Possible Secondary Indirect Effects of Copper on *Mya arenaria* and Benthic Pelagic Coupling

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Abstract:

Because of the way bivalves not only feed, but also the way they process and deposit their waste as pseudofeces, they are important in benthic-pelagic coupling. The purpose of these experiment were to determine what the secondary non-lethal effects of copper were on *M. arenaria* and whether of not these effects differed among different copper compounds and also to see whether these effects differed when the *M. arenaria* was coupled with a pelagic organism (*Fundulus heteroclitus*). The presence of copper decreased respiration and filtration of the *M. arenaria* as compared to the treatments that received no copper. There was no effect of copper sulfate on the *M. arenaria*. It was also determined that the *M. arenaria* incorporated the copper into their body tissue and that in the presence of copper the *M. arenaria* release a protein that increases the turbidity of the water.

Key words: *Mya arenaria*, *Fundulus heteroclitus*, copper, benthic pelagic coupling

Introduction:

The ways in which ecosystems interact and the ways in which species in those ecosystems interact are very important to understanding our planet. One important interaction in marine systems is benthic-pelagic coupling, or simply the interactions between benthic organisms and pelagic ones.

Because of the way bivalves not only feed, but also the way they process and deposit their waste as pseudofeces, they are important in benthic-pelagic coupling. In areas of dense populations bivalves are very important in the cycling of both carbon and nitrogen through the ecosystems. These dense aggregations of bivalves also increase the structural and functional sustainability of their estuarine ecosystems (Dame 1996).

The effects of heavy metals have been studied on numerous species. In bivalves, heavy metal exposure has been shown to affect lipid peroxidation (Romeo et al 1997), and the depletion of glutathione (Viarengo et al. 1990), Zn, Cd, and Se assimilation has been shown to directly correlate with carbon assimilation (Wang & Fisher 1996) and in high concentration they cause death. They have also been shown to indirectly cause oxidative stress which leads to other toxic processes (Winston et al. 1991). Copper in particular has been shown to alter the functional response of hemocytes in bivalves (Matozzo et al. 2001). Copper is used in marine ecosystems to control algae blooms, and in large concentrations as a mollusk-acide. The bottoms of boats are often also painted with paint which contains copper and acts as an anti-fouling agent.

Mya *arenaria*, a soft shell clam exposed to Cu in the sediment has been shown to incorporate it into its tissue (Veinott et al. 2001). M. *arenaria* is a commercially important bivalve species to the east coast. A filter feeder, M. *arenaria* is capable of filtering vast amounts of water over time in order to filter enough nutrients to maintain itself. In fact models have shown that M. *arenaria* along with the other bivalves in the Chesapeake are capable of filtering and removing up to 50% of the annual phytoplankton primary production in the bay (Dame 1996) and in Maine M. *arenaria* along with *Miytilus edulis* are plentiful enough in Lowes Cove to filter half of the over half of the tidal volume (Carlson et al. 1984).

The purpose of these experiment were to determine what the secondary non-lethal effects of copper were on *M. arenaria* and whether of not these effects differed among different copper compounds. Another purpose was to examine how the benthic pelagic coupling of *M. arenaria* and *Fundulus heteroclitus* may influence the effect of copper on the *M. arenaria*. As indicators of coppers effect on *M. arenaria* I examined respiration rate, filtration rate, total copper concentration in body tissues, time spent siphoning, and turbidity as a measure of the proteins that the *M. arenaria* released.

I hypothesized that both respiration and filtration rates would decrease with an increased copper concentration because of the toxicity of the copper and the potential stress to the animals it would be inducing. Similarly, I hypothesize that the time the clams spend filtering would also decrease with the increased copper, and the copper concentration in the body tissues of the *M. arenaria* would increase as the concentration of copper introduced was increased.

Methods:

Overview-

All *M. arenaria* used in the experiments were purchased from the Falmouth Fish Market in Falmouth. All clams were on the small end of legal collecting size ranging from about 2 to 3 inches and from twenty to forty grams wet weight. Preliminary trials were run at different concentrations of copper to determine an appropriate dosing range. Copper was introduced into these tanks by adding Cupramine™, a chemical commonly used to treat ectoparasites in aquarium fish. Cupramine is copper bound to an amine group and ionizes fully into Cu^{2+} in water. In the second trial copper was also introduced in the form of copper sulfate ($CuSO_4$), which also fully ionizes in water, in equivalent concentrations to the Cupramine™. *Mya* were feed approximately every other day with Instant Algae and all tanks were given equal amounts. All data was analyzed by calculating standard error and multiplying it by two to give ninety five percent confidence.

Experimental Design-

Trial One-

Eight ten gallon tanks were filled to eight gallons with Instant Ocean™, an artificial seawater powered which was mixed with De-ionized water. The salinity of each tank was 30ppm and the tanks were allowed to sit over night to reach ambient temperature. The next day the tanks were dosed with 4 different amounts of copper and two replicates per dose; two tanks received 0 Cu^{2+} L^{-1} copper, two tanks received 1.34mg Cu^{2+} L^{-1}, two tanks received 2.68mg Cu^{2+} L^{-1}, and two tanks received 4.02 Cu^{2+} L^{-1} from a stock solution of Cupramine™ with an equivalent copper concentration of 10,000mg Cu^{2+} L^{-1}. After mixing each tank thoroughly fifteen *M. arenaria* were added to each tank and in four of the tanks an additional fifteen *F. heteroclitus* were added also. Thus four tanks (one of each concentration) contained only *M. arenaria* and four (also one at each concentration) contained fifteen *M. arenaria* and fifteen *F. heteroclitus*. On the same day as the organisms were placed in their aquariums four *M. arenaria* that were straight from the fish market were run through all examinations. At day four two *M. arenaria* from each tank which still contained live organisms were tested. In the interest of the small amount of time allotted to this experiment each *M. arenaria* in the tanks will serve as a pseudo replicate.

Trial Two-

After viewing and discussing the results of trial one I decided to examine whether the secondary effects of the treatment were due to the copper in the Cupramine™ or the amine group. Nine plastic tanks were set up and filled with eight liters of seawater. One tank served as a control, four with the same concentrations of copper from Cupramine™ as trial one, and four with the same concentrations of copper from trial one but from copper sulfate instead. After the containers were filled they sat over night and the next day the copper was added, the water was mixed and then four *M. arenaria* were added to each tank. Time zeros were again run on four new *M. arenaria*. The water on all nine tanks was changed completely everyday. Two *M. arenaria* were sampled after three days and again at five days following the procedure outlined above.

Measured Responses-

Respiration-

The respiration of the *M. arenaria* was determined by incubation in sealed static chambers. Erlenmeyer flasks were carefully filled with Instant Ocean™ at ambient temperature in order to ensure no air bubbles remained inside. The dissolved oxygen (DO) in the water was measured with a DO probe and then a *M. arenaria* was placed inside, sealed with a rubber stopper, at which point the entire Erlenmeyer was placed into an aquarium filled with water (in order to maintain a constant temperature) and was incubated for not less than one hour. After incubation the DO was again measured using the DO probe. A control Erlenmeyer that contained only instant ocean was also incubated to make sure the change in DO was due to the *M. arenaria*. The change in DO was divided by the time of the incubation and then standardized for the size of the organisms dry body weight.

Filtration / Feeding-

The filtration rate of *M. arenaria* was measured by measuring th concentration of chlorophyll a in a water sample before and after incubation with one clam. Each individual was placed into an individual 500mL beaker which was filled with 300mL of seawater at ambient temperature and 20mL of diluted Instant Algae™. An addition control beaker that contained no *M. arenaria* was also incubated to make sure that the change in chlorophyll was due to the *M. arenaria* filtration and not another process. The beakers were left to sit for no less than thirty minuets at which time 150mL of the water was filtered on a GFF vacuum filter and the chlorophyll was extracted (see SES handout for procedure) and measured on a spectrophotometer. The rate of filtration was calculated based on the chlorophyll a concentration and the time the beaker was incubated and was then standardized by the dry body weight of the individuals.

