

**Secondary Indirect Effects of Copper on Mummichogs  
(*Fundulus heteroclitus*) and its Implications on Benthic-  
Pelagic Food Coupling**

Marissa Sue  
Wellesley College  
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### ABSTRACT

The behavioral, physical, and biological reaction of a fish to pollutants such as copper can not only have a negative effect on individual survival, but also on a population and ecosystem level. While copper is necessary at low levels for all organisms, exceeding a certain amount of copper in an environment can be toxic. Copper was introduced in the form of Cupramine™ at levels of 0.6674 mg copper L<sup>-1</sup>, 2.0021 mg copper L<sup>-1</sup>, and 6.0064 mg copper L<sup>-1</sup> with two treatment types. The results were analyzed for the significance of having *Mya arenaria* present. Additionally, copper sulfate was used to study benthic-pelagic relationships with a fish alone treatment at differing concentrations in order to further examine the indirect effects of copper contamination introduced as a different form of copper. Cupramine™ had a negative impact on *Fundulus heteroclitus*. The nutritional state/fish condition, respiration, packed cell volume, and prey capture efficiency all demonstrated a general decrease with increased time and concentration. Respiration and gill content of copper was more severe for the copper sulfate tanks. This study concluded that three-day trials of the copper sulfate and Cupramine™ with *Mya arenaria* were insufficient to determine interactive effects because of the close proximity of the testing period to the initial dosing. However, it is clear that even with relatively low, sublethal levels of copper, long-term exposure has significant effects on secondary indirect effects.

### INTRODUCTION

The functioning of ecosystems is dependent on the interactions of biotic and abiotic entities. These processes and fluxes are key factors in determining how an ecosystem will work and react to change. Anthropogenic inputs to aquatic systems have greatly altered these important interactions. While anthropogenic influences occur frequently across all ecosystems, one interaction that has greatly been effected by several types of human actions is feeding behavior of individual species and food webs. The flow of nutrients and energy from primary producers to top carnivores is key in maintaining trophic structure. However, pollutants such as PCBs, methane, lead, zinc and cadmium have the potential to trophically flow through contaminated systems (Weis et al. 2003). Bioaccumulation is a problem for copper since it becomes strongly concentrated with each successive trophic level (Extension 2003).

Copper plays important role as an essential minor metal, but excessive copper can also have negative biological, chemical, and physiological effects on organisms. While low concentrations of copper are an important component of enzymes, copper can also catalyze highly reactive hydroxyl radicals. These radicals have the potential to cause oxidative damage (Gaetke 2003). Fully ionic Cu<sup>+2</sup> is the bioavailable, destructive form of copper (Meador 1993). In most natural environments, this form is mitigated since copper is decreased in seawater by chelators, which bind the metal and complex it with organic matter, ligands, and some biological species (Weis 1993; Moffett et al. 1997). Anthropogenic sources of copper are mainly introduced to aquatic systems through wood treated with chromated copper arsenate, which is 18.5% copper oxide (Weis 1998). Another input is via runoff of copper sulfate, which is used as a fungicide in both commercial and organic farms (Smolowitz 2003).

Toxicity caused by high concentrations of copper and other heavy metals has both direct and secondary indirect effects on organisms. While direct effects eventually lead to mortality if the compound is at high enough concentrations, secondary indirect effects are a manifestation of direct effects and often cause altered behaviors. Examples of these include respiratory rates, prey capture, social interactions, ability to escape predation, and swimming performance (Weis 2001). Several studies have marked these sets of behaviors as a potential “biomarker” for pollution and other toxicants (Weis et al. 2003). Lowered prey capture rate has been associated in mummichogs with reduced energy uptake that directly affects individual growth and survival. These changes in behavior can cause a higher mortality rate than a natural baseline and thus cause for ecological concern. Increased death rate of one species has the potential to alter the dynamics of an entire ecosystem through interspecific interactions and alteration of niche roles (Weis 2001).

Ecosystem interactions are oftentimes central in altering behavior, physiology of individuals, and environmental conditions of the habitat. *Mya arenaria* are naturally occurring in the mummichog’s environment. They are used in one of the treatment types to examine the trophic dynamic and benthic-pelagic coupling of the two species. The *M. arenaria* both filter, which is a potential source of decreased ionic copper in the tank and deposit pseudo feces, which can be ingested by the mummichogs. Since mummichogs are opportunistic and detrital feeders, they have the potential of high heavy metal loading due to trophic transfer. The coupling of the two have the potential to alter the concentration of copper found in the mummichogs.

Since polluted areas generally do not have one chemical source of contamination, but rather several, this study uses aquaria to examine the isolated effects of copper on *F. heteroclitus* in the form of Cupramine™ and Copper sulfate. Juveniles were used since they are highly sensitive to pollutants. Use of juveniles allows the results to be extrapolated to predict longer exposure times at the same concentrations (Schreck 1990).

## METHODS

In aquaria, introduction of copper is often in the form of Cupramine™, which is used to kill protozoan ectoparasites. Cupramine™ is used in both salt and freshwater aquaria and contains 10,000 mg copper/L (Seachem 2003). The therapeutic dose is 0.3-0.35 ppm while manufacturers claim a fish tolerance of up to 0.8 ppm (Treating 2003). Since the cellular structure of invertebrates is similar to the target parasite, it is advised to remove all invertebrates before treatment. However, studies are not available to show the sensitivity of invertebrates to copper in the form of Cupramine™. While Cupramine™ contains two amine groups, which may factor into how a species may react to the chemical, copper sulfate does not. A comparison of the two through different treatment types may illustrate the degree of toxicity of each by display of more or less severe secondary indirect effects.

The setup of this experiment consisted of ten tanks. Eight ten-gallon tanks were used for study of Cupramine™ with four containing only *F. heteroclitus* and four containing *F. heteroclitus* with *M. arenaria*. Each of the treatment tanks held 15 fish. The tanks were dosed and held at constant concentration. In addition to the control, the dosed tanks were maintained at 0.67 mg copper L-1, 2.00 mg copper L-1 or 6.01 mg

copper L-1 level. Two five-gallon tanks with five fish each were dosed with copper sulfate at 0.67 mg copper L-1 or 6.01 mg copper L-1, which spans the range of the highest and lowest Cupramine™ dosing. Each fish within a tank acted as a pseudo replicate due to limitations in time and space. Fish were obtained from Great Sippewissett via minnow traps with the assumption that background copper levels were insignificantly different.

Each tank was filled using Instant Ocean™ in order to ensure constant background levels of copper in the water. Salinity, temperature, pH, nitrite, nitrate and ammonia are vital signs of the tank that were tested on a daily basis. Water changes and redosing occurred when ammonia levels exceeded 0.5 ppm, pH dropped below 7.0, or time lapsed more than three days. Air stones were used for aeration and filters were not used to prevent potential sources of copper uptake due to absorption of copper (SeaChem 2003). Two fish from a tank were randomly selected at each sampling point and tested for prey capture rate, respiratory rate, fish condition, packed cell volume, and copper. Four sampling points ending at day sixteen were obtained for the Cupramine™ tank with only fish. The Cupramine™ mixed tank with fish and clam and the copper sulfate tank with fish only had one sampling point at day three.

Statistical analysis was made using two-way ANOVA tests to evaluate all data sets obtained. The effect of day and concentration of copper on the fish only Cupramine™ tanks was analyzed. Source of copper and concentration was tested for significance in the copper sulfate with fish only tanks. The concentration and presence of clams was evaluated in the mixed tank treated with Cupramine™.

