. Example of a typical fitted model is as follows:

$$p_i = \frac{\exp(\beta_0 + \beta_1 h_i + \beta_2 t_i + \beta_3 C_i)}{1 + \exp(\beta_0 + \beta_1 h_i + \beta_2 t_i + \beta_3 C_i)}$$

The terms of this model are interpreted in the following way:

- p_i is the ith cumulative observed proportion of dead dreissenids observed by time t_i. The modeled proportion was calculated after pooling the data from three replicate mesh bags together.
- β_1 is the effect of time (h), i.e. a coefficient showing how much the proportion of dead molluscs increases when time increments by 1 hour.
- β_2 is the effect of water temperature (t). The temperature was centered by subtracting its mean value from each of the measured individual temperature values, making the mean of the resulting values equal to zero. The main reason for performing such a transformation was to ease the interpretation of the model intercept, β_0 (see below). After centering, the meaning of the coefficient β_2 is as follows: it indicates how much the

proportion of dead molluscs changes when temperature deviates (i.e., increases or declines) from its mean value by one centigrade degree.

- β_3 is the effect of algaecide concentration (C), which was treated as a categorical predictor and thus was coded in the model as a "dummy variable". Thus, with the two concentrations tested, the variable "concentration" takes on only two possible values – 0 if it is "low", and 1 if it is "high". When relevant, other categorical predictors (e.g., month) were coded similarly.
- β_0 is the model intercept. Taking into account the aforementioned meanings of the other model parameters, the intercept is interpreted as the expected dreissenid mortality at the "high" algaecide concentration at time 0, with the water temperature kept at its mean level.

The analysed mortality values demonstrated an excessive variance as compared to the variance that would be expected from a variable with binomial distribution. To account for this overdispersion, we fitted logistic regression models of the so called "quasibinomial family" using the maximum likelihood methods of parameter estimation (Venables and Ripley 2002). The deviance test (Venables and Ripley 2002) was employed to decide which parameters were to be kept in the model and which of them had only minor effect on mortality and should be left out. In the Results section, we present only the final models, whose parameters were found to have significant statistical association with dreissenid mortality. Adequacy of the final models was checked by examining the distribution of their standardised residuals (i.e. differences between the actual observations and model-fitted values, divided by their standard deviation). The residuals of the fitted models are assumed to be normally distributed. This assumption was checked using the quantile-quantile plots (Q-Q plots), which visualise the actual quantiles of residuals against the theoretically expected normal quantiles. If the normality assumption is met, data points on a Q-Q plot form approximately a 45° diagonal line (Venables and Ripley 2008). To further support this visual check quantitatively, we also used the Shapiro-Wilks' test for normality (Shapiro and Wilk 1965).

GAMs represent a class of semi-parametric statistical models, which are particularly suitable for modeling non-linear relationships. An example GAM fitted to the dreissenid mortality data from this study is as follows:

$$p_i = \frac{\exp(\beta_0 + f(h_i) + \beta_1 t_i + \beta_1 C_i)}{1 + \exp(\beta_0 + f(h_i) + \beta_1 t_i + \beta_1 C_i)},$$

where all parameters have the same interpretation as in the GLMs described above, except for the nonparametric term $f(h_i)$. In this particular example, $f(h_i)$ represents a spline smoother that models a nonlinear response of mortality to time. This smoothing function was estimated as a cubic regression spline (Wood 2006). Residuals of the fitted GAMs were allowed to be correlated through the following submodel ("autoregressive model of order 1", AR-1), which relates a residual at time h to the residual at time $h - 1$, along with "noise" η_h" (Wood 2006):

$$\varepsilon_h = \rho \varepsilon_{h-1} + \eta_h$$

The parameter ρ is unknown, and is to be estimated from the data. The AR-1 model corresponds to the following correlation structure:

$$cor(\varepsilon_s, \varepsilon_h) = \begin{cases} 1 & if \ \ s = h \\ \rho^{|h-s|} & else \end{cases}$$

Suppose, $\rho = 0.5$ and $h = s + 1$. Then the correlation between residuals separated by one hour is 0.5. If the separation is two units in time, then the correlation is $0.5^2 = 0.25$. The further apart two residuals are, the less they are correlated. This autocorrelation structure was applied on the mortality time series for each algaecide concentration separately (e.g., "high" and "low"), and then one average estimate of ρ was obtained for both time series (Wood 2006). Adequacy diagnostics of the fitted GAMs was performed using the same tools as for the GLMs (see above).

GLMs were fitted using standard functionality of the R v2.15.0 statistical software (R Development Core Group 2012). GAMs were fitted with the help of add-on package *mgcv* for R (Wood 2006).

4.0 Results

4.1 Effects of Green Clean products on dreissenid mussels

The experiment started on May 21, 2012. The test chemicals were the GreenClean Sodium Carbonate Peroxyhydrate Powder (27.6% Hydrogen Dioxide by weight) and Green Clean Liquid (27% Hydrogen Dioxide). Both products were tested at the maximum strength approved for use in open water. This translates to the equivalent treatment of 90 lbs/acre-foot for the powder and 30 gal/acre-foot for the liquid. Over the course of the experiment, the experimental temperature ranged from 25.3ºC to 28.0ºC.

This product dissipates within 12 hours in the environment. On the advice of the manufacturer we limited the experiment to a 12 hour exposure of adult mussels.

No mortality was recorded in either of the experimental treatments or in the control. Further, there was no de-clumping of the adult mussels (de-clumping is a sign of mussels under stress from the environment). None of the shells of the adults were bleached or compromised in any way.

Table 2. Environmental parameters during Green Clean experiment

	Start – 10am			Finish-10pm		
Drum	**Temp**	**DO**	**pH**	**Temp**	**DO**	**pH**
A	25.3	9.47	8.71	27.1	9.3	8.64
C	26.3	9.39	8.74	28.0	8.53	8.53
D	25.7	9.54	8.7	27.2	9.33	9.11

Due to lack of efficacy, these products were not tested at San Justo Reservoir on zebra mussels.

4.2 Effects of copper sulfate on dreissenid mussels

Two separate exposure experiments using copper sulfate were carried out on zebra mussels. The first experiment was conducted in July 2012. The second experiment was carried out in December 2012. The reason for repeating the experiments in December was the addition of two copper algaecides to the testing protocol after July 2012. To be able to compare results for all four algaecides tested under the same temperature regime, the experiment with copper sulfate was repeated.

It is important to remember that the high concentration of copper sulfate is in fact half the g/mL copper equivalent of other algaecides, based on recommended application rates.

4.2.1 Copper sulfate and zebra mussel mortality during 96h exposure in July

The first experiment started on July 9th, 2012 at San Justo Reservoir. The results of the July recovery experiment are shown in Figure 6. Temperatures in the drum with the low copper level averaged 18.5°C, with a minimum of 15.7°C and a maximum of 22.8°C. The drum with high copper level had an average temperature of 18.6°C with a minimum of 15.9°C and maximum of 22.8°C. For the complete record of environmental parameters see Appendix 1.

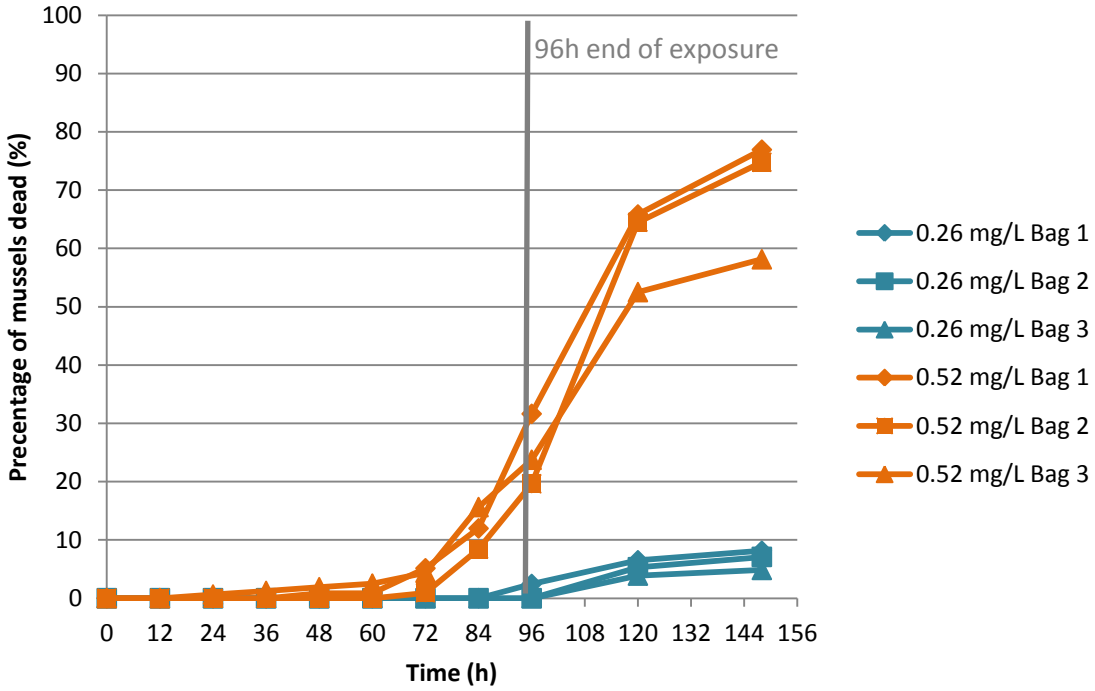

Figure 6. Zebra mussel mortality when exposed to copper sulfate in July

The July experiment had an average mortality of 0.8% after 96h at the low copper concentration, and an average mortality of 25.0% after 96h at high copper levels. It was noted that mussels were

continuing to experience mortality after they were removed from the experimental coolers and placed in recovery chambers. Recovery chambers continued to be monitored and after 148h mortalities rose to 6.7% for low and 70.0% for high copper.

4.2.2 Effect of copper sulfate on zebra mussel mortality during 96h exposure in December

The experiment using copper sulfate was repeated in December, 2012. After the 96 hour exposure to low copper concentration the maximum mortality varied from 4.5 to 23%. At the high copper concentration the maximum mortality varied from 29 to 47%. After an additional 60 hours in the recovery cooler the maximum mortality for low copper rose to a minimum of 5.26% and a maximum of 31%. At the high copper level the minimum mortality increased to 40% and maximum mortality reached 72% (Figure 7). During the experimental exposure the ambient water temperature varied from a high of 21.5ºC to a low of 15.6 ºC.

There was no mortality recorded in the control.

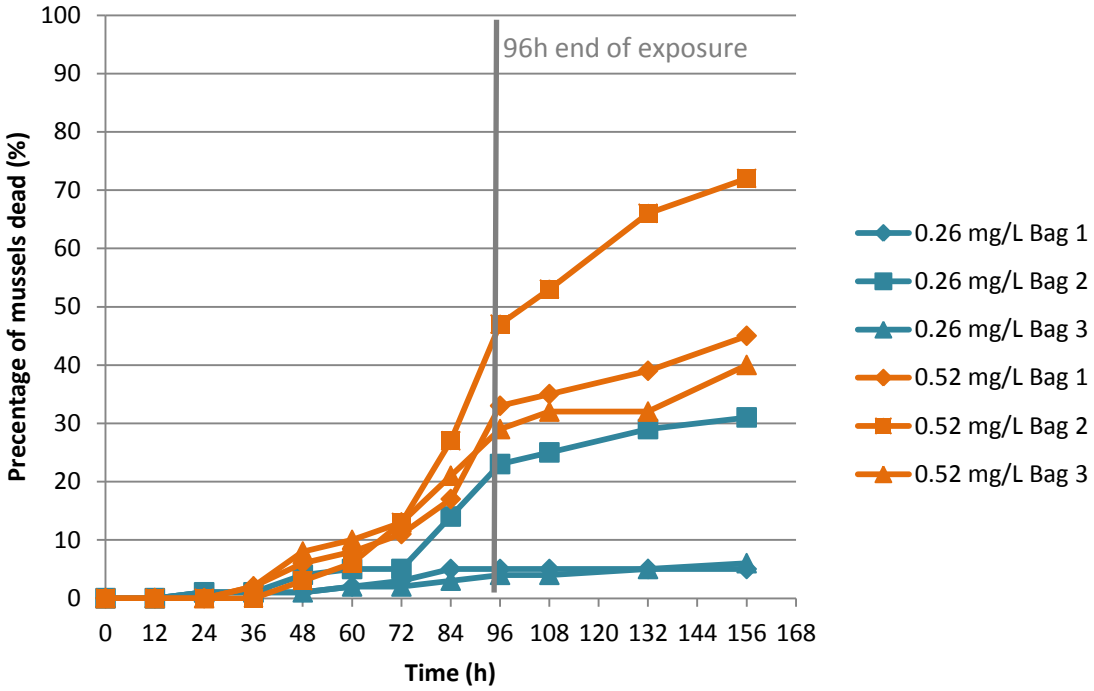

Figure 7. Zebra mussel mortality when exposed to copper sulfate in December

4.2.3 Effect of copper sulfate on zebra mussel mortality following 12h exposure in December

A short term exposure/recovery experiment was also conducted in December. Zebra mussels were exposed to copper sulfate at high copper level for 12h, and then observed during a 36h recovery period. There was no mussel mortality recorded in this experiment or in the controls.

4.2.4 Effect of copper sulfate on quagga mussel mortality during 96h exposure in November

In November, an experiment exposing quagga mussels to copper sulfate was conducted over 96h. This experiment was carried out at Davis Dam, starting on November 14th, 2012. The ambient temperature during the experiment fluctuated between 18.2ºC and 16.7 ºC. The average temperature was 17.2 ºC. The results are shown in Figure 8.

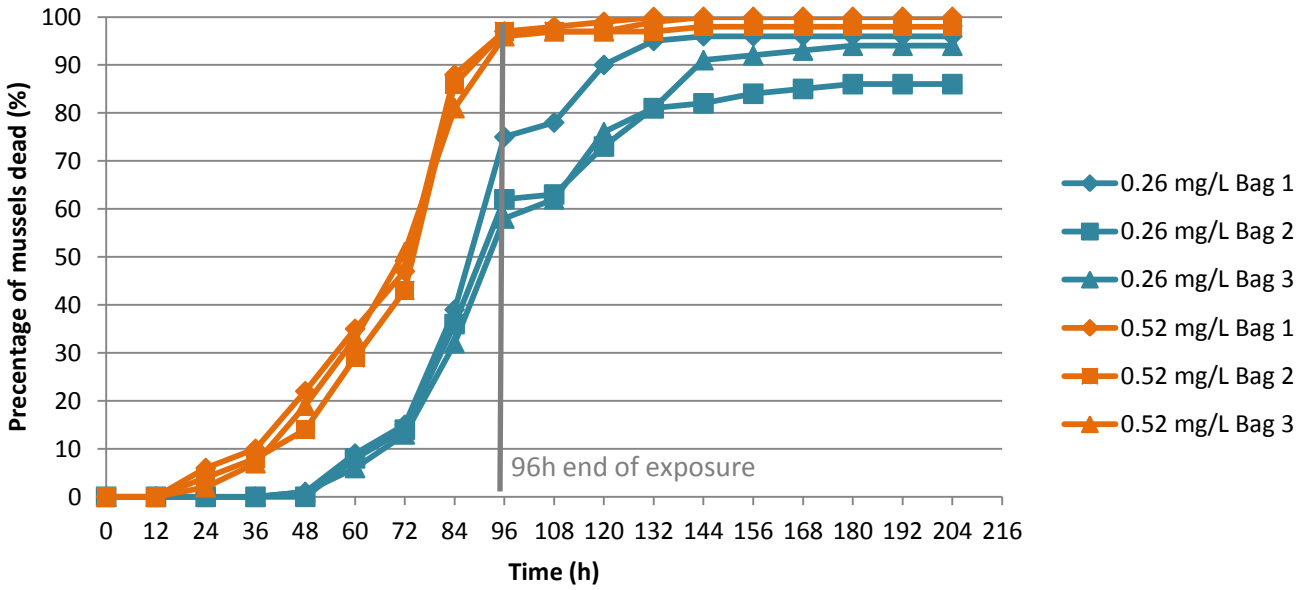

Figure 8. Quagga mussel mortality when exposed to copper sulfate

The mortality of the quagga mussels after 96 hours (Fig. 8) ranged from 58 to 74% at low copper concentration. At the high copper concentration the maximum mortality varied from 96.2% to 96.5%. After an additional 60 hours in the recovery cooler the maximum mortality for mussels exposed to a low copper concentration rose to a minimum of 84.5% and a maximum of 95.6%. At the high copper concentration level, the minimum mortality increased to 98.30% and maximum mortality reached 100%. We continued to observe the mussels in the recovery tank for an additional 50 hours. The mortality in the adults exposed to low copper continued to increase slightly. There were no mortalities recorded in the control group.

4.2.5 Effect of copper sulfate on quagga mussel mortality following 12h exposure in November

In November, a recovery experiment was conducted where quagga mussels were exposed to copper sulfate at high copper levels for 12h, and then observed during a 36h recovery period. There was no mussel mortality recorded in this experiment or in the controls.

4.2.6 Modeling the response of zebra mussels to copper sulphate at 96h exposure

The following logistic regression model was found to adequately fit the data on mortality of the zebra mussels after their exposure to copper sulfate for 96 h (see also Figures 9 and 10):

$$p_i = \frac{\exp(-9.03 + 0.04h_i + 0.19[t-18.4]_i + 3.55C_i + 2.04Month_i - 0.57Period - 1.83[C_i \times Month_i])}{1 + \exp(-9.03 + 0.04h_i + 0.19[t-18.4]_i + 3.55C_i + 2.04Month_i - 0.57Period - 1.83[C_i \times Month_i])}$$

All variables included into this model considerably contributed to prediction of the zebra mussel mortality (Table 3). As is seen from the equation, the cumulative mortality, in addition to its increase over time, was positively associated with the concentration of copper sulfate, water temperature, and overall was also higher in December compared to July. However, there was a significant interaction between the copper sulfate concentration and month, i.e. mussel mortality at the high copper concentration was lower in December compared to July. In addition, we found that the increase in mussel mortality was generally slower in the post-exposure period (i.e., after 96 h; see the "Period" term in the equation) in December compared to July.