Determination of Tissue Copper-

After the filtration and respiration rates were tested the clams were weighed and then cut into body and shell and dried overnight in a drying oven at approximately 60^{o}C. After drying the shell and body were reweighed. The *M. arenaria* bodies were placed in scintvials and ashed at 550^{o}C for ten total hours. After ashing 10mL of 1N HCl was added to each scintvial to dissolve the tissue. The liquid was then analyzed on an Atomic absorption machine and the copper concentration was calculated using the absorbance and a standard curve and standardized by the dry body weight of the individuals.

Protein Assay-

Based on the cloudiness of the water in trial one it was suggested that the clams may be releasing a protein due the stress they under that may be the cause of the cloudiness. To test this water from the tanks in trial two was sampled three times, once after a water change when the copper additives had been added but the clams had not, and on two different days right before the water change when the clams had been in the water for approximately 24 hours. The two 24 hour samples were blank corrected using the water with chemicals but without the clams in order to examine only the absorbance caused by the clams. The samples were run on a spectrophotometer at 280nm, the wavelength which usually detects proteins, and it was determine that these actually spiked at 266nm so all samples were rerun at 266nm.

Results:

<u>*Trial One:*</u>

At day two of exposure the water in all tanks, except the controls, became cloudy, this cloudiness seemed to increase as the exposure time increased. After four days of exposure all clams in the 4.02mg Cu^{2+} L^{-1} in the *M. arenaria* only tanks were dead and all but one were dead in the joint tanks. The single live clam from the highest copper exposure, along with two clams from each of the other tanks were sampled and run through the tests.

Respiration-

In the *M. arenaria* only tanks the second highest dose had a significant affect on the respiration of the sampled clams as it was much lower than that of the clams from the control tank and that of the time zero clams (Figure One). In the joint tanks the Cupramine™ treatment significantly lowered the respiration of the sampled dosed clams as compared to the controls and time zeros (Figure Two).

Filtration Rate-

With regards to filtrations rates, the treatments of Cupramine™ significantly lowered the filtration rates of the *M. arenaria* in both the *M. arenaria* only tanks as compared to the time zero measurements (Figure Three) and the joint tanks in which all treated tanks had lower respiration rates than both the time zeros and the control tank (Figure Four).

Copper in Body Tissues-

The concentration of copper in the body tissues of the clams, in the *Mya* only tanks only the middle two doses showed a significant difference from the time zero clams, but neither of those showed a statistically significant difference from the control tank (Figure Five). In the joint tanks the two highest concentrations showed a statistically significant difference from the control tank and the time zero measurements (Figure Six).

Trial Two:

After three days of exposure to Cupramine™ or Copper Sulfate the health of the *M. arenaria* seemed to be deteriorating. The clams at the higher concentrations had flaccid siphons and were noticeably slower to respond to touch and perturbation. Therefore two clams from each tanks were sampled.

Respiration:

Both copper sulfate and Cupramine™ affected the clams respiration by decreasing it relative to the time zero clams and the control (no exposure) clams at day five (Figures Seven and Eight). In the *M. arenaria* exposed to Cupramine™ the respiration decreased more as the concentration of exposure increased (Figure Seven). After 5 days of exposure the remaining clams in the second, third and fourth highest doses of copper sulfate were dead, with only the controls and the lowest exposure dose surviving (Figure Seven). Comparatively the respiration in the controls was much higher than that of the remaining copper sulfate exposed clams. Similarly the clams in the second lowest and highest Cupramine™ doses also died and the remaining clams exposed to Cupramine™ had much lower respiration than their control counterparts (Figure Eight). With both types of exposures at the second lowest level (1.34mg Cu^{2+} L^{-1}) the respiration was higher than the controls at day 3, but at day five the remaining clams were dead.

Filtration:

There was no significant difference among any of the treatments from the control *Mya* or the time zero measurements in the copper sulfate treated tanks. (Figure Nine) In the Cupramine treated clams there was a significant difference between the .67mg per L treatment, the control treatment (no copper) and the 2.86mg per L treatment at day five. But there was no significant statistical difference across the treatments at day three (Figure Ten).

Copper in Body Tissues-

In the both the copper sulfate and Cupramine treated *Mya* there was no significant difference across treatments at time zero. At day three there was a significant difference in the 4.02mg per L dose and the 2.68mg per L as compared to the control. At five days all treatments were different from the 0mg per L treatment (Figures Eleven and Twelve).

Protein Assay:

The absorbance in the water from the tanks with clams and copper sulfate and Cupramine™ were much higher than the water with *M. arenaria* and no copper additive which were in fact negative. At both day three (Figure Thirteen) and day five (Figure Fifteen) in the water from the Cupramine treated tanks had a much higher absorbance than the non-treated water. This is true for all copper sulfate treated tanks also, except for the tank that received the highest concentration of copper sulfate at day three which had a negative absorbance (Figures Fourteen and Sixteen).

Discussion:

Ecosystem studies is an ever-increasing field. Two important aspects of ecosystem studies is the study of the relationships between organisms and the way in which those organisms respond to perturbation by humans. Humans perturb natural marine environment in numerous ways; from boat traffic, dumping, and harvesting of animals the oceans are just as at risk as the land.

Bivalves are important in marine systems because they couple the benthic processes with pelagic ones. Bivalves are important in forming the structure of some marine ecosystems along with cycling carbon, nitrogen and ecosystem stability (Dame 1996). *Mya arenaria* are a commercially important bivalve on the east coast. Known locally as "steamers", *M. arenaria* is a soft shell clam common in the mud and sand flats of the north eastern Atlantic coast. *M. arenaria* are especially vulnerable to pollution in the form of heavy metals such as Se, Zn, and Cd (Wang & Fisher 1996). *M. arenaria* has also been shown to incorporate copper into it's tissues when the copper is present in the sediments that *M. arenaria* inhabits.

The purpose of this experiment was to examine the affects copper has on *M. arenaria* respiration rate, and filtration rate, to examine how much copper is incorporated into the body tissues of the clams and to see whether these affects differ depending on what form of copper (Cupramine or copper sulfate) is used to introduce the copper. And also to see if the affects differ when *M. arenaria* is in the presence of *Fundulus heteroclitus*.

Respiration rate of *M. arenaria*, overall, decreases with copper because organisms systems begin to shut down, dissimilar from traditional expected response of rapid respiration under stress. The *M. arenaria* from the joint tanks had a lower respiration than that of the *Mya* from the clam only treatments. This may be because the fish often disturbed the Mya by swimming into them and nipping at their siphons, I further observed that in the joint tanks the *Mya* spent less time with their siphons extended than their *Mya* only counterparts (Bowie personal observation). Generally the filtration rates of the *M. arenaria* at different treatment levels of Cupramine followed the same patterns as the respiration rate. This makes sense since the *M. arenaria* filter feed and respire through the same external organ, their siphon. However the copper sulfate did not have a statistically significant effect on filtration, this may be cause by copper sulfate being processed differently than Cupramine, or the filtration affects may be a result of the amine group in the Cupramine.

Both copper from cupramine and copper from copper sulfate was fatal to most clams at the prescribed doses after five days. And both forms were stored in the tissues of the clams however the Cupramine was incorporated in lower concentrations than the copper sulfate. Copper concentration in the body tissues did not seem to be affected by whether *M. arenaria* was in the presence of *F. heteroclitus* or not.

As evident from the protein assay, some protein is being released by the *M. arenaria* in the presence of both copper sulfate and Cupramine that is not being released in the absence of copper. What the structure of the protein is, or its function is I was unable to determine and would require further study.

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References:

Dame, Richard F. Ecology of Marine Bivalves: An Ecosystem approach. 1996 CRC Press, Boca Raton, FL.

Hynes, David et al. 1998. *The Use of Transplanted, Cultured Mussels (Mytilus edulis) to Monitor Pollutants along the Ninety Mile Beach, Victoria, Austalia III. Heavey Metals.* Marine Pollution Bulletin. Vol. 36 No. 5 pp. 396-399.

Matozzo, V. et al. 2001 *Effects of Copper and Cadmium Exposure on Functional Hemocytes in the Clam, Tapes philippinarum.* Arch. Environ. Contam. Toxicol 41, p 163-170.

