EFFECTS OF COPPER AND LIGHT EXPOSURE ON THE DEVELOPMENT AND SURVIVAL OF THE WOOD FROG TADPOLE (*RANA SYLVATICA*)

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Colleen C.R. Sharp

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EFFECTS OF COPPER AND LIGHT EXPOSURE ON THE DEVELOPMENT AND SURVIVAL OF THE WOOD FROG TADPOLE (*RANA SYLVATICA*)

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Thesis

Approved:	Accepted:
_____	_____
Advisor Dr. Francisco Moore	Department Chair Dr. Bruce Cushing
_____	_____
Committee Member Dr. Brian Bagatto	Dean of the College Dr. Ronald Levant
_____	_____
Committee Member Dr. Timothy Matson	Dean of the Graduate School Dr. George Newkome
_____	_____
Committee Member Dr. Peter Niewiarowski	Date

ABSTRACT

Both concern over amphibian declines (Dunson et al., 1992; Blaustein, 1994) and the potential of amphibians as indicators (Phillips, 1990; Dunson et al., 1992; Boyer and Grue, 1995) of ecosystem health provided the impetus for this study. Utilizing amphibians as indicator species is comparable to the use of "canaries in a coal mine" when assessing the quality of an aquatic environment (Barinaga, 1990). Embryos, tadpoles, and adults are considered to be sensitive to environmental contaminants in part due to their unshelled eggs and permeable skins (Bridges et al., 2002; Blaustein et al., 2003; Kiesecker et al., 2004; Hogan et al., 2006). Amphibians offer a unique biphasic life cycle for studying water and land habitats as well as the interactions between the two environments. This study investigated the interaction between two human-mediated environmental changes on the development of a common North American anuran, (*Rana sylvatica*) the Wood Frog.

Anthropogenic changes have increased copper and sunlight in many amphibian habitats. Human disturbance often leads to a decrease in canopy cover, which thereby reduces shade for developing embryos and tadpoles of certain anuran species within the aquatic environments below (Werner and Glennemeier, 1999; Skelly et al., 2002). Water runoff from impermeable surfaces and agricultural and residential properties transport toxins

and excess nutrients into bodies of water, leading to algal blooms. In ponds, a common algaecide utilized to eliminate the ensuing blooms is copper sulfate.

This work investigated the effects of copper sulfate and increased solar radiation on the developmental rate and survival of Wood Frog tadpoles in high pH ponds. pH levels in Northeastern Ohio ponds are higher (7.0-8.5; Matson et al., unpublished data, 2006) than in many other areas of Wood Frog study and research is lacking in non-lab environments at these pH levels. A field study used cattle tanks for testing the influence of increased light, increased copper, and for interactions between these environmental perturbations. Copper was a significant source of variation in measures of tadpole developmental rate and marginally significant in tadpole survival. Shade was not a significant source of variation in survival, but did significantly slow development. In addition, copper and shade interacted in their effect on developmental rate as measured by an increase in body mass in ambient copper treatments. These results are beneficial in understanding whether the use of copper sulfate is a contributor to amphibian decline.

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CHAPTER I

INTRODUCTION

Considerable work has been done on the influence of heavy metals and solar radiation on pond-dwelling amphibians. However, research is lacking in ponds with higher levels of pH such as those found in Northeastern Ohio (Horne and Dunson, 1995b; Matson, personal communication). Effects of metals may differ under these circumstances since pH is expected to alter the toxicity of metals (Horne and Dunson, 1995a, 1995b). Copper sulfate, a commonly used algaecide, is an over-the-counter product used to kill the algae causing algal blooms in ponds. The effects copper sulfate has on nontarget species is important to understand. Additionally, removal of vegetation in and around ponds allows increased levels of solar radiation to reach the water below. Since increased solar radiation is known to be harmful, it was incorporated into this study. A controlled field study was conducted to test the influence of copper sulfate and shade loss in neutral to high pH environments (7.0-8.5) on the development and survival of Wood Frog, *Rana sylvatica*, tadpoles. Concerns over amphibian declines and the importance of amphibians as bioindicators of ecosystem health provided the impetus for this study.

A worldwide debate over declining amphibian populations has been noted for several decades (Barinaga, 1990; Phillips, 1990; Dunson et al., 1992;

Stebbins and Cohen, 1995; Stuart et al., 2004). As knowledge of this phenomenon becomes more widespread, additional attention has been directed toward its understanding (Phillips, 1990; Dunson et al., 1992; Stebbins and Cohen,1995; Sparling et al., 2002; Stuart et al., 2004; Baud and Beck, 2005). Some suggest the decline in many amphibians may be a symptom of larger phenomena (Phillips, 1990; Kiesecker et al., 2004). Anthropogenic factors such as global warming, ultraviolet radiation, invasive species, disease, habitat loss, and pollution have been linked to amphibian decline (Phillips, 1990; Wyman, 1990; Wake, 1991; Stuart et al., 2004; Pounds et al., 2006). It is therefore important to understand the impact humans have on the natural environment. As human populations increase, more demands are made upon dwindling natural resources. These demands may launch events that disturb a delicately balanced ecosystem leading to a loss of diversity through extirpation or extinction of various forms of wildlife.

Amphibians serve important roles as herbivores, prey, and predators (Blaustein et al., 1994). As prey items, their abundance provides energy and nutrients for higher trophic levels. In some impoverished human societies, anurans provide an important source of animal protein (Stebbins and Cohen, 1995). In some developed countries frog legs are a common food. Consumers in France ate 3000 to 4000 tons of frog legs in 1990 alone (Phillips, 1990). Herbivorous anuran tadpoles reduce the growth of algae and other aquatic plants in the course of their feeding activity (Berven, 1990; Stebbins and Cohen, 1995). As predators, amphibians contribute to the control of insect populations (Phillips,

1990). For these reasons the presence of amphibians in ponds is of considerable importance to community and ecosystem dynamics.

Amphibian biological indicator species may be comparable to “canaries in a coal mine” when assessing the quality of an environment (Barinaga, 1990). Due to their biphasic life cycle, amphibians may be used to study water and land components of ecosystems as well as the relationships between the two habitats (Dunson et al., 1992; Boyer and Grue, 1995; Stebbins and Cohen, 1995; Sparling et al., 2002). For many amphibians, life begins in a pond as a sedentary embryo encased within a vulnerable, clear jelly egg mass or film. In time, the embryo will hatch into a free-swimming aquatic larva, commonly known as a tadpole in frogs. Tadpoles undergo growth and differentiation and eventually transform into a terrestrial juvenile by undergoing metamorphosis (Zug, 1993). These traits, as well as their permeable skin and limited dispersal and home range, support the use of anurans as invaluable tools in monitoring the welfare of our water, land, air, and the interactions between them (Boyer and Grue, 1995; Stebbins and Cohen, 1995; Sparling et al., 2002).

Rainfall, groundwater, and surface runoff are the major sources of water in temporary ponds (Mitsch and Gosselink, 2000). If water sources are contaminated, receiving ponds will likely be negatively impacted. Anthropogenic contributions to source water pollution include greenhouse gas emissions, fertilizers, herbicides, pesticides, deforestation, sedimentation, various metals, ultraviolet radiation, and deicing road salt (Foos, 2003; Thunqvist, 2004; Relyea, 2004, 2005a, 2005b). Pollutants in breeding ponds can be toxic to developing

frog embryos and tadpoles, causing sublethal effects or mortality (Sadinski and Dunson, 1992; Horne and Dunson, 1995a, 1995b; Pahkala et al., 2002). Since anuran tadpoles are herbivores, detrivores, and scavengers that forage from different levels of the water column (Zug, 1993), ingestion of toxins is likely to occur. Combining pollutants with each other or with various levels of environmental factors such as temperature, pH, or hardness may transform water once deemed “safe” into a toxic medium for anurans (Horne and Dunson, 1995a, 1995b; Zaga et al, 1998; Bridges and Boone, 2003; Damm, 2003; Semlitsch and Bridges, 2005).

Toxicology of ponds as a result of anthropogenic impacts is a major factor in amphibian conservation. Ponds provide essential habitat in which many amphibians congregate in the spring to mate (Zug, 1993; Stebbins and Cohen, 1995). Resulting embryos remain in the ponds surrounded by protective gelatinous membranes. These permeable membranes leave embryos vulnerable to toxins that are present in the pond. A number of studies have revealed correlations between delayed development and mortality in embryos and hatchlings exposed to various levels of pH (3.9-7.6), metals, and ultraviolet radiation (Freda and Dunson, 1986; Freda and McDonald, 1993; Horne and Dunson, 1995b; Pahkala et al., 2002; Baud and Beck, 2005).

The Wood Frog occurs throughout much of the northeastern United States, as far north as Alaska, Quebec, and British Columbia, and south to northern Georgia (Conant and Collins, 1998; Redmer and Trauth, 2005). There has recently been some taxonomic uncertainty regarding Wood Frogs. Frost et

al. (2006) place Wood Frogs with *Lithobates*. In this study, due to tradition and more recent work by Hillis (2007), I will use the genus *Rana*. Wood Frogs are tolerant of ponds with low pH, although the hatching of embryos does not correlate with hatching success (Freda and Dunson, 1986). Wood Frogs have an impressive ability to withstand low temperatures, down to -5˚C. Glucose prevents tissue damage when portions of their body fluids freeze (Stebbins and Cohen, 1995). Wood Frogs are among the first amphibians each year to utilize ponds for breeding due to their tolerance of cold conditions. The adults have an explosive breeding strategy, laying their gametes in large communal masses within a few days in early spring, and then leaving the ponds (Duellman and Trueb, 1994). One female is capable of laying thousands of eggs at one time (Stebbins and Cohen, 1995). Wood Frogs migrate to their natal ponds in forests to breed, sometimes when ice still covers them.

Copper occurs naturally in surface waters. It is present primarily as the copper (II) ion, also known as the cupric ion, or Cu^{2+}. Cupric ions are highly reactive and are usually found as part of compounds in water (Stumm and Morgan, 1981). Copper salts, such as copper sulfate ($CuSO_4$), are used as herbicides, fungicides, and algaecides (Herkovits and Helguero, 1998; De Oliveira-Filho et al., 2004). Copper sulfate is highly soluble (Montag et al, 2006). Due to the binding affinity of Cu, only a portion of Cu from an herbicide attaches to the target species, making it important to learn of its effects on nontarget species (De Oliveira-Filho et al., 2004). Previous laboratory studies have shown detrimental effects on amphibians (embryos and tadpoles) as a result of Cu

(Horne and Dunson, 1995b; Herkovits and Helguero, 1998; Parris and Baud, 2004; Baud and Beck, 2005) in terms of developmental rate and survival.

Metals and pH have a complex relationship. In low pH solutions, metals are more soluble. However, while fewer metal ions may be present in high pH solutions, those present may have elevated toxic effects because of increased biological availability (Horne and Dunson, 1994). Horne and Dunson (1995b) concluded in their research that 15 µg/l of Cu was more toxic to Wood Frogs when in a higher pH (5.50) than at a lower pH (4.50) solution, even though Cu concentrations are lower at higher pH (Stiff, 1971). A study by Freda (1991) shows water hardness reduces the toxic effects of low pH and toxic metals and Stiff (1971) revealed fewer Cu ions are available with increased water hardness. Given that the skin of amphibians is permeable to ion exchange (Zug, 1993; Stebbins and Cohen, 1995), pH may significantly alter the uptake and turnover of toxic compounds in amphibians. The Northeast Ohio region demonstrates a higher pH in temporary breeding ponds than found in most previous studies (Matson, unpublished data, 2005). Shortly after snowmelt in March 2006, the mean pH of eight Mentor Marsh ponds, located east of Cleveland, was 6.99 (Matson et al., unpublished data). The need to understand how pH affects the availability and toxicity of metals in breeding ponds and the toxicity of those metals to anurans provided the impetus for this research. There are no published field studies addressing the toxicity of metals on amphibians at higher levels of pH such as are often found in the field in Northeast Ohio.

Another anthropogenic impact that has been demonstrated to be harmful to amphibians is increased sunlight received in many breeding ponds (Blaustein et al., 1998; Baud and Beck, 2005). Natural events as well as anthropogenic pollutants have removed some of the stratospheric ozone layer that protects the surface of the earth from these harmful rays (Blaustein et al., 2003; Boone et al., 2003). The thinner ozone layer allows more sunlight to reach the earth's surface. Effects of ultraviolet radiation on anurans can include retinal damage in adults, morphological abnormalities, embryonic, larval, and post-metamorphic mortalities, and delayed growth in embryos (Pahkala et al., 2002; Bridges and Boone, 2003; Ankley et al., 2004; Baud and Beck, 2005; Blaustein and Belden, 2005; Blaustein et al., 2005). Perhaps even more important for certain breeding anurans however is the loss of shade due to deforestation either during lumbering or residential development (Phillips, 1990; Egan and Paton, 2004). Vegetation also provides those amphibians that lay their eggs in the water a place to attach their eggs (Egan and Paton, 2004).

In this study, I focused on two anthropogenic perturbations that are common in Northeast Ohio. The first is copper sulfate in its use as an algaecide, and also its use in residential areas where pond vegetation has been cleared for recreational enjoyment. I hypothesized that due to the high pH of local ponds, Cu would be an even more important toxin in Northeast Ohio than in previous studies. Knowing that many ponds may experience both high [Cu] and increased natural light, I tested my hypothesis across differing environments.

