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Modeling Pulse Exposures From Copper Algaecides and Effects on Target and Non-Target Species

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MODELING PULSE EXPOSURES FROM COPPER ALGAECIDES AND EFFECTS ON TARGET AND NON-TARGET SPECIES

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by
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ABSTRACT

Pulse exposures are fundamentally different from static exposures in that they temporarily exceed pretreatment concentrations. Given this fundamental difference, responses of organisms to pulse exposures should also differ from responses to static exposures. In this dissertation, copper-based algaecides were studied because they have notable characteristics that can be used to ask questions regarding pulse exposure durations and consequent effects. Specifically, the time, location and applied concentration are predetermined allowing for measurement of copper exposures in situ. Additionally, algaecides are intended to result in effects to noxious species so that responses can be measured.

For the first experiment in this dissertation, copper dissipation rates following an algaecide application were calculated and modeled both physically and mathematically by characterizing individual fate processes for copper (i.e. algal sorption, sediment sorption, copper precipitation and dilution). After characterizing copper pulse exposures, the influence of exposure duration on organism responses was discerned. The target cyanobacterium *Lyngbya wollei* and the non-target fish *Pimephales promelas* were used to demonstrate relationships between pulse-exposure durations and organism responses. For *Lyngbya wollei,* initial cyanobacterial biomass may be another characteristic that alters responses of the cyanobacterium in addition to exposure duration.

From the characterization of copper pulse exposures, aqueous phase copper concentrations dissipated rapidly (half-life = 0.03 days) in situ. This rapid half-life was comparable to the half-life for dilution (half-life = 0.03 days), whereas the half-lives for

sediment sorption (half-life = 3 days), copper precipitation (no significant differences in aqueous copper concentrations in 13 days) and algal sorption (no significant differences in aqueous copper concentrations in 8 days) were greater, indicating that dissipation was mainly due to dilution. Mathematic and physical (mesocosm) models of copper pulse exposures resulted in similar dissipation half-lives (mathematic half-life = 0.03 days and mesocosm half-life = 0.02 days) relative to half-lives calculated from copper concentrations measured in situ (half-life = 0.03 days) demonstrating that these approaches could be used to predict exposure durations.

In terms of *L. wollei* responses to copper pulse exposures, the biomass of the cyanobacterium at exposure initiation is a variable in addition to exposure duration and copper concentration that drives responses. For a series of initial cyanobacterial biomasses from 13 g wet weight (WW)/m^2 to 1,558 g WW/m^2 exposed to the same copper concentration and exposure duration (1 mg Cu/L for 24 hours), responses of *L. wollei* range from the maximum response ($\geq$ 90% response in terms of percent damaged trichomes) to non-detect demonstrating the impact of biomass at exposure initiation. A model was subsequently designed using initial biomass, copper concentration and exposure duration to predict responses of *L. wollei* to a copper algaecide. For the non-target fish, *P. promelas*, copper exposures with 1.5 hour half-lives resulted in LC_{50s} (calculated from percent survival measured 96 hours following exposure initiation) that exceeded those of static exposures by an order of magnitude (i.e. 164 μg Cu/L to 1,134 μg Cu/L). LC_{50s} (calculated from measurements 96 hours following exposure initiation) for copper pulses with half-lives of 4 and 8 hours exceeded static exposures by a factor of

approximately 3 while half-lives of 15 hours resulted in comparable *P. promelas* responses relative to 96 hour static LC_{50s}. The experiments presented in this dissertation provided approaches to understand the limits and bounds of pulse exposures in terms of exposure durations and effects on both target and non-target organisms.

DEDICATION

I dedicate this dissertation to my parents whom by example taught the discipline, perseverance and commitment necessary to complete this degree. And to Mike Eck for support and encouragement throughout this process.

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ORGANIZATION OF DISSERTATION

This dissertation consists of five chapters including the Introduction (Chapter One), three independent manuscripts (Chapters Two, Three and Four), and Conclusions (Chapter Five). The manuscripts are formatted for publication in scientific journals and some redundancy of material is required.

CHAPTER II: Characterization of Copper Algaecide (Copper Ethanolamine) Dissipation Rates Following Pulse Exposures

Published in Water, Air & Soil Pollution

CHAPTER III: *Lyngbya wollei* responses to Copper Algaecide Exposures Predicted using a Concentration-Exposure Time (CET) Model: Influence of Initial Biomass

To be Submitted to Journal of Aquatic Plant Management

CHAPTER IV: Relationship Among Aqueous Copper Half-lives and Responses of *Pimephales promelas* to a Series of Copper Sulfate Pentahydrate Concentrations

In Review at Ecotoxicology

CHAPTER ONE

Introduction

Exposures are characterized by concentration, form, frequency and importantly duration, therefore pulse exposures are fundamentally different from static exposures. Given this fundamental difference, organism responses to pulse exposures also differ from responses to static exposures. Investigation of copper-based algaecides provides the opportunity to study pulse exposures and consequent effects on organisms because the location, timing and applied concentration of copper are known prior to treatment. Additionally, algaecides are intended to elicit effects allowing for responses to exposures to be discerned.

Copper-based algaecides are applied to lakes, ponds, reservoirs, canals, etc. when algal and cyanobacterial growths impede water resource uses (US EPA 2006). Some common copper algaecide formulations include a copper salt ($CuSO_4 \bullet 5H_2O$) and copper chelates (e.g. citrate, gluconate, ethanolamine) that are applied at concentrations ranging from 0.1-1.0 mg Cu/L to aquatic systems (US EPA 2006). Within these legal application concentrations, copper exposures in situ are influenced by different fate processes (i.e. dilution, sorption and precipitation), resulting in pulse exposures that range in duration from minutes to days in situ. Relative to standard toxicity experiments conducted with static exposures, pulse exposures can alter consequent responses of target algae and cyanobacteria, and non-target algae, fish and invertebrates (Reinert et al. 2002). In order to interpret potential for ecological risks for copper-based algaecides, models are needed to predict durations of copper based algaecide exposures in situ. Subsequently,

understanding the influence of pulse exposures on responses of target and non-target species is necessary. The experiments in this dissertation were designed to: 1) characterize fate processes to model copper-pulse exposures in situ; 2) evaluate effects of copper pulse exposures on responses of a target species (i.e. cyanobacterium, *Lyngbya wollei*); and 3) evaluate effects of copper pulse exposures on responses of a non-target species (i.e. fish, *Pimephales promelas*) (Figure 1.1).

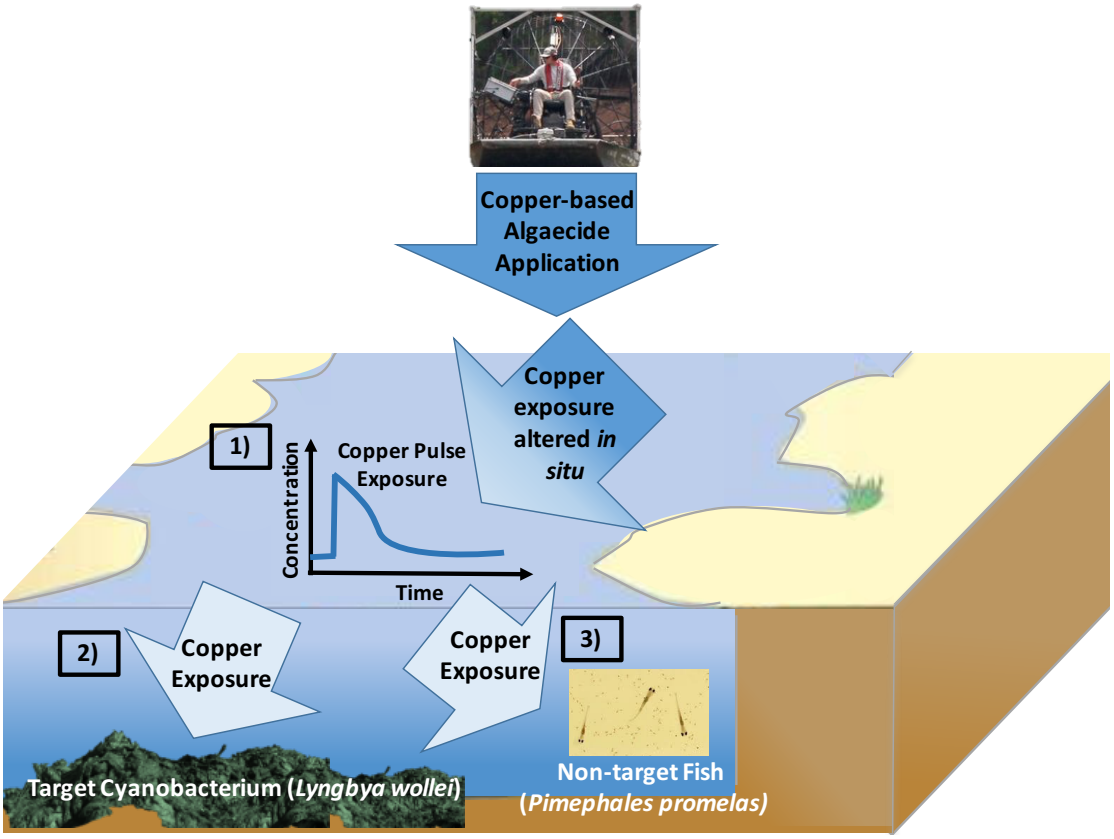

Figure 1.1 Conceptual model for experiments in dissertation.

Factors influencing copper dissipation rates can range based on inter and intra-lake variation resulting in copper pulse exposures of different durations (Button et al 1977, Anderson 1999, McNevin and Boyd 2004, Liu et al. 2006). Therefore, models

characterizing copper dissipation rates need to be site-specific. To address this issue, scaled experiments (i.e. in situ, laboratory and mesocosm) were used to demonstrate an approach to model copper fate processes for a site and predict exposures in situ.

The fundamental premise for the scaled approach used in this experiment is that copper dissipation at a given scale is only impacted by the physical and chemical reactions captured within that scale. Processes that can influence copper fate in situ after algaecide applications include algal sorption, precipitation, sediment sorption and dilution (US EPA 2006). Some of these processes may play a dominant role in overall dissipation while others may have minimal roles within relevant timescales for dominant fate processes. By characterizing these dominant fate processes, copper dissipation rates from copper-based algaecide applications can be predicted using mathematic and physical models.

Once pulse exposures are characterized, the next step is understanding the influence of pulse exposures on organism responses. Pulse copper exposures are anticipated to impact target and non-target species differently. Target algae and cyanobacteria are the impetus for an algaecide treatment, and are consequently exposed to a copper pulse. However, the relative mass or density of these organisms can also be an intrinsic exposure modifying factor that can alter copper exposures via sorption of copper to algae. Non-target species, on the other hand, are incidentally exposed to a copper pulse during algaecide treatments.

Exposures of copper-based algaecides to the target cyanobacterium, *L. wollei* were used to demonstrate the influence of exposure duration (resulting from dissipating

copper pulse exposures) on cyanobacterial responses. A potentially analogous situation in which exposure duration is incorporated into organism response models is concentration-exposure time models (CET). CET models are historically used to predict responses of vascular plants to herbicide concentrations that have exposure times altered by site characteristics (Van and Conant 1988, Getsinger et al. 1991). Because this situation is potentially analogous but not equivalent, an additional parameter modifying cyanobacterial responses to copper pulse exposures is initial biomass. Sequential experiments were necessary to parse the influence of copper concentration, exposure duration and initial cyanobacterial biomass on *L. wollei* responses. Initial experiments were designed to discern an algaecide with sufficient potency to ask these questions. Subsequently, a CET model was developed altering exposure duration and copper concentration to measure the effects on cyanobacterial response. From this CET model for *L. wollei*, a copper concentration and exposure duration resulting in maximum response ($\geq$ 90%) was applied to *L. wollei* with a series of cyanobacterial biomasses at initiation of exposure. If cyanobacterial biomass at initiation of exposure results in responses different from those predicted from the CET model, a new model will be developed. This new model will incorporate the variables of copper concentration, exposure duration and cyanobacterial biomass to predict responses of *L. wollei*.

Responses of non-target species are also important when characterizing risks from copper-based algaecide exposures. For the final experiment of this dissertation, *P. promelas* responses to $CuSO_4 \bullet 5H_2O$ were used to demonstrate the relationship between exposure duration and fish (*P. promelas*) responses to $CuSO_4 \bullet 5H_2O$ exposures.

Responses of *P. promelas* to $CuSO_4 \cdot 5H_2O$ concentrations with different exposure durations were compared to responses elicited for standard toxicity experiments with static exposures. The results from this experiment can be used to demonstrate the relative conservatism of static toxicity experiments when applied to pulse exposure scenarios for the non-target fish, *P. promelas*.

Collectively, the experiments in this dissertation characterize copper pulse exposures and their effects to both target (*L. wollei*) and non-target (*P. promelas*) organisms from copper-based algaecides. The specific objectives for the three independent manuscripts were prepared for specific journals, and the objectives of each experiment are listed below:

Chapter 2: Characterization of Copper Algaecide (Copper Ethanolamine) Dissipation Rates Following Pulse Exposures

Submitted to *Water, Air & Soil Pollution*

Specific objectives: 1) calculate the copper dissipation rate from copper concentrations measured in situ following a pulse exposure of algaecide (i.e. Cutrine-Plus®, copper ethanolamine), 2) isolate and calculate individual copper dissipation rates from fate processes (i.e. algal sorption, sediment sorption, copper precipitation and dilution), 3) calculate the copper dissipation rate based on copper concentrations measured in mesocosms incorporating individual fate processes (i.e. algal sorption, sediment sorption, precipitation and dilution), and 4) compare individual and cumulative (i.e. mesocosm and in situ) copper dissipation rates and rank individual copper dissipation rates using a mathematical model.

Chapter 3: *Lyngbya wollei* Responses to Copper Algaecide Exposures Predicted Using a Concentration-exposure Time (CET) Model: Influence of Initial Biomass

Targeted Journal: *Journal of Aquatic Plant Management*

Specific objectives: 1) measure and compare responses of *L. wollei* from a 44,500 m^2 pond in Spartanburg County, South Carolina to a series of copper concentrations (0.1-1.0 mg Cu/L) in separate laboratory experiments using copper as Clearigate®, Cutrine® Ultra, Cutrine-Plus® and Algimycin® PWF to determine a copper-based algaecide that has sufficient potency (i.e. relationship between copper concentration and response to $\geq$ 90 % cyanobacterial response) to measure a change in *L. wollei* responses within legal label concentrations, 2) measure and compare responses of *L. wollei* from the same pond to a series of exposure durations and concentrations of copper as a copper-based algaecide (with sufficient potency based on the previous experiment) to develop the *L. wollei* CET model , 3) measure and compare responses of *L. wollei* (from the pond) for a series of initial biomasses (0.25 g/m^2 – 1,508 g/m2) to an exposure duration and concentration of the copper-based algaecide that result in $\geq$ 90 % cyanobacterial response in the CET model to discern the influence of initial cyanobacterial biomasses on responses of *L. wollei*, and 4) measure and compare copper concentrations (within legal label concentrations, 0.1-1.0 mg Cu/L) that result in $\geq$ 90 % cyanobacterial response for a series of exposure durations and initial cyanobacterial biomasses to develop a new model for *L. wollei*.

Chapter 4: Relationship Among Aqueous Copper Half-lives and Responses of *Pimephales promelas* to a Series of Copper Sulfate Pentahydrate Concentrations

Submitted to *Ecotoxicology*

Specific objectives: 1) measure responses of < 24 hour old *P. promelas* in terms of survival exposed to a series of copper concentrations as copper sulfate pentahydrate in toxicity experiments with four different half-lives and to static exposures, 2) measure sorbed copper to *P. promelas* at completion of the toxicity experiment (i.e. 96 hours after exposure initiation) as a parameter to indicate the consequence of different exposure conditions (i.e. dissipating and static) and 3) calculate the relationship between 1/half-lives and responses of *P. promelas* to copper sulfate pentahydrate concentrations.

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CHAPTER TWO

CHARACTERIZATION OF COPPER ALGAECIDE (COPPER ETHANOLAMINE) DISSIPATION RATES FOLLOWING PULSE EXPOSURES

Abstract

Dissipation rates of copper following algaecide treatments resulting in pulse exposures can be accurately modeled if the component dissipation rates are known. Scaled experiments (in situ, laboratory and mesocosm) were used to parse and rank dominant processes from concurrent processes affecting copper fate in pulse exposures. Cumulative copper dissipation rates were calculated based on copper concentrations measured in situ and in mesocosms. Individual copper dissipation rates were calculated from copper concentrations measured in laboratory experiments. Predictions of the influence of individual dissipation rates on the cumulative dissipation rate were assessed mathematically. In situ aqueous copper concentrations dissipated rapidly following an algaecide treatment, with a calculated half-life of 0.03 days. Based on laboratory experiments, the most rapid copper fate process was dilution with a half-life of 0.03 days, followed by sediment sorption with a half-life of approximately 3 days. Mesocosm experiments incorporating physical characteristics of the site (i.e. dilution, sediment, algae and site water) resulted in similar copper half-lives (0.02 days) relative to the in situ copper half-life. Prediction of the fate of copper from algaecide treatments requires incorporation of accurate estimates of dominant fate processes that can be determined physically and mathematically.

