RESPONSES OF PROBLEMATIC CYANOBACTERIA TO EXPOSURES OF COPPER CONTAINING ALGAECIDES

O'niell Tedrow
Clemson University, otedrow@clemson.edu

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RESPONSES OF PROBLEMATIC CYANOBACTERIA TO EXPOSURES OF COPPER CONTAINING ALGAECIDES

A Thesis
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science
Forest Resources

by
O’Niell Roy Tedrow
August 2007

Accepted by:
Dr. John H. Rodgers, Jr., Committee Chair
Dr. John J. Hains, Jr.
Dr. Thomas E. Schwedler
ABSTRACT

Cyanobacteria (blue-green algae) are associated with production of potent hepatotoxins (*Microcystis*; microcystin) and dense surface and benthic mats (*Lyngbya*), which impede critical water resource usages. Water resource managers are in need of effective and efficient treatment techniques for these problematic algae in field situations. Applications of algaecides are considered in situations where algal problems become acute or when critical water usages are threatened. However, laboratory data are needed that accurately predict responses of algae prior to field-scale algaecide applications. Site water and algae were used in laboratory algal toxicity experiments to predict responses of the target algae following an algaecide exposure and to develop a site-specific treatment strategy. Laboratory results were used to guide field-scale applications of specific algaecides in specific water resources. With strategic monitoring programs and efficient use of effective algaecides, problems caused by cyanobacteria can be managed and critical water resource usages can be restored and maintained.
DEDICATION

I would like to dedicate this Thesis to my family; Judy, Jerry, and Noel Tedrow. Research in this Thesis could not have been completed without the incredible support of all my family, and friends, who put up with my long hours, missed Holidays, short vacations, and cancelled plans. I truly owe my sanity to their understanding; especially my Dad. He is my inspiration and the reason for me achieving this degree.

Do good, be kind, and goodness and kindness will follow.

“Every silver lining’s got a touch of gray. We will get by, we will survive.

- Robert Hunter.
ACKNOWLEDGMENTS

I would like to acknowledge Applied Biochemists, a division of Advantis, Inc., the Nebraska Game and Parks Commission, the City of High Point, North Carolina, and Midwest APMS for funding the research presented in this Thesis. I would also like to thank 3M Corporation and the Midwest SETAC organization for the monetary travel award for Best Student Presentation (March 2006) at the annual Midwest SETAC meeting in St. Cloud, Minnesota.

I also thank my advisor Dr. John H. Rodgers, Jr., for his patience, support, and willingness to make time for all of his students, regardless of his own responsibilities. For this, our entire team is thankful. I thank my committee members, Dr. John Hains and Dr. Thomas Schwedler for helpful suggestions in preparing these manuscripts. I also thank Dr. Louwanda Jolley and Dr. Wayne Chao for their invaluable analytical assistance. Also, I would like to thank the entire Forestry Department faculty and all of the graduate students in this Forestry Department that have come and gone during my time here.

I also thank Dr’s. Matthew Julius and Heiko Schoenfuss of the Saint Cloud State University Biological Sciences Department (Saint Cloud, Minnesota). Without their support and encouragement, I would never have presented at Midwest SETAC and would have never had this opportunity.

And how could I forget every Cracker Barrel that I marked off on my map to and from field sites and the countless pounds of ribs from Sardies that kept me from withering into oblivion.
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CHAPTER 1

INTRODUCTION

Algae can interfere with water resource usages by attaining densities or producing and releasing compounds (i.e. toxins, taste-and-odor) that impede use of the water resource (Hallegraeff 1993; WHO 2003). In situations where this resource is used for critical purposes such as production of potable water, livestock watering, or aquaculture facilities, managers may need to initiate control strategies to mitigate risks caused by problematic algal densities. The research described in this Thesis is part of an ongoing effort developing effective and efficient approaches to control problematic algal growths of planktonic and benthic algae. This research focused on using site water, site collected algae, and realistic laboratory algaecide exposures to predict field responses of algae to an algaecide exposure.

Some species of cyanobacteria (blue-green algae) have been implicated in production and release of secondary compounds that affect taste and odor of infested water bodies. Taste-and-odor causing compounds taint farm-raised fish and cost catfish farmers in the United States millions of dollars per year (Walker and Higginbotham 2000; Lawton et al. 2003). Certain cyanobacterial species may produce potent toxins at or above concentrations of concern (Sivonen 1996; WHO 2003). Production and release of microcystin has caused numerous and repeated public-use warnings in lakes and reservoirs across the United States. Cyanobacterial toxins, such as microcystin, present in concentrations of concern
(≥ 1.0 μg L⁻¹; WHO 2003) can pose threats to humans, domestic pets and livestock, and potentially other wildlife that may use the affected water resource for potable water or other purposes (Falconer 1996, 1999; Carmichael et al. 2001; Briand et al. 2003; Malbrouck and Kestemont 2006).

Other species of cyanobacteria, such as *Lyngbya* sp. can be associated with production of toxins, but primarily cause aesthetic and water use problems associated with excessive growth (i.e. benthic and surface mats of *Lyngbya*). In many reservoirs in the southeastern United States, *Lyngbya* growths have impeded water resources for public and private use. Surface-mat infestations of *Lyngbya* can reach densities restricting use of water resources (Speziale et al. 1991; Speziale and Dyck 1992).

An approach used to control the growth of these cyanobacteria is treatment using copper sulfate or chelated-copper containing algaecides. However, reports of release of toxins or taste-and-odor compounds following treatment of cyanobacteria with copper containing algaecides, such as Coptrol® (Jones and Orr 1994; Peterson et al. 1995; Lawton et al. 2003) have been published and have caused water resource managers concern.

In practical situations (i.e. field applications), release of secondary compounds has not been observed (Mastin et al. 2002). Observations in Indianapolis (Eagle Creek Reservoir) have suggested that specifically determined applications of copper-based formulations (e.g. Cutrine®-Plus or Cutrine®-Ultra) do not result in algal release, or elevation of aqueous concentrations of geosmin (GSM) or 2-methylisoborneol (MIB) following a field algaecide treatment. It is
likely that some concentrations of copper as algaecide would target algal cell membranes, resulting in lysis and leakage of cytoplasm and intracellular constituents. However, it is also likely that some concentrations or formulations of copper as algaecide would target intracellular metabolic processes resulting in cell death without release of intracellular compounds. If a specific treatment (i.e. concentration and duration of exposure) of copper as algaecide does not cause release and subsequent increase in ambient water concentration of either GSM or MIB but controls growth of nuisance algae, the same may be true for algal toxins such as microcystin.

Experimental Objectives

Initially, this research focused on *Microcystis aeruginosa* in Pawnee Reservoir, Nebraska, which produces microcystin. Site water collected from Pawnee Reservoir served as the source of *Microcystis* that produces microcystin. Pawnee Reservoir has a surface area of approximately 300 hectares (USACE; Jay Woltemath, pers. comm., Nebraska Game and Parks Commission). Watershed soils around Pawnee Reservoir are susceptible to erosion and are rich in nutrients such as nitrogen and phosphorus. Areas surrounding Pawnee Reservoir are used extensively for agricultural purposes such as crop production and pastureland. Although this reservoir was developed primarily for flood control, recreational uses have increased and generate public and private revenue. These uses are impaired due to problematic algal growths such as *Microcystis*. Over 200 campsites and recreation areas are located around Pawnee Reservoir (USACE;
Jay Woltemath, pers. comm.). I tested the hypothesis that there is no statistically significant increase in aqueous or total microcystin, above background microcystin concentration, following exposure of *Microcystis* to different formulations of copper as strongly chelated forms (Cutrine®-Ultra and Clearigate®), a weakly chelated form (Algimycin® PWF), and a copper salt (copper sulfate pentahydrate) at 0.2, 0.4, 0.6, 0.8, and 1.0 mg Cu L⁻¹ in site water after a 96 hour exposure duration.

This research also focused on field confirmation of laboratory results. Primary objectives of this study were to: 1) measure the time-course responses of *Microcystis* following an application of Cutrine®-Ultra in Pawnee Reservoir, and 2) compare similar laboratory and field exposures in Pawnee Reservoir and responses of *Microcystis* to those exposures. Response parameters were measured as chlorophyll *a* concentrations, *Microcystis* cell densities, and microcystin concentrations. Pre-treatment measurements included chlorophyll *a* concentrations, algal cell densities, and microcystin concentrations. Water characteristics [i.e. *pH* (SU), total alkalinity (mg L⁻¹ as CaCO₃), total hardness (mg L⁻¹ as CaCO₃), dissolved oxygen (mg O₂ L⁻¹), conductivity (μS cm⁻²), and temperature (°C)] were measured according to APHA (1998) methods pre- and post- treatment as described by a pre-determined sampling / monitoring protocol. Responses of *Microcystis* after treatment using Cutrine®-Ultra were measured in terms of chlorophyll *a* concentrations, algal cell densities, and aqueous and total microcystin concentrations.
Finally, this research focused on field confirmation of laboratory results using Algimycin® PWF to control growth of *Lyngbya* present in a potable water supply (City Lake) in High Point, North Carolina. *Lyngbya* is a benthic, filamentous cyanobacterium (blue-green alga), which forms dense benthic mats in infested water bodies. These dense algal mats may float to the water’s surface. These surface mats of *Lyngbya* may be many meters thick (Speziale et al. 1991; Speziale and Dyck 1992). *Lyngbya* and site water were sampled from City Lake. Following laboratory exposures to two different forms of copper (Algimycin® PWF and Clearigate®) and a sequential algaecide technique (PAK™ 27, 24 hours prior to Algimycin® PWF with Cide-Kick® II), Algimycin® PWF was chosen for treatment of *Lyngbya* in City Lake. Control of this alga in laboratory experiments was measured as µg chlorophyll *a* gram⁻¹ of *Lyngbya* after a 96 hour exposure. Measured field responses of this alga included chlorophyll *a* concentrations and algal biomass pre- and post- treatment with Algimycin® PWF.

**Organization of this Thesis**

This Thesis is organized into five chapters, including introductory information as Chapter One and Summary of Conclusions as Chapter Five. Chapters Two and Four are formatted for submission to the *Journal of Aquatic Plant Management*, and Chapter Three is formatted for submission to *Environmental Toxicology*. Some replication of information was therefore necessary.
Literature Cited


CHAPTER 2
RESPONSES OF MICROCYSTIS AERUGINOSA TO COPPER-CONTAINING ALGAECIDE EXPOSURES AND THE POTENTIAL FOR POST-TREATMENT RELEASE OF MICROCYSTIN

Abstract
Laboratory algaecide exposures were conducted using water from Pawnee Reservoir, Nebraska, to assess the efficacy of four copper-containing algaecides (Cutrine®-Ultra, Clearigate®, Algimycin® PWF, and copper sulfate pentahydrate) for treatment of Microcystis aeruginosa (Kütz. em. Elenkin) and to determine the potential for post-treatment release of microcystin. Three techniques (freezing-and-thawing, grinding, and sonication) to extract cell-associated microcystin were compared, and sonication was used to measure the time-course release of microcystin after exposure to Cutrine®-Ultra. Five concentrations (0.2, 0.4, 0.6, 0.8, and 1.0 mg Cu L⁻¹) of copper as algaecide and an unexposed control were used for laboratory exposures. Responses measured after exposures included chlorophyll a concentrations, cell densities, and microcystin concentrations. After a 96 hour exposure, control of this alga was achieved using 0.2 mg Cu L⁻¹ as Cutrine®-Ultra, five times less than the maximum label application concentration. Of the techniques tested, sonication was most effective for measurement of total microcystin based on internal standard additions of microcystin-LR. Aqueous microcystin concentrations increased from ~0.4 to ~3.5 μg L⁻¹, six hours after
exposure to $\geq 0.2$ mg Cu L$^{-1}$ as Cutrine$^\text{®}$-Ultra. However, aqueous microcystin concentrations decreased to background after 48 hours of exposure. Increases in aqueous and total microcystin after treatment were transitory and not of ecological or human health significance. With strategic monitoring programs and efficacious use of appropriate algaecides, risks due to microcystin can be managed and water resource usages can be restored.

*Key Words:* Pawnee Reservoir, toxin, cyanobacteria, algae control, Cutrine$^\text{®}$-Ultra, copper.

**Introduction**

That the incidence of Harmful Algal Blooms (HABs) is increasing with climate change is debatable (Hallegraeff 1993), but increasing demands on impacted water resources have focused attention on reservoirs and other aquatic systems that serve as domestic water supplies or for other important purposes such as livestock watering, irrigation, and recreation. Pawnee Reservoir in Emerald, Nebraska (Lancaster County) has experienced extensive and repeated “blooms” of *Microcystis aeruginosa* (Kütz. em. Elenkin) accompanied by total concentrations of microcystin at, or above, levels of concern for human health (WHO 2003). In situations where water resources are used for domestic water supply, agricultural water supply, or contact recreation, production of a potent hepatotoxin such as microcystin by *Microcystis* can prohibit use of the resource and pose risks for humans, pets, livestock, and potentially other wildlife (Falconer
1996, 1999; Carmichael et al. 2001; Briand et al. 2003; Malbrouck and Kestemont 2006). Often the onset of a problem due to Microcystis is signaled and measured in terms of algal cell density in water samples as well as total microcystin concentrations, or the presence of microcystin-synthetase genes in an algal sample (Saker et al. 2006).

If the water resource usage is necessary or essential and the presence of Microcystis obviates that usage, then water resource managers may be compelled to take action. Importantly, the course of action to mitigate a problem caused by the HAB should not result in a more severe problem. Frequently, water resource managers carefully evaluate the onset of a problem such as a HAB, its potential progression, and consequences of a control action such as application of algaecide. To make these management decisions, resource managers may evaluate all practical and legal alternatives such as mechanical, biological, and physical as well as chemical control strategies (including nutrient source control). Applications of algaecides are often the most cost effective and environmentally sound approaches for managing HABs such as Microcystis (Mastin et al. 2002).

Reports of releases of toxins and taste-and-odor compounds following treatment of cyanobacteria with copper-containing algaecides, such as Coptrol® and other chemicals such as chlorine, copper sulfate, potassium permanganate, calcium hydroxide, and hydrogen peroxide used in potable water production facilities, have been published and have caused water resource managers concern (Kenefick et al. 1993; Jones and Orr 1994; Peterson et al. 1995). The veracity and universal application of conclusions drawn in these reports have not been
evaluated. In practical situations (i.e. field applications), production of taste-and-odor compounds and release of toxins have not been observed after treatments (David Isaacs, pers. comm.; Mastin et al. 2002). Given the current prevalence and the potential magnitude of these problems, the question deserves further study.

To make sound risk-based decisions, accurate measurements of microcystin are important, especially in situations where human health may be at risk. Although measurement of aqueous microcystin signals post-treatment release of this hepatotoxic endotoxin (Lam et al. 1995; Sangolkar et al. 2006) from algal cells, total microcystin measurements are used to make risk-based decisions regarding continued utilization of water resources (WHO 2003). Several cell disruption methods have been used for extraction of total microcystin including: 1) freezing-and-thawing to rupture cells and extract cell-associated microcystin (John Lund, pers. comm., Nebraska Department of Environmental Quality; Frank 2002; Grützmacher 2002), 2) grinding cells using a tissue grinder to rupture cells (Bernard et al. 2004), and 3) sonication to disrupt cells (Ross et al. 2006; Zhang et al. 2006). These methods expose microcystin from ruptured cells to potential degradative enzymes and processes, and may cause a method-dependent difference in measured concentrations of microcystin. Data are needed to discern differences in these methods for extraction of cell-associated microcystin. Analytical methods for detection and measurement of microcystin include high pressure liquid chromatography (HPLC), liquid chromatography / mass spectroscopy (LC / MS), and enzyme linked immunosorbent assay (ELISA) techniques (Sangolkar et al. 2006; Yuan et al. 2006). In these experiments, ELISA
was used due to its sensitivity, specificity, and efficacy relative to the other available analytical methods.

A “bloom” of *Microcystis aeruginosa* in Pawnee Reservoir provided an opportunity to further evaluate the potential for release of microcystin following an algaecide treatment. Since all algaecide exposures do not elicit the same response from the target algal species, it is necessary to identify an efficacious algaecidal treatment in terms of the minimum exposure concentration and duration required to achieve the desired level of control (Fitzgerald and Jackson 1979; Murray-Gulde et al. 2002). For the present laboratory study, four copper-containing algaecides (Cutrine®-Ultra, Clearigate®, Algimycin® PWF, and copper sulfate pentahydrate) were used for treatment of *Microcystis* in water sampled on several dates from Pawnee Reservoir. Therefore, the objectives of the present study were to: 1) identify an efficacious algaecide and treatment concentration to eliminate *Microcystis* from Pawnee Reservoir water in laboratory exposures, 2) measure responses of *Microcystis* in terms of chlorophyll *a* concentrations, algal cell densities, and microcystin concentrations in water samples following individual exposures to four copper containing algaecides, 3) evaluate three techniques used for extraction and measurement of total microcystin, and 4) measure the time-course release of microcystin from *Microcystis* after an exposure to Cutrine®-Ultra.
Materials and Methods

Site Characteristics: Pawnee Reservoir, Nebraska (Algae and Water Source)

Pawnee Reservoir has a surface area of approximately 300 hectares and a maximum and mean depth of approximately 12.8 and 3.4 meters, respectively. Water retention time of Pawnee Reservoir is ~1.4 years with only brief periods of thermal stratification during summer months due to wind action and recreational use of this resource (USACE; USEPA 2001; Popp et al. 1996; Holz et al. 1997). Watershed soils around Pawnee Reservoir are highly susceptible to erosion and are rich in nutrients. Areas surrounding this reservoir are used extensively for agricultural purposes such as crop production and pastureland (Popp et al. 1996; Holz et al. 1997). Two primary beneficial uses of Pawnee Reservoir that are impaired due to problematic algal growths such as Microcystis include primary contact recreation and agricultural water supply. Over 200 campsites and recreation areas are located around Pawnee Reservoir (USACE).

For laboratory algaecide exposures, three water samples were collected (June, 2005; August, 2005; August, 2006) from Pawnee Reservoir that contained Microcystis that produced problematic concentrations of microcystin (≥ 20 µg L⁻¹; Nebraska Department of Health and Human Services 2006). The first sample (June 2005), approximately 40 liters, had a Microcystis cell density (> 10⁵ cells mL⁻¹) in excess of normal densities for this reservoir. Since this sample was collected from in-shore areas of windward accumulations, it was not representative of field densities of Microcystis, and the sample was diluted prior to laboratory experiment initiation. Responses of algae to exposures of algaecides

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are density dependent (Murray-Gulde et al. 2002) and the sample was diluted to obtain an initial experimental *Microcystis* cell density of $2.17 \times 10^5$ cells mL$^{-1}$. This dilution of Pawnee Reservoir water was used for the initial algal toxicity experiment with the algaecide Cutrine®-Ultra. The second sample (August 2005), approximately 20 liters, was representative of field densities of *Microcystis* in this reservoir ($3.12 \times 10^5$ cells mL$^{-1}$) and was used as collected in algal toxicity experiments with three algaecides: Clearigate®, Algimycin® PWF, and copper sulfate pentahydrate. A third sample (August 2006) was collected from Pawnee Reservoir for use in the time-course release of microcystin experiment following exposure of *Microcystis* to Cutrine®-Ultra. This sample was representative of field densities of *Microcystis* and was used as collected.