Table 3. Analysis of deviance table for parameters of the GLM that describes the response of zebra mussels to treatment with copper sulfate for 96 hours. Deviance of the null model (i.e. model that has no predictors included) is 4302.6, with 45 degrees of freedom.

Parameter	Deviance explained by a parameter (added into the model (sequentially)	Degrees of freedom	Residual degrees of freedom	Deviance left unexplained	P-value for the chi-squared test
h (time)	2533.5	1	44	1769.1	<<0.001
t (temperature)	191.8	1	43	1577.4	<<0.001
C (concentration)	1201.6	1	42	375.8	<<0.001
Month[a]	62.7	1	41	313.1	<<0.001
Period[b]	24.0	1	40	289.1	0.015
C × Month	116.4	1	39	172.7	<<0.001

[a] Month was coded in the model as a dummy variable that takes a value of 0 for July and 1 for December

[b] Period was coded in the model as a dummy variable that takes a value of 0 for the period from 0 to 96 hours (including), and 1 for the remaining duration of the experiment.

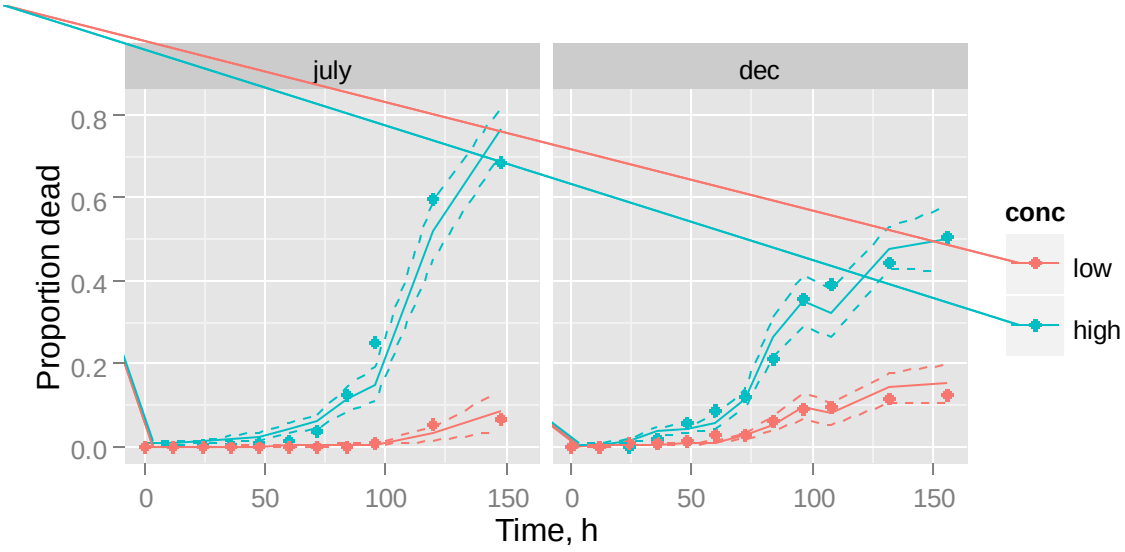

Figure 9. Fit of the model which describes the response of zebra mussels to treatment with copper sulfate for 96 hours. Dots show the actual cumulative proportions of molluscs found to be dead by a certain time since the beginning of the experiment. Solid lines correspond to the fitted values. Dashed lines denote 95% confidence intervals for the fitted values. Pearson correlation coefficient for the observed vs. fitted values is 0.985 ($P << 0.001$), suggesting that the model fits the data very well.

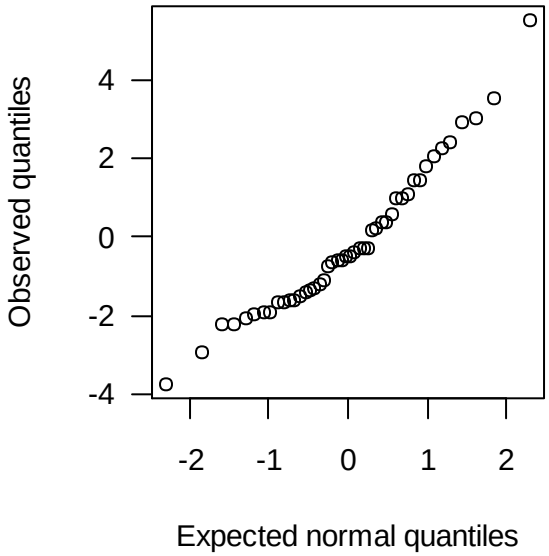

Figure 10. Q-Q plot for residuals of the model that describes the response of zebra mussels to treatment with copper sulfate for 96 hours. The dots on this plot form approximately a diagonal line, suggesting normality of the residuals. This is further confirmed by the non-significant Shapiro-Wilk's test for normality ($W = 0.956$, $P = 0.083$).

It should be noted that the results of this model are to be interpreted with caution as the particular mechanism behind the month effect is not clear and could potentially be confounded by temperature. As is seen from Figure 11, temperature demonstrated mirrored temporal profiles in July compared to December. Thus, it is not clear whether it was actually the seasonal effect or the temperature effect that caused the observed differences in mortality rates increase between the months.

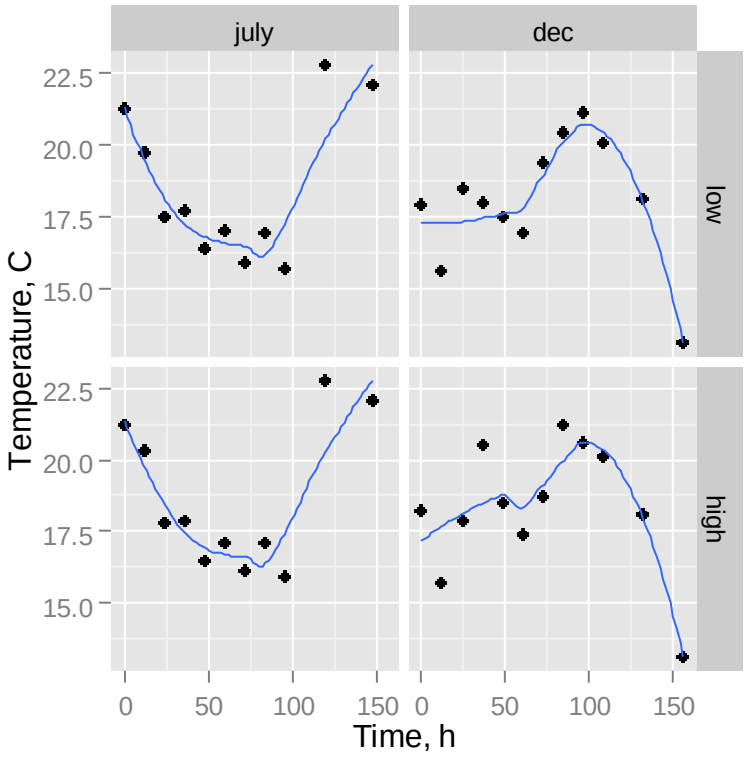

Figure 11. Dynamics of the water temperature over the duration of experiments on mortality of zebra mussels exposed to copper sulfate in different months.

4.2.7 Modeling the response of quagga mussels to copper sulphate at 96h exposure

As the data from this experiment demonstrated an autocorrelation (not shown here), we fitted a generalized additive model with correlated residuals to these data. The following GAM was found to fit the data adequately (see Figures 12 and 13):

$$p_i = \frac{\exp(\beta_0 + f(h_i) + \beta_1 t_i + \beta_1 C_i)}{1 + \exp(\beta_0 + f(h_i) + \beta_1 t_i + \beta_1 C_i)}$$

All variables included into this model considerably contributed to prediction of the quagga mussels' mortality (Table 4). As is seen from the equation, the cumulative mortality was nonlinearly related to time (see Figure 11), and was positively associated with the concentration of copper sulfate. F-stat represents the variance within groups divided by the variance between groups. If there is no difference between the groups of data, then the F-statistic value would be 0, the higher the value the more difference there is between the groups. Therefore time plays a very important role in mortality of quaggas when exposed to copper sulfate

We observed no statistically significant relation of mortality rate to the water temperature, a finding that could be related to a low variation of the recorded temperature values (min 16.4°C, max 18.2°C). The estimated parameter of residual autocorrelation made up ρ = 0.412, and was statistically significantly different from 0 (lower 95% confidence limit = 0.036, upper 95% confidence limit = 0.686).

Table 4. Analysis of variance table for parameters of the GAM that describes the response of quagga mussels to treatment with copper sulfate for 96 hours (n = 30).

Parameter	Degrees of freedom	F-statistic	P-value for the F-test
C (concentration)	1	25.4	<<0.001
f(h) (time)	1.99	683.0	<<0.001

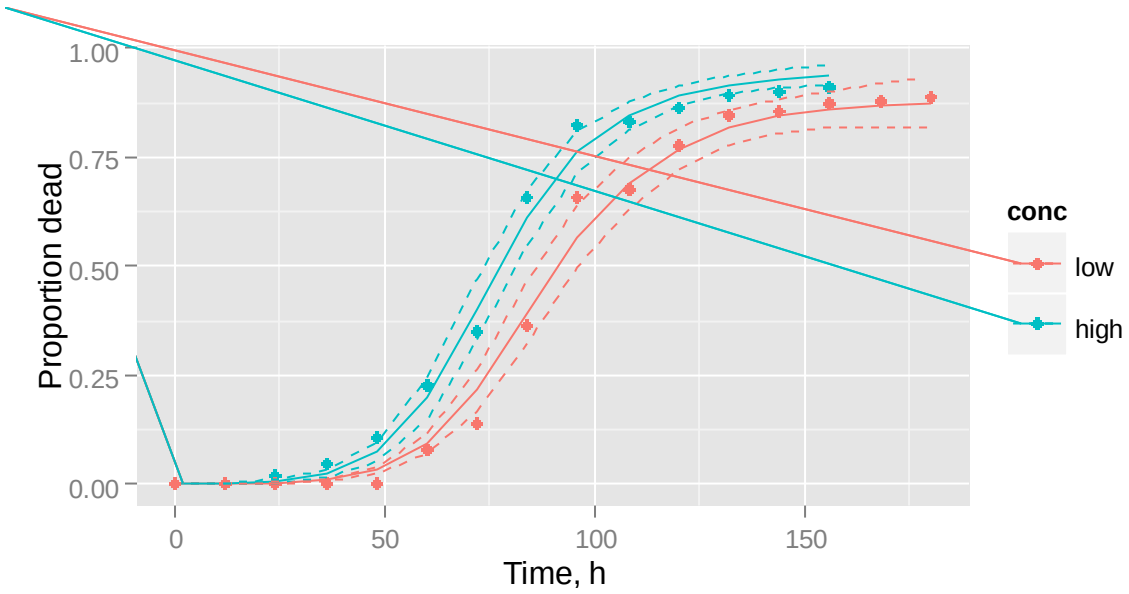

Figure 12. Fit of the model which describes the response of quagga mussels to treatment with copper sulfate for 96 hours. Dots show the actual cumulative proportions of mussels found to be dead by a certain time since the beginning of the experiment. Solid lines correspond to the fitted values. Dashed lines denote 95% confidence intervals for the fitted values. Pearson correlation coefficient for the observed vs. fitted values is 0.996 (P << 0.001), suggesting that the model fits the data very well.

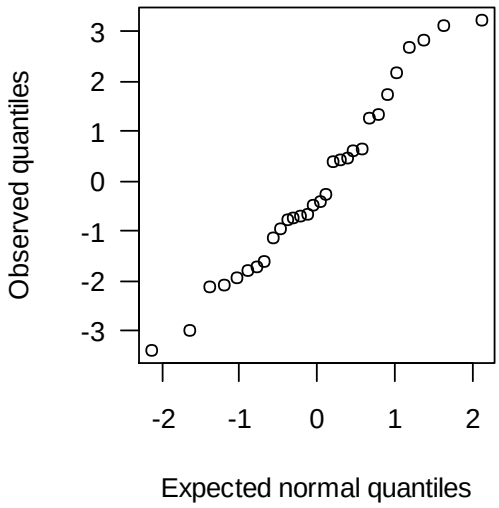

Figure 13. Q-Q plot for residuals of the model that describes the response of quagga mussels to treatment with copper sulfate for 96 hours. The dots arrange approximately into a diagonal line, suggesting normality of the residuals. This is further confirmed by the non-significant Shapiro-Wilk's test for normality (W = 0.996, P = 0.443).

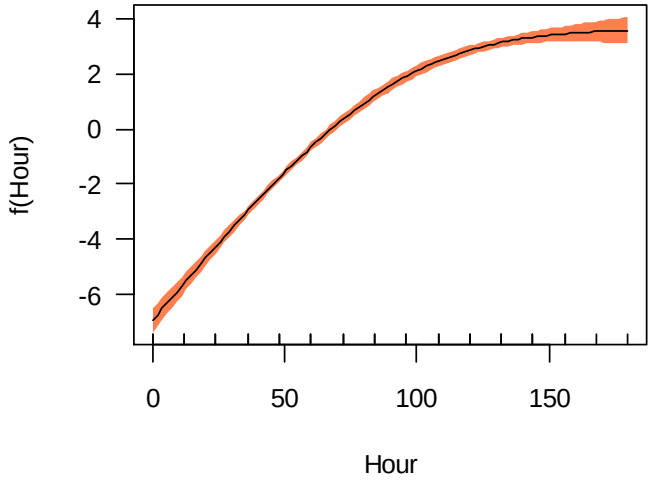

Figure 14. Estimated smoothing function for time in the model that describes the response of quagga mussels to treatment with copper sulfate for 96 hours. The smoother is shown as a solid line, along with its 95% point-wise confidence bands. The Y-axis reflects the contribution of the smoother to the fitted mortality values.

4.3 Effects of Natrix™ on dreissenid mussels

Similar to the copper sulfate experiment, 96h exposure mortalities for zebra mussels were monitored in both July and December 2012.

4.3.1 Effect of Natrix™ on zebra mussel mortality during 96h exposure in July

The first experiment started on July 9, 2012 observing zebra mussel mortality during exposure to Natrix™. Temperatures in the drum with the low copper averaged 17.9°C, with a minimum of 15.1°C and a maximum of 22.8°C. The drum with high copper had an average temperature of 18.0°C with a minimum of 15.2°C and maximum of 22.8°C.

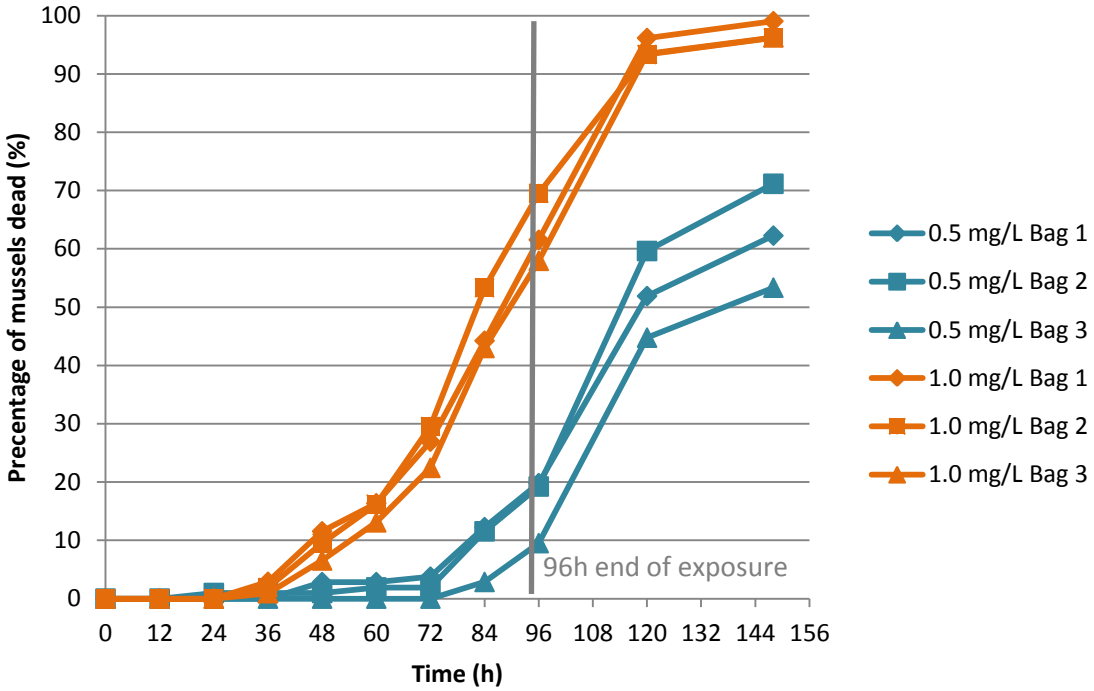

Figure 15. Zebra mussel mortality when exposed to Natrix™ in July

The July experiment (Figure 15) had an average mortality of 16.2% after 96h at low copper, and an average mortality of 63.0% after 96h at high copper. The experiment was continued as mussels continue to experience mortality after being placed in recovery chambers with fresh water, and mortalities rose after 148h to 62.3% for low and 97.2% for high copper.