### ***Prey Capture Rate***

The testing area consisted of a brown box holding four 1000 mL beakers filled to the 550 mL line with ocean water. Each fish was allowed to sit for at least five minutes to acclimate to the surroundings. While *F. heteroclitus* are opportunistic eaters, they often feed upon crustaceans, annelids, and grass shrimp. Brine shrimp, *Artemia Salina* were used in this study to simulate a prey capture scenario where food is motile. Thirty brine shrimp were introduced and bites were classified into three categories: miscues, strikes, and spits. Miscues occurred when the individual aimed for a brine shrimp but missed, strikes were successful captures, and spits were catch and release of the brine shrimp. The *F. heteroclitus* was timed for six minutes per trial. The fish were consistently fed the night before as in the daily routine with trials occurring the next morning or early afternoon without their morning feeding. Prey capture efficiency was calculated by total successful strikes divided by total strikes (the sum of miscues, strikes, and spits) (Schreck 1990).

### ***Respirometry***

Respiration of an individual fish took place in 250-mL Biological Oxygen Demand (BOD) bottle. Instant Ocean™ was used in order to eliminate respiration or photosynthesis by algae and bacteria. A dissolved oxygen probe was used to measure the DO in each bottle at the beginning and end of approximately an hour-long period. The time period was determined by ensuring that dissolved oxygen did not fall below 2.00 mg L-1 in order to avoid artificially low respiration rates due to hypoxia (Stierhoff 2003).

Between these measurements, the BOD bottles with the fish were placed in an aquarium in order to simulate tank conditions as to minimize erratic behavior and keep temperature constant. The goal was to measure basal aerobic metabolism. Large fish consume more total oxygen and small fish consume more oxygen on a unit-weight basis. The relationship between size and oxygen consumption is a linear line with a slope of less than one. The equation  $Y = a(X^b)$  with Y equal to oxygen consumption, a equal to mass coefficient, X equal to body mass, and b equal to mass exponent, is used to calculate the oxygen consumption independent of size (Schreck 1990).

### ***Fish Condition***

The nutritional state of the fish, or “well being” was measured using the Fulton’s condition factor,  $K = \text{wet weight}/\text{length}^3 \times 100,000$  (Schreck 1990). These measurements were taken after anaesthetizing the fish using tricaine in the form of MS222 in 400 ppt in accordance with the 2000 American Veterinary Medical Association Panel on Euthanasia (American 2000).

### ***Packed Cell Volume***

Blood was taken from the spinal artery/vein with an anticoagulated hematocrit tube by making a ventral incision forward of the caudal peduncle and slightly behind the anal fin. The hematocrit tubes were spun for five minutes at 2600 RPMs in a refrigerated centrifuge. Packed cell volume was determined by looking at percent of red blood cells to serum. The tubes were Xeroxed at 200% to more accurately measure the packed cell volume.

### ***Copper Analysis***

The liver, gills, and tissue (including bones) were dissected from each individual fish and dried at 60° C overnight. After weights were taken to four decimal points, the parts were ashed at 500° C in 20-mL scintillation vials for at least five hours. Five mL of 10% hydrochloric acid was added to each scintillation vial. Atomic absorption was used to determine the concentration of copper per gram of dry weight of liver, gill, or tissue.

## Results

### ***Fish alone treated with Cupramine™***

The fish condition factors were extremely similar for all concentrations of copper for all days with the exception of day six (Figure 1). While the values showed fluctuations with no general trend, on day six there was a 0.3 to 0.6 increase from day three levels. There was a significant difference in change with day when the fish condition was tested ( $p=0.0023$ ), but not by concentration of copper.

There was a significant difference in change in the prey capture efficiency when evaluated by concentration of copper ( $p=0.0024$ ), but not by day. There was a visible difference in the behavior of the *F. heteroclitus*. While the fish in the control tank actively searched for the prey with precise movement, the fish in the treated tanks were increasingly sluggish. They also showed high gilling rates and lack of fine motor skills or premeditated, coordinated movement. The treatment fish were more likely to wait for the brine shrimp to enter their range of vision before attacking. Prey capture efficiency of the control tanks stayed at a relatively stable level around 100% with a slight decline in

the first sampling point and the last sampling point (Figure 2). The prey capture efficiency of the 0.67 mg L<sup>-1</sup> and 2.00 mg L<sup>-1</sup> treatment tanks showed a fluctuation over time. The two highest concentration groups demonstrated an increase from the day zero sampling point. In the highest concentration, this was preceded by a large drop in the prey capture efficiency from day zero to day six.

Respiration decreased with concentration and time (Figure 3). There was a strong downward trend when comparing number of days after initial dosing to a decrease in the respiration. Change in respiration showed a significant difference with concentration ( $p=0.0024$ ). Sampling points from day six to day sixteen showed the expected trend of decreased respiration with increased concentration.

The change in packed cell volume evaluated by concentration was statistically significant ( $p=0.0199$ ). Packed cell volume displayed a trend of lower percent red blood cells at higher concentrations of copper which is particularly evident when comparing day zero to day sixteen (Figure 4). However, day six and day twelve packed cell volumes for the fish treated with 0.67 mg copper L<sup>-1</sup> and 2.00 mg copper L<sup>-1</sup> showed even lower packed cell volumes than the highest concentration. Nonetheless, a downward trend was clearly seen from day zero to day sixteen.

There was no common trend for copper concentration in the liver between the individual treatments over time (Figure 5). The highest concentration showed an initial decrease in copper concentration followed by an increase. The day sixteen level of copper was highest for the highest concentration of copper in the water. The control, low, and medium concentration tanks increased before reaching their final copper concentration level at day sixteen. The 2.00 mg copper L<sup>-1</sup> tank had over a five-fold change from day zero to day six followed by a decline in liver copper. However, there was a significant difference in copper concentration of the liver between the different treatments dosed with Cupramine™. The copper concentration in the gills for all tanks displayed a “u”-shaped curve and there was no significant effect of time interval or concentration of copper on the copper content of the gills (Figure 6). The concentration of copper in the tissues remained at a fairly stable level throughout time and by tank concentration (Figure 7).

### ***Fish alone treated with copper sulfate***

The fish conditions of the copper sulfate treated tanks were not significantly different for the two treatment concentrations on day 3 (Figure 8). Fish condition was within the range of the day three results for the Cupramine™ fish alone treated tanks.

There was a threefold difference between prey capture efficiency of the highest and lowest concentrations on day three which were comparable efficiencies to the Cupramine™ treated tank (Figure 9).

The respiration for the copper sulfate treated fish for the highest concentration of copper was more than three times less than the equivalently dosed Cupramine™ tank (Figure 10 & 3), leading to a significant difference in respiration from copper source ( $p=0.016$ ). The lowest concentration of copper via copper sulfate had a similar respiration rate as the equivalently dosed Cupramine™ tank.

Even though packed cell volume was lower for the higher concentration, a significant difference was not found when looking at the connection between copper source as well as concentration and packed cell volume (Figure 11).

The copper concentration in the livers of the fish increased by 100 mg copper g dry weight<sup>-1</sup> from day zero to day three for the high treatment while the low concentration showed a slight decrease (Figure 12). Both of these values were within the range of the day three values for the Cupramine™ treated tanks. Similarly, the amount of copper in the gills for the copper sulfate treated fish was similar to the Cupramine™ treated fish (Figure 13). The day three levels of copper in the gills were similar for the high and low treatments. There was a significant difference in copper concentration of the *F. heteroclitus* tissue with different copper sources (Figure 14 & 7). The equivalent amount of copper administered via copper sulfate had more of an effect on the copper concentration of the tissues than it did via Cupramine™. There was a larger difference in the amount of copper in the tissues between copper sources for the higher concentration of copper in the water. While seemingly natural mortality of two fish occurred in the fish treated with Cupramine™ tanks, three out of five fish were found dead on day four in the 6.0064 mg copper L<sup>-1</sup>, resulting in total total mortality in the tank.