Regoli, F. et al. 1995. *Gluthathione, glutathione-dependent and antioxidant enzymes in mussel, Mytilus galloprovincialis, exposed to metals under field and laboratory conditions: implications for the use of biochemical biomarkers.* Aquatic Toxicology 31 pp. 143 – 164.

Regoli, F. et al. 1998. *Trace Metals and Variations of Anitoxidant Enzymes in Arctic Bivalve Populations.* Archives of Environmental Toxicology. 35, 594 - 601

Roemeo, M. et al. 1997 *Effect of Heavy Metals on Lipid Peroxidation in the Mediterranean Clam Ruditapes decussates.* Comp. Biochem. Physiol. 1, pp. 33-37.

Wang, W. et al. 1996. *Assimilation of trace elements and carbon by the mussel Mytilus edulis: Effects of food composition.* Limnology and Oceanogaphy 41 (2) pp. 197 - 207

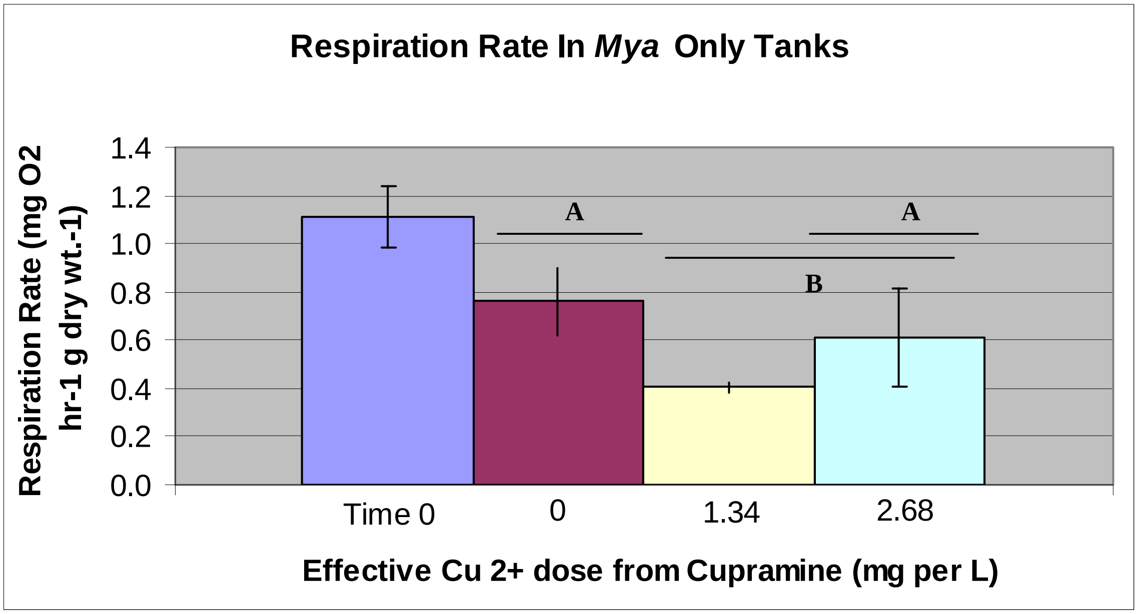

Figure One: Respiration of *Mya arenaria* from *Mya* only tanks after 4 days of incubation in different concentrations of copper. Error bars represent two times the standard error. There is no significant difference between the zero dose and the 2.68mg / L^{-1} dose (A), or between the 1.34mg / L^{-1} dose (B).

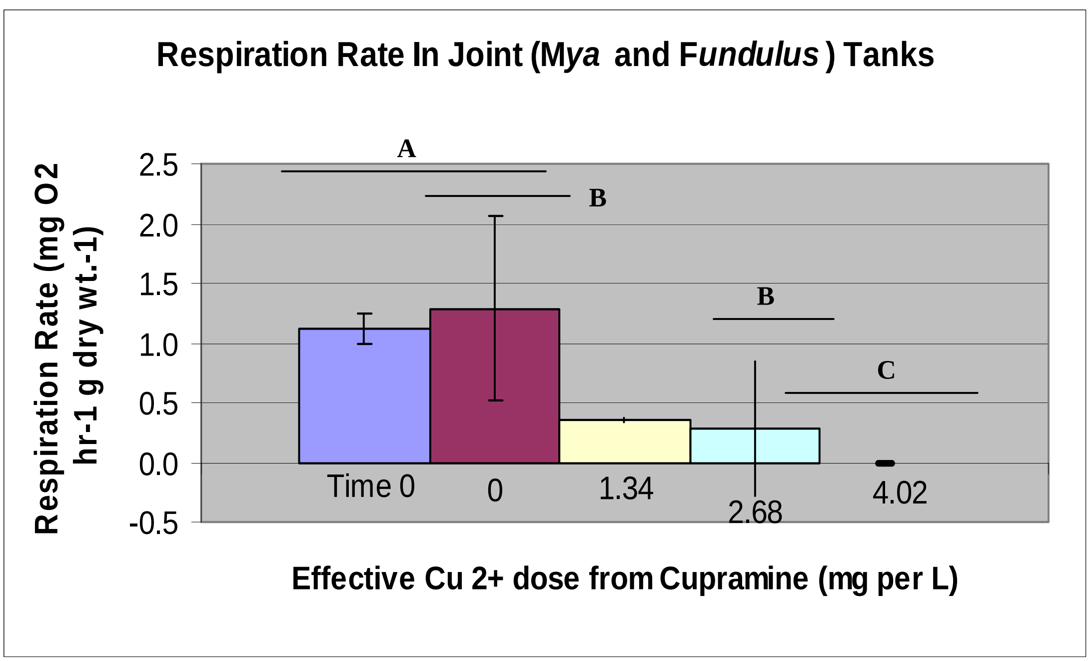

Figure Two: Respiration of *Mya arenaria* from joint tanks after 4 days of incubation in different concentrations of copper. Error bars represent two times the standard error. There is no significant difference between the zero dose and the time zero controls (A), nor is there a difference between the 2.68mg / L^{-1} dose and the zero dose (B), or between the 4.02mg / L^{-1} dose and the 2.68mg / L^{-1} dose(C).

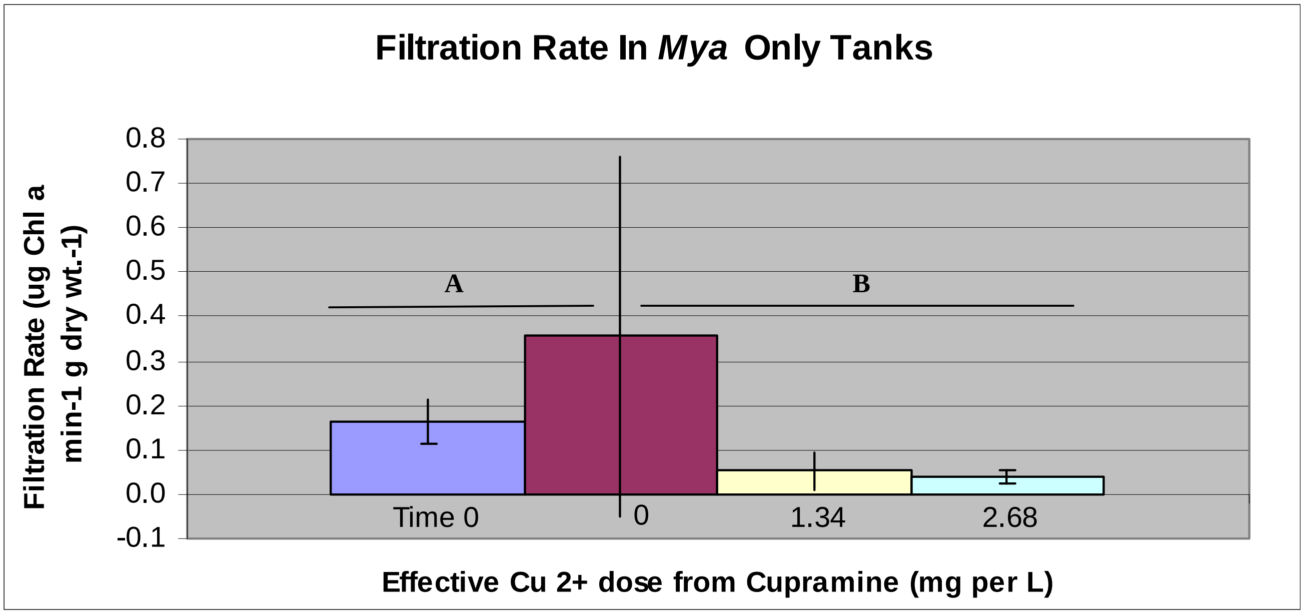

Figure Three: Filtration rate of *Mya arenaria* from *Mya* only tanks after 4 days of incubation in different concentrations of copper. Error bars represent two times the standard error. There is no significant difference between the zero dose and the time zero controls (A), nor is there a difference between the zero dose, the 1.34mg / L^{-1} dose and the 2.68mg / L^{-1} dose (B).