**Preparation of Stock Algaecide Solutions for Experiments**

Three copper-containing algaecides (Cutrine®-Ultra, Clearigate®, and Algimycin® PWF) and copper sulfate pentahydrate were evaluated for efficacy in controlling *Microcystis* from water sampled from Pawnee Reservoir. Cutrine®-Ultra is a chelated-copper containing compound, with an elemental copper concentration of 9% in the form of mixed copper mono- and tri- ethanolamine complexes plus an emulsified surfactant, D-limonene [Applied Biochemists 2002c]. Clearigate® contains 3.825% elemental copper by weight, which is formulated as ethanolamine complexes, plus an emulsified surfactant, D-limonene (Applied Biochemists 2005). Algimycin® PWF has a copper concentration of 5% in the form of copper sulfate pentahydrate, copper citrate, and other gluconate
chelates (Applied Biochemists 2002a, b). Copper sulfate pentahydrate (~25.5% copper; Sigma Chemical Co., P.O. Box 14508, St. Louis, MO 63178 USA) is a non-chelated form of copper and is representative of the common copper salt used as an algaecide. Stock algaecide solutions (100 mg Cu L\(^{-1}\)) for treatments were prepared using NANOpure™ water within four hours prior to experiment initiation.

**Algal Toxicity Experiments**

Algal toxicity experiments using each of the four algaecides were initiated using four replicates of treatment concentrations of 0.2, 0.4, 0.6, 0.8, and 1.0 mg Cu L\(^{-1}\) as algaecide, and an untreated control. Treatment concentrations were achieved by adding relatively small volumes of stock algaecide solutions (stock = 100 mg Cu L\(^{-1}\)) to site water containing *Microcystis* and completing the dilution to volume in a 1-L volumetric flask using site water. Experimental chambers consisted of static, non-renewal 200 mL volumes of treated or untreated site water containing the target algae in Erlenmeyer flasks that were swirled once daily by hand. Exposure duration for each experiment was 96 hours. A 16:8 hour light-dark cycle was used, with “cool white” fluorescent lighting at an intensity of 86 ± 8.6 μE m\(^{-2}\) s\(^{-1}\). Exposure chambers were maintained at a temperature of 22 ± 2°C (modified from APHA 1998). Total copper concentrations were measured using a Perkin-Elmer 5100 PC flame and graphite furnace atomic absorption (AA) spectrophotometer (method 3010-B; APHA 1998) (Table 2.1). Approximately 200 μL trace-metal grade nitric acid (Fisher Scientific, Fair Lawn, NJ 07410) was
used to acidify all aqueous copper samples (~12 mL; $pH \leq 2$) (total) $\geq 24$ hours prior to measurement. Water characteristics [$pH$ (Standard Units), dissolved oxygen (D.O.; mg O$_2$ L$^{-1}$), conductivity (μS cm$^{-2}$), total alkalinity (mg L$^{-1}$ as CaCO$_3$), total hardness (mg L$^{-1}$ as CaCO$_3$), and temperature ($^\circ$C)] were measured according to APHA (1998) methods (Tables 2.2, 2.3). Characteristics of Pawnee Reservoir water used for the algal toxicity tests ranged from moderately-hard to hard (Table 2.2) (Sawyer et al. 1994).

**Algal Response Parameters**

Response parameters measured for the algal toxicity experiments included chlorophyll $a$ concentrations, algal cell densities, and microcystin concentrations. Chlorophyll $a$ was extracted according to United States Environmental Protection Agency (USEPA) method 445.0 (Arar and Collins 1997). Chlorophyll $a$ was measured fluorometrically using a SpectraMax® 190 Gemini 96 well plate spectrofluorometer (Molecular Devices Corporation, Sunnyvale, CA 94089). Fluorometric measurements of test samples were calibrated with fluorescence values of known chlorophyll $a$ standards. Chlorophyll $a$ standards were prepared from a stock solution of 4000 μg chlorophyll $a$ L$^{-1}$ (Sigma C-5753; range = 10-1280 μg chlorophyll $a$ L$^{-1}$), kept protected from light and stored in a -20°C freezer. Algal cell densities were measured using an Improved Neubauer hemacytometer (DiBartolomeis and Mone´ 2003).

Concentrations of aqueous and total microcystin were measured using an enzyme-linked immunosorbent assay (ELISA) kit (Envirologix®, Portland, ME
04103) specific for variants of the algal toxins microcystin and nodularin. Samples for aqueous microcystin measurement were collected from each treatment replicate, filtered using a 0.45 μm filter, and refrigerated in glass vials wrapped in foil. In the initial experiments using Clearigate®, Algimycin® PWF, and copper sulfate pentahydrate to determine an efficacious algaecide treatment, samples for total microcystin measurement were collected from each treatment replicate. Samples of water containing Microcystis were frozen and thawed (using a 30-35°C water bath) three times prior to measurement of total microcystin (Frank 2002; Grützmacher et al. 2002). After freezing-and-thawing, samples were filtered using a cellulose membrane filter (0.45 μm) prior to measurement. Absorbance of the antibody-linked microcystin was measured using a Molecular Devices “Emax” precision microplate reader (Molecular Devices, Menlo Park, CA).

Cell-Associated Microcystin Extraction Techniques for Total Microcystin Measurements

In a subsequent experiment, three techniques for extraction of cell-associated microcystin were evaluated to discern potential method-dependent differences in measured total microcystin concentrations: 1) a freezing-and-thawing technique (described above), 2) grinding of Microcystis filtered from Pawnee Reservoir water using a mechanical tissue grinder with an all glass mortar and pestle followed by reconstitution with an appropriate volume of water, and 3) sonication of the whole water sample at 47 kHz for 90 minutes using a
Bransonic® sonicator model 5210 (Danbury, CT 06813-1961). Analytical recovery of microcystin was verified by addition of microcystin-LR (CyanoHAB Services Dayton, OH 45435) to water samples as an internal standard (APHA 1998).

*Algaecide Influence on Aqueous Microcystin Measurement*

To assess the potential influence or interference of an addition of algaecide on aqueous microcystin measurements as well as microcystin stability, 1.0 mg Cu L\(^{-1}\) as Cutrine\(^{®}\)-Ultra, Clearigate\(^{®}\), Algimycin\(^{®}\) PWF, and copper sulfate pentahydrate was amended to filtered (≤ 0.45 µm) Pawnee Reservoir water samples. Aqueous microcystin was measured following a 96 incubation period. These treatments were tested simultaneously with algal toxicity experiments.

*Time-Course Release of Microcystin after Cutrine\(^{®}\)-Ultra Treatments*

To measure the time-course release of microcystin, a laboratory experiment was conducted exposing *Microcystis* in Pawnee Reservoir water to a series of copper concentrations as Cutrine\(^{®}\)-Ultra. Aqueous and total microcystin were measured pre-exposure, six, 24, 48, and 96 hours after exposure. Microcystin concentrations (aqueous and total) were measured within 24 hours of collection in this experiment. Total microcystin was measured following sonication at 47 kHz for 90 minutes for extraction of cell-associated microcystin.
**Statistical Analyses**

A one-way analysis of variance (ANOVA) was used to determine statistically significant differences in chlorophyll $a$ concentrations, algal cell densities, and microcystin concentrations between treatments and untreated controls. Significant differences were discerned further using a Dunnett’s test. If data did not meet the assumptions for parametric testing, then a non-parametric ANOVA on ranked data followed by a Dunn's test was used. All data were analyzed using SigmaStat version 3.1 for Windows (alpha = 0.05) (Systat Software, Inc., Point Richmond, CA 94804-2028).

**Results and Discussion**

*Algicidal Efficacy: Algal Toxicity Experiments*

Responses of *Microcystis* to algaecide exposures are reported based on nominal copper concentrations due to insignificant differences between nominal and measured copper concentrations (Table 2.1). Cutrine®-Ultra and Clearigate® were the more effective of the four algaecides evaluated for controlling *Microcystis* in water samples from Pawnee Reservoir (Figures 2.1, 2.2). Chlorophyll $a$ concentrations in exposures of $\geq 0.2$ mg Cu L$^{-1}$ as Cutrine®-Ultra were significantly less than in untreated controls (Figure 2.1). Algal cell densities in Cutrine®-Ultra exposures were below detection limits (Figure 2.2). Chlorophyll $a$ concentrations and algal cell densities in exposures of $\geq 0.2$ mg Cu L$^{-1}$ as Clearigate® were below detection limits (Figures 2.1, 2.2). Chlorophyll $a$ concentrations in exposures of $\geq 0.2$ mg Cu L$^{-1}$ as Algimycin® PWF and copper
sulfate pentahydrate were below detection limits (Figure 2.1). However, exposures of 0.2-0.6 mg Cu L\(^{-1}\) as Algimycin\(^{®}\) PWF and copper sulfate pentahydrate contained countable densities of \textit{Microcystis} cells at experiment conclusion (Figure 2.2). The purpose of this laboratory study was to discern an efficacious treatment for \textit{Microcystis} that could be applied in Pawnee Reservoir. “Efficacy” in this case was defined as an effective algal treatment at a minimum effective algaecide concentration, as well as a treatment that would result in minimal release of microcystin.

\textit{Chlorophyll a Measurements}

For the purpose of these experiments, control of \textit{Microcystis} based on chlorophyll \textit{a} concentration was defined as the presence of less than 10 \(\mu\)g chlorophyll \textit{a} L\(^{-1}\) following exposure to a specific form of copper (algaecide). This level of chlorophyll \textit{a} approaches the limit of detection for the spectrofluorometer under these experimental conditions and is essentially oligotrophic in terms of chlorophyll biomass (Wetzel 2001). The initial chlorophyll \textit{a} concentration in site water used for the Cutrine\(^{®}\)-Ultra experiment was 356 \(\mu\)g L\(^{-1}\) (± 27 \(\mu\)g L\(^{-1}\)). The chlorophyll \textit{a} concentration of untreated controls was 693 \(\mu\)g L\(^{-1}\) (± 17 \(\mu\)g L\(^{-1}\)) after the 96 hour exposure duration. Chlorophyll \textit{a} concentrations in exposures of \(\geq 0.2\) mg Cu L\(^{-1}\) as Cutrine\(^{®}\)-Ultra significantly decreased compared to unexposed controls, ranging from 60 \(\mu\)g L\(^{-1}\) (± 18 \(\mu\)g L\(^{-1}\)) in the 0.2 mg Cu L\(^{-1}\) exposure concentration to 31 \(\mu\)g L\(^{-1}\) (± 1 \(\mu\)g
L\(^{-1}\)) in the 0.4 mg Cu L\(^{-1}\) exposure concentration (Figure 2.1). The initial chlorophyll \(a\) concentration was 91 \(\mu g\) L\(^{-1}\) (± 11 \(\mu g\) L\(^{-1}\)) in Pawnee Reservoir water used for the Clearigate®, Algimycin® PWF, and copper sulfate pentahydrate experiments. The chlorophyll \(a\) concentration in the untreated control was 61 \(\mu g\) L\(^{-1}\) (± 30 \(\mu g\) L\(^{-1}\)) after 96 hours. Chlorophyll \(a\) concentrations in exposures of \(\geq 0.2\) mg Cu L\(^{-1}\) as Clearigate®, Algimycin® PWF, and copper sulfate pentahydrate, were not measurable (< 10 \(\mu g\) L\(^{-1}\)) after 96 hours (Figure 2.1).

Microcystis Cell Density Measurements

For the purpose of these experiments, control of Microcystis based on cell density was defined as less than 5.0 \(x\) 10\(^{2}\) cells mL\(^{-1}\) following the 96 hour exposure duration. Using the low density counting method for the Improved Neubauer hemacytometer (DiBartolomeis and Mone’ 2003) and counting each treatment replicate four times results in a detection limit of 5.0 \(x\) 10\(^{2}\) cells mL\(^{-1}\). The initial Microcystis cell density for the Cutrine®-Ultra experiment was 2.17 \(x\) 10\(^{5}\) cells mL\(^{-1}\) (± 2.0 \(x\) 10\(^{4}\) cells mL\(^{-1}\)). The Microcystis cell density was 1.88 \(x\) 10\(^{5}\) cells mL\(^{-1}\) (± 4.4 \(x\) 10\(^{4}\) cells mL\(^{-1}\)) in untreated controls after 96 hours. Exposures of \(\geq 0.2\) mg Cu L\(^{-1}\) as Cutrine®-Ultra contained no countable or visible Microcystis cells after 96 hours (Figure 2.2-A).

The initial Microcystis cell density for the Clearigate®, Algimycin® PWF, and copper sulfate pentahydrate experiments was 3.12 \(x\) 10\(^{5}\) cells mL\(^{-1}\) (± 2.1 \(x\) 10\(^{4}\) cells mL\(^{-1}\)). The Microcystis cell density was 5.57 \(x\) 10\(^{5}\) cells mL\(^{-1}\) (± 4.03 \(x\) 10\(^{4}\) cells mL\(^{-1}\)) in untreated controls after 96 hours. Exposures of \(\geq 0.2\) mg Cu L\(^{-1}\)
as Clearigate® contained no countable or visible *Microcystis* cells after 96 hours (Figure 2.2-B).

The cell densities in treatments of 0.2, 0.4 and 0.6 mg Cu L\(^{-1}\) as Algimycin® PWF were 5.2 x 10^4 cells mL\(^{-1}\) (± 1.1 x 10^4 cells mL\(^{-1}\)), 2.1 x 10^4 cells mL\(^{-1}\) (± 3.5 x 10^3 cells mL\(^{-1}\)), and 1.1 x 10^4 cells mL\(^{-1}\) (± 3.8 x 10^3 cells mL\(^{-1}\)), respectively. Exposure concentrations of ≥ 0.8 mg Cu L\(^{-1}\) as Algimycin® PWF contained no countable or visible *Microcystis* cells after 96 hours (Figure 2.2-C).

*Microcystis* cell densities in exposures of 0.2, 0.4 and 0.6 mg Cu L\(^{-1}\) as copper sulfate pentahydrate were 5.5 x 10^4 cells mL\(^{-1}\) (± 4.8 x 10^3 cells mL\(^{-1}\)), 2.85 x 10^4 cells mL\(^{-1}\) (± 1.28 x 10^4 cells mL\(^{-1}\)), and 1.7 x 10^4 cells mL\(^{-1}\) (± 2.0 x 10^3 cells mL\(^{-1}\)), respectively. Exposure concentrations of ≥ 0.8 mg Cu L\(^{-1}\) as copper sulfate pentahydrate contained no countable or visible *Microcystis* cells after 96 hours (Figure 2.2-D).

**Aqueous Microcystin Concentration Measurements**

Aqueous microcystin measurements indicate release of this hepatotoxic endotoxin from algal cells (Lam et al. 1995; Sangolkar et al. 2006). The pre-exposure aqueous microcystin concentration in Pawnee Reservoir water used for the initial Cutrine®-Ultra experiment was 3.10 ± 0.21 μg L\(^{-1}\). The aqueous microcystin concentration was 3.90 ± 0.32 μg L\(^{-1}\) in unexposed controls after 96 hours. The aqueous microcystin concentrations in exposures of ≥
0.2 mg Cu L\(^{-1}\) as Cutrine\(^\circledR\)-Ultra ranged from 1.40 µg L\(^{-1}\) (± 0.12 µg L\(^{-1}\)) in the 0.2 mg Cu L\(^{-1}\) exposure concentration to 1.10 µg L\(^{-1}\) (± 0.25 µg L\(^{-1}\)) in the 0.4 mg Cu L\(^{-1}\) exposure concentration after 96 hours (Figure 2.3-A). All aqueous microcystin concentrations measured in exposures of Cutrine\(^\circledR\)-Ultra were significantly less than initial concentrations in site water and concentrations in unexposed controls, and there were no statistically significant differences in aqueous microcystin concentrations in exposures ranging from 0.2-1.0 mg Cu L\(^{-1}\) (Figure 2.3-A).

The initial aqueous microcystin concentration in Pawnee Reservoir water used for the Clearigate\(^\circledR\), Algimycin\(^\circledR\) PWF, and copper sulfate pentahydrate experiments was 0.49 µg L\(^{-1}\) (± 0.06 µg L\(^{-1}\)). The aqueous microcystin concentration was 0.38 µg L\(^{-1}\) (± 0.05 µg L\(^{-1}\)) in untreated controls after 96 hours. Microcystin concentrations in exposures of ≥ 0.2 mg Cu L\(^{-1}\) as Clearigate\(^\circledR\) ranged from 0.39 µg L\(^{-1}\) (± 0.18 µg L\(^{-1}\)) in the 1.0 mg Cu L\(^{-1}\) exposure concentration to 0.31 µg L\(^{-1}\) (± 0.02 µg L\(^{-1}\)) in the 0.4 mg Cu L\(^{-1}\) exposure concentration after 96 hours (Figure 2.3-B). No statistically significant differences in aqueous microcystin concentrations were observed between unexposed controls and Clearigate\(^\circledR\) exposures. Aqueous microcystin concentrations for treatments of ≥ 0.2 mg Cu L\(^{-1}\) as Algimycin\(^\circledR\) PWF ranged from 0.46 µg L\(^{-1}\) (± 0.17 µg L\(^{-1}\)) in the 0.8 mg Cu L\(^{-1}\) exposure concentration to 0.20 µg L\(^{-1}\) (± 0.01 µg L\(^{-1}\)) in the 0.4 mg Cu L\(^{-1}\) exposure concentration at experiment conclusion (Figure 2.3-C). Aqueous microcystin concentrations in exposures of ≥ 0.2 mg Cu L\(^{-1}\) as copper sulfate pentahydrate ranged from 0.28 µg L\(^{-1}\) (± 0.09 µg L\(^{-1}\)) in the 1.0 mg Cu L\(^{-1}\) exposure concentration to 0.24 µg L\(^{-1}\) (± 0.02 µg L\(^{-1}\)) in the 0.2 and 0.4 mg Cu L\(^{-1}\)
exposure concentrations at experiment conclusion (Figure 2.3-D). No statistically significant differences in aqueous microcystin concentrations were observed between unexposed controls or exposures of copper sulfate pentahydrate.

**Total Microcystin Concentration Measurements**

Total microcystin measurements have been used to make risk-based decisions regarding utilization of water resources for example the World Health Organization (2003) recommended potable water guideline ($\leq 1.0 \mu g \ L^{-1}$). Prior to treatment, the total microcystin concentration measured in Pawnee Reservoir water for the Clearigate®, Algimycin® PWF, and copper sulfate pentahydrate experiments was $0.18 \mu g \ L^{-1}$ ($\pm 0.05 \mu g \ L^{-1}$). Total microcystin concentrations in the unexposed controls and exposures of $\geq 0.2 \ mg \ Cu \ L^{-1}$ as Clearigate® ranged from $0.21 \mu g \ L^{-1}$ ($\pm 0.07 \mu g \ L^{-1}$) in unexposed controls to non-detect in exposures of 0.6, 0.8, and 1.0 mg Cu L$^{-1}$ as Clearigate® after 96 hours (Figure 2.4-A). Total microcystin concentrations in Algimycin® PWF exposures ranged from $0.24 \mu g \ L^{-1}$ ($\pm 0.01 \mu g \ L^{-1}$) in the 0.4 mg Cu L$^{-1}$ exposures to non-detect in exposures of 0.2 and 1.0 mg Cu L$^{-1}$ exposures after 96 hours (Figure 2.4-B). Total microcystin concentrations in exposures of $\geq 0.2 \ mg \ Cu \ L^{-1}$ as copper sulfate pentahydrate ranged from $0.16 \mu g \ L^{-1}$ in the 0.8 mg Cu L$^{-1}$ exposures to non-detect in exposures of 0.2, 0.4, 0.6, and 1.0 mg Cu L$^{-1}$ exposures after 96 h (Figure 2.4-C).