#### ***Mixed (fish with clams) treated with Cupramine™***

The *M. Arenaria* provided some structure for the *Fundulus*, which induced differences in the behavior of the fish. While the fish in the tanks without the clams grouped together in a corner, the fish with the clams were able to hid amongst the bivalves. Additionally, as time increased there was a substantial difference in the bahavior of the fish at increasing concentrations. Higher concentration fish had a lower response to tapping on the glass. While the fish in the control tanks would follow the person feeding them to either side of the tank and wait at the surface, the fish in the treated tanks would remain at the bottom of the tank. The grouping seen at the beginning of the experiment in the dosed tanks disappeared and the fish spread out with no apparent sense of danger. The visible difference between tanks would suggest that the varying results reflect visible changes in treatments. However, statistical analysis showed that there was no difference in the secondary indirect effects with or without the *M. arenaria*.

When *F. heteroclitus* was placed in a tank with *M. arenaria*, fish condition showed similar values as the *F. heteroclitus* only tanks (Figure 15). There was no significant difference in fish condition related with presence of *M. arenaria* or concentration.

Prey capture efficiency declined with increasing copper concentration as in the fish only tanks (Figure 16). Prey capture efficiency was down to 0% for the tank dosed with the highest amount of copper. Behavioral observations show that active searching only occurred in the lowest concentration of copper consistently throughout the sampling points. Presence of *M. arenaria* and concentration of copper did not induce significant change on the prey capture efficiency.

The changes in respiration were overall lower for the mixed tanks than the fish only tanks (Figure 17). The respiration rates of the fish in the mixed dosed tanks showed similar values as the control tank.

While packed cell volume decreased at day three in the mixed tank as it did in the fish only tank, the general decrease in packed cell volume was greater for the mixed tank (Figure 18). The control showed a significant trend, which matched the change in packed cell volume of the middle concentration of copper.

The copper concentration of the liver showed increased concentration with any amount of copper in the water (Figure 19). However, there was no trend between the concentration of copper and the concentration of copper in the liver. This was also true for the copper concentration of the gills (Figure 20) and the tissue (Figure 21).

This study was ended after the first sampling point due to mass mortality of the clams due to copper toxicity occurring on day four. While benthic pelagic food coupling of psuedo feces to fish does occur found through visual observations, this would not be occurring at the given copper concentrations of the clams post mortem.

### Discussion

One of the parameters of this experiment is acclimation of the fish to the tank. Some factors that influence their adjustment include ammonia level, pH, lack of structure in the tanks, and food. Stressors on the fish may physically or behaviorally act as an additive effect and will be indicated in the results. Therefore, the controls act as a guide to determine the magnitude of the potential additive effects. One of the factors in this experiment that affected all fish was the switch from natural diet to flake food and brine shrimp, which was fed ad libitum. This could have a potential influence on the condition factor of the fish through a change in the amount of weight gain. The fish were being fed twice a day consistently, which is potentially much more than the fish would obtain in the wild. Weight gain by increased food intake would exceed the weight loss from copper toxicity and would mask the true effect of the copper (Schreck 1990). This may explain the increased fish condition from the initial state levels. The copper effect eventually outweighs the altered feeding habits and results in decreased fish condition. However, growth and weight loss/gain can occur over a relatively long period of time and it is possible that no significant change would take place over a sixteen-day period. Therefore, the differences found in the condition factor could be correlated with the initial fish condition rather than the treatment types. This is particularly true, as supported by the statistical analysis, for the three-day period treatments of the mixed Cupramine™ tanks and the copper sulfate tanks.

It is a significant finding that prey capture efficiency declines with concentration of copper. Capture is a measure of both appetite and complex coordination of both movement and sight. Reaction to toxicants at sublethal levels such as swimming performance can be displayed through changes in behavior before physiological damage occurs (Schreck 1990). Many miscues would indicate that appetite is high and coordination is less than adequate, while low amount of miscues and strikes would indicate low motivation to feed. In this experiment, spitting of the brine shrimp was counted as a miscue (strike with no capture), because the important factor being examined is expenditure of energy. Energy metabolism of an individual is critical to survival. While energy to reproduce is allocated last after all other energy needs are met, reproduction is nonetheless important in propagation of the species and population (Schreck 1990). Unfruitful expenditure of precious energy results in potential decline of the individual's health and survival, which will consequently decrease the population and alter the ecosystem.

While organisms tend to show an increase in respiration when stressed, there was a decrease in respiration with increased concentration. Fish gills serve several functions including excretion of electrolytes, oxygen-blood exchange, and osmoregulatory

excretion. Chloride cells regulate the interior amount of liquid by sequestering and excreting salts (Hoar 1947). Damage in the gills could result in a decreased efficiency of oxygen extraction from the water leading to lower respiration. Gill damage would also affect the percent packed cell volume of the blood. One of the possible explanations for a decreased packed cell volume with increased concentration is that the osmoregulatory mechanism is not functioning properly. Fish must drink water and excrete the salts to stay hydrated. If the chloride cells were damaged, then the internal salt content would increase leading to an uptake of water. The amount of serum in the blood would increase because of the influx of liquids into the body and bloodstream (Smolowitz 2003). The amount of red blood cells would appear to be decreasing due to increase of serum. However, one of the results of copper toxicosis is hemolysis in which copper directly affects the cell membranes. Reduction of blood cells can occur within a few days suggesting that the results seen could occur within the timeframe of testing. In sheep, destruction of the hemoglobin can be so extreme that the animal falls into hemolytic crisis. Copper toxicosis in both humans (Wilson's disease) and sheep is a result of genetics, which inhibits the production of a copper transporting protein in the liver (Bremner 1998). However, the symptoms such as dullness and respiratory distress can also be expected in fish.

Since copper sulfate is fully ionic, the fish were expected to have a more severe reaction to the copper sulfate treatment than the Cupramine™ treatment. Studies have shown that copper is highly toxic to fish and may cause anoxia with the amount of dead biomass produced by its application (Extension 2003). Fish fry are more resistant to copper sulfate than eggs or adults and can cause mutagenesis at levels of 400 to 1,000 ppm over long periods of time. The most distinct changes occur in the spleen, liver and kidneys (Jones 1983). Respiration and the copper in the gills were the two effects dependent on copper source. Change in respiration and tissue copper indicates that the mechanism of impact by the copper via copper sulfate is related to these two secondary indirect effects. Additionally, since total mortality of the fish at the highest copper sulfate tank occurred at day four, this suggests that the LD50 for the copper sulfate is higher than the Cupramine™. Cupramine™ is not as lethal as copper sulfate and may not affect the fish by the same mechanism. However, fish treated with either chemical display common secondary indirect effects.

The fish tanks treated with Cupramine™ had significant change in all secondary indirect effects measured excluding copper in the gills and tissue. While the addition of *M. arenaria* was hypothesized to have a buffering effect on the magnitude of the sublethal dosing of the copper, this was not the case for the day three trials. Even though there was no effect with presence of *M. arenaria*, this does not suggest that the benthic-pelagic interactions are trivial in copper contaminated areas. A three-day period is insufficient in determining a clear interaction between fish and bivalve. The mass mortality of the clams prevented study of this interaction. However, it is possible that if a bivalve other than *M. arenaria* was used with higher tolerance to copper, then there would be a significant interaction between the two with an increased time interval of cohabitation.

Secondary indirect effects can act as an important set of indicators to detect pollution and toxicants. Nevertheless, care must be taken in using biomarkers to determine the overall health of an environment since species can build a tolerance to

heavy metals and chemicals (Bello 1999). This could have a significant effect on biomagnification since higher tolerance would make the starting level of copper in the base of the food chain much greater. Not only would this impact human consumption of fish, but this would also have enormous impacts on the environment. On a genetic scale, copper pollution selects for certain characteristics and thus would decrease the genetic diversity. While secondary indirect effects may not be the ultimate indicator of pollution, these behavior and physiological changes are important in determining how an ecosystem will be altered by anthropogenic inputs.