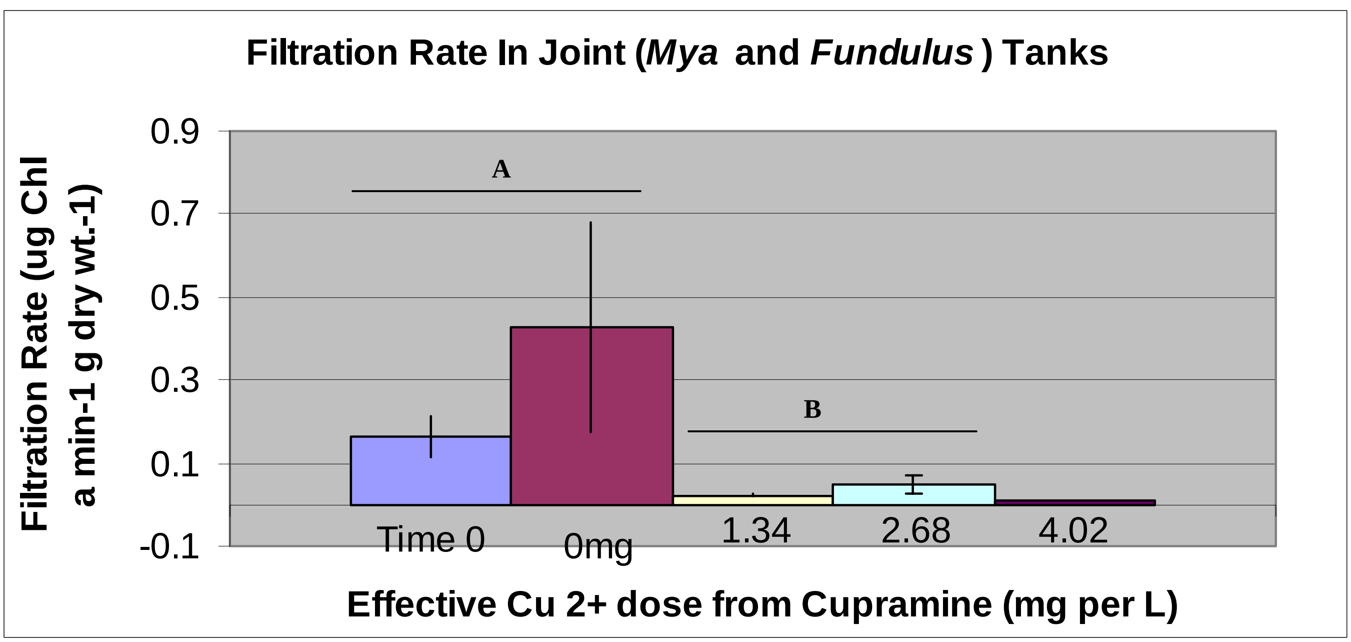

Figure Four: Filtration rate of *Mya arenaria* from *Mya* only tanks after 4 days of incubation in different concentrations of copper. Error bars represent two times the standard error. There is no significant difference between the zero dose and the time zero controls (A), nor is there a difference between the 1.34mg / L^{-1} dose and the 2.68mg / L^{-1} dose (B).

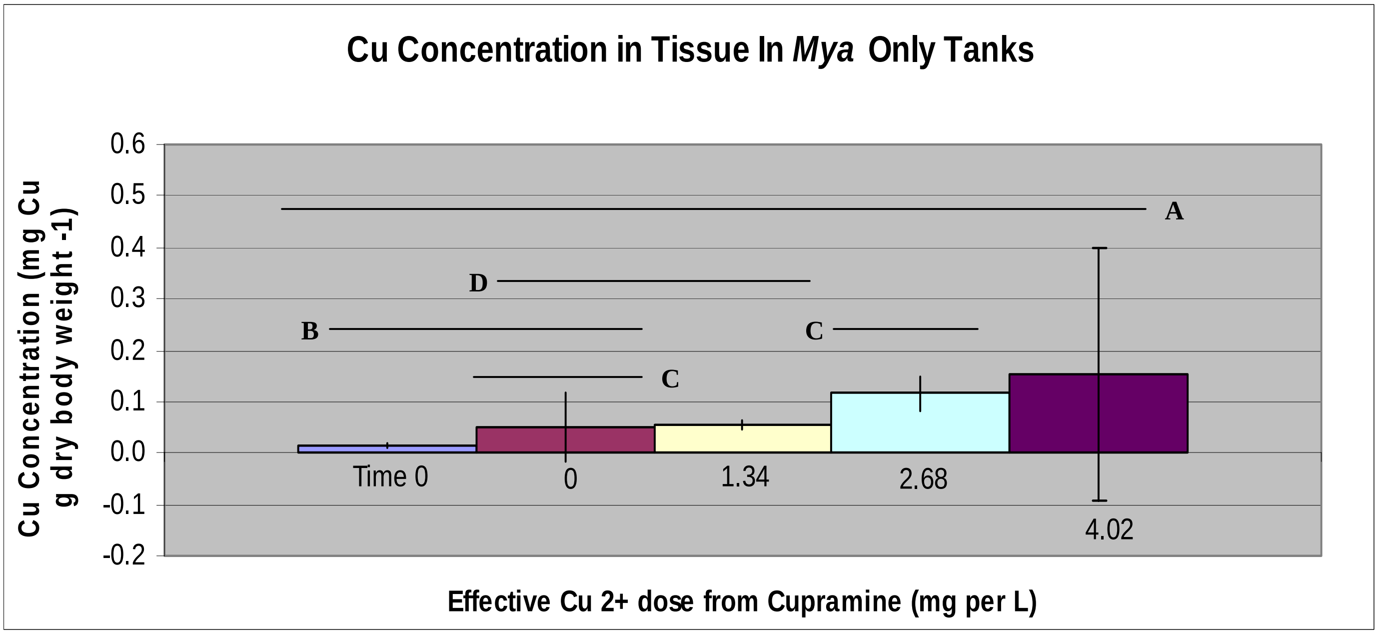

Figure Five: Concentration of copper in the body tissue of *Mya arenaria* from *Mya* only tanks after 4 days of incubation in different concentrations of copper. Error bars represent two times the standard error. The only significant differences are between the time zero controls and the 1.34mg per L and 2.68mg per L doses.

