The Toxicology of Saratoga's Drinking Water: Herbicides impact aquatic animals

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Abstract

Copper sulfate, an algicide often applied to drinking water reservoirs, controls algal blooms by inhibiting nitrogen fixation by those organisms. Once added, the reactive form of copper released by the algicidal compound accumulates in the sediments. Copper additions can also influence non-target organisms, including aquatic animals.This study asks whether copper sulfate impacts the metabolism of three benthic organisms: dragonfly nymphs (order *Odonata*), pond snails (gastropod mollusks), and leeches (phylum *Annelida*). We exposed individuals of the three species to one of the following copper sulfate concentrations over a two-week period: 0, 325, 650, and 1300 μg/L. Metabolic functioning was deduced by measuring individual organism's oxygen consumption over time (respiration). Water column and sediment copper concentrations were determined via atomic absorption spectrophotometry. All three groups of test organisms experienced increased mortality and altered metabolic responses in the copper treatments compared to control. We conclude that the metabolic responses could be magnified during sensitive stages of organism development, such as the molt of a dragonfly nymph, and animal groups with exposed permeable surfaces, such as leeches, are particularly vulnerable.

Introduction

The city of Saratoga Springs has been adding the commonly-used algicide, copper sulfate ($CuSO_4$), to the drinking reservoir, Loughberry Lake, for decades (Eliot et al. 2008). By inhibiting their ability to fix nitrogen, copper sulfate decreases blue green algae populations that reduce the quality of drinking water (Elder & Horne, 1978; Van Hullebusch et al. 2003; Eliot et al. 2008; Stoner & White, 2008). Controlling the algal blooms is important in maintaining good tasting, odorless drinking water. Copper's reactive nature, in tandem with its natural abundance and its addition via anthropogenic activities, has lead to increased concentrations in the water column and sediments of lakes. The increase in the water column is short-lived because more copper accumulates in sediments due to its high reactivity with both inorganic and organic species. Copper has accumulated in the sediments of Loughberry Lake, reaching concentrations as high as 3819 µg/g (Eliot et al. 2008). Copper (II), the reactive component of copper sulfate, has a large propensity to enter into complexation, adsorption, and precipitation reactions with inorganic species found in sediments (Effler et al. 1980; Sanchez & Lee, 1978; Sylva, 1976). In uncontaminated freshwater water bodies, the concentration of copper in the water column is generally below 5 µg/L, but in the sediments it ranges from 0.8 to 50 µg/g (dry-weight basis) (Flemmings & Trevors 1989). In a Wisconsin lake experiencing copper sulfate treatment, the water column copper concentrations was only 3 µg/L, while concentrations in the sediments ranged from 320 to 1093 µg/g (Sanchez & Lee 1978).

Copper is a required micronutrient for an assortment of metabolic processes in freshwater species. For example, it is essential for the synthesis of metalloproteins, a group of at least 30 enzymes that act as redox catalysts or dioxygen carriers (Brown et al.1992, Flemmings & Trevors 1989). It is also found in enzymes that are involved in free radical protection, the function of neurotransmitters, and the biosynthesis of connective tissue (Cyrino de Oliveira et al. 2004). However, copper's ability to participate in oxidation and reduction reactions allows it to catalyze the formation of harmful free radicals (Gaetke & Chow 2003). In environments with elevated concentrations, organisms may uptake more copper than they require, which leaves copper free to catalyze these detrimental reactions. The free radical species then carry out oxidative damage on important molecules, such as DNA and proteins. These damaging effects at the cellular level are seen through varying degrees of impaired function (the extreme being death) at the level of the whole organism. Although copper is necessary for organisms to thrive, elevated concentrations have been shown to negatively impact the biotic community of lakes.

Lethal effects on organisms are often observed when copper concentrations are high. Past research has documented lethal effects of elevated concentrations on dragonfly larvae, daphnia, freshwater mussels, pond snails, and fish species including rainbow trout and fathead minnows (Tollett *et al.* 2008, Brown *et al.* 1974, Pagenkopf *et al.* 1974, White & Stoner 2008, Cyrino de Olivera-Filho et al. 2004, Sherba et al. 2000, Gupta et al. 1981, Keller & Zam 1991). In two studies involving crayfish (*Orconectes rusticus* and *Cherax destructor)*, copper sensitivity was greater in juveniles (Hubschman 1967, Khan & Nugeogda 2007). However, copper tolerance was documented in mayfly larvae, which possess exoskeletons that have been shown to accumulate high levels of copper and other toxic metals (Eisler 1998, Tollet *et al.* 2009). These studies illustrate the differences in copper toxicity experienced across taxa and developmental stages of aquatic animals. While mayfly larvae may not experience negative impacts of copper exposure, the accumulation of copper on their exoskeletons could lead to further consequences for the organisms that feed on them.

Three recent studies investigated copper sulfate in Loughberry Lake. Those works demonstrated that crayfish experienced copper bioaccumulation, which was higher in areas were sediment concentrations were higher (Alley 2008). Bioaccumulation was also observed in five species at high trophic levels—crayfish, pumpkinseed sunfish, yellow perch, bluegill sunfish, and largemouth bass—that inhibit Loughberry Lake (Gouin and Helterline 2008). The copper content of these species' muscle and organ tissues was determined. The results followed a trend opposite the expected trend of accumulation at higher levels: bottom feeders such as pumpkinseed sunfish and crayfish had higher levels of copper in their tissues than did pelagic species such as largemouth bass. While the results from these studies were only supported by a small sample size, they provide a foundation for further studies on $CuSO_4$ treatments in Loughberry Lake.

Bioaccumulation does not necessarily indicate that the organisms are experiencing problems from copper exposure – copper can also be stored in inert locations within the organism. Copper challenges organisms when it interferes with the functioning of the organism. Two explanations for copper toxicity have been identified: at low concentrations, it was hypothesized that copper interferes with cell maintenance and repair, while at high concentrations, copper interferes with the respiratory system by inhibiting enzymes (Hubschman 1967). This direct impact on respiration was also seen in zebra mussels; mussels exposed to higher concentrations of copper experienced decreased respiration (Prasada Rao & Khan 2000). Investigating the precise impacts of copper on these biochemical pathways is time consuming and difficult. Instead, we chose to measure the sublethal consequences of copper sulfate treatment through monitoring the respiration of three aquatic animals as an overall indicator of their metabolic functioning.