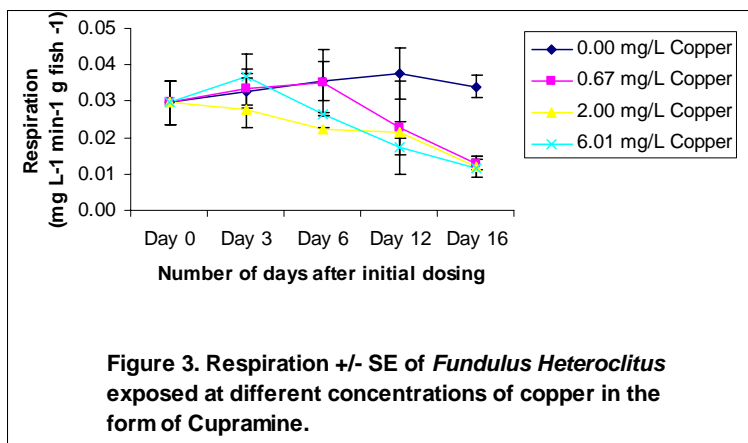
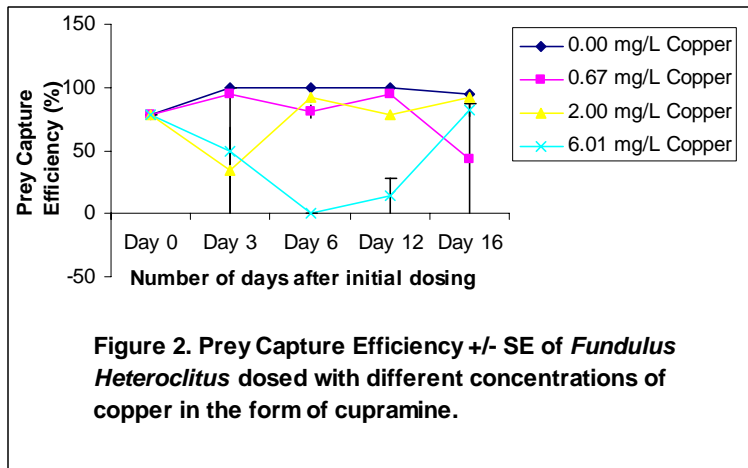
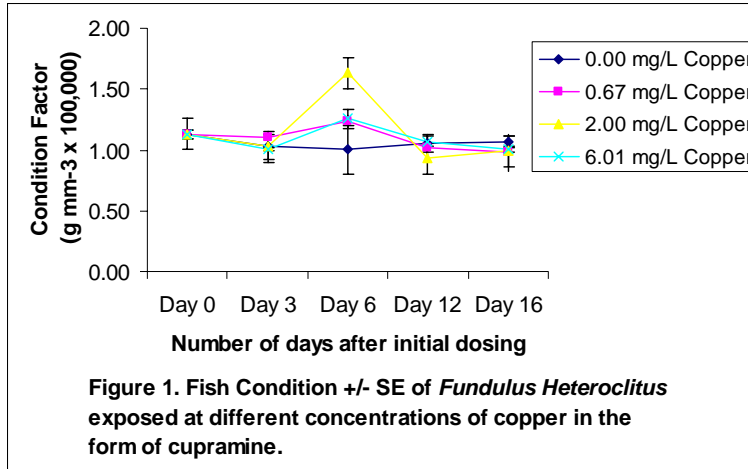
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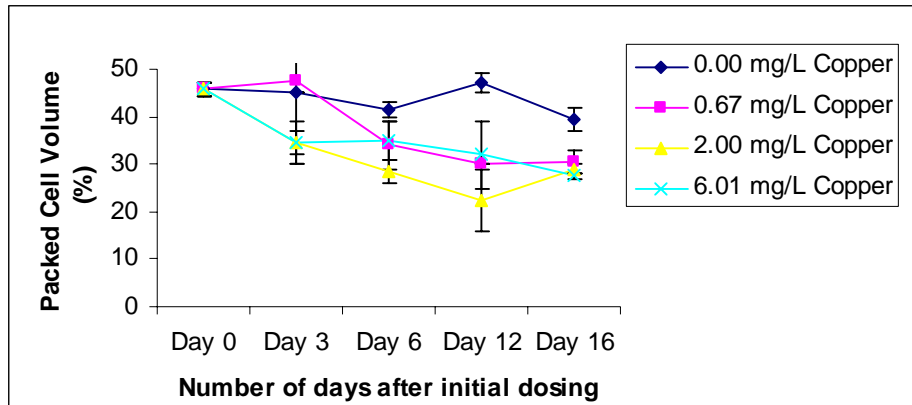
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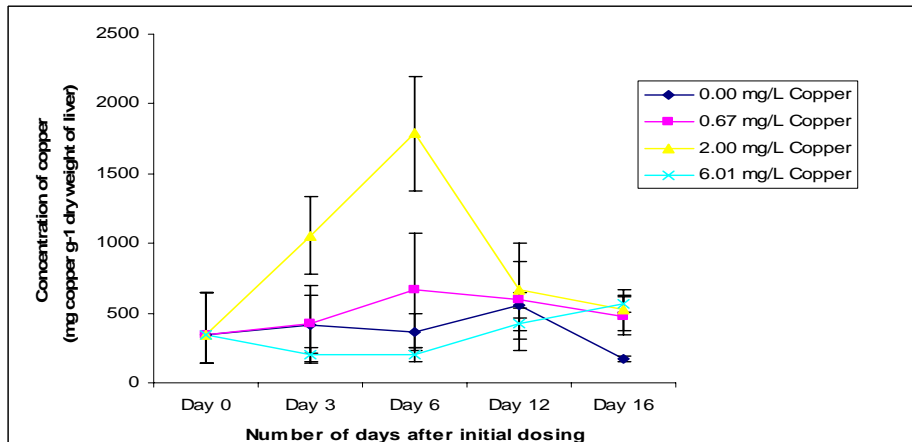
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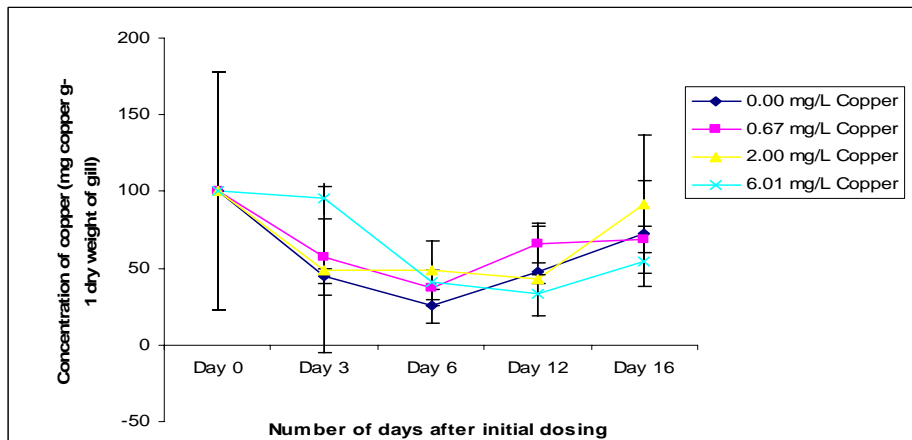