Introduction

Physical models at different scales (e.g. in situ, laboratory and mesocosm) are often used to study and predict the fate of materials and exposures in aquatic systems (Giesy 1980, Johnson and Rodgers 2006). Copper-based algaecide treatments involve purposeful introduction of copper into aquatic systems and provide an opportunity to measure the fate of copper in an aquatic system as the timing, mass and location of applications are known. Copper concentrations following an algaecide treatment are anticipated to rapidly dissipate as a function of relevant fate processes (i.e. algal sorption, precipitation, sediment sorption and dilution) resulting in a pulse exposure. The influences of individual fate processes on overall copper dissipation rates in aquatic systems are relatively unstudied. Fundamentally, delimitation or isolation of individual fate processes that influence copper dissipation rates in an aquatic system is necessary to improve understanding and prediction of copper fate. In the present study, scaled (i.e. in situ, laboratory and mesocosm) experiments were used to characterize copper fate following an algaecide treatment in terms of copper dissipation rate coefficients and half-lives. Data (i.e. rate coefficients) from laboratory experiments used to isolate individual fate processes parameterized a mathematic model to identify dominant copper fate processes in an aquatic system by comparison to the cumulative copper dissipation rate coefficient calculated from in situ measurements.

Because of the elemental nature of copper, processes influencing copper fate in an aquatic system are limited. Processes anticipated to drive copper fate for algaecides include sorption (i.e. algal and sediment), precipitation and dilution. In the context of an

algaecide treatment, sources of ligands for copper sorption include sediment and organic matter of algal origin. Copper can bind with sulfides [$K_{sp} = 10^{-16}$ covellite (CuS); Stumm and Morgan 1996], iron and manganese oxyhydroxides and organic matter ($Kd_{oc} = 10^{5.62} – 10^{6.41}$ L/kg; Mahoney et al. 1996) associated with sediments. Planktonic algal densities that have initiated algaecide treatments range from $1x10^4$ (Rodgers et al. 2010) to $1x10^6$ cells/mL (Isaacs et al. 2013, Greenfield et al. 2014). Algae can sorb copper from the aqueous phase with reported algal sorption capacities ranging from 0.056 mg Cu/g to 133 mg Cu/g (Bishnoi et al. 2004, Gupta et al. 2006, Flouty and Estephane 2012). Based on chemical equilibria in environmentally relevant conditions of freshwater environments (pH = 6-8 and Eh > 0.0) precipitation of copper complexes can occur through binding of copper ions (Cu^{2+}) with dissolved anions of oxygen, sulfur, carbon and hydrogen (Cu_2O, CuO, CuOH, CuSOH, $Cu_2(OH)_2CO_3$) (Brookins 1988) forming relatively insoluble products. Dilution is also assumed to be an important process because of the heterogeneous nature of algaecide treatments and because applications of algaecides cannot exceed 50% of the surface area of a water resource (i.e. pursuant to the Federal Insecticide, Fungicide and Rodenticide Act). Physical processes in lentic systems that result in dilution include advection, diffusion and dispersion (Burns et al. 1982) and are spatially and temporally specific.

Currently, computer modeling (i.e. EXAMS) of copper fate from algaecide treatments predict maximum copper concentrations of 990 μg/L with a 21-day average of 522 μg/L, a 60-day average of 234 μg/L and a 90-day average of 158 μg/L (US EPA 2005a, US EPA 2006). These predictions are based on an initial application of copper of 990 μg/L

and assume that the dominant copper fate process in aquatic systems is sediment sorption with a half-life of 10 days. The modeled copper concentrations following algaecide treatment likely overestimate the time copper remains in the aqueous phase and thus exposures of non-target species (i.e. co-occurring organisms that are not problematic) would be concomitantly overestimated. Based on measured aqueous copper concentrations following algaecide treatments in situ, concentrations of copper decrease to pretreatment concentrations in hours to days (Button et al. 1977, Anderson 2003). The difference between predicted and measured copper dissipation can be attributed to incongruity in fate processes incorporated in the computer model and fate processes influencing copper in aquatic systems. In this case, there is a relatively large divergence between predicted and measured copper dissipation, therefore the process or processes that are being discounted likely result in rapid copper half-lives in situ. If the dominant fate process or processes are identified, predictions of copper fate for algaecides will be more accurate.

The fundamental premise for the scaled approach used in the current experiment is that copper dissipation at a given scale is limited to the physical and chemical reactions captured within that scale. For example, in a beaker containing only site-water (algae and sediment removed) and applied copper, copper dissipation can be attributed to precipitation from the reaction of dissolved copper with anions and ligands in site-water. Alternatively, an aquatic environment containing algae, water and sediment will result in a copper dissipation rate, attributed to the sum of copper fate processes. As such, the influence of individual processes cannot be discerned using copper measurements in an

aquatic environment alone. Using a conservative dye such as rhodamine WT (Fox et al. 1991), site specific parameters such as dilution can be measured in situ. Laboratory experiments are used to isolate and parse individual fate processes by limiting chemical interactions with other factors than the reaction or specific process being studied. Finally, by designing mesocosm scale experiments with sufficient site specific characteristics (i.e. algae, site water and sediment) simulated copper dissipation rates will be predictive of the rate observed in situ.

Comparisons of individual fate processes can be used to rank dissipation rates for the purpose of discerning dominant copper fate processes. A mathematic fate model was used to discern the role of individual dissipation rates (i.e. algal sorption, precipitation, sediment sorption and dilution) on the overall copper dissipation rate calculated from copper concentrations measured in situ (K_{tot}) (equation 1).

$$K_{tot} = K_{algae} + K_{precip.} + K_{sediment} + K_{dilution} \quad [Equation\ 1]$$

Where K_{tot}= overall copper dissipation rate coefficient in situ
K_{algae}= copper dissipation rate coefficient from sorption to algae (calculated from copper concentrations measured in the laboratory)
$K_{precip.}$= copper dissipation rate coefficient from precipitation (calculated from copper concentrations measured in the laboratory)
$K_{sediment}$= copper dissipation rate coefficient from sorption to sediment (calculated from copper concentrations measured in the laboratory)
$K_{dilution}$= copper dissipation rate coefficient from dilution (estimated in situ using rhodamine WT)

With equation 1, fate processes that do not result in a measurable decrease in aqueous copper concentrations at relevant times for other processes (i.e. dominant fate processes)

can be removed from the equation. The remaining process or processes will have the greatest influence on copper fate in an aquatic system (i.e. dominant fate process).

Identification of dominant fate processes has advantages for design and implementation of subsequent experiments. With knowledge of the fate process or processes that drive exposures in an aquatic system, the relevant processes can be characterized for other sites or at different times to predict exposures from copper-based algaecide treatments. In order to measure and rank fate processes influencing copper from an algaecide treatment, the specific objectives of this study were to 1) calculate the copper dissipation rate from copper concentrations measured in situ following a pulse exposure of algaecide (i.e. Cutrine-Plus®, copper ethanolamine), 2) isolate and calculate individual copper dissipation rates from fate processes (i.e. algal sorption, sediment sorption, copper precipitation and dilution), 3) calculate the copper dissipation rate based on copper concentrations measured in mesocosms incorporating individual fate processes (i.e. algal sorption, sediment sorption, precipitation and dilution), and 4) compare individual and cumulative (i.e. mesocosm and in situ) copper dissipation rates and rank individual copper dissipation rates using a mathematical model.

Materials and Methods

Study Site and Characteristics

The pond (34º34'48.75" N, 82º43'40.28" W) used for in situ measurement of aqueous copper concentrations was approximately 6,000 m^2 with an average depth of 1.2 m. Copper ethanolamine (Table 2.2) was applied to 126 m^2 for treatment of a problematic density ($8.7x10^4$ cells/mL) of *Ankistrodesmus falcatus* in a section of the pond. A relatively small surface area of the pond was treated (i.e. 2%) as this was the section of the pond where *A. falcatus* was located. Typically, problematic algae are heterogeneously distributed and algaecide treatments are applied to the fraction of the aquatic system containing the algae (Geer et al. 2017, Calomeni et al. 2015). Water inputs to the pond included an ephemeral creek connecting other ponds in series and agricultural/ livestock runoff. Sediments in this pond had organic ligands from livestock as well as grass clippings and hay deposited from runoff. Because dissipation rates depend on water and sediment characteristics (Murray-Gulde et al. 2002), site-specific aqueous and sediment characteristics were measured using the methods outlined in Table 2.1.

Aqueous and Sediment Copper Measurement, Extraction and Statistical Analyses

Copper present in suspended and dissolved forms in the aqueous phase was measured as soluble copper (US EPA 1992) and acid-soluble copper (US EPA 1991). Sediment samples were collected pre- and post-treatment in the laboratory and in situ. Sediment copper concentrations were measured using a Perkin Elmer (Waltham, MA) Optima 3100RL inductively coupled plasma-optical emission spectrometer (ICP-OES)

following hot acid extraction (US EPA 1996). QA/QC included replicate samples, standards, and blank matrix spike recovery. Pre- and post-treatment sediment copper concentrations were compared statistically using linear contrasts (JMP Pro V.12).

Measurement of Copper Concentrations for the in situ Copper Dissipation Rate Following a Pulse Exposure (i.e. Copper Ethanolamine Treatment)

Copper ethanolamine was applied as a liquid surface treatment at a concentration of 1 mg Cu/L to 2% of the surface area of the pond. Water samples (20 L, n=3) were collected within the treatment area 0.04, 0.06, 0.08, 0.10, 0.13 and 1d post- treatment for copper analysis. Pre- and post-treatment sediment samples (approximately 1 cm depth) were collected within the treatment area and sediment copper concentrations were measured.

Measurement of Copper Concentrations for Isolation of Individual Copper Fate Processes (i.e. Algal Sorption, Sediment Sorption Precipitation and Dilution)

Copper dissipation rate coefficients and half-lives resulting from algal sorption, precipitation and sediment sorption were calculated from copper concentrations measured in the laboratory. Experimental chambers consisted of 500 mL wide mouth borosilicate glass jars. A stock copper ethanolamine solution was prepared in Nano-pure® water at a concentration of 1,000 mg Cu/L. The appropriate volume of this stock solution was amended to experimental chambers to a targeted copper concentration of 1 mg Cu/L.

The aqueous copper dissipations rate coefficient and half-life from algal sorption were calculated from aqueous copper concentrations measured from jars containing site water and algae (initial cell density of $8.7x10^4$ cells/mL). At experiment initiation, 300 mL site water containing algae was added to three replicate experimental chambers.

Aqueous samples (4 mL, n=3) were collected for copper analysis immediately post-copper amendment to confirm copper concentrations and at 0.2, 0.3, 1.1, and 8.0 days post amendment.

To measure precipitation of copper from the aqueous phase, 400 mL of filtered site water was added to three replicate experimental chambers amended with copper (as previously described). Site water was filtered through glass fiber filters (Fisherbrand ™ < 3 μm) to remove algal cells (*A. falcatus,* length 30 – 40 μm). Four mL aqueous samples were collected immediately post- amendment and at 1.0, 2.3, 4.9, 5.3, 10.7, 13.0 days (n=3).

The copper dissipation rate coefficient and half-life due to sediment sorption were calculated from copper concentrations measured from filtered (as previously described to remove algal cells) site collected water and sediment. To initiate experiments, 115 mL of sediment and 300 mL of site water were added to three replicate experimental chambers. Copper was amended to achieve a concentration of 1 mg Cu/L. Aqueous samples (4 mL) were collected immediately post copper amendment, 1.3, 4.1, 7.3, 11.0, 15.3, and 18.0 days post amendment (n=3). When aqueous copper concentrations achieved pre-amendment concentrations, the top 1 cm of sediment was skimmed from the experimental chambers and the remaining mass of sediment was homogenized and removed from chambers (approximately 4 cm). Copper was then extracted from the sediments (1 cm and 4 cm sediment depth) and measured as previously described.

The site specific dilution rate coefficient and half-life for copper were estimated using rhodamine WT (a fluorescent dye, Cole-Parmer, Vernon Hills, IL) in situ. The dye

was tank mixed with the copper-based algaecide and applied simultaneously. Dye concentrations were applied at a concentration sufficient to capture more than 3 aqueous half-lives prior to achieving the detection limit (i.e. approximately 0.5 μg/L) within the treated area. The rhodamine WT dye was quantified measuring fluorescence (Ex 555/ Em 560) using matrix matched standards with linear regression to calculate a standard curve. Water samples (20 L, n=3) were collected for rhodamine WT analysis at 0.04, 0.06, 0.08, 0.10, 0.13 and 1d post-initiation of application within the copper ethanolamine treatment area.

Measurement of Copper Concentrations for the Mesocosm Copper Dissipation Rate

High-density polyethylene, 30-gallon drums were modified and utilized as mesocosms within the pond. The bottoms of the drums were removed and the open-ended drums were placed into the pond and secured into pond sediments (~5-10 cm into sediments). To replicate dilution, half the volume of the mesocosms was removed at every aqueous half-life (based on dilution rates calculated from in situ measurements using the dye). Aqueous samples (50 mL, n=3) were collected following each dilution step and copper concentrations (soluble and acid soluble) were measured.

Calculation of Rate Coefficients and Comparison of Copper Fate Processes Using a Mathematic Model

A first order rate equation (Equation 2) was used to calculate copper and rhodamine WT dissipation rate coefficients based on the premise that one of the reactants (i.e. copper or rhodamine WT dye) was limiting while the other reactants (e.g. sediment ligands, anions, algal ligands) were present in excess. Previous publications have identified first order rates for copper dissipation (Goulet et al. 2001, Murray-Gulde et al.

2005) and dye dilution rates (Fox et al. 1991). Pre-treatment acid soluble copper concentrations at this site ranged from 0.005 to 0.032 mg Cu/L. To calculate dissipation rate coefficients, copper concentrations that were below the detection limit (0.020 mg Cu/L) of the ICP-OES for copper were recorded as 0.020 mg Cu/L.

$$C_t = C_i\, e^{-Kt} \qquad [Equation\ 2]$$

where: C_t = the average aqueous copper concentration (mg Cu/L) at time t
C_i = the average initial aqueous copper concentration (mg Cu/L)
K = rate coefficient
t = time (days)

Rate coefficients for independent fate processes influencing copper dissipation calculated using Equation 2 were used to parameterize Equation 1.

Results and Discussion

Sediment and Water Characteristics

Characteristics of sediments used in experiments (i.e. in situ, laboratory and mesocosm) were comparable in terms of percent organic matter (OM), pH, acid volatile sulfides (AVS) and oxidation-reduction potential (Table 2.3). Sediments had high OM percentages (2.5-10.6 %) compared to calculated OM percentages in sediments across the continental United States 0.48% (25^{th} interquartile range) - 2.67% (75^{th} interquartile range) (calculated as the product of 1.724 and organic carbon percentage; Nelson and Sommers 1996) (Suedel and Rodgers 1991). The sediment pH was less than neutral (pH 6.4-6.7). Sediment oxidation reduction potential ranged from -69.8 to 155.4 mV indicating relatively oxic sediment conditions (Mitsch and Gosselink 2000). This was confirmed by the low AVS concentrations in sediments (ND to 0.024 μmol sulfide/ g) relative to reported AVS concentrations in freshwater sediments (Besser et al. 1996, US EPA 2005b).

Similar water characteristics were measured in situ, in the laboratory and in mesocosms (Table 2.4). The measured water hardness (64 – 80 mg/L as CaCO3) is characteristic of moderately hard waters (Briggs and Ficke 1977).

In Situ Copper Dissipation Rate Following a Pulse Exposure (i.e. Copper Ethanolamine Treatment)

In situ, copper concentrations were 1.105 $\pm$ 0.570 mg Cu/L and 0.724 $\pm$ 0.202 mg Cu/L for acid soluble and soluble copper, respectively following treatment (0.04 days collected from the treatment area [2% surface area of pond]; Figure 2.1). First order rate

coefficients, half-lives and coefficients of correlation (R^2) were calculated using data from 0.04 days (0.96 hour) to 0.125 days (3 hours) post-amendment and are presented in Table 2.5. Copper concentrations decreased to 0.015 $\pm$ 0.003 mg Cu/L as acid soluble copper and 0.012 $\pm$ 0.002 mg Cu/L as soluble copper (measured using GFAAS) at the following sampling interval (1 day). These copper concentrations (0.015 $\pm$ 0.003 mg Cu/L as acid soluble copper and 0.012 $\pm$ 0.002 mg Cu/L as soluble copper) are within the range of pretreatment copper concentrations at this site, 0.005 to 0.032 mg Cu/L.

In situ sediment copper concentrations measured pre- and post-treatment were not significantly different ($p = 0.9551$, $\alpha = 0.05$). Pretreatment sediment copper concentrations were 18 $\pm$ 17 mg Cu/kg and post-treatment sediment copper concentrations were 19 $\pm$ 9 mg Cu/kg.