4.3.2 Effect of Natrix™ on zebra mussel mortality during 96h exposure in December

The experiment was repeated in December 2012 (Figure 16). After the 96 hour exposure to low copper as Natrix™, the maximum mortality varied from 49.6 to 63.8%. At the high level of copper as Natrix™, the maximum mortality varied from 56.4 to 80%. After additional 60 hours in the recovery cooler, the maximum mortality for low copper rose to a minimum of 58.7% and a maximum of 72.4%. At the high copper level, the minimum mortality increased to 73.2% and maximum mortality reached 93.9% (Figure 16). During the experimental exposure, the ambient water temperature varied from a high of 22.3ºC to a low of 13.1 ºC . The average temperature for the low copper treatment was 19.3 ºC and 18.7 ºC for the high copper treatment. There was no mortality recorded in the control.

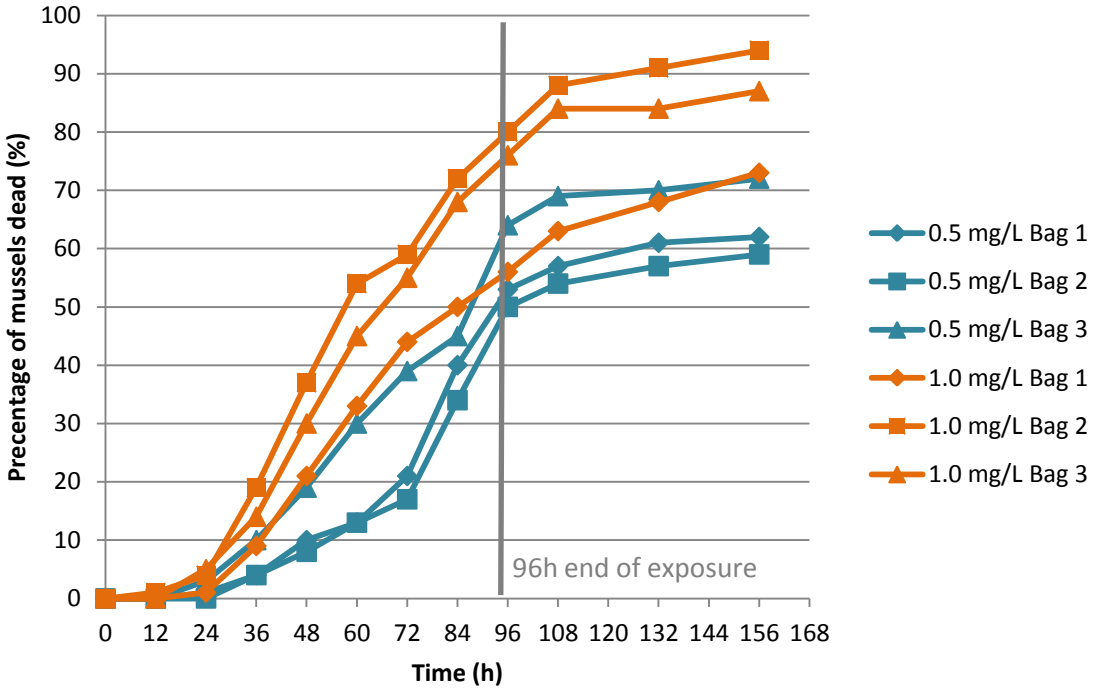

Figure 16. Zebra mussel mortality when exposed to *Natrix*™

4.3.3 Effect of Natrix™ on zebra mussel mortality following 12h exposure in December

In December, a recovery experiment was also completed. Zebra mussels were exposed for 12h to high copper solution (1.0 mg/L) created with Natrix™. Following exposure, the mussels were placed in a recovery chamber and observed for 36h for mortality. There was some post exposure mortality noted during this experiment, from 1.89% to 5.88% after 36h of recovery (Figure 17). The temperature averaged 17.4°C, with a minimum of 16.4°C and a maximum of 19.0°C.

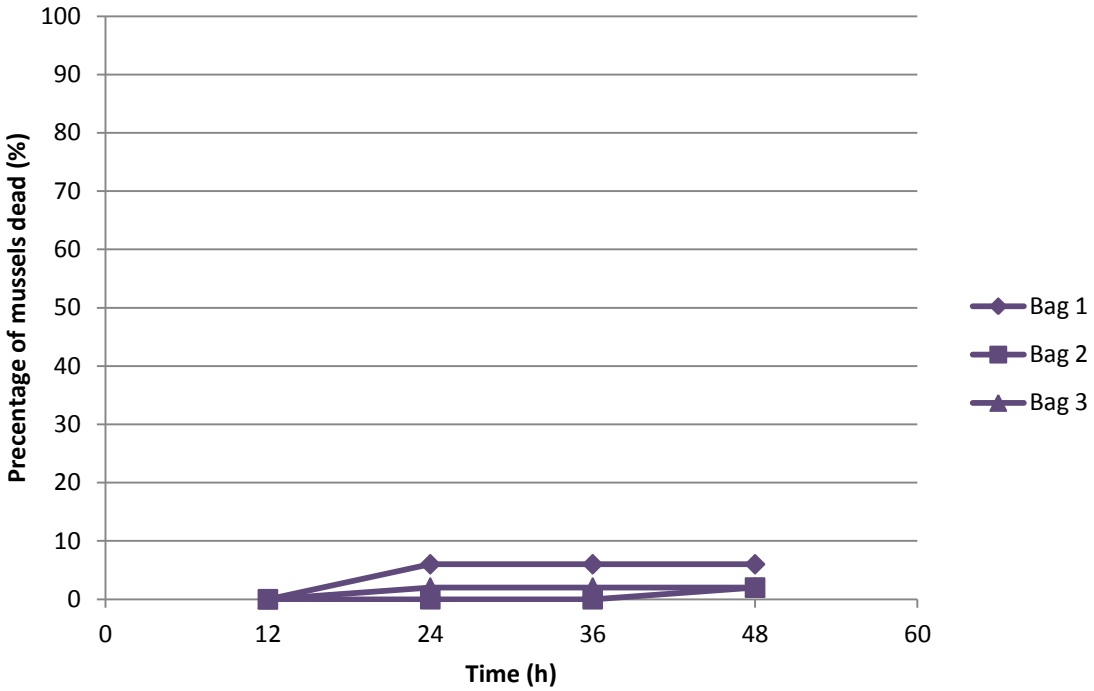

Figure 17. Zebra mussel mortality following removal from *Natrix*™ solution

4.3.4 Effect of Natrix™ on quagga mussel mortality during 96h exposure in November

This experiment was carried out at Davis Dam, starting on November 14, 2012. The ambient temperature ranged from 18.2ºC and 16.7 ºC. The average temperature was 17.1 ºC for the low copper exposure and 17.2 ºC for the high copper exposure.

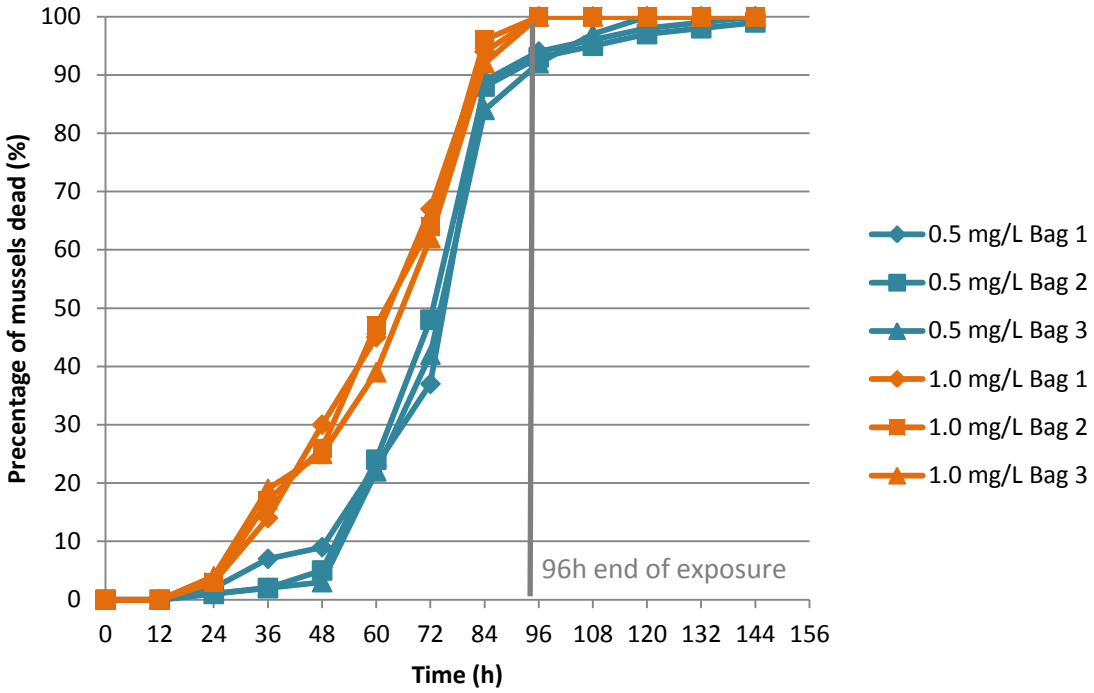

Figure 18. Quagga mussel mortality when exposed to *Natrix*™

The mortality of the quagga mussels after 96 hours ranged from 92.2 to 93.9% at low copper as Natrix™ (Figure 18). At the high copper concentration all three bags reached 100% mortality at 96 hours. The survivors from the high copper concentration were observed for an additional 48 hours during which time the mortality continued to increase. At the end of this 48 recovery period the mortality ranged from to 98.9 to 100%.

There was no mortality in the control groups.

4.3.5 Effect of Natrix™ on quagga mussel mortality following 12h exposure in December

A recovery experiment was completed in December. There was no mortality of the quagga mussels after the 12 hour exposure to high copper from a Natrix™ solution. However, mortality was observed after a 24 hour recovery period. At the end of the experiment, 36 hours after exposure, mortality ranged from 2.0% to 13.5% (Figure 19). The ambient temperature ranged from 18.2ºC and 16.7 ºC. The average temperature was 17.1 ºC.

There was no mortality observed in the control group.

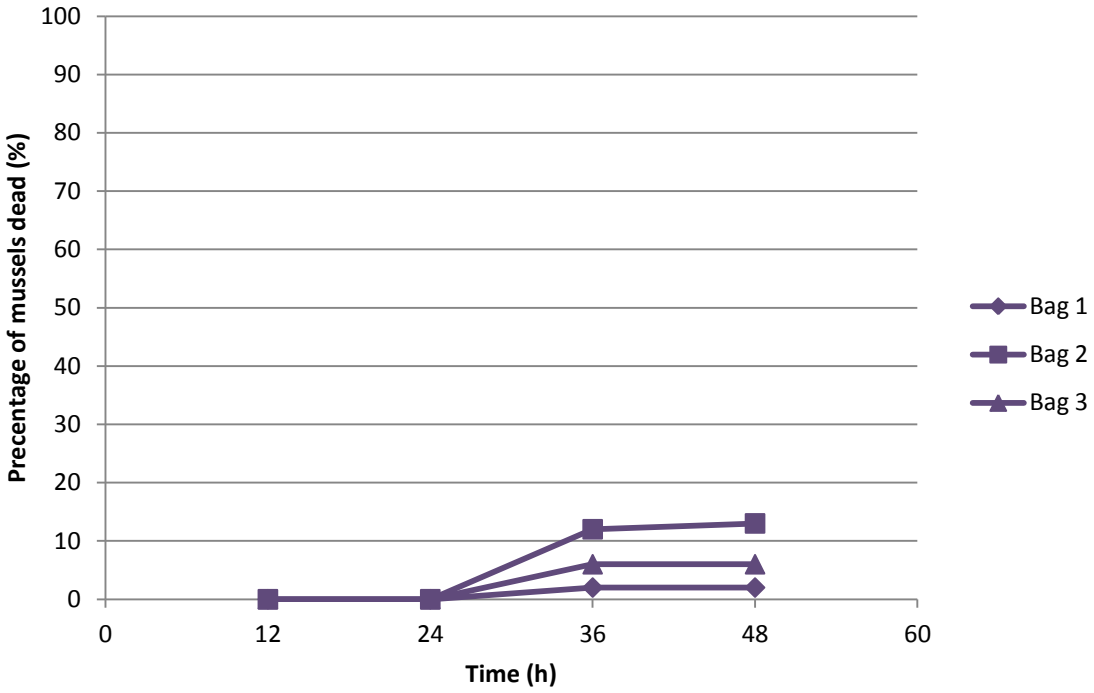

Figure 19. Quagga mussel mortality following removal from *Natrix*™ solution

4.3.6 Modeling the response of zebra mussels to Natrix™ at 96h exposure

The following logistic regression model was found to adequately fit the data on mortality of the zebra mussels after their exposure to copper sulfate for 96 h (see also Figures 20 and 21):

$$p_i = \frac{\exp(-6.39 + 0.05\mathrm{h}_i + 0.21[\mathrm{t}-18.5]_i + 2.30\mathrm{C}_i + 1.18\mathrm{Month}_i - 0.82\mathrm{Period}_i - 1.13[\mathrm{C}_i \times \mathrm{Month}_i])}{1+\exp(-6.39 + 0.05\mathrm{h}_i + 0.21[\mathrm{t}-18.5]_i + 2.30\mathrm{C}_i + 1.18\mathrm{Month}_i - 0.82\mathrm{Period}_i - 1.13[\mathrm{C}_i \times \mathrm{Month}_i])}$$

All variables included into this model considerably contributed to prediction of the zebra mussel mortality (Table 5). As is seen from the equation, the cumulative mortality, in addition to its increase over time, was positively associated with the concentration of Natrix, water temperature, and overall was also higher in December compared to July. However, there was a significant interaction between the Natrix concentration and month, i.e. in December the mortality of molluscs exposed to higher concentration overall was lower than at this same concentration in July. In addition, we found that the increase of the mussels' mortality was

generally slower in the post-exposure period (i.e., after 96 h; see the "Period" term in the equation).

Table 5. Analysis of deviance table for parameters of the GLM that describes the response of zebra mussels to treatment with Natrix™ for 96 hours. The deviance of the null model (i.e. model that has no predictors included) is 8022, with 45 degrees of freedom.

Parameter	Deviance explained by a parameter (added into the model sequentially)	Degrees of freedom	Residual degrees of freedom	Deviance left unexplained	P-value for the chi-squared test
h (time)	6053	1	44	1969	<<0.001
C (concentration)	789	1	43	1180	<<0.001
Month[a]	281	1	42	899	<<0.001
t (temperature)	337	1	41	561	<<0.001
Period[b]	111	1	40	451	<0.001
C × Month	116.4	1	39	339	<0.001

[a] Month was coded in the model as a dummy variable that takes a value of 0 for July and 1 for December

[b] Period was similarly coded as a dummy variable that takes a value of 0 for the period from 0 to 96 hours (including), and 1 for the remaining duration of the experiment.

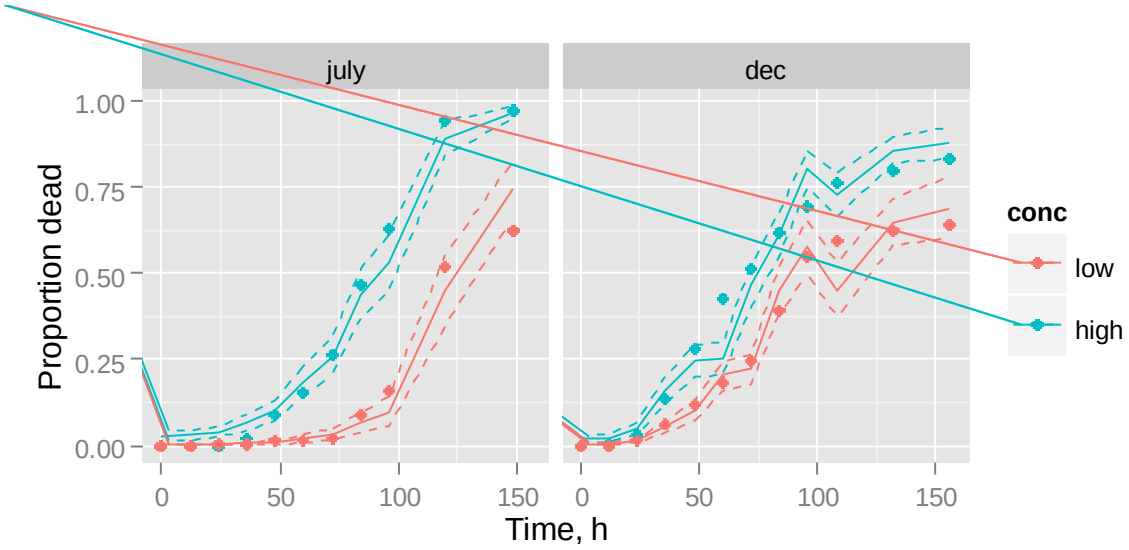

Figure 20. Fit of the model which describes the response of zebra mussels to treatment with Natrix for 96 hours. Dots show the actual cumulative proportions of molluscs found to be dead by a certain time since the beginning of the experiment. Solid lines correspond to the fitted values. Dashed lines denote 95% confidence intervals for the fitted values. Pearson correlation coefficient for the observed vs. fitted values is 0.985 (P << 0.001), suggesting that the model fits the data very well.

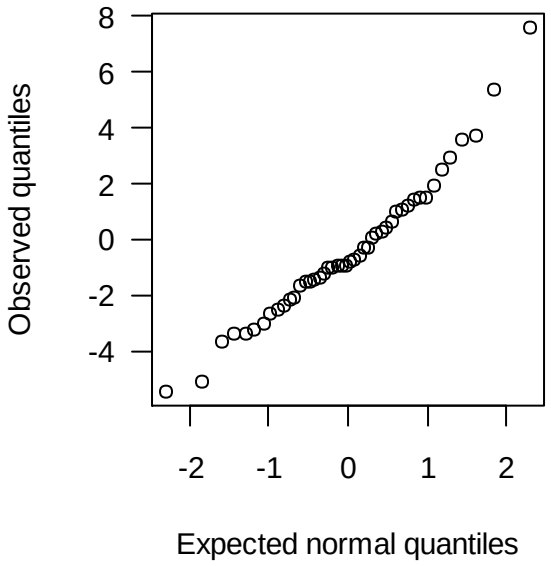

Figure 21. Q-Q plot for residuals of the model that describes the response of zebra mussels to treatment with Natrix for 96 hours. The dots on this plot form approximately a diagonal line, suggesting normality of the residuals. This is further confirmed by the non-significant Shapiro-Wilk's test for normality (W = 0.965, P = 0.182).