Metabolism is a good indicator of the state of functioning in an organism because it integrates multiple cellular processes. The metabolism of an animal includes all of the chemical reactions that sustain its many cells, tissues, and organs. An organism's metabolism can be inferred by measuring its respiration. The majority of metabolic reactions require energy, which in cases involving aerobic respiration is supplied via other reactions in which oxygen is directly involved. Therefore, there is a direct functional relationship (and hence correlation) between oxygen consumption during aerobic respiration and energy metabolism. By measuring respiration, the magnitude of copper impairment can be gauged enough for comparison between copper treatments without the need for determining the specific cellular functions copper interferes with and how.

The aim of our study is to determine how copper sulfate treatments alter the metabolic processes of three different taxa of aquatic animals. Benthic organisms will be the focus of this study because they interact with the sediments where copper accumulates in high concentrations. Loughberry Lake is well above the higher of two effect levels for metal concentrations in sediment (as designated by the New York State Department of Health). The lowest effect level (LEL) is 16 µg/g and the severe effect level is 110 µg/g. At concentrations above the SEL, the biota can be severely impacted (NYSDEC 1999).

The three study organisms are dragonfly nymphs (order *Odonata* and predators of smaller invertebrates), leeches (phylum *Annelida* and also predators of small invertebrates), and freshwater pond snails (gastropod, mollusk, and scrapers of plant material and detritus). These organisms represent three distinct families of aquatic organisms and therefore could provide a scope of possible implications for ecosystem function. Each set of organisms will be exposed to one of four treatments: three copper sulfate treatments (below the EPA limit of 1300 µg/L copper sulfate) and a control treatment.. Over a fourteen day period, each individual organism's metabolism will be measured in terms of their respiration (determined through oxygen consumption). Mortality will also be recorded during this time.

Methods

Organisms

The three species of test organisms—pond snails, dragonfly nymphs, and leeches—were obtained from Carolina Biological. The pond snails are protected by a hard shell but also have a permeable foot that protrudes from that shell. They ranged in mass from 0.0401 to 0.7708 g. The within-tank location of the snails once introduced to their tanks varied: some tended to reside on walls of their tank, while others remained on the sand at the bottom of their tank. Overall, the snails were the least active of the three. The dragonfly nymphs were more active than the snails, often seen swimming across the bottom of the tank, or sometimes up a wall before returning down closer to the sand. Like other insects, these immature dragonflies have an exoskeleton that they shed several times before emerging from their juvenile nymph stage. Our dragonfly masses ranged from 0.007 and 0.724 g and the leeches ranged from 1.17 to 2.94 g in mass. Leeches have entirely permeable bodies, with no shell or exoskeleton for potential protections. When they were not quickly maneuvering across the sand at the bottom of the tank, they would stay relatively still, wrapped and tangled up around one another.

Creation and monitoring of experimental setting

The experimental tanks included an initial application of 0 (hereafter described as control), 325, 650, or 1300 µg/L copper sulfate. These concentrations were chosen based on the EPA limitation of 1300 µg/L of copper in freshwater lakes. For each treatment, we prepared two tanks with 2,500g of sand, 15L of water at the appropriate copper concentration, and an aerating bubbler. Three to five individuals of each test species were placed in each tank. The first 14-day trial began on 2-11-12 and ended on 2-26-12; it included both the pond snails and the first group of dragonfly nymphs. The second trial, which focused on leeches, began on 3-7-12, metabolic readings were only taken on days zero and twelve, but deaths were monitored through day 18, on 3-25-12. The leech trial consisted of two treatments: a control and a copper tank. The initial concentration in the copper tank was 325 µg/L copper sulfate. On day 1, a supplementary dose of copper was added, bringing the total concentration to 975 µg/L. After the leech trial, we returned to the initial experimental set-up to complete a second dragonfly nymph trial, which began on 3-26-12 and ended on 4-10-12.

During the first and last trials, we sampled both the water column and the sediment of all 8 tanks on days 1, 7, and 15 to monitor the movement of copper between the two. Copper was extracted from the sediment using a 1:10 sediment to 0.1 M HCl solution over 16 hours, during which the extractions were agitated on a rocker. After the 16 hours, the extractions were centrifuged at 4,000 rpm for 20 minutes and then filtered through 0.45 µm cellulose membrane filters. The water column samples were acidified in a 1:10 ratio of water to 0.1 M HCl and then filtered through 0.45 µm cellulose membrane filters. The resulting filtered samples were analyzed with a copper flame method on an Atomic Absorption spectrophotometer.

Measurements of metabolism

All organisms were given at least one day to recover from their shipping trip before initial masses and respiration readings were recorded, and they were introduced to their treatment tanks. Respiration was measured individually by placing an organism in a 30 or 60 mL glass chamber with a stopper containing either dionized water (for initial measurements) or water from their respective tank (for all subsequent measurements). For the dragonflies and leeches, the dissolved oxygen (DO) of the water was measured with a DO meter every 30 minutes over an hour; the DO in snail chambers was measured every hour for two hours. For each reading, the chamber was agitated for 20 seconds and then the DO value was recorded 20 seconds after agitation ceased. We standardized the respiration in the larger chambers to match those in the smaller chambers by doubling their total respiration.

For the snail and both dragonfly trials, initial metabolic readings were taken immediately prior to treatment exposure and then again 24=hours post exposure (day 1). Additional readings for the snails were taken on days 2, 4, 6, 10, and 15 and for the dragonfly nymphs on days 2, 3, 5, 7, 11, and 15. Metabolic readings were taken on days 0, 1, 3, 5, 7, 9, 11, 13, and 15 for the second dragonfly trial. For the leech trial, which only consisted of a one control and one copper tank, readings were taken at 0 hours, 6 hours, and then at 12 days post-treatment.