Data from this experiment indicated that the measurements of aqueous microcystin often exceeded the total microcystin concentrations under these experimental conditions (Figures 2.3 and 2.4). To determine whether the
discrepancy between total measurements and aqueous measurements in this study were methodologically related, an experiment was conducted to examine three techniques for extraction of cell-associated microcystin.

**Cell-Associated Microcystin Extraction Experiment**

*(Measurement of Total Microcystin)*

A laboratory experiment was conducted to measure total microcystin following three techniques for cell-associated microcystin extraction, along with additions of internal standards of microcystin-LR to measure analytical recovery (APHA 1998) (Figures 2.5-2.7). The freezing-and-thawing technique for measurement of total microcystin did not affect the measurement of aqueous microcystin (Figure 2.5). Total microcystin measured after freezing-and-thawing did not differ significantly from the aqueous measurement. However, recovery of total microcystin was ~70% based on analysis of the internal standard of microcystin-LR (~1 μg L⁻¹) (APHA 1998). The grinding technique did not affect measurement of aqueous microcystin (Figure 2.6). Total microcystin measured after grinding was significantly greater (~3x) than the aqueous microcystin concentration after grinding. However, the internal standard of microcystin (~1 μg L⁻¹) was not measurable indicating significant interference. The sonication technique did not affect measurement of aqueous microcystin and the total microcystin measurement was significantly greater (~3x) than the aqueous microcystin (Figure 2.7). Recovery of the internal standard of microcystin-LR (~1 μg L⁻¹) was ~100%. Therefore, sonication of the sample at 47 kHz for 90 minutes
was the most efficacious of the three methods tested under these experimental conditions (Figure 2.7).

*Algaecide Influence on Aqueous Microcystin Measurement*

Concentrations of aqueous microcystin measured in the algaecide amended samples were not significantly different from the unamended sample (data not shown). Algaecide amendments did not affect the measurement of aqueous microcystin. No significant difference in aqueous microcystin concentration was observed between the initial aqueous microcystin concentration and measured aqueous microcystin concentrations from algaecide amendments after 96 hours (data not shown).

*Time-Course Release of Microcystin after Cutrine®-Ultra Exposures*

In all exposure concentrations of Cutrine®-Ultra (0.2-1.0 mg Cu L⁻¹), aqueous microcystin concentrations increased significantly from ~0.4 μg L⁻¹ (pretreatment) to ~3.5 μg L⁻¹ (6 hours post-treatment) (Figure 2.8). Aqueous microcystin concentrations remained elevated for 24 hours post-treatment in exposures of Cutrine®-Ultra ≥ 0.4 mg Cu L⁻¹ (Figure 2.8). However, aqueous microcystin concentrations returned to background within 48 hours after exposure to Cutrine®-Ultra (Figure 2.8). Total microcystin concentrations decreased significantly less than 48 hours after exposure to Cutrine®-Ultra. Relatively rapid decline in total microcystin concentrations post-treatment has been observed in
previous studies that concluded microcystin can be readily degraded following release from algal cells (Jones and Orr 1994; Lam et al. 1995; Christofferson et al. 2002).

Summary

As expected, *Microcystis aeruginosa* in Pawnee Reservoir water was relatively sensitive to all of the copper algaecides within the range of USEPA registered product use rates. Responses of this cyanobacterium in terms of chlorophyll *a* and cell density were generally proportional to the exposures (i.e. concentration, duration). Importantly, this alga in Pawnee Reservoir water was most sensitive to the Cutrine®-Ultra exposures with control obtained at 0.2 mg Cu L⁻¹ [as Cutrine®-Ultra; 2.17 x 10⁵ cells mL⁻¹, 356 µg chlorophyll *a* L⁻¹ (pre-treatment)]. This concentration of copper is five times less than would be allowed as the maximum treatment permitted by the label and is ~50% less than the amount required to achieve the same level of control using copper sulfate pentahydrate or the weakly-chelated algaecide Algimycin® PWF. This sensitivity of algae in site water emphasizes the utility and importance of laboratory studies prior to large field-scale applications in order to efficiently use effective algaecides while maximizing the potential margin of safety to non-target species (Fitzgerald and Jackson 1979, Mastin et al. 2002, Murray-Gulde et al. 2002). For this laboratory study, chlorophyll *a* and cell density measurements correlated in terms of controlling this alga in this water. However, due to the nature of these
two parameters, both chlorophyll $a$ and cell density must be measured to accurately assess control of algae following algaecide exposures.

One reason for measuring aqueous microcystin in this study was to determine if release of microcystin occurred following algaecide exposures. Aqueous microcystin measurements assess the potential for significant microcystin release, and increases in concentrations, following algaecide exposures. While measurements of aqueous microcystin are important for signaling release of microcystin following algaecide treatment, measurements of total microcystin are used to guide policy and regulatory or management decisions (i.e. WHO 2003). In this case, measurement of total microcystin was complicated or confounded by the technique used to extract microcystin from algal cells. Based on these limited data, sonication was the most reliable technique for release of cell-associated microcystin under these conditions. For critical water resources, where decisions regarding usage are made based upon total microcystin measurements, further study may be needed regarding the potential for microcystin release post-treatment as well as the technique used for measuring total microcystin. Regardless, we recommend utilization of internal and surrogate standards during total microcystin measurements (APHA 1998). Following the time-course microcystin release experiment post-exposure to Cutrine®-Ultra, increases in aqueous and total microcystin were transitory and were not of ecological or health significance.

Previous studies (i.e. Kenefick et al. 1993; Jones and Orr 1994; Peterson et al. 1995) have concluded that intracellular contents of algae are released
following exposure to algaecides and chemicals used for potable water production. These studies are often cited as reliable evidence that release of microcystin after treatment could pose significant risks to humans or animals for weeks. Kenefick and co-workers (1993) studied algae (including *Microcystis*) that had been concentrated from Coal Lake in single un-replicated treatments (10.7-L) in the laboratory. The algal biomass was increased to 12 times the mass in Coal Lake and the chemical treatment (copper sulfate of unspecified exposure concentration) was indicated as “higher chemical doses than commonly used in the treatment of surface waters.” In this and a subsequent experiment with cultured algae, microcystin was released following a copper sulfate treatment. Jones and Orr (1994) studied *Microcystis aeruginosa* in an Australian reservoir that produced concentrations of microcystin of 1300-1800 μg L⁻¹. The algaecide treatment consisted of “spot-sprayed” Coptrol® (a copper-containing algaecide). The dense growth of algae was controlled within 2-3 days after treatment. Release of microcystin was observed in this situation, but persistence was ephemeral (2-9 days) with a microcystin degradation rate of approximately 500 μg L⁻¹ day⁻¹. Although the actual treatment exposure concentration of copper was unspecified, it is unlikely that this treatment is related in any way to current legal or practical management practices. Peterson et al. (1995) studied *Aphanizomenon flos-aquae* in the laboratory in culture medium. This alga was treated with chlorine, potassium permanganate, ferric chloride, calcium hydroxide, hydrogen peroxide, and copper sulfate. At relatively high concentrations, ferric chloride, copper sulfate, and potassium permanganate caused cell membrane damage and release
of dissolved organic carbon and geosmin. Clearly, these studies and their results are not applicable for prediction of responses of algae to algaecide exposures used in current practices by water resource managers. With strategic monitoring programs and efficient use of effective algaecides, risks can be managed and water resource usages can be restored (Mastin et al. 2002). In this specific situation in Pawnee Reservoir, ingestion of microcystin through swimming and other water sports is of concern and the presence of microcystin in either aqueous or intracellular phases would be of concern due to its toxicity. With a timely monitoring program, water resource managers can intervene before *Microcystis* achieves densities and releases microcystin that can pose risks to people and other animals.
Acknowledgements

We gratefully acknowledge financial and field assistance from Applied Biochemists, a division of Advantis Technologies, Inc., and the Nebraska Game and Parks Commission. We also thank the University of Nebraska-Lincoln Water Center and the Nebraska Department of Environmental Quality for their support. And we thank Dr. Louwanda Jolley and Dr. Wayne Chao for assistance with copper measurements.
Literature Cited


United States Army Corps of Engineers (USACE). Pawnee Reservoir information page. 


Table 2.1: Nominal and measured initial exposure concentrations of copper (total) as Cutrine®-Ultra, Clearigate®, Algimycin® PWF, and copper sulfate pentahydrate (mean ± one standard deviation).

<table>
<thead>
<tr>
<th>Nominal Exposure Concentrations (mg Cu L⁻¹)</th>
<th>Cutrine®-Ultra</th>
<th>Clearigate®</th>
<th>Algimycin® PWF</th>
<th>Copper Sulfate Pentahydrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unexposed Controls</td>
<td>0.023 ± 0.002</td>
<td>0.049 ± 0.002</td>
<td>0.049 ± 0.002</td>
<td>0.049 ± 0.002</td>
</tr>
<tr>
<td>0.2</td>
<td>0.213 ± 0.002</td>
<td>0.209 ± 0.002</td>
<td>0.289 ± 0.003</td>
<td>0.243 ± 0.002</td>
</tr>
<tr>
<td>0.4</td>
<td>0.440 ± 0.005</td>
<td>0.484 ± 0.004</td>
<td>0.473 ± 0.004</td>
<td>0.456 ± 0.002</td>
</tr>
<tr>
<td>0.6</td>
<td>0.673 ± 0.008</td>
<td>0.736 ± 0.009</td>
<td>0.674 ± 0.010</td>
<td>0.700 ± 0.003</td>
</tr>
<tr>
<td>0.8</td>
<td>0.901 ± 0.011</td>
<td>0.981 ± 0.011</td>
<td>0.962 ± 0.049</td>
<td>0.873 ± 0.016</td>
</tr>
<tr>
<td>1.0</td>
<td>1.090 ± 0.001</td>
<td>1.160 ± 0.014</td>
<td>1.136 ± 0.020</td>
<td>1.148 ± 0.016</td>
</tr>
</tbody>
</table>
Table 2.2: Characteristics of Pawnee Reservoir water used for algal toxicity tests with Cutrine®-Ultra. Initial measurements were made at the beginning of the experiment, and the other measurements (range) were made following 96 hours of exposure.

<table>
<thead>
<tr>
<th>Water Characteristic Parameter</th>
<th>Exposure Concentration (mg Cu L⁻¹ as Cutrine®-Ultra)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
</tr>
<tr>
<td>pH (SU)</td>
<td>8.1</td>
</tr>
<tr>
<td>Dissolved Oxygen (mg O₂ L⁻¹)</td>
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</tr>
<tr>
<td>Total Alkalinity (mg L⁻¹ as CaCO₃)</td>
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<tr>
<td>Total Hardness (mg L⁻¹ as CaCO₃)</td>
<td>62</td>
</tr>
<tr>
<td>Conductivity (µS cm⁻²)</td>
<td>283</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>22</td>
</tr>
</tbody>
</table>
Table 2.3: Characteristics of Pawnee Reservoir water used for algal toxicity tests with Clearigate®, Algimycin® PWF, and copper sulfate pentahydrate. Initial measurements were made at the beginning of the experiment, and the other measurements (range) were made following 96 hours of exposure.

<table>
<thead>
<tr>
<th>Water Characteristic Parameter</th>
<th>Initial</th>
<th>Unexposed Controls</th>
<th>0.2</th>
<th>0.4</th>
<th>0.6</th>
<th>0.8</th>
<th>1.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH (SU)</td>
<td>8.1</td>
<td>8.7-9.0</td>
<td>8.4-8.7</td>
<td>8.5-8.7</td>
<td>8.6-8.7</td>
<td>8.7</td>
<td>8.7-8.8</td>
</tr>
<tr>
<td>Dissolved Oxygen (mg O₂ L⁻¹)</td>
<td>7.9</td>
<td>7.7-8.7</td>
<td>7.5-8.0</td>
<td>7.6-7.9</td>
<td>7.6-8.0</td>
<td>7.8-8.0</td>
<td>7.5-8.0</td>
</tr>
<tr>
<td>Total Alkalinity (mg L⁻¹ as CaCO₃)</td>
<td>200</td>
<td>200</td>
<td>186-192</td>
<td>188-194</td>
<td>188-194</td>
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<td>186-196</td>
</tr>
<tr>
<td>Total Hardness (mg L⁻¹ as CaCO₃)</td>
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<td>160</td>
<td>156-160</td>
<td>160-164</td>
<td>160-200</td>
<td>120-172</td>
<td>160-172</td>
</tr>
<tr>
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<tr>
<td>Temperature (°C)</td>
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<td>24</td>
<td>24</td>
<td>24</td>
<td>24</td>
<td>24</td>
<td>24</td>
</tr>
</tbody>
</table>
List of Figures

Figure 2.1: Chlorophyll $a$ concentrations in Pawnee Reservoir water after 96 hour exposures to: A) Cutrine®-Ultra, B) Clearigate®, C) Algimycin® PWF, and D) copper sulfate pentahydrate. Bars represent one standard deviation. Asterisks indicate chlorophyll $a$ concentrations below the detection limit. Detection limit = 10 $\mu$g chlorophyll $a$ L$^{-1}$.

Figure 2.2: *Microcystis* cell densities in Pawnee Reservoir water after 96 hour exposures to: A) Cutrine®-Ultra, B) Clearigate®, C) Algimycin® PWF, and D) copper sulfate pentahydrate. Bars represent one standard deviation. Asterisks indicate cell densities below the detection limit. Detection limit = 5.0 x 10$^2$ cells mL$^{-1}$.

Figure 2.3: Aqueous microcystin concentrations in Pawnee Reservoir water after 96 hour exposures to: A) Cutrine®-Ultra, B) Clearigate®, C) Algimycin® PWF, and D) copper sulfate pentahydrate. Bars represent one standard deviation.

Figure 2.4: Total microcystin concentrations in Pawnee Reservoir water after 96 hour exposures to: A) Clearigate®, B) Algimycin® PWF, and C) copper sulfate pentahydrate. Cell-associated microcystin was extracted using the freezing-and-thawing technique. Bars represent one standard deviation. Asterisks indicate microcystin concentrations below the detection limit. Detection limit = 0.16 $\mu$g L$^{-1}$.
**Figure 2.5:** Aqueous microcystin concentration and microcystin concentrations in Pawnee Reservoir water prior to, and after, the freezing-and-thawing technique for extraction of cell-associated microcystin. Bars represent one standard deviation. Treatments coded with the same letter are not significantly different (p = 0.05). Treatments not letter-coded were amended with an internal standard of microcystin-LR and not included in statistical analyses.¹

¹ **Figure 2.5:**

- Aqueous = aqueous microcystin concentration (pre- and post- treatment);
- Aqueous +1 = aqueous microcystin concentration amended with 1 µg microcystin-LR L⁻¹ (pre- and post- treatment);
- Total = total microcystin concentration after the freezing-and-thawing technique;
- Total +1 = total microcystin concentration amended with 1 µg microcystin-LR L⁻¹ after the freezing-and-thawing technique.
Figure 2.6: Aqueous microcystin concentration and microcystin concentrations in Pawnee Reservoir water prior to, and after, the grinding technique for extraction of cell-associated microcystin. Bars represent one standard deviation. Treatments coded with the same letter are not significantly different (p = 0.05). Treatments not letter-coded were amended with an internal standard of microcystin-LR and not included in statistical analyses.2

2 Figure 2.6: **Aqueous** = aqueous microcystin concentration (pre- and post- treatment); **Aqueous +1** = aqueous microcystin concentration amended with 1 microcystin-LR L⁻¹ after grinding (pre- and post- treatment); **NANO +1** = 1 µg microcystin L⁻¹ in NANOpure™ water after grinding; **Total** = total microcystin concentration after grinding of *Microcystis* filtered from Pawnee Reservoir water; **Total +1** = total microcystin concentration amended with 1 µg microcystin-LR L⁻¹ after grinding of *Microcystis* filtered from Pawnee Reservoir water.
Figure 2.7: Aqueous microcystin concentration and microcystin concentrations in Pawnee Reservoir water prior to, and after, the sonication technique for extraction of cell-associated microcystin. Bars represent one standard deviation. Treatments coded with the same letter are not significantly different (p = 0.05). Treatments not letter-coded were amended with an internal standard of microcystin-LR and not included in statistical analyses.³

³ Figure 2.7: Aqueous= aqueous microcystin concentration (pre- and post- treatment); Aqueous +1= aqueous microcystin concentration amended with 1 μg microcystin-LR L⁻¹ (pre- and post-treatment); NANO +1= 1 μg microcystin-LR L⁻¹ in NANOpure™ water after sonication; Total= total microcystin concentration after sonication of unfiltered Pawnee Reservoir water; Total +1= total microcystin concentration after sonication of unfiltered Pawnee Reservoir water, amended with 1 μg microcystin-LR L⁻¹.
Figure 2.8: Time-course measurements of A) aqueous and B) total microcystin concentrations in Pawnee Reservoir water pre-exposure, six, 24, 48, and 96 hours post-exposure to Cutrine®-Ultra. Total microcystin was measured after sonication of the water sample. Bars represent one standard deviation.
Figure 2.1

A. Initial chlorophyll a concentration.
B. Chlorophyll a concentration after 96 hours.
C. Initial chlorophyll a concentration.
D. Chlorophyll a concentration after 96 hours.
Figure 2.2

A. Initial cell density vs. cell density after 96 hours for Microcystis sp. exposed to different copper concentrations in Cutrine-Ultra.

B. Initial cell density vs. cell density after 96 hours for Microcystis sp. exposed to different copper concentrations in Clearigate.

C. Initial cell density vs. cell density after 96 hours for Microcystis sp. exposed to different copper concentrations in Algimycin PWF.

D. Initial cell density vs. cell density after 96 hours for Microcystis sp. exposed to different copper concentrations in CuSO₄ · 5H₂O.
Figure 2.3

**A**

Exposure Concentration (mg Cu / L as Cutrine-Ultra)

Initial aqueous microcystin concentration. Aqueous microcystin concentration after 96 hours.

**B**

Exposure Concentration (mg Cu / L as Clearigate)

**C**

Exposure Concentration (mg Cu / L as Algimycin PWF)

**D**

Exposure Concentration (mg Cu / L as CuSO4 x 5H2O)
Figure 2.4

A

Exposure Concentration (mg Cu/L as Clirigate)

Total Microcystin (ug/L)

B

Exposure Concentration (mg Cu/L as Algimycin PWF)

Total Microcystin (ug/L)

C

Exposure Concentration (mg Cu/L as CuSO4 x 5H2O)

Total Microcystin (ug/L)

Initial total microcystin concentration.

Total microcystin concentrations after 96 hrs.
Figure 2.5

Initial aqueous microcystin concentrations.

Microcystin concentrations after freezing-and-thawing.

Initial aqueous microcystin concentrations.

Microcystin concentrations after freezing-and-thawing.
Figure 2.6

Initial aqueous microcystin concentrations.
Microcystin concentrations after grinding.

Initial aqueous microcystin concentrations.
Microcystin concentrations after grinding.

Microcystin (µg/L)

Treatments

Aqueous, Aqueous+1, NANO+1, Aqueous+1, Total, Total+1

Microcystin concentrations after grinding.
Figure 2.7

Initial aqueous microcystin concentrations.

Microcystin concentrations after sonication.

Initial aqueous microcystin concentrations.

Microcystin concentrations after sonication.

Treatments

Microcystin (ug / L)

Aqueous
Aqueous +1
Aqueous
Aqueous +1
NANO +1
Total
Total +1

Initial aqueous microcystin concentrations.