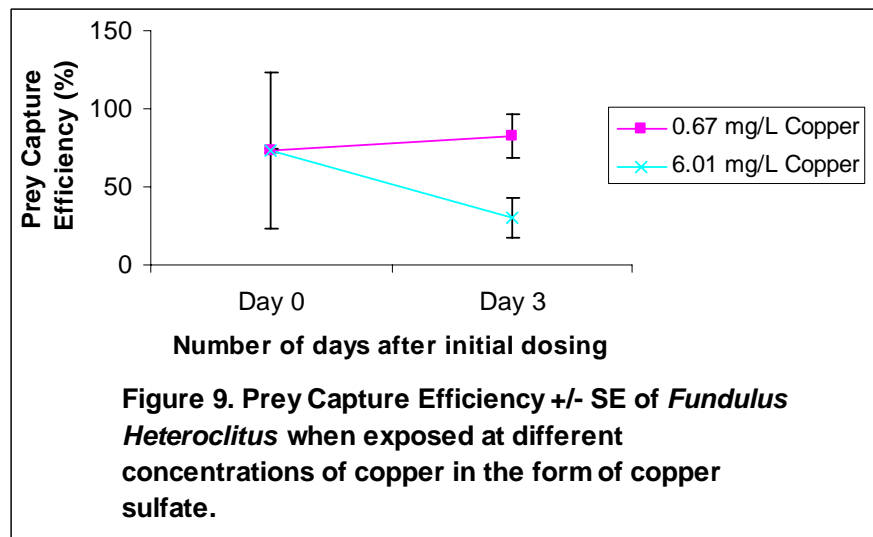
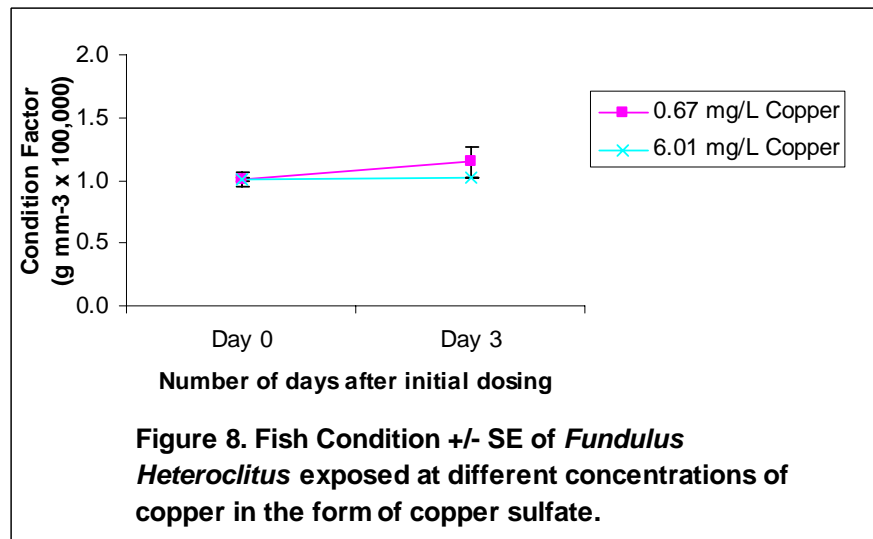
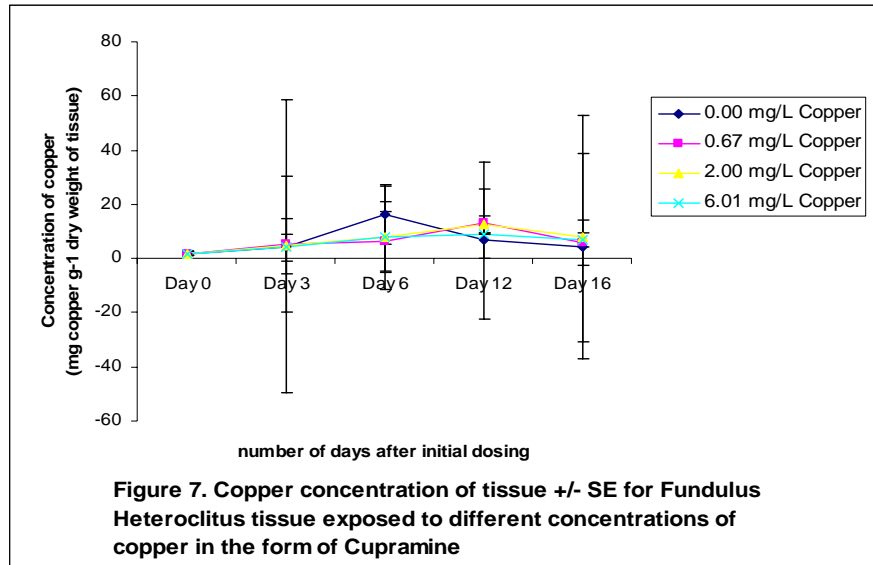
**Figure 4. Packed Cell Volume +/- SE of *Fundulus Heteroclitus* exposed at different concentrations of copper in the form of Cupramine.**

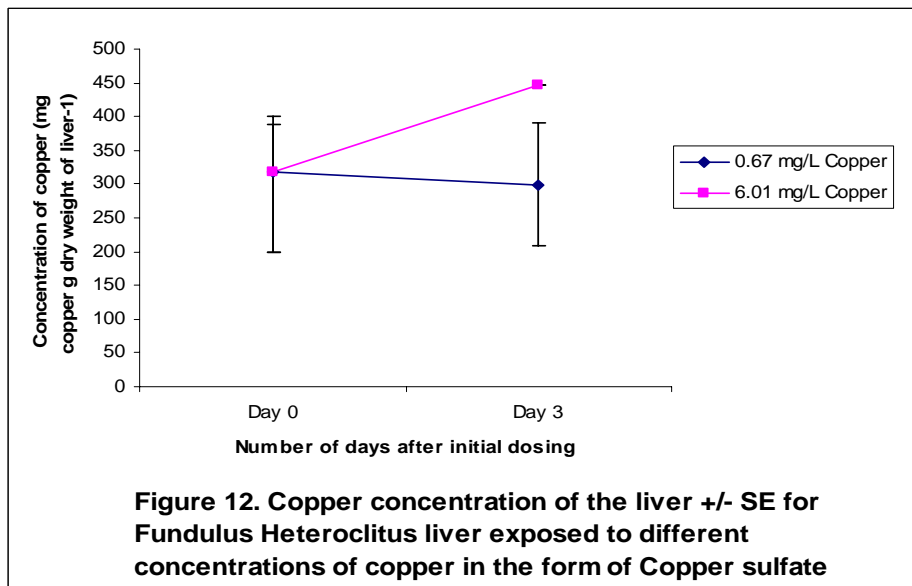
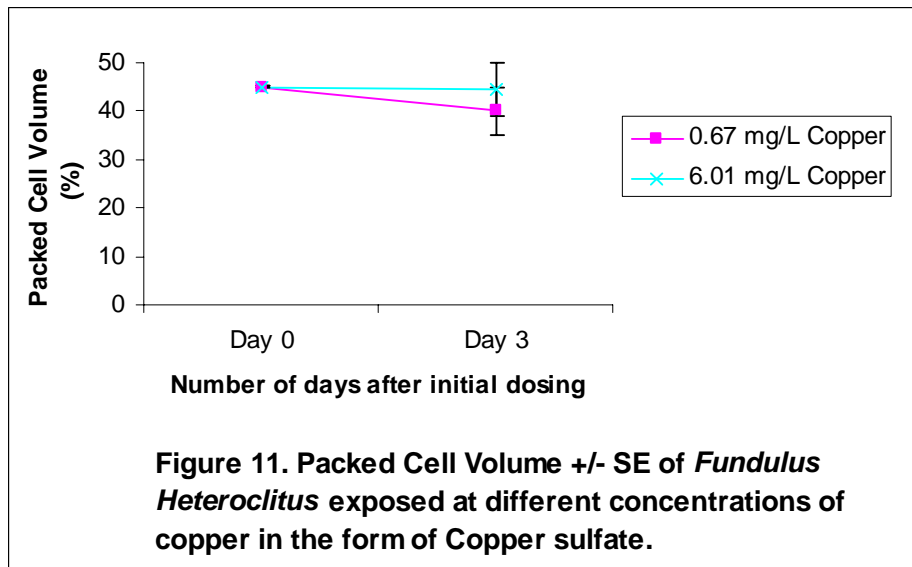
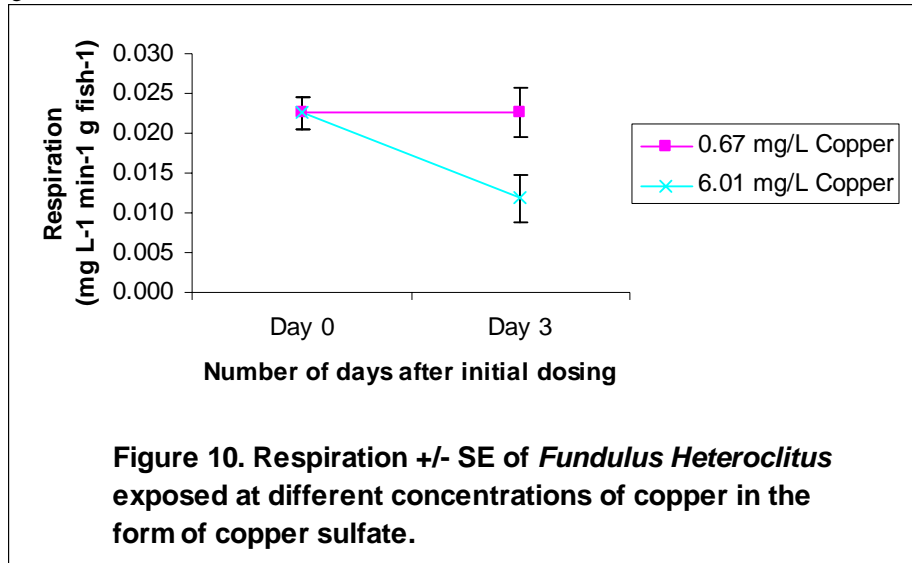