This study tested for developmental effects of typical levels of anthropogenic Cu and increased sunlight on developmental rate, consisting of mass and developmental stage, and survival in natural field conditions on Wood Frog tadpoles. I hypothesized that excess Cu and sunlight in the water column would have a negative impact on the development and survival of Wood Frog tadpoles. In order to test the environmental specificity of either of those effects, I included crossed treatment factors which allowed me to test for interactions between sunlight and Cu. I hypothesized that the influence of Cu on anuran development and survival would vary directly with sunlight levels.

CHAPTER II

MATERIALS AND METHODS

Study Organism

Wood Frogs were chosen as a transplant species in this study due to an observed decline in populations following embryo transplantation into mitigated wetlands at Mentor Marsh and Mentor Marsh State Nature Preserve northeast of Cleveland (Matson, unpublished data). Transplants occurred each year between 2001 and 2005. In June of 2001 and subsequently in 2003 and 2004, Wood Frog tadpoles were abundant. A calling male population began in 2003 and increased in size in 2004; however, no tadpoles were netted from the wetlands in May of 2005. Water chemistries revealed [Cu] ranging from 30-80 µg/L at a pH of 6.66-6.87 (Matson, unpublished data). Wood Frogs are known to be tolerant of low pH (Freda and Dunson, 1985; Freda and Dunson, 1986), which made it interesting to see how they would do in a higher pH environment.

Study site

The location for this study was at the University of Akron's Martin Center for Field Studies and Environmental Education located in the Bath Nature Preserve in Bath Township, Ohio (41˚9'29" N, 81˚38'0" W). The preserve is

located approximately 3 km west of the Cuyahoga Valley National Park between the greater metropolitan areas of Cleveland and Akron. The field station is situated within the 160 protected ha of the nature preserve.

Pond mesocosms

Sixteen 380-liter cattle tanks (Rubbermaid, model no. 4242) located at the field station in the Bath Nature Preserve were set into the ground to act as reservoirs for the pond mesocosms created in this study. The upper lips of the cattle tanks were installed slightly above ground level to prevent water runoff from flowing into the tanks. The tanks were positioned in a 4 tank wide by 4 tank long square array with approximately 1 m buffers between each tank.

A screen cover with 1.25 cm^2 mesh was attached to a wooden frame placed above each cattle tank allowing airflow, sunlight, and rainwater in as well as keeping predators out. The wooden frame rested on four legs to prevent the screen from resting on the surface of the water. The tanks were filled to within 40 liters of full with water from Bath Pond, located on the preserve, to create natural pond mesocosms in a controlled environment. Subsequent rainfall contributed to natural fluctuations in water levels in the tanks.

Transplantation

Freshly laid Wood Frog egg masses (< 24 h old) were collected from woodland ponds in the Grand River drainage system. The ponds are located within Natural Areas (41˚42’36” N, 80˚52’47” W) owned by the Cleveland

Museum of Natural History. The egg masses were placed into four 380-liter aboveground cattle tanks located at the field station partially filled with pond water from a neighboring pond (Bath Pond) located on the preserve. The eggs were observed on a daily basis for hatchlings. Upon hatching, 4,800 living tadpoles were removed from the aboveground tanks and randomly distributed in groups of 300 into each of the sixteen buried cattle tanks. The tadpoles were allowed one day to adjust to their new surroundings to prevent undue stress without treatment applications to the pond mesocosms. No mortality was observed during this accommodation period.

Shade Treatment

Shade and ambient light were the two light treatments applied in this study. A 60% high density polyethylene shade cloth (National Tool Grinding) placed onto the screen cover approximately 10 cm above the water in eight of the sixteen tanks represented ponds with shade. The remaining eight tanks did not have a shade cloth, allowing them to receive ambient sunlight. Measures of visible wavelength light both above and below the shade cloth verified the 60% shade cloth rating.

Copper Treatment

This study utilized two Cu treatments, typical treatment Cu (high [Cu]) and ambient [Cu]. Eight high [Cu] (four with shade and four without) and eight ambient [Cu] (four with shade and four without shade) tanks were used. Copper

tests (LaMotte TesTab Water Investigation Kit, Model AM-12, Code 5849) from Bath Pond indicated that [Cu] was below detection limits of the kit (0.33 mg/l). The Cu treatment ponds were dosed with over-the-counter $CuSO_4 \cdot 5H_20$ (Brand Copper Sulfate Crystals, Applied Biochemists). For algae control Applied Biochemists recommends using 1 to 2 mg/l of crystals. The upper limit of 2 mg/l (copper sulfate as crystals, 0.5 mg/l as Cu) was used in this study to represent the level used by a nonprofessional who wanted to aggressively treat algae. Aqueous stock solution of $CuSO_4$ was added to the high [Cu] treatment tanks by slowly pouring and stirring with a plastic pipe to spread it evenly throughout the tanks. Subsequent testing of Cu indicated that treatment levels were achieved and not exceeded.

Treatment Application

A random number generator selected which tanks would receive which treatment. Tank numbers 1-4 were on the north side of the tank system and tanks 13-16 made up the south side. Tank numbers 1, 2, 4, 7, 10, 11, 14, and 15 received high [Cu] treatments (Figure 1). Shade treatments were applied to tanks 1, 4, 5, 6, 11, 12, 15, and 16. This setup allowed there to be four treatments with four replicates per treatment.

Application of Cu to each of the eight high [Cu] treatment tanks was made on April 3, 2007. After addition of the Cu the shade cloth was attached to the eight shade treatment tanks. Throughout the study, the tanks were checked every few days to look for dead tadpoles and to take readings of water

temperature, dissolved oxygen (DO), and pH. The temperature and DO probes were taped to a bamboo pole at three depths to consistently take the readings near the bottom, middle, and top of the water column in the center of each tank. The bottom reading was taken 8.0 cm from the bottom of the tank, the middle reading was taken 28.0 cm from the tank bottom, and the top reading was 48.0 cm from the tank bottom, approximately 8.0 cm below the water's surface depending on water level. Averages were taken of these three measurements for temperature and DO. These averages were used in later calculations. Water was sampled from the middle only of the water column to test for pH.

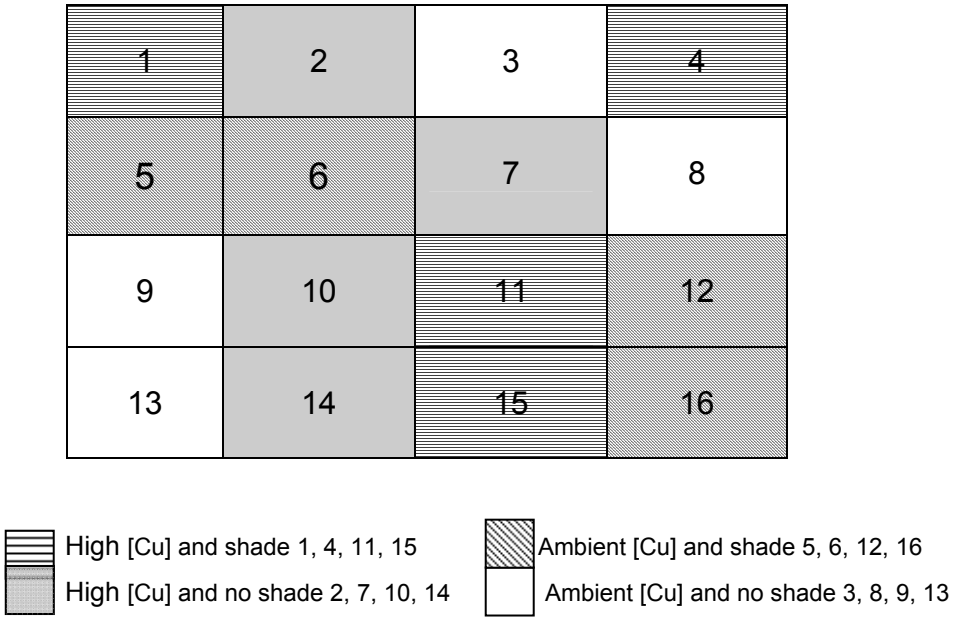

Figure 1. Treatment setup of the 16 pond mesocosms.

A Fluke thermometer (51-54 Series II) was used to take water temperature readings. The DO meter was a Milwaukee SM600 Dissolved Oxygen Meter. pH papers (pHydrion Papers) were used to test pH on four dates (4/7, 4/8, 4/11, and

4/15) on water samples taken from the middle of the water column. This method was switched to using LaMotte pH Wide Range TesTabs (LaMotte TesTab Water Investigation Kit, Model AM-12, Code 5849) for more accuracy. Dead tadpoles were never found in the tanks.

Water Chemistry

Four rounds of water chemistry measures were taken from the sixteen tanks. Two rounds were taken at the beginning of the study and two near the end of the study. During each round, two samples of water were retrieved from each tank, and designated samples A and B. "A" samples were taken from the north end of the tank and "B" samples from the south end. The samples were taken midway in the water column approximately 36 cm below the surface of the water. Samples for pH were taken in the center of the tanks from the tank edges in the middle of the water column. Air bubbles were prevented from forming in the containers by placing the lid on the container under water. The water samples were immediately refrigerated and tested within three days. A LaMotte TesTab Water Investigation Kit (Model AM-12, Code 5849) was used to test for levels and presence of hardness, alkalinity, pH, and [Cu]. The first and second rounds of samples were taken within four days of each other. This allowed examination of variation between water samples.

Data Collection

The study was terminated before metamorphosis began in order to allow consistency in interpretation of tadpole mass data. On Day 31 (May 4, 2007) of the study the tadpoles were euthanized in a solution of 0.25 ml Eugenol (clove oil) and 1.0 ml of 70% ethyl alcohol in 1 liter of water. Tadpoles were then fixed in 10% formalin and preserved in jars labeled with their tank number in 70% ethyl alcohol. For four days following the end of the study, any remaining tadpoles were netted from the tank until no additional tadpoles were found in successive inspections. The tadpoles caught after Day 31 of the study were used in analysis for survival only and not in developmental rate.

Tadpoles were viewed under a dissecting scope to determine their stage of development using Gosner's method (1960). Once stage was determined, the tadpoles were placed into a drying oven (VWR International) at 41˚C overnight. The next day each tadpole was placed individually on a Cahn 25 automatic electrobalance to determine its dry mass. Tadpoles appearing conspicuously deformed as well as a representative of each developmental stage for each tank were preserved for later analysis and were not placed in the drying oven.

Statistical Analysis

Statistical analyses were all conducted using SAS software (Version 9.1, SAS Institute, Inc.). Two-way ANOVA models (The GLM Procedure, SAS) were used to test for significant sources of variation in dry mass, developmental stage, and survival. ANCOVA models (The GLM Procedure, SAS) including

temperature and DO as covariates were also used to separate the direct influence of Cu and shade on the response variables from their indirect influence through temperature and DO. Conformity of data to parametric assumptions was confirmed for each statistical model (The Univariate Procedure, SAS). Post hoc tests for differences between means were performed using Tukey's multiple comparison tests (The GLM Procedure, SAS). Unless otherwise indicated, significance is indicated by a p-value ≤ 0.05.

CHAPTER III

RESULTS

Data Collection

Post-mortem measurements taken on tadpoles are shown in Table 1. The variables measured were dry mass, developmental stage using Gosner's method (1960), and percent survival.

Table 1. Summary of tadpole measurement means and standard errors across tanks for mass, developmental stage, and percent survival. "Sh" represents shade treatments and "NSh" represents no shade treatments.

Treatment	Mass (mg)	Stage (Gosner, 1960)	Survival (percent)
High [Cu]/Sh	0.7082 ± 0.0481	25.04 ± 0.02	82.25 ± 4.63
High [Cu]/NSh	1.0939 ± 0.1275	25.15 ± 0.05	81.75 ± 5.10
Ambient [Cu]/Sh	4.3083 ± 0.2632	26.61 ± 0.17	88.50 ± 2.14
Ambient [Cu]/NSh	5.6578 ± 0.2773	26.98 ± 0.09	92.50 ± 1.75

Influence of Copper and Shade

Calculations were done to determine the influence copper and shade had on tadpole mass, developmental stage, survival, water temperature, and dissolved oxygen levels. The following sections contain these results.

Tadpole Dry Mass

Shade and Cu were both significant sources of variation in tadpole dry mass (Table 2). High [Cu] significantly decreased dry mass in both shade and no shade tanks (Figure 2). Shade decreased tadpole dry mass and high [Cu] appeared to alter that influence of shade (marginally significant Copper x Shade interaction, Table 2) such that shade significantly decreased dry mass in ambient [Cu] but did not significantly reduce the dry mass in high [Cu] (Figure 2).

Table 2. Results of two-way ANOVA for dry mass (Type III SS).