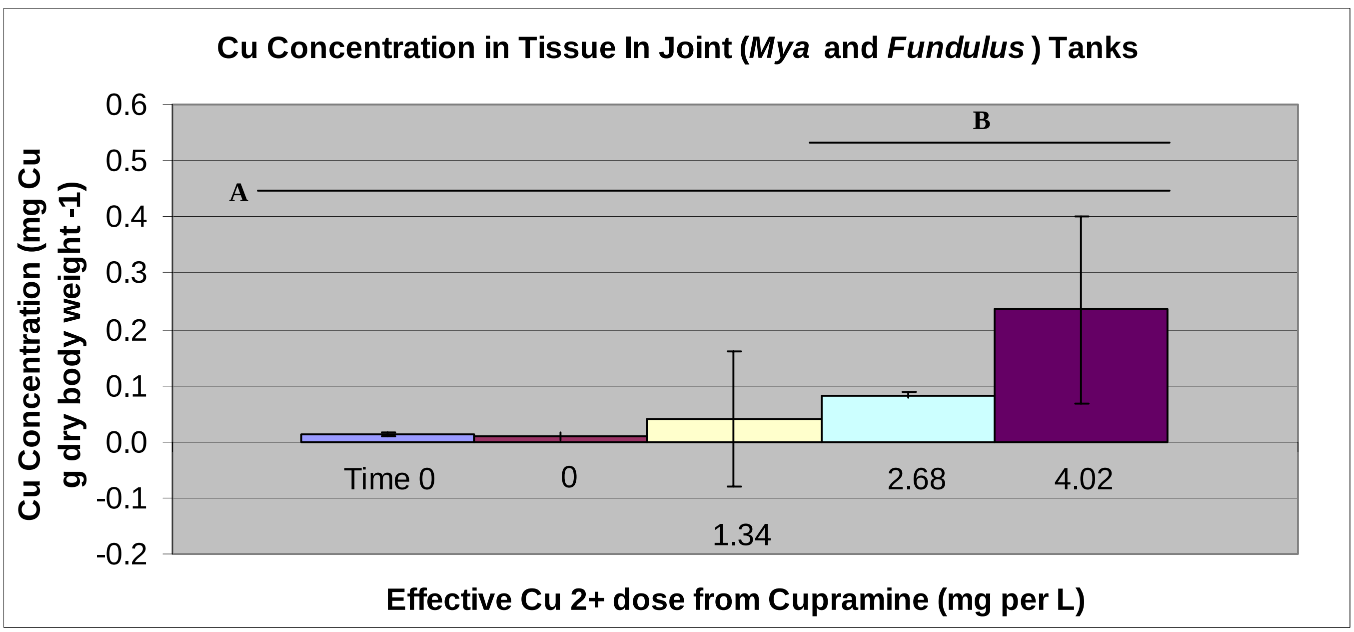

Figure Six: Concentration of copper in the body tissue of *Mya arenaria* from joint tanks after 4 days of incubation in different concentrations of copper. Error bars represent two times the standard error. There is no significant difference between the 4.02mg per L dose and the 2.68mg per L dose, nor is there a statistical difference of the 1.34mg per L dose and any other dose.

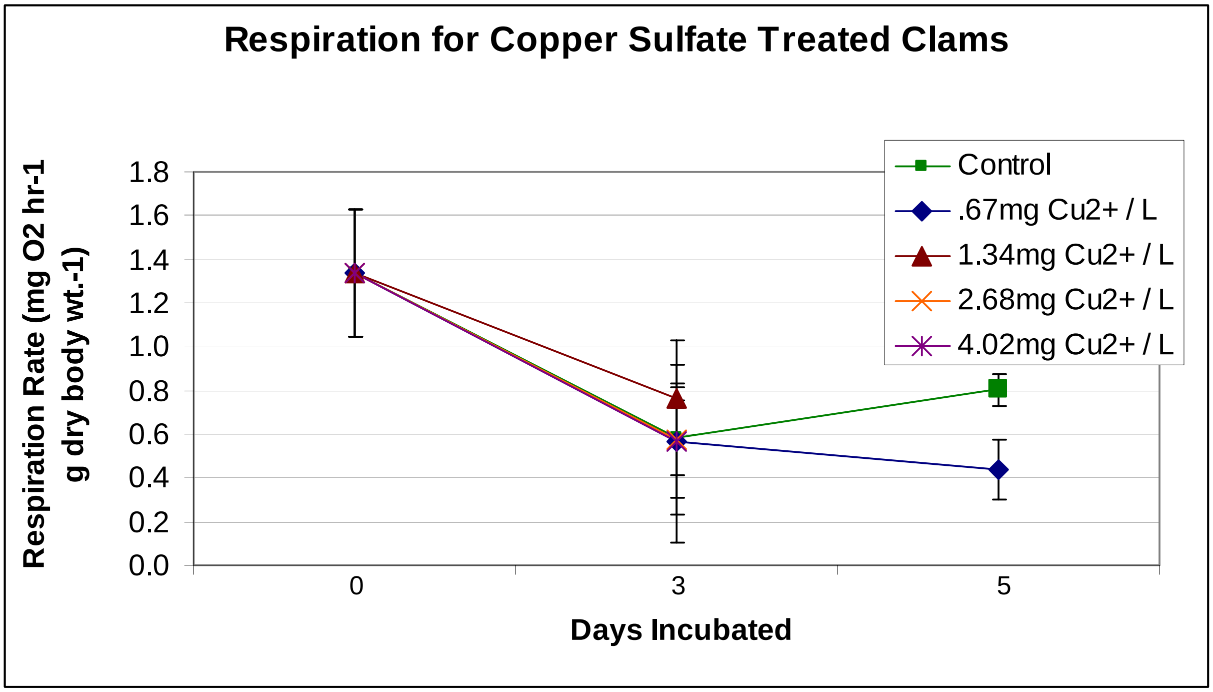

Figure Seven: Respiration rate for *Mya arenaria* incubated in different concentrations of copper from copper sulfate at time zero, after three days and five days. There is no significant difference across treatments at time zero or day three. There is a significant difference between the .67mg per L treatment and the control treatment (no copper) at day five. The lack of data points for some treatments at day five are because of the mortality to all remaining specimens.

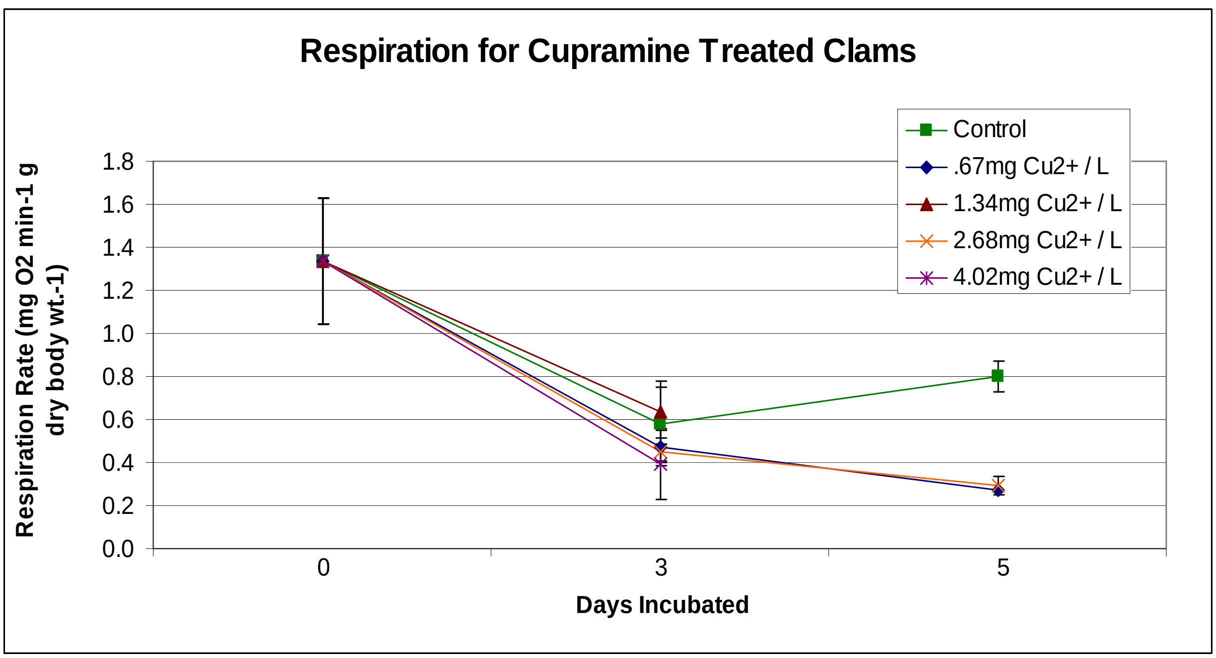

Figure Eight: Respiration rate for *Mya arenaria* incubated in different concentrations of copper from Cupramine at time zero, after three days and five days. There is no significant difference across treatments at time zero or day three. There is a significant difference between the .67mg per L treatment, the control treatment (no copper) and the 2.86mg per L treatment at day five. Also, there is no significant difference between the 2.86mg per L treatment and the .67mg per L treatment at 5 days. The lack of data points for some treatments at day five are because of the mortality to all remaining specimens.