Individual Copper Fate Processes (i.e. Algal Sorption, Sediment Sorption, Precipitation, and Dilution)

In laboratory experiments, fate processes were controlled to isolate their individual influence on copper dissipation rates. For the experiment measuring copper dissipation as a result of algal sorption, aqueous copper concentrations were 1.019 $\pm$ 0.051 mg Cu/L and 0.717 $\pm$ 0.251 mg Cu/L as acid soluble and soluble copper concentrations respectively following amendment. Maximum measurable copper sorption to algal cells typically occurs less than a day following exposure (Pradham and Rai 2002, Gupta et al. 2005, Flouty and Estephane 2012). Significant differences in initial aqueous concentrations were not discerned relative to concentrations measured 8

days post-amendment as acid soluble ($p = 0.7193$; $\alpha = 0.05$) or soluble ($p = 0.4817$; $\alpha = 0.05$) copper (Figure 2.2A).

In laboratory experiments evaluating precipitation of copper alone, copper in the aqueous phase did not decrease significantly ($p = 0.1826$ and 0.1410 for acid soluble and soluble copper, respectively; $\alpha = 0.05$) throughout the 13 day experiment duration. For acid soluble copper, the concentration was 1.064 ± 0.024 mg Cu/L following amendment and the final copper concentration was 0.994 ± 0.071 mg Cu/L (Figure 2.2B). Following amendment, the initial copper concentration was 0.979 ± 0.017 and the final was 1.007 ± 0.071 mg Cu/L for soluble copper. Soluble copper concentrations averaged 93% of acid soluble copper concentrations throughout the 13 day experiment.

Experiments conducted to calculate the copper dissipation rate from sorption to sediments resulted in decreased aqueous copper concentrations to approximately 0.020 mg/L (soluble and acid soluble copper) by 15 days post-amendment (Figure 2.2C). Copper concentrations were 0.958 ± 0.082 mg Cu/L as acid soluble and 0.629 ± 0.147 mg Cu/L as soluble copper following amendment, indicating that immediately post-amendment, 34% of copper partitioned to suspended sediment. The first order rate coefficient, half-life and coefficient of correlation (R^2) were calculated using data from 0 – 15 days post-amendment and are presented in Table 2.5. Calculated sediment sorption half-lives in this experiment (2.89 days for soluble copper and 2.67 days for acid soluble copper) were comparable to those previously reported for copper ethanolamine amended to mesocosms containing sediment and a cyanobacterium (i.e. *Microcystis aeruginosa*) (2.6 – 5.5 days; Murray-Gulde et al. 2002).

Copper that partitioned to the sediment phase in this laboratory sediment sorption experiment was heterogeneously distributed as a function of sediment depth. Surficial (1 cm) pre- (44 $\pm$ 1 mg Cu/kg) and post- amendment (60 $\pm$ 1 mg Cu/kg) sediment copper concentrations were significantly different ($p = 0.0016$, $\alpha = 0.05$). Assuming all copper partitioned to the surficial sediment layer (1 cm), copper extracted from the sediment accounted for 72 % of the amended copper in experimental chambers. Significant differences in sediment copper concentrations were not measured ($p = 0.213$, $\alpha = 0.05$) when all sediment within the experimental chambers were homogenized and extracted (approximately 4 cm depth). For the 0-4 cm sediment depth, pre-amendment sediment copper concentrations were 15 $\pm$ 2 mg Cu/kg and post- amendment were 19 $\pm$ 4 mg Cu/kg.

The site specific dilution rate were estimated using rhodamine WT dye. The initial measured rhodamine WT concentration was 45 $\pm$ 26 μg/L and decreased to 0.8 $\pm$ 0.1 μg/L by 1 day post- application (Figure 2.3). The aqueous half-life, rate coefficient and coefficient of correlation are presented in Table 2.5 and were calculated using dye concentrations from 0.04 days (0.96 hour) to 0.1 days (2.5 hours).

Copper Dissipation Rate from Mesocosms

Mesocosm experiments were used to test the hypothesis that in situ copper dissipation rates can be physically modeled (with mesocosms) with sufficient simulation of in situ conditions (i.e. dilution, sediment, site water and algae). Based on the calculated in situ dilution half-life, water (i.e. half the volume of the mesocosm) was exchanged at a rate of approximately 0.03 days. Initial copper concentrations were 0.389

$\pm$ 0.029 mg Cu/L and 0.182 $\pm$ 0.060 mg Cu/L for acid soluble and soluble copper respectively (Figure 2.4). Within the mesocosms, half the water was removed and replaced with un-amended water from the ponds for four replacements (4 half-lives). Final copper concentrations were 0.024 mg Cu/L for acid soluble copper and below the detection limit for soluble copper (0.020 mg Cu/L). First order rate coefficients, half-lives and coefficients of correlation (R^2) were calculated using data from 0 to 0.08 days (1.9 hours) post amendment (Table 2.5).

Copper half-lives calculated from concentrations measured in mesocosms that excluded dilution as a fate process resulted in copper dissipation half-lives of 2.6 – 5.7 days (Murray-Gulde et al. 2002; Table 2.6). This dissipation half-life is similar to the dissipation half-life calculated from the current laboratory experiment from sediment sorption alone (2.89 - 2.67 days, Table 2.5). By incorporating dilution as a fate process in the mesocosm, copper half-lives were more accurate (i.e. similar to the half-life calculated using in situ copper concentrations). Calculated copper half-lives in situ were 0.03 days relative to copper half-lives in mesocosms (including dilution) of 0.02 days (Table 2.5). The convergence of copper half-lives (i.e. mesocosm and in situ) by adding dilution into the mesocosm physical model corroborates the importance of dilution as a dominant fate process driving copper dissipation rates at this site. Additionally, the congruence of copper dissipation rates (coefficients and half-lives) in situ and using this mesocosm design (incorporating dilution) demonstrate the importance of capturing sufficient site specific characteristics so that mesocosm experiments are similar to in situ conditions.

Comparison of Copper Fate Processes Using a Mathematic Model

To synthesize data for in situ and laboratory copper dissipation rate coefficients, a mathematical model was used (equation 1). Copper dissipation rate coefficients were not calculated for fate processes (i.e. algal sorption and precipitation measured in the laboratory) that did not result in significant decreases in aqueous copper concentrations at relevant durations for other fate processes. Relevant durations in this context refer to the time required for copper concentrations to decrease to pre-treatment concentrations (i.e. 1- 15 days post amendment). Algal sorption and precipitation were therefore removed from equation 1 yielding equation 3.

$$K_{tot} = K_{sediment} + K_{dilution} \qquad [Equation\ 3]$$

Where K_{tot}= overall copper dissipation rate coefficient in situ (soluble = 23.79 ln [Cu]/day, acid soluble = 24.63 ln [Cu]/day)
$K_{sediment}$= copper dissipation rate coefficient from sorption to sediment (soluble = 0.24 ln [Cu]/day, acid soluble = 0.26 ln [Cu]/day)
$K_{dilution}$= copper dissipation rate coefficient from dilution (27.64 ln [dye]/day)

The calculated overall copper dissipation rate coefficient (i.e. sum $K_{sediment}$ and $K_{dilution}$) using the site-specific dilution rate coefficient (estimated using dye) and copper sediment sorption rate coefficient (calculated from copper concentrations measured in the laboratory) are 27.88 ln [Cu]/day and 27.90 ln [Cu]/day for soluble and acid soluble copper respectively. These calculated rates are between 117% -113% the calculated copper dissipation rate coefficient in situ (23.79 ln [Cu]/day, soluble copper and 24.63 ln [Cu]/day, acid soluble copper). If the laboratory derived copper sediment sorption rate coefficient had been the only rate considered, 1% of the in situ copper

dissipation rate would have been anticipated. At this site, copper dilution and dispersion accounted for the majority of copper dissipation.

Dilution rates from site to site and temporally are anticipated to range widely (half-lives <0.06 days – 8.3 days; Table 2.6) relative to sediment sorption rates. Relatively small shallow lakes exposed to wind, similar to the study site in the current experiment are typically well mixed (Imboden and Wüest 1995). Treatments applied to bays or coves that are enclosed may have slower dilution rates as mixing is limited by topography (Fox et al. 2002). Additionally, dilution rates likely range as a function of the relative volume in which the algaecide is applied based on the laws of mass balance and mass action. Subsequently, if the algaecide is applied to a relatively small fraction of the volume of the aquatic system (e.g. 2% in the current experiment), one would anticipate more rapid dissipation rates relative to 50% of the surface area (i.e. maximum legal application). Based on legal label restrictions that copper-based algaecides cannot be applied to greater than 50% of an aquatic system, dilution will be a fate process influencing copper dissipation rates in situ. Because dilution rates range widely and can be the driving fate process for copper dissipation rates, site -specific dilution rates should be considered in predictions of copper fate in situ. If dilution half-lives approach copper sediment sorption half-lives (2.6 days – 11.8 days; Table 2.6), sediment sorption would be a significant fate process.

Ultimately, synthesis of data from the current experiment can be used to identify considerations for design of future predictive experiments (e.g. laboratory and mesocosm as well as computer and mathematic models). For physical models (e.g. laboratory,

mesocosm), dissipation rates at this small scale will be comparable to in situ dissipation rates by incorporating site characteristics that are driving the dominant dissipation rate (e.g. dilution water in this experiment). For computer or mathematic models, incorporation of dominant fate processes is needed for accurate predictions of in situ dissipation rates. Experiments designed with consideration of dominant fate processes result in accurate predictions of dissipation rates.

Conclusions

In the current experiment, physical and mathematic models were used to isolate and rank individual fate processes that drive aqueous copper dissipation rates following pulse exposures of copper-based algaecides. In this study, dilution resulted in the most rapid half-life (0.03 days, estimated using rhodamine WT dye), followed by sediment sorption (2.89 and 2.67 days for soluble and acid soluble copper respectively). Other fate processes anticipated to influence copper fate in situ (i.e. algal sorption and precipitation) did not result in significant decreases in copper concentrations 8 (algal sorption) and 13 (precipitation) days post copper amendment.

The two dominant fate processes (i.e. dilution and sediment sorption) that contributed to the copper dissipation rate in situ were used to parameterize a mathematical model comparing the sum of individual fate processes to the copper dissipation rate in situ. This model is based on the hypothesis that the sum rate coefficients of individual fate processes will be equivalent to the in situ copper dissipation rate coefficient. Comparison of individual fate processes to the copper dissipation rate coefficient in situ indicated that the dilution rate coefficient alone accounted for almost 100 % of the in situ copper dissipation rate coefficient. The sediment sorption rate coefficient contributed approximately 1%.

Mesocosm experiments incorporating dilution and containing sediment, site water, algae and amended copper were compared to the in situ copper dissipation rate. These half-lives were similar (0.02 and 0.03 days for the mesocosm and in situ experiments respectively) demonstrating the importance of capturing sufficient site

characteristics that drive fate processes. The approach taken in this experiment serves as a model to identify and interpret dominant fate processes for constituents in situ that can be captured by interpretation of rates (i.e. mathematic models) and through physical modeling.

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Table 2.1 Analytical methods, citations and method detection limits for sediment and water characteristics.

Parameter	Methods	Method Detection Limit/ Variance
Sediment Characteristics		
Percent Organic Matter	Loss-on-ignition at 360°C (Schulte 1995, Salehi et al. 2011)	0.02%
pH	Electrometric method 4500-H^+ B: Orion model 420A (APHA 2012, McLean 1982)	0.01 S.U.
Acid Volatile Sulfides (AVS)	Modified diffusion method (Leonard et al. 1996)	0.004 μmol sulfide/ g
Oxidation-reduction (Eh)	Modified standard method 2580B: Accumet® calomel reference electrode, Fluke® 77 III voltage meter (Faulkner et al. 1989)	10 mV
Water Characteristics		
pH	Electrometric method 4500-H^+ B: Orion model 420A (APHA 2012)	0.01 S.U.
Temperature	Direct measurement HOBO pendant® Data Logger	$\pm$ 0.53°C
Dissolved Oxygen	Direct measurement Hach® HQ30d Portable Meter with IntelliCAL™ LDO101 Standard Luminescent/Optical Dissolved Oxygen (LDO) Probe	$\pm$ 0.1 mg/L
Conductivity	Direct measurement 2510 B:YSI 30 (APHA 2012)	0.1 µS/cm
Alkalinity	Titration method 2320 B (APHA 2012)	2 mg/L as $CaCO_3$
Hardness	EDTA Titrimetric Method 2340 C (APHA 2012)	2 mg/L as $CaCO_3$

Table 2.2 Physical and chemical characteristics of Cutrine-Plus®.

Characteristics	
Active ingredient	9 % Cu
Maximum label concentration (mg Cu/L)	1.0 [a]
Composition of ingredients	Triethanolamine (19-29%) [b] Ethanolamine (15-25%) [b] Basic Copper Carbonate (11-21%) [b]
Water solubility	Miscible [b]
Specific gravity (g/cm^3)	1.1 (gravimetric measurement)
pH (S.U.)	10.3-10.5 [b]

[a]Applied Biochemists (Arch Chemicals a Lonza Business, Alpharetta, GA). Specimen label. Cutrine-Plus®

[b]Applied Biochemists (Arch Chemicals a Lonza Business, Alpharetta, GA). Safety data sheet. Cutrine-Plus®

Table 2.3 Measured physical and chemical characteristics of sediment in situ, laboratory, and mesocosm experiments.

Parameter	In Situ	Laboratory	Mesocosm
Percent Organic Matter (%) (n=1)	4.1	2.5	10.6
pH S.U. (n=1)	6.7	6.7	6.4
Acid Volatile Sulfides (AVS) μmol sulfide/ g (n=1)	0.024	ND	ND
Oxidation-reduction (Eh; mV) (minimum and maximum, n=3)	16-154.2	-69.8-68.4	26-155.4

Table 2.4 Measured physical and chemical characteristics of water in situ, laboratory and mesocosm experiments. Parameters measured at experiment completion (n=1) unless otherwise noted.

Parameter	In Situ	Laboratory	Mesocosm
pH S.U.	7.27	7.77 [a] - 7.75 [b]	7.06
Temperature °C	19	25	27 - 35 [c]
Dissolved Oxygen mg/L as O_2	12.30	9.56 [a]	5.48 - 10.00 [c]
Conductivity µS/cm	301	314[a] - 302 [b]	291
Alkalinity mg/L as $CaCO_3$	76	76 [a] - 84 [b]	92
Hardness mg/L as $CaCO_3$	72	72 [a] - 80 [b]	64

[a] Measured in filtered water samples (e.g. precipitation and sediment sorption experiments)
[b] Measured for algal sorption experiment
[c] Minimum and maximum over four hours

Table 2.5 Aqueous copper rate coefficients, half-lives and correlation coefficients calculated using average copper concentrations (n = 3). [Cu] denotes copper concentration in mg Cu/L.

		Rate Coefficient (ln [Cu]/day)	Half-life (day)	R^2
In Situ Dissipation Rate				
	Acid Soluble	24.63	0.03	0.98
	Soluble	23.79	0.03	0.99
Individual Dissipation Rates (Laboratory Experiments)				
Algal Sorption	Acid Soluble	-	-	-
	Soluble	-	-	-
Precipitation	Acid Soluble	-	-	-
	Soluble	-	-	-
Sediment Sorption	Acid Soluble	0.26	2.67	0.98
	Soluble	0.24	2.89	0.93
Dilution (Rhodamine Dye)	NA	27.64[a]	0.03	1.00
Mesocosm Dissipation Rate				
	Acid Soluble	33.57	0.02	0.97
	Soluble	28.64	0.02	0.93

[a] Estimated using rhodamine dye concentrations units are as ln [dye]/day.

Table 2.6 Half-lives for dilution estimated using Rhodamine dye and copper dissipation from sediment sorption from peer-reviewed literature.