It should be noted that the results of this model are to be interpreted with caution as the particular mechanism behind the month effect is not clear and could potentially be confounded by temperature. As is seen from Figure 22, temperature demonstrated mirrored temporal profiles in July compared to December. Thus, it is not clear whether it was actually the seasonal effect or the temperature effect that caused the observed differences in rates of mortality's increase between the months.

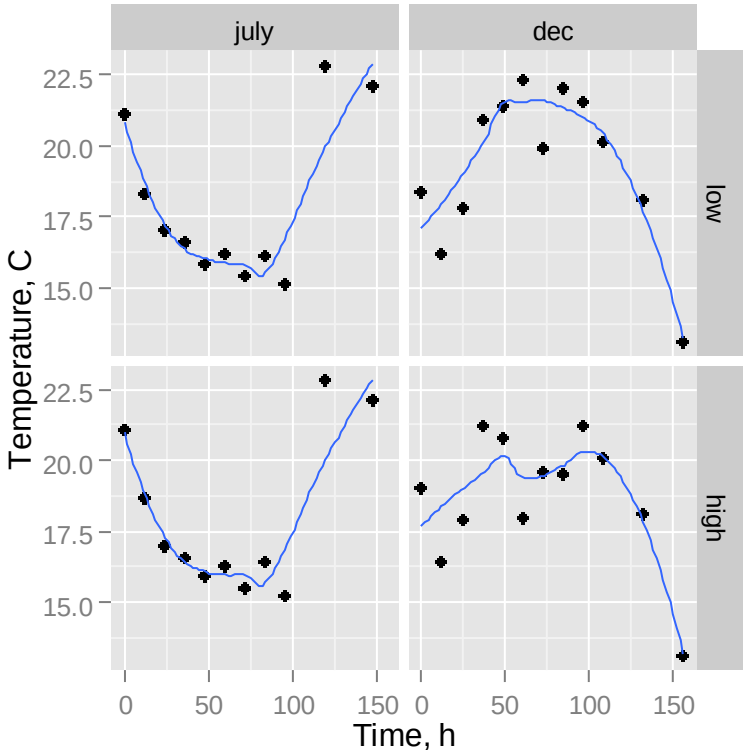

Figure 22. Dynamics of the water temperature over the duration of experiments on mortality of zebra mussels exposed to Natrix in different months.

4.3.7 Modeling the response of quagga mussels to Natrix™ at 96h exposure

The following logistic regression model was found to adequately fit the data on mortality of quagga mussels after 96 h exposure to copper sulfate (see also Figures 23 and 24):

$$p_i = \frac{\exp(-7.07 + 0.10\mathrm{h}_i + 1.19\mathrm{C}_i)}{1 + \exp(-7.07 + 0.10\mathrm{h}_i + 1.19\mathrm{C}_i)}$$

All variables included into this model considerably contributed to prediction of the quagga mussel mortality (Table 6). As is seen from the equation, the cumulative mortality, in addition to its increase over time, was positively associated with the concentration of Natrix.

Table 6. Analysis of deviance table for parameters of the GLM that describes the response of quagga mussels to treatment with Natrix for 96 hours. The deviance of the null model (i.e. model that has no predictors included) is 7911, with 24 degrees of freedom.

Parameter	Deviance explained by a parameter (added into the model (sequentially)	Degrees of freedom	Residual degrees of freedom	Deviance left unexplained	P-value for the chi-squared test
h (time)	7579	1	23	332	<<0.001
C (concentration)	193	1	22	139	<<0.001

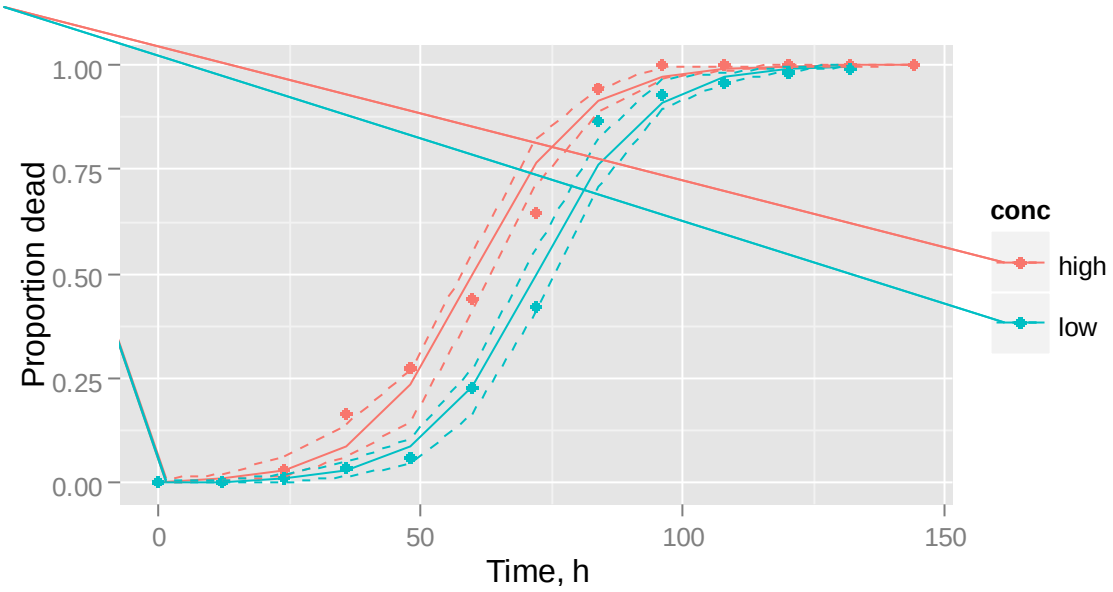

Figure 23. Goodness of fit of the model that describes the response of quagga mussels to treatment with Natrix for 96 hours. Dots show the actual cumulative proportions of molluscs found to be dead by a certain time since the beginning of the experiment. Solid lines correspond to the fitted values. Dashed lines denote 95% confidence intervals for the fitted values. Pearson correlation coefficient for the observed and fitted values is 0.995 (P << 0.001), suggesting that the model fits the data very well.

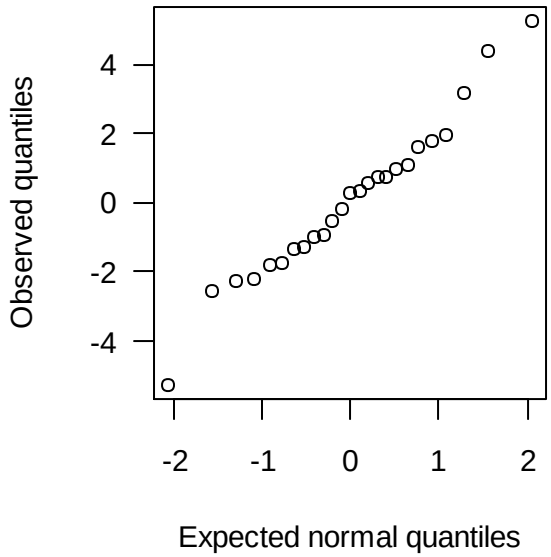

Figure 24. Q-Q plot for residuals of the model that describes the response of quagga mussels to treatment with Natrix for 96 hours. The dots on this plot form approximately a diagonal line, suggesting normality of the residuals. This is further confirmed by the non-significant Shapiro-Wilk's test for normality (W = 0.976, P = 0.794).

4.4 Effects of Captain™ liquid copper algaecide on dreissenid mussels

4.4.1 Captain™ and zebra mussel mortality during 96h exposure in December

Zebra mussels were exposed to a low and high concentration of Captain™ (0.5 mg/L and 1.0 mg/L) for 96 hours in December 2012 (Figure 25). First mortality in adults was noted after 12 hours in the high copper solution. At the end of the 96 hour exposure period, the low treatment achieved maximum mortality between 20.3 and 32.7% while the high treatment achieved mortality ranging from 66.2 to 78.3%. We continued to observe the surviving mussels for an additional 60 hours in a recovery tank. The mortalities continued to increase during the recovery period. At the end of the 60 hour recovery, the low treatment achieved maximum mortality between 31.8% and 55.8% while the high treatment achieved mortality ranging from 80.28 to 95.3%. The minimum temperature during the experiment was 13.1 ºC, maximum was 21.3 ºC, and the average temperature was 17.7°C in the low treatment and 18.02 ºC in the high treatment.

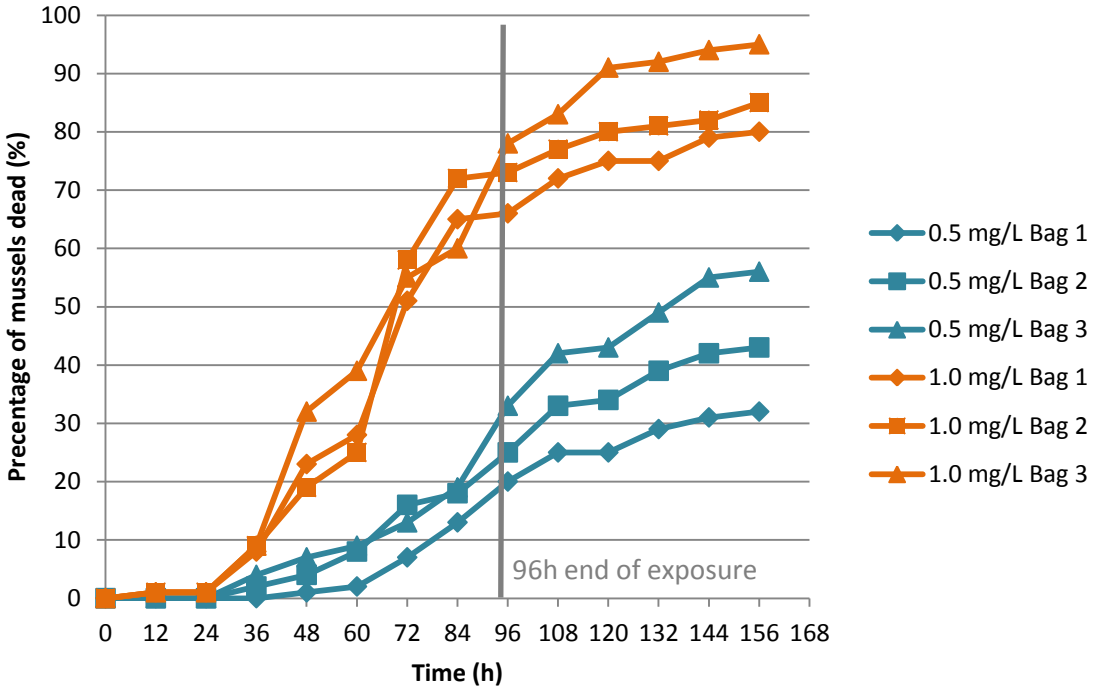

Figure 25. Zebra mussel mortality when exposed to Captain™ as low copper and high copper for 96 hours

4.4.2 Captain™ and zebra mussel mortality following 12h exposure in December

The recovery experiment observed zebra mussel survival for a 36h recover period, after removal from a 12h exposure to high copper solution of Captain™. The 12-hour exposure resulted in no mortality. At the end of the 36-hour recovery period, the maximum mortality was 3% (Figure 26). The minimum temperature during the experiment was 15.5ºC, maximum was 21.0ºC and the average temperature was 19.0ºC.

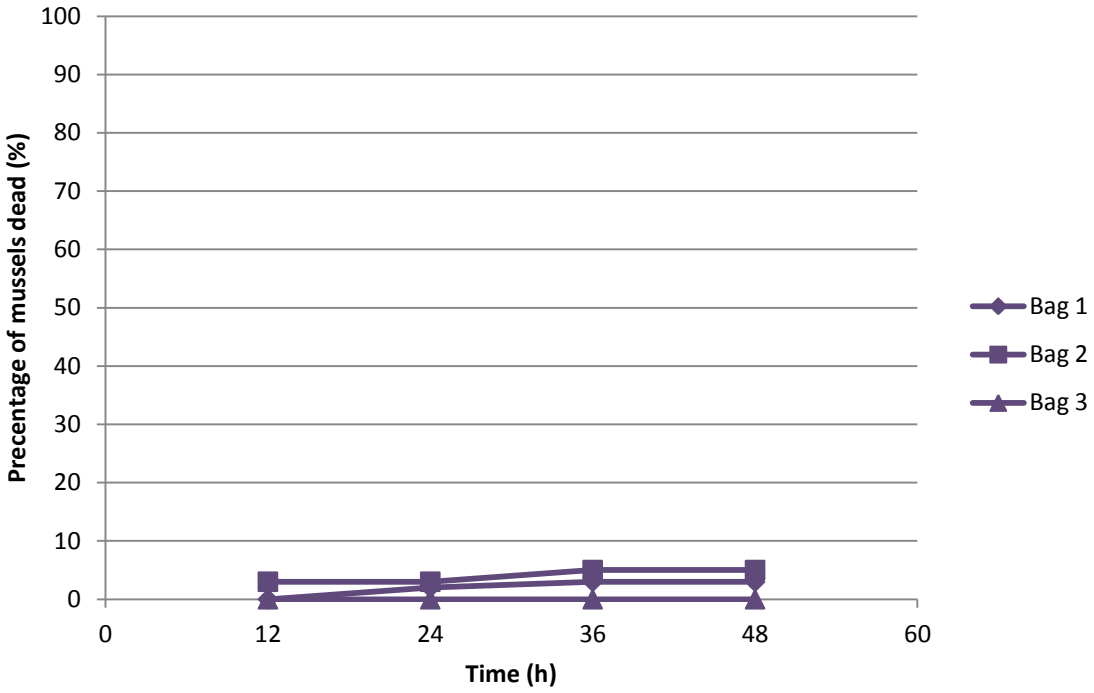

Figure 26. Zebra mussel mortality after 12h exposure to Captain™ as high copper equivalent (1 mg/L)

4.4.3 Effect of Captain™ on quagga mussel mortality during 96h exposure in December

Quagga mussels were exposed to a low and high concentration of Captain™ (0.5 mg/L and 1.0 mg/L) for 96 hours in December 2012 (Figure 27). First mortality in adults was noted after 12 hours in the high copper solution. Mortality did not occur in the low copper solution until the 24 hour mark. There continued to be higher cumulative mortality observed in the group exposed to high copper solution until the 84 hour mark. After that point, the cumulative mortality in the low copper treatment was essentially equivalent that of the high copper treatment. The low treatment achieved 92 to 95% mortality in 96 hours, while the high treatment achieved 91 to 96% mortality. The minimum temperature during the experiment was 16.8 ºC, maximum was 18.0 ºC and the average temperature was 17.2 ºC.

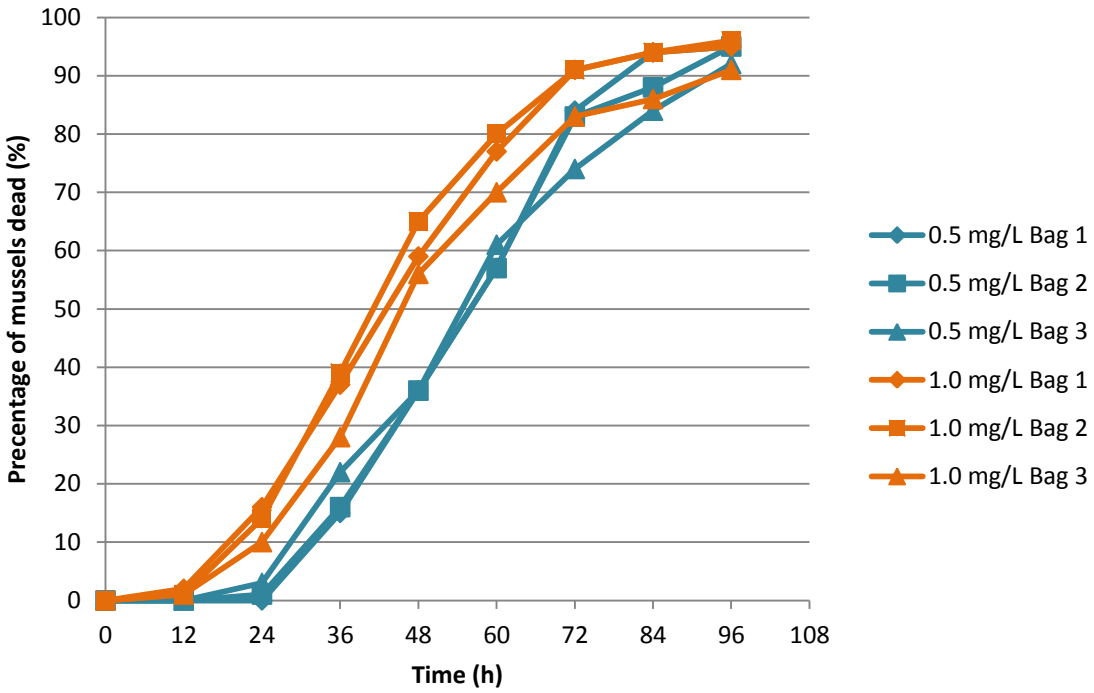

Figure 27. Quagga mussel mortality when exposed to Captain™ for 96 hours

4.4.4 Effect of Captain™ on quagga mussel mortality following 12h exposure in December

The recovery experiment looked at effects of short term exposure (12h) to a high copper Captain™ solution on quagga mussels during a recovery period of 36h (Figure 28). Some mortality was observed following the initial 12 hour exposure. The mortality increased during the 36 hour recovery period. The maximum mortality achieved at the end of the 36 hour recovery period was 10%. The temperature averaged 17.5°C, with a minimum of 17.1°C and a maximum of 18.0°C.