Source	df	MS	F	Pr > F
Copper	1	66.651	303.44	< 0.0001
Shade	1	3.011	13.71	0.0030
Shade x Copper	1	0.929	4.23	0.0622
Error	12	0.220		

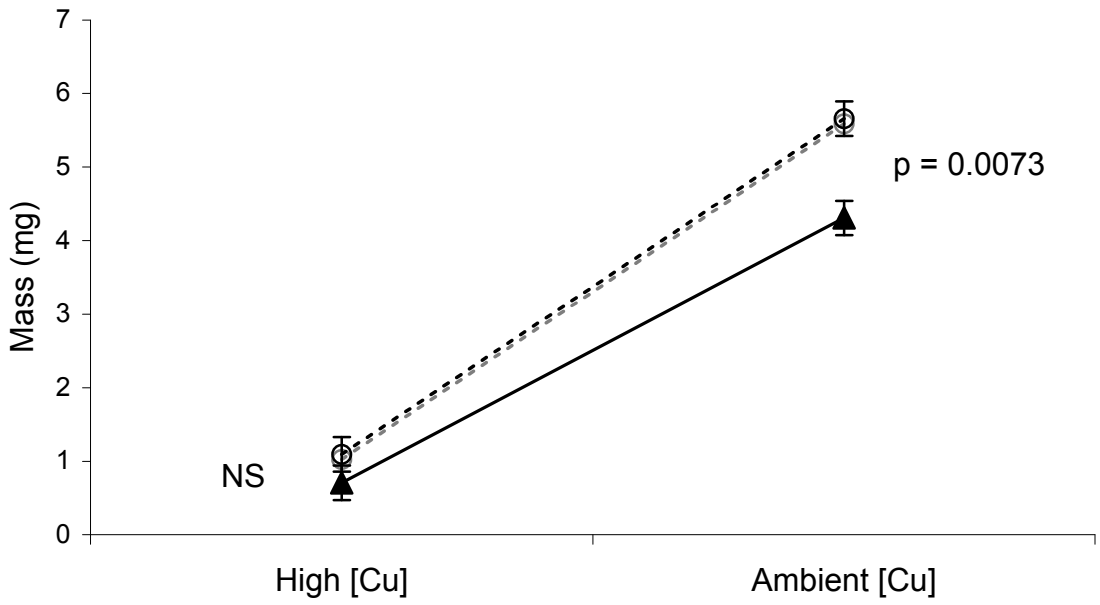

Figure 2. Least Square (LS) means for dry mass of surviving tadpoles at 31 days in high [Cu] and ambient [Cu] treatments. Open symbols represent no shade treatments and solid symbols represent shade treatments. Error bars indicate ± one standard error. Significance of post hoc comparisons within each Cu treatment are indicated next to compared data points.

Tadpole Developmental Stage

Copper was a significant and shade was a marginally significant source of variation in tadpole developmental stage (Table 3). There was no significant interaction between Cu and shade in their effect on developmental stage. Gosner (1960) developmental stage for tadpoles in ambient [Cu] tanks was significantly higher than in high [Cu] treatments (Table 3, Figure 3).

Table 3. Results of two-way ANOVA for developmental stage (Type III SS).

Source	df	MS	F	Pr > F
Copper	1	11.560	223.81	< 0.0001
Shade	1	0.237	4.58	0.0535
Shade x Copper	1	0.071	1.37	0.2645
Error	15	0.052		

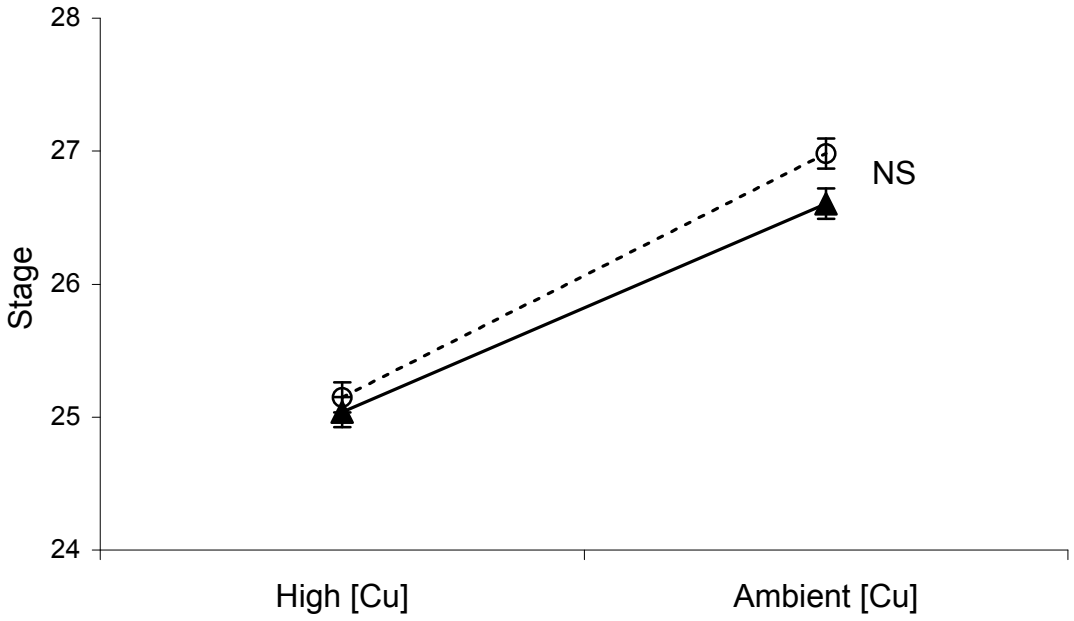

Figure 3. LS means for developmental stage of surviving tadpoles at 31 days in high [Cu] and ambient [Cu] treatments. Open symbols represent no shade treatments and solid symbols represent shade treatments. Error bars indicate ±

one standard error. Significance of post hoc comparisons within each Cu treatment are indicated next to compared data points.

Tadpole Survivorship

Copper was marginally a significant source of variation in tadpole survivorship (Table 4). Shade was not a significant source of variation either through its main effect or via an interaction with high [Cu]. Shade and Cu did not interact to alter tadpole survival (Table 4, Figure 4).

Table 4. Results of two-way ANOVA for survivorship (Type III SS).

Source	df	MS	F	Pr > F
Copper	1	2678.063	3.91	0.0715
Shade	1	105.063	0.15	0.7023
Shade x Copper	1	189.063	0.28	0.6090
Error	12	685.563		

Water Temperature

Shade and Cu were both significant sources of variation in water temperature (Table 5). Water temperature was lower in shade relative to no shade treatments (Figure 5). Copper appeared to alter the influence of shade treatments on water temperature (Table 5, Figure 5) although this influence was marginally significant.

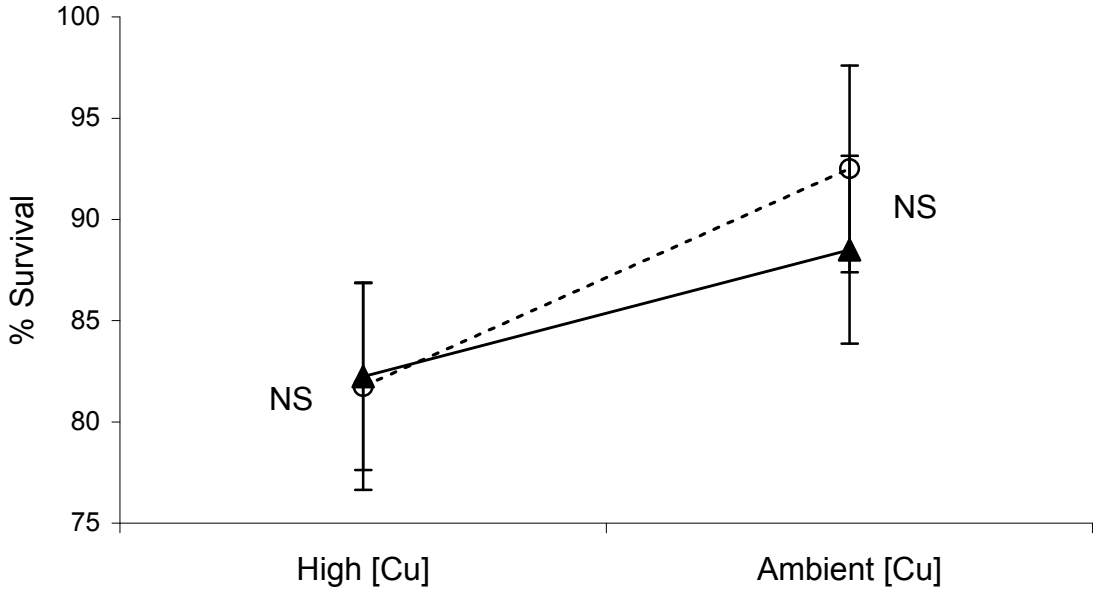

Figure 4. LS means for number of surviving tadpoles at 31 days in high [Cu] and ambient [Cu] treatments. Open symbols represent no shade treatments and solid symbols represent shade treatments. Error bars indicate ± one standard error. Significance of post hoc comparisons within each Cu treatment are indicated next to compared data points.

Table 5. Results of two-way ANOVA for temperature (Type III SS).

Source	df	MS	F	Pr > F
Copper	1	0.243	5.30	0.0401
Shade	1	7.139	155.79	< 0.0001
Shade x Copper	1	0.209	4.56	0.0540
Error	12	0.046		

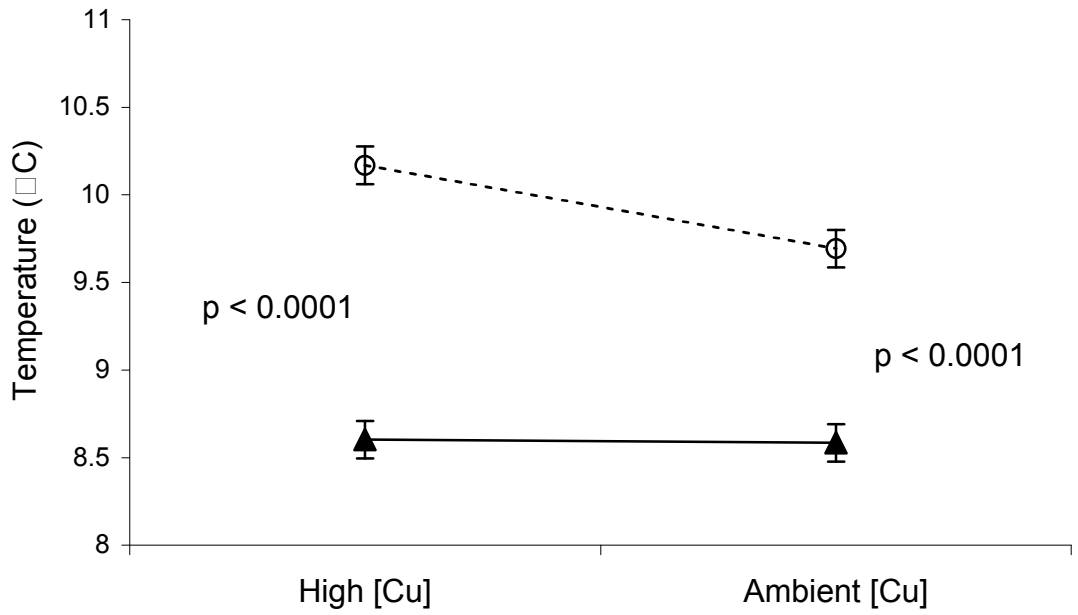

Figure 5. Shade and Cu effects on temperature. Open symbols represent no shade treatments and solid symbols represent shade treatments. Error bars indicate ± one standard error. Significance of post hoc comparisons within each Cu treatment are indicated next to compared data points.

Dissolved Oxygen

Copper was a significant source of variation in DO levels (Table 6). High [Cu] resulted in decreased levels of DO in both shade and no shade tanks (Figure 6). Shade did not influence DO either directly or through an interaction with Cu.

Table 6. Results of two-way ANOVA for DO (Type III SS).

Source	df	MS	F	Pr > F
Copper	1	1.778	16.62	0.0015
Shade	1	0.076	0.71	0.4169
Shade x Copper	1	0.214	2.00	0.1827
Error	12	0.107		

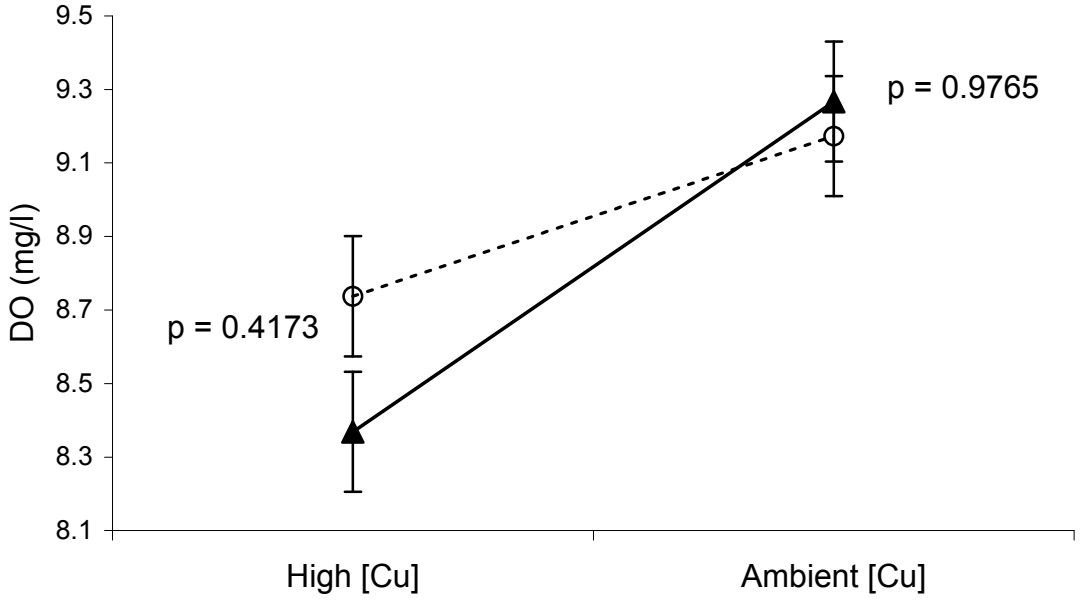

Figure 6. Shade and Cu effects on DO. Open symbols represent no shade treatments and solid symbols represent shade treatments. Error bars indicate ± one standard error. Significance of post hoc comparisons within each Cu treatment are indicated next to compared data points.