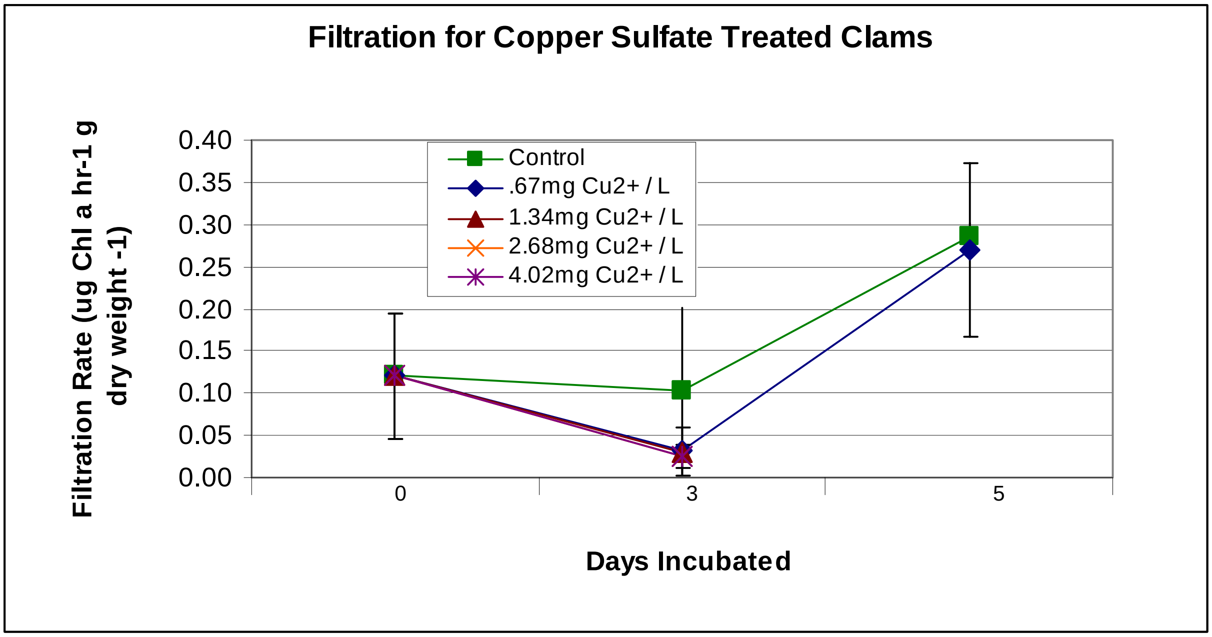

Figure Nine: Respiration rate for *Mya arenaria* incubated in different concentrations of copper sulfate at time zero, after three days and five days. There is no significant difference across treatments at time zero or day three or day five. The lack of data points for some treatments at day five are because of the mortality to all remaining specimens.

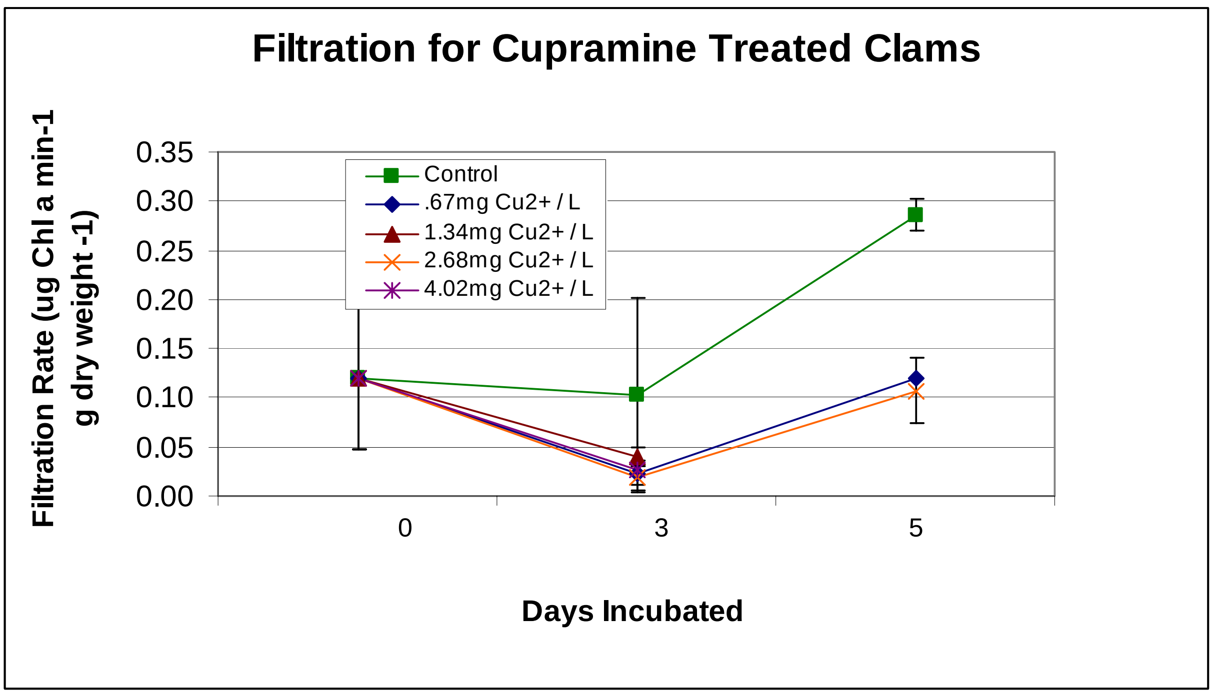

Figure Ten: Respiration rate for *Mya arenaria* incubated in different concentrations of copper from Cupramine at time zero, after three days and five days. There is no significant difference across treatments at time zero or day three. There is a significant difference between the .67mg per L treatment, the control treatment (no copper) and the 2.86mg per L treatment at day five. Also, there is no significant difference between the 2.86mg per L treatment and the .67mg per L treatment at 5 days. The lack of data points for some treatments at day five are because of the mortality to all remaining specimens.