An average metabolic response value was calculated for each treatment group for each day of reading. Using the pre-copper exposure (initial) readings of all individuals plus the daily readings for the control groups, a power equation describing the expected total respiration based on the individual's mass was determined. Total respiration is the amount of oxygen consumed over an hour (ppm/hr). For each day, a total respiration calculated using this equation to obtain a predicted value for how each individual organism would respond in the absence of copper. The actual total respiration was divided by the organism's predicted value. This was the individual's metabolic response value. The calculate an average metabolic response value for the entire treatment group for each day, the natural log of the individual metabolic response was averaged. The exponential function was then used for each of these average values. The resulting number was the average metabolic response of a specific treatment group. This number is used to describe how the treatment group differed from an identical group that was not exposed to copper. If the value was above 1, then the treatment group performed above the predicted value and if it was below 1, it performed below the predicted value.

Death records

Death tolls were recorded every day for the snail and both dragonfly trials and on days 1, 3 and 12 for the leech trial. Proportions of living organisms remaining in each treatment tank were calculated for each test organism over the course of our metabolic measurement trials. All copper tanks were pooled together to review the overall effect on mortality that the presence of copper has on these organisms.

Results

Copper concentrations

The copper concentrations in the water column decreased over a 15-day span (Figure 1a; table 1). During that same time span, the copper concentrations in the sediment increased (Figure 1b; table 2). In all three copper treatment concentrations, the sediment copper concentration dropped slightly between days 7 and 15.

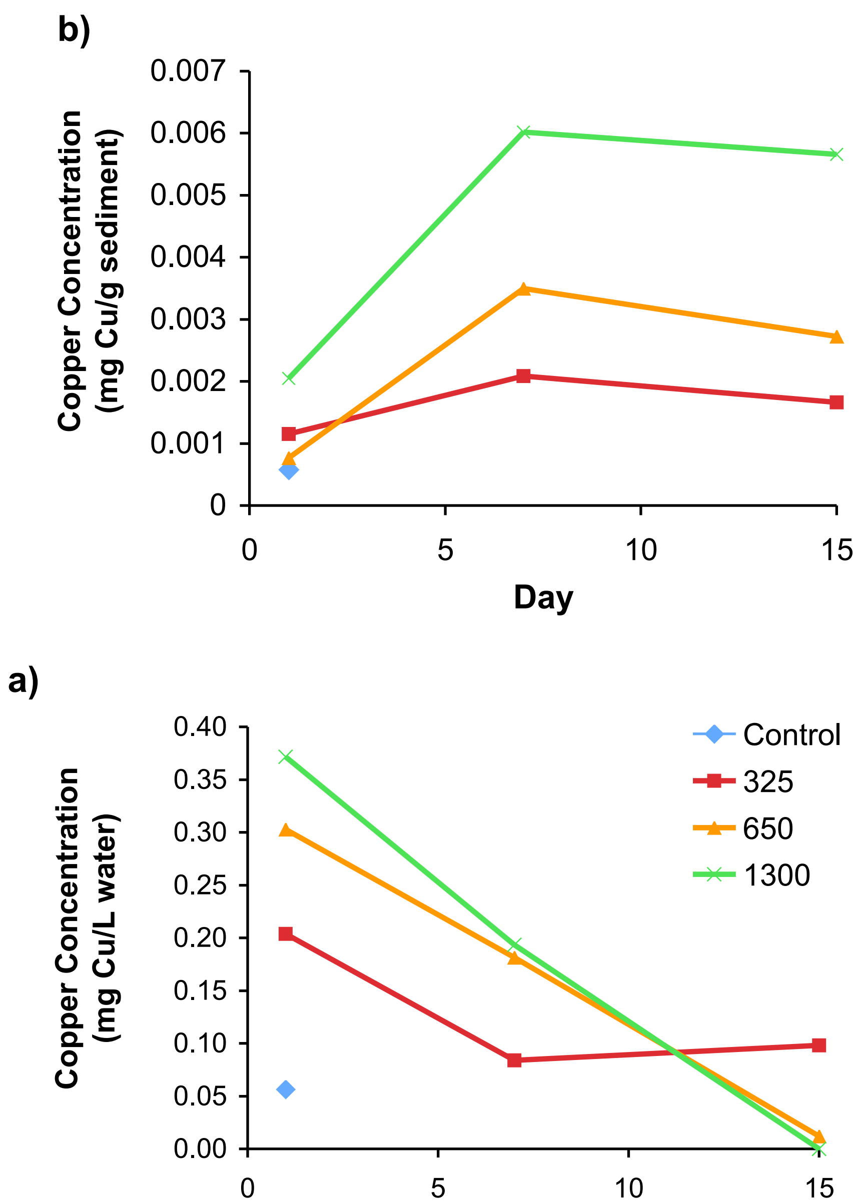


Figure 1. Copper movement between water column and sediment between days 1, 7 and 15 of dragonfly nymph and snail trial. (1a) shows the copper concentration in mg of Cu per L of water in the water column. (1b) shows copper concentration in mg of Cu per g of tank sediment.

Table 1. The water column copper concentration for the four treatments over the course of the dragonfly and snail experiment. The concentration is represented in micrograms copper per liter of water.

	Control		325 µg/L		650 µg/L		1300 µg/L	
Day	Avg (µg/L)	Std. Dev (µg/L)	Avg (µg/L)	Std. Dev (µg/L)	Avg (µg/L)	Std. Dev (µg/L)	Avg (µg/L)	Std. Dev (µg/L)
1	56.5	92.5	204	188	303	76.0	371	114
7			83.9	236	181	47.1	193	107
15			98.0	149	12.0	225	0	359

Table 2. The sediment copper concentration for the four treatments over the course of the dragonfly and snail experiment. The concentration is represented in micrograms copper per gram of dry sediment.