Influence of Temperature and Dissolved Oxygen

Linear regressions of temperature on tadpole developmental rate and survival indicated that temperature had no influence on any of those variables (Figures 7, 8, and 9). DO is a significant source of variation on dry mass (Figure 10) and developmental stage (Figure 11). DO is not a significant source of variation on tadpole survival ($p = 0.2153$, Figure 12).

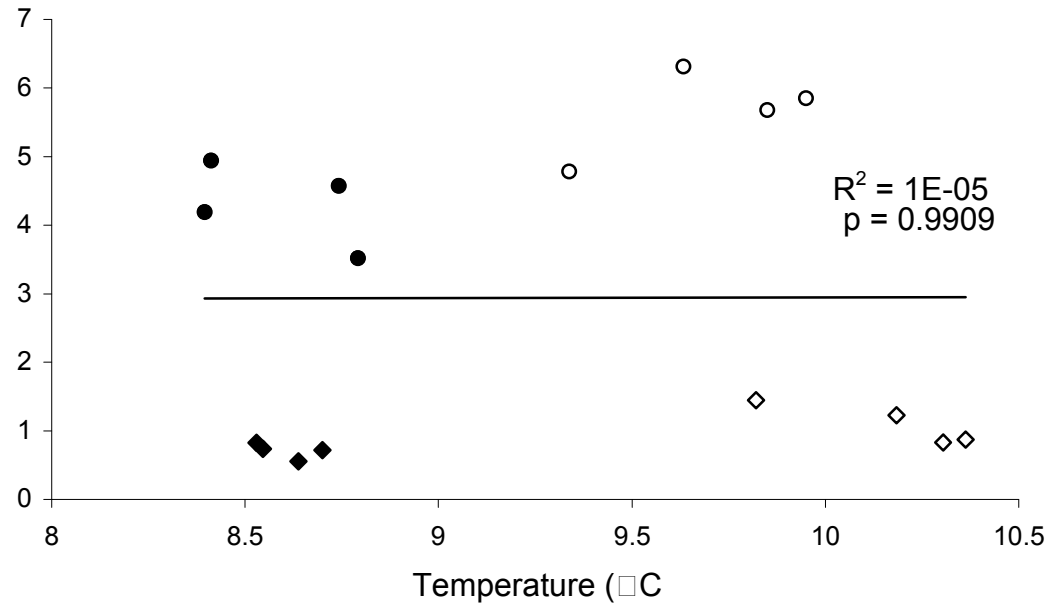

Figure 7. Effects of temperature on tadpole dry mass. Open symbols represent no shade treatments and solid symbols represent shade treatments. Diamonds represent high [Cu] and circles represent ambient [Cu].

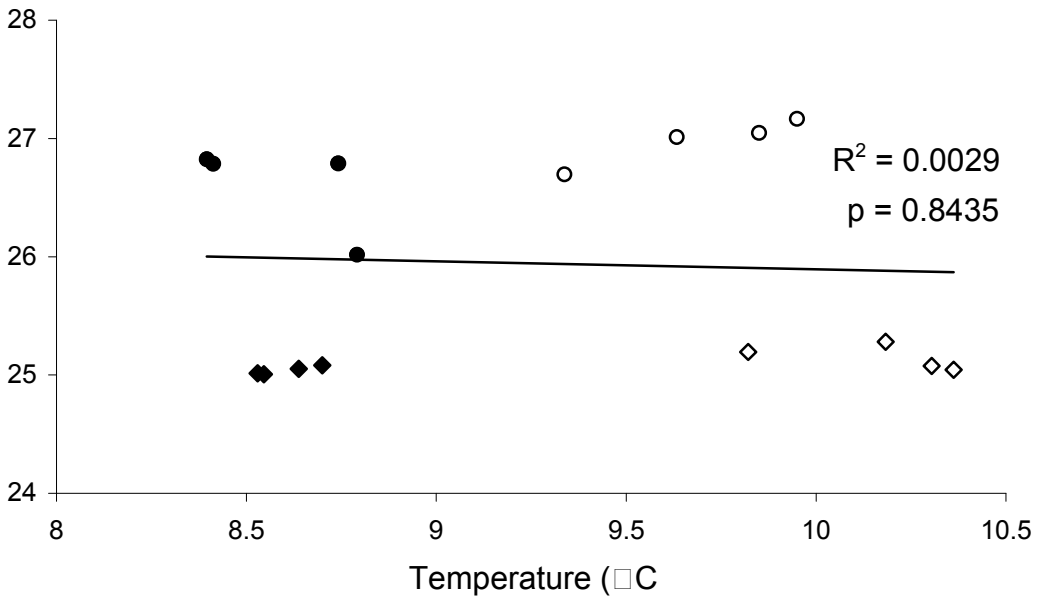

Figure 8. Effects of temperature on tadpole developmental stage. Open symbols represent no shade treatments and solid symbols represent shade treatments. Diamonds represent high [Cu] and circles represent ambient [Cu].

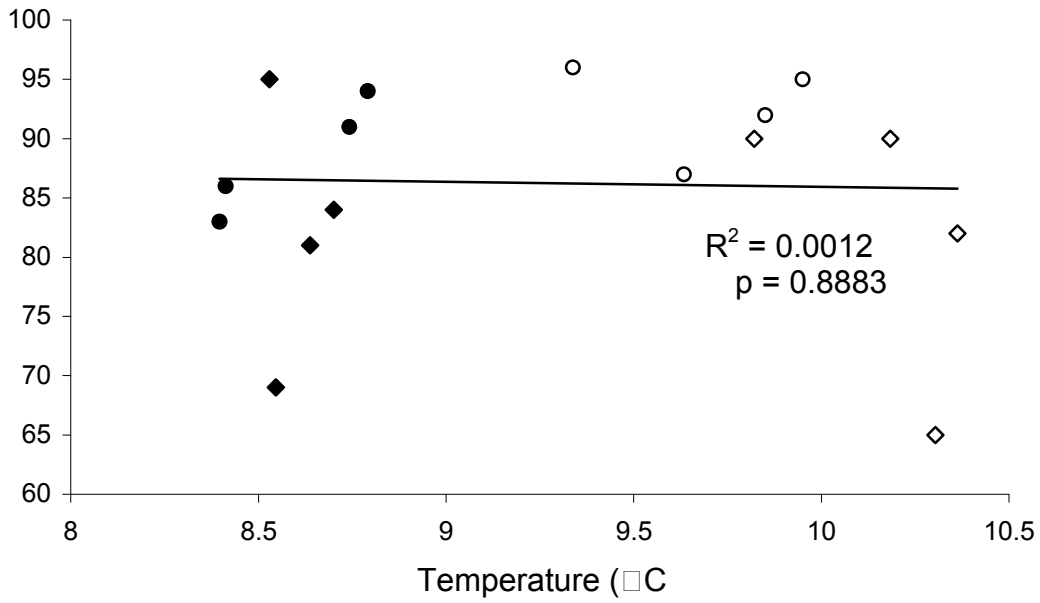

Figure 9. Effects of temperature on tadpole survival. Open symbols represent no shade treatments and solid symbols represent shade treatments. Diamonds represent high [Cu] and circles represent ambient [Cu].

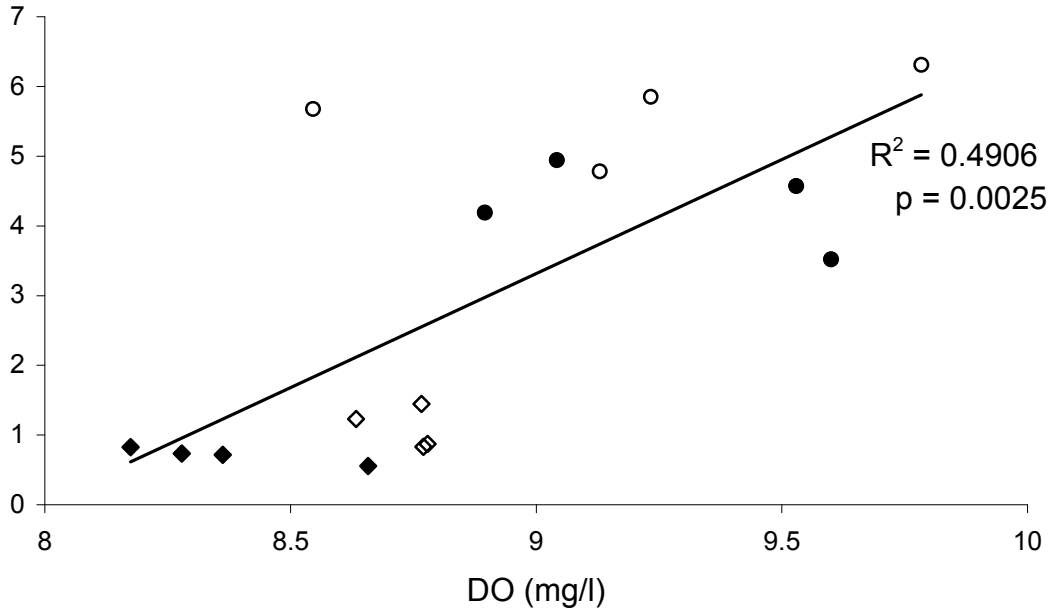

Figure 10. Effects of DO on tadpole dry mass. Open symbols represent no shade treatments and solid symbols represent shade treatments. Diamonds represent high [Cu] and circles represent ambient [Cu].

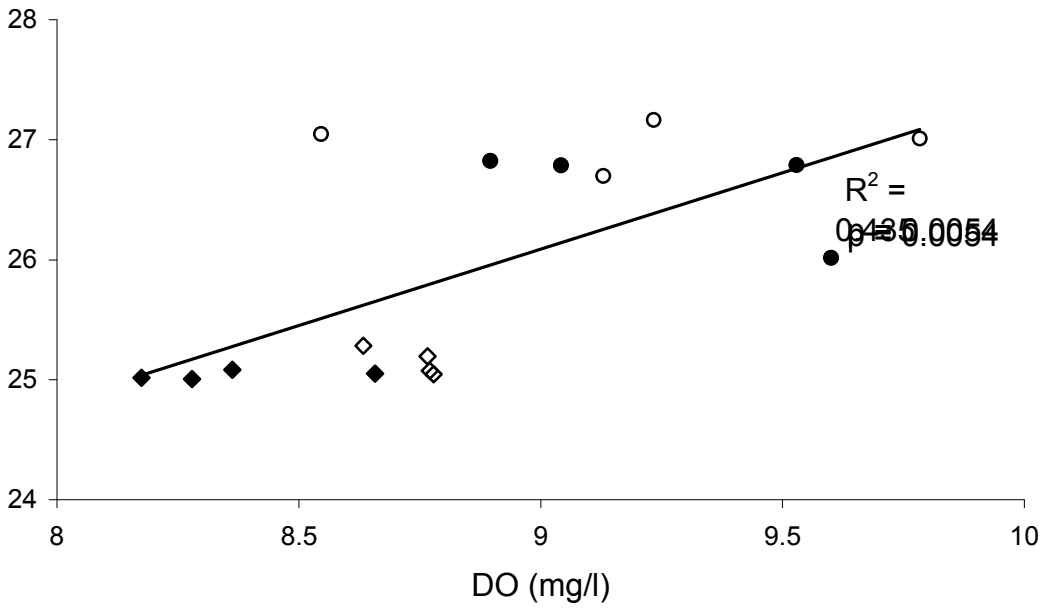

Figure 11. Effects of DO on tadpole developmental stage. Open symbols represent no shade treatments and solid symbols represent shade treatments. Diamonds represent high [Cu] and circles represent ambient [Cu].

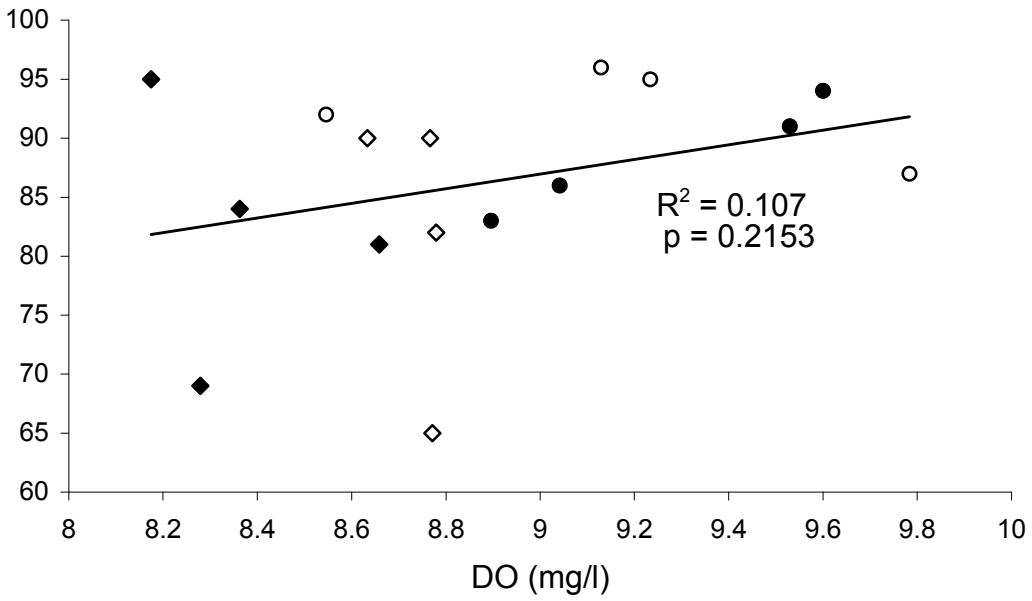

Figure 12. Effects of DO on tadpole survival. Open symbols represent no shade treatments and solid symbols represent shade treatments. Diamonds represent high [Cu] and circles represent ambient [Cu].