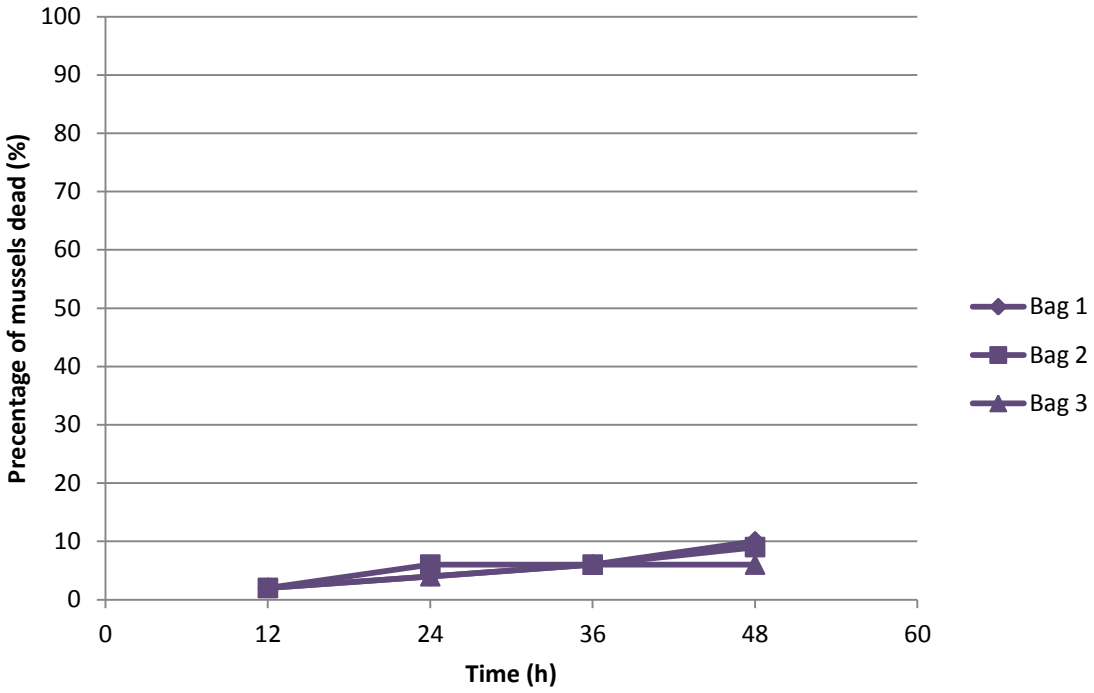

Figure 28. Quagga mussel mortality following 12 hour exposure to Captain™ solution, high copper equivalent (1.0 mg/L)

4.4.5 Modeling the response of zebra mussels to Captain™ at 96h exposure

The following logistic regression model was found to adequately fit the data on mortality of the zebra mussels after their exposure to Captain™ for 96h (see also Figures 29 and 30):

$$p_i = \frac{\exp(-5.32 + 0.04\mathrm{h}_i + 0.23[\mathrm{t} - 17.9]_i + 2.06\mathrm{C}_i)}{1 + \exp(-5.32 + 0.04\mathrm{h}_i + 0.23[\mathrm{t} - 17.9]_i + 2.06\mathrm{C}_i)}$$

All variables included into this model considerably contributed to prediction of zebra mussel mortality (Table 7). As is seen from the equation, the cumulative mortality, in addition to its increase over time, was positively associated with the concentration of Captain™ and water temperature.

Table 7. Analysis of deviance table for parameters of the GLM that describes the response of zebra mussels to treatment with Captain™ for 96 hours. The deviance of the null model (i.e. model that has no predictors included) is 3860, with 23 degrees of freedom.

Parameter	Deviance explained by a parameter (added into the model sequentially)	Degrees of freedom	Residual degrees of freedom	Deviance left unexplained	P-value for the chi-squared test
h (time)	2310	1	22	1550	<<0.001
C (concentration)	789	1	21	457	<<0.001
t (temperature)	311	1	20	146	<<0.001

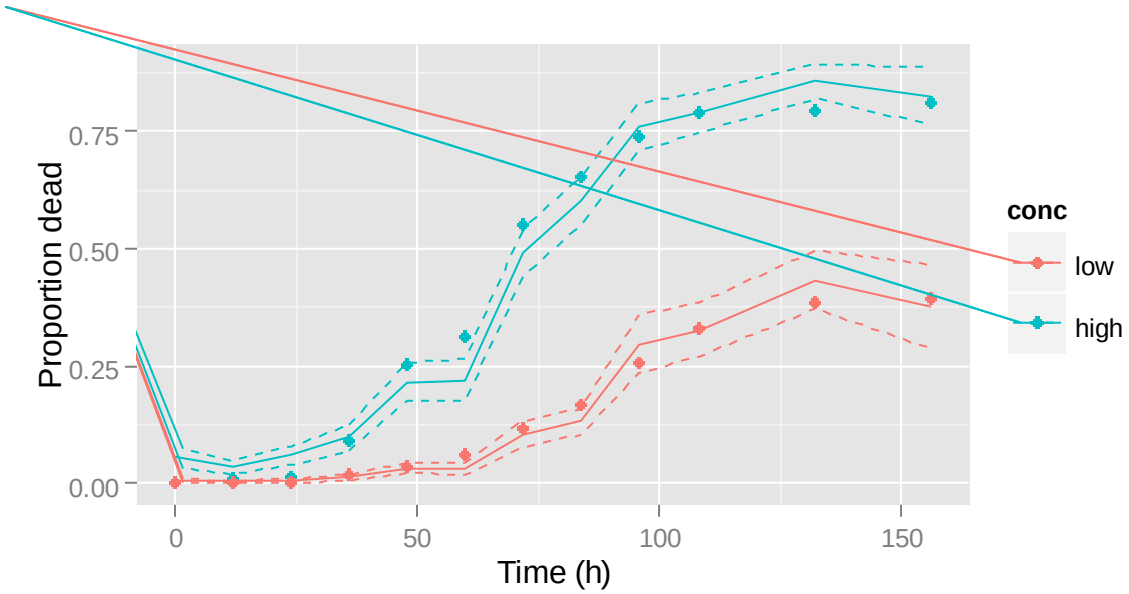

Figure 29. Goodness of fit of the GLM that describes the response of zebra mussels to treatment with Captain™ for 96 hours. Dots show the actual cumulative proportions of molluscs found to be dead by a certain time since the beginning of the experiment. Solid lines correspond to the fitted values. Dashed lines denote 95% confidence intervals for the fitted values. Pearson correlation coefficient for the observed vs. fitted values is 0.992 (P << 0.001), suggesting that the model fits the data very well.

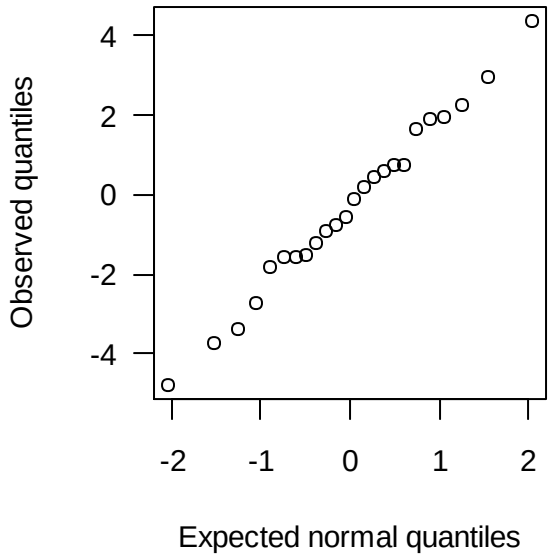

Figure 30. Q-Q plot for residuals of the GLM that describes the response of zebra mussels to treatment with Captain for 96 hours. The dots on this plot form a diagonal line, suggesting normality of the residuals. This is further confirmed by the non-significant Shapiro-Wilk's test for normality (W = 0.991, P = 0.998).

4.4.6 Modeling the response of quagga mussels to Captain™ at 96h exposure

The following logistic regression model was found to adequately fit the data on mortality of the quagga mussels after 96 h exposure to Captain™ (see also Figures 31 and 32):

$$p_i = \frac{\exp(-4.70 + 0.08\mathrm{h}_i + 0.84\mathrm{C}_i)}{1 + \exp(-4.70 + 0.08\mathrm{h}_i + 0.84\mathrm{C}_i)}$$

All variables included into this model considerably contributed to prediction of quagga mussel mortality (Table 8). As is seen from the equation, the cumulative mortality, in addition to its increase over time, was positively associated with the concentration of Captain™.

Table 8. Analysis of deviance table for parameters of the GLM that describes the response of quagga mussels to treatment with Captain™ for 96 hours. The deviance of the null model (i.e. model that has no predictors included) is 3778.1, with 17 degrees of freedom.

Parameter	Deviance explained by a parameter (added into the model sequentially)	Degrees of freedom	Residual degrees of freedom	Deviance left unexplained	P-value for the chi-squared test
h (time)	3564.3	1	16	213.8	<<0.001
C (concentration)	102.1	1	15	111.6	<<0.001

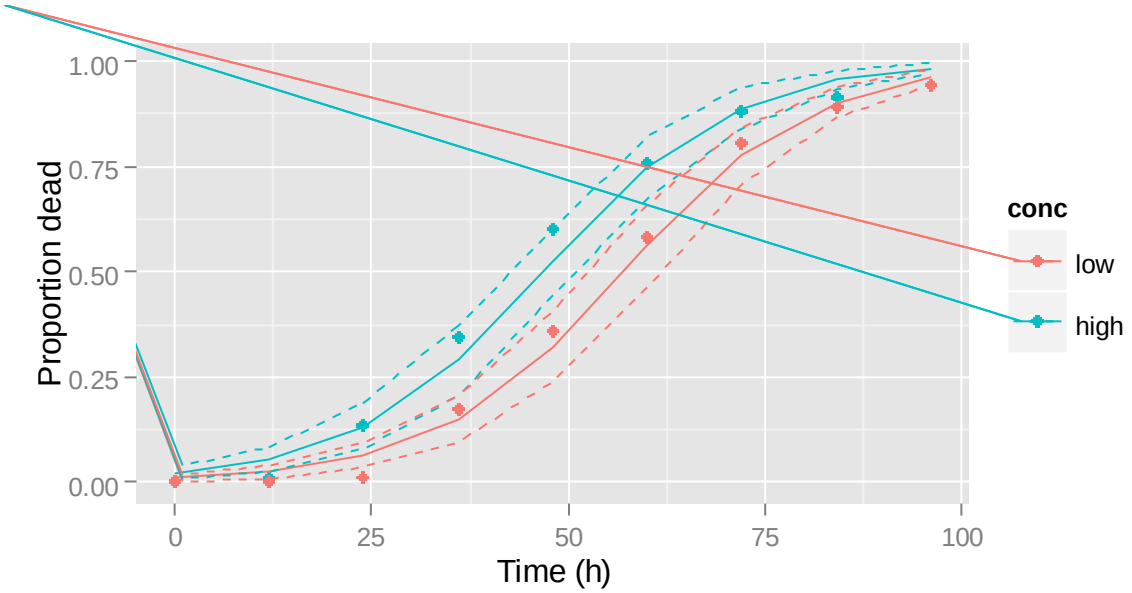

Figure 31. Goodness of fit of the GLM that describes the response of quagga mussels to treatment with Captain™ for 96 hours. Dots show the actual cumulative proportions of molluscs found to be dead by a certain time since the beginning of the experiment. Solid lines correspond to the fitted values. Dashed lines denote 95% confidence intervals for the fitted values. Pearson correlation coefficient for the observed vs. fitted values is 0.996 ($P << 0.001$), suggesting that the model fits the data very well.

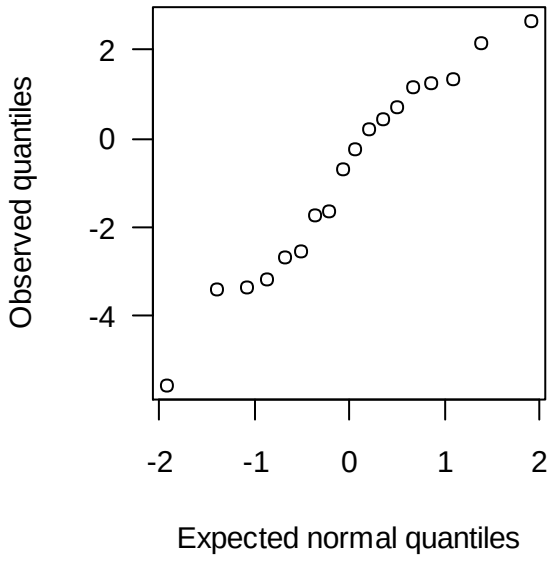

Figure 32. Q-Q plot for residuals of the GLM that describes the response of quagga mussels to treatment with Captain™ for 96 hours. The dots on this plot form approximately a diagonal line, suggesting normality of the residuals. This is further confirmed by the non-significant Shapiro-Wilk's test for normality ($W = 0.960$, $P = 0.598$)

4.5 Effects of EarthTec® algaecide on dreissenid mussels

4.5.1 Effect of EarthTec® on zebra mussel mortality during 96h exposure in December

Zebra mussels were exposed to low and high concentration of EarthTec® for 96h in December (Figure 33). The minimum temperature during the experiment was 16.5 ºC, the maximum temperature was 17.9 ºC and the average temperature was 17.2 ºC. First mortality in adults was noted after 12 hours and greater mortality was observed in the high copper solution. There continued to be higher cumulative mortality observed in the high copper treatment until the 60 hour mark. After 72 hours, cumulative mortality in the low copper treatment was nearly equivalent to the high copper treatment. The low copper treatment achieved 100% mortality in 84 hours while the high copper treatment achieved 100% mortality in 72 hours.

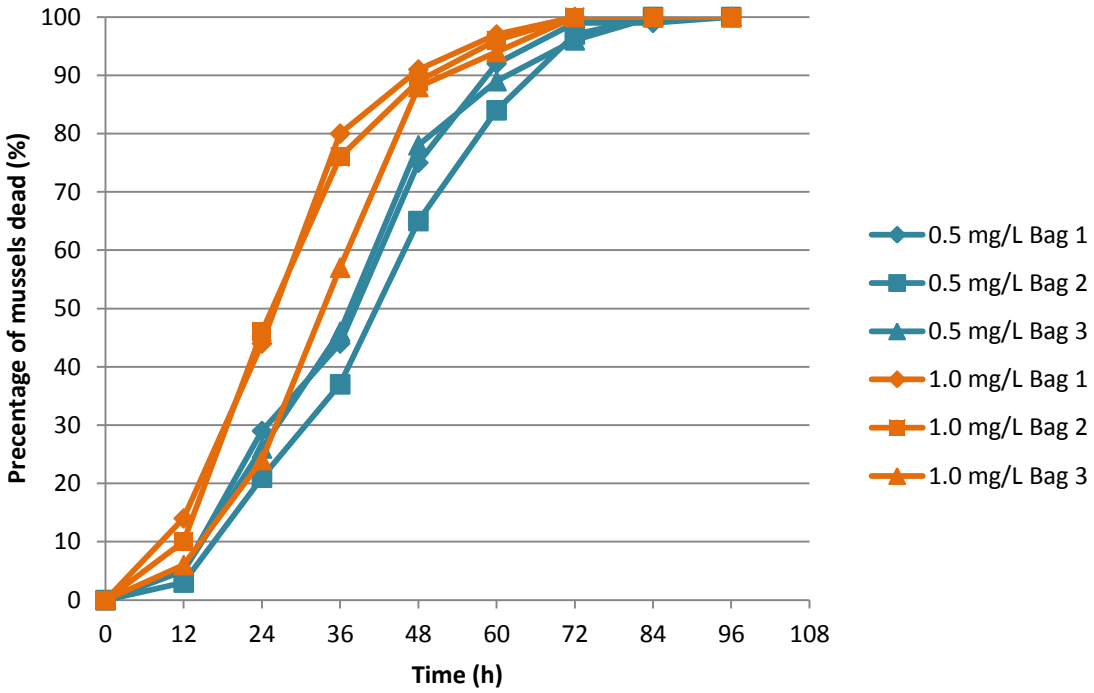

Figure 33. Zebra mussel mortality when exposed to EarthTec® for 96 hours

4.5.2 Effect of EarthTec® on zebra mussel mortality following 12h exposure in December

The recovery experiment with zebra mussels was limited to a 12-hour exposure period in the high treatment. The minimum temperature during the experiment was 16.5 ºC, maximum was 22 ºC and the average temperature was 19.6 ºC. The 12-hour exposure resulted in a total mortality between 37 and 60% at the end of the 36 hour exposure period (Figure 34). Mortality in the adults continued to increase during the entire recovery period.