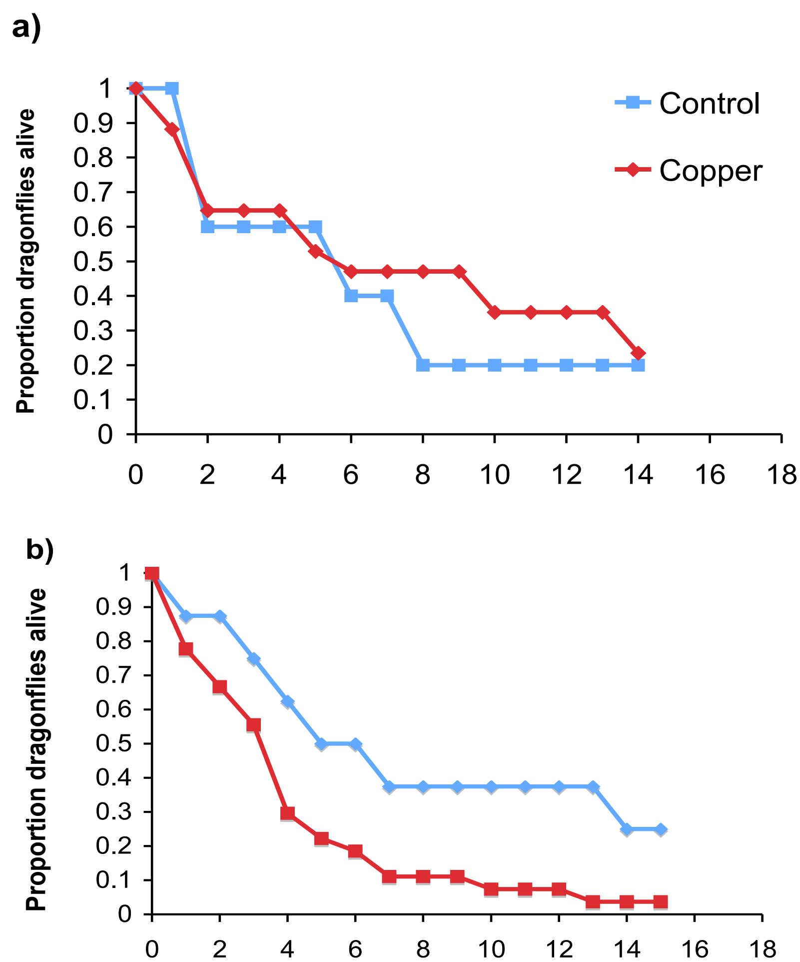
	Control		325 µg/L		650 µg/L		1300 µg/L	
Day	Avg (µg/g)	Std. Dev (µg/g)	Avg (µg/g)	Std. Dev (µg/g)	Avg (µg/g)	Std. Dev (µg/g)	Avg (µg/g)	Std. Dev (µg/g)
1	0.577	0.0970	1.12	0.296	0.768	0.262	2.05	0.434
7			2.09	0.372	3.50	1.09	6.02	0.396
15			1.66	0.380	2.73	0.569	5.66	1.84

Death rates

The death rates for the first group of dragonflies (dragonflies I) were similar in the control and copper tanks (Figure 2a). An average daily death rate representing the average number of individuals who did not survive through any given day revealed only a slight increased death rate between control and copper treated dragonflies I, with daily rates of 0.08 and 0.09, respectively (Figure 3a). The death rates of the second group of copper-exposed dragonflies (dragonflies II), however, were different from those of dragonflies I (Figure 2b and 3a). While the average daily death rate for control dragonflies II was the same as control dragonflies I (0.08), the copper daily death rate for dragonflies II was almost double that of copper dragonflies I with an average of 0.17 dragonflies that died per day. Additionally, the daily death rates between copper and control in the second dragonfly trial were significantly different (Figure 3a).

The snails exposed to copper began to die within 24 hours of exposure, whereas the entire cohort of control snails remained alive until day 9 (Figure 2c). The average daily death rates for control and copper exposed snails were 0.05 and 0.12, respectively (Figure 3b).

The control and copper death rates for leeches were similar for the first three days of the trial. Overall, however, the leeches exhibited the greatest and most significant difference between control and copper daily death rates, which were 0.006, and 0.140, respectively (Figure 3c).



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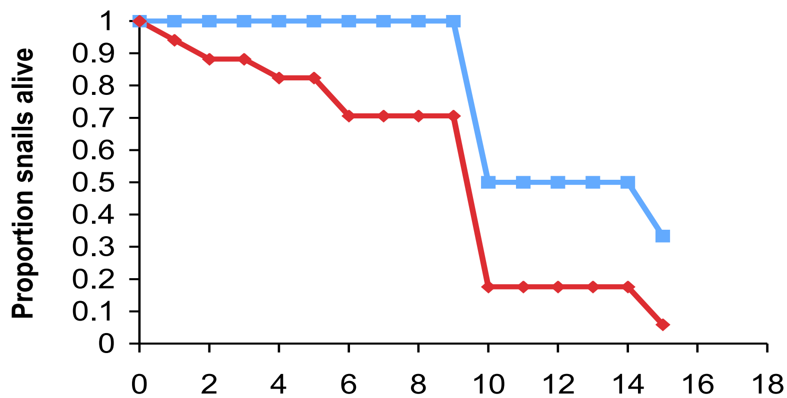

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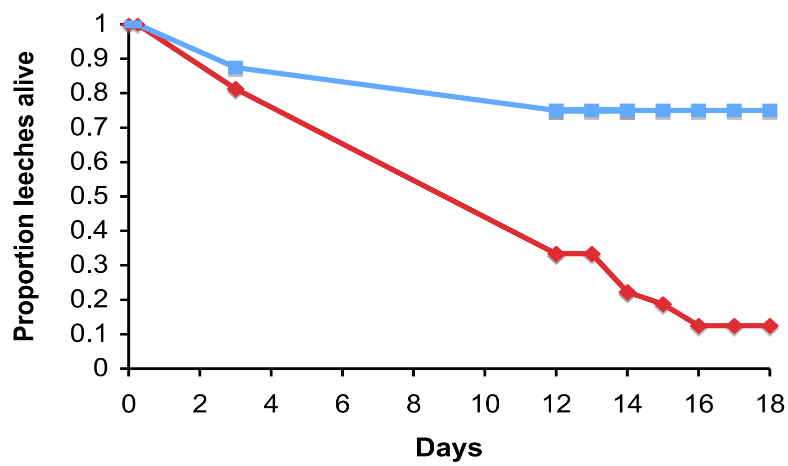


Figure 2. Proportions of living organisms in control and aggregate copper tanks for (a) dragonflies I, (b) dragonflies II, (c) snails and (d) leeches from day zero to 14, 15, and 18, respectively.