Influence of Shade and Copper with Dissolved Oxygen's Influence Removed

Calculations were done to determine the influence copper and shade had on tadpole mass and developmental stage with the removal of any influence by dissolved oxygen. The following section contains these results.

Tadpole Mass and Stage

When the influence of DO was removed from the model, the effects of shade and Cu on mass remained significant (Table 7). When the influence of

DO was removed from the model, the effects of shade and Cu on developmental stage remained significant (Table 8).

Table 7. Results of ANCOVA with DO as covariate for mass (Type I SS).

Source	df	MS	F	Pr > F
DO	1	35.9235	150.05	< 0.0001
Copper	1	30.7326	128.37	< 0.0001
Shade	1	3.1081	12.98	0.0041
Shade x Copper	1	0.8289	3.46	0.0897
Error	11	0.2394		

Table 8. Results of ANCOVA with DO as covariate for stage (Type I SS).

Source	df	MS	F	Pr > F
DO	1	5.4319	106.75	< 0.0001
Copper	1	6.1730	121.32	< 0.0001
Shade	1	0.2986	5.87	0.0339
Shade x Copper	1	0.0236	0.46	0.5098
Error	11	0.0509		

Water Chemistry

Table 9 shows the means for water chemistry measures for the 16 pond mesocosms. Means were calculated for each treatment across the experiment. Water temperature and dissolved oxygen represent the means of measurements taken at three depths in the middle of each tank throughout the study. pH

calculations represent data from when water temperature and DO were recorded as well as when the four rounds of water chemistries were done. The measurements shown for pH in Table 9 represent results using the LaMotte TesTab kit. pH measurements from the dates 4/7, 4/8, 4/11, and 4/15 using pH papers were removed since every measurement had a pH of 6, which was questionable. Tables listing all individual water chemistry measurements were included in Appendix C and D. Copper, hardness, and alkalinity represent the means of four rounds of chemistries taken approximately 8 cm below the surface of the water. Dissolved oxygen was corrected for temperature. A calibration test of the LaMotte TesTab Water Investigation Kit (Appendix A) indicated a minimum detection limit of 0.33 mg/l. Hardness and alkalinity are expressed as mg/l of calcium carbonate ($CaCO_3$). Hardness was in the moderately hard range (60-120 mg/l) and alkalinity was in the typical range of freshwater systems (20-200 mg/l).

Table 9. Summary of treatment means and standard errors across tanks for water temperature, DO, pH, copper, hardness, and alkalinity. “Sh” represents shade treatments and “NSh” represents no shade treatments.

Treatment	Water Temp. (˚C)	DO (mg/l)	pH	Copper (mg/l)	Hardness (mg/l)	Alkalinity (mg/l)
High [Cu]/Sh	8.6 ± 0.0	8.4 ± 0.1	7.8 ± 0.0	0.85 ± 0.06	76.88 ± 0.96	72.19 ± 1.47
High [Cu]/NSh	10.2 ± 0.1	8.7 ± 0.0	7.8 ± 0.1	0.58 ± 0.04	77.34 ± 0.64	72.50 ± 1.73
Ambient [Cu]/Sh	8.6 ± 0.1	9.3 ± 0.2	8.0 ± 0.1	0 ± 0	73.28 ± 0.89	72.97 ± 1.20
Ambient [Cu]/ NSh	9.7 ± 0.1	9.2 ± 0.2	8.0 ± 0.0	0.03 ± 0.02	72.50 ± 0.91	72.50 ± 0.96

CHAPTER IV

DISCUSSION

R. sylvatica is a widespread species that is likely to occur in many suburban areas. My research shows that Wood Frog tadpoles will be impacted if the ponds they choose to breed in are treated with high [Cu] or if the surrounding vegetation is altered. This study demonstrates that used as an algaecide at normal treatment levels (2 mg/l), copper sulfate significantly decreases tadpole developmental rate (Tables 2 and 3) and is marginally significant ($p = 0.0715$) as a source of variation in survivorship (Table 4) in high pH field conditions. Shade significantly decreased tadpole developmental rate (Tables 2 and 3) under those same conditions.

Decreases in developmental rate might be explained by a reduction in primary productivity. Since copper sulfate kills algae, primary production is reduced, which decreases the amount of food available to herbivorous tadpoles. Reduced primary productivity may also decrease levels of dissolved oxygen that would otherwise have been produced by the algae and increase the water temperature by removing algae cover. Kiffney and Richardson (2001) looked at the wet mass of Tailed Frog tadpoles (*Ascaphus truei*) and their relationships with nutrients and periphyton. They found the amount of periphyton and wet mass of tadpoles to be significantly greater in nutrient-enriched treatments.

These results indicate that as Cu decreases primary productivity, the mass of tadpoles in Cu treatments would also decrease.

Studies conducted on amphibians from polluted locations indicate that in a polluted environment more time is spent searching for food and, hence, more energy is expended (Rowe et al., 2001). If energy is not directed toward growth, tadpoles retain a smaller body mass. Smaller body mass at metamorphosis may decrease reproductive fitness and survival (Berven, 1990; Newman and Dunham, 1994). However, tadpoles with a smaller body mass at metamorphosis exiting a pond with a short hydroperiod display higher survival than those taking a longer time to develop to a larger body mass and risking the pond drying before development is complete (Newman, 1988a, 1989). The combination of a significantly lower body mass and lower developmental stage in tadpoles from bodies of water treated with Cu could be detrimental to the survival of the slower developing tadpoles and the smaller terrestrial anurans exiting those ponds.

It is difficult to determine if the tadpoles in my study took longer to reach specific developmental stages than what is shown in tables for natural Wood Frog tadpole development (Pollister and Moore, 1937; Moore, 1939). The studies conducted to create these tables (Pollister and Moore, 1937; Moore, 1939) for Wood Frog tadpoles did not continue their tables throughout metamorphosis, or even to stage 26, the average stage in my study across all treatments. Moore (1939) concluded it took 11-14 days to get to stage 20 using tables constructed by Pollister and Moore (1937) at 10˚C (Table 10), the temperature closest to the average temperature in my study of 9˚C. My study

was allowed to continue for 31 days, at which time stage 26 (Gosner, 1960) was the average developmental stage. To clarify, stage 20 in tables created by both Pollister and Moore (1937) and Gosner (1960) represent the same physical stage of tadpole development. Since Gosner does not include the number of days it takes to reach each of the 46 developmental stages on his tables, it was hard to compare developmental rates in my study to natural developmental rates.

Table 10. Number of days to reach stage 20 of development (Moore, 1939).

	Temperature (˚C)				
	10.0 ± 0.6	15.3± 0.3	18.5 ± 0.2	19.9 ± 0.1	23.7 ± 0.2
Days	11-14	5-6	4	3-4	2

Since systems with algae problems are usually located in open canopy areas where vegetation has been purposely removed, it was important for this study to include a sun/shade component along with Cu. My findings revealed shade is important in determining developmental rate but is not as important when Cu is present (Tables 2-4, Figures 2-4). Shade findings in my research agree with those of other studies (Skelly et al., 2002; Baud and Beck, 2005) that vegetation removal, or increased light, from aquatic areas has an effect on tadpole development. However, in contrast, when Bridges and Boone (2003) looked at effects of UV-B and the insecticide carbaryl on the metamorphic mass and length of larval period of the Southern Leopard Frog tadpole (*Rana sphenocephala*), they found UV-B intensity was not a significant source of variation in either variable, but carbaryl was a significant source of variation on

metamorphic mass. Bridges and Boone explained that this may be due to the tendency of carbaryl to promote algal blooms and provide additional food for the tadpoles.

Previous studies have shown Cu to produce negative impacts on nontarget species such as invertebrates and fish (Hawkins and Griffiths, 1987; De Oliveira-Filho et al., 2004). A study of copper sulfate used as an algaecide in a tropical drinking water reservoir revealed no zooplanton in samples taken 4 and 12 days after the addition of Cu (Hawkins and Griffiths, 1987). It was not until day 47 that the arthropod community had been re-established. In another study *Daphnia similis* proved more susceptible to lower LC50 (concentration at which 50% of the organisms exhibit lethal responses) levels of Cu (0.013 mg/l) than the target green alga species *Raphidocelis subcapitata* (0.119 mg/l) at two days after the addition of Cu (De Oliveira-Filho et al., 2004). After two days, the study concluded that mortality of copper-based pesticides proved to be nearly as toxic to nontarget zebrafish (*Danio rerio*) (0.063 mg Cu/l) as to the target snail (*Biomphalaria glabrata*) (0.191 mg Cu/l). Since more Cu was used in my research (0.5 mg/l) than in the LC50s in the De Oliveira-Filho study, I would predict there to be effects on nontarget species at recommended levels of $CuSO_4$ when used as an algaecide.

Bridges et al. (2002) compared sensitivity to Cu between two species of tadpoles (*Rana utricularia* and *Bufo boreas*) and three fish species (*Lepomis macrochirus*, *Pimephales promelas*, and *Oncorhynchus mykiss*). The 96-hour LC50s for Cu were considerably lower for the tadpoles (0.12- 0.23 mg/l) than for

all three fish species (0.47-7.3 mg/l). Clearly toxicity in fish is not a good indicator of safe dosage levels for amphibians given the increased sensitivity relative to fish and the large influence on fish as nontargets. The susceptibility of anurans to this chemical therefore is of concern if Cu was applied at concentrations required to be an effective algaecide.

Ozone depletion due to human impacts has raised questions regarding the effects it has on amphibians and its potential contribution toward their declines (Blaustein et al., 2005). One study providing a link between these categories was conducted in Central and South America by comparing Total Ozone Mapping Spectrometer satellite data with information provided by the Declining Amphibian Populations Task Force (DAPTF) as well as other publications (Middleton et al., 2001). They found that UV-B exposure was higher in the Central America sites where amphibian declines had been most severe. These increased levels of UV-B when combined with the high temperatures of the area and anthropogenic inputs adding to air pollution such as biomass burning can have a synergistic effect creating unfavorable conditions for amphibians to survive.

Most research investigating the effects of solar radiation have been conducted using anuran embryos exposed to artificial UV-B (Blaustein et al., 1998; Rasanen et al., 2003; Baud and Beck, 2005; Blaustein and Belden, 2005). One study concerned with solar radiation in three species of anuran tadpoles (*Rana septentrionalis*, *Rana pipiens*, and *Rana clamitans*) determined that ambient sunlight (290-700 nm) caused 80-100% mortality soon after hatching (4,

7, and 10 days respectively; Tietge et al., 2001). Little mortality occurred in all three species in treatments filtering out UV-B (290-320 nm) and UV-A (320-380 nm). My results, however, point out that developmental rate is accelerated by increased light and that the harmful influence of UV may sometimes be counteracted by this quickened development.

Low statistical power due to unusually high survivorship across all treatments in my study prevented detection of any significant trends in survival. These data should therefore not be taken as any indication of lack of treatment effects on survival in general. The highest mortality periods (hatching and metamorphosis) were excluded from this experiment leading to low mortality in all treatments. UV-B exposure is linked to high mortality in embryos and is evident as sublethal effects in tadpoles and postmetamorphic anurans (Blaustein et al, 2005). It has also been observed that survival is higher in cattle tank pond mesocosms than in natural ponds (Boone et al., 2004). Additionally, the hardness of the water used in my study from Bath Pond is considered moderately hard at an average of 75 mg/l (LaMotte TesTab Water Investigation Kit). As other studies have shown, increasing water hardness decreases toxic effects of Cu (Stiff, 1971; Horne and Dunson, 1995b). Even though the results of my study show no significance in survival due to Cu and shade treatments, a change in water chemistry or type of water body may show otherwise.

A study by Baud and Beck (2005) presented perhaps the closest parallel to this study, but it followed only survival. They looked at the effects of ultraviolet radiation and Cu in Spring Peeper embryo and tadpole (*Pseudacris crucifer*)

survival. The study consisted of a 2 x 3 factorial design with three levels of UV-B exposure (zero, low UV-B, and high UV-B/ambient light) and two levels of [Cu] (zero and 2.6 mg/l) at a consistent water temperature (18˚C) and pH (7.5). Baud and Beck found both Cu and UV-B to be significant sources of variation in survival as well as the interaction between them (Table 11). Copper was a marginally significant source of variation on survival in my study, but shade (light) or the interaction of Cu and shade were not significant. Upon further comparison, our studies are similar when considering what I show as sublethal effects, they show as survival (Table 11). Differences may be due to the more natural pond mesocosms in my study with use of natural sunlight and diurnal changes, the utilization of pond water, lower levels of [Cu], fluctuation in water temperature, and the discontinuation of the study before survival in any treatment became less than 10%. This demonstrates the potential importance of possible interactive effects of different factors.

Table 11. Results of two-way ANOVA for tadpole survival and developmental rate. Significant p-values are in bold.