Dilution			
Location	**Characteristics of site**	**Half-life (day)**	**Citation**
Sacramento-San Joaquin delta, CA	7 sites – sloughs, harbors and marinas (surface area not specified)	0.08 - 1	Anderson 1999
Lake Seminole, GA	2 sites – 40,000 m^2 plots in Lake Seminole	<0.06 – 0.18	Fox et al. 1992
Lake Minnetonka, MN	2 sites – 65,000 m^2 plots in separate bays	3.9 – 7.8	Fox et al. 2002
Crystal River, FL	4 sites – 10,000 m^2 canals	0.5 – 8.3	Fox et al. 1991[a]
Sediment sorption			
		Half-life (day)	**Citation**
Laboratory		7.8 – 11.8	Jones et al. 2008
Mesocosm		2.6 – 5.7	Murray-Gulde et al. 2002

[a] Rhodamine dye mixed 1:1 with methanol prior to application

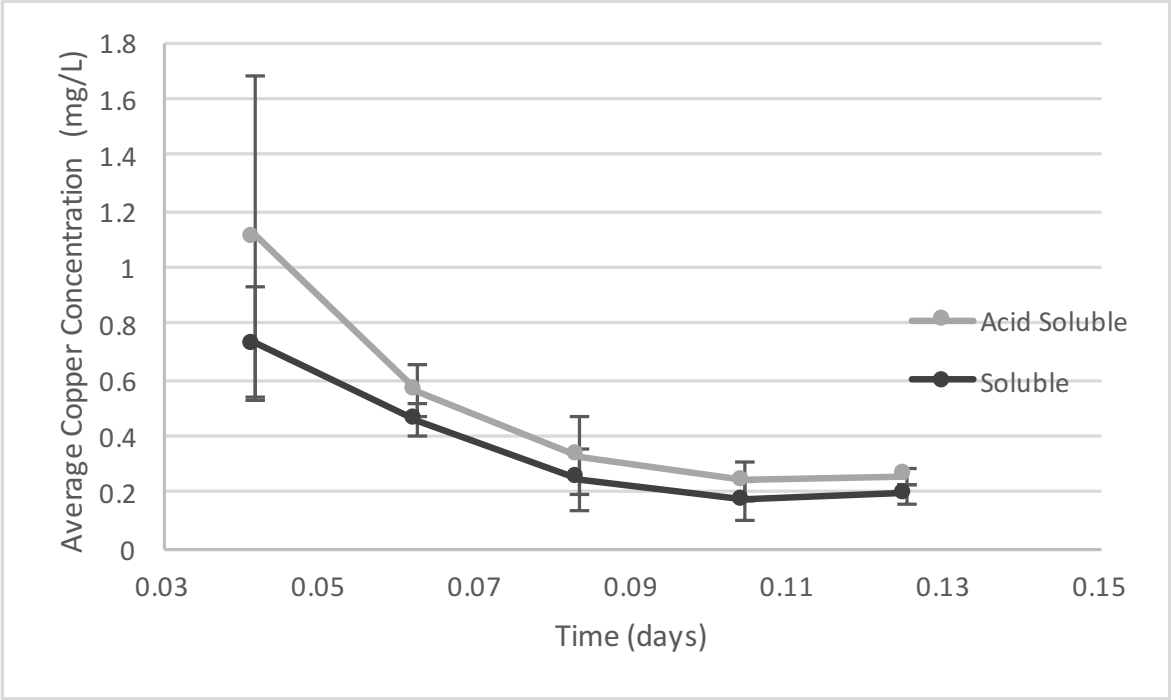

Figure 2.1 Average aqueous concentrations as soluble and acid soluble copper over time in situ (34º34'48.75" N, 82º43'40.28" W); n=3; error bars indicate $\pm$1 standard deviation.

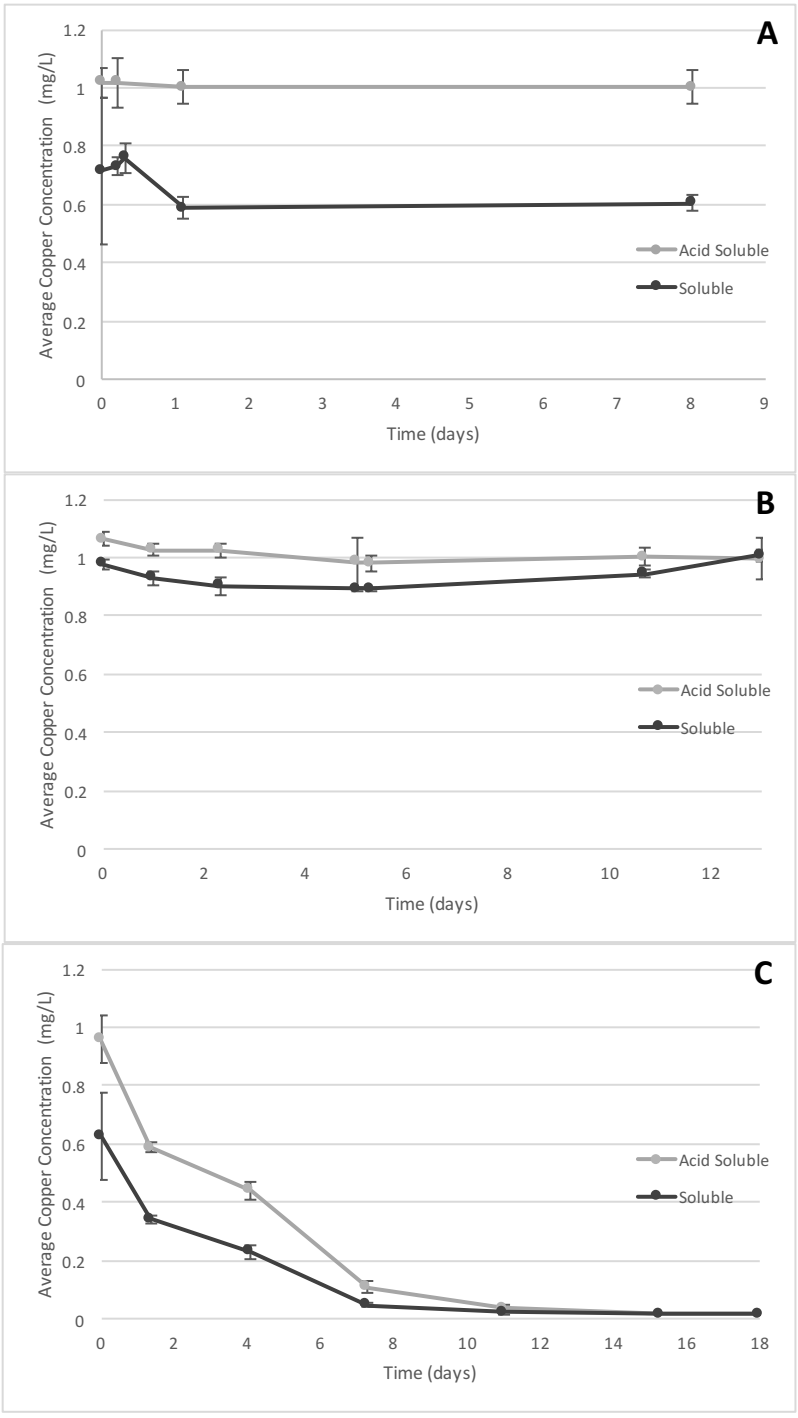

Figure 2.2 Average aqueous concentrations as soluble and acid soluble copper over time for algal sorption (A), precipitation (B) and sediment sorption (C); n=3; error bars indicate $\pm$1 standard deviation.

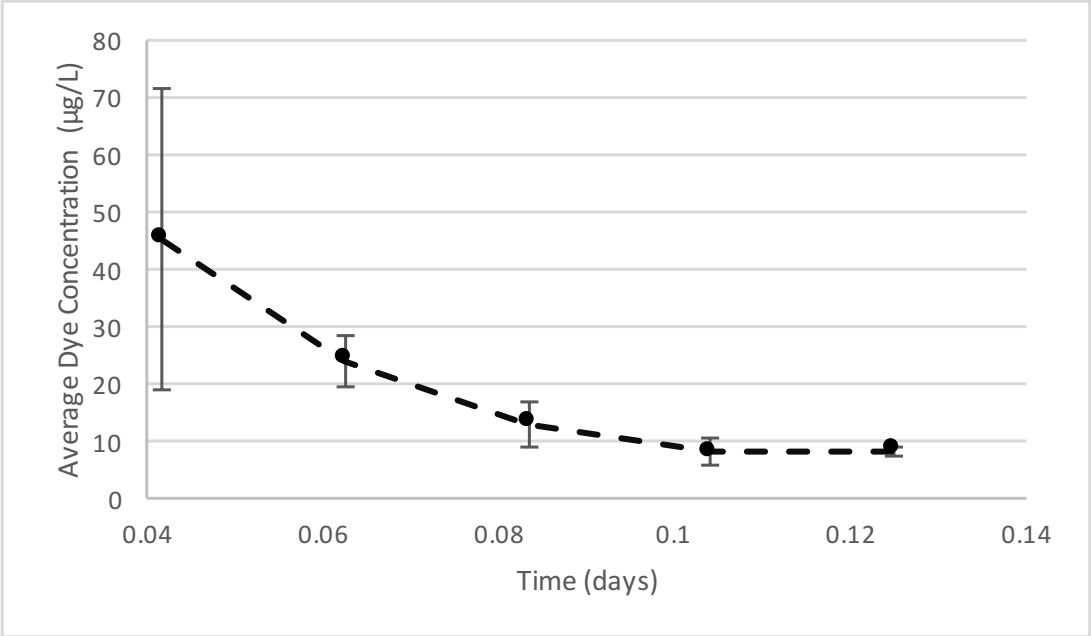

Figure 2.3 Average rhodamine WT dye concentrations over time; n=3; error bars indicate $\pm$1 standard deviation.

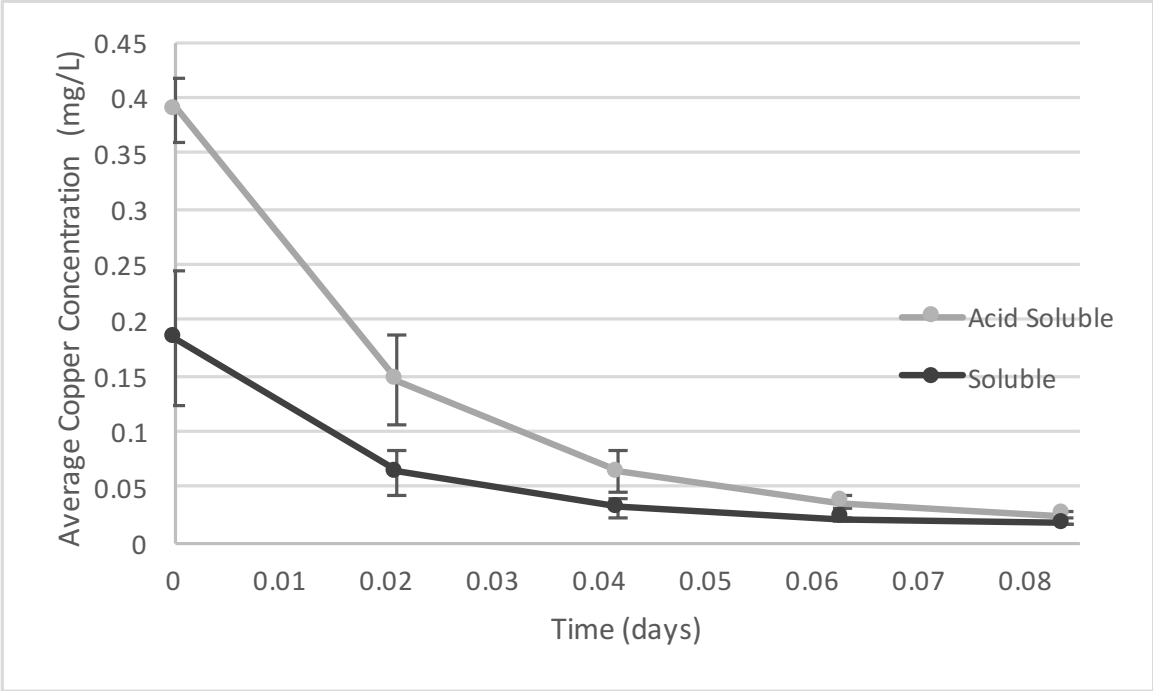

Figure 2.4 Average copper concentration over time in mesocosm; n=3; error bars indicate $\pm$1 standard deviation.

CHAPTER THREE

LYNGBYA WOLLEI RESPONSES TO COPPER ALGAECIDE EXPOSURES PREDICTED USING A CONCENTRATION-EXPOSURE TIME (CET) MODEL: INFLUENCE OF INITIAL BIOMASS

Abstract

Concentration-exposure time models (CET) are used to predict responses of aquatic vascular plants to herbicides and could be applicable for cyanobacterial responses to algaecides if appropriate variables are captured by the model. For the cyanobacterium, *Lyngbya wollei*, initial biomass upon application may be an important parameter driving responses to copper algaecides. Specific objectives of this study were to 1) discern an algaecide formulation with sufficient potency and relationship between copper concentration and response for a CET model for *L. wollei*, 2) develop a CET model for the algaecide and *L. wollei*, 3) determine the influence of initial biomass on measured responses vs. effects predicted using the CET model, and 4) develop a new model with *L wollei* biomass as a variable. The algaecide, Clearigate®, had sufficient potency ($\geq$ 90% response) and strength of relationship between copper concentration and response (R^2 = 0.99 from 0.1-0.7 mg Cu/L) to develop a CET model. Exposures of 0.4, 0.7 and 1.0 mg Cu/L for 24 hours, exposures of 0.4, 0.7 and 1.0 mg Cu/L for 8 hours, and exposures of 0.7 and 1.0 mg Cu/L for 1 hour resulted in 87-100 % response of *L. wollei* (percent damaged trichomes). Initial biomasses greater than those used for the CET model (52 g WW/m^2) decreased responses to non-detect (1,558 g WW/m^2). Because initial biomass influenced predicted responses, a new model was developed with biomass as a variable (BDC model). The BDC model increased the range of initial biomasses (13 -104 g

WW/m^2) in which performance of Clearigate® for controlling the growth of *L. wollei* could be predicted.

Introduction

Prior to treatment of problematic algae or cyanobacteria in water resources with algaecides, predictions of effective exposures are necessary to guide management decisions, since characteristics of the infested site (e.g. pH, conductivity, alkalinity, hardness, dissolved and particulate organic matter, water mixing) and innate sensitivity of the target algal population(s) can alter the concentration of algaecide needed to achieve control (Rodgers et al. 2010, Bishop and Rodgers 2011, Greenfield et al. 2013, Isaacs et al. 2013 and Calomeni et al. 2015). Herbicide treatments for vascular plants are generally analogous situations to algaecide treatments for algae and cyanobacteria. For specific couplets of an herbicide and vascular plant, concentration-exposure time (CET) models have been useful for prediction of herbicide exposures necessary to result in consequent responses of a vascular plant (Getsinger et al. 1991, Van and Conant 1988, Getsinger and Netherland 1997). The fundamental basis for CET models is the theory that sufficient contact time is necessary to achieve a critical concentration of herbicide within the target vascular plant for herbicides that have exposure durations altered by site characteristics (e.g. lotic systems, large aquatic systems with relatively small treatment areas, etc.) (Van and Conant 1988, Netherland 1991). These models may also be applicable for algaecide treatments for problematic algae and cyanobacteria. To test this hypothesis, a CET model was developed for the problematic cyanobacterium, *Lyngbya wollei*, and exposures of a copper-based algaecide. For cyanobacteria, an additional independent variable, specifically the influence of cyanobacterial biomass at initiation of exposure on the outcome of an algaecide treatment, may be necessary to accurately

predict responses to algaecide exposures. As an extension of the theory driving the CET model, the biomass of a cyanobacterial population can alter the concentration of algaecide within an alga influencing the response. If initial biomass is an important exposure characteristic for accurate prediction of cyanobacterial responses to algaecide exposures, a different model that includes initial biomass as a variable will be needed.

Similar to vascular plant CET models (Getsinger and Netherland 1997), unique properties of a cyanobacterium (e.g. sensitivity) and an algaecide (e.g. potency, mechanisms of action, formulation) necessitate a specific CET model for each cyanobacterium-algaecide combination. Initially, CET model development for cyanobacteria should logically be prioritized for common and ubiquitous problematic cyanobacteria and frequently used algaecides. *L. wollei* is a problematic cyanobacterium that interferes with designated water resource uses throughout North America from Florida (Foss et al. 2012) to Canada (Vis et al. 2008). This cyanobacterium forms aesthetically objectionable growths and evidence indicates that *Lyngbya* can produce paralytic shellfish poisons (Carmichael et al. 1997, Foss et al. 2012, Lajeunese et al. 2012) and extirpate native species within infested areas (Mastin et al. 2002). In the current experiment, *L. wollei* that was impeding recreational uses (i.e. fishing, swimming, boating) was collected from a 44,500 m^2 pond in Spartanburg County, South Carolina. Copper-based algaecides have been used to treat algae and cyanobacteria producing taste and odor compounds in potable water for more than a century (Moore and Kellerman 1905) and continue to be used in irrigation canals, ponds, lakes and reservoirs (Netherland 2014). Exposures of copper-based algaecides result in aqueous copper

concentrations exceeding pre-treatment concentrations for minutes to days following application (Button et al 1977, Anderson 1999, McNevin and Boyd 2004, Liu et al. 2006, Calomeni et al. in press) depending on site characteristics. Wide-ranging copper exposure durations (i.e. minutes to days) are anticipated to have a similarly wide-ranging influence on responses, supporting development of a unique cyanobacterial CET model specifically for copper-based algaecides.

To develop the *L. wollei* CET model, laboratory experiments were conducted sequentially. Laboratory-scale experiments are appropriate for discerning the influences of specific exposure characteristics (i.e. concentration, exposure duration and initial cyanobacterial biomass) on response, while allowing replication to discern differences in responses as a function of exposures (Giesy and Odum 1980). Initial experiments were designed to determine an effective algaecide for controlling the growth of this accession of *L. wollei*. Several copper-based products are available for use as algaecides and responses of *L. wollei* to copper based algaecides range as a function of formulation (Calomeni et al. 2015). Because of the robust structure of *L. wollei* (i.e. mucilaginous sheath) and the theory that algae are more sensitive to chelated copper formulations relative to non-chelated (e.g. copper sulfate pentahydrate), chelated copper formulations were evaluated (Bishop and Rodgers 2011, Rodgers et al. 2010, Calomeni et al. 2014). Chelated copper algaecide formulations include 1) copper citrate and gluconate (e.g. Algimycin® PWF), 2) copper ethanolamine (e.g. Cutrine-Plus®), and 3) copper ethanolamine mixed with different percentages of the adjuvant D-limonene (e.g. Cutrine® Ultra and Clearigate®) (Table 3.1). Based on decisions made during their registration

with the US Environmental Protection Agency (US EPA), algaecides have restrictions in terms of the concentration of active ingredient that can be applied to an aquatic system during a treatment. These restrictions are specified on the product label, termed "legal label concentration" throughout this manuscript. To develop the CET model, a sufficiently potent copper algaecide formulation is needed resulting in significant (i.e. ≥ 90%) as well as a range of cyanobacterial responses for legal label concentrations (0.1-1.0 g Cu/L), so that differences in cyanobacterial responses due to exposure duration can be discerned.