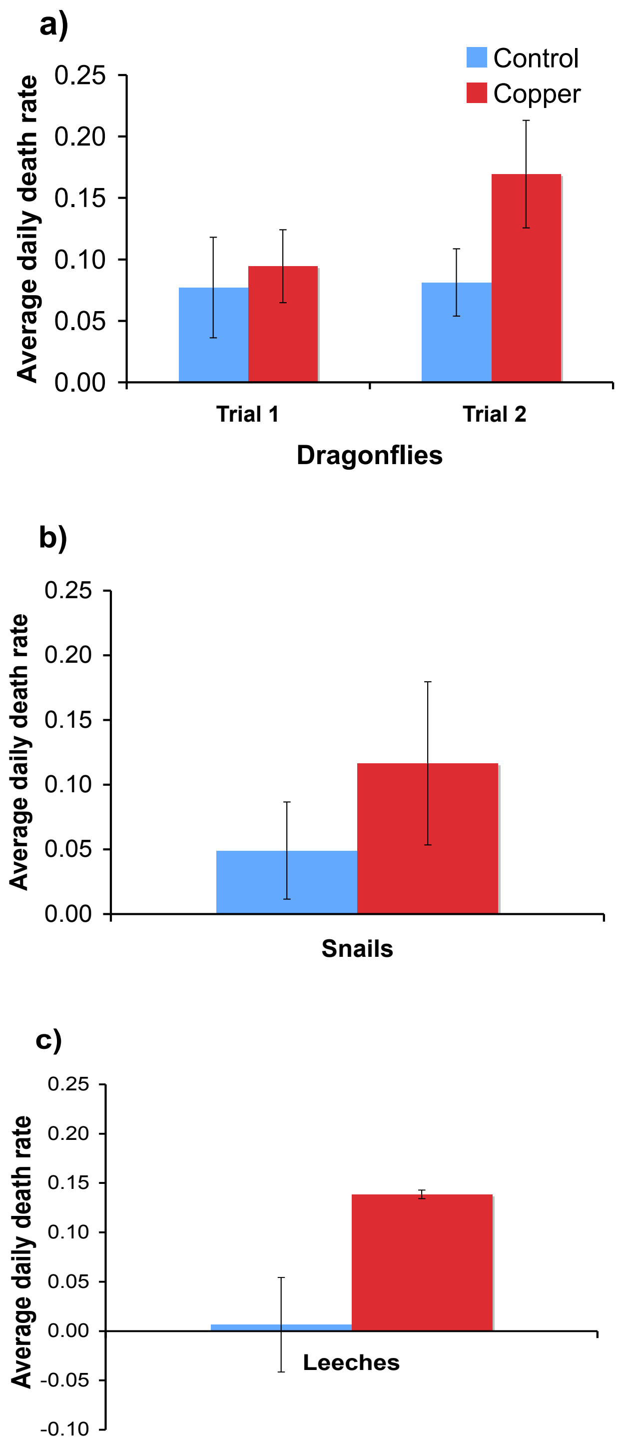


Figure 3. Average daily death rates and standard errors for a) both dragonfly trials, b) snails, and c)

Snail metabolism

Baseline respiration readings were taken on day 0, however, oxygen consumption measurements were only reliable beginning on day 1, 24 hours after treatment exposure. The first day of data showed a mix of average metabolic responses within control and each copper concentration: 1300 µg/L had the lowest response (below y=1, also indicating that it was lower than expected based on our expected response in the absence of copper), control and 325µg/L tanks exhibited very similar responses, and the 650µg/L individuals performed the highest responses (Figure 4a).