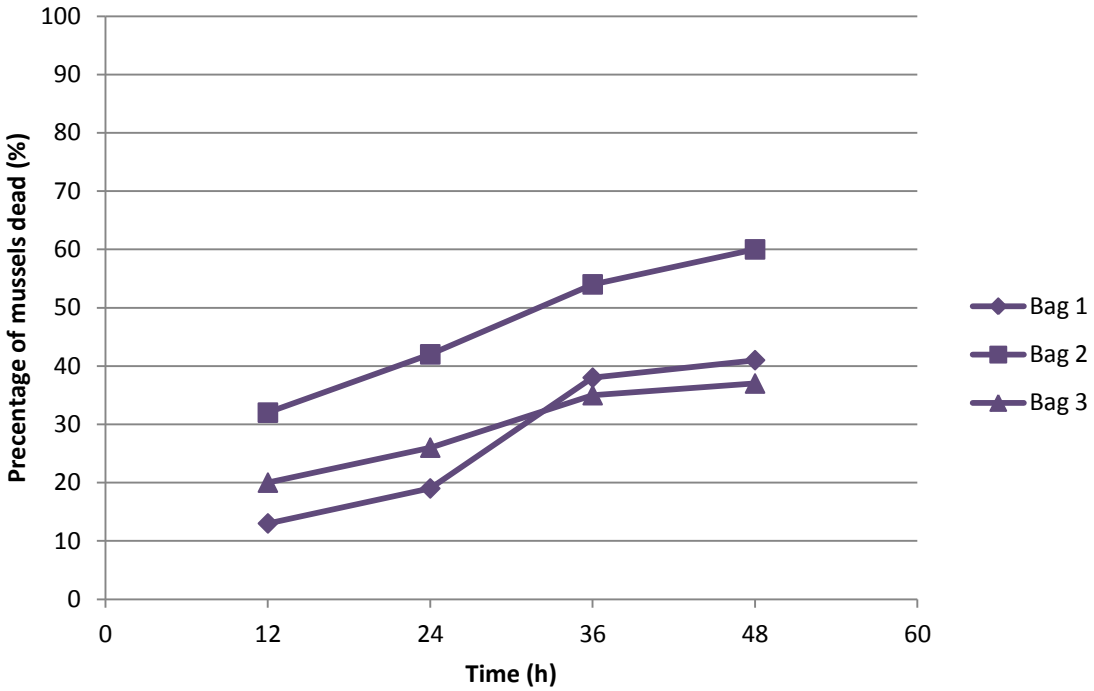

Figure 34. Zebra mussel mortality following exposure of 12 hours to EarthTec® solution, high copper equivalent

4.5.3 Effect of EarthTec® on quagga mussel mortality during 96h exposure in December

Quagga mussels were exposed to low and high concentration of EarthTec® for 96h in December (Figure 35). The minimum temperature during the experiment was 16.5 ºC, maximum was 17.9 ºC and the average temperature was 17.2 ºC. First mortality in adults was noted after 24 hours, with greater mortality observed in the high copper solution. There continued to be higher mortality observed in the group exposed to high of copper solution until the 80 hour mark. After that point, the low copper treatment was essentially equivalent to the high copper treatment. The low copper treatment achieved 97% mortality while the high copper treatment achieved 99% mortality.

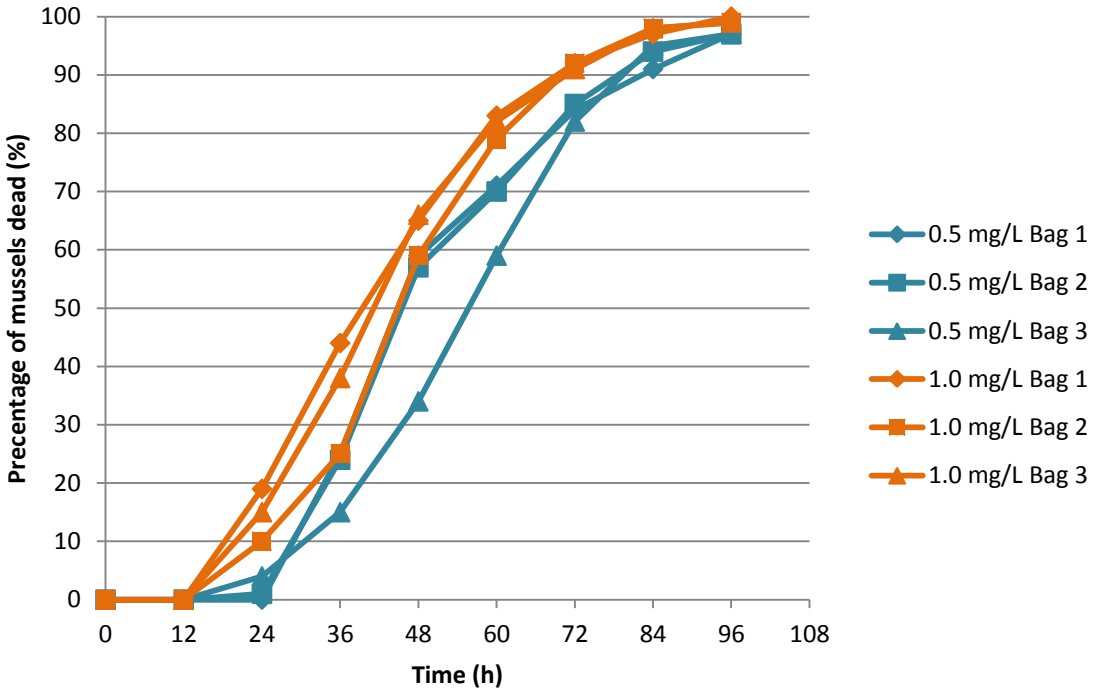

Figure 35. Quagga mussel mortality when exposed to EarthTec® for 96 hours

4.5.4 Effect of EarthTec® on quagga mussel mortality following 12h exposure in December

The EarthTec® recovery experiment involved observation of quagga mussels during a 36h recovery period following a 12 hour exposure to high copper solution. The average temperature was 17.63°C, with a minimum of 17.10°C and a maximum of 18.30°C. Following the initial exposure, adult mussels experienced some mortality during the recovery period (Figure 36). The maximum mortality achieved at the end of the 36 hour recovery period was 12%.

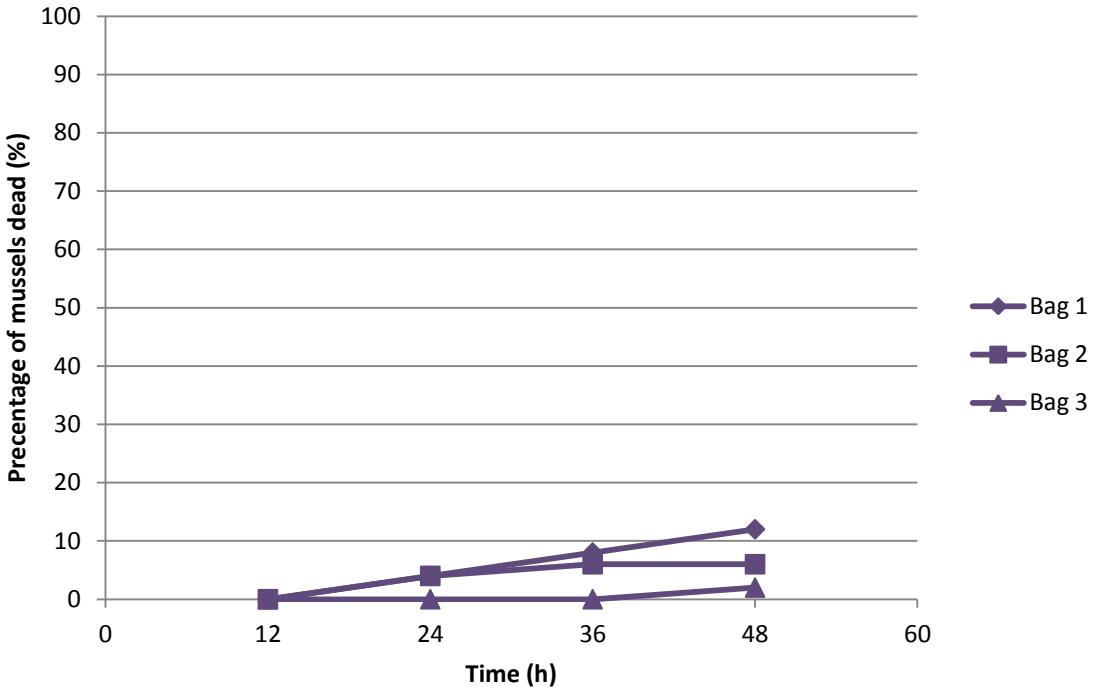

Figure 36. Quagga mussel mortality following removal from EarthTec® solution, high copper equivalent

4.5.5 Modeling the response of zebra mussels to EarthTec® at 96 h exposure

The following logistic regression model was found to adequately fit the data on mortality of the zebra mussels after their exposure to EarthTec® for 96h (see also Figures 37 and 38):

$$p_i = \frac{\exp(-4.36 + 0.11h_i + 1.02C_i)}{1 + \exp(-4.36 + 0.11h_i + 1.02C_i)}$$

All variables included into this model considerably contributed to prediction of the zebra mussel mortality (Table 9). As is seen from the equation, the cumulative mortality, in addition to its increase over time, was positively associated with the concentration of EarthTec®.

Table 9. Analysis of deviance table for parameters of the GLM that describes the response of zebra mussels to treatment with EarthTec® for 96 hours. The deviance of the null model (i.e. model that has no predictors included) is 4709.4, with 15 degrees of freedom.

Parameter	Deviance explained by a parameter (added into the model sequentially)	Degrees of freedom	Residual degrees of freedom	Deviance left unexplained	P-value for the chi-squared test
h (time)	4493.3	1	14	216.1	<<0.001
C (concentration)	138.3	1	13	77.8	<<0.001

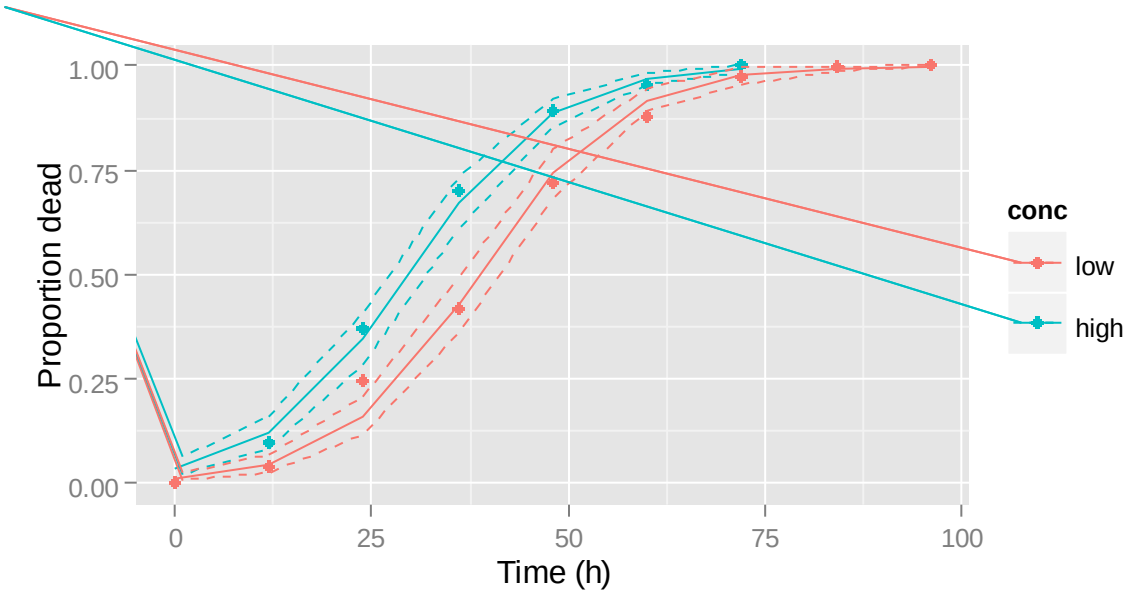

Figure 37. Goodness of fit of the GLM that describes the response of zebra mussels to treatment with EarthTec® for 96 hours. Dots show the actual cumulative proportions of molluscs found to be dead by a certain time since the beginning of the experiment. Solid lines correspond to the fitted values. Dashed lines denote 95% confidence intervals for the fitted values. Pearson correlation coefficient for the observed vs. fitted values is 0.997 (P << 0.001), suggesting that the model fits the data very well.

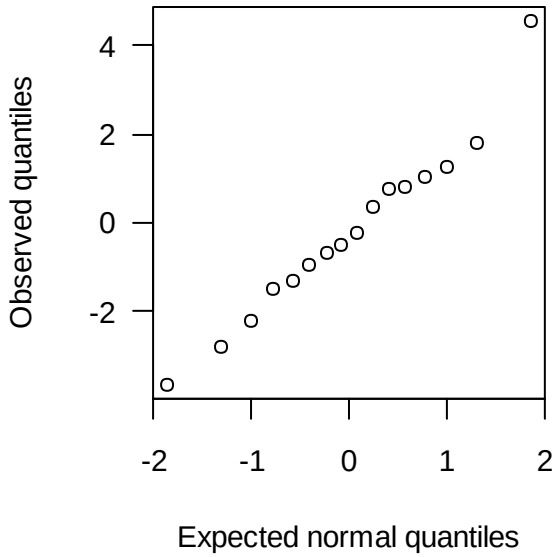

Figure 38. Q-Q plot for residuals of the GLM that describes the response of zebra mussels to treatment with EarthTec® for 96 hours. The dots on this plot form a diagonal line, suggesting normality of the residuals. This is further confirmed by the non-significant Shapiro-Wilk's test for normality (W = 0.912, P = 0.864).

4.5.6 Modeling the response of quagga mussels to EarthTec® at 96 h exposure

The following logistic regression model was found to adequately fit the data on mortality of the quagga mussels after 96h exposure to EarthTec® (see also Figures 39 and 40):

$$p_i = \frac{\exp(-5.04 + 0.10\mathrm{h}_i + 0.82\mathrm{C}_i)}{1 + \exp(-5.04 + 0.10\mathrm{h}_i + 0.82\mathrm{C}_i)}$$

All variables included into this model considerably contributed to prediction of the quagga mussel mortality (Table 10). As is seen from the equation, the cumulative mortality, in addition to its increase over time, was positively associated with the concentration of EarthTec®.

Table 10. Analysis of deviance table for parameters of the GLM that describes the response of quagga mussels to treatment with EarthTec® for 96 hours. The deviance of the null model (i.e. model that has no predictors included) is 4397.4, with 17 degrees of freedom.

Parameter	Deviance explained by a parameter (added into the model sequentially)	Degrees of freedom	Residual degrees of freedom	Deviance left unexplained	P-value for the chi-squared test
h (time)	4208.5	1	16	189.0	<<0.001
C (concentration)	87.7	1	15	101.3	<<0.001

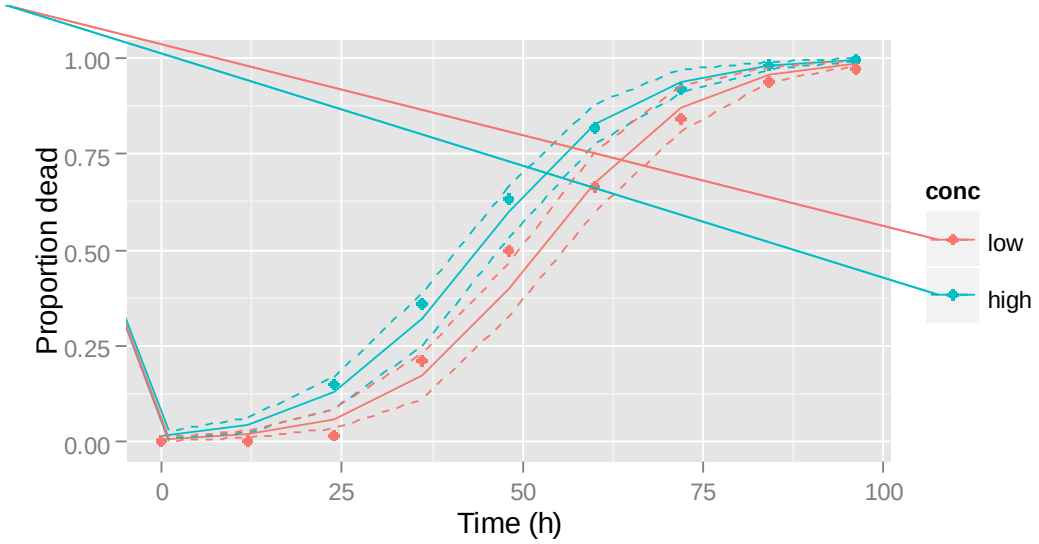

Figure 39. Goodness of fit of the GLM that describes the response of quagga mussels to treatment with EarthTec® for 96 hours. Dots show the actual cumulative proportions of molluscs found to be dead by a certain time since the beginning of the experiment. Solid lines correspond to the fitted values. Dashed lines denote 95% confidence intervals for the fitted values. Pearson correlation coefficient for the observed vs. fitted values is 0.997 ($P << 0.001$), suggesting that the model fits the data very well.

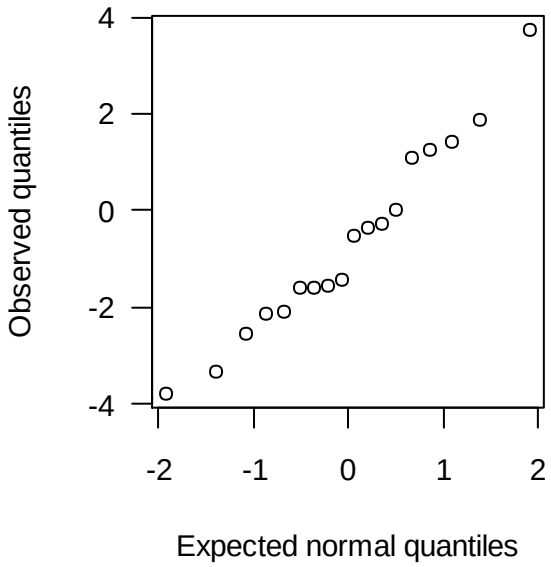

Figure 40. Q-Q plot for residuals of the GLM that describes the response of quagga mussels to treatment with EarthTec® for 96 hours. The dots on this plot form a diagonal line, suggesting normality of the residuals. This is further confirmed by the non-significant Shapiro-Wilk's test for normality ($W = 0.967$, $P = 0.736$).