Source	Baud and Beck (2005) Survival	Sharp (2008) Survival	Sharp (2008) Developmental Rate Mass / Stage
Copper	**p = 0.005**	p = 0.0715	**p < 0.0001** / **p < 0.0001**
Shade	**p < 0.001**	p = 0.7023	**p = 0.0030** / p = 0.0535
Copper x Shade	**p = 0.03**	p = 0.6090	p = 0.0622 / p = 0.2645

Previous studies found higher water temperatures resulted in faster development and growth in anurans when other factors such as the availability of food, temperature tolerance of the species, and density were removed (Pollister

and Moore, 1937; Moore, 1939; Newman, 1998; Browne and Edwards, 2003). I wanted to see if the high [Cu] and shade treatments influenced the water temperature in the pond mesocosms. My results show that shade and [Cu] were significant sources of variation for water temperature (Table 5). It is expected that shade treatments would filter out a portion of the sunlight and lower the water temperature below, which is what occurred in this study (Figure 5). The influence of Cu addition was not apparent in shade treatments, but Cu did slightly increase water temperature in no shade treatments (Figure 5). This may be due to less algae in the water to absorb the sun's energy for photosynthesis.

I found no influence of temperature on tadpole developmental rate and survival. Even though the influence of [Cu] on temperature ($p = 0.0401$) was negligible, it may have ameliorated some of the positive effects of temperature due to the significant source of variation by Cu on dry mass ($p < 0.0001$; Figure 7) and developmental stage ($p < 0.0001$; Figure 8). Thermoregulation may also explain why temperature was not significant. Anurans use behavior to regulate their body temperatures by selecting the temperature that best suits them in the thermal gradients available (Duellman and Trueb, 1994). Water temperatures in my research were taken at various depths and did vary by several degrees. Since they had a choice of where to spend their time in the cattle tanks, I would expect tadpoles to have taken this opportunity to remain in water temperatures most comfortable (Wu et al, 2007).

It is important to address the effects of DO and correlated influence of primary productivity on development of Wood Frogs. Since an algaecide kills

algae, less oxygen and food should be available in the water column for larval amphibians to consume (Hota, 1994; Relyea, 2005b). The decomposing algae also removes oxygen from the water column. Since photosynthesis requires sunlight for the process of creating oxygen, shade would be expected to have an effect on DO levels. Copper was a significant source of variation on DO ($p = 0.0015$) in this study (Figures 10-12), indicating DO to be a good measurement for primary productivity. Shade was not a significant source of variation ($p = 0.4169$) nor was the interaction between Cu and shade ($p = 0.1827$) on DO. DO was a significant source of variation on tadpole mass ($p = 0.0025$) and stage ($p = 0.0054$), but not on survival ($p = 0.2153$). However, when effects of DO were removed using ANCOVA (Tables 7 and 8) for both measures, Cu and shade remained significant sources of variation (Table 7), and their interaction remained marginally significant ($p = 0.0897$) on tadpole mass. For developmental stage, Cu and shade were still significant sources and once again their interaction was not significant when DO effects were removed (Table 8). DO did not appear to influence the treatment effects in this study.

This study determined Cu to be a significant source of variation of developmental rate and tadpole survival of a common North American anuran. Although the use of copper sulfate as an algaecide is not the single factor responsible for the worldwide decline of amphibians, it does play a part in damaging their local environment. Shade was also a significant source of variation in tadpole mass and developmental stage. We should make it a priority to discover and utilize alternatives to better conserve tadpole habitat. More

research is required to look at the interconnections within freshwater systems and the effects pesticides have on them. Although there was not a significant interaction between the effects of Cu and shade across all response variables, there was a marginally significant interaction in developmental rate as measured by body mass increase in ambient [Cu]. By removing the direct effects of temperature and DO, we can better visualize the harmful influence of Cu and various light treatments.

LITERATURE CITED

Ankley, G. T., Degitz, S. J., Diamond, S. A. and Tietge, J. E. (2004). Assessment of environmental stressors potentially responsible for malformations in North American anuran amphibians. *Ecotoxicology and Environmental Safety* 58, 7-16.

Barinaga, M. (1990). Where have all the froggies gone? *Science* 247, 1033-1034.

Baud, D. R. and Beck, M. L. (2005). Interactive effects of UV-B and copper on spring peeper tadpoles (*Pseudacris crucifer*). *Southeastern Naturalist* 4, 15-22.

Berven, K. A. (1990). Factors affecting population fluctuations in larval and adult stages of the wood frog (*Rana sylvatica*). *Ecology* 71, 1599-1608.

Blaustein, A. R. (1994). Chicken little or Nero's fiddle? A perspective on declining amphibians populations. *Herpetologica* 50, 85-97.

Blaustein, A. R. and Belden, L. K. (2005). Ultraviolet Radiation. In M. Lannoo (Ed.), *Amphibian declines: The conservation status of United States species* (pp. 87-88). Berkeley: University of California Press.

Blaustein, A. R., Kiesecker, J. M., Chivers, D. P., Hokit, D. G., Marco, A., Belden, L. K. and Hatch, A. (1998). Effects of ultraviolet radiation on amphibians: Field experience. *American Zoology* 38, 799-812.

Blaustein, A. R., Romansic, J. M., Kiesecker, J. M. and Hatch, A. C. (2003). Ultraviolet radiation, toxic chemicals, and amphibian population declines. *Diversity and Distributions* 9, 123-140.

Blaustein, A. R., Romansic, J. M. and Scheessele, E. A. (2005). Ambient levels of ultraviolet-B radiation cause mortality in juvenile western toads, *Bufo boreas*. *American Midland Naturalist* 154, 375-382.

Blaustein, A. R., Wake, D. B. and Sousa, W. P. (1994). Amphibian declines: Judging stability, persistence, and susceptibility of populations to local and global extinctions. *Conservation Biology* 8, 60-71.

Boone, M. D., Corn, P. S., Donnelly, M. A., Little, E. E. and Niewiarowski, P. H. (2003). Physical Stressors. In G. Linder, S. K. Krest and D. W. Sparling (Eds.), *Amphibian Decline: An integrated analysis of multiple stressor effects* (pp. 129-151). Raleigh: SETAC Press.

Boone, M. D., Semlitsch, R. D., Fairchild, J. F. and Rothermel, B. B. (2004). Effects of an insecticide on amphibians in large-scale experimental ponds. *Ecological Applications* 14, 685-691.

Boyer, R. and Grue, C. E. (1995). The need for water quality criteria for frogs. *Environmental Health Perspectives* 103, 352-357.

Bridges, C. M. and Boone, M. D. (2003). The interactive effects of UV-B and insecticide exposure on tadpole survival, growth, and development. ` *Biological Conservation* 113, 49-54.

Bridges, C. M., Dwyer, F. J., Hardesty, D. K. and Whites, D. W. (2002). Comparative contaminant toxicity: Are amphibian larvae more sensitive than fish? *Bulletin of Environmental Contamination and Toxicology* 69, 562-569.

Browne, R. K. and Edwards, D. L. (2003). The effect of temperature on the growth and development of the endangered green and golden bell frog (*Litoria aurea*). *Journal of Thermal Biology* 28, 295-299.

Conant, R. and Collins, J. T. (1998). *A field guide to reptiles and amphibians of Eastern and Central North America*. New York: Houghton Mifflin Company.

Damm, S. A. (2003). *Effects of aluminum, copper, and lead on the development and survival of gray treefrog (Hyla versicolor) larvae*. Unpublished master's thesis, University of Akron, Akron, OH.

De Oliveira-Filho, Lopes, R. M. and Paumgartten, F. J. R. (2004). Comparative study on the susceptibility of freshwater species to copper-based pesticides. *Chemosphere* 56, 369-374.

Duellman, W. E., and Trueb, L. (1994). *Biology of amphibians*. Baltimore: The Johns Hopkins University Press.

Dunson, W. A., Wyman, R. L. and Corbett, E. S. (1992). A symposium on amphibian declines and habitat acidification. *Journal of Herpetology* 26, 349-352.

Egan, R. S. and Paton, P. W. C. (2004). Within-pond parameters affecting oviposition by wood frogs and spotted salamanders. *Wetlands* 24, 1-13.

Foos, A. (2003). Spatial distribution of road salt contamination of natural springs and seeps, Cuyahoga Falls, Ohio, USA. *Environmental Geology* 44, 14-19.

Freda, J. (1991). The effects of aluminum and other metals on amphibians. *Environmental Pollution* 71, 305-328.

Freda, J. and Dunson, W. A. (1985). The effect of acidic precipitation on amphibian breeding in temporary ponds in Pennsylvania. U.S. Fish and Wildlife Service, Eastern Energy and Land Use Team. *Biology of Reproduction* 80, 1-86.

Freda, J. and Dunson, W. A. (1986). Effects of low pH and other chemical variables on the local distribution of amphibians. *Copeia* 2, 454-466.

Freda, J. and McDonald, D. G. (1993). Toxicity of amphibian breeding ponds in the Sudbury region. *Canadian Journal of Fisheries and Aquatic Sciences* 50, 1497-1503.

Frost, D. R., Grant, T., Faivovich, J., Bain, R. H., Haas, A., Haddad, C. F. B., De Sa, R. O., Channing, A., Wilkinson, M., Donnellan, S. C., Raxworthy, C. J., Campbell, J. A., Blotto, B. L., Moler, P., Drewes, R. C., Nussbaum, R. A., Lynch, J. D., Green, D. M., and Wheeler, W. C. (2006). The amphibian tree of life. *Bulletin of the American Museum of Natural History* 297, 1-370.

Gosner, K. L. (1960) A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* 16, 183-190.

Hawkins, P. R. and Griffiths, D. J. (1987). Copper as an algicide in a tropical reservoir. *Water Reservoir* 21, 475-480.

Herkovits, J. and Helguero, L. A. (1998). Copper toxicity and copper-zinc interactions in amphibian embryos. *The Science of the Total Environment* 221, 1-10.

Hillis, D. M. (2007). Constraints in naming parts of the tree of life. *Molecular Phylogenetics and Evolution* 42, 331-338.

Hogan, N. S., Lean, D. R. S. and Trudeau, V. L. (2006). Exposures to estradiol, ethinylestradiol and octylphenol affect survival and growth of *Rana pipiens* and *Rana sylvatica* tadpoles. *Journal of Toxicology and Environmental Health, Part A* 69, 1555-1569.

Horne, M. T. and Dunson, W. A. (1994) Exclusion of the Jefferson salamander, *Ambystoma jeffrersonianum*, from some potential breeding ponds in Pennsylvania: Effects of pH, temperature, and metals on embryonic development. *Archives of Environmental Contamination and Toxicology* 27, 323-330.

Horne, M. T. and Dunson, W. A. (1995a). The interactive effects of low pH, toxic metals, and DOC on a simulated temporary pond community. *Environmental Pollution* 89, 155-161.

Horne, M. T. and Dunson, W. A. (1995b). Effects of low pH, metals, and water hardness on larval amphibians. *Archives of Environmental Toxicology* 29, 500-505.

Hota, A. K. (1994). Growth in amphibians. *Gerontology* 40, 147-160.

Kiesecker, J. M., Belden, L. K., Shea, K. and Rubbo, M. J. (2004). Amphibian decline and emerging disease. *American Scientist* 92, 138-147.

Kiffney, P. M. and Richardson, J. S. (2001). Interactions among nutrients, periphyton, and invertebrate and vertebrate (*Ascaphus truei*) grazers in experimental channels. *Copeia* 2, 422-429.

Middleton, E. M., Herman, J. R., Celarier, E. A., Wilkinson, J. W., Carey, C. and Rusin, R. J. (2001). Evaluating ultraviolet radiation exposure with satellite data at sites of amphibian declines in Central and South America. *Conservation Biology* 15, 914-929.

Mitsch, W. J. and Gosselink, J. G. (2000). *Wetlands*. New York: John Wiley & Sons, Inc.

Montag, J., Schreiber, L. and Schonherr, J. (2006). An *in vitro* study of the nature of protective activities of copper sulphate, copper hydroxide and copper oxide against conidia of *Venturia inaequalis*. *Journal of Phytopathology* 154, 474-481.

Moore, J. A. (1939). Temperature tolerance and rates of development in the eggs of amphibia. *Ecology* 20, 459-478.

Newman, R. A. (1988a). Genetic variation for larval anuran (*Scaphiopus couchii*) development time in an uncertain environment. *Evolution* 42, 763-773.

Newman, R. A. (1989). Developmental plasticity of *Scaphiopus couchii* tadpoles in an unpredictable environment. *Ecology* 70, 1775-1787.

Newman, R. A. (1998). Ecological constraints on amphibian metamorphosis: Interactions of temperature and larval density with responses to changing food level. *Oecologia* 115, 9-16.

Newman, R. A. and Dunham, A. E. (1994). Size at metamorphosis and water loss in a desert anuran (*Scaphiopus couchii*). *Copeia* 2, 372-381.

Pahkala, M., Laurila, A. and Merila, J. (2002). Effects of ultraviolet-B radiation on common frog *Rana temporaria* embryos from along a latitudinal gradient. *Oecologia* 133, 458-465.

Parris, M. J. and Baud, D. R. (2004). Interactive effects of a heavy metal and Chytridiomycosis on gray treefrog larvae (*Hyla chrysoscelis*). *Copeia* 2, 344-350.

Phillips, K. (1990). Where have all the frogs and toads gone? *BioScience* 40, 422-424.

Pollister, A. W. and Moore, J. A. (1937). Tables for the normal development of *Rana sylvatica*. *The Anatomical Record* 68, 489-496.

Pounds, J. A., Bustamante, M. R., Coloma, L. A., Consuegra, J. A., Fogden, M. P. L., Foster, P. N., La Marca, E., Masters, K. L., Merino-Viteri, A., Puschendorf, R., Ron, S. R., Sanchez-Azofeifa, G. A., Still, C. J. and Young, B. E. (2006). Widespread amphibian extinctions from epidemic disease driven by global warning. *Nature* 439, 161-167.

Rasanen, K., Pahkala, M., Laurila, A. and Merila, J. (2003). Does jelly envelope protect the common frog *Rana temporaria* embryos from UV-B radiation? *Herpetologica* 59, 293-300.