Microcystin concentrations after sonication.
Figure 2.8

(A) Aqueous Microcystin (µg / L) vs. Exposure Concentration (mg Cu / L as Cutrine-Ultra) for different time periods (Initial, 6 Hours, 24 Hours, 48 Hours, 96 Hours).

(B) Total Microcystin (µg / L) vs. Exposure Concentration (mg Cu / L as Cutrine-Ultra) for different time periods (Initial, 6 Hours, 24 Hours, 48 Hours, 96 Hours).
CHAPTER 3
LABORATORY AND FIELD RESPONSES OF *MICROCYSTIS AERUGINOSA* FOLLOWING AN ALGAECIDE APPLICATION

Abstract

Pawnee Reservoir in Nebraska has experienced repeated blooms of toxin producing *Microcystis aeruginosa*. To discern an efficacious algaecide treatment, water sampled from Pawnee Reservoir containing *Microcystis* was used in a laboratory algal toxicity test with Cutrine®-Ultra and the laboratory results were used to guide an application in Pawnee Reservoir. In both situations, similar laboratory and field exposures and responses were compared. Growth of *Microcystis* was controlled in the laboratory algal toxicity test after a 96 hour exposure to 0.2 mg Cu L⁻¹ as Cutrine®-Ultra (~2.4x10⁵ cells mL⁻¹; 34μg chlorophyll a L⁻¹, pre-treatment) in terms of chlorophyll a (<10 μg L⁻¹) and cell density (< 5x10² cells mL⁻¹). Aqueous microcystin, from treated *Microcystis*, increased six hours post-treatment from ~0.31 μg L⁻¹ to ~3.51 μg L⁻¹. However, aqueous microcystin decreased to background within 48 hours after exposure in the laboratory study. Based on pre-treatment cell densities, and total copper concentrations four hours after treatment in Pawnee Reservoir, the laboratory exposure of 0.2 mg Cu L⁻¹ was compared with field exposures in the southwest treatment site (~0.85 mg Cu L⁻¹) and the marina treatment site (~0.60 mg Cu L⁻¹). In both treatment sites, a decrease in chlorophyll a and cell density was observed.
four hours after treatment, along with an increase in aqueous microcystin from 
\(~0.4 \ \mu g \ L^{-1}\) to \(~3.9 \ \mu g \ L^{-1}\). However, aqueous microcystin concentrations
decreased to background (\(~0.4 \ \mu g \ L^{-1}\)) 48 hours post-treatment. Using site water
and realistic algaecide exposures, accurate and reliable predictions of field
responses of algae were developed in the laboratory. This strategy was useful to
mitigate risks from toxin producing algae and to restore use of this critical water
resource.

*Key Words:* Algae control; cyanobacteria; toxin; microcystin; copper; Pawnee
Reservoir.

**Introduction**

As water resources become more extensively used and to a greater
intensity, problematic growths of algae have become more severe and perhaps
more prevalent (Hallegraeff 1993; Zurawell et al. 2005). Algae can interfere with
critical water resource usages by producing excessive densities, toxins, or taste-
and-odor causing compounds (WHO 2003). Algae, such as *Microcystis
eaeruginosa* (Kütz. em. Elenkin 1933) that produce potent toxins can rapidly
achieve densities and toxin production requiring intervention by water resource
managers. When these algae become a sufficient problem and interfere with
critical water usages, resource managers often initiate control actions. The most
efficient and effective control actions to alleviate acute problems, such as toxin
production, may involve application of an algaecide.
Pawnee Reservoir in Lancaster County, Nebraska, has experienced repeated problematic densities of *Microcystis* along with corresponding production and release of the potent toxin, microcystin. Microcystin concentrations in Pawnee Reservoir have prompted warnings and water use restrictions for contact recreation (i.e. swimming, skiing) over the past several years. Pawnee Reservoir has a surface area of approximately 300 hectares (741 acres) and was completed July 16, 1964 (USACE). The watershed area of Pawnee Reservoir encompasses approximately 9,453 hectares (23,359 acres; Jay Woltemath, pers. comm., Nebraska Game and Parks Commission). Pawnee Reservoir is susceptible to exceptional sedimentation due to extensive agricultural practices and highly erodible soils in the watershed (Holz et al. 1997). Although this reservoir was developed primarily for flood control, recreational uses have increased and generate significant public and private revenue (Popp et al. 1996; Jay Woltemath, pers. comm.). Over 200 campsites and recreation areas surround Pawnee Reservoir (USACE). However, recreational uses of this water resource are impaired due to problematic densities of *Microcystis* with associated microcystin production and release (Jay Woltemath, pers. comm.).

Over the past few years, concentrations of total microcystin measured in Pawnee Reservoir have exceeded 20 μg L⁻¹ during the summer months (Nebraska Health and Human Services System 2006) [WHO (2003) recommended guideline for potable water is ≤ 1 μg L⁻¹]. Elevated concentrations of this hepatotoxin in Pawnee Reservoir pose significant risks for contact recreational users (Falconer 1996, 1999; Chorus et al. 2000; Briand et al. 2003; Carmichael et al. 2001).
During the summer months, beaches and other public recreational areas are often closed, and water use restrictions are issued weekly after measurement of elevated microcystin concentrations (Nebraska Health and Human Services System 2006). In 2006, densities of *Microcystis* and production of microcystin prompted water resource managers to consider an algaecide application to restore use of this reservoir.

Frequently, water resource managers carefully evaluate the onset of a problem such as a HAB (Watzin et al. 2006), its potential progression, as well as consequences of a control action such as an algaecide application. To make these management decisions, resource managers usually evaluate all practical and legal alternatives such as mechanical, biological, physical, and chemical control strategies (including nutrient source control). Algaecide applications are often the most cost effective and environmentally sound approaches for managing HABs such as *Microcystis* (Mastin et al. 2002).

Since all algaecide exposures do not elicit the same response from a target algal species, it is necessary to identify an efficacious algaecidal treatment in terms of the minimum exposure concentration and duration required to achieve the desired level of control (Fitzgerald and Jackson 1979; Gulde et al. 2002). Laboratory studies can facilitate predictions of effective field treatments, but these laboratory results must be confirmed in subsequent field studies. Particularly with treatments of copper-containing algaecides, water characteristics and other site conditions influence the speciation and bioavailability of copper, and thus the algaecidal effectiveness of an application (Haughey et al. 2000). Utilization of site
water containing the associated algae in laboratory studies minimizes the opportunity for ambiguity in translating the laboratory results directly to the field situation (Fitzgerald and Jackson 1979).

A laboratory study and subsequent application of Cutrine®-Ultra in Pawnee Reservoir, Nebraska, provided an opportunity to measure algal responses in laboratory exposures and to compare those responses with observations in the field. Cutrine®-Ultra is a chelated-copper containing algaecide, with a copper concentration of 9% in the form of mixed copper mono- and tri- ethanolamine complexes with an emulsified surfactant, D-limonene (Applied Biochemists 2002). Specific objectives of this research were to: 1) measure time-course responses of *Microcystis* following laboratory exposures to Cutrine-Ultra in terms of chlorophyll *a* concentrations, cell densities, and microcystin concentrations, 2) measure time-course responses of *Microcystis* after a field application of Cutrine®-Ultra, and 3) compare responses of *Microcystis* following a field application of Cutrine®-Ultra with responses measured in laboratory exposures to Cutrine®-Ultra.

**Materials and Methods**

*Laboratory Algal Toxicity Test*

Water sampled from Pawnee Reservoir containing *Microcystis* was used for the time-course laboratory algal toxicity test. In the laboratory algal toxicity test, four replicates of exposure concentrations of 0.2, 0.4, 0.6, 0.8, and 1.0 mg Cu L⁻¹ as Cutrine®-Ultra and an unexposed control were tested. Treatment
concentrations were achieved by adding relatively small volumes of stock algaecide solutions (stock = 100 mg Cu L\(^{-1}\)) to site water containing \textit{Microcystis} and completing the dilution to volume in a 1-L volumetric flask using Pawnee Reservoir water. Exposures consisted of static, non-renewal 200 mL volumes of treated or untreated site water containing the target algae in 250 mL Erlenmeyer flasks that were swirled once daily by hand. Exposure duration for the laboratory algal toxicity test was 96 hours. A 16h:8h light-dark cycle was used, with “cool white” fluorescent lighting at an intensity of 86 ± 8.6 \(\mu\)E m\(^{-2}\) s\(^{-1}\). Exposure chambers were maintained at a temperature of 21 ± 2°C (modified from APHA 1998). To verify exposures, total copper concentrations were measured using a Perkin-Elmer 5100 PC flame and graphite furnace atomic absorption (AA) spectrophotometer (method 3010-B; APHA 1998). To measure total copper, approximately 200 \(\mu\)L trace-metal grade nitric acid (Fisher Scientific, Fair Lawn, NJ 07410) was used to acidify all aqueous copper samples (~12 mL; \(pH \leq 2\)).

Water characteristics \([pH \text{ (Standard Units), dissolved oxygen (D.O.; mg O}_2 \text{ L}^{-1}), conductivity (\mu S \text{ cm}^{-2}), \text{total alkalinity (mg L}^{-1} \text{ as CaCO}_3), \text{total hardness (mg L}^{-1} \text{ as CaCO}_3), \text{and temperature (°C)})\] were measured according to APHA methods (1998).

Response parameters measured for the laboratory algal toxicity test were chlorophyll \(a\) concentrations, algal cell densities, and aqueous and total microcystin concentrations. Chlorophyll \(a\) and cell density in Pawnee Reservoir water were measured pre-exposure, and from each exposure replicate after 24, 48 and 96 hours of treatment. Chlorophyll \(a\) was extracted according to USEPA
method 445.0 (Arar and Collins 1997). Fluorometric measurements of test samples were calibrated with fluorescence values of known chlorophyll $a$ standards. Chlorophyll $a$ standards were prepared from a stock solution of 4000 $\mu$g chlorophyll $a$ L$^{-1}$ (Sigma C-5753; range = 10-1280 $\mu$g chlorophyll $a$ L$^{-1}$), kept protected from light and stored in a -20°C freezer. Algal cell densities were measured using an Improved Neubauer hemacytometer (DiBartolomeis and Mone’ 2003).

Aqueous and total microcystin concentrations were measured pre-exposure, and twice from two composite samples from each treatment after six, 24, 48, and 96 hours of exposure. Total microcystin samples were sonicated at 47 kHz for 90 minutes to extract cell-associated microcystin prior to measurement (Ross et al. 2006; Zhang et al. 2006) using a Branson® sonicator model 5210 (Danbury, CT 06813-1961). Aqueous and total microcystin concentrations were measured using an enzyme-linked immunosorbent assay (ELISA) (Envirologix®, Portland, ME 04103) specific for variants of microcystin and nodularin. Absorbance of antibody-linked microcystin was measured using a Molecular Devices “Emax” precision microplate reader (Molecular Devices, Menlo Park, CA).

**Pawnee Reservoir: Algaecide Application Study**

On June 6, 2006, Cutrine®-Ultra was applied over approximately 71 hectares (175.7 acres) along the west beach and southwest shore, along the dam shoreline, inside the marina area, and along the east beach in Pawnee Reservoir,
Nebraska. Two sites within the treatment area were monitored pre- and post-treatment: 1) along the southwest corner (an open water site), and 2) the marina site (a semi-enclosed area with limited open-water access to the reservoir body) (Figure 3.1). Approximately 1,597 liters (422 gallons) of Cutrine®-Ultra were applied to the total treatment area. Due to extreme algal densities observed in Pawnee Reservoir prior to treatment, a target concentration of 0.4-0.5 mg Cu L⁻¹ was selected for the entire treatment area. For the algaecide treatment, reservoir water was inducted and mixed with Cutrine®-Ultra concentrate immediately prior to application and injected just below the water’s surface using trailing hoses.

Water samples were collected from the two treatment sites (the southwest corner treatment site and the marina treatment site) as well as an untreated reference site (a northwest cove of the reservoir) pre- and post- treatment for measurement of total copper concentrations, algal cell densities, chlorophyll $a$ concentrations, and aqueous and total microcystin concentrations. Samples were collected from the southwest corner treatment site pre-treatment, four-hours, 24 hours, 48 hours, and 6 days after treatment. Samples were collected from the marina treatment site pre-treatment, four hours, 24 hours, 48 hours, six days and nine days after treatment. Samples were collected from the untreated reference site pre-treatment, 24 hours, 48 hours, six days and nine days after treatment. Composite samples were obtained from these three sites along a 20-30 meter transect.

Samples for chlorophyll $a$ and aqueous microcystin concentrations were filtered using Swinnex® adapter; 25 mm, cellulose membrane filters (0.45 μm).
and stored on ice, protected from light. Samples for cell density measurements were collected, kept on ice, and protected from light. Samples for total microcystin measurement were un-filtered water samples and were stored in glass culture tubes, on ice, protected from light. To extract cell-associated microcystin, total microcystin samples were frozen-and-thawed three times prior to measurement (Frank 2002; Grützmacher et al. 2002). After freezing-and-thawing, samples were filtered using a cellulose membrane filter (0.45 μm) prior to measurement. Microcystin concentrations were measured using an enzyme-linked immunosorbent assay (ELISA) kit (Envirologix®, Portland, ME 04103). Water characteristics [pH (Standard Units), dissolved oxygen (D.O.; mg O₂ L⁻¹), conductivity (μS cm⁻²), total alkalinity (mg L⁻¹ as CaCO₃), total hardness (mg L⁻¹ as CaCO₃), and temperature (°C)] were measured according to APHA methods (1998).

Comparison of Laboratory and Pawnee Reservoir Results

The laboratory algal toxicity test was intended to provide information regarding responses of Microcystis in Pawnee Reservoir water to exposures of Cutrine®-Ultra. The primary purpose of this laboratory experiment was to provide predictions of responses of Microcystis in Pawnee Reservoir to a field application of Cutrine®-Ultra. In order to reliably predict responses of algae in field treatments from laboratory results, comparison of similar exposures is necessary (Fitzgerald and Jackson 1979). In this situation, the laboratory experiment was conducted using Pawnee Reservoir water with a mean pre-treatment Microcystis
cell density of approximately 2.4 x 10^5 cells mL^{-1}. Control of Microcystis was achieved using an exposure of 0.2 mg Cu L^{-1}. For the field application in Pawnee Reservoir, the mean pre-treatment Microcystis cell density was 4.6 x 10^5 cells mL^{-1} (~2x the pre-treatment cell density of the laboratory experiment). Since the cell density can influence responses of algae after an exposure, the targeted application concentration was increased to ~0.4-0.5 mg Cu L^{-1} in the entire treatment area based upon consideration of the algal accumulation on the day of treatment. In both situations, similar laboratory and field exposures and responses to those exposures were compared.

**Statistical Analyses**

A one-way analysis of variance (ANOVA) was used to determine statistically significant differences in chlorophyll a concentrations, algal cell densities, and aqueous and total microcystin concentrations. Significant differences were discerned further using a Dunnett’s multiples range test. If data did not meet the assumptions for parametric testing, then a non-parametric ANOVA on ranked data followed by a Dunn's multiple range test was used. All data were analyzed using SigmaStat version 3.1 for Windows (alpha = 0.05) (Systat Software, Inc., Point Richmond, CA 94804-2028).
Results and Discussion

Laboratory Algal Toxicity Test

The primary purpose of the laboratory algal toxicity test was to identify an efficacious algaecide and treatment (concentration and duration of exposure) that would control the growth of *Microcystis aeruginosa* in Pawnee Reservoir water. For this study, control was defined as chlorophyll *a* concentrations and cell densities below detection limits (< 10 μg chlorophyll *a* L⁻¹; < 5.0 x 10² cells mL⁻¹, respectively). An additional consideration was decreased total and aqueous microcystin concentrations to background concentrations in 96 hours or less.

The exposures of copper as Cutrine®-Ultra used in the laboratory algal toxicity test were verified by measuring total copper concentrations from treatments (Table 3.1). Measured concentrations of copper in these exposures were approximately 95-110% of nominal concentrations. For these exposures in Pawnee Reservoir water, pH, alkalinity, and hardness were indicative of hard water (Table 3.2) (Sawyer et al. 1994). Laboratory exposures were conducted at a temperature of 21 ± 2°C (Table 3.2).

In the laboratory algal toxicity test, Cutrine®-Ultra controlled *Microcystis aeruginosa* in water sampled from Pawnee Reservoir at an exposure concentration of 0.2 mg Cu L⁻¹ (potential algaecide dose = ~9.1 x 10⁻⁷ ug Cu cell⁻¹). After the 96 hour exposure duration, the chlorophyll *a* concentration was not measurable (< 10 ug L⁻¹), the *Microcystis* cell density was not measurable (< 5 x 10² cells mL⁻¹), and the aqueous microcystin concentration decreased to approximately background concentration (~0.4 μg L⁻¹). The aqueous microcystin concentration
increased to approximately 3.51 µg L\(^{-1}\) six hours after treatment and decreased to the background concentration (~0.4 µg L\(^{-1}\)) by 48 hours after treatment (Figure 3.2).

In all laboratory algal toxicity test exposure concentrations of Cutrine\(^\text{®}\)-Ultra (0.2-1.0 mg Cu L\(^{-1}\)), aqueous microcystin concentrations increased significantly from ~0.4 µg L\(^{-1}\) (pre-treatment) to ~3.51 µg L\(^{-1}\) (six hours post-treatment). Aqueous microcystin concentrations remained elevated for 24 hours post-treatment in exposures of Cutrine\(^\text{®}\)-Ultra ≥ 0.4 mg Cu L\(^{-1}\). However, aqueous microcystin concentrations returned to background within 48 hours after exposure to Cutrine\(^\text{®}\)-Ultra. Less than 48 hours after exposure to Cutrine\(^\text{®}\)-Ultra, total microcystin concentrations also decreased significantly. Relatively rapid decline in total microcystin concentrations post-treatment has been observed in previous field studies that concluded microcystin can be readily degraded following release from algal cells (Jones and Orr 1994; Lam et al. 1995; Christofferson et al. 2002).

**Pawnee Reservoir: Algaecide Application Study**

Approximately 1,597 liters (422 gallons) of Cutrine\(^\text{®}\)-Ultra were applied over ~71 hectares (175 acres) in Pawnee Reservoir, Nebraska (Figure 3.1) on June 6, 2006. Approximately 416 liters (110 gallons) of Cutrine\(^\text{®}\)-Ultra were applied to the southwest treatment site and approximately 53 liters (14 gallons) were applied to the marina treatment site. The remaining algaecide was applied to the rest of the treatment area (Figure 3.1). Cutrine\(^\text{®}\)-Ultra was applied below the water’s surface using trailing hoses, beginning along the shoreline and increasing in
distance from the shoreline as the application progressed. *In situ* measurements of water characteristics (*pH*, dissolved oxygen, temperature and conductivity) were acquired in the southwest corner treatment site, marina treatment site and the untreated reference site, immediately prior to treatment (pre-treatment), and four hours, 24 hours, and 48 hours post-treatment. No significant differences were observed in *pH*, dissolved oxygen, temperature or conductivity after treatment relative to pretreatment values or the reference site values.

Approximately four hours after treatment, measured total copper concentrations in samples from the southwest treatment site and the marina treatment site were in excess of the 0.4-0.5 mg Cu L⁻¹ target treatment concentration (Table 3.3). However, total copper concentrations did not significantly differ from background (pre-treatment) concentrations in samples from these two treatment sites 24 hours after treatment nor did they differ from measurements in the reference site (Table 3.3). Total copper concentrations did not significantly increase in the untreated reference site on any sampling occasion after application of Cutrine®-Ultra in Pawnee Reservoir (Table 3.3).