5.0 Discussion

5.1 Possible confounding factors: Temperature and Water Quality

Both temperature and water quality varied with location, as did the mussel species. Zebra mussel experiments were conducted in somewhat warmer waters (Table 11).

Table 11: Summary of all temperature data collected at both experimental locations.

Location	Mussel Species	Average Temperature	Maximum Temperature	Minimum Temperature
San Justo Reservoir	zebra	18.3°C	22.8°C	13.1°C
Davis Dam	quagga	17.3°C	19.1°C	15.9°C

Ambient water temperature was controlled during these experiments to the best of our ability. For quagga mussels, temperature variations were found to be insignificant. For zebra mussels, because of the two different time periods for the test, temperature may have played some role in final mortality observed. Generally, copper-based algaecides have greater efficacy at higher temperatures. The slightly higher temperature during the zebra mussel experiments may have contributed somewhat to increase mortality.

In lake waters that are high in alkalinity, copper solubility decreases as pH increases. The alkalinity at San Justo Reservoir was between 80 and 85 mg/$CaCO_3$ and the calcium concentration was between 18 and 20 mg Ca/L, pH varied from 7.9 to 8.6. At Davis Dam, the alkalinity was 130 mg/$CaCO_3$, the calcium concentration was approximately 80 mg/L and the pH varied from 7.9 to 8.6. Based on this environmental data, the copper-based algaecides would be expected to be more effective at San Justo Reservoir then at Davis Dam. High alkalinity due to calcium levels has been shown to inhibit copper efficacy. Copper toxicity to algae is known to be regulated by alkalinity, hardness and pH of water (Masuda and Boyd, 1993). Copper toxicity to freshwater fish has been shown to be dependent on a number of factors including pH, DOC, alkalinity, and hardness (Pagenkopf, 1983; Di Toro et.al, 1997). High concentrations of calcium, a major component of hardness, is thought to limit copper toxicity by protecting the ion-regulating mechanisms at the gills of fish from the disruptive effects of copper (Pagenkopf, 1983). Similar effect of decreased copper toxicity could be expected in dreissenids. This effect was not observed. Quagga mussels, in water with high alkalinity and high calcium levels, died more readily than zebra mussels in water with relatively low calcium. To determine if this is a species specific effect, side by side evaluations of quagga and zebra mussels would have to be carried out in the same water source.

5.2 Seasonal variation in zebra mussel mortality

Different mortality curves were obtained for zebra mussels treated with algaecides (copper sulfate and Natrix™) in July compared to December (see sections 4.2.1 and 4.3.1 for July and 4.2.2 and 4.3.2 for December). Although these seasonal changes could potentially be associated with certain differences in physiological condition of the mussels (e.g. reproduction vs. post-reproduction period) or metabolic rate, it is difficult to conclude with confidence whether the seasonal effect indeed took place. The differences in mortality of zebra mussels in July and December are shown in Figures 41 and 42. Although the results suggest seasonal difference in response, it is important to remember that the ambient temperature profiles were quite different in July compared to December (see Figure 11). Therefore, temperature is an important confounding variable in these results and precludes any conclusions of seasonal differences in response to chemical control.

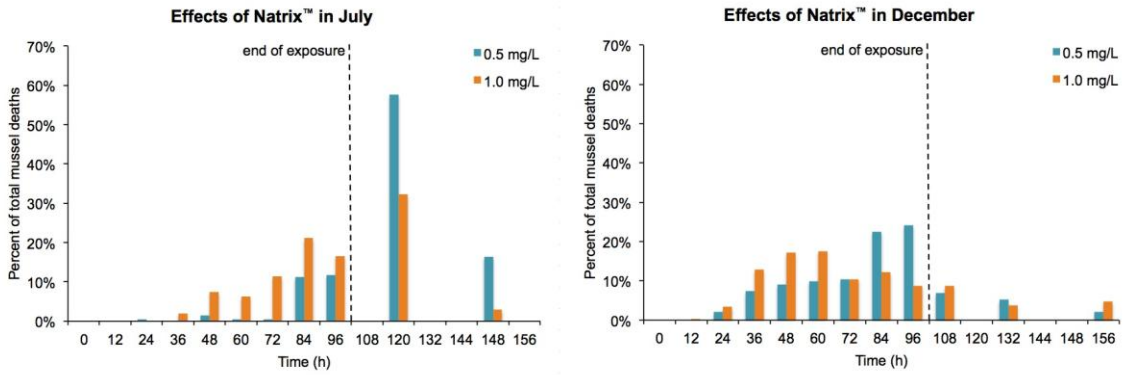

Figure 41. Mortality plots for zebra mussels exposed to Natrix™ in July and December

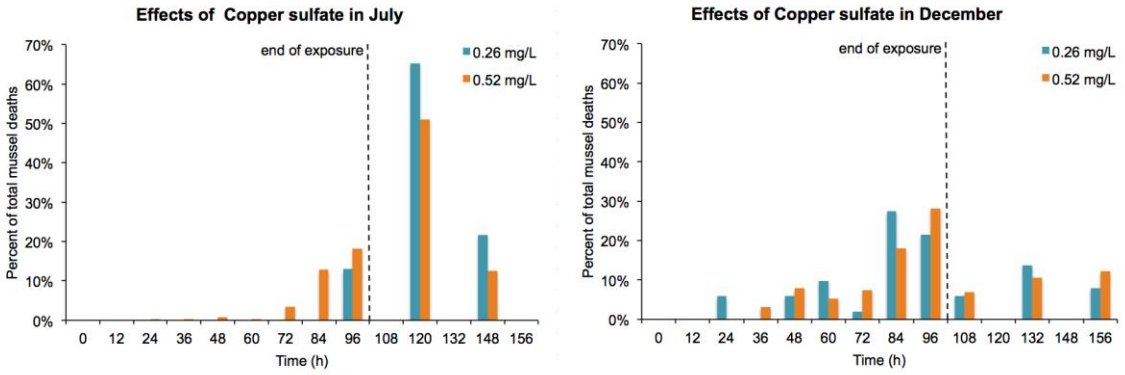

Figure 42. Mortality plots for zebra mussels exposed to copper sulfate in July and December

The statistical modeling of both copper sulphate and Natrix™ effects suggests that the temperature explains more of the deviance than the month (see Tables 3 and 5). Therefore, temperature plays a larger role in mortality than the time of year at which the algaecide is used. This suggests that mussels may be affected regardless of the point in their life cycle when the algaecide is applied. Additional experiments conducted in different months but at constant

temperature regimes could help clarify the importance of season on the differential mortality of zebra mussels treated with algaecides.

5.3 Differences in effect of algaecides: quagga vs. zebra mussels

From our experiments, it would appear that quagga mussels were more susceptible to copper-based algaecides than zebra mussels despite higher calcium present in the ambient water of the quagga experiments (Table 13). The only exception to this trend was shown by EarthTec® algaecide which appears equally effective in inducing mortality in both dreissenid species (Figure 43).

Table 13. Average percent mortality after 96h of exposure, and mortality following maximum recovery time allowed. When very high mortality was reached in 96h, mussels were not transferred to recovery coolers. July trial experiments were only conducted for zebra mussels with copper sulphate and Natrix™

	Low Concentration				**High Concentration**			
	Zebra		**Quagga**		**Zebra**		**Quagga**	
Algaecide	**96h**	**Max**	**96h**	**Max**	**96h**	**Max**	**96h**	**Max**
Copper Sulfate (July)	0.8%	6.7%	–	–	25.0%	69.9%	–	–
Copper Sulfate (Nov)	10.4%	14.2%	65.0%	92.4%	36.3%	52.1%	96.5%	99.4%
Natrix™ (July)	16.2%	62.2%	–	–	63.0%	97.2%	–	–
Natrix™ (Nov)	55.3%	64.4%	93.0%	99.6%	70.7%	84.6%	100%	–
Captain™	26.0%	43.5%	94.1%	–	72.5%	86.7%	94.1%	–
EarthTec®	**100%**	–	**97.1%**	–	**100%**	–	**99.3%**	–

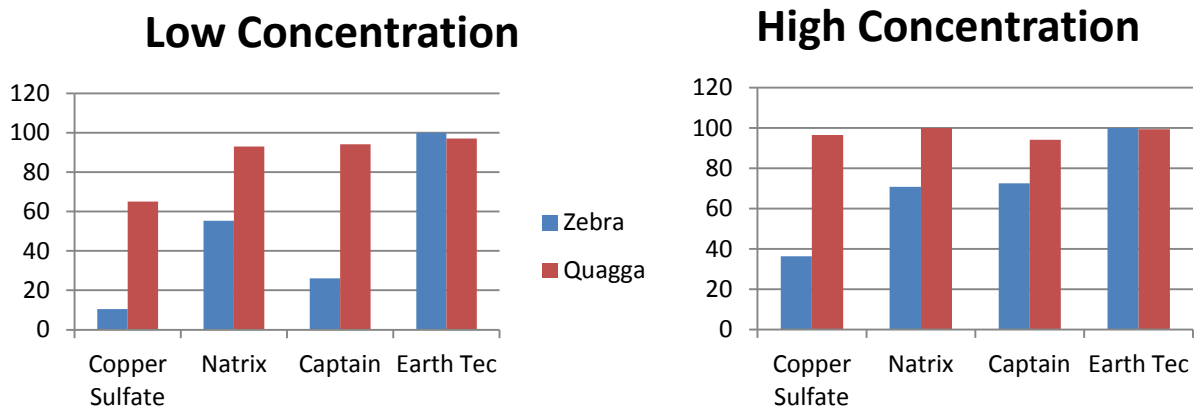

Figure 43. Average percent mortality after 96h of exposure to low and high algaecide levels

It is important to remember that the high concentration of copper sulfate is in fact comparable to the "low" concentrations of all other products, as the maximum suggested application rate is half the g/mL copper equivalent of other algaecides.

5.4 Role of retention time

Our statistical modeling suggests that the retention time is a vital variable to consider for mussel mortality. In every model, for both mussel species and all different algaecides tested, both concentration and exposure time were able to explain significant amounts of deviance in the mortality data. In some cases the exposure time was more important than the concentration.

The clearest example of this is the experiment using EarthTec®. The lower concentration of this product (0.5 mg/L copper equivalent) achieved the same result as the higher concentration (1 mg/L copper equivalent) in a 96 hour exposure. Therefore, in reservoirs where there is at least a 96h retention time, our results suggest that using a dose of 0.5 mg/L copper equivalent would be just as effective as a dose of 1 mg/L (Figure 10). Therefore for EarthTec® the recommended dosage for mussel treatment would be 0.5mg/L copper equivalent for 96h retention time, decreasing product cost for consumers and reducing the environmental impact.

5.5 Effect of temperature

Temperature was not included in final models for quagga mussels, suggesting that we successfully controlled this variable.

However, our control of temperature in the zebra mussel experiments was imperfect, and temperature was found to be significantly associated with mortality of the zebra mussels treated with copper sulfate, Natrix™, and Captain™. Using the model parameters developed for each of these algaecides, it was possible to predict the mortality of zebra mussels at both 20ºC and 25ºC after 96h exposure to high algaecide level.

Table 12. Modeled zebra mussel mortality after 96h exposure to high levels of copper equivalent (see Table 1), with 95% confidence intervals given in parentheses.

Algaecide	**20ºC**	**25ºC**
Copper Sulfate	July: 28% (22.2% - 33.9%) December: 32.5% (26.7% - 38.3%)	July: 50.7% (40.9% - 60.5%) December: 55.9% (46.0% - 65.9%)
Natrix™	July: 75.3% (68.0% - 82.5%) December: 76.2% (70.5% - 81.9%)	July: 89.6% (84.1% - 95.0%) December: 90.0% (85.9% - 94.2%)
Captain	70.1% (65.4% - 74.9%)	88.0% (83.2% - 92.9%)

This finding suggests that higher ambient water temperatures during treatment will result in higher mortality of adult mussels than observed in our experiments. While our model was constructed for zebra mussels only, we would expect the same results in treatments of quagga mussels.

6.0 Conclusions and Management Recommendations

Algaecide products based on sodium carbonate peroxyhydrate (GreenClean) do not appear to affect adult quagga mussels even when applied at the maximum permissible level for algae control.

The use of copper-based algaecides is a viable tool for managing zebra and quagga mussel infestations, particularly in water bodies which require the use of these chemicals for the control of algae or aquatic plants. In 2013, EarthTec was issued a USEPA label for use against dreissenids in open water up to 1.0 mg/L as copper. Other copper products (copper sulfate and certain copper carbonates or chelates) can be used to control mollusks in open water systems, but require a Special Local Need Label (also known as a Section 24-c) issued by the USEPA. A Special Local Need Label is currently available for the use of the copper carbonate formulation, Natrix™, for control of invasive and exotic aquatic mussels, snails, oysters and clams in Idaho, Georgia, Missouri, South Carolina and Texas (http://tirmsdev.com/SePRO-Corporation-Natrix-p64533).

The results for the copper sulfate experiments were remarkable given that copper sulfate was applied at 50% of the copper equivalent level used for the other algaecides. Copper sulfate is relatively inexpensive compared to the other products tested. Figure 44 illustrates how the "high" concentration of copper sulfate compared to the "low" concentrations of other algaecides (equivalent copper level) appears to have almost identical effect. For quagga mussels, copper sulfate is an obvious choice due to effectiveness and low cost. For zebra mussels the choice is more complex. A lake-wide application of copper sulfate (as pentahydrate crystals) was applied

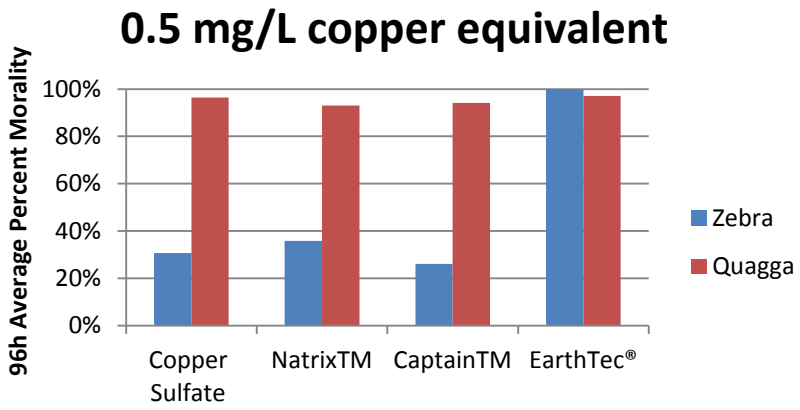

Figure 44. Average percent mortality after 96h of exposure to algaecides at 0.5 mg/L copper equivalent

to Lake Offutt, Offutt Air Force Base, Nebraska, in 2008, under a Special Local Need Label, in an attempt to eradicate zebra mussels (http://www.aquaticnuisance.org/wordpress/wp-content/uploads/2009/01/OAFB-ZM-Final-Summary-Report.pdf). Copper sulfate applied at a rate of 1 part per million (ppm) was effective for controlling zebra mussels in Lake Offutt; however, some non-target fish mortality was observed following treatment. If copper sulfate was applied at a rate of 1 mg/L copper equivalent as granules, without mixing, it would likely sink to

the bottom of the treated water body where the majority of dreissenid mussels are found. This type of application would allow the treatment to take effect over a longer period of time, as the granules dissolved. The granules on the bottom will not be easily flushed out during water exchange in a reservoir and very high mortalities of adult dreissenids are likely to be achieved.

The higher the ambient temperature of the water during treatment, the more effective all treatments are likely to be. When evaluating an effectiveness of an algaecide treatment, mussels should be observed for at least 5 days after the treatment to take into account the post exposure mortality of mussels.

There are significant differences in the response of quagga mussels compared to zebra mussels to treatment with copper based algaecides. In a majority of the experiments, quagga mussels had much greater mortality following exposure to the test products. The exception was the product EarthTec® which appeared to cause equal mortalities in both dreissenid species.

7.0 References

Di Toro, D.M., R.C. Santore, and P.R. Paquin. 1997. Chemistry of Copper Bioavailability I: Model of Acute Copper Toxicity to Fish. Presented at the 1997 Annual SETAC Conference, San Francisco, CA.

Hosea, R.C. and B. Finlayson. 2005. Controlling the spread of New Zealand mud snails on wading gear. Administrative Report 2005-02. California Department of Fish and Game, Office of Spill Prevention and Response, Sacramento, CA. 38 pp

Jenner, H.A. and J.P.M. Jansses-Mommem. 1993. Monitoring and Control of Dreissena polymorpha and other macrofouling bivalves in the Neatherlands. In: Zebra Mussels: biology, Impacts and Control, T.F.Nalepa and D.W.Schloesser, eds.pp.537-553. Boca Raton:Lewis Publishers.

Masuda, K. and Boyd, C.E. 1993. Comparative evaluation of the solubility and algal toxicity of copper sulfate and chelated copper. Aquaculture, 117: 287-302.

Pagenkopf, G.K. 1983. Gill surface interaction model for trace-metal toxicity to fishes: Role of complexation, pH, and water hardness. Environ. Sci. Technol. 17:342-347. Playle, R.C., D.G. Dixon, K. Burnison. 1993a. Copper and cadmium binding to fish gills: Estimates of metal-gill stability constants and modelling of metal accumulation. Can. J. Fish. Aquat. Sci. 50:2678-2687.

R Development Core Team. 2012. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0. Available at: http://www.R-project.org

Rajagopala, S.,G. van der Veld, and H. A. Jenner. 2002. Effects of low-level chlorination on zebra mussel, Dreissena polymorpha. Water Research 36 (2002) 3029–3034

Shapiro, S. S. and Wilk, M. B. 1965. An analysis of variance test for normality (complete samples). Biometrika 52 (3-4): 591-611

Venables W. N., and Ripley B. D. 2002. Modern Applied Statistics with S, 4th edition. Springer.

Wood, S. 2006. Generalized additive models: an introduction with R. CRC Press.