Once questions regarding a sufficiently potent algaecide formulation are resolved, exposure duration is the next critical independent variable to evaluate. Similar to vascular plants (Getsinger and Netherland 1997), exposure duration is likely positively correlated with cyanobacterial responses until exposure durations exceed a threshold duration, at which time there is no further relationship with cyanobacterial response. As copper dissipation rates range widely (i.e. minutes to days) in aquatic systems and can influence cyanobacterial responses, the influence of exposure duration is anticipated to be an important independent variable for the *L. wollei* CET model.

For *L. wollei*, a CET model needs to be sufficiently robust to be predictive of cyanobacterial responses with wide ranging cyanobacterial biomasses. *L. wollei* biomasses can differ as a function of location and season (i.e. winter to summer) (Beer et al. 1986, Bridgeman et al. 2011). Based on peer reviewed literature, the average *L. wollei* biomass observed is 307 g dry weight (DW)/m^2 (n=61) with minimum and maximum biomasses ranging from 0.25 g-DW/m^2 to 1, 508 g-DW/m^2 in aquatic systems (Beer et al.

1986, Speziale et al. 1991, Cowell and Botts 1994, Macbeth 2004, Vis et al. 2008, Bridgeman et al. 2011, Panek 2012, Lévesque et al. 2015). To evaluate a *L. wollei* CET model, a range of cyanobacterial biomasses were exposed to copper concentrations and exposure durations anticipated to result in ≥ 90% cyanobacterial response. The biomass of *L. wollei* at initiation of copper exposures is termed "initial cyanobacterial biomass" throughout this manuscript. If cyanobacterial responses deviate from those predicted by the CET model, initial cyanobacterial biomass will be added to the model and the accuracy of subsequent predictions will be evaluated.

To incorporate initial cyanobacterial biomass in the *L. wollei* CET model, a model with two independent variables (i.e. exposure duration and concentration) and one dependent variable (i.e. cyanobacterial response) would be expanded to include three independent variables (i.e. exposure duration, concentration and initial cyanobacterial biomass as well as the dependent variable, cyanobacterial response). As a means to simplify this model, the copper concentration that results in the intended cyanobacterial response (≥ 90%) following an algaecide application is the focus of the new model. Site characteristics, specifically exposure duration and initial cyanobacterial biomass are then independent variables while the copper concentration resulting in ≥ 90% cyanobacterial response is the dependent variable. Using this approach, a new model can be developed that includes initial cyanobacterial biomass as a variable to predict performance of a copper algaecide for *L. wollei*.

The overall objective of this experiment was to discern the influence of a series of initial cyanobacterial (*L. wollei*) biomasses on responses of *L. wollei* predicted from a

CET model. Specific objectives were to 1) measure and compare responses of *L. wollei* from a 44,500 m^2 pond in Spartanburg County, South Carolina to a series of copper concentrations (0.1-1.0 mg Cu/L) in separate laboratory experiments using copper as Clearigate®, Cutrine® Ultra, Cutrine-Plus® and Algimycin® PWF to determine a copper-based algaecide that has sufficient potency (i.e. relationship between copper concentration and response to $\geq$ 90 % cyanobacterial response) to measure a change in *L. wollei* responses within legal label concentrations, 2) measure and compare responses of *L. wollei* from the same pond to a series of exposure durations and concentrations of copper as a copper-based algaecide (with sufficient potency based on the previous experiment) to develop the *L. wollei* CET model, 3) measure and compare responses of *L. wollei* (from the pond) for a series of initial biomasses (0.25 g/m^2 – 1,508 g/m2) to an exposure duration and concentration of the copper-based algaecide that result in $\geq$ 90 % cyanobacterial response in the CET model to discern the influence of initial cyanobacterial biomasses on responses of *L. wollei*, and 4) measure and compare copper concentrations (within legal label concentrations, 0.1-1.0 mg Cu/L) that result in $\geq$ 90 % cyanobacterial response for a series of exposure durations and initial cyanobacterial biomasses to develop a new model for *L. wollei*.

Materials and Methods

Responses of L. wollei to a Series of Concentrations of Copper Algaecides

L. wollei and water samples were collected during the summer of 2017 from a 44,500 m^2 pond in Spartanburg County, South Carolina, USA. This pond was originally used for recreation including fishing, swimming and boating, until recent growths of *L. wollei* impeded these uses. Once transported to the laboratory, cyanobacterial and water samples (average [n=6] $\pm$ standard deviation; pH = 6.9 $\pm$ 0.4 S.U., conductivity = 76.2 $\pm$ 4.8 μS/cm, alkalinity = 35 $\pm$ 7 mg/L as $CaCO_3$, hardness = 29 $\pm$ 5 mg/L as $CaCO_3$, dissolved oxygen = 9 $\pm$ 2 mg O_2/L) were maintained at 22$\pm$1˚C with an 18:6 h light: dark cycle provided by cool-white fluorescent bulbs (Residential Ecolux 40 W, GE) at 2,660 lx prior to and during experiments.

To determine a copper-based algaecide to use to develop a *L. wollei* CET model, the cyanobacterium was exposed to a series of copper concentrations from different copper-based algaecides (Table 3.1). *L. wollei* was exposed in 250 mL borosilicate glass beakers by adding appropriate volumes of 1,000 mg Cu/L stock solutions of each algaecide separately to 200 mL site water containing the cyanobacterium. A series of copper concentrations (0.1, 0.4, 0.7 and 1.0 mg Cu/L) for each algaecide were arrayed with three replicates per concentration. Copper concentrations were confirmed immediately following addition of copper algaecide measuring soluble (US EPA 1992) and acid soluble (US EPA 1991) copper concentrations with a Perkins Elmer (Waltham, MA) Optima 3100RL inductively coupled plasma-optical emission spectrometer (ICP-OES, method detection limit = 0.050 mg Cu/L) and graphite furnace atomic absorption

spectrometry (GFAAS, Varian Inc. AA 280FS Fast Sequential Atomic Absorption Spectrometer, method detection limit = 0.005 mg Cu/L) when applicable (APHA 2012). Quality assurance and control included replicate samples, standards, and blank matrix spike recovery.

Cyanobacterial responses (i.e. percent damaged trichomes and percent difference in *L. wollei* mass [wet weight]) to these copper concentrations were measured 7 days post-initiation. Based on preliminary experiments, this was sufficient time for responses of *L. wollei* to be manifested. Percent damaged trichomes indicates the viability of cells following exposure based on microscopic observations of cellular structure and pigment although, for differences to be discerned in terms of percent difference in mass, cells have to be non-viable and also decompose. Multiple response measures were used to discern responses of *L. wollei* to copper concentrations because cyanobacterial responses are characteristically complex (Calomeni and Rodgers 2015).

To discern percent damaged trichomes, cells within trichomes were evaluated at 400x magnification with a Leica ATC2000 Binocular Compound Microscope. Because a fraction of cells within a trichome (even in untreated controls) can be damaged and the designation of damaged and not-damaged is a binary parameter, a criterion was established for identifying a damaged trichome. The criterion was, if more than 50% of cells within the trichome segment for a field of view (400x magnification) were damaged, the trichome was recorded as damaged. Cells were damaged if they were absent, brown, or chlorotic (Figure 3.1). Ten trichome segments were enumerated per replicate for a total of approximately 530 cells (1,590 cells/exposure). To measure the difference in *L.*

wollei mass (wet weight), at the completion of the 7-day experiment, the remaining mass of cyanobacterium was removed from beakers and blotted dry with Kimteck wipes (KimWipes™) prior to weighing (Wong et al. 2009, Saunders et al. 2012, Burns et al. 2015). Responses of *L. wollei* were expressed as percentages using the following equations (Eqs. 1 and 2). The copper-based algaecide that resulted in increasing responses of *L. wollei* to $\geq$ 90 % within legal label concentrations (Table 3.1) was used in subsequent experiments. Linear regression and correlation coefficients (R^2) were used to determine the strength of the relationship between copper concentration and response.

$$P_{trichome} = \frac{T_{exposure}}{10} \times 100\% \quad (Eq. 1)$$

Where $P_{trichome}$ = percent damaged trichomes
$T_{exposure}$ = number of damaged trichome segments out of ten trichome segments discerned 7 days after experiment initiation

$$P_{mass} = \frac{\left(M_{control} - M_{exposure}\right)}{M_{coontrol}} \times 100\% \quad (Eq. 2)$$

Where P_{mass} = percent difference in *L. wollei* mass
$M_{control}$ = weight (g) of *L. wollei* "mat" in untreated control measured 7 days after experiment initiation
$M_{exposure}$ = weight (g) of *L. wollei* "mat" measured 7 days after experiment initiation (algaecide treatment)

L. wollei CET Model

To develop the *L. wollei* CET model, the cyanobacterium was exposed to a series of copper concentrations and durations of exposure. Exposure durations were arrayed to capture durations likely to result from copper algaecide applications *in situ* (i.e. minutes to days) but also producing a range of cyanobacterial responses based on preliminary experiments. The copper-based algaecide was applied at 0.1, 0.4, 0.7 and 1.0 mg Cu/L

for 0.25 hour, 1 hour, 8 hours and 24 hours in separate experimental chambers with three replicates per exposure (i.e. concentration and exposure duration). Beakers containing algae and site-water without copper treatment were included as untreated controls. At the end of each exposure duration, all beakers including the untreated controls were drained, site water (untreated) was used to rinse the beaker and the *L. wollei* mass three times and the beakers were refilled with untreated site water. Copper concentrations were measured immediately following addition of algaecide to site water containing algae and at the end of the exposure duration using methods detailed previously. Cyanobacterial responses were measured 7 days following exposure initiation to allow sufficient time for responses to manifest. Cyanobacterial responses were compared using analysis of variance (ANOVA) and linear contrasts (JMP Pro V.12).

Influence of Initial Biomass on the L. wollei CET Model

The initial cyanobacterial biomass in the *L. wollei* CET model was 52 g wet weight (WW)/m^2. To discern the influence of initial cyanobacterial biomass on measured responses of *L. wollei*, different initial cyanobacterial biomasses were exposed to the copper concentration and exposure duration resulting in $\geq$ 90% response from the *L. wollei* CET model. Nine initial *L. wollei* biomasses (three replicates per biomass) were arrayed (0.25 g WW/m^2, 13 g WW/m^2, 26 g WW/m^2, 52 g WW/m^2, 130 g WW/m2, 182 g WW/m^2, 260 g WW/m^2, 519 g WW/m^2 and 1,558 g WW/m^2) capturing the four orders of magnitude difference in *L. wollei* biomasses observed *in situ*. Copper concentrations were confirmed and responses of *L. wollei* were measured as previously described. Responses of *L. wollei* with different initial biomasses were compared to responses

measured using comparable exposures to the *L. wollei* CET model (initial cyanobacterial biomass = 52 g WW/m^2, copper concentration = 1.0 mg Cu/L as Clearigate® and 24 hours exposure duration) using ANOVA and Student's *t*-test.

Development of a New Model for Responses of L. wollei to a Copper Algaecide

If responses of *L. wollei* among a range of initial biomasses to the same copper exposures differed relative to those predicted from the *L. wollei* CET model, initial cyanobacterial biomass would be a necessary parameter to add to a new model that predicts responses of *L. wollei* to copper exposures. This new model incorporated initial cyanobacterial biomass and exposure duration as independent variables and the copper concentration resulting in $\geq$ 90% response as the dependent variable. To develop the new model, termed biomass, duration and concentration model (BDC), results from the previous objectives were used to bound exposures. Specifically, a series of exposure durations (objective 2) and initial cyanobacterial biomasses (objective 3) were arrayed that resulted in $\geq$ 90% response for legal label copper concentrations. *L. wollei* was exposed to a series of copper concentrations (i.e. bounded by the maximum legal label concentration of copper) for each combination of exposure duration and biomass. Responses of *L. wollei* for each copper concentration, exposure duration and biomass were compared statistically (ANOVA and linear contrasts) to maximum responses of *L. wollei* measured (initial cyanobacterial biomass = 52 g WW/m^2, copper concentration = 1.0 mg Cu/L as Clearigate® and 24 hours exposure duration). The lowest copper concentration for each combination of biomass and exposure duration that resulted in a

comparable response relative to this maximum response of *L. wollei* was used for the BDC model.

Results and Discussion

Confirmation of Copper Exposure Concentrations

Copper concentrations (acid soluble and soluble) measured in untreated site water (pretreatment copper concentrations) ranged from non-detect (< 0.005 mg Cu/L) to 0.020 mg Cu/L (Table 3.2). Following addition of copper-based algaecide, average percent differences for acid soluble copper concentrations were 3$\pm$17% and for soluble copper concentrations were 31$\pm$15% (Table 3.2). Because measured acid soluble copper concentrations were comparable to targeted copper concentrations, the targeted copper concentration was used for comparisons throughout this experiment. For those experiments in which copper exposures were replaced with untreated site water following the completion of specific exposure durations, soluble copper concentrations decreased to non-detect to 0.061 mg Cu/L (initial algal biomass = 1,558 g wet weight/m^2).

Responses of L. wollei to a Series of Concentrations of Copper Algaecides

For subsequent experiments, a copper algaecide was needed with the following characteristics, 1) sufficiently potent to result in $\geq$ 90% cyanobacterial response and 2) a positive linear relationship between copper concentration and responses of *L. wollei* within legal label concentrations. All algaecides evaluated resulted in $\geq$ 90% cyanobacterial response in terms of percent damaged trichomes 7 days post-addition of algaecide (Figure 3.2A). Exposures of 0.7 mg Cu/L for Clearigate®, 1.0 mg Cu/L for Cutrine® Ultra, 0.7 mg Cu/L for Cutrine-Plus® and 0.4 mg Cu/L for Algimycin® PWF resulted in the greatest response achieved in terms of percent damaged trichomes for each

algaecide. The *L. wollei* within untreated controls had 0 ± 0% damaged trichomes at completion of the 7-day experiment.

In terms of percent difference in dry mass from untreated controls, the maximum measured response for *L. wollei* was 63 ± 4% following an exposure of Clearigate® at 0.7 mg Cu/L (Figure 3.2B). Percent damaged trichomes was a more sensitive measurement of responses of *L. wollei* in this experiment (Figures 3.2A and 3.2B), likely because individual cells within trichomes respond to exposures rapidly allowing for responses to be readily apparent. This is relative to percent difference in mass that requires sufficient time for enough cells to become non-viable and decompose for responses to be discerned.

Correlation coefficients were measured and used to quantify the strength of the linear relationship between copper concentration for each formulation and response within legal label concentrations. Correlation coefficients in terms of percent damaged trichomes were 0.99 (Clearigate®, 0.1 to 0.7 mg Cu/L), 0.19 (Cutrine® Ultra, 0.1 to 1.0 mg Cu/L), 0.57 (Cutrine-Plus®, 0.1 to 1.0 mg Cu/L) and 0.15 (Algimycin® PWF, 0.1 to 1.0 mg Cu/L). For percent difference in mass, correlation coefficients were 0.99 (Clearigate®, 0.1 to 0.7 mg Cu/L), 0.27 (Cutrine® Ultra, 0.1 to 1.0 mg Cu/L), 0.43 (Cutrine-Plus®, 0.1 to 1.0 mg Cu/L) and 0.19 (Algimycin® PWF, 0.1 to 1.0 mg Cu/L). Based on the magnitude of cyanobacterial response and correlation coefficient, the ethanolamine chelated algaecide formulated with D-limonene, Clearigate®, was used for subsequent experiments. Since exposures of Clearigate® resulted in a series of increasing responses of the cyanobacterium within legal label concentrations, this algaecide could be used to develop the *L. wollei* CET model.

***L. wollei* CET Model**

In terms of percent damaged trichomes, exposures of 0.4 and 0.7 mg Cu/L for a 24-hour duration, exposures of 0.4, 0.7 and 1.0 mg Cu/L for 8 hours, and exposures of 0.7 and 1.0 mg Cu/L for 1 hour resulted in statistically similar algal responses (87 – 100 % response) as the maximum exposure evaluated (1.0 mg Cu/L for 24 hours = 94 % response) (Figure 3.3A, $p \geq 0.05$). Responses of *L. wollei* in untreated controls had 13 $\pm$ 14% damaged trichomes at completion of the 7-day experiment. For percent differences in wet weight, the exposures of 0.1 mg Cu/L for 24 hours and exposures of 0.4 mg Cu/L and 1.0 mg Cu/L for 0.25 hours were also similar (13 – 43% response) to responses to the maximum exposure concentration and duration evaluated (39% response; Figure 3.3B). To decrease variance associated with responses of *L. wollei* in terms of percent difference in wet weight, responses throughout the remainder of this experiment were also measured in terms of dry weight (dried for 24 h at 100˚C). Responses of *L. wollei* in terms of dry weight corroborated the results in terms of percent damaged trichomes for exposures that resulted in responses not significantly different than responses to the maximum exposure (Figures 3.3A and 3.3C). Exposures consisting of concentrations and exposure durations less than those reported above would result in significantly less responses than can be achieved using the maximum exposure evaluated.