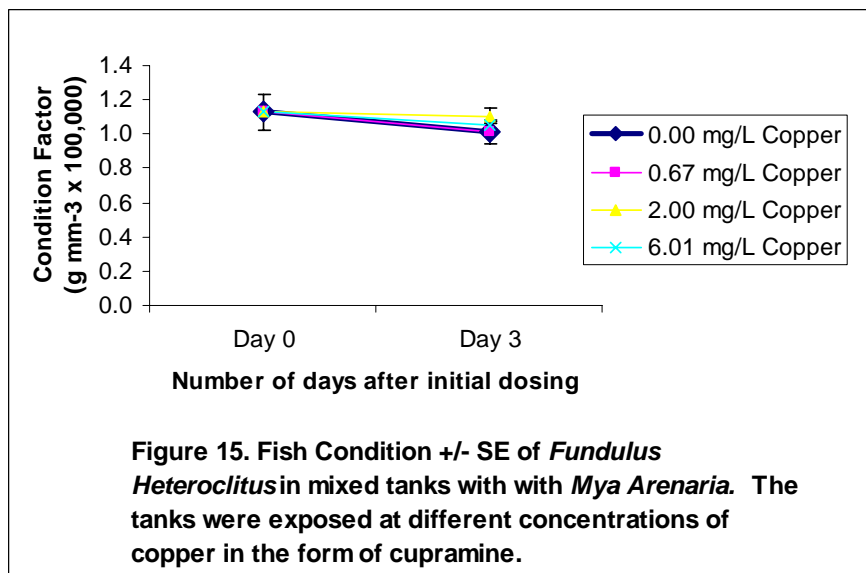
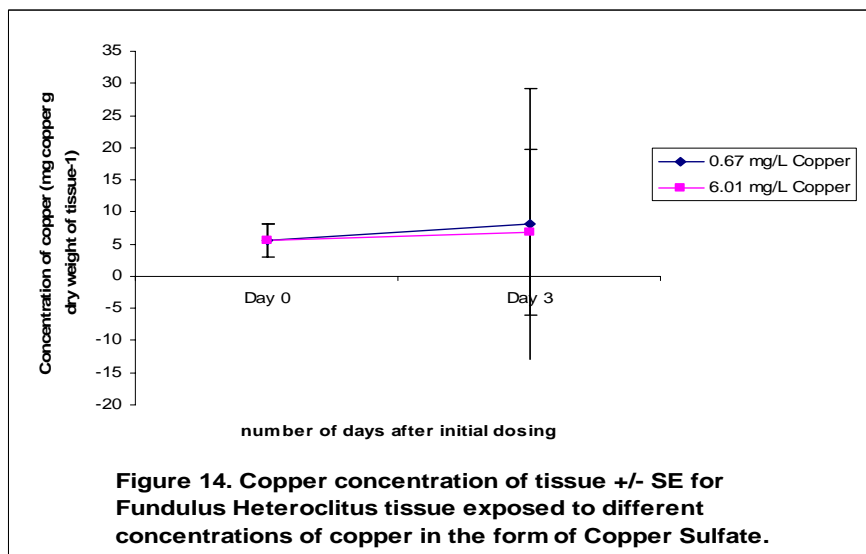
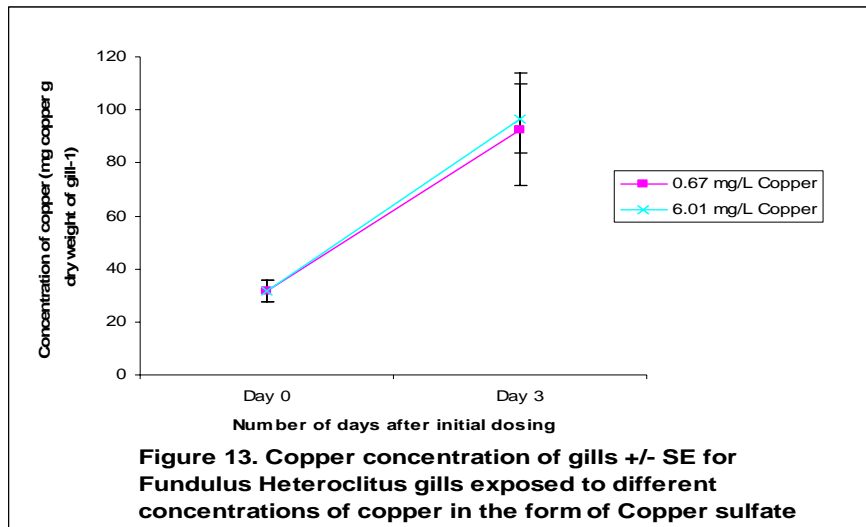
**Figure 5. Copper concentration of the liver +/- SE for *Fundulus Heteroclitus* livers exposed to different concentrations of copper in the form of Cupramine**