Redmer, M. and Trauth, S. E. (2005). *Rana sylvatica,* wood frog. In M. Lannoo (Ed.), *Amphibian declines: The conservation status of United States species* (pp. 590-593). Berkeley: University of California Press.

Relyea, R. A. (2004). Synergistic impacts of malathion and predatory stress on six species of North American tadpoles. *Environmental Toxicology and Chemistry* 23, 1080-1084.

Relyea, R. A. (2005a). The lethal impacts of Roundup and predatory stress on six species of North American tadpoles. *Archives of Environmental Contamination and Toxicology* 48, 351-357.

Relyea, R. A. (2005b). The impact of insecticides and herbicides on the biodiversity and productivity of aquatic communities. *Ecological Application*s 15, 618-627.

Rowe, C. L., Hopkins, W. A. and Congdon, J. D. (2001). Integrating individual-based indices of contaminant effects. *The Scientific World* 1, 703-712.

Sadinski, W. J. and Dunson, W. A. (1992). A multilevel study of effects of low pH on amphibians of temporary ponds. *Journal of Herpetology* 26, 413-422.

Semlitsch, R. and Bridges, C. M. (2005). Amphibian ecotoxicology. In M. Lannoo (Ed.), *Amphibian declines: The conservation status of United States species* (pp. 241-243). Berkeley: University of California Press.

Skelly, D. K., Freidenburg, L. K. and Kiesecker, J. M. (2002). Forest canopy and the performance of larval amphibians. *Ecology* 83, 983-992.

Sparling, D. W., Richter, K. O., Calhoun, A. and Micacchion, M. (2002). *Using amphibians in bioassessments of wetlands: What is the value of using amphibians in bioassessments?* (822-R-02-022) . United States Environmental Protection Agency.

Stebbins, R. C. and Cohen, N. W. (1995). *A natural history of amphibians*. Princeton: Princeton University Press.

Stiff, M. J. (1971). The chemical states of copper in polluted fresh water and a scheme of analysis to differentiate them. *Water Research Pergamon Press* 5, 585-599.

Stuart, S. N., Chanson, J. S., Cox, N. A., Young, B. E., Rodrigues, A. S. L., Fischman, D. L. and Waller, R. W. (2004). Status and trends of amphibian declines and extinctions worldwide. *Science* 306, 1783-1786.

Stumm, W. and Morgan, J. J. (1981). *Aquatic Chemistry*. New York: John Wiley & Sons.

Thunqvist, E. (2004). Regional increase of mean chloride concentration in water due to the application of deicing salt. *Science of the Total Environment* 325, 29-37.

Tietge, J. E., Diamond, S. A., Ankley, G. T., DeFoe, D. L., Holcombe, G. W., Jensen, K. M., Degitz, S. J., Elonen, G. E. and Hammer, E. (2001). Ambient solar UV radiation causes mortality in larvae of three species of *Rana* under controlled exposure conditions. *Photochemistry and Photobiology* 74, 261-268.

Wake, D. B. (1991). Declining amphibian populations. *Science* 253, 860.
Werner, E. E. and K. S. Glennemeier. (1999). Influence of forest canopy cover on the breeding pond distributions of several amphibian species. *Copeia* 1, 1-12.

Werner, E. E. and Glennemeier, K. S. (1999). Influence of forest canopy cover on the breeding pond distributions of several amphibian species. *Copeia* 1, 1-12.

Wu, H. J., Yen, C. F. and Kam, Y. C. (2007). Metabolic compensation and behavioral thermoregulation of subtropical rhacophorid (*Polypedates megacephalus*) tadpoles in container habitats. *Comparative Biochemistry and Physiology, Part B* 146, 101-106.

Wyman, R. L. (1990). What's happening to the amphibians? *Conservation Biology* 4, 350-352.

Zaga, A., Little, E., Rabeni, C. and Ellersieck, M. (1998). Photoenhanced toxicity of a carbamate insecticide to early life stage anuran amphibians. *Environmental Toxicology and Chemistry* 17, 2543-2553.

Zug, G. R. (1993*). Herpetology: An introductory biology of amphibians and reptiles*. San Diego: Academic Press, Inc.

APPENDICES

APPENDIX A

COPPER CALIBRATION TEST

The LaMotte TesTab Water Investigation Kit (Model AM-12, Code 5849) was calibrated using six treatment levels in mg/l (0, 0.25, 0.5, 1.0, 1.25, and 1.5). Three trials were conducted at each level and then graphed. The minimum detection level of the kit was calculated as 0.33 mg Cu/l using the trendline linear equation.

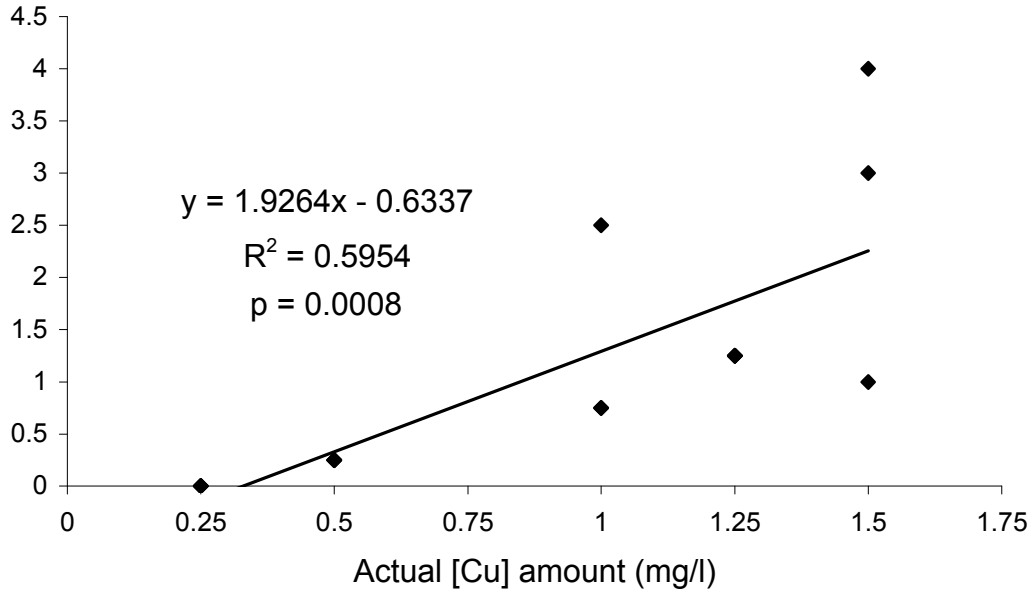

Figure 13. Test results for copper calibration analysis of the LaMotte TesTab Water Investigation Kit.

APPENDIX B

ANOVA FOR ALKALINITY AND HARDNESS

A one-way ANOVA was performed to test pond water used in this study for alkalinity and hardness using data collected from four rounds of water chemistry testing. No significant difference was found between treatments in alkalinity (Table 12). A significant difference was found between treatments in hardness (Table 13).

Table 12. Single Factor ANOVA for alkalinity.

Source of Variation	df	MS	F	p-value
Treatment	3	0.4150	0.0414	0.9882
Error	12	10.0179		

Table 13. Single Factor ANOVA for hardness.

Source of Variation	df	MS	F	p-value
Treatment	3	24.2839	6.1399	0.0090
Error	12	3.9551		

APPENDIX C

WATER TEMPERATURE, DO, AND PH

Table 14. Water chemistry data for water temperature, DO, and pH. “Bottom” refers to reading taken 6.0 cm from the bottom of the tank, “Middle” 28.0 cm from the bottom, and “Top” 48.0 cm from the bottom. All readings were taken in the center of the tanks from the tank edges.

		Air Temp.	Water Temperature ūC			Dissolved Oxygen			pH
Tank No.	Date	(ūC)	Bottom	Middle	Top	Bottom	Middle	Top	Middle
1	8-Apr	1.2	4.3	3.6	2.3	8.4	8.7	9.2	6.0
1	12-Apr	7.6	5.4	5.9	6.3	9	9.6	8.6	7.5
1	15-Apr	2.8	4.2	4.3	3.9	9.7	9.6	8.8	6.0
1	18-Apr	9.4	6	6.3	6.7	7.8	10	11.5	8.0
1	21-Apr	18.1	7.9	8	9	8.4	10.3	9.5	8.0
1	24-Apr	21.8	11.2	13.4	15.5	4.3	4.8	7.2	8.0
1	27-Apr	17.6	10.7	12.6	13.6	5.5	9.3	9.7	8.3
1	30-Apr	14	12.3	14.5	16.8	3.9	3.7	8.7	7.8
2	8-Apr	1.2	4.3	4.6	3.4	7.1	7.5	8.4	6.0
2	12-Apr	7.6	6	6.5	6.7	9.8	10.4	9.7	7.9
2	15-Apr	2.8	4.2	4.2	4.2	9.9	10.3	9.3	6.0
2	18-Apr	9.4	7.2	7.8	8.2	7.8	11.1	9.9	8.0
2	21-Apr	18.1	10	10.4	12.8	9.3	9.9	9.6	8.0
2	24-Apr	21.8	14.5	16.7	20.6	5.3	10.3	10	8.0
2	27-Apr	17.6	12.5	14.3	15.5	4.9	9.5	10.8	8.0
2	30-Apr	14	15	19.1	20	4.6	4.9	10.4	8.5
3	8-Apr	1.2	4.3	4.5	2.8	6.7	8.7	9.2	6.0
3	12-Apr	7.6	6	6.2	6.5	7	12	10.6	8.0
3	15-Apr	2.8	4.3	4.4	4.3	8.3	11.2	10.1	6.0
3	18-Apr	9.4	6.9	7.2	7.2	7.1	12.6	11.8	8.3
3	21-Apr	18.1	9	9.1	10.3	5.4	8.9	10.3	8.0
3	24-Apr	21.8	14.2	15.5	18.6	5.3	6	12.1	8.5
3	27-Apr	17.6	12	13.7	14.9	5.1	6.6	10.7	8.3
3	30-Apr	14	15.8	18	20.7	3.6	5.1	10.7	7.3

Table 14. Water chemistry data for water temperature, DO, and pH. "Bottom" refers to reading taken 6.0 cm from the bottom of the tan, "Middle" 28.0 cm from the bottom, and "Top" 48.0 cm from the bottom. All readings were taken in the center of the tanks from the tank edges. (continued)

		Air Temp.	Water Temperature ūC			Dissolved Oxygen			pH
Tank No.	Date	(ūC)	Bottom	Middle	Top	Bottom	Middle	Top	Middle
4	8-Apr	1.2	4.2	4.4	3	6.6	8.8	8.3	6.0
4	12-Apr	7.6	5.5	5.9	6.1	9.2	9.3	8.6	7.8
4	15-Apr	2.8	4.3	4.3	3.8	9	9.5	8.6	6.0
4	18-Apr	9.4	5.9	6.1	6.3	8.7	10.5	9.2	8.0
4	21-Apr	18.1	8.1	8.3	9.3	8.3	10	9.4	8.0
4	24-Apr	21.8	11.6	13	15.6	6.2	7.9	9.5	8.0
4	27-Apr	17.6	10.6	12.5	13.6	5.5	9.4	9.4	7.5
4	30-Apr	14	12.3	14.3	16.1	4.1	4.1	8.6	7.0
5	8-Apr	1.2	3.8	4.2	3.8	7.2	8.7	9.4	6.0
5	12-Apr	7.6	5.5	5.9	6.1	8.6	10.7	9.5	8.0
5	15-Apr	2.8	4.3	4.3	3.8	8	11.3	10.2	6.0
5	18-Apr	9.4	5.8	6.1	6.2	8.1	11.9	10.1	8.0
5	21-Apr	18.1	7.5	7.8	8.1	7.4	12	10.7	8.0
5	24-Apr	21.8	10.8	13.1	14.5	6.7	11	10.5	8.0
5	27-Apr	17.6	10.6	12.4	13.9	5.2	10.3	10.4	8.0
5	30-Apr	14	12.5	14.9	16	4	5.1	10	7.8
6	8-Apr	1.2	4.2	4.4	3.7	7.9	8.3	9.1	6.0
6	12-Apr	7.6	5.5	5.8	6	7.4	10.5	9.5	7.8
6	15-Apr	2.8	4.4	4.3	4.2	8.3	11.6	10.4	6.0
6	18-Apr	9.4	5.9	6	6	7.6	10.9	10.9	8.0
6	21-Apr	18.1	7.5	7.6	8	6.4	10.1	9.8	8.0
6	24-Apr	21.8	11.3	12.9	14.5	6.4	9.5	10.6	8.3
6	27-Apr	17.6	10.8	12.4	13.3	4.8	10.3	10.3	8.3
6	30-Apr	14	12.6	14.3	15.9	3.8	8.8	10.3	8.0

Table 14. Water chemistry data for water temperature, DO, and pH. “Bottom” refers to reading taken 6.0 cm from the bottom of the tan, “Middle” 28.0 cm from the bottom, and “Top” 48.0 cm from the bottom. All readings were taken in the center of the tanks from the tank edges. (continued)