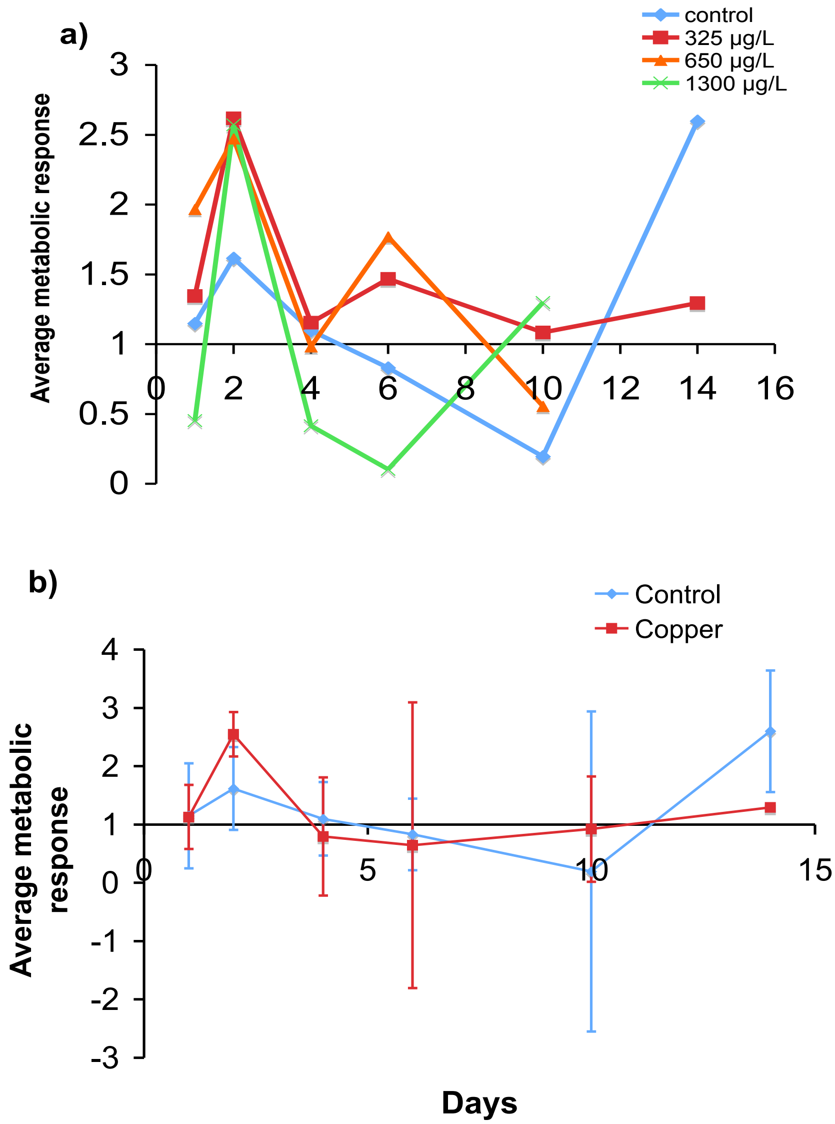


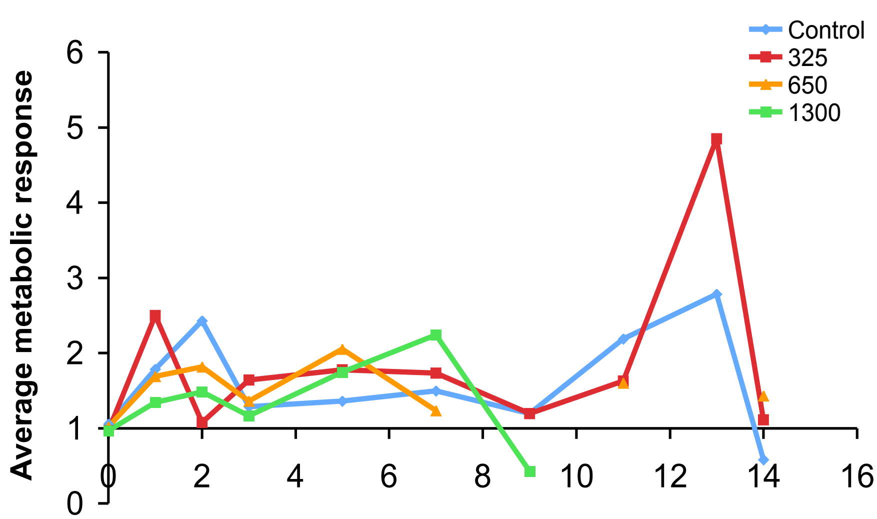
Figure 4. Average metabolic responses across days of the experiment for snails in (a) control, 325, 650, and 1300 µg/L treatment tanks and (b) control and pooled copper tanks, with standard error bars.

By day 2, responses in each treatment increased greatly. All copper tanks rose to almost identical average metabolic responses while individuals from the control tanks exhibited an average response that was 33% less than any of the copper tanks. The 1300 µg/L tanks experienced the greatest increase in respiration from day 1 to day 2. For the remainder of the snail trial, responses within the four treatments continued to increase and decrease in a varied fashion. To simplify the data, Figure 4b depicts the average metabolic responses in all of the copper tanks pooled together versus the control. The standard deviations of these averages indicate that there is no significant difference between average metabolic responses in either the presence or absence of copper.

Dragonfly metabolism

On day 0, before any individuals were exposed to copper treatments, all dragonfly nymphs performed as expected in the absence of copper (Figure 5a). After day 2, copper tanks generally performed higher than controls. By day 7, 1300 µg/L individuals performed their highest metabolic response over the course of experiments, just two days before they were all dead.

a)



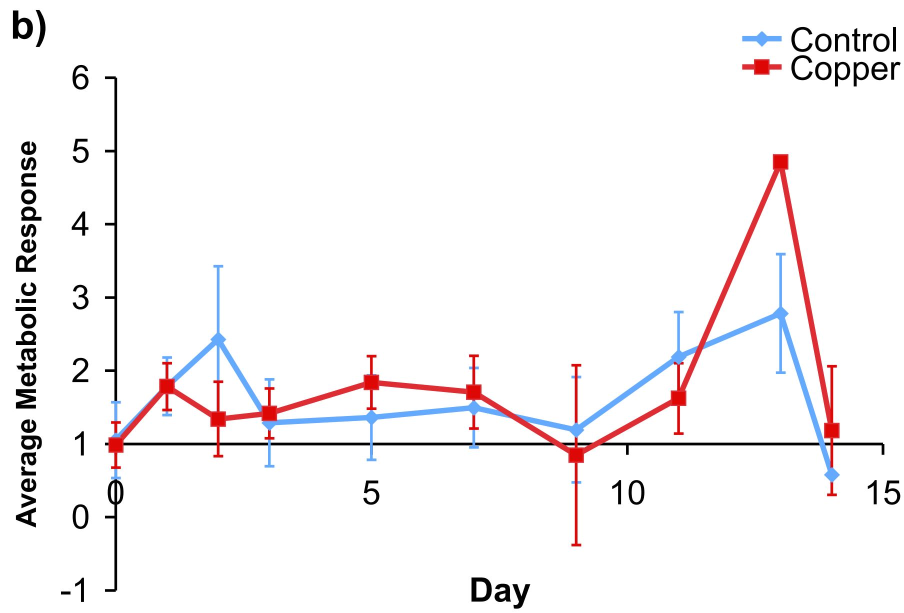


Figure 5. Average metabolic responses across days of the experiment for dragonflies I and II in (a) control, 325, 650, and 1300 µg/L treatment tanks and (b) control and pooled copper tanks, with standard error bars.

When looking at all copper treatments compared to control (Figure 5b), both increased at the same rate from their initial readings to their 24-hour readings. Control continued to increase on to day 2, while copper began to drop. For the remainder of dragonfly trials, both copper and control treatments performed varying responses, all altered from those expected (no significant difference existed between copper and control treatments).

Leech metabolism

All leeches exhibited similar metabolic responses before copper exposure (day 0; Fig 6). After six hours of exposure to copper (day 0.25), copper individuals had an average metabolic response greater to that of the control leeches.