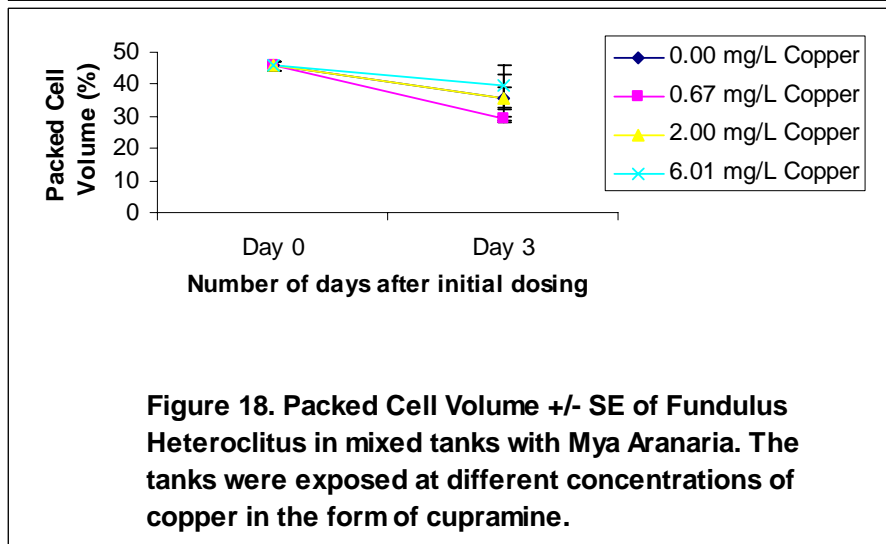
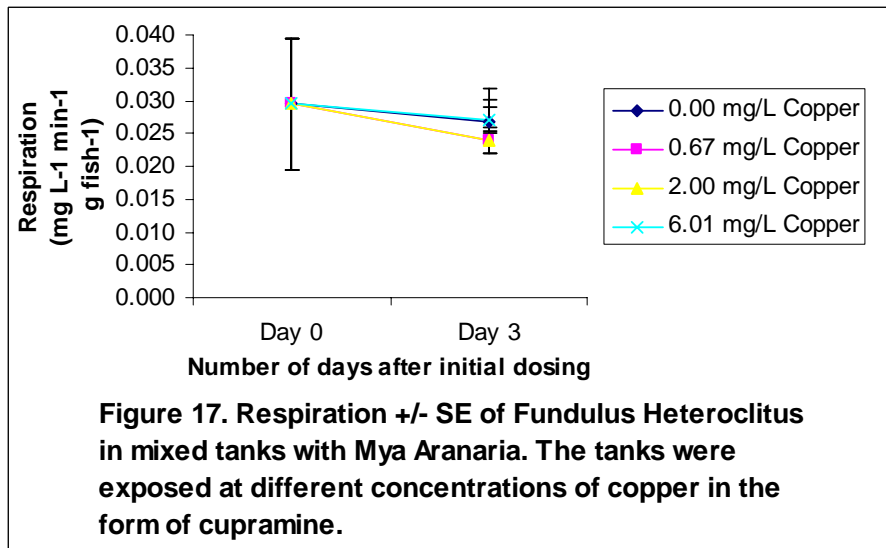
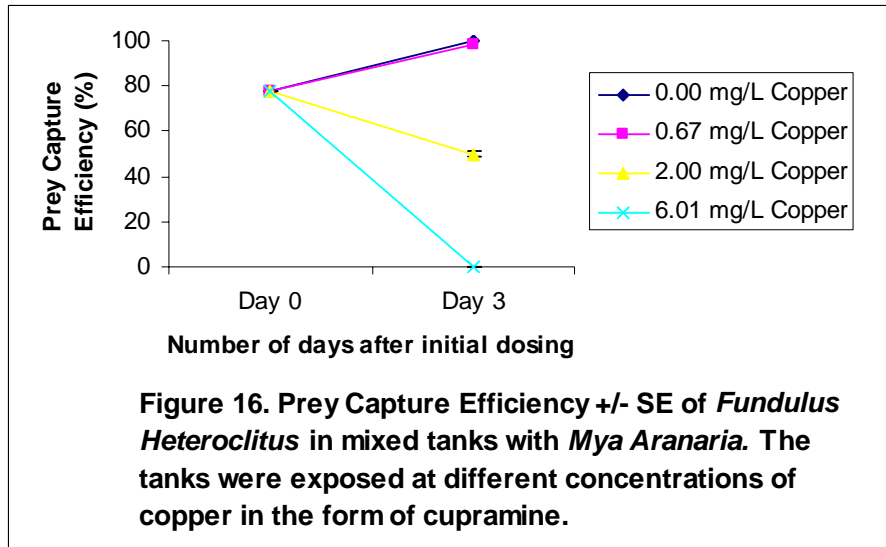


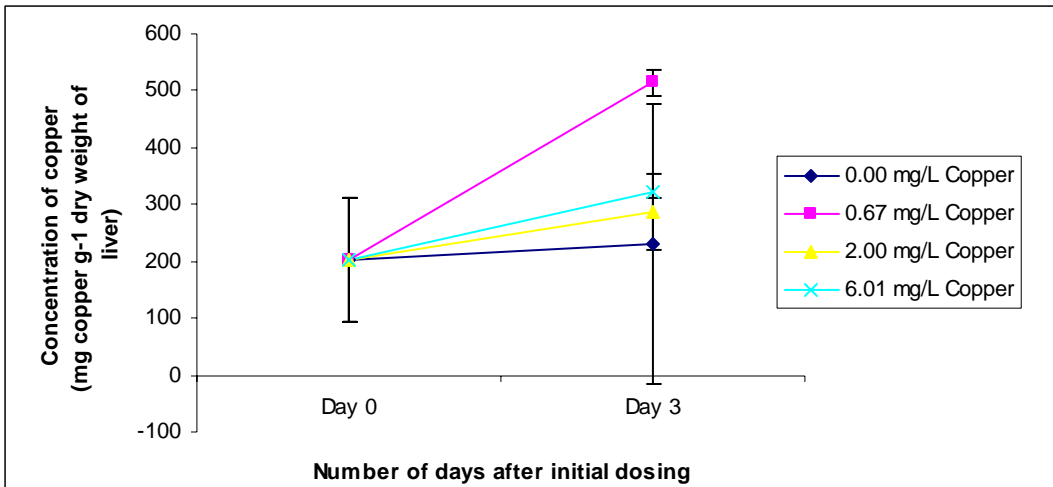
**Figure 6. Copper concentration of *Fundulus Heteroclitus* gills exposed to different concentrations of copper in the form of Cupramine**