Water characteristics measured from samples in the southwest treatment site and the marina treatment site did not differ significantly from the untreated reference site (Table 3.4). Measured values for *pH*, alkalinity and hardness indicated hard water (similar to the laboratory toxicity test measurements). The ionic strength (conductivity) measured from the southwest treatment site and the marina treatment site was not significantly different from the conductivity of the
water in the laboratory algal toxicity test. The water temperature measured in the two treatment sites was 26 ± 2°C.

**Algal Responses: Southwest Corner Treatment Site**

Pre-treatment *Microcystis* cell density and chlorophyll a concentration in the southwest corner treatment site of Pawnee Reservoir were 8.4 x 10^5 cells mL\(^{-1}\) (± 3.2 x 10^5) and 49 µg L\(^{-1}\) (± 10), respectively. Pre-treatment aqueous and total microcystin concentrations were 0.25 µg L\(^{-1}\) (± 0.01) and 0.31 µg L\(^{-1}\) (± 0.01), respectively (Figure 3.3-A). A significant decrease in *Microcystis* cell density was observed 48 hours and six days after treatment. Chlorophyll a concentrations were below detection 48 hours and six days after treatment (Figure 3.3-A). A significant increase in aqueous microcystin concentration was observed four hours after treatment and remained elevated for 24-48 hours (Figure 3.3-A). The aqueous microcystin concentration decreased to approximately the pre-treatment concentration within 48 hours after treatment. Total microcystin concentrations measured four hours, 24 hours, 48 hours, and six days, after treatment, were significantly less than the pre-treatment total microcystin concentration (Figure 3.3-A).

**Algal Responses: Marina Treatment Site**

Pre-treatment *Microcystis* cell density and chlorophyll a concentration in the marina treatment site were 7.4 x 10^4 cells mL\(^{-1}\) (± 1.3 x 10^4) and 64 µg L\(^{-1}\) (± 6), respectively. Pre-treatment aqueous and total microcystin concentrations in the
marina treatment site were approximately 0.98 µg L⁻¹ (± 0.02) and 0.28 µg L⁻¹ (± 0.01), respectively. The freezing-and-thawing technique was used for extraction of cell-associated microcystin in Pawnee Reservoir samples prior to measurement of total microcystin, and the discrepancy in aqueous vs. total microcystin measurements has been observed and investigated previously (Tedrow et al. in review). It is likely that this technique underestimates the concentration of total microcystin. No significant difference in Microcystis cell density was observed between any of the sampling periods throughout this study (Figure 3.3-B).

Chlorophyll \( a \) concentrations measured four and 48 hours after treatment were significantly decreased compared to the pre-treatment concentration (Figure 3.3-B). The aqueous microcystin concentration increased significantly four hours after treatment and remained elevated for 24-48 hours. The aqueous microcystin concentration decreased to approximately the pre-treatment concentration within 48 hours after treatment (Figure 3.3-B). Total microcystin concentrations measured 48 hours, six days, and nine days after treatment, were significantly less than the pre-treatment total microcystin concentration (Figure 3.3-B).

Based on field observations in Pawnee Reservoir, Microcystis distribution was strongly influenced by weather conditions (i.e. wind and wave action). As observed during this field treatment conducted under bright sunlit conditions, Microcystis became buoyant and rose to the surface of the water column. Dense algal accumulations were observed along windward shores and bays, approximately 24 hours after treatment in the marina treatment site. Prevailing winds from the northwest influenced the movement of Microcystis and an
increase in density in the marina treatment site. This observation is supported by measurements of cell density and chlorophyll $a$ within the marina treatment site 24 hours after treatment (Figure 3.3-B).

**Algal Parameters: Untreated Reference Site**

Initial *Microcystis* cell density and chlorophyll $a$ concentration in the untreated reference site were $4.2 \times 10^4$ cells mL$^{-1}$ ($\pm 6.7 \times 10^3$) and $38 \mu$g L$^{-1}$ ($\pm 7$), respectively. Aqueous and total microcystin concentrations measured on June 6, 2006, were $0.27 \mu$g L$^{-1}$ ($\pm 0.01$) and $0.22 \mu$g L$^{-1}$ ($\pm 0.01$), respectively (Figure 3.3-C). A significant decrease in *Microcystis* cell densities was observed 24 hours and nine days after treatment (Figure 3.3-C). The chlorophyll $a$ concentration and *Microcystis* cell density were not measurable six days after treatment (Figure 3.3-C). A significant decrease in aqueous microcystin concentration was observed between initial samples and samples obtained after 24 hours, 48 hours, six days, and nine days. A significant decrease in the total microcystin concentration was observed after six and nine days (Figure 3.3-C).

**Comparison of Laboratory and Pawnee Reservoir Results**

Factors such as $p$H, hardness, alkalinity and conductivity can strongly influence the speciation and performance of an algaecide (Haughey et al. 2000). Since the water characteristics were similar in the laboratory and Pawnee Reservoir studies, the speciation and bioavailability of the copper in the algaecide should be similar (Erickson et al. 1996). In both cases (the laboratory and Pawnee
Reservoir), *Microcystis* was growing in essentially the same water which should minimize ambiguity in translating laboratory results to the field.

In the laboratory, *Microcystis* in Pawnee Reservoir water was controlled using an exposure of 0.2 mg Cu L⁻¹ as Cutrine®-Ultra (Figure 3.2; Table 3.5). In order to reasonably compare responses of algae, the laboratory and field exposures must be similar. Responses of algae to an algaecide exposure often depend on the cell density (Murray-Gulde et al. 2002), so the exposures in this case were adjusted for *Microcystis* cell densities. Since pre-treatment *Microcystis* cell density in the laboratory algal toxicity experiment was ~2.4 x 10⁵ cells mL⁻¹ (± 1.9 x 10⁴ cells mL⁻¹), and control was achieved using 0.2 mg Cu L⁻¹, a potential algaecide dose of ~9.1 x 10⁻⁷ µg Cu cell⁻¹ was estimated. Potential algaecide doses in the southwest corner treatment site and the marina treatment site were based on pre-treatment *Microcystis* cell densities and total copper concentrations measured approximately four hours after application. These potential algaecide doses were estimated as ~1.0 x 10⁻⁶ µg Cu cell⁻¹ and ~1.4 x 10⁻⁶ µg Cu cell⁻¹ in the southwest corner treatment site and the marina treatment site, respectively. These estimated algaecide doses were approximately one order of magnitude greater than the laboratory exposure. Responses of *Microcystis* in the southwest corner treatment site were similar to responses measured in the laboratory algal toxicity experiment (Figures 3.2 and 3.3-A; Table 3.5).
Summary

*Microcystis aeruginosa* in Pawnee Reservoir water was sensitive to the Cutrine®-Ultra exposures with control obtained at 0.2 mg Cu L⁻¹ [as Cutrine®-Ultra; 2.4 x 10⁵ cells mL⁻¹, 34 μg chlorophyll *a* L⁻¹ (pre-treatment)]. This concentration of copper is five times less than would be allowed as the maximum treatment permitted by the label. This sensitivity of algae in site water emphasizes the utility and importance of laboratory studies prior to large field-scale applications in order to efficiently use algaecides while maximizing the potential margin of safety to non-target species (Fitzgerald and Jackson 1979, Mastin et al. 2002, Murray-Gulde et al. 2002).

As expected, *Microcystis* in Pawnee Reservoir was sensitive and responded to the algaecide treatment in terms of algal cell densities, chlorophyll *a* concentrations, and aqueous microcystin concentrations. Total copper concentrations decreased to background in both treatment sites, and did not differ from the untreated reference site 24 hours after treatment, likely due to algal uptake. In this study, aqueous microcystin was measured to determine whether microcystin was released from algal cells after treatment. Aqueous microcystin measurements assess the degree of microcystin release and subsequent increases in concentrations after an algaecide treatment. Measurements of aqueous microcystin are critical for signaling release of microcystin after treatment; however, total microcystin measurements are used to guide policy, and regulatory or management decisions (i.e. WHO 2003). In the Pawnee Reservoir field study, measurement of total microcystin was complicated or confounded by the
technique used to extract microcystin from algal cells. In critical situations where water usage decisions are determined based on total microcystin concentrations, further study is needed regarding the capacity for microcystin release after treatment, as well as the technique used to extract cell-associated microcystin prior to total microcystin measurement. Regardless, we recommend using internal and surrogate standards of microcystin during measurement of total microcystin (APHA 1998). After treatment of *Microcystis* in Pawnee Reservoir with Cutrine®-Ultra, aqueous and total microcystin increases were ephemeral and not of ecological or health significance in terms of concentration or persistence. Lack of persistence of microcystin in waters where *Microcystis* is common has been observed by Jones and Orr (1994), Lam et al. (1995) and Christofferson et al. (2002).

Previous studies (i.e. Kenefick et al. 1993; Jones and Orr 1994; Peterson et al. 1995) have observed release of intracellular contents of algae following exposure to algaecides and potable water treatment chemicals. These studies are commonly cited as evidence that release of microcystin following exposure to these chemicals could pose significant risks to humans or animals for extended periods of time. These studies and their results are not applicable for prediction of responses of algae to algaecide exposures used in current practices by water resource managers (Tedrow et al. in review). By developing strategic monitoring programs and efficient use of effective algaecides, risks from problematic cyanobacteria can be managed and water resource usages can be restored and maintained (Mastin et al. 2002).
With a timely monitoring program, water resource managers can intervene before *Microcystis* achieves densities and microcystin releases that could pose risks to people and other animals.
Acknowledgements

The authors thank Applied Biochemists and the Nebraska Game and Parks Commission for providing the algaecide concentrates used for this field treatment, collecting water samples used for laboratory exposures and for providing financial support for this research. We also thank the University of Nebraska-Lincoln Water Center and the Nebraska Department of Environmental Quality for their support. We also thank Rob Ruskamp and Jay Woltemath of the Nebraska Game and Parks Commission for their generous contribution of time and resources. And we thank Dr. Louwanda Jolley and Dr. Wayne Chao for assistance with copper measurements.
Literature Cited


Table 3.1: Nominal and measured (mean) initial exposure concentrations of copper (total) as Cutrine®-Ultra in the laboratory algal toxicity test with *Microcystis* in Pawnee Reservoir water.

<table>
<thead>
<tr>
<th>Nominal Exposure Concentrations (mg Cu L⁻¹)</th>
<th>Measured (mean) Total Copper Concentrations (mg Cu L⁻¹)</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.03</td>
<td>± 0.02</td>
</tr>
<tr>
<td>0.2</td>
<td>0.22</td>
<td>± 0.03</td>
</tr>
<tr>
<td>0.4</td>
<td>0.44</td>
<td>± 0.01</td>
</tr>
<tr>
<td>0.6</td>
<td>0.67</td>
<td>± 0.05</td>
</tr>
<tr>
<td>0.8</td>
<td>0.87</td>
<td>± 0.01</td>
</tr>
<tr>
<td>1.0</td>
<td>0.99</td>
<td>± 0.03</td>
</tr>
</tbody>
</table>
Table 3.2: Characteristics of Pawnee Reservoir water used for the laboratory algal toxicity test with *Microcystis* exposed to Cutrine®-Ultra.

<table>
<thead>
<tr>
<th>Water Characteristic</th>
<th>Units</th>
<th>Laboratory Exposures (Cutrine®-Ultra)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>pH</em></td>
<td>(SU)</td>
<td>8.1</td>
</tr>
<tr>
<td>Dissolved Oxygen</td>
<td>(mg O₂ L⁻¹)</td>
<td>8.4</td>
</tr>
<tr>
<td>Total Alkalinity</td>
<td>(mg L⁻¹ as CaCO₃)</td>
<td>184</td>
</tr>
<tr>
<td>Total Hardness</td>
<td>(mg L⁻¹ as CaCO₃)</td>
<td>160</td>
</tr>
<tr>
<td>Conductivity</td>
<td>(µS cm⁻²)</td>
<td>432</td>
</tr>
<tr>
<td>Temperature</td>
<td>(°C)</td>
<td>21</td>
</tr>
</tbody>
</table>
Table 3.3: Pre- and post- treatment total copper concentrations measured in Pawnee Reservoir water sampled from the southwest corner treatment site, marina treatment site and the untreated reference site (mean ± one standard deviation).

<table>
<thead>
<tr>
<th>Sampling Times</th>
<th>Southwest Corner Treatment Site</th>
<th>Marina Treatment Site</th>
<th>Untreated Reference Site</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-treatment</td>
<td>0.02 ± 0.01</td>
<td>0.05 ± 0.01</td>
<td>0.03 ± 0.01</td>
</tr>
<tr>
<td>4 HAT&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.85 ± 0.01</td>
<td>0.60 ± 0.01</td>
<td>NS</td>
</tr>
<tr>
<td>24 HAT</td>
<td>0.03 ≤ 0.01</td>
<td>0.04 ± 0.01</td>
<td>0.02 ± 0.01</td>
</tr>
<tr>
<td>48 HAT</td>
<td>0.01 ≤ 0.01</td>
<td>0.03 ± 0.02</td>
<td>0.02 ± 0.01</td>
</tr>
<tr>
<td>6 DAT&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.04 ± 0.02</td>
<td>0.03 ± 0.01</td>
<td>0.03 ± 0.01</td>
</tr>
<tr>
<td>9 DAT&lt;sup&gt;c&lt;/sup&gt;</td>
<td>NS</td>
<td>0.03 ± 0.02</td>
<td>0.03 ± 0.01</td>
</tr>
</tbody>
</table>

<sup>a</sup> Hours after treatment. <sup>b</sup> Days after treatment. <sup>c</sup> Not sampled.
Table 3.4: Measured (mean) characteristics of Pawnee Reservoir water obtained from the southwest corner treatment site, marina treatment site, and the untreated reference site pre- and post- treatment.

<table>
<thead>
<tr>
<th>Water Characteristic</th>
<th>Sampling Sites and Times</th>
<th>Southwest Corner Treatment Site</th>
<th>Marina Treatment Site</th>
<th>Untreated Reference Site</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pre&lt;sup&gt;a&lt;/sup&gt; 4 HAT&lt;sup&gt;b&lt;/sup&gt; 24 HAT 48 HAT 6 DAT&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Pre 4 HAT 24 HAT 48 HAT 6 DAT</td>
<td>Pre 24 HAT 48 HAT 6 DAT 9 DAT</td>
</tr>
<tr>
<td>pH (SU)</td>
<td></td>
<td>8.9 8.1 8.2 8.4 8.1</td>
<td>8.7 8.3 8.0 8.2 8.2 8.3</td>
<td>8.4 8.3 8.3 8.3 8.3</td>
</tr>
<tr>
<td>Dissolved Oxygen (mg O₂ L⁻¹)</td>
<td></td>
<td>9.0 8.4 11.1 8.6 8.5</td>
<td>8.7 8.6 7.9 8.7 8.8 8.8</td>
<td>8.8 10.1 8.5 9.0 8.6</td>
</tr>
<tr>
<td>Total Alkalinity (mg L⁻¹ as CaCO₃)</td>
<td></td>
<td>170 176 170 168 170</td>
<td>170 184 150 174 170 174</td>
<td>180 167 170 172 176</td>
</tr>
<tr>
<td>Total Hardness (mg L⁻¹ as CaCO₃)</td>
<td></td>
<td>158 192 160 188 156</td>
<td>154 182 157 164 156 154</td>
<td>164 163 174 164 154</td>
</tr>
<tr>
<td>Conductivity (µS cm⁻²)</td>
<td></td>
<td>342 346 410 347 372</td>
<td>323 334 425 347 372 381</td>
<td>343 420 356 377 382</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td></td>
<td>25 27 26 27 23</td>
<td>25 26 27 26 23 21</td>
<td>26 27 27 23 21</td>
</tr>
</tbody>
</table>

<sup>a</sup> Pre-treatment. <sup>b</sup> Hours after treatment. <sup>c</sup> Days after treatment.
Table 3.5: Comparing laboratory and field measurements of Pawnee Reservoir water pre- and post-treatment using Cutrine®-Ultra. Measurements included water characteristics, total copper concentrations, algal cell densities, chlorophyll \(a\) concentrations, and aqueous and total microcystin concentrations.

### Exposures

<table>
<thead>
<tr>
<th>Water Characteristic</th>
<th>Laboratory Study</th>
<th>Pawnee Reservoir Treatment Sites mean (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH (SU)</td>
<td>8.1</td>
<td>8.8 (8.7-8.9)</td>
</tr>
<tr>
<td>Dissolved Oxygen (mg O(_2) L(^{-1}))</td>
<td>8.4</td>
<td>8.9 (8.7-9.0)</td>
</tr>
<tr>
<td>Total Alkalinity (mg L(^{-1}) as CaCO(_3))</td>
<td>184</td>
<td>170 (both sites)</td>
</tr>
<tr>
<td>Total Hardness (mg L(^{-1}) as CaCO(_3))</td>
<td>160</td>
<td>156 (154-158)</td>
</tr>
<tr>
<td>Conductivity ((\mu)S cm(^{-2}))</td>
<td>432</td>
<td>333 (323-342)</td>
</tr>
<tr>
<td>Temperature ((^\circ)C)</td>
<td>21</td>
<td>25 (both sites)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Laboratory Study Exposures (nominal) (mg Cu L(^{-1}))</th>
<th>Laboratory Study mean ± one S.D. (mg Cu L(^{-1}))</th>
<th>Pawnee Reservoir Sampling Times</th>
<th>Pawnee Reservoir Treatment Sites mean (range) (mg Cu L(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unexposed Control</td>
<td>0.03 ± 0.02</td>
<td>Pre(^a)</td>
<td>0.35 (0.02-0.05)</td>
</tr>
<tr>
<td>0.2</td>
<td>0.22 ± 0.03</td>
<td>4 HAT(^b)</td>
<td>0.73 (0.60-0.85)</td>
</tr>
<tr>
<td>0.4</td>
<td>0.44 ± 0.01</td>
<td>24 HAT</td>
<td>0.04 (0.03-0.04)</td>
</tr>
<tr>
<td>0.6</td>
<td>0.67 ± 0.05</td>
<td>48 HAT</td>
<td>0.02 (0.01-0.03)</td>
</tr>
<tr>
<td>0.8</td>
<td>0.87 ± 0.01</td>
<td>6 DAT(^c) (both sites)</td>
<td>0.03</td>
</tr>
<tr>
<td>1.0</td>
<td>0.99 ± 0.03</td>
<td>9 DAT (marina)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

\(^a\) Pre-treatment. \(^b\) Hours after treatment. \(^c\) Days after treatment.