Influence of Biomass on the L. wollei CET Model

To discern the influence of initial cyanobacterial biomass on measured responses of *L. wollei* to copper algaecide exposures, different initial cyanobacterial biomasses were exposed to the greatest copper concentration and exposure duration from the *L.*

wollei CET model. Percent damaged trichomes ranged from 62 – 97% 7 days post-exposure initiation for initial *L. wollei* biomasses from 13 g WW/m^2 to 519 g WW/m^2, (Figure 3.4A) and were similar to responses measured using the maximum exposure from the *L. wollei* CET model (initial biomass = 52 g WW/m^2, copper concentration = 1 mg Cu/L and exposure duration = 24 hours, response = 81 %). For the initial cyanobacterial biomass of 1,558 g WW/m^2, damaged trichomes 7 days post-exposure initiation decreased to 23 $\pm$ 6%. Responses of *L. wollei* at 1,558 g WW/m^2 were significantly less than the response of *L. wollei* (81 % response, $p < 0.0016$, α=0.05) measured with an initial biomass of 52 g WW/m^2 used in the CET model exposed to the same copper concentration (1 mg Cu/L) for the same duration (24 hours). Percent damaged trichomes 7 days post-exposure initiation for 1,558 g WW/m^2 were not significantly different than the response for untreated controls (12 $\pm$ 11%, $p = 0.2161$, $\alpha = 0.05$).

In terms of percent difference in mass, significantly less cyanobacterial response (i.e. percent difference in wet and dry weight) resulted from an exposure of 1 mg Cu/L for 24 hours for the initial cyanobacterial biomass of 130 g WW/m^2 ($p < 0.0023$ wet weight and $p < 0.001$ dry weight, α=0.05) relative to the *L. wollei* biomass used in the CET model (Figure 3.4B). At the initial biomass of 130 g WW/m^2, responses of *L. wollei* decreased from 52$\pm$6% (CET model exposure) to 15$\pm$8% as wet weight and 58$\pm$1% (CET model exposure) to 39$\pm$2% as dry weight. Responses of *L. wollei* continued to decrease in terms of percent difference in mass with an initial biomass of 1,558 g WW/m^2 resulting in no measureable difference relative to the untreated control in terms of wet weight (0%) and 7% for dry weight.

For the experimental conditions of this study, initial cyanobacterial biomasses less than 52 g WW/m^2 were overexposed meaning that less copper exposure (i.e. duration, concentration or both) would result in the same response. Alternatively, for this accession of *L. wollei*, initial biomasses of 130 g WW/m^2 and larger would require a greater copper exposure to result in the same response as that predicted from the *L. wollei* CET model. Since the concentration applied is the maximum legal label concentration (1.0 mg Cu/L) and an initial biomass greater than and equal to 130 g WW/m^2 would require a copper exposure in exceedance of the maximum label concentration, responses of *L. wollei* comparable to those predicted from the CET model cannot be achieved with one algaecide application. This emphasizes the importance of treating prior to peak cyanobacterial biomass or early in a *L. wollei* infestation so that the greatest response can be achieved with one application. If the *L. wollei* biomass is equivalent to or greater than 130 g WW/m^2 for this site, several algaecide treatments may be required to incrementally decreased the biomass.

For this *L. wollei* from a 44,500 m^2 pond in Spartanburg County, SC, cyanobacterial responses can range from the predicted response (CET model) to no measurable response (relative to the untreated control) as a function of initial cyanobacterial biomass. Because of the range of cyanobacterial responses elicited using the same exposure parameters (i.e. copper concentration and exposure duration), the results from this experiment demonstrate that initial *L. wollei* biomass is an important exposure parameter driving responses of *L. wollei*. For this accession of *L. wollei*, the initial cyanobacterial biomasses that this CET model is applicable for is 52 g WW/m^2. To

expand predictions beyond a single initial biomass, a new model is necessary where initial cyanobacterial biomass is a significant contributor to the performance of Clearigate® for *L. wollei*.

Development of a New Model for Responses of L. wollei to a Copper Algaecide

The new model (BDC) for responses of *L. wollei* to Clearigate® has exposures bounded by previous results in this experiment. The exposure duration (from objective 2) of 0.25 hour did not result in $\geq$ 90% responses of *L. wollei* under the conditions of this experiment. Since for the BDC model exposures are arrayed to capture $\geq$ 90% responses, the exposure duration of 0.25 hour was excluded from the model. In terms of initial cyanobacterial biomass, responses of *L. wollei* with an initial biomass of 0.25 g WW/m^2 could not be detected because the mass at completion of the experiment was below the method detection limit (< 0.15 g WW/m^2). This initial biomass (0.25 g WW/m^2) was therefore not included in the BDC model and the lowest biomass included was 13 g WW/m^2, the second lowest biomass evaluated in this experiment. Based on the results of the third objective, the initial cyanobacterial biomass of 130 g WW/m^2 did not result in $\geq$ 90% responses of *L. wollei* to an exposure of 1.0 mg Cu/L for 24 hours. Therefore, there may be an initial cyanobacterial biomass between 52 g WW/m^2 and 130 g WW/m^2 that can be treated with 1.0 mg Cu/L for 24 hours and result in $\geq$ 90% responses of *L. wollei*. An additional biomass of 104 g WW/m^2, was therefore evaluated for the BDC model.

As expected from the results of the objective evaluating the influence of initial cyanobacterial biomasses on responses, the initial cyanobacterial biomasses of 13 g WW/m^2 and 26 g WW/m^2 were overexposed and required a lower copper concentration

than 1 mg Cu/L to achieve the maximum response. For 13 g WW/m^2 these exposures were 0.3 mg Cu/L for a 1 hour exposure duration and 0.07 mg Cu/L for 8 and 24 hours (Fig. 5). A concentration of 0.4 mg Cu/L for a 1 hour exposure duration and 0.2 mg Cu/L for 8 and 24 hours resulted in the maximum response for the initial cyanobacterial biomass of 26 g WW/m^2.

Results from the BDC model demonstrate that as initial cyanobacterial biomass increases the copper concentration necessary to result in $\geq$ 90% response increases (Fig. 5). On a theoretical basis, this result is anticipated. In theory, there is a mass of copper per exposed algal population required to result in a specific response (De Schamphelaere et al. 2005). An exaggerated effect for benthic algae relative to planktonic algae is that as the mass of the cyanobacterial population increases, a greater copper concentration would then be necessary to result in a comparable response (Geer 2016, Kinley et al. 2017). The BDC model also indicated that as exposure duration increases a lower copper concentration is needed to result in $\geq$ 90% response of *L. wollei*. This has also been demonstrated in CET models for exposed vascular plants (Netherland 1991) and for the CET model in the current experiment.

Similar to CET models, the BDC model is specific to couplets of the exposed organism and algaecide. Conditions that may require careful consideration prior to use of the specific BDC model include different water characteristics (e.g. pH, hardness, alkalinity, conductivity, particulate and dissolved organic matter) and different algae and cyanobacteria (e.g. genera, species, accessions). This experiment demonstrated an

approach for development of BDC models that can be used for additional couplets of algaecides to exposed algae and cyanobacteria.

Conclusions

The purpose of this experiment was to determine the influence of initial cyanobacterial biomass on responses of the commonly problematic cyanobacterium, *L. wollei* predicted from a specific CET model developed for a copper-based algaecide. Of the copper based algaecides evaluated, Clearigate®, a formulation with D-limonene and chelated with ethanolamine had sufficient potency and captured a range of cyanobacterial responses within legal label concentrations to be used to develop the CET model. Using the *L. wollei* CET model, exposures of 0.4, 0.7 and 1.0 mg Cu/L for 24-hours, exposures of 0.4, 0.7 and 1.0 mg Cu/L for 8 hours, and exposures of 0.7 and 1.0 mg Cu/L for 1 hour resulted in maximum cyanobacterial responses measured at completion of the 7-day experiment (87-100% percent damaged trichomes, 13 – 43% wet weight and 47 – 63% dry weight). The initial cyanobacterial biomass used in the CET model was 52 g WW/m^2 and initial biomasses exceeding this value resulted in responses less than those predicted from the CET model. At the initial cyanobacterial biomass of 1,558 g WW/m^2, cyanobacterial responses to the same copper concentration and duration resulting in maximum cyanobacterial response based on the CET model were non-detect (percent damaged trichomes and wet weight) to 7% (dry weight) relative to untreated controls. Initial cyanobacterial biomass was subsequently captured in a new model for *L. wollei* exposed to Clearigate®, the BDC model. A useful feature of the BDC model relative to the CET model for Clearigate® exposed to *L. wollei* is that it expands the range of initial cyanobacterial biomasses (13 g WW/m^2 to 104 g WW/m^2) for which the model is predictive of performance.

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Table 3.1 Physical and chemical properties of copper-based algaecides.

Product	Clearigate®	Cutrine® Ultra	Cutrine-Plus®	Algimycin® PWF
Composition	D-limonene Triethanolamine Ethanolamine Basic copper carbonate	D-limonene Triethanolamine Ethanolamine Basic copper carbonate	Triethanolamine Ethanolamine Basic copper carbonate	Copper gluconate Copper citrate
Active ingredient	3.8 % Cu	9 % Cu	9 % Cu	9 % Cu
Minimum application concentration	0.1 mg Cu/L	0.1 mg Cu/L	0.1 mg Cu/L	0.1 mg Cu/L
Maximum application concentration	1.0 mg Cu/L	1.0 mg Cu/L	1.0 mg Cu/L	1.0 mg Cu/L
Physical description	Viscous blue liquid	Viscous blue liquid	Blue liquid	Blue liquid
pH	9.7-10.0	10.2-10.3	10.3-10.5	1.5-2.5

Table 3.2 Targeted and measured acid soluble and soluble copper concentrations (n=1) for experiments.

Objective	Targeted Copper Concentration (mg Cu/L)		Acid Soluble Copper Concentration (mg Cu/L)	Soluble Copper Concentration (mg Cu/L)
1. Responses of *L. wollei* to different copper algaecides	**Algaecide**	**Concertation**		
	Clearigate®	Untreated control	0.010	0.007
		0.1	0.117	0.089
		0.4	0.488	0.267
		0.7	0.793	0.555
		1.0	1.094	0.769
	Cutrine® Ultra	Untreated control	0.010	0.007
		0.1	0.118	0.083
		0.4	0.445	0.277
		0.7	0.703	0.567
		1.0	1.047	0.716
	Cutrine-Plus®	Untreated control	0.010	0.007
		0.1	0.090	0.073
		0.4	0.406	0.259
		0.7	0.763	0.532
		1.0	1.114	0.827
	Algimycin® PWF	Untreated control	0.010	0.007
		0.1	0.102	0.084
		0.4	0.322	0.260
		0.7	0.636	0.559
		1.0	0.919	0.829
2. CET model [a]		Untreated control	ND	ND
		0.1	0.092	0.075
		0.4	0.323	0.246
		0.7	0.604	0.436
		1.0	0.798	0.666

3. Responses of *L. wollei* to copper exposures with different initial biomasses [b]		Untreated control 1.0		0.018 0.980	0.020 0.857
4. BDC model[c]	Initial algal Biomass (g wet weight/m^2)	Exposure Duration (hour)	Targeted Copper Concentration (mg Cu/L)		
	Untreated control	-	-	ND	ND
	13	1	0.3	0.303	0.204
		8	0.07	0.079	0.058
		24	0.07	0.079	0.058
	26	1	0.4	0.517	0.195
		8	0.2	0.243	0.088
		24	0.2	0.243	0.088
	52	1	1.0	1.330	0.505
		8	0.7	0.916	0.390
		24	0.4	0.517	0.195
	104	1	1.0	0.711	0.370
		8	0.7	0.503	0.284
		24	0.7	0.503	0.284

[a] Following completion of the exposure duration and replacement of the exposure with unamended site water, soluble copper concentrations ranged from ND (<0.005 mg Cu/L) to 0.020 mg Cu/L.

[b] Following completion of the exposure duration and replacement of the exposure with unamended site water, soluble copper concentrations ranged from 0.011 mg Cu/L (initial algal biomass = 26 g wet weight/m^2) to 0.061 mg Cu/L (initial algal biomass = 1,558 g wet weight/m^2).

[c] Following completion of the exposure duration and replacement of the exposure with unamended site water, soluble copper concentrations were non-detect (<0.005 mg Cu/L).

Table 2.1 Analytical methods, citations and method detection limits for sediment and water characteristics.

Parameter	Methods	Method Detection Limit/ Variance
Sediment Characteristics		
Percent Organic Matter	Loss-on-ignition at 360°C (Schulte 1995, Salehi et al. 2011)	0.02%
pH	Electrometric method 4500-H^+ B: Orion model 420A (APHA 2012, McLean 1982)	0.01 S.U.
Acid Volatile Sulfides (AVS)	Modified diffusion method (Leonard et al. 1996)	0.004 μmol sulfide/ g
Oxidation-reduction (Eh)	Modified standard method 2580B: Accumet® calomel reference electrode, Fluke® 77 III voltage meter (Faulkner et al. 1989)	10 mV
Water Characteristics		
pH	Electrometric method 4500-H^+ B: Orion model 420A (APHA 2012)	0.01 S.U.
Temperature	Direct measurement HOBO pendant® Data Logger	$\pm$ 0.53°C
Dissolved Oxygen	Direct measurement Hach® HQ30d Portable Meter with IntelliCAL™ LDO101 Standard Luminescent/Optical Dissolved Oxygen (LDO) Probe	$\pm$ 0.1 mg/L
Conductivity	Direct measurement 2510 B:YSI 30 (APHA 2012)	0.1 µS/cm
Alkalinity	Titration method 2320 B (APHA 2012)	2 mg/L as $CaCO_3$
Hardness	EDTA Titrimetric Method 2340 C (APHA 2012)	2 mg/L as $CaCO_3$

Table 2.2 Physical and chemical characteristics of Cutrine-Plus®.

Characteristics	
Active ingredient	9 % Cu
Maximum label concentration (mg Cu/L)	1.0 [a]
Composition of ingredients	Triethanolamine (19-29%) [b] Ethanolamine (15-25%) [b] Basic Copper Carbonate (11-21%) [b]
Water solubility	Miscible [b]
Specific gravity (g/cm^3)	1.1 (gravimetric measurement)
pH (S.U.)	10.3-10.5 [b]

[a]Applied Biochemists (Arch Chemicals a Lonza Business, Alpharetta, GA). Specimen label. Cutrine-Plus®

[b]Applied Biochemists (Arch Chemicals a Lonza Business, Alpharetta, GA). Safety data sheet. Cutrine-Plus®

Table 2.3 Measured physical and chemical characteristics of sediment in situ, laboratory, and mesocosm experiments.

Parameter	In Situ	Laboratory	Mesocosm
Percent Organic Matter (%) (n=1)	4.1	2.5	10.6
pH S.U. (n=1)	6.7	6.7	6.4
Acid Volatile Sulfides (AVS) μmol sulfide/ g (n=1)	0.024	ND	ND
Oxidation-reduction (Eh; mV) (minimum and maximum, n=3)	16-154.2	-69.8-68.4	26-155.4

Table 2.4 Measured physical and chemical characteristics of water in situ, laboratory and mesocosm experiments. Parameters measured at experiment completion (n=1) unless otherwise noted.

Parameter	In Situ	Laboratory	Mesocosm
pH S.U.	7.27	7.77 [a] - 7.75 [b]	7.06
Temperature °C	19	25	27 - 35 [c]
Dissolved Oxygen mg/L as O_2	12.30	9.56 [a]	5.48 - 10.00 [c]
Conductivity µS/cm	301	314[a] - 302 [b]	291
Alkalinity mg/L as $CaCO_3$	76	76 [a] - 84 [b]	92
Hardness mg/L as $CaCO_3$	72	72 [a] - 80 [b]	64

[a] Measured in filtered water samples (e.g. precipitation and sediment sorption experiments)
[b] Measured for algal sorption experiment
[c] Minimum and maximum over four hours

Table 2.5 Aqueous copper rate coefficients, half-lives and correlation coefficients calculated using average copper concentrations (n = 3). [Cu] denotes copper concentration in mg Cu/L.

		Rate Coefficient (ln [Cu]/day)	Half-life (day)	R^2
In Situ Dissipation Rate				
	Acid Soluble	24.63	0.03	0.98
	Soluble	23.79	0.03	0.99
Individual Dissipation Rates (Laboratory Experiments)				
Algal Sorption	Acid Soluble	-	-	-
	Soluble	-	-	-
Precipitation	Acid Soluble	-	-	-
	Soluble	-	-	-
Sediment Sorption	Acid Soluble	0.26	2.67	0.98
	Soluble	0.24	2.89	0.93
Dilution (Rhodamine Dye)	NA	27.64[a]	0.03	1.00
Mesocosm Dissipation Rate				
	Acid Soluble	33.57	0.02	0.97
	Soluble	28.64	0.02	0.93

[a] Estimated using rhodamine dye concentrations units are as ln [dye]/day.