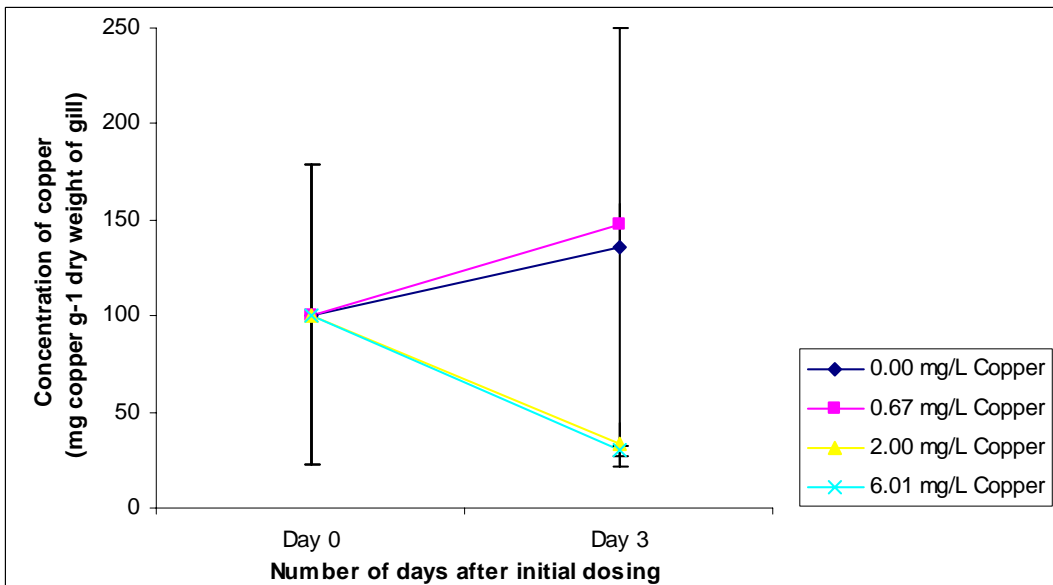




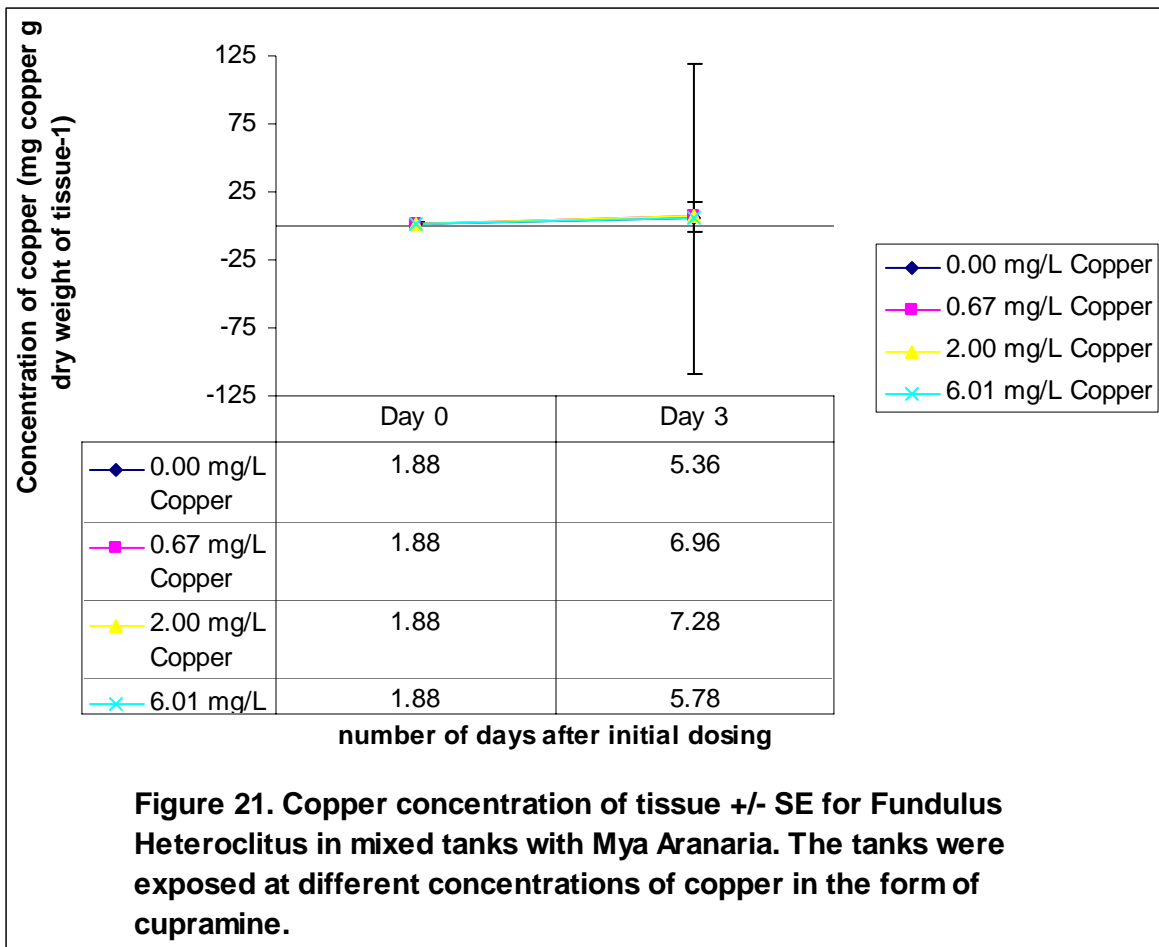




**Figure 19. Copper concentration of liver +/- SE for *Fundulus Heteroclitus* in mixed tanks with *Mya Aranaria*. The tanks were exposed at different concentrations of copper in the form of cupramine.**



**Figure 20. Copper concentration of the gills +/- SE for *Fundulus Heteroclitus* in mixed tanks with *Mya Aranaria*. The tanks were exposed at different concentrations of copper in the form of cupramine.**



Appendix

Table 1. Lengths and Weights of all fish used in the trials. A) fish alone treated with Cupramine™ B) fish alone treated with copper sulfate C) mixed treated with Cupramine™

a) Fish alone treated with Cupramine

Concentration of Copper (mg/L)	Day 0			Day 3		Day 6		Day 12		Day 16	
	Length (mm)	Weight (g)		Length (mm)	Weight (g)	Length (mm)	Weight (g)	Length (mm)	Weight (g)	Length (mm)	Weight (g)
0.0000 mg/L Copper	50	1.32	0.0000 mg/L Copper	50	1.15	40	0.77	58	2.21	52	1.57
0.0000 mg/L Copper	65	2.98	0.0000 mg/L Copper	45	1.03	59	1.65	49	1.15	64	2.67
0.0000 mg/L Copper	55	2.01	0.6674 mg/L Copper	45	0.95	51	1.71	45	0.84	51	1.5
0.0000 mg/L Copper	52.5	1.7	0.6674 mg/L Copper	50	1.44	52	1.67	46	1.09	59	1.7
			2.0021 mg/L Copper	52.5	1.48	45	1.65	47	1.03	59	1.94
			2.0021 mg/L Copper	52.5	1.49	48	1.61	49	1.02	57	1.93
			6.0064 mg/L Copper	56	1.74	61	2.67	58	2.01	63	2.45
			6.0064 mg/L Copper	54	1.6	0.5	1.67	52	1.56	56	1.81

b) Fish alone treated with Copper sulfate

Concentration of Copper (mg/L)	Day 0		Concentration of Copper (mg/L)	Fish	Day 3	
	Length (mm)	Weight (g)			Length (mm)	Weight (g)
0.00 mg/L Copper	1.24	0.248	0.67 mg/L Copper	1	1.32	0.280851
0.00 mg/L Copper	2.41	0.401667	0.67 mg/L Copper	2	1.2	0.244898
0.00 mg/L Copper	1.65	0.3	6.01 mg/L Copper	1	1.52	0.286792
0.00 mg/L Copper	1.7	0.298246	6.01 mg/L Copper	2	1.37	0.268627

c) Fish with clams treated with Cupramine

Concentration of Copper (mg/L)	Day 0		Concentration of Copper (mg/L)	Fish	Day 3	
	Length (mm)	Weight (g)			Length (mm)	Weight (g)
0.00 mg/L Copper	50	1.32	0.00 mg/L Copper	1	63	2.36
0.00 mg/L Copper	65	2.98	0.00 mg/L Copper	2	50	1.35
0.00 mg/L Copper	55	2.01	0.67 mg/L Copper	1	51	1.28
0.00 mg/L Copper	52.5	1.7	0.67 mg/L Copper	2	58	2.06
			2.00 mg/L Copper	1	65	2.91
			2.00 mg/L Copper	2	65	3.16
			6.01 mg/L Copper	1	54	1.62
			6.01 mg/L Copper	2	56	1.89

Table 2. P-values for a 2 way ANOVA statistical test. A) fish alone treated with Cupramine™ B) mixed treated with Cupramine™ C) fish alone treated with copper sulfate

A)			
Fish Alone treated with Cupramine			
	Concentration	Day	Concentration and Day
Fish condition	0.4776	0.0023	0.054
Prey capture efficiency	0.0024	0.8951	0.0543
Respiration	0.0024	0.8951	0.0543
Packed cell volume	0.0199	0.2083	0.7488
Cu in liver	0.0027	0.2706	0.0995
Cu in gills	0.2235	0.2781	0.7323
Cu in tissue	0.6725	0.0859	0.545

B)			
Fish with Mya treated with Cupramine			
	Concentration	Presence of Mya	Concentration and Presence of Mya
Fish condition	0.8057	0.8607	0.476
Prey capture efficiency	0.0588	0.7049	0.6633
Respiration	0.6533	0.0614	0.9037
Packed cell volume	0.9074	0.3373	0.4508
Cu in liver	0.1398	0.2632	0.122
Cu in gills	0.6372	0.8462	0.3499
Cu in tissue	0.6564	0.0688	0.6328

C)			
Fish Alone treated with Copper sulfate			
	Concentration	Copper Source	Concentration and Copper Source
Fish condition	0.4178	0.5522	0.9868
Prey capture efficiency	0.2217	0.3594	0.9291
Respiration	0.5561	0.0016	0.1524
Packed cell volume	0.64	0.8824	0.3969
Cu in liver	0.3898	0.766	0.7333
Cu in gills	0.0423	0.0994	0.0702
Cu in tissue	0.2075	0.02	0.2977