* Water characteristics and total copper concentrations measured from the untreated reference site did not significantly differ from pre-treatment concentrations measured from the treatment sites.
### Responses

<table>
<thead>
<tr>
<th>Laboratory Study Sampling Times: Chlorophyll $a$</th>
<th>Laboratory Exposure of 0.2 mg Cu L$^{-1}$ mean ± one S.D. ($\mu g$ chl $a$ L$^{-1}$)</th>
<th>Pawnee Reservoir Sampling Times: Chlorophyll $a$</th>
<th>Pawnee Reservoir Treatment Sites mean (range) ($\mu g$ chl $a$ L$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre$^a$</td>
<td>34 ± 2</td>
<td>Pre</td>
<td>57 (49-64)</td>
</tr>
<tr>
<td>24 HAT$^b$</td>
<td>ND$^c$</td>
<td>4 HAT</td>
<td>19 (15-23)</td>
</tr>
<tr>
<td>48 HAT</td>
<td>ND</td>
<td>24 HAT</td>
<td>37 (12-62)</td>
</tr>
<tr>
<td>96 HAT</td>
<td>ND</td>
<td>48 HAT</td>
<td>12 (ND-12)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6 DAT$^d$</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9 DAT (marina)</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reference Site</td>
<td>28 (ND-54)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Laboratory Study Sampling Times: Cell Densities</th>
<th>Laboratory Exposure of 0.2 mg Cu L$^{-1}$ mean ± one S.D. (cells mL$^{-1}$)</th>
<th>Pawnee Reservoir Sampling Times: Cell Densities</th>
<th>Pawnee Reservoir Treatment Sites mean (range) (cells mL$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre</td>
<td>2.4x10$^5$ ± 1.9x10$^4$</td>
<td>Pre</td>
<td>4.6x10$^5$ (7.4x10$^4$-8.4x10$^5$)</td>
</tr>
<tr>
<td>24 HAT</td>
<td>3.1x10$^4$ ± 1.1x10$^4$</td>
<td>4 HAT</td>
<td>1.1x10$^5$ (3.6x10$^4$-1.9x10$^5$)</td>
</tr>
<tr>
<td>48 HAT</td>
<td>1.3x10$^5$ ± 9.3x10$^3$</td>
<td>24 HAT</td>
<td>1.6x10$^5$ (3.2x10$^4$-2.8x10$^5$)</td>
</tr>
<tr>
<td>96 HAT</td>
<td>ND</td>
<td>48 HAT</td>
<td>2.0x10$^4$ (1.7x10$^3$-2.3x10$^4$)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6 DAT</td>
<td>7.4x10$^3$ (1.1x10$^3$-3.8x10$^3$)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9 DAT (marina)</td>
<td>4.6x10$^4$ (1.0x10$^3$-8.1x10$^4$)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reference Site</td>
<td>2.3x10$^4$ (ND-4.2x10$^4$)</td>
</tr>
</tbody>
</table>

$^a$ Pre-treatment. $^b$ Hours after treatment. $^c$ Not detectable (see text). $^d$ Days after treatment.
## Responses

<table>
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<tr>
<th>Laboratory Study Sampling Times: Aqueous Microcystin</th>
<th>Laboratory Exposure of 0.2 mg Cu L⁻¹ mean ± one S.D. (µg L⁻¹)</th>
<th>Pawnee Reservoir Sampling Times: Aqueous Microcystin</th>
<th>Pawnee Reservoir Treatment Sites mean (range) (µg L⁻¹)</th>
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<tr>
<td>Pre&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.31 ± NC&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Pre</td>
<td>0.62 (0.26-0.98)</td>
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<tr>
<td>4 HAT&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.51 ± 0.17</td>
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<td>3.85 (3.31-4.38)</td>
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<td>0.56 ± 0.11</td>
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<td>1.70 (1.21-2.19)</td>
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<td>0.46 ± 0.16</td>
<td>48 HAT</td>
<td>0.22 (0.19-0.24)</td>
</tr>
<tr>
<td>96 HAT</td>
<td>0.38 ± 0.04</td>
<td>6 DAT&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.16 (ND-0.16)</td>
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<tr>
<td>9 DAT (marina)</td>
<td></td>
<td></td>
<td>0.16</td>
</tr>
<tr>
<td>Reference Site</td>
<td></td>
<td></td>
<td>0.18 (ND-0.28)</td>
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<table>
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<tr>
<th>Laboratory Study Sampling Times: Total Microcystin</th>
<th>Laboratory Exposure of 0.2 mg Cu L⁻¹ mean ± one S.D. (µg L⁻¹)</th>
<th>Pawnee Reservoir Sampling Times: Total Microcystin</th>
<th>Pawnee Reservoir Treatment Sites mean (range) (µg L⁻¹)</th>
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</thead>
<tbody>
<tr>
<td>Pre</td>
<td>2.80 ± 0.55</td>
<td>Pre</td>
<td>0.30 (0.28-0.32)</td>
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<td>4 HAT</td>
<td>4.66 ± 0.10</td>
<td>4 HAT</td>
<td>0.22 (both sites)</td>
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<tr>
<td>24 HAT</td>
<td>0.45 ± 0.08</td>
<td>24 HAT</td>
<td>0.31 (0.22-0.39)</td>
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<tr>
<td>48 HAT</td>
<td>0.43 ± 0.09</td>
<td>48 HAT</td>
<td>0.16 (ND&lt;sup&gt;f&lt;/sup&gt;-0.18)</td>
</tr>
<tr>
<td>96 HAT</td>
<td>0.62 ± 0.03</td>
<td>6 DAT</td>
<td>0.16 (ND-0.18)</td>
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<tr>
<td>9 DAT (marina)</td>
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<td></td>
<td>0.16</td>
</tr>
<tr>
<td>Reference Site</td>
<td></td>
<td></td>
<td>0.18 (ND-0.22)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Pre-treatment.  <sup>b</sup> Hours after treatment.  <sup>c</sup> Days after treatment.  <sup>d</sup> Not calculatable.  
<sup>e</sup> Measured after sonication.  <sup>f</sup> Measured after freezing-and-thawing.  <sup>g</sup> Not detectable (detection limit = ≥ 0.16 µg L⁻¹).
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Figure 3.1: Contour map of Pawnee Reservoir, Nebraska, depicting the entire treatment area (outlined in red), along with the two monitored treatment sites (the southwest corner treatment site and the marina treatment site) and the untreated reference site.

Figure 3.2: Laboratory measurements of *Microcystis* in Pawnee Reservoir water pre- and post- treatment using 0.2 mg Cu L⁻¹ as Cutrine®-Ultra. Cell-associated microcystin was extracted using sonication, prior to measurement of total microcystin (HAT = Hours after treatment; DAT = Days after treatment).

Figure 3.3: Measurements of *Microcystis* in Pawnee Reservoir water pre- and post- treatment in A) the southwest corner treatment site, B) the marina treatment site, and C) the untreated reference site (HAT = Hours after treatment; DAT = Days after treatment).
Figure 3.1

Untreated Reference Site

Marina Treatment Site.

SW Corner Treatment Site.
Figure 3.2

Sampling Times
(* Not measured.)
(** Not measurable.)
Figure 3.3

A

Sampling Times
(SW Corner)

** Not measurable

B

Sampling Times
(Marina Area)

C

Sampling Times
(Reference Area)

** Not measurable
CHAPTER 4
LABORATORY AND FIELD RESPONSES OF *LYNGBYA* SP.
FOLLOWING EXPOSURES TO COPPER-CONTAINING ALGAECIDES

Abstract

Based upon results from laboratory algal toxicity tests, I predicted responses of *Lyngbya* to algaecide exposures in City Lake (High Point, North Carolina) were confirmed. In this research, I sought an efficacious treatment for *Lyngbya* using laboratory algal toxicity tests, evaluated the treatment in the field, and compared similar exposures and responses from the laboratory with exposures and responses in City Lake. *Lyngbya* in City Lake water was susceptible to Algimycin® PWF (copper-citrate and copper-gluconate chelated algaecide) in laboratory exposures. Following two applications of Algimycin® PWF, *Lyngbya* biomass in City Lake decreased (~77%). When similar algaecide exposures in the laboratory were compared with exposures in City Lake, responses of *Lyngbya* were comparable. Uses of this reservoir (i.e. potable water, recreation, and fishing) were restored and maintained by application of an effective algaecide. Early detection of *Lyngbya* growth lead to development and implementation of an effective management strategy, restoring and maintaining critical water resource usages.
Key words: Algae control, copper, Algimycin®, PWF, Clearigate®, PAK™ 27, cyanobacteria, City Lake.

Introduction

As water resources become more intensely and extensively utilized, the impacts of nuisance and alien aquatic species become more acute and widespread (Hallegraeff 1993). Excessive growths of algae can cause significant disruption of water resource usages. In freshwater reservoirs throughout the United States, benthic, mat-forming algae, such as *Lyngbya*, have caused problems ranging from eroding property values, and production and release of taste-and-odor compounds, to avoidance behavior demonstrated by some fish species (Speziale and Dyck 1992; Gross and Martin 1996; Doyle and Smart 1998; Mastin et al. 2002; Cowell and Dawes 2004). If benthic algae interfere with critical water resource usages and problems become severe, water resource managers often initiate management practices such as applications of algaecides. Based on economic and environmental considerations, copper algaecide formulations are often used to control excessive growths of *Lyngbya* (Mastin et al. 2002).

The filamentous cyanobacterium (blue-green alga), *Lyngbya*, forms dense benthic and surface mats with cells protected by an external sheath comprised of polysaccharides, peptidoglycans and minerals such as calcium carbonate (Speziale et al. 1991; Speziale and Dyck 1992; Hoiczyk and Baumeister 1995; Gross and Martin 1996; Doyle and Smart 1998; Pentecost 2003; Wehr and Sheath 2003). *Lyngbya* can thrive at extreme temperatures ranging from melt-water lakes
and streams to hot springs (Graham and Wilcox 2000). This alga also contains photosynthetic accessory pigments (i.e. phycobilins) which permit growth in low light conditions (i.e. < 2% incident photosynthetically active radiation). *Lyngbya* can grow in waters with low nitrogen concentrations (≤ 0.07 mg NO₃-N L⁻¹) due to its ability to fix nitrogen (Cowell and Dawes 2004). *Lyngbya* can achieve densities of ≥ 1.14 Kg m⁻² (dry weight) with ~40-100% of this biomass existing as benthic mats (Speziale and Dyck 1992; Doyle and Smart 1998). In situations where *Lyngbya* accumulates extreme benthic biomass, single applications of an algaecide are unlikely to achieve control.

Given the costs of treatments of benthic mat forming algae, such as *Lyngbya*, as well as potential environmental impacts, it is logical that water resource managers would apply the minimum amount of an efficacious algaecide required to gain control of the target algae (Tubea et al. 1981). To efficiently and effectively treat *Lyngbya*, it is important to accurately predict field responses of algae based upon laboratory exposures to algaecides and measurements of responses to those exposures. We have used laboratory algaecide exposures involving algae in site waters to predict efficacious treatments (form and treatment concentration) to achieve control of target algae (Mastin et al. 2002; Murray-Gulde et al. 2002). For copper-containing algaecides, water characteristics and other site parameters influence the speciation of copper and thus the bioavailability and efficacy of an algaecide application (Erickson et al. 1996; Haughey et al. 2000). Use of site water with associated algae in the laboratory algal toxicity tests minimizes the potential for ambiguity in applying
laboratory results directly to a field situation (Fitzgerald and Jackson 1979). An infestation of *Lyngbya* in City Lake (High Point, North Carolina) provided an opportunity to test laboratory predictions of responses of *Lyngbya* in this approximately 138 hectare (~341 acre) drinking water reservoir after applications of a copper-containing algaecide.

The dam forming City Lake was completed in 1928. The primary designated use of this reservoir was storage of potable water for the City of High Point, North Carolina (NC). However, recreational uses such as boating and fishing have become increasingly important as a significant source of public and private revenue. City Lake has a surface area of approximately 138 hectares (341 acres), a drainage basin of ~15,902 hectares (39,278 acres) and a mean depth of approximately three meters. Water retention time of City Lake is approximately 26 days. This reservoir has hosted professional fishing tournaments and sustains viable bass and carp fisheries. Local firefighters and associated emergency personnel use City Lake to practice diving techniques and simulate emergency rescue situations such as drowning victims. Surface and benthic mats of *Lyngbya* in City Lake appeared following an input of nutrients from a nearby sewage pipeline prior to July, 2000. These *Lyngbya* mats covered the periphery of the reservoir extending approximately 5-8 meters (~15-24 feet) from the shoreline. Use of this resource for fishing, recreational and other activities conducted by local emergency personnel was restricted due to these algal mats (William Frazier, pers. comm., City of High Point, NC).
Three chemical control tactics were evaluated in laboratory tests for efficacy in controlling *Lyngbya* from City Lake, High Point, NC. The algaecide, Algimycin\textsuperscript{®} PWF, has a copper concentration of 5% in the form of copper sulfate pentahydrate, copper citrate, and other copper gluconate chelates (Applied Biochemists 2002, 2005b). Clearigate\textsuperscript{®} contains 3.825% elemental copper by weight, which is formulated as ethanolamine complexes, along with the adjuvant D-limonene [Applied Biochemists 2005(a)]. PAK\textsuperscript{TM} 27 is a granular, hydrogen peroxide-based algaecide, with an active ingredient (sodium carbonate peroxyhydrate) concentration of 85% (Solvay Chemicals 2004). Cide-Kick\textsuperscript{®} II is an adjuvant comprised of D-limonene, terpene hydrocarbon and nonylphenol polyethylene glycol ether (Brewer International 2000), and was used with Algimycin\textsuperscript{®} PWF in the sequential algaecide exposure (Duke et al. in review).

The overall objective of this research was to compare similar laboratory and field exposures of *Lyngbya* to Algimycin\textsuperscript{®} PWF, and compare responses to those exposures. Specific objectives of this research were to: 1) measure responses of *Lyngbya* in laboratory exposures of Algimycin\textsuperscript{®} PWF, Clearigate\textsuperscript{®}, and a sequential algaecide treatment using PAK\textsuperscript{TM} 27 prior to Algimycin\textsuperscript{®} PWF with Cide-Kick\textsuperscript{®} II, 2) measure responses of *Lyngbya* to treatments of Algimycin\textsuperscript{®} PWF in City Lake, High Point, NC, in terms of *Lyngbya* biomass (g m\textsuperscript{-2}), chlorophyll *a* concentration (mg m\textsuperscript{-2}), and chlorophyll *a* concentrations (\(\mu\)g g\textsuperscript{-1} *Lyngbya*), and 3) compare responses of *Lyngbya* in laboratory exposures of Algimycin\textsuperscript{®} PWF to responses after field applications of Algimycin\textsuperscript{®} PWF.
Materials and Methods

Laboratory Algal Toxicity Tests

Stock algaecide solutions (1000 mg Cu L\(^{-1}\)) for laboratory exposures were made using NANOpure™ water within four hours prior to initiation of experiments. Exposure concentrations of copper as Algimycin® PWF and Clearigate® were prepared by adding the appropriate volume of stock algaecide solution (1000 mg Cu L\(^{-1}\)) to each exposure replicate to achieve the desired exposure concentration. Concentrations of copper as Algimycin® PWF and Clearigate® for these laboratory exposures were 10, 100, 200, and 300 mg Cu g\(^{-1}\) Lyngbya.

Exposure duration was 96 hours using a photo-period of 16h: 8h (light: dark). Light intensity was \(\sim\)3077 lux, using cool white fluorescent lighting. Exposure chambers were maintained at a temperature of 21°C ± 2°C. Algaecide exposures were verified by measuring total copper concentrations from exposure vessels. To measure total copper, approximately 200 μL trace-metal grade nitric acid (Fisher Scientific, Fair Lawn, NJ 07410) was used to acidify all aqueous copper samples (\(\sim\)12 mL; \(pH \leq 2\)). Total copper concentrations were measured using a Perkin-Elmer 5100 PC flame and graphite furnace atomic absorption (AA) spectrophotometer (method 3010-B; APHA 1998). Water quality characteristics [\(pH\) (Standard Units), dissolved oxygen (D.O.; mg O\(_2\) L\(^{-1}\)), conductivity (μS cm\(^{-2}\)), total alkalinity (mg L\(^{-1}\) as CaCO\(_3\)), total hardness (mg L\(^{-1}\) as CaCO\(_3\)), and temperature (°C)] were measured according to APHA methods (1998).
For these laboratory algaecide exposures, a volume of approximately 20 liters of water was collected from City Lake in High Point, NC. Samples of *Lyngbya* were collected from benthic mats. These samples of water and *Lyngbya* were used for all laboratory algaecide toxicity tests. Because responses of algae to exposures of algaecides are density dependent (Murray-Gulde et al. 2002), laboratory exposures used 0.1 g *Lyngbya* per exposure replicate. Exposures of *Lyngbya* to Algimycin® PWF were initiated using 200 mL site water and 0.1 g (wet weight) *Lyngbya* in 250 mL Erlenmeyer flasks. Exposures of *Lyngbya* to Clearigate® were developed using 100 mL site water and 0.1 g (wet weight) *Lyngbya* in 125 mL Erlenmeyer flasks. The sequential algaecide treatment exposures were started using 1-L of site water in 2-L Erlenmeyer flasks with 0.1 g (wet weight) *Lyngbya* exposed to 6.2 mg PAK™ 27, 24 hours prior to addition of 1.0 mg Cu L⁻¹ as Algimycin® PWF with 6 μL Cide-Kick® II.

Algal response measured for the laboratory algal toxicity experiments was chlorophyll *a* concentration after 96 hour exposures. Samples for pre-exposure chlorophyll *a* measurement (0.1 g wet weight *Lyngbya*) were collected and frozen at -20°C for ≥ 24 hours prior to grinding the algal mass using a ceramic mortar and pestle. Algal material was filtered from each exposure replicate using a 25 mm cellulose membrane filter (0.45 μm) at experiment conclusion and frozen for ≥ 24 hours. Filtered algal material was macerated using a ceramic mortar and pestle. Chlorophyll *a* was extracted from the ground algal material using 20 mL of 90% buffered acetone according to United States Environmental Protection Agency (USEPA) method 445.0 (Arar and Collins 1997). Chlorophyll *a*
concentrations were measured fluorometrically using a SpectraMax® 190 Gemini 96 well plate-reading spectrofluorometer (Molecular Devices Corporation, Sunnyvale, CA 94089). Fluorometric measurements of test samples were calibrated with fluorescence values of known chlorophyll $a$ standards. Chlorophyll $a$ standards were prepared from a stock solution of 4000 $\mu$g chlorophyll $a$ L$^{-1}$ (Sigma C-5753; range = 10-1280 $\mu$g chlorophyll $a$ L$^{-1}$), kept protected from light and stored in a -20°C freezer.

City Lake: Algaecide Application Study

To determine the treatment effectiveness of Algimycin® PWF for *Lyngbya* in City Lake, two sites were monitored: 1) an untreated reference area (~3 acres; 1.2 hectares) northwest of the treatment area, and 2) a treatment area (~3 acres; 1.2 hectares) (Figure 4.1). Composite water samples were collected along a 20 meter transect in each monitoring site. The untreated reference site and the treatment site were similar in terms of average depth, lengths of the sampling transect, and distance of the sampling transect from shore. Water characteristics ($p$H, dissolved oxygen, conductivity and temperature) were measured prior to and periodically after treatment. Due to the biomass of *Lyngbya* observed in City Lake, multiple applications of Algimycin® PWF were recommended. For comparison with results obtained in the laboratory test, results from City Lake were evaluated after two applications as explained below.

The first field application of Algimycin® PWF was initiated on April 27, 2006. Approximately 76 liters (20 gallons) were applied beneath the water’s
surface using trailing hoses. The Algimycin® PWF concentrate was diluted using site water prior to application [~76 liters (20 gallons) of Algimycin® PWF concentrate and ~114 liters (30 gallons) of site water]. In addition to pre-treatment samples, samples for aqueous total copper concentrations were collected from the treatment site four hours, 24 hours, three days, five days, and eight days post-treatment.