Table 2.6 Half-lives for dilution estimated using Rhodamine dye and copper dissipation from sediment sorption from peer-reviewed literature.

Dilution			
Location	**Characteristics of site**	**Half-life (day)**	**Citation**
Sacramento-San Joaquin delta, CA	7 sites – sloughs, harbors and marinas (surface area not specified)	0.08 - 1	Anderson 1999
Lake Seminole, GA	2 sites – 40,000 m^2 plots in Lake Seminole	<0.06 – 0.18	Fox et al. 1992
Lake Minnetonka, MN	2 sites – 65,000 m^2 plots in separate bays	3.9 – 7.8	Fox et al. 2002
Crystal River, FL	4 sites – 10,000 m^2 canals	0.5 – 8.3	Fox et al. 1991[a]
Sediment sorption			
		Half-life (day)	**Citation**
Laboratory		7.8 – 11.8	Jones et al. 2008
Mesocosm		2.6 – 5.7	Murray-Gulde et al. 2002

[a] Rhodamine dye mixed 1:1 with methanol prior to application

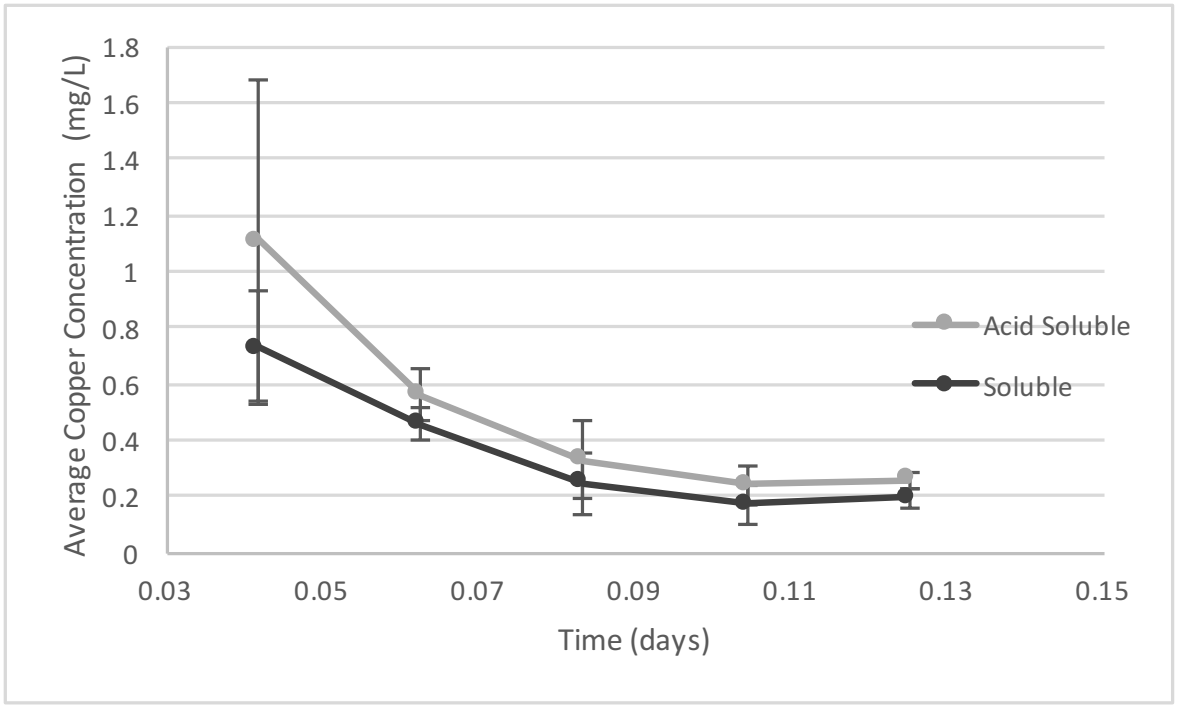

Figure 2.1 Average aqueous concentrations as soluble and acid soluble copper over time in situ (34º34'48.75" N, 82º43'40.28" W); n=3; error bars indicate $\pm$1 standard deviation.

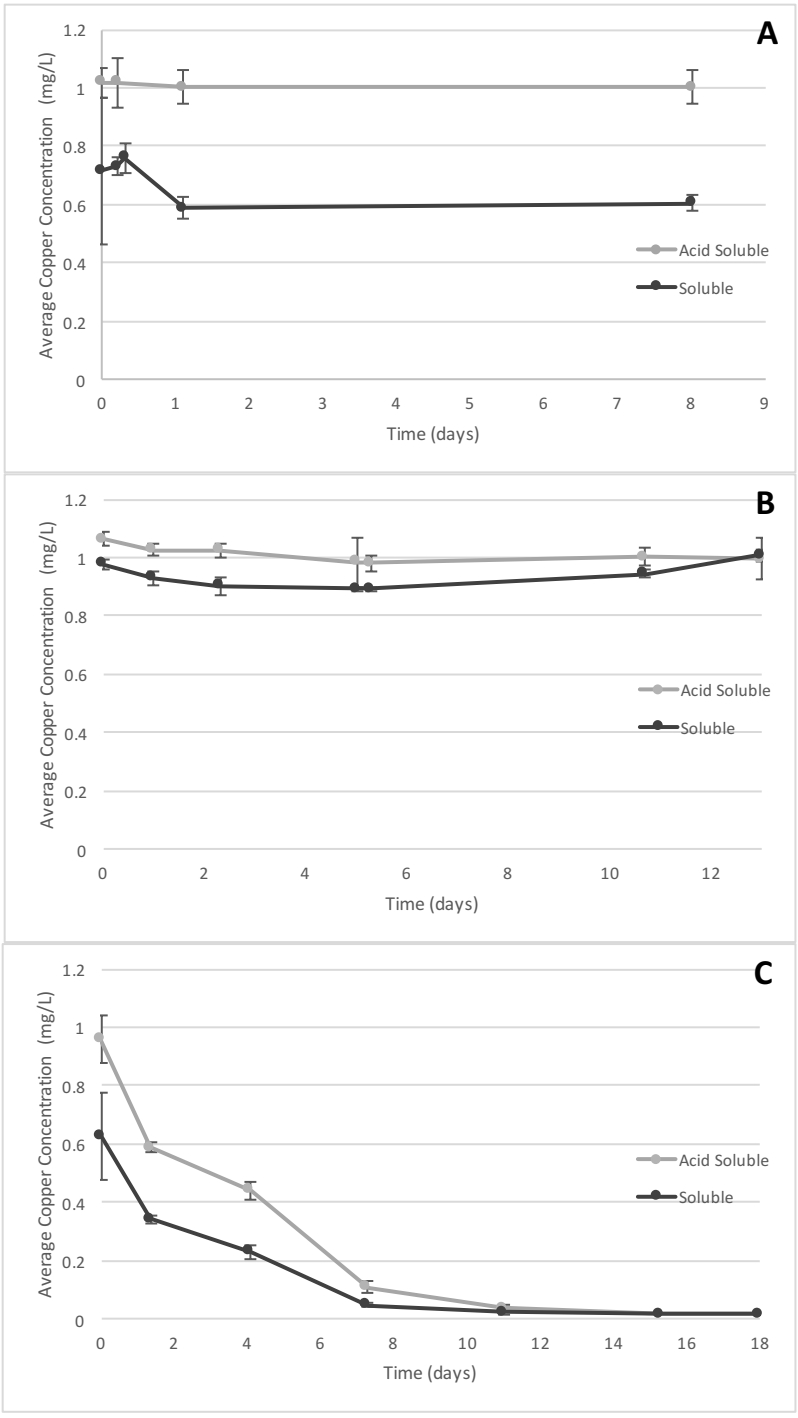

Figure 2.2 Average aqueous concentrations as soluble and acid soluble copper over time for algal sorption (A), precipitation (B) and sediment sorption (C); n=3; error bars indicate $\pm$1 standard deviation.

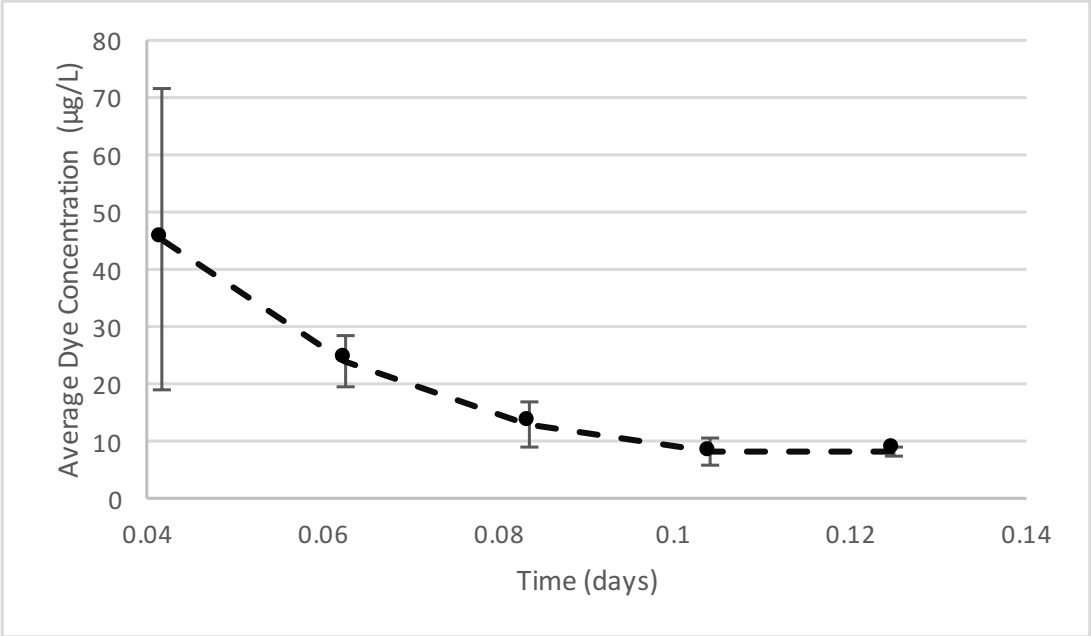

Figure 2.3 Average rhodamine WT dye concentrations over time; n=3; error bars indicate ±1 standard deviation.

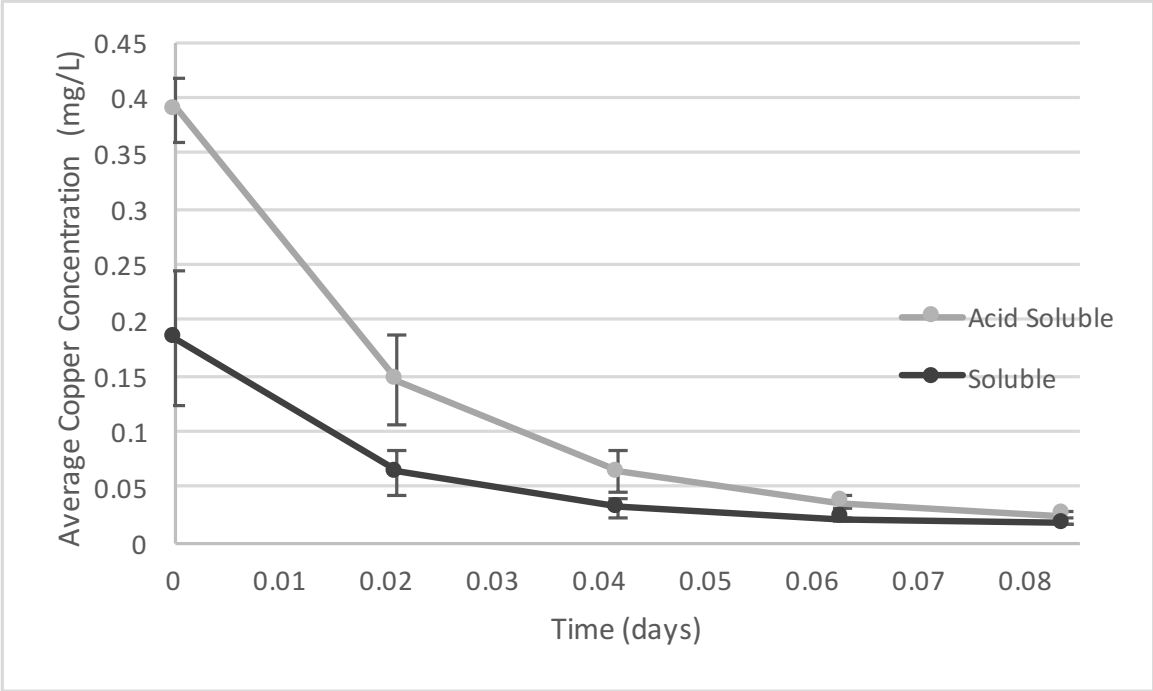

Figure 2.4 Average copper concentration over time in mesocosm; n=3; error bars indicate $\pm$1 standard deviation.

CHAPTER THREE

LYNGBYA WOLLEI RESPONSES TO COPPER ALGAECIDE EXPOSURES PREDICTED USING A CONCENTRATION-EXPOSURE TIME (CET) MODEL: INFLUENCE OF INITIAL BIOMASS

Abstract

Concentration-exposure time models (CET) are used to predict responses of aquatic vascular plants to herbicides and could be applicable for cyanobacterial responses to algaecides if appropriate variables are captured by the model. For the cyanobacterium, *Lyngbya wollei*, initial biomass upon application may be an important parameter driving responses to copper algaecides. Specific objectives of this study were to 1) discern an algaecide formulation with sufficient potency and relationship between copper concentration and response for a CET model for *L. wollei*, 2) develop a CET model for the algaecide and *L. wollei*, 3) determine the influence of initial biomass on measured responses vs. effects predicted using the CET model, and 4) develop a new model with *L wollei* biomass as a variable. The algaecide, Clearigate®, had sufficient potency (≥ 90% response) and strength of relationship between copper concentration and response (R^2 = 0.99 from 0.1-0.7 mg Cu/L) to develop a CET model. Exposures of 0.4, 0.7 and 1.0 mg Cu/L for 24 hours, exposures of 0.4, 0.7 and 1.0 mg Cu/L for 8 hours, and exposures of 0.7 and 1.0 mg Cu/L for 1 hour resulted in 87-100 % response of *L. wollei* (percent damaged trichomes). Initial biomasses greater than those used for the CET model (52 g WW/m^2) decreased responses to non-detect (1,558 g WW/m^2). Because initial biomass influenced predicted responses, a new model was developed with biomass as a variable (BDC model). The BDC model increased the range of initial biomasses (13 -104 g

WW/m^2) in which performance of Clearigate® for controlling the growth of *L. wollei* could be predicted.

Introduction

Prior to treatment of problematic algae or cyanobacteria in water resources with algaecides, predictions of effective exposures are necessary to guide management decisions, since characteristics of the infested site (e.g. pH, conductivity, alkalinity, hardness, dissolved and particulate organic matter, water mixing) and innate sensitivity of the target algal population(s) can alter the concentration of algaecide needed to achieve control (Rodgers et al. 2010, Bishop and Rodgers 2011, Greenfield et al. 2013, Isaacs et al. 2013 and Calomeni et al. 2015). Herbicide treatments for vascular plants are generally analogous situations to algaecide treatments for algae and cyanobacteria. For specific couplets of an herbicide and vascular plant, concentration-exposure time (CET) models have been useful for prediction of herbicide exposures necessary to result in consequent responses of a vascular plant (Getsinger et al. 1991, Van and Conant 1988, Getsinger and Netherland 1997). The fundamental basis for CET models is the theory that sufficient contact time is necessary to achieve a critical concentration of herbicide within the target vascular plant for herbicides that have exposure durations altered by site characteristics (e.g. lotic systems, large aquatic systems with relatively small treatment areas, etc.) (Van and Conant 1988, Netherland 1991). These models may also be applicable for algaecide treatments for problematic algae and cyanobacteria. To test this hypothesis, a CET model was developed for the problematic cyanobacterium, *Lyngbya wollei*, and exposures of a copper-based algaecide. For cyanobacteria, an additional independent variable, specifically the influence of cyanobacterial biomass at initiation of exposure on the outcome of an algaecide treatment, may be necessary to accurately

predict responses to algaecide exposures. As an extension of the theory driving the CET model, the biomass of a cyanobacterial population can alter the concentration of algaecide within an alga influencing the response. If initial biomass is an important exposure characteristic for accurate prediction of cyanobacterial responses to algaecide exposures, a different model that includes initial biomass as a variable will be needed.