Appendix 1 – Tables of Environmental Parameters

Table A1 Dissolved oxygen levels (mg/L) in quagga mussel 96h experiments.

	Copper sulfate			Natrix™			Captain			EarthTec®		
Time (h)	**Control**	**0.26 mg/L**	**0.52 mg/L**	**Control**	**0.5 mg/L**	**1 mg/L**	**Control**	**0.5 mg/L**	**1 mg/L**	**Control**	**0.5 mg/L**	**1 mg/L**
0	6.32	8.17	8.17	6.32	8.33	8.43	8.24	8.63	8.64	8.24	8.26	8.22
12	7.30	8.15	8.18	7.30	8.05	8.18	8.01	8.96	8.73	8.01	8.44	8.35
24	6.51	7.32	7.78	6.51	7.87	7.63	7.53	8.48	8.62	7.53	8.48	8.62
36	6.01	7.54	6.00	6.01	6.84	7.68	7.63	8.34	8.14	7.63	8.25	8.51
48	6.03	5.81	7.12	6.03	4.90	7.19	6.12	8.31	8.67	6.12	8.67	8.9
60	5.49	5.80	7.01	5.49	6.39	5.13	7.00	7.42	8.35	7.00	8.89	9.1
72	6.29	4.99	6.47	6.29	7.05	5.20	6.32	6.30	6.79	6.32	8.28	8.68
84	5.96	5.32	7.14	5.96	5.89	5.60	9.00	8.93	17	9.00	16.9	
96	5.13	5.88	5.62	5.13	5.98	6.27	6.03	5.12	4.53	6.03	8.14	
108	6.47	6.47	6.47	6.47	6.47	6.47	6.47	6.47	6.47	6.47	6.47	6.47
132	9.37	9.37	9.37	9.37	9.37	9.37	9.37	9.37	9.37	9.37	9.37	9.37
156	11.01	11.01	11.01	11.01	11.01	11.01	11.01	11.01	11.01	11.01	11.01	11.01

Table A2 Temperature levels (°C) in quagga mussel 96h experiments.

	Copper sulfate			Natrix™			Captain			EarthTec®		
Time (h)	**Control**	**0.26 mg/L**	**0.52 mg/L**	**Control**	**0.5 mg/L**	**1 mg/L**	**Control**	**0.5 mg/L**	**1 mg/L**	**Control**	**0.5 mg/L**	**1 mg/L**
0	20.5	17.9	18.2	20.5	18.4	19.0	20.4	19.5	20.0	20.4	21.3	22.0
12	16.5	15.6	15.7	16.5	16.2	16.4	15.5	15.5	15.5	15.5	16.0	16.5
24	20.0	18.5	17.9	20.0	17.8	17.9	15.3	15.7	16.1	15.3	16.4	16.7
36	19.2	18.0	20.5	19.2	20.9	21.2	16.8	16.4	16.5	16.8	16.3	16.2
48	19.3	17.5	18.5	19.3	21.4	20.8	19.4	18.3	18.6	19.4	17.6	16.8
60	17.8	16.9	17.4	17.8	22.3	18.0	16.8	16.3	16.7	16.8	16.1	15.9
72	16.8	19.4	18.7	16.8	19.9	19.6	20.5	19.9	20.1	20.5	20.2	20.3
84	18.9	20.4	21.2	18.9	22.0	19.5	19.2	19.0	20.1	19.2	19.5	
96	19.3	21.1	20.6	19.3	21.5	21.2	19.3	21.5	21.3	19.3	19.1	
108	20.1	20.1	20.1	20.1	20.1	20.1	20.1	20.1	20.1	20.1	20.1	20.1
132	18.1	18.1	18.1	18.1	18.1	18.1	18.1	18.1	18.1	18.1	18.1	18.1
156	13.1	13.1	13.1	13.1	13.1	13.1	13.1	13.1	13.1	13.1	13.1	13.1

Table A3 pH levels in quagga mussel 96h experiments.

	Copper sulfate			Natrix™			Captain			EarthTec®		
Time (h)	**Control**	**0.26 mg/L**	**0.52 mg/L**	**Control**	**0.5 mg/L**	**1 mg/L**	**Control**	**0.5 mg/L**	**1 mg/L**	**Control**	**0.5 mg/L**	**1 mg/L**
0	8.10	8.14	8.22	8.10	8.27	8.38	8.23	8.32	8.32	8.23	8.26	8.27
12	8.00	8.32	8.22	8.00	8.30	8.38	8.28	8.38	8.21	8.28	7.71	7.61
24	8.08	8.15	8.17	8.08	8.24	8.33	8.15	8.33	8.49	8.15	7.96	7.70
36	8.11	8.21	8.25	8.11	8.33	8.39	8.20	8.32	8.42	8.20	8.03	7.77
48	8.01	8.03	8.05	8.01	8.15	8.34	8.30	8.32	8.37	8.30	8.03	7.89
60	7.99	8.04	8.12	7.99	8.28	8.23	7.96	8.55	8.49	7.96	8.06	7.95
72	7.98	8.02	8.10	7.98	8.16	8.21	8.10	8.39	8.29	8.10	8.19	8.00
84	8.04	8.08	8.15	8.04	8.15	8.19	8.11	8.29	8.25	8.11	8.14	0.00
96	7.95	8.07	8.09	7.95	8.16	8.19	8.01	8.08	8.08	8.01	8.23	0.00
108	7.97	7.97	7.97	7.97	7.97	7.97	7.97	7.97	7.97	7.97	7.97	7.97
132	8.61	8.61	8.61	8.61	8.61	8.61	8.61	8.61	8.61	8.61	8.61	8.61
156	8.53	8.53	8.53	8.53	8.53	8.53	8.53	8.53	8.53	8.53	8.53	8.53

Table A4 Dissolved oxygen levels (mg/L) in zebra mussel 96h experiments.

	Copper sulfate			Natrix™			Captain			EarthTec®		
Time (h)	**Control**	**0.26 mg/L**	**0.52 mg/L**	**Control**	**0.5 mg/L**	**1 mg/L**	**Control**	**0.5 mg/L**	**1 mg/L**	**Control**	**0.5 mg/L**	**1 mg/L**
0	9.20	9.10	9.13	9.20	9.16	9.11	8.11	9.16	9.45	8.11	9.42	8.44
12	7.05	8.70	9.28	7.05	9.20	9.31	9.26	9.27	9.26	9.26	9.36	9.44
24	7.47	8.64	9.22	7.47	9.23	9.23	8.50	9.02	9.26	8.50	9.31	17.10
36	7.76	8.93	9.18	7.76	9.20	9.21	9.01	8.73	8.78	9.01	8.63	8.44
48	7.57	9.00	9.30	7.57	9.44	9.41	9.11	8.86	8.76	9.11	8.86	17.90
60	7.88	8.62	9.14	7.88	9.27	9.31	9.25	8.81	8.71	9.25	8.82	9.42
72	6.67	8.49	9.02	6.67	9.00	9.14	8.48	8.81	8.88	8.48	9.15	8.41
84	7.18	8.33	8.59	7.18	8.50	8.92	9.00	8.93	17.00	9.00	16.90	17.40
96	8.65	8.29	8.58	8.65	7.60	8.52	9.14	9.15	16.50	9.14	16.50	9.32
108	8.11	9.22	9.22	8.11	9.02	9.02						8.37
120	9.26	9.22	9.22	9.26	9.26	9.26						17.60
132	8.50	9.22	9.22	8.50	8.73	8.73						8.98
144	9.33	9.33	9.33	9.33		9.34						8.37
156	9.23	9.23	9.23									17.20
168	9.33	9.33										9.05
180	9.20	9.20										8.38

Table A5 Temperature levels (°C) in zebra mussel 96h experiments.

	Copper sulfate			Natrix™			Captain			EarthTec®		
Time (h)	**Control**	**0.26 mg/L**	**0.52 mg/L**	**Control**	**0.5 mg/L**	**1 mg/L**	**Control**	**0.5 mg/L**	**1 mg/L**	**Control**	**0.5 mg/L**	**1 mg/L**
0	18.2	18..2	18.2	18.2	18.3	18.2	17	17	17	17	17.2	
12	16.9	17.1	17.3	16.9	17.3	17.3	17.9	18	17.8	17.9	17.7	
24	17.1	17.4	17.5	17.1	17.3	17.4	17.2	17.2	17	17.2	17.2	
36	17.3	17	17.1	17.3	16.7	16.9	17.7	17.7	17.7	17.7	17.7	
48	17.1	16.7	16.4	17.1	15.9	15.9	16.8	17	17.1	16.8	17.1	
60	16.7	16.5	16.4	16.7	16.3	16.4	17.5	17.6	17.5	17.5	17.4	
72	17.5	17	17	17.5	16.6	16.9	16.9	16.9	16.7	16.9	16.5	
84	17.4	17.4	17.2	17.4	17	17.1	17.2	17.1	9.06	17.2	9.18	
96	17.8	17.9	17.9	17.8	18	18.5	16.7	16.8	9.26	16.7	9.33	
108	17	17.1	17.1	17	17.1	17.1						
120	17.9	17.5	17.5	17.9	17.5	17.5						
132	17.2	17.1	17.1	17.2	17.1	17.1						
144	17.4	17.4	17.4	17.4		17.5						
156	17	17	17									
168	17.4	17.4										
180	17.1	17.1										

Table A6 pH levels in zebra mussel 96h experiments.

	Copper sulfate			Natrix™			Captain			EarthTec®		
Time (h)	**Control**	**0.26 mg/L**	**0.52 mg/L**	**Control**	**0.5 mg/L**	**1 mg/L**	**Control**	**0.5 mg/L**	**1 mg/L**	**Control**	**0.5 mg/L**	**1 mg/L**
0	8.48	8.47	8.45	8.48	8.49	8.45	8.43	8.50	8.57	8.43	8.54	17.40
12	8.13	8.36	8.42	8.13	8.50	8.58	8.52	8.52	8.59	8.52	8.56	9.23
24	8.22	8.29	8.34	8.22	8.45	8.48	8.46	8.46	8.52	8.46	8.49	8.42
36	8.32	8.35	8.41	8.32	8.57	8.51	8.51	8.48	8.49	8.51	8.46	16.60
48	7.40	8.31	8.39	7.40	8.50	8.48	8.50	8.48	8.52	8.50	8.45	9.23
60	8.32	8.27	8.41	8.32	8.49	8.47	8.51	8.50	8.48	8.51	8.43	8.40
72	8.30	8.32	8.35	8.30	8.48	8.43	8.48	8.48	8.50	8.48	8.48	9.40
84	8.28	8.26	8.32	8.28	8.39	8.35	8.29	8.34	8.42	8.29	8.42	16.90
96	8.43	8.26	8.28	8.43	8.36	8.43	8.45	8.53	8.56	8.45	8.54	8.50
108	8.43	8.50	8.50	8.43	8.48	8.48						9.47
120	8.52	8.52	8.52	8.52	8.49	17.50						16.60
132	8.46	8.50	8.50	8.46	8.52	8.52						
144	8.52	8.52	8.52	8.52		8.46						
156	8.52	8.52	8.52									
168	8.52	8.52										
180	8.49	8.49										

Table A7 Temperature (°C) in zebra mussel recovery experiments. Note that Control I was for both copper sulfate and Natrix experiments, while Control II was for Captain and EarthTec®.

Time (h)	Control I	Copper sulfate	Natrix™	Control II	Captain	EarthTec®
0	17.1	18.2	19.0	20.4	20.0	22.0
12	16.5	15.7	16.4	15.5	15.5	16.5
24	17.2	17.1	17.1	21.0	21.0	21.0
36	17.5	17.6	17.5	19.2	19.2	19.2
48	17.1	17.1	17.1	19.3	19.3	19.3

Table A8 Dissolved oxygen (mg/L) in zebra mussel recovery experiments. Note that Control I was for both copper sulfate and Natrix experiments, while Control II was for Captain and EarthTec®.

Time (h)	Control I	Copper sulfate	Natrix™	Control II	Captain	EarthTec®
0	8.17	8.17	8.43	8.23	8.32	8.27
12	7.30	8.18	8.18	8.01	8.73.	8.35
24	8.90	9.22	9.26	6.51	6.51	6.51
36	9.10	9.16	9.26	6.01	6.01	6.01
48	9.22	9.21	8.73	6.03	6.03	9.32

Table A9 pH levels in zebra mussel recovery experiments. Note that Control I was for both copper sulfate and Natrix experiments, while Control II was for Captain and EarthTec®.

Time (h)	Control I	Copper sulfate	Natrix™	Control II	Captain	EarthTec®
0	8.28	8.22	8.38	8.28	8.21	7.61
12	8.00	8.22	8.38	8.28	8.21	7.61
24	8.48	8.50	8.50	8.08	8.08	8.08
36	8.50	8.52	8.49	8.11	8.11	8.11
48	8.50	8.49	8.52	8.01	8.01	8.01

Table A10 Temperature (°C) in quagga mussel recovery experiments. Note that Control I was for both copper sulfate and Natrix experiments, while Control II was for Captain and EarthTec®.

Time (h)	Control I	Copper sulfate	Natrix™	Control II	Captain	EarthTec®
0	17.4	17.0	17.0	17.4	17.6	17.6
12	18.9	18.8	18.9	17.5	17.8	17.9
24	17.2	17.1	17.1	17.3	17.2	17.2
36	17.5	17.6	17.5	17.5	17.5	17.5
48	17.1	17.1	17.1	17.1	17.1	17.1

Table A11 Dissolved oxygen (mg/L)in quagga mussel recovery experiments. Note that Control I was for both copper sulfate and Natrix experiments, while Control II was for Captain and EarthTec®.

Time (h)	Control I	Copper sulfate	Natrix™	Control II	Captain	EarthTec®
0	7.18	8.99	9.12	7.18	9.20	9.30
12	8.88	9.10	9.16	8.88	9.26	9.42
24	8.90	9.22	9.26	8.95	9.02	9.02
36	9.10	9.16	9.26	9.16	9.16	9.16
48	9.22	9.21	8.73	9.32	9.32	9.32

Table A12 pH levels in quagga mussel recovery experiments. Note that Control I was for both copper sulfate and Natrix experiments, while Control II was for Captain and EarthTec®.

Time (h)	Control I	Copper sulfate	Natrix™	Control II	Captain	EarthTec®
0	8.28	8.48	8.56	8.28	8.59	8.54
12	8.40	8.41	8.39	8.40	8.59	8.44
24	8.48	8.50	8.50	8.48	8.45	8.45
36	8.50	8.52	8.49	8.51	8.51	8.51
48	8.50	8.49	8.52	8.53	8.53	8.53

Appendix 2 – Algaecide Calculation Tables

Table A13 Calculation tables for use of copper sulfate.

conversion	7.480519	gal per cubic ft
water density	62.3	lbm/cubic ft
Weight	8.07116E-06	Copper Sulphate/ppm/Gal
convert	453.5924	from lbs to gm

	Test Volume	Based on One Acre-Foot
Volume (gal)	50.00	325851.43
volume (cubic ft)	6.68	43560.00
Mass of water (lbm)	416	2713788

concentration of copper sulphate (ppm)	Amount of Test Product		lbs/acre-Foot
	lbs	gm	
0.25	0.00010089	0.05	0.66
0.5	0.000201779	0.09	1.3
1	0.000403558	0.18	2.6
1.5	0.000605337	0.27	3.9
2	0.000807116	0.37	5.3
5	0.002017791	0.92	13.2
32	0.012913861	5.86	84.2

Table A14 Calculation tables for Natrix™.

conversion	7.480519	gal per cubic ft
water density	62.3	lbm/cubic ft

	Test Volume	Based on One Acre-Foot
Volume (gal)	50.00	325851.43
volume (cubic ft)	6.68	43560.00
Mass of water (lbm)	416	2713788

concentration of copper (ppm)	Amount of Test Product		Vol/acre-Foot
	Gal	mL	Gal
0.4	0.000184133	0.70	1.2
0.5	0.000230166	0.87	1.5
0.6	0.000276199	1.05	1.8
0.7	0.000322233	1.22	2.1
0.8	0.000368266	1.39	2.4
0.9	0.000414299	1.57	2.7
1	0.000460332	1.74	3

Table A15 Calculation tables for Captain™.

conversion	7.480519	gal per cubic ft
water density	62.3	lbm/cubic ft
volume	9.20665E-06	Captain/ppm/Gal

	Test Volume	Based on One Acre-Foot
Volume (gal)	50.00	325851.43
volume (cubic ft)	6.68	43560.00
Mass of water (lbm)	416	2713788

concentration of copper (ppm)	Amount of Test Product			Vol/acre-Foot
	Gal	mL	ppm	Gal
0.4	0.000184133	0.70	3.7	1.2
0.5	0.000230166	0.87	4.6	1.5
0.6	0.000276199	1.05	5.5	1.8
0.7	0.000322233	1.22	6.4	2.1
0.8	0.000368266	1.39	7.4	2.4
0.9	0.000414299	1.57	8.3	2.7
1	0.000460332	1.74	9.2	3

Table A16 Calculation tables for EarthTec®.

conversion	7.480519	gal per cubic ft
water density	62.3	lbm/cubic ft
Volume of Earthtec	1.66667E-05	Earthtec/ppm/Gal

	Test Volume	Based on One Acre-Foot
Volume (gal)	50.00	325851.43
volume (cubic ft)	6.68	43560.00
Mass of water (lbm)	416	2713788

concentration of copper (ppm)	Amount of Test Product			Vol/acre-Foot
	Gal	mL	ppm	Gal
0.4	0.000333333	1.26	6.7	2.17
0.5	0.000416667	1.58	8.3	2.72
0.6	0.0005	1.89	10.0	3.26
0.7	0.000583333	2.21	11.7	3.80
0.8	0.000666667	2.52	13.3	4.34
0.9	0.00075	2.84	15.0	4.89
1	0.000833333	3.15	16.7	5.43