The second field application of Algimycin® PWF was conducted on June 19, 2006, and essentially duplicated the treatment described above. Samples for pre-treatment aqueous total copper concentrations were obtained from the untreated reference site and the treatment site immediately prior to treatment. Samples for aqueous total copper concentrations were collected from the treatment site four hours, three days, five days, and eight days post-treatment.

Samples for measurement of pre-treatment Lyngbya biomass and chlorophyll a concentration were collected immediately prior to the first algaecide application on April 27, 2006 from the untreated reference site and the treatment site (Figure 4.1). Samples of Lyngbya were collected from the untreated reference site and the treatment site on June 19 and July 26, 2006, for measurement of Lyngbya biomass and chlorophyll a concentrations (approximately one month after each application). Three Lyngbya samples from each of three points along the 20 meter transect across each site were collected using a posthole digger (diameter sampled 10.77 cm, area 0.0092 m²). All Lyngbya samples were collected as benthic samples in the untreated reference site and the treatment site. Three aliquots of 0.1 g Lyngbya were used from each algal sample for chlorophyll
a measurement. The remaining *Lyngbya* was dried at 60°C for 12-14 days for measurement of dry weight algal biomass (Speziale et al. 1991).

*Comparison of Laboratory and City Lake Results*

The laboratory study provided information regarding responses of *Lyngbya* in City Lake water to exposures of Algimycin® PWF, and predictions of responses of *Lyngbya* in City Lake after field applications of Algimycin® PWF. To reliably predict responses of algae in field treatments from laboratory results, comparison of similar exposures is necessary (Fitzgerald and Jackson 1979). A range of laboratory algaecide exposures was selected to encompass potential treatments that could be used in City Lake. Since *Lyngbya* was treated with algaecide in the field as a benthic mat and the algaecide is applied to the overlying water, it is important to compare the appropriate laboratory exposure to accommodate for the differences in water: algal mass relationships from the laboratory to the field. With the conditions in this situation, the laboratory exposure of ~10.0 mg Cu g⁻¹ *Lyngbya* closely resembled field exposures in terms of mass of copper applied to mass of *Lyngbya* treated after two applications. In both situations, similar exposures (~10 mg Cu g⁻¹ *Lyngbya*) and responses of *Lyngbya* to those exposures were compared.
Statistical Analyses

A one-way analysis of variance was used to discern differences in chlorophyll $a$ concentrations in laboratory treatments with differences identified through multiple range testing (Dunnett’s). A t-test procedure was used to determine statistically significant differences in *Lyngbya* biomass measurements and chlorophyll $a$ concentrations between sampling dates at the treatment site and the untreated reference site. In situations where variances between groups tested were not equal, a Mann-Whitney rank sum test was performed. All data were analyzed using SigmaStat version 3.1 for Windows (alpha = 0.05) (Systat Software, Inc., Point Richmond, CA 94804-2028).

Results and Discussion

Laboratory Algal Toxicity Tests

The primary purpose of the laboratory algal toxicity tests was to identify an efficacious algaecide and treatment (concentration and duration of exposure) that would control the growth of *Lyngbya* sp. in City Lake water. Exposures of Algimycin® PWF and Clearigate® in the laboratory algal toxicity tests were verified by measuring total copper concentrations from exposure chambers (Table 4.1). Measured concentrations of copper as Algimycin® PWF in these laboratory exposures were approximately 99-122% of nominal concentrations (Table 4.1). Measured concentrations of copper as Clearigate® in these laboratory exposures were approximately 94-174% of nominal concentrations (Table 4.1). The measured concentration of copper as Algimycin® PWF used in the sequential
laboratory algaecide exposure treatment was 80% of the nominal concentration. For these laboratory exposures, City Lake water characteristics (i.e. pH, alkalinity, hardness, conductivity) were indicative of soft water (Table 4.2) (Sawyer et al. 1994). Laboratory exposures were conducted at a temperature of 21 ± 2°C (Table 4.2).

Responses of *Lyngbya* to laboratory algaecide treatments are reported based upon nominal exposure concentrations (Table 4.1; Figure 4.2). The pre-treatment chlorophyll *a* concentration for these algal toxicity experiments was 300 (± 35) µg chlorophyll *a* g⁻¹ *Lyngbya*. After 96 hours, the chlorophyll *a* concentration in untreated controls increased significantly to 423 (± 35) µg chlorophyll *a* g⁻¹ *Lyngbya* (Figure 4.2-A, B). Following 96 hour exposures to 10, 100, 200, and 300 mg Cu g⁻¹ *Lyngbya* as Algimycin® PWF and Clearigate®, chlorophyll *a* concentrations decreased significantly (average = 76%) compared to pre-treatment chlorophyll *a* concentrations (Figure 4.2-A, B). In the sequential algaecide exposure, the chlorophyll *a* concentration decreased significantly to 78 (± 18) µg chlorophyll *a* g⁻¹ *Lyngbya* after 96 hours (Figure 4.2-A). In these laboratory experiments, *Lyngbya* in water sampled from City Lake responded sensitively to exposures of Algimycin® PWF with 71% decrease in chlorophyll *a* after exposure to ~10 mg Cu g⁻¹ *Lyngbya*. Based upon these laboratory data, a management strategy was developed for City Lake in the spring of 2006 using Algimycin® PWF.
City Lake: Algaecide Application Study

On the first day of treatment (April 27, 2006), concentrations of copper in City Lake ranged from approximately 0.006 mg L\(^{-1}\) to approximately 0.187 mg Cu L\(^{-1}\) four hours post-treatment (Table 4.3). For the second treatment on June 19, 2006, measured copper concentrations ranged from non-detect (∆ 0.001 mg Cu L\(^{-1}\)) to approximately 0.213 mg Cu L\(^{-1}\) four hours post-treatment. Total copper concentrations in the treatment site returned to background (∆ 0.002 mg Cu L\(^{-1}\)) within three to five days post-treatment. Characteristics of City Lake water (i.e. pH, alkalinity, hardness, conductivity) did not differ significantly between the treatment site and the untreated reference site either pre- or post-treatment (Table 4.4).

Lyngbya biomass measurements were obtained from benthic samples from the untreated reference site and the treatment area. Initial Lyngbya biomass measured in the untreated reference site was 900 (± 414) g Lyngbya (dry weight) m\(^{-2}\). Measurements of Lyngbya biomass in the untreated reference site did not differ significantly for any of the sampling dates (i.e. did not change during the course of this study) (Figure 4.3-A). In the treatment site, pre-treatment biomass was 2238 (± 961) g Lyngbya m\(^{-2}\), and significant decreases in biomass were observed after the first (June 19, 2006) and second (July 26, 2006) algaecide treatments (Figure 4.3-A).

The initial chlorophyll \(a\) concentration per square meter from benthic mat samples of Lyngbya from the untreated reference site was 110 (± 47) mg m\(^{-2}\). There was no significant difference in chlorophyll \(a\) concentration per square
meter for any of the samples from the untreated reference site throughout this study (Figure 4.3-B). Pre-treatment chlorophyll \( a \) concentration in the treatment site was 247 (± 86) mg m\(^{-2}\). In samples from the treatment site, a significant decrease in chlorophyll \( a \) concentration per square meter was measured after the second algaecide application compared to the pre-treatment measurement (Figure 4.3-B).

Initial chlorophyll \( a \) concentration in the untreated reference site was 129 (± 28) \( \mu \)g g\(^{-1}\) *Lyngbya*. The chlorophyll \( a \) concentration in samples from the benthic mat (\( \mu \)g g\(^{-1}\) *Lyngbya*) was significantly greater (~44%) at the mid-point sampling (June 19, 2006) than measurements earlier or later in the growing season (April 27, 2006, and July 26, 2006) (Figure 4.3-C). Pre-treatment chlorophyll \( a \) concentration in the treatment area was 120 (± 31) \( \mu \)g chlorophyll \( a \) g\(^{-1}\) *Lyngbya* and a significant decrease was observed in chlorophyll \( a \) concentration (\( \mu \)g g\(^{-1}\) *Lyngbya*) between measurements from June 19, 2006, and July 26, 2006, after the second treatment (Figure 4.3-C).

**Comparison of Laboratory and City Lake Results**

Water characteristics such as \( p \)H, hardness, alkalinity and conductivity can significantly alter speciation and bioavailability of copper (Haughey et al. 2000). Since the water characteristics were similar in the laboratory study and the City Lake field study, the speciation and bioavailability of the copper in Algimycin\(^{\circledR}\) PWF should be similar (Erickson et al. 1996). In both the laboratory study and the City Lake field study, *Lyngbya* was growing in essentially the same water which
should minimize uncertainty in translating laboratory results to the field (Table 4.5).

For this research, the laboratory experiment was conducted using City Lake water and *Lyngbya*, with a mean pre-treatment *Lyngbya* chlorophyll *a* concentration of ~300 µg g⁻¹ *Lyngbya*. The potential algaecide burden under these laboratory conditions was ~10 mg Cu g⁻¹ *Lyngbya*. The potential burdens under these field conditions ranged from 6.5-13.3 mg Cu g⁻¹ *Lyngbya*, based on total copper concentrations measured four hours after treatment and pre-treatment *Lyngbya* biomass measurements (Table 4.5).

Control of *Lyngbya* in the laboratory algal toxicity test was achieved using an exposure of Algimycin® PWF producing a potential burden of ~10.0 mg Cu g⁻¹ *Lyngbya*). For the first and second field applications of algaecide in City Lake, the mean pre-treatment *Lyngbya* biomass was ~2238 g *Lyngbya* m⁻² and 764 g *Lyngbya* m⁻², and the concentrations of copper measured four hours after treatment were ~0.187 µg Cu L⁻¹ and ~0.213 µg Cu L⁻¹, respectively. The two field algaecide treatments monitored represented potential Algimycin® PWF burdens of approximately 6.5 and 13.3 mg Cu g⁻¹ *Lyngbya* (Table 4.5). Consequently, it is not unexpected that *Lyngbya* responded to exposures of Algimycin® PWF in City Lake. Due to the mass of algae accumulated in City Lake, multiple treatments were necessary to achieve control.
Summary

*Lyngbya* in site water from City Lake in High Point, NC, was sensitive to Algimycin® PWF after a 96 hour exposure (pre-treatment chlorophyll a concentration 300 μg g⁻¹ *Lyngbya*) and a treatment strategy for *Lyngbya* in City Lake was developed based on laboratory results. These laboratory data emphasize the value and utility of using site water and site algae for laboratory algal toxicity experiments. Using this approach for predicting field responses of algae to algaecide applications permits more efficient use of effective algaecides.

Field treatments were initiated on April 27, 2006, and responses of *Lyngbya* to treatments of Algimycin® PWF in City Lake closely resembled responses measured in the laboratory experiment. Laboratory exposures used a predetermined volume of water in an exposure chamber and contact between the algaecide and *Lyngbya* was maximized. During field treatments, contact between the algaecide and *Lyngbya* was maximized by applying the algaecide below the water’s surface. Algaecide application techniques included treatment of the *Lyngbya* infested area from the shoreline to deeper water and only partial treatment of the infested area. These application techniques decrease potential exposures for non-target species, increase potential margins of safety for fish and invertebrates, and provided refugia during treatment.

Mastin et al. (2002) measured responses of *Lyngbya* from a north Louisiana reservoir to laboratory exposures of Clearigate® in an aqueous culture medium with a mean pH, alkalinity, and hardness of 7.4, 176 mg L⁻¹ as CaCO₃, and 92 mg L⁻¹ as CaCO₃, respectively. In this study, *Lyngbya* responded to
exposures of 0.3 and 0.6 mg Cu L\(^{-1}\) as Clearigate\textsuperscript{®}, with a 75 and 78 % decrease in *Lyngbya* after treatment. Duke et al. (in review) studied responses of *Lyngbya* sampled from two Alabama reservoirs (Lay and Jordan Reservoirs) to laboratory algaecide exposures in site waters with a mean pH, alkalinity, and hardness of 8.2, 54 mg L\(^{-1}\) as CaCO\(_3\), and 60 mg L\(^{-1}\) as CaCO\(_3\), respectively. *Lyngbya* used for this laboratory study responded to the sequential algaecide exposure technique (also used in the present study) with a \(>99\)% decrease in chlorophyll *a* concentration. Multiple applications of this sequential algaecide technique were required due to the density of *Lyngbya* in the treatment areas of Lay and Jordan Reservoirs. After treatments, algal biomass decreased (67-93%) in Lay and Jordan Reservoirs. In the present laboratory study, *Lyngbya* sampled from City Lake in site water with a mean pH, alkalinity, and hardness of 7.4, 50 mg L\(^{-1}\) as CaCO\(_3\), and 44 mg L\(^{-1}\) as CaCO\(_3\), respectively, responded to exposures of Algimycin\textsuperscript{®} PWF with a significant decrease (~71%) in chlorophyll *a*. Multiple applications of Algimycin\textsuperscript{®} PWF were required due to the density of *Lyngbya* observed in City Lake, and *Lyngbya* biomass decreased significantly (~77%) after two field algaecide treatments.

It is apparent from these studies that responses of *Lyngbya* to algaecide exposures vary widely. Although genetic, physiological and morphological variation can explain some of the differences observed, water characteristics that influence speciation and bioavailability can significantly alter exposures of copper-containing algaecides and subsequent responses of target algae. This further emphasizes the importance and utility of using laboratory algal toxicity
tests with site water and site algae to predict responses of target algae in field-scale applications. The laboratory information on sensitivity to algaecides, coupled with early detection of growth leading to development of extreme *Lyngbya* densities, can assist implementation of an effective management strategy that can restore and maintain critical water resource usages.
Acknowledgements

We would like to thank Applied Biochemists, a division of Advantis Technologies, Inc., and the City of High Point, North Carolina, personnel for providing funding for this research, and time and equipment for sampling through the summer of 2006. We also thank Laura Calloway for assistance with collection of water and algae samples and City of High Point GIS for mapping assistance.


Table 4.1: Laboratory algal toxicity experiments: Nominal and measured (mean) total concentrations of copper as Algimycin® PWF, Clearigate® and the sequential algaecide treatment using Algimycin® PWF (± one standard deviation).

<table>
<thead>
<tr>
<th>Nominal Exposure Concentration (mg Cu L⁻¹)</th>
<th>Measured Exposure (mg Cu L⁻¹)</th>
<th>Potential Algaecide Burden (mg Cu g⁻¹ Lyngbya)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Algimycin® PWF</td>
<td>Clearigate®</td>
</tr>
<tr>
<td>Unexposed Controls</td>
<td>NDᵃ</td>
<td>ND</td>
</tr>
<tr>
<td>5.0</td>
<td>6.1 ± 0.4</td>
<td>8.7 ± 0.2</td>
</tr>
<tr>
<td>50.0</td>
<td>49.7 ± 1.7</td>
<td>46.8 ± 2.8</td>
</tr>
<tr>
<td>100.0</td>
<td>106.0 ± 3.3</td>
<td>109.1 ± 8.37</td>
</tr>
<tr>
<td>150.0</td>
<td>176.6 ± 19.1</td>
<td>167.7 ± 9.2</td>
</tr>
<tr>
<td>1.0 (Sequential Algaecide Treatment using Algimycin® PWF)</td>
<td>0.8 ± 0.1</td>
<td>NTᵇ</td>
</tr>
</tbody>
</table>

ᵃ Not detectable (Detection limit = 0.001 mg Cu L⁻¹).ᵇ Not tested.
Table 4.2: Laboratory algal toxicity experiments: Measurements of pre-treatment water characteristics of City Lake (High Point, North Carolina) water used for Algimycin® PWF, Clearigate®, and the sequential algaecide treatments.

<table>
<thead>
<tr>
<th>Water Characteristic Parameter</th>
<th>Algimycin® PWF</th>
<th>Clearigate®</th>
<th>Sequential Algaecide Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH (SU)</td>
<td>7.4</td>
<td>7.3</td>
<td>7.4</td>
</tr>
<tr>
<td>Dissolved Oxygen (mg O₂ L⁻¹)</td>
<td>7.4</td>
<td>7.4</td>
<td>7.4</td>
</tr>
<tr>
<td>Total Alkalinity (mg L⁻¹ as CaCO₃)</td>
<td>50</td>
<td>44</td>
<td>48</td>
</tr>
<tr>
<td>Total Hardness (mg L⁻¹ as CaCO₃)</td>
<td>44</td>
<td>40</td>
<td>48</td>
</tr>
<tr>
<td>Conductivity (μS cm⁻²)</td>
<td>125</td>
<td>144</td>
<td>133</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>21</td>
<td>22</td>
<td>21</td>
</tr>
</tbody>
</table>
Table 4.3: City Lake (High Point, North Carolina) treatments: Measured total copper concentrations pre- and post-treatment using Algimycin® PWF on April 27, 2006 and June 19, 2006 (mean ± one standard deviation).

<table>
<thead>
<tr>
<th>Sampling Times</th>
<th>1st Treatment</th>
<th>2nd Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.006 ± 0.003</td>
<td>ND&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>4 HAT&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.187 ± 0.070</td>
<td>0.213 ± 0.025</td>
</tr>
<tr>
<td>24 HAT</td>
<td>0.070 ± 0.006</td>
<td>NS</td>
</tr>
<tr>
<td>3 DAT&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.078 ± 0.002</td>
<td>0.058 ± 0.012</td>
</tr>
<tr>
<td>5 DAT</td>
<td>NS&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.001 ± 0.001</td>
</tr>
<tr>
<td>8 DAT</td>
<td>0.006 ± 0.006</td>
<td>ND</td>
</tr>
</tbody>
</table>

<sup>a</sup> Pre-treatment.  <sup>b</sup> Hours after treatment.  <sup>c</sup> Days after treatment.  <sup>d</sup> Not sampled.  <sup>e</sup> Not detectable (Detection limit = 0.001 mg Cu L<sup>-1</sup>).
<table>
<thead>
<tr>
<th>Water Characteristic Parameter</th>
<th>Reference Site</th>
<th>Treatment Site</th>
</tr>
</thead>
<tbody>
<tr>
<td>$pH$ (SU)</td>
<td>7.1</td>
<td>7.7</td>
</tr>
<tr>
<td>Dissolved Oxygen (mg O₂ L⁻¹)</td>
<td>9.5</td>
<td>8.0</td>
</tr>
<tr>
<td>Conductivity (µS cm⁻²)</td>
<td>195</td>
<td>103</td>
</tr>
<tr>
<td>Total Alkalinity (mg L⁻¹ as CaCO₃)</td>
<td>40</td>
<td>32</td>
</tr>
<tr>
<td>Total Hardness (mg L⁻¹ as CaCO₃)</td>
<td>44</td>
<td>38</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>20</td>
<td>23</td>
</tr>
</tbody>
</table>

Table 4.4: Characteristics measured in City Lake (High Point, North Carolina) water from the untreated reference site and the treatment site prior to application of Algimycin® PWF on April 27, 2006 and June 19, 2006.
Table 4.5: Comparing laboratory and field measurements of City Lake (High Point, North Carolina) pre- and post- treatment using Algimycin® PWF. Measurements included water characteristics, total copper concentrations, *Lyngbya* biomass and chlorophyll *a* concentrations.