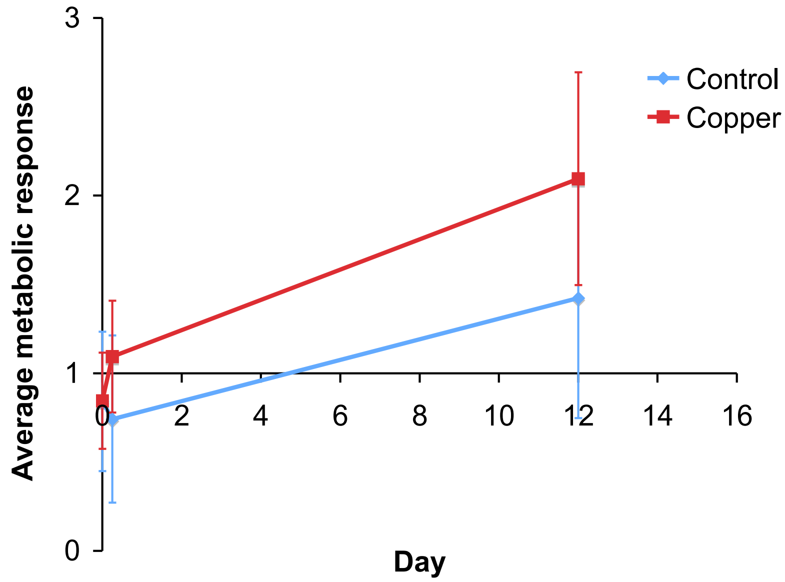


Figure 6. Average metabolic response of leeches at 0, 0.25 and 12 days with standard errors.

On day 12, both the control and copper tanks exhibited responses higher to those they showed on days 0 and 0.25, and greater than predicted responses based on day 0 data (fig 5). Based on the standard deviations of these average responses, no significant difference existed between the control and copper-exposed leeches.

Discussion

All three organisms experienced increased rates of mortality in the copper treatments. While the first cohort of dragonfly nymphs only experienced a slightly higher daily death rate in the copper treatments, the second cohort that was undergoing molting exhibited a significant difference in daily death rate compared to the control treatment. The metabolic response of the organisms to copper exposure was less consistent: the snails experienced an increased metabolic response within the first two days of copper exposure, while the copper-exposed leeches exhibited an increase six hours after exposure. Over the duration of the experiment, the copper concentration in the water column decreased as the copper accumulated in the sediments, which indicated the movement of the copper ions from the water column to the sediments.

Snails

Baseline metabolic data (from day 0) could not be included in any of our analyses or data depictions due to evolving methodologies between days 0 and 1. On day 0, over 50% of our snails' metabolic readings were negative (exhibiting an *increase* in dissolved oxygen concentrations within their concentrations) likely because their changes in DO were measured within 60-ml chambers that were too large for the small and relatively sedentary organisms. Additionally, chambers were disrupted for readings at 10-minute intervals and their oxygen consumption was only measured over 1 hour. After extending snail metabolic readings to two hours (with only one measurement in between at 1 hour), oxygen consumption measurements were more consistently positive (exhibiting a decrease in DO) and reliable.

The copper-exposed snails in all three concentrations of copper sulfate exhibited a higher increased metabolic response after 48 hours than those snails in the control treatment. The difference between the control and copper treatments were not statistically significant; however this may be due to the grouping of the three distinct copper sulfate treatments into one copper group. If sample sizes were larger, statistical analysis of each individual copper treatment may have yielded significant results. Beyond the initial 48 hours, however, the metabolic responses in all treatments decreased greatly. The considerable increase early on in the experiment could be attributed to a possible temporary inhibition of copper regulating mechanisms within the snails (Pyatt *et al.* 2003), when the Cu concentrations are still high in the water column. Beyond the first few days, Cu concentrations diminish in the water column as it accumulates in the sediments (see Figure 1a-b, and tables 1 and 2), and the need to deal with Cu regulation decreases as well. Another explanation for the lack of a steady trend in metabolic response in any of the given treatments or clear-cut result of increasing copper exposure could be due to the varying and unknown life stages of the snails studied. Other studies have investigated Cu bioaccumulation in different tissues of freshwater snails, as well as the effect of Cu on embryo development and survival (Pyatt *et al.* 2003, Khangarot & Das 2010, Das & Khangarot 2011, Ng *et al.* 2011), but not metabolic effects. These reports all support the inference that Cu is more likely to affect the performance of organisms at certain, perhaps sensitive, periods of their lives. During most life stages, the snails are able to deal with a certain level of excess copper via ion regulatory processes (Pyatt *et al.* 2003).

Even though the surviving snails appear to "recover" metabolically from copper treatments after a few days (and also likely due to decreased Cu water column concentrations), they still had higher daily death rates, although not significantly so, when exposed to Cu as opposed to no Cu. The increased death rates for copper treated snails might be due to an overall biological stress that Cu induces via the creation of excess reactive oxygen species (ROS) in the organism (Pyatt *et al.* 2003). Perhaps this overall stress does not get expressed through the organism's metabolic processes, or at least not through the basic metabolic process of oxygen consumption. While respiratory processes recover, other biological processes could be continuously under attack, leading to a decreased lifespan for the snail. Further, the changes in metabolic response over the course of the experiment may not be solely due to copper exposure - the composition of the measured group changes as individuals die. As each treatment population becomes smaller, fewer individuals determine the group response. Since we could not track *individual* responses over the course of the experiment, it is difficult to control for metabolic idiosyncrasies.

Dragonflies

The first cohort of dragonflies exhibited only a slight difference in mortality between copper and control treatments. However, in the second cohort of dragonflies, where we observed several of them molting their exoskeletons, the copper exposed dragonflies experienced nearly double the daily death rates of those in the first trial. Past research on the sensitivity of Odonate larvae is limited; to date only one study has investigated the mortality of larvae in response to copper exposure. A high rate of mortality was observed in another experiment's copper treatments when the sensitivity of three species of Odonate larvae—*Pachydiplax longipennis, Erythemis simplicicollis*, and *Ladona deplanata*—to varying concentrations of cadmium, copper, and lead were explored (Tollet *et al.* 2009). These concentrations were well above the concentrations found in the environment. The authors attributed this high mortality to the accumulation of copper by metal-binding proteins. The lowest copper concentrations in the Tollet *et al.*

study was 2860 μg copper/L which is much higher than the larger concentration of 518 μg Cu/L used in our study. At the sublethal concentrations utilized in our study, the dragonfly larvae demonstrate copper tolerance.