Similar to vascular plant CET models (Getsinger and Netherland 1997), unique properties of a cyanobacterium (e.g. sensitivity) and an algaecide (e.g. potency, mechanisms of action, formulation) necessitate a specific CET model for each cyanobacterium-algaecide combination. Initially, CET model development for cyanobacteria should logically be prioritized for common and ubiquitous problematic cyanobacteria and frequently used algaecides. *L. wollei* is a problematic cyanobacterium that interferes with designated water resource uses throughout North America from Florida (Foss et al. 2012) to Canada (Vis et al. 2008). This cyanobacterium forms aesthetically objectionable growths and evidence indicates that *Lyngbya* can produce paralytic shellfish poisons (Carmichael et al. 1997, Foss et al. 2012, Lajeunese et al. 2012) and extirpate native species within infested areas (Mastin et al. 2002). In the current experiment, *L. wollei* that was impeding recreational uses (i.e. fishing, swimming, boating) was collected from a 44,500 m^2 pond in Spartanburg County, South Carolina. Copper-based algaecides have been used to treat algae and cyanobacteria producing taste and odor compounds in potable water for more than a century (Moore and Kellerman 1905) and continue to be used in irrigation canals, ponds, lakes and reservoirs (Netherland 2014). Exposures of copper-based algaecides result in aqueous copper

concentrations exceeding pre-treatment concentrations for minutes to days following application (Button et al 1977, Anderson 1999, McNevin and Boyd 2004, Liu et al. 2006, Calomeni et al. in press) depending on site characteristics. Wide-ranging copper exposure durations (i.e. minutes to days) are anticipated to have a similarly wide-ranging influence on responses, supporting development of a unique cyanobacterial CET model specifically for copper-based algaecides.

To develop the *L. wollei* CET model, laboratory experiments were conducted sequentially. Laboratory-scale experiments are appropriate for discerning the influences of specific exposure characteristics (i.e. concentration, exposure duration and initial cyanobacterial biomass) on response, while allowing replication to discern differences in responses as a function of exposures (Giesy and Odum 1980). Initial experiments were designed to determine an effective algaecide for controlling the growth of this accession of *L. wollei*. Several copper-based products are available for use as algaecides and responses of *L. wollei* to copper based algaecides range as a function of formulation (Calomeni et al. 2015). Because of the robust structure of *L. wollei* (i.e. mucilaginous sheath) and the theory that algae are more sensitive to chelated copper formulations relative to non-chelated (e.g. copper sulfate pentahydrate), chelated copper formulations were evaluated (Bishop and Rodgers 2011, Rodgers et al. 2010, Calomeni et al. 2014). Chelated copper algaecide formulations include 1) copper citrate and gluconate (e.g. Algimycin® PWF), 2) copper ethanolamine (e.g. Cutrine-Plus®), and 3) copper ethanolamine mixed with different percentages of the adjuvant D-limonene (e.g. Cutrine® Ultra and Clearigate®) (Table 3.1). Based on decisions made during their registration

with the US Environmental Protection Agency (US EPA), algaecides have restrictions in terms of the concentration of active ingredient that can be applied to an aquatic system during a treatment. These restrictions are specified on the product label, termed "legal label concentration" throughout this manuscript. To develop the CET model, a sufficiently potent copper algaecide formulation is needed resulting in significant (i.e. ≥ 90%) as well as a range of cyanobacterial responses for legal label concentrations (0.1-1.0 g Cu/L), so that differences in cyanobacterial responses due to exposure duration can be discerned.

Once questions regarding a sufficiently potent algaecide formulation are resolved, exposure duration is the next critical independent variable to evaluate. Similar to vascular plants (Getsinger and Netherland 1997), exposure duration is likely positively correlated with cyanobacterial responses until exposure durations exceed a threshold duration, at which time there is no further relationship with cyanobacterial response. As copper dissipation rates range widely (i.e. minutes to days) in aquatic systems and can influence cyanobacterial responses, the influence of exposure duration is anticipated to be an important independent variable for the *L. wollei* CET model.

For *L. wollei*, a CET model needs to be sufficiently robust to be predictive of cyanobacterial responses with wide ranging cyanobacterial biomasses. *L. wollei* biomasses can differ as a function of location and season (i.e. winter to summer) (Beer et al. 1986, Bridgeman et al. 2011). Based on peer reviewed literature, the average *L. wollei* biomass observed is 307 g dry weight (DW)/m^2 (n=61) with minimum and maximum biomasses ranging from 0.25 g-DW/m^2 to 1, 508 g-DW/m^2 in aquatic systems (Beer et al.

1986, Speziale et al. 1991, Cowell and Botts 1994, Macbeth 2004, Vis et al. 2008, Bridgeman et al. 2011, Panek 2012, Lévesque et al. 2015). To evaluate a *L. wollei* CET model, a range of cyanobacterial biomasses were exposed to copper concentrations and exposure durations anticipated to result in ≥ 90% cyanobacterial response. The biomass of *L. wollei* at initiation of copper exposures is termed "initial cyanobacterial biomass" throughout this manuscript. If cyanobacterial responses deviate from those predicted by the CET model, initial cyanobacterial biomass will be added to the model and the accuracy of subsequent predictions will be evaluated.

To incorporate initial cyanobacterial biomass in the *L. wollei* CET model, a model with two independent variables (i.e. exposure duration and concentration) and one dependent variable (i.e. cyanobacterial response) would be expanded to include three independent variables (i.e. exposure duration, concentration and initial cyanobacterial biomass as well as the dependent variable, cyanobacterial response). As a means to simplify this model, the copper concentration that results in the intended cyanobacterial response (≥ 90%) following an algaecide application is the focus of the new model. Site characteristics, specifically exposure duration and initial cyanobacterial biomass are then independent variables while the copper concentration resulting in ≥ 90% cyanobacterial response is the dependent variable. Using this approach, a new model can be developed that includes initial cyanobacterial biomass as a variable to predict performance of a copper algaecide for *L. wollei*.

The overall objective of this experiment was to discern the influence of a series of initial cyanobacterial (*L. wollei*) biomasses on responses of *L. wollei* predicted from a

CET model. Specific objectives were to 1) measure and compare responses of *L. wollei* from a 44,500 m^2 pond in Spartanburg County, South Carolina to a series of copper concentrations (0.1-1.0 mg Cu/L) in separate laboratory experiments using copper as Clearigate®, Cutrine® Ultra, Cutrine-Plus® and Algimycin® PWF to determine a copper-based algaecide that has sufficient potency (i.e. relationship between copper concentration and response to $\geq$ 90 % cyanobacterial response) to measure a change in *L. wollei* responses within legal label concentrations, 2) measure and compare responses of *L. wollei* from the same pond to a series of exposure durations and concentrations of copper as a copper-based algaecide (with sufficient potency based on the previous experiment) to develop the *L. wollei* CET model, 3) measure and compare responses of *L. wollei* (from the pond) for a series of initial biomasses (0.25 g/m^2 – 1,508 g/m2) to an exposure duration and concentration of the copper-based algaecide that result in $\geq$ 90 % cyanobacterial response in the CET model to discern the influence of initial cyanobacterial biomasses on responses of *L. wollei*, and 4) measure and compare copper concentrations (within legal label concentrations, 0.1-1.0 mg Cu/L) that result in $\geq$ 90 % cyanobacterial response for a series of exposure durations and initial cyanobacterial biomasses to develop a new model for *L. wollei*.

Materials and Methods

Responses of L. wollei to a Series of Concentrations of Copper Algaecides

L. wollei and water samples were collected during the summer of 2017 from a 44,500 m^2 pond in Spartanburg County, South Carolina, USA. This pond was originally used for recreation including fishing, swimming and boating, until recent growths of *L. wollei* impeded these uses. Once transported to the laboratory, cyanobacterial and water samples (average [n=6] $\pm$ standard deviation; pH = 6.9 $\pm$ 0.4 S.U., conductivity = 76.2 $\pm$ 4.8 μS/cm, alkalinity = 35 $\pm$ 7 mg/L as $CaCO_3$, hardness = 29 $\pm$ 5 mg/L as $CaCO_3$, dissolved oxygen = 9 $\pm$ 2 mg O_2/L) were maintained at 22$\pm$1˚C with an 18:6 h light: dark cycle provided by cool-white fluorescent bulbs (Residential Ecolux 40 W, GE) at 2,660 lx prior to and during experiments.

To determine a copper-based algaecide to use to develop a *L. wollei* CET model, the cyanobacterium was exposed to a series of copper concentrations from different copper-based algaecides (Table 3.1). *L. wollei* was exposed in 250 mL borosilicate glass beakers by adding appropriate volumes of 1,000 mg Cu/L stock solutions of each algaecide separately to 200 mL site water containing the cyanobacterium. A series of copper concentrations (0.1, 0.4, 0.7 and 1.0 mg Cu/L) for each algaecide were arrayed with three replicates per concentration. Copper concentrations were confirmed immediately following addition of copper algaecide measuring soluble (US EPA 1992) and acid soluble (US EPA 1991) copper concentrations with a Perkins Elmer (Waltham, MA) Optima 3100RL inductively coupled plasma-optical emission spectrometer (ICP-OES, method detection limit = 0.050 mg Cu/L) and graphite furnace atomic absorption

spectrometry (GFAAS, Varian Inc. AA 280FS Fast Sequential Atomic Absorption Spectrometer, method detection limit = 0.005 mg Cu/L) when applicable (APHA 2012). Quality assurance and control included replicate samples, standards, and blank matrix spike recovery.

Cyanobacterial responses (i.e. percent damaged trichomes and percent difference in *L. wollei* mass [wet weight]) to these copper concentrations were measured 7 days post-initiation. Based on preliminary experiments, this was sufficient time for responses of *L. wollei* to be manifested. Percent damaged trichomes indicates the viability of cells following exposure based on microscopic observations of cellular structure and pigment although, for differences to be discerned in terms of percent difference in mass, cells have to be non-viable and also decompose. Multiple response measures were used to discern responses of *L. wollei* to copper concentrations because cyanobacterial responses are characteristically complex (Calomeni and Rodgers 2015).

To discern percent damaged trichomes, cells within trichomes were evaluated at 400x magnification with a Leica ATC2000 Binocular Compound Microscope. Because a fraction of cells within a trichome (even in untreated controls) can be damaged and the designation of damaged and not-damaged is a binary parameter, a criterion was established for identifying a damaged trichome. The criterion was, if more than 50% of cells within the trichome segment for a field of view (400x magnification) were damaged, the trichome was recorded as damaged. Cells were damaged if they were absent, brown, or chlorotic (Figure 3.1). Ten trichome segments were enumerated per replicate for a total of approximately 530 cells (1,590 cells/exposure). To measure the difference in *L.*

wollei mass (wet weight), at the completion of the 7-day experiment, the remaining mass of cyanobacterium was removed from beakers and blotted dry with Kimteck wipes (KimWipes™) prior to weighing (Wong et al. 2009, Saunders et al. 2012, Burns et al. 2015). Responses of *L. wollei* were expressed as percentages using the following equations (Eqs. 1 and 2). The copper-based algaecide that resulted in increasing responses of *L. wollei* to $\geq$ 90 % within legal label concentrations (Table 3.1) was used in subsequent experiments. Linear regression and correlation coefficients (R^2) were used to determine the strength of the relationship between copper concentration and response.

$$P_{trichome} = \frac{T_{exposure}}{10} \times 100\% \quad (Eq. 1)$$

Where $P_{trichome}$ = percent damaged trichomes
$T_{exposure}$ = number of damaged trichome segments out of ten trichome segments discerned 7 days after experiment initiation

$$P_{mass} = \frac{\left(M_{control} - M_{exposure}\right)}{M_{coontrol}} \times 100\% \quad (Eq. 2)$$

Where P_{mass} = percent difference in *L. wollei* mass
$M_{control}$ = weight (g) of *L. wollei* "mat" in untreated control measured 7 days after experiment initiation
$M_{exposure}$ = weight (g) of *L. wollei* "mat" measured 7 days after experiment initiation (algaecide treatment)

L. wollei CET Model

To develop the *L. wollei* CET model, the cyanobacterium was exposed to a series of copper concentrations and durations of exposure. Exposure durations were arrayed to capture durations likely to result from copper algaecide applications *in situ* (i.e. minutes to days) but also producing a range of cyanobacterial responses based on preliminary experiments. The copper-based algaecide was applied at 0.1, 0.4, 0.7 and 1.0 mg Cu/L

for 0.25 hour, 1 hour, 8 hours and 24 hours in separate experimental chambers with three replicates per exposure (i.e. concentration and exposure duration). Beakers containing algae and site-water without copper treatment were included as untreated controls. At the end of each exposure duration, all beakers including the untreated controls were drained, site water (untreated) was used to rinse the beaker and the *L. wollei* mass three times and the beakers were refilled with untreated site water. Copper concentrations were measured immediately following addition of algaecide to site water containing algae and at the end of the exposure duration using methods detailed previously. Cyanobacterial responses were measured 7 days following exposure initiation to allow sufficient time for responses to manifest. Cyanobacterial responses were compared using analysis of variance (ANOVA) and linear contrasts (JMP Pro V.12).

Influence of Initial Biomass on the L. wollei CET Model

The initial cyanobacterial biomass in the *L. wollei* CET model was 52 g wet weight (WW)/m^2. To discern the influence of initial cyanobacterial biomass on measured responses of *L. wollei*, different initial cyanobacterial biomasses were exposed to the copper concentration and exposure duration resulting in $\geq$ 90% response from the *L. wollei* CET model. Nine initial *L. wollei* biomasses (three replicates per biomass) were arrayed (0.25 g WW/m^2, 13 g WW/m^2, 26 g WW/m^2, 52 g WW/m^2, 130 g WW/m2, 182 g WW/m^2, 260 g WW/m^2, 519 g WW/m^2 and 1,558 g WW/m^2) capturing the four orders of magnitude difference in *L. wollei* biomasses observed *in situ*. Copper concentrations were confirmed and responses of *L. wollei* were measured as previously described. Responses of *L. wollei* with different initial biomasses were compared to responses

measured using comparable exposures to the *L. wollei* CET model (initial cyanobacterial biomass = 52 g WW/m^2, copper concentration = 1.0 mg Cu/L as Clearigate® and 24 hours exposure duration) using ANOVA and Student's *t*-test.

Development of a New Model for Responses of L. wollei to a Copper Algaecide

If responses of *L. wollei* among a range of initial biomasses to the same copper exposures differed relative to those predicted from the *L. wollei* CET model, initial cyanobacterial biomass would be a necessary parameter to add to a new model that predicts responses of *L. wollei* to copper exposures. This new model incorporated initial cyanobacterial biomass and exposure duration as independent variables and the copper concentration resulting in $\geq$ 90% response as the dependent variable. To develop the new model, termed biomass, duration and concentration model (BDC), results from the previous objectives were used to bound exposures. Specifically, a series of exposure durations (objective 2) and initial cyanobacterial biomasses (objective 3) were arrayed that resulted in $\geq$ 90% response for legal label copper concentrations. *L. wollei* was exposed to a series of copper concentrations (i.e. bounded by the maximum legal label concentration of copper) for each combination of exposure duration and biomass. Responses of *L. wollei* for each copper concentration, exposure duration and biomass were compared statistically (ANOVA and linear contrasts) to maximum responses of *L. wollei* measured (initial cyanobacterial biomass = 52 g WW/m^2, copper concentration = 1.0 mg Cu/L as Clearigate® and 24 hours exposure duration). The lowest copper concentration for each combination of biomass and exposure duration that resulted in a

comparable response relative to this maximum response of *L. wollei* was used for the BDC model.

Results and Discussion

Confirmation of Copper Exposure Concentrations

Copper concentrations (acid soluble and soluble) measured in untreated site water (pretreatment copper concentrations) ranged from non-detect (< 0.005 mg Cu/L) to 0.020 mg Cu/L (Table 3.2). Following addition of copper-based algaecide, average percent differences for acid soluble copper concentrations were 3$\pm$17% and for soluble copper concentrations were 31$\pm$15% (Table 3.2). Because measured acid soluble copper concentrations were comparable to targeted copper concentrations, the targeted copper concentration was used for comparisons throughout this experiment. For those experiments in which copper exposures were replaced with untreated site water following the completion of specific exposure durations, soluble copper concentrations decreased to non-detect to 0.061 mg Cu/L (initial algal biomass = 1,558 g wet weight/m^2).

Responses of L. wollei to a Series of Concentrations of Copper Algaecides

For subsequent experiments, a copper algaecide was needed with the following characteristics, 1) sufficiently potent to result in $\geq$ 90% cyanobacterial response and 2) a positive linear relationship between copper concentration and responses of *L. wollei* within legal label concentrations. All algaecides evaluated resulted in $\geq$ 90% cyanobacterial response in terms of percent damaged trichomes 7 days post-addition of algaecide (Figure 3.2A). Exposures of 0.7 mg Cu/L for Clearigate®, 1.0 mg Cu/L for Cutrine® Ultra, 0.7 mg Cu/L for Cutrine-Plus® and 0.4 mg Cu/L for Algimycin® PWF resulted in the greatest response achieved in terms of percent damaged trichomes for each

algaecide. The *L. wollei* within untreated controls had 0 ± 0% damaged trichomes at completion of the 7-day experiment.

In terms of percent difference in dry mass from untreated controls, the maximum measured response for *L. wollei* was 63 ± 4% following an exposure of Clearigate® at 0.7 mg Cu/L (Figure 3.2B). Percent damaged trichomes was a more sensitive measurement of responses of *L. wollei* in this experiment (Figures 3.2A and 3.2B), likely because individual cells within trichomes respond to exposures rapidly allowing for responses to be readily apparent. This is relative to percent difference in mass that requires sufficient time for enough cells to become non-viable and decompose for responses