### Exposures

<table>
<thead>
<tr>
<th>Water Characteristics</th>
<th>Laboratory Study</th>
<th><em>City Lake Treatment Site mean (range)</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>pH (SU)</td>
<td>7.4</td>
<td>7.4 (7.2-7.6)</td>
</tr>
<tr>
<td>Dissolved Oxygen (mg O₂ L⁻¹)</td>
<td>7.4</td>
<td>8.8 (8.0-9.5)</td>
</tr>
<tr>
<td>Total Alkalinity (mg L⁻¹ as CaCO₃)</td>
<td>50</td>
<td>33 (24-42)</td>
</tr>
<tr>
<td>Total Hardness (mg L⁻¹ as CaCO₃)</td>
<td>44</td>
<td>40 (30-50)</td>
</tr>
<tr>
<td>Conductivity (µS cm⁻²)</td>
<td>125</td>
<td>101 (74-128)</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>21</td>
<td>22 (21-23)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>mg Cu g⁻¹ <em>Lyngbya</em> in the Laboratory Study Exposures (nominal)</th>
<th>mg Cu g⁻¹ <em>Lyngbya</em> in the Laboratory Study mean ± one S.D. (measured)</th>
<th>City Lake Sampling Dates</th>
<th>mg Cu g⁻¹ <em>Lyngbya</em> at the City Lake Treatment Site (4 h after treatment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unexposed Control</td>
<td>NDa</td>
<td>June 19, 2006</td>
<td>~6.5</td>
</tr>
<tr>
<td>10</td>
<td>12 ± 0.9</td>
<td>July 26, 2006</td>
<td>~13.3</td>
</tr>
<tr>
<td>100</td>
<td>9.9 ± 3.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>212 ± 6.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>300</td>
<td>353 ± 38.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Not detectable (Detection limit = 0.001 mg Cu L⁻¹). b Pre-treatment. c Hours after treatment. d Days after treatment.

Water characteristics and total copper concentrations measured from the untreated reference site did not significantly differ from pre-treatment measurements from the treatment site.
(Table 4.5 continued)

<table>
<thead>
<tr>
<th>Laboratory Samples</th>
<th>ug chl a g(^{-1}) Lyngbya Laboratory Study</th>
<th>City Lake Sampling Dates</th>
<th>g <em>Lyngbya</em> m(^{-2}) DW(^{b}) in City Lake Treatment Site</th>
<th>mg chl a m(^{-2}) in City Lake Treatment Site</th>
<th>ug chl a g(^{-1}) Lyngbya in City Lake Treatment Site</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-treatment</td>
<td>300 ± 36</td>
<td>Pre-treatment</td>
<td>2238 ± 961</td>
<td>247 ± 86</td>
<td>120 ± 31</td>
</tr>
<tr>
<td>96 HAT(^{a}) (10 mg Cu g(^{-1}) <em>Lyngbya</em>)</td>
<td>88 ± 5</td>
<td>After First Treatment</td>
<td>764 ± 329</td>
<td>159 ± 112</td>
<td>187 ± 83</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(June 19, 2006)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>After Second Treatment</td>
<td>535 ± 327</td>
<td>59 ± 38</td>
<td>112 ± 28</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(July 26, 2006)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^{a}\) Hours after treatment. \(^{b}\) Dry weight.
List of Figures

Figure 4.1: Map of City Lake (High Point, North Carolina) indicating the untreated reference site and the monitored treatment site.

Figure 4.2: Chlorophyll a concentrations (µg g⁻¹ Lyngbya) measured after 96 hour laboratory exposures to A) Algimycin® PWF and the sequential algaecide treatment (mg Cu g⁻¹ Lyngbya) and B) Clearigate® (mg Cu g⁻¹ Lyngbya). “Sequential Trt” refers to the sequential algaecide treatment used in laboratory exposures. “Pre” refers to the pre-treatment measurement. Bars represent one standard deviation.

Figure 4.3: Mean measurements of A) algal biomass (dry weight; g Lyngbya m⁻²), B) mg chlorophyll a m⁻², and C) µg chlorophyll a g⁻¹ of Lyngbya measured pre- and post- treatment with Algimycin® PWF in City Lake (High Point, North Carolina).
Figure 4.2

A

B

Exposure Concentration
(mg Cu / g Lyngbya as Algimycin PWF)

Exposure Concentration
(mg Cu / g Lyngbya as Clearigate)

Chlorophyll a (ug / g Lyngbya)

Pre-treatment.

96 Hour.
Figure 4.3

A

B

C

Lyngbya Biomass (g / m²)

Chlorophyll a (mg / m²)

Chlorophyll a (µg / g Lyngbya)

Sampling Dates

Reference Site

Treatment Site

April 27, 2006

June 19, 2006

July 26, 2006

Pre-treatment.

Post-1st treatment.

Post-2nd treatment.

Pre-treatment.

Post-1st treatment.

Post-2nd treatment.

Pre-treatment.

Post-1st treatment.

Post-2nd treatment.
CHAPTER 5
SUMMARY AND CONCLUSIONS

Worldwide, HABs are increasingly impeding critical water resource usages. These algae achieve problematic densities or produce and release toxins or taste-and-odor compounds, which pose risks to humans and other animals (Briand et al. 2003; WHO 2003). Often, cyanobacteria (blue-green algae) are the cause of these problems (Hallegraeff 1993; WHO 2003). In situations where use of the water resource is critical (i.e. potable water production, livestock watering, aquaculture), managers may evaluate all control options for remediating the algal problem (i.e. mechanical, physical, biological, chemical). Frequently, application of an algaecide is an effective and efficient option. However, one must first discern that a problem exists and that an algaecide application will not result in a more critical situation.

Research presented in this Thesis used site water, site-collected algae, and realistic measured laboratory algaecide exposures to predict responses of algae to a specific field algaecide application. Utilization of site water with associated algae in laboratory algal toxicity tests minimized the potential for ambiguity in translating the laboratory results directly to the field situation (Fitzgerald and Jackson 1979; Mastin et al. 2002; Duke et al. in review; Tedrow et al. in review). Laboratory results were used to guide field applications of different algaecides, in different water resources with different physical and chemical characteristics and
primary uses, infested with different types of algae [i.e. planktonic (*Microcystis aeruginosa*) and benthic (*Lyngbya* sp.)].

Chapter Two of this Thesis focused on microcystin-producing *Microcystis aeruginosa* sampled from Pawnee Reservoir, Nebraska. Samples of site water containing *Microcystis* were collected and used in laboratory algal toxicity tests to evaluate the efficacy of four copper-containing algaecides (Cutrine®-Ultra, Clearigate®, Algimycin® PWF, and copper sulfate pentahydrate) for controlling this alga in this reservoir. Control of *Microcystis* in Pawnee Reservoir water for this laboratory study was defined as chlorophyll *a* and cell density below detection ($< 10 \mu g$ chlorophyll *a* L$^{-1}$ and $< 5 \times 10^2$ cells mL$^{-1}$).

The primary use of Pawnee Reservoir is for flood control. However, recreational activities generate significant public and private revenue. Pawnee Reservoir has experienced repeated dense growths of *Microcystis* associated with production and release of microcystin above levels of concern, which have impacted use of this resource. Therefore water resource managers evaluated control options, including algaecide application. Specific objectives of this research were to: 1) identify an efficacious algaecide and treatment concentration to eliminate *Microcystis* from Pawnee Reservoir water in laboratory algal toxicity tests, 2) measure responses of *Microcystis* in terms of chlorophyll *a*, algal cell density, and microcystin concentrations in water samples following individual exposures to four copper containing algaecides, 3) evaluate three techniques used for extraction and measurement of total microcystin, and 4) measure the time-
course release of microcystin from *Microcystis* after an exposure to Cutrine®-Ultra.

*Microcystis aeruginosa* in Pawnee Reservoir water was sensitive to all four copper algaecides. Responses of this cyanobacterium in terms of chlorophyll *a* and cell density were generally proportional to the exposures (concentration and duration of exposure). Importantly, *Microcystis* in Pawnee Reservoir water was most sensitive to Cutrine®-Ultra with control achieved after a 96 hour exposure to 0.2 mg Cu L⁻¹ (pre-treatment = \(~2.17 \times 10^5\) cells mL⁻¹; \(~356\ \mu g\) chlorophyll *a* L⁻¹). After 96 hours, chlorophyll *a* and *Microcystis* cell density were below detection and aqueous microcystin significantly decreased (\(~67\%\)) after the 96 hour exposure. This concentration of copper (0.2 mg Cu L⁻¹) is five times less than would be allowed as the maximum treatment permitted by the label.

Aqueous microcystin was measured in this study was to determine if significant release of microcystin occurred after exposure. While measurement of aqueous microcystin is important to determine release of microcystin after treatment, measurement of total microcystin is used to guide policy, and regulatory or management decisions (i.e. WHO 2003). Total microcystin concentrations were measured from treatment replicates after exposure to Clearigate®, Algimycin® PWF, and copper sulfate pentahydrate. In this case, measurement of total microcystin was complicated or confounded by the technique used to extract microcystin from *Microcystis*. Therefore, three techniques were evaluated for extraction of cell-associated microcystin: 1) freezing-and-thawing, 2) grinding, and 3) sonication. Based on measurement of
aqueous and total microcystin, and recovery of an internal standard (APHA 1998) of microcystin-LR, sonication was the most reliable technique for extraction of cell-associated microcystin under these conditions. For critical water resources, where decisions regarding usage are made based upon total microcystin measurements, further study may be needed regarding the potential for microcystin release post-treatment as well as the technique used for release of cell-associated microcystin. Regardless, we recommend using internal and surrogate microcystin standards for total microcystin measurements to verify recovery (APHA 1998).

For the time-course experiment, exposure concentrations of 0.2, 0.4, 0.6, 0.8, and 1.0 mg Cu L\(^{-1}\) as Cutrine\textsuperscript{®}-Ultra and untreated controls were tested. Microcystin concentrations (aqueous and total) were measured pre-treatment and six, 24, 48, and 96 hours after exposure. Following the time-course microcystin release experiment, increases in aqueous and total microcystin (six-24 hours after exposure) were transitory after exposure to Cutrine\textsuperscript{®}-Ultra and were not of ecological or health significance in terms of concentration or persistence. Using this approach emphasizes the utility and importance of laboratory studies prior to field-scale applications in order to use effective algaecides efficiently, while maximizing the potential margin of safety to non-target species (Fitzgerald and Jackson 1979, Mastin et al. 2002, Murray-Gulde et al. 2002).

Chapter Three of this Thesis focused on using results from a laboratory algal toxicity test with Pawnee Reservoir water and *Microcystis aeruginosa* to guide a field application of Cutrine\textsuperscript{®}-Ultra in Pawnee Reservoir. Specific
objectives of this research were to: 1) measure time-course responses of Microcystis following laboratory exposures to Cutrine®-Ultra in terms of chlorophyll a concentrations, cell densities, and microcystin concentrations, 2) measure time-course responses of Microcystis after a field application of Cutrine®-Ultra, and 3) compare responses of Microcystis following a field application of Cutrine®-Ultra with responses measured in laboratory exposures to Cutrine®-Ultra.

In the time-course laboratory study, Microcystis in Pawnee Reservoir water was exposed to 0.2, 0.4, 0.6, 0.8, and 1.0 mg Cu L\(^{-1}\) as Cutrine®-Ultra for 96 hours. Chlorophyll a concentration and cell density were measured pre-treatment and 24, 48, and 96 hours after exposure. Aqueous and total microcystin concentrations were measured pre-treatment and six, 24, 48, and 96 hours after exposure. Growth of Microcystis was controlled using a 96 hour exposure of 0.2 mg Cu L\(^{-1}\) as Cutrine®-Ultra (pre-treatment = \(\sim 2.4 \times 10^5\) cells mL\(^{-1}\), \(\sim 34\) µg chlorophyll a L\(^{-1}\)). After 96 hours, chlorophyll a concentration and cell density were below detection (< 10 µg chlorophyll a L\(^{-1}\) and < 5.0 \(\times 10^2\) cells mL\(^{-1}\), respectively), and aqueous and total microcystin concentrations decreased to \(\sim 0.4\) µg L\(^{-1}\).

On June 6, 2006, Cutrine®-Ultra was applied to Pawnee Reservoir to treat Microcystis aeruginosa. Two sites were monitored for algaecidal efficacy in Pawnee Reservoir: 1) the southwest corner treatment site (an open water site), and 2) the marina treatment site (a semi-enclosed area with limited open-water access to the reservoir body), along with an untreated reference site. Exposure
concentrations in Pawnee Reservoir ranged from ~0.85 and ~0.60 mg Cu L⁻¹ as Cutrine®-Ultra in the southwest corner treatment site and the marina treatment site, respectively. After approximately 48 hours, Microcystis in Pawnee Reservoir responded to treatment using Cutrine®-Ultra in terms of algal cell densities, chlorophyll a concentrations, and aqueous microcystin concentrations. Potential algaecide burdens in Pawnee Reservoir (southwest corner site = ~1.0 x 10⁻⁶ μg Cu cell⁻¹; marina treatment site = ~1.4 x 10⁻⁶ μg Cu cell⁻¹) were approximately one order of magnitude greater than the laboratory study (~9.1 x 10⁻⁷ μg Cu cell⁻¹). Therefore, similar laboratory and field exposures and responses were compared.

Aqueous microcystin was measured pre- and post- treatment to determine whether microcystin was released from Microcystis. In the Pawnee Reservoir field study, measurement of total microcystin was confounded by the technique used to extract microcystin from cells (freezing-and-thawing total microcystin samples). After treatment of Microcystis in Pawnee Reservoir with Cutrine®-Ultra, increases of aqueous microcystin were ephemeral and not of ecological or health significance in terms of concentration or persistence. Lack of persistence of microcystin in waters where Microcystis and microcystin are common has been observed by Lam et al. (1995), Christofferson et al. (2002), Jones and Orr (1994) and Tedrow et al. [in review (a)].

Chapter Four of this Thesis focused on predicting responses of a benthic cyanobacterium (Lyngbya sp.) to exposures of algaecides based upon laboratory results. Lyngbya forms dense benthic mats (Speziale et al. 1991; Speziale and Dyck 1992), can thrive at extreme temperatures (Graham and Wilcox 2000), and
can achieve densities of $\geq 1.14$ Kg m$^{-2}$ (dry weight) with $\sim$40-100% of this biomass existing as benthic mats (Speziale and Dyck 1992; Doyle and Smart 1998). *Lyngbya* for this study was sampled from City Lake in High Point, North Carolina (NC). The designated use of City Lake is potable water retention for the City of High Point, NC. However, recreational activities have developed and generate significant public and private revenue (William Frazier, pers. comm., City of High Point, NC). Use of City Lake for potable water, along with recreational and other activities, was impeded due to dense growths of *Lyngbya*. Resource managers evaluated control options, which included the application of an algaecide. Preliminary laboratory algal toxicity experiments were performed to determine an efficacious algaecidal treatment for *Lyngbya* in City Lake water. Specific objectives of this research were to: 1) measure responses of *Lyngbya* in laboratory exposures of Algimycin® PWF, Clearigate®, and a sequential algaecide technique using PAK™ 27 prior to Algimycin® PWF with Cide-Kick® II, 2) measure responses of *Lyngbya* to field exposures of Algimycin® PWF in City Lake, High Point, NC, as *Lyngbya* biomass (g m$^{-2}$), chlorophyll $a$ concentration (mg m$^{-2}$), and chlorophyll $a$ concentrations (µg g$^{-1}$ *Lyngbya*), and 3) compare responses of *Lyngbya* in laboratory exposures of Algimycin® PWF to two field applications of Algimycin® PWF.

Exposure concentrations of copper as Algimycin® PWF and Clearigate® were 10, 100, 200, and 300 mg Cu g$^{-1}$ *Lyngbya*. The exposure concentration of copper as Algimycin® PWF used in the sequential algaecide technique was 10 mg Cu g$^{-1}$ *Lyngbya*. A significant decrease in chlorophyll $a$ concentration was
measured after 96 hour exposures to all three algaecidal techniques used (pre-treatment chlorophyll \(a\) concentration was \(\sim 300 \mu g \ g^{-1} Lyngbya\)). However, \textit{Lyngbya} in City Lake water was most sensitive to Algimycin\textsuperscript{®} PWF after 96 hours (post-exposure to \(\sim 10 \ mg \ Cu \ g^{-1} Lyngbya\) chlorophyll \(a\) concentration was \(\sim 88 \mu g \ chlorophyll \ a \ g^{-1} Lyngbya; \sim 71\% \ decrease\)). Based on these laboratory results, Algimycin\textsuperscript{®} PWF was chosen for treatment of \textit{Lyngbya} in City Lake, High Point, NC.

Two sites were monitored during the treatments: 1) a treated site and 2) an untreated reference site. Field treatments were initiated on April 27, 2006 (first treatment) and June 19, 2006 (second treatment). Exposure concentrations of copper as Algimycin\textsuperscript{®} PWF in field treatments were \(\sim 0.187 \ mg \ Cu \ L^{-1} \ (\sim 6.5 \ mg \ Cu \ g^{-1} Lyngbya; \ first \ treatment)\) and \(\sim 0.213 \ mg \ Cu \ L^{-1} \ (\sim 13.3 \ mg \ Cu \ g^{-1} Lyngbya; \ second \ treatment)\), measured four hours after algaecide application. \textit{Lyngbya} biomass decreased significantly in the treatment site after the first and second applications compared to the pre-treatment biomass measurement (\(\sim 67\%\) and \(77\%, \) respectively).

With the conditions in this situation, the laboratory exposure of \(\sim 10.0 \ mg \ Cu \ g^{-1} Lyngbya\) closely resembled field exposures in terms of mass of copper applied to mass of \textit{Lyngbya} treated. The estimated potential algaecide burden under these laboratory conditions was \(\sim 10 \ mg \ Cu \ g^{-1} Lyngbya\). The estimated potential algaecide burdens under these field conditions ranged from 6.5-13.3 mg Cu g\(^{-1}\) \textit{Lyngbya}, based on aqueous acid-extractable copper concentrations measured four hours after treatment and pre-treatment \textit{Lyngbya} biomass.
measurements. Consequently, it is not unexpected that *Lyngbya* responded to application of Algimycin® PWF in City Lake. Due to the mass of algae accumulated in City Lake, multiple treatments were necessary to maintain control.

During field treatments of *Lyngbya* in City Lake, contact between Algimycin® PWF and *Lyngbya* was maximized by applying the algaecide below the water’s surface. Algaecide application techniques included treatment of the infested area from the shoreline to deeper water and only partial treatment of the water resource. These application techniques decreased potential exposures for non-target species, increased potential margins of safety for fish and invertebrates, and provided refugia during treatment.

Laboratory algal toxicity tests using site water and site algae have demonstrated a robust ability to predict field responses of algae to a specific algaecide (Fitzgerald and Jackson 1979; Mastin et al. 2002; Duke et al. in review; Tedrow et al. in review). Timely responses to acute algal problems are necessary. Realistic laboratory exposures using site water and site algae have provided answers to questions of algaecidal efficacy. However, similar laboratory and field exposures must be measured to accurately compare responses of algae.

For comparison with results obtained in the laboratory test, results from City Lake were evaluated after two applications of Algimycin® PWF. By comparing similar exposures, and responses to those exposures, in site water with characteristics similar to water used for laboratory exposures, uncertainty regarding speciation, and thusly bioavailability, of copper (as algaecide) can be controlled (Erickson et al. 1996; Haughey et al. 2000). Careful and accurate
laboratory exposures lead to efficient use of effective algaecides in critical water-use situations. This decreases the necessary amount of algaecide while simultaneously increasing the potential margin of safety for non-target species. We have used laboratory algal toxicity experiments to accurately predict responses of target algae and apply those results directly to the field situation (Mastin et al. 2002; Murray-Gulde et al. 2002; Duke et al. in review; Tedrow et al. in review). However, with early detection of algal growths and early implementation of an effective and efficient control strategy prior to development of an acute problem, water resource usages can be restored and maintained.
Literature Cited