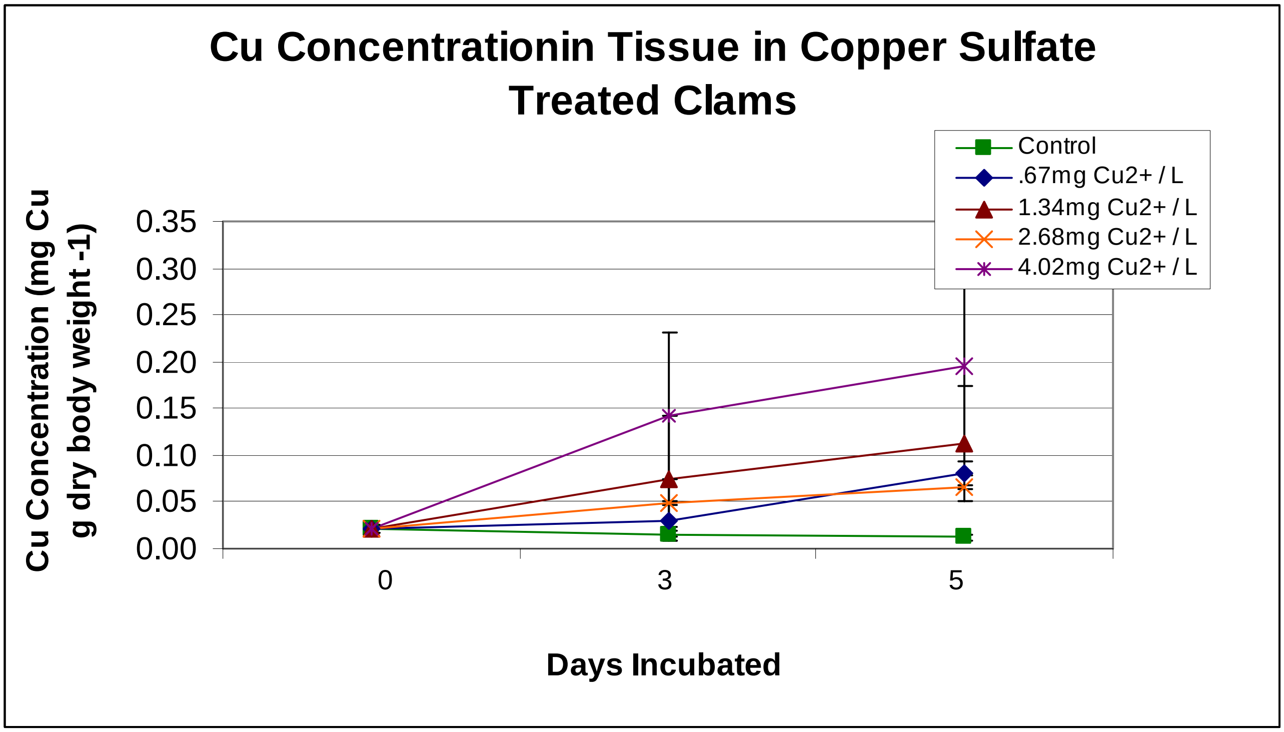

Figure Eleven: Cu concentration in body tissues of *Mya arenaria* incubated in different concentrations of copper from copper sulfate at time zero, after three days and five days. There is no significant difference across treatments at time zero. At day three there is a significant difference in the 4.02mg per L dose and the 2,68mg per L as compared to the control. At five days all treatments are different from the 0mg per L treatment and the 4.02mg per L treatment is also significantly different from the .62mg per L and 2.68mg per L treatments.

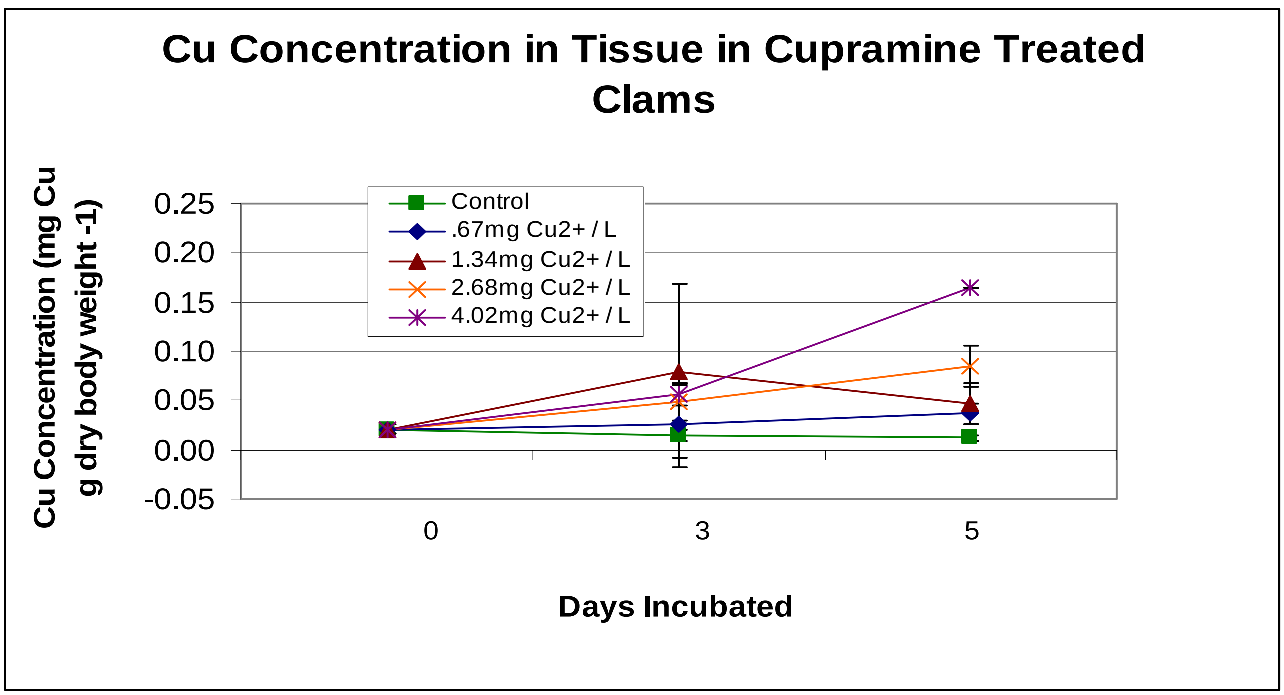

Figure Twelve: Cu concentration in body tissues of *Mya arenaria* incubated in different concentrations of copper from Cupramine at time zero, after three days and five days. There is no significant difference across treatments at time zero. At day three there is a significant difference from the control in the 4.02, and 2.68mg per L doses. At five days all treatments are different from the 0mg per L treatment and the 4.02mg per L treatment is also significantly different from all the other doses.

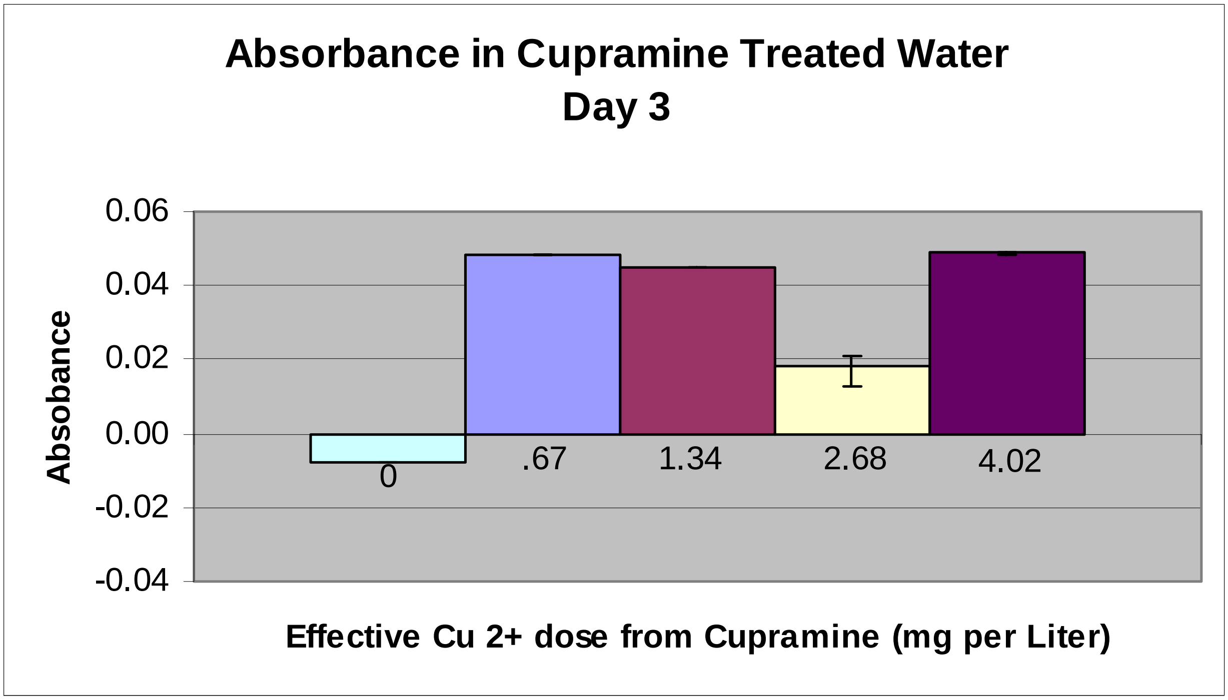

Figure Thirteen: Absorbance in Cupramine treated water on day three. There is a significant difference between the control (0mg per L) and all other copper concentrations.