		Air Temp.	Water Temperature ūC			Dissolved Oxygen			pH
Tank No.	Date	(ūC)	Bottom	Middle	Top	Bottom	Middle	Top	Middle
7	8-Apr	1.2	4.3	4.4	3.7	7.1	8.4	8.5	6.0
7	12-Apr	7.6	6	6.4	6.8	9.1	9.9	9.2	7.9
7	15-Apr	2.8	4.2	4.3	4.3	9	9.9	8.9	6.0
7	18-Apr	9.4	7.2	7.6	7.8	9.1	10.4	9.8	8.0
7	21-Apr	18.1	10	10.6	11.9	9.2	10	9.5	7.8
7	24-Apr	21.8	14.8	16.9	20.4	7.2	9.4	9.6	7.5
7	27-Apr	17.6	12.4	13.9	15.6	5.4	9.5	10.7	8.3
7	30-Apr	14	15.4	18.6	19.8	4.5	5.5	10.7	6.5
8	8-Apr	1.2	3.9	4.4	4.4	7.4	9	9.6	6.0
8	12-Apr	7.6	6.2	6.2	6.5	8.1	10.7	9.9	8.0
8	15-Apr	2.8	4.6	4.6	4.6	8.7	11.8	10.6	6.0
8	18-Apr	9.4	7	7.3	7.3	7.2	11.3	10.4	8.3
8	21-Apr	18.1	9.2	9.4	10	9.4	10.8	10.3	8.0
8	24-Apr	21.8	14.2	15.7	18.4	6.8	13.1	10.8	8.3
8	27-Apr	17.6	12.4	13.6	14.7	5.7	10.3	10.7	8.0
8	30-Apr	14	16	17.6	20.6	4.2	4.5	10.3	7.5
9	8-Apr	1.2	4.9	5	4.5	6.7	11	10	6.0
9	12-Apr	7.6	5.7	6.2	6.3	8.7	10.1	10.2	7.8
9	15-Apr	2.8	4.4	4.3	3.9	8.2	11.4	10.9	6.0
9	18-Apr	9.4	6.6	7	7.1	8.8	12.7	11.7	8.3
9	21-Apr	18.1	8.9	9.3	10.2	7.1	12	11.7	8.3
9	24-Apr	21.8	13.2	15	17.6	9.7	11.4	11.3	8.3
9	27-Apr	17.6	11.3	13.2	14.3	5.6	11	10.4	8.3
9	30-Apr	14	14.8	17.8	19.7	4.2	8.9	11.1	7.8

Table 14. Water chemistry data for water temperature, DO, and pH. "Bottom" refers to reading taken 6.0 cm from the bottom of the tan, "Middle" 28.0 cm from the bottom, and "Top" 48.0 cm from the bottom. All readings were taken in the center of the tanks from the tank edges. (continued)

		Air Temp.	Water Temperature ūC			Dissolved Oxygen			pH
Tank No.	Date	(ūC)	Bottom	Middle	Top	Bottom	Middle	Top	Middle
10	8-Apr	1.2	4.7	4.9	4.9	7	8.7	8.7	6.0
10	12-Apr	7.6	5.8	6.2	6.8	8.7	9.6	9.3	7.6
10	15-Apr	2.8	4.4	4.3	4.3	8.4	9.5	8.9	6.0
10	18-Apr	9.4	7.1	7.4	7.6	9	9.9	9.2	8.0
10	21-Apr	18.1	9.7	10.3	11.9	8.1	9.7	9.1	8.0
10	24-Apr	21.8	14.3	16.5	20.1	7.4	9.4	9.6	7.5
10	27-Apr	17.6	11.8	13.9	15.1	5.5	9.3	9.7	8.0
10	30-Apr	14	14.7	18.3	19.4	4.1	6.9	11.5	7.3
11	8-Apr	1.2	4.6	4.6	4.5	8.7	8	10	6.0
11	12-Apr	7.6	5.2	5.7	6.1	9	9.6	9.1	8.4
11	15-Apr	2.8	4.3	4.2	3.4	8.2	9.6	8.9	6.0
11	18-Apr	9.4	5.8	6.2	6.3	9.4	10.2	9.6	8.0
11	21-Apr	18.1	8	8.3	9.3	8.3	10.3	9.5	8.0
11	24-Apr	21.8	11.6	13.6	16.3	6.4	9.3	9.6	7.5
11	27-Apr	17.6	10.4	12.2	13.8	4.9	9.7	10.5	8.0
11	30-Apr	14	12.1	14.6	16.2	4.1	5.4	9.5	7.8
12	8-Apr	1.2	4.8	4.8	4.8	7.5	10.5	9	6.0
12	12-Apr	7.6	5.6	5.9	6.1	9	11.4	10.5	8.0
12	15-Apr	2.8	4.4	4.3	4.2	9.7	11.7	10	6.0
12	18-Apr	9.4	5.8	6.2	6.2	9.8	12.5	10.7	8.3
12	21-Apr	18.1	8.1	8.3	9.6	7.6	11.6	10.7	8.0
12	24-Apr	21.8	11.8	13.8	15.9	6.8	12.5	11.5	8.3
12	27-Apr	17.6	10.6	12.4	13.6	4.9	10.3	10.4	8.0
12	30-Apr	14	12.5	14.6	16.7	4.1	6.7	11	8.0

Table 14. Water chemistry data for water temperature, DO, and pH. “Bottom” refers to reading taken 6.0 cm from the bottom of the tan, “Middle” 28.0 cm from the bottom, and “Top” 48.0 cm from the bottom. All readings were taken in the center of the tanks from the tank edges. (continued)

		Air Temp.	Water Temperature ūC			Dissolved Oxygen			pH
Tank No.	Date	(ūC)	Bottom	Middle	Top	Bottom	Middle	Top	Middle
13	8-Apr	1.2	4.6	4.7	4.8	7.4	11.1	9.3	6.0
13	12-Apr	7.6	5.6	6.2	6.5	8.5	10.6	9.9	7.5
13	15-Apr	2.8	4.3	4.3	3.5	8.6	11.1	10	6.0
13	18-Apr	9.4	6.3	6.8	6.9	9	11.4	10	8.0
13	21-Apr	18.1	8.3	8.7	9.8	7.5	10.2	9.7	8.0
13	24-Apr	21.8	12.6	14.3	16.9	7.1	10.4	10	8.0
13	27-Apr	17.6	10.9	13	14.5	5.8	10	10.2	8.3
13	30-Apr	14	14	17.4	19.2	4.3	6.9	10.1	7.8
14	8-Apr	1.2	4.7	4.6	4.4	9.1	9.2	10.3	6.0
14	12-Apr	7.6	5.6	6.2	6.4	8.7	9.8	9.8	8.0
14	15-Apr	2.8	4.3	4.3	4.2	7.5	9.3	9.1	6.0
14	18-Apr	9.4	6.6	7.1	7.3	10.6	10.8	9.6	8.0
14	21-Apr	18.1	9.5	10.1	11.5	7.7	9.7	9.1	8.0
14	24-Apr	21.8	13.4	16.2	19.1	6.6	8.8	9.6	7.5
14	27-Apr	17.6	11.8	13.9	14.9	5.3	8.6	10.2	7.8
14	30-Apr	14	13.8	17.2	18.6	3.9	5.9	11.2	7.8
15	8-Apr	1.2	4.7	4.7	4.6	8.1	8.9	9.6	6.0
15	12-Apr	7.6	5.1	5.6	6	8.7	10	9.8	7.9
15	15-Apr	2.8	4.3	4.3	3.5	7.8	10.2	9.1	6.0
15	18-Apr	9.4	5.9	6.2	6.5	10	10.7	10	8.3
15	21-Apr	18.1	8.2	8.9	9.3	5.4	5.9	10.5	8.0
15	24-Apr	21.8	11.6	13.5	16.2	6.7	9.8	10.3	7.5
15	27-Apr	17.6	10.6	12.3	13.6	4.3	4.6	9	7.8
15	30-Apr	14	12.3	14.7	16.2	4.3	4.6	12.4	7.8
16	8-Apr	1.2	4.5	4.6	3.1	7.6	10.8	10.5	6.0
16	12-Apr	7.6	5.4	5.9	6.2	9.2	11.4	10.6	8.0
16	15-Apr	2.8	4.3	4.3	3.6	9.4	11.4	10.8	6.0
16	18-Apr	9.4	5.8	6.3	6.4	9.7	13.1	11.3	8.3
16	21-Apr	18.1	7.9	8.2	9.2	8.7	10.7	11.4	8.3
16	24-Apr	21.8	11.5	13.5	16	7.2	12.5	11.5	8.3
16	27-Apr	17.6	11	13.2	13.9	4.6	6	10.3	8.3
16	30-Apr	14	12.5	15	17.5	4.2	4.6	11.2	7.8
16	4-May	21.9	12.4	12.6	12.9	3.5	3.4	3.3	8.3

APPENDIX D

FOUR ROUNDS OF WATER CHEMISTRY DATA

Table 15. Four rounds of water chemistry data for [Cu], hardness, alkalinity, and pH.

Tank No.	Date	[Cu] "A"	[C] "B"	Hardness "A"	Hardness "B"	Alkalinity "A"	Alkalinity "B"	pH "C"
1	7-Apr	0.00	0.75	80	80	50	70	6.0
1	11-Apr	1.00	0.75	80	80	80	80	6.0
1	29-Apr	0.75	0.50	80	80	70	80	7.8
1	3-May	0.75	1.00	80	80	80	80	8.0
2	7-Apr	0.00	0.75	80	80	50	50	6.0
2	11-Apr	0.50	0.75	80	80	65	80	6.0
2	29-Apr	0.50	0.25	75	80	80	80	8.5
2	3-May	0.50	0.75	80	80	70	60	8.3
3	7-Apr	0.00	0.00	80	80	50	60	6.0
3	11-Apr	0.25	0.00	75	80	80	80	6.0
3	29-Apr	0.00	0.00	70	60	80	80	7.3
3	3-May	0.00	0.00	65	45	65	70	7.8
4	7-Apr	0.50	0.88	80	80	50	65	6.0
4	11-Apr	0.75	0.75	80	80	75	80	6.0
4	29-Apr	1.00	0.75	70	80	80	80	7.0
4	3-May	1.00	1.00	75	70	65	60	8.0
5	7-Apr	0.00	0.00	80	80	45	75	6.0
5	11-Apr	0.00	0.00	80	80	75	80	6.0
5	29-Apr	0.00	0.00	70	65	80	80	7.8
5	3-May	0.00	0.00	70	65	70	65	7.5

Table 15. Four rounds of water chemistry data for [Cu], hardness, alkalinity, and pH. (continued)

Tank No.	Date	[Cu] "A"	[C] "B"	Hardness "A"	Hardness "B"	Alkalinity "A"	Alkalinity "B"	pH "C"
6	7-Apr	0.00	0.00	80	80	45	75	6.0
6	11-Apr	0.00	0.00	80	80	80	80	6.0
6	29-Apr	0.00	0.00	60	70	80	75	8.0
6	3-May	0.00	0.00	70	65	60	65	7.5
7	7-Apr	0.00	0.75	80	80	45	80	6.0
7	11-Apr	1.00	1.00	80	80	80	80	6.0
7	29-Apr	0.50	0.75	75	70	80	80	6.5
7	3-May	0.75	0.75	70	75	80	80	8.3
8	7-Apr	0.00	0.00	80	80	45	80	6.0
8	11-Apr	0.00	0.00	80	80	80	80	6.0
8	29-Apr	0.00	0.00	60	70	80	70	7.5
8	3-May	0.00	0.00	65	75	75	80	8.3
9	7-Apr	0.00	0.00	80	80	45	75	6.0
9	11-Apr	0.00	0.00	80	80	80	80	6.0
9	29-Apr	0.00	0.00	65	75	80	80	7.8
9	3-May	0.00	0.00	60	70	80	80	8.3
10	7-Apr	0.50	0.75	80	80	45	65	6.0
10	11-Apr	1.00	0.00	80	80	70	80	6.0
10	29-Apr	0.50	0.50	80	75	80	80	7.3
10	3-May	0.75	0.50	65	70	80	80	7.8

Table 15. Four rounds of water chemistry data for [Cu], hardness, alkalinity, and pH. (continued)

Tank No.	Date	[Cu] "A"	[C] "B"	Hardness "A"	Hardness "B"	Alkalinity "A"	Alkalinity "B"	pH "C"
11	7-Apr	0.50	0.75	80	80	45	70	6.0
11	11-Apr	0.75	1.00	80	80	65	80	6.0
11	29-Apr	1.00	1.00	70	80	80	80	7.8
11	3-May	1.25	1.25	70	60	65	70	8.0
12	7-Apr	0.00	0.00	80	80	45	75	6.0
12	11-Apr	0.00	0.00	65	80	80	80	6.0
12	29-Apr	0.00	0.00	65	75	80	80	8.0
12	3-May	0.00	0.00	80	80	80	80	8.0
13	7-Apr	0.00	0.00	80	80	45	70	6.0
13	11-Apr	0.00	0.50	75	80	80	80	6.0
13	29-Apr	0.00	0.00	70	65	80	80	7.8
13	3-May	0.00	0.25	55	80	65	65	8.0
14	7-Apr	0.50	0.50	80	80	45	70	6.0
14	11-Apr	0.75	0.75	80	80	75	80	6.0
14	29-Apr	0.50	0.50	75	75	80	90	7.8
14	3-May	0.75	0.75	80	70	80	80	8.0
15	7-Apr	0.50	0.75	80	80	40	70	6.0
15	11-Apr	0.75	1.00	80	70	80	90	6.0
15	29-Apr	1.00	0.75	75	80	80	90	7.8
15	3-May	1.00	1.25	65	75	80	80	7.8
16	7-Apr	0.00	0.00	80	80	65	75	6.0
16	11-Apr	0.00	0.00	80	80	80	80	6.0
16	29-Apr	0.00	0.00	55	60	80	65	7.8
16	3-May	0.00	0.00	60	70	80	80	8.3