The dragonfly larvae in the copper treatment exhibited a spike in their average metabolic response on day one, which correlates with the highest water column copper concentration. Uptake of copper can occur in the gut or rectal gills of dragonfly larvae, as well as through permeable surfaces (Eisler 1998, Smita *et al.* 2010). Uptake through the gills of the larvae is dependent on the water column concentration, so as the copper transitioned from the water column to the sediment, the uptake via gills decreased. The increased metabolic response could be explained by metabolic stress caused through the production of ROS or due to damage to the gill epithelial. Disruption to both the smooth lining and epithelial layers of the rectal gills of dragonfly larvae exposed to lethal concentrations of copper sulfate has also been documented via scanning electron microscopy (Smita *et al*. 2010). Since sublethal concentrations were used in our study it is difficult to say whether this damage occurred in our larvae; however, it could explain the highest metabolic response corresponding with the highest water column concentration.

Leeches

Copper exposed leeches exhibited higher metabolic responses than control leeches and the difference in death rates between control and copper leeches were more dramatic than either snails or dragonflies and were significantly different. One explanation for that more pronounced response is the benthic-feeding nature of the leeches (Pyle & Mirza 2007). Until three days post exposure, all copper and control leeches remained alive, and this was likely while copper concentrations were still high in the water column as opposed to the sediments (again, refer to Figure 1a-b and tables 1 and 2). After a few days, the copper began moving quickly into the sediments, which is where the leeches spend their time, usually foraging and feeding (even though we did not provision them with any food). Therefore, their increasing death rates recorded by day 12 may be due to increased sediment Cu concentrations. The dramatic difference in leech death rate when compared to the dragonfly larvae and snails could be attributed to the leech's lack of an exoskeleton or hard shell that limits copper exposure and absorption.

The Bigger Picture

The sediments of Loughberry Lake range from 2199 to 3819 µg/g copper, which is well above the New York State Department of Health's severe effect level (SEL) for metal sediment concentration of 110 µg/g (NYSDEC 1999, Eliot *et al.* 2008). At metal concentrations above the SEL, the aquatic biota are at risk. In our study, the highest sediment concentration measured was 6.02 µg/g in the 1300 µg/L copper sulfate treatment on day seven. The copper accumulation in Loughberry Lake sediments has occurred over decades and the volume of copper sulfate applied was much greater than in our experiment, which would explain why our experimental sediment had much lower concentrations.

On average, the city of Saratoga Springs applies 206 kilograms of copper sulfate to Loughberry Lake four times during the spring and early summer (Alley 2008). Based on this average and the entire volume of the lake, the water column concentration immediately after application would be 215 µg/L. While this value is less than our lowest concentration, these applications are concentrated in the north end of the lake where algal blooms primarily occur, and thus the actual water column concentration is probably much higher in this region (Eliot *et al.* 2008). It is more likely that the water column concentration in this region of the lake is closer to the 650 µg/L treatment and therefore the organisms that inhabit this area may experience a brief metabolic response after each application. Considering the elevated sediment copper concentration in Loughberry Lake, it is reasonable to assume that the trend of increased mortality in our copper treatments translates to the organisms inhabiting Loughberry, specifically the northern region (Figure 2). Currently no species census of the lake exists, so populations of dragonfly nymphs, snails, and leeches may be minimal or nonexistent.

Loughberry Lake's purpose is to serve as a drinking water reservoir, so one can argue that it does not matter if the functioning of the biotic community is being severely impacted by the application of copper sulfate. However, Loughberry is not a closed system—the copper sulfate applied could move downstream into other aquatic ecosystems. More importantly, if the sediments were disturbed due to depressed water levels due to overuse and/or drought, large amounts of copper could be released into the water column (Sutherland *et al.* 1988). For these reasons, it is important to consider the negative impacts that copper sulfate application in Loughberry Lake. Additionally, copper sulfate application is not limited to drinking water reservoirs; it has been applied to recreational lakes to control nuisance algal blooms that make the water look unappealing (Haughey *et al.* 2000). The functioning of the biotic communities in these lakes is arguably very important, especially ones that are located outside of cities and are more closely tied to the surrounding terrestrial ecosystem.

Conclusions

There was a consistent lethal effect of copper sulfate across the three aquatic invertebrates and evidence suggesting that sublethal metabolic effects may also be occurring. Our data indicated at the very least a temporary metabolic responses to copper sulfate application. The brief impact of the copper sulfate in terms of metabolic response is most likely because the water column copper sulfate concentration was not maintained over the course of the experiment. Rarely does an aquatic environment experience a constant concentration of a contaminant; instead they occur in pulses following rain events or industrial spills (Johnston & Keough 2000). Our experiment was designed to mimic the pulse of copper sulfate that a lake experiences after the application of the algicide. If this pulse were to occur during the sensitive period of an organism's development, it could have more serious impacts on the organism and result in either death or abnormal development. In the case of organisms in which larvae hatch and progress through development in unison, an ill-timed application could result in the loss of an entire cohort. The exposure of snail eggs to copper concentrations above 56 μg/L can either entirely inhibit or disrupt embryo development (Das & Khangarot 2011). Dragonfly larvae undergoing molts may be more susceptible to increased water column copper concentrations because they loose the ability to accumulate copper on their exoskeleton and may experience higher rates of copper uptake due to the increased exposure of permeable surfaces.

Future studies should focus on the effects of repeated pulses of copper sulfate, which may occur if algicide application to lakes occurs multiple times during the spring. The length of the intervals between these applications may impact whether the individuals can recover from the prior application. This information could be used in recommending a minimum period of time between applications to minimize the impact on the aquatic invertebrates. Additionally, more research should be done on the metabolic responses to copper sulfate treatment over multiple life stages, for example sensitive periods in development.

Based on the results presented in this study, there is evidence that the application of copper sulfate does impact both the metabolism and lifespan of the three organisms we studied. These consequences may be magnified in the lake due to the elevated sediment copper concentrations and concentrated applications. While our results do suggest that leeches are very copper sensitive; as an individual species, their absence would not greatly impact the ecosystem functioning. Pond snails also experienced an increased mortality in the copper treatments. Pond snails do provide an important service to the ecosystem as scrapers; they feed on detritus and assist with decomposition within the lake. Since algicide application is localized to the north end of the lake, it is possible that the negative impact of copper is limited to a small enough proportion of the pond snail population that there would be no significant detriment to the ecosystem. Further investigation of these topics will lend more evidence to whether the algicide regime should be altered or halted.

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