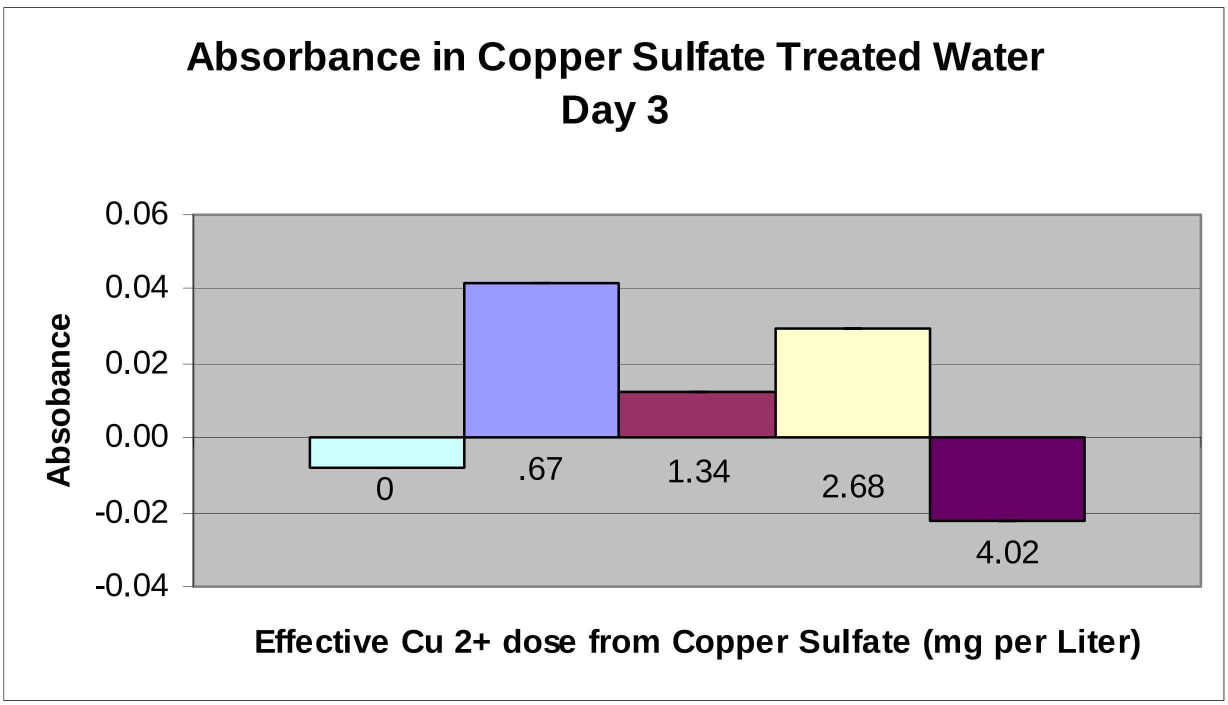

Figure Fourteen: Absorbance in copper sulfate treated water on day three. There is a significant difference between the control (0mg per L) and all other copper concentrations except the 4.02mg per L dose.

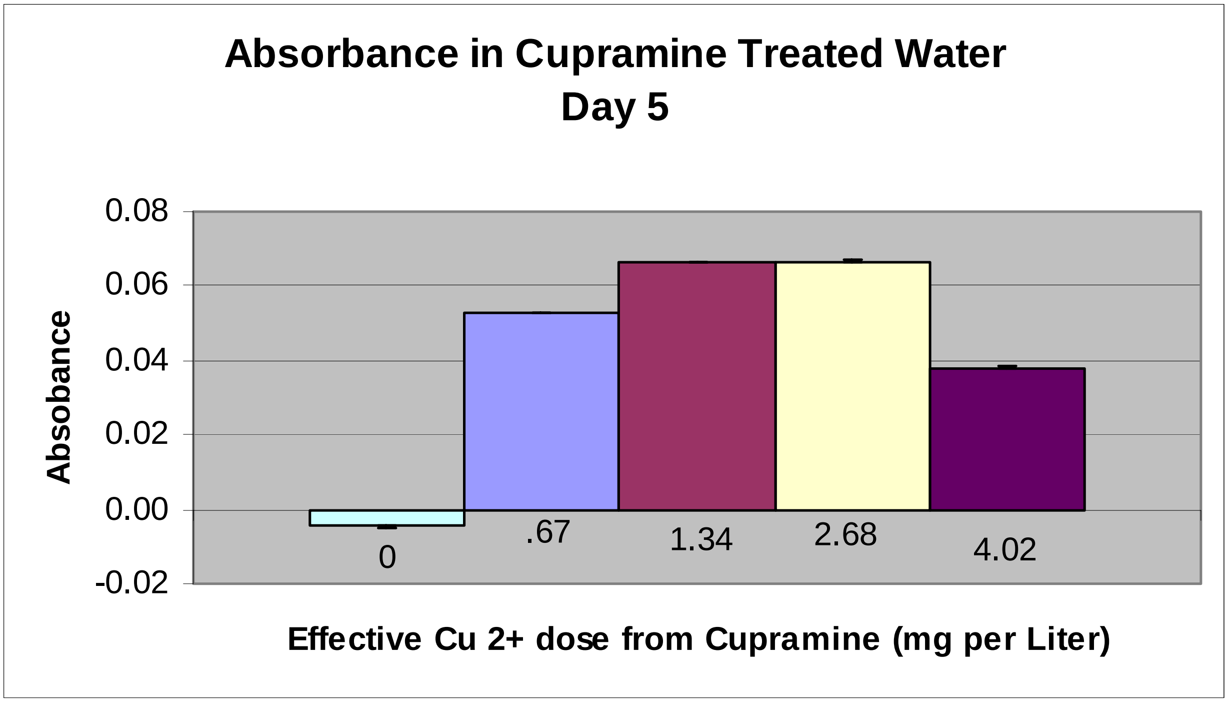

Figure Fifteen: Absorbance in Cupramine treated water on day five. There is a significant difference between the control (0mg per L) and all other copper concentrations.

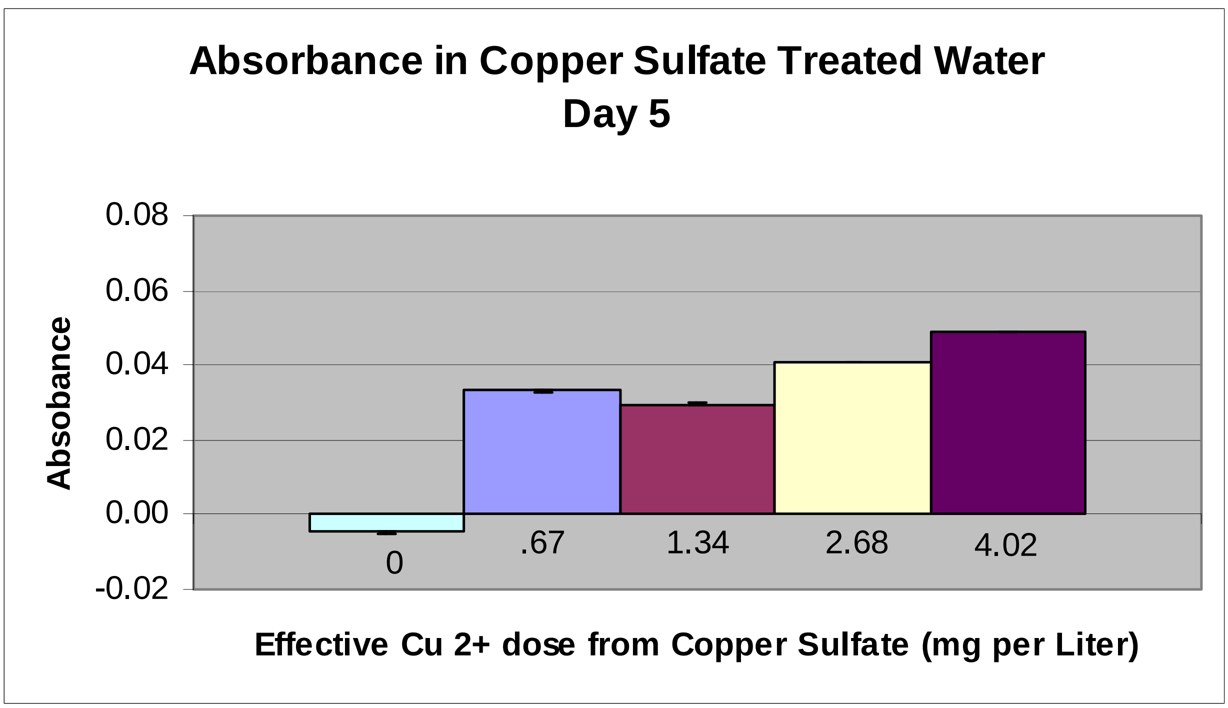

Figure Sixteen: Absorbance in copper sulfate treated water on day three. There is a significant difference between the control (0mg per L) and all other copper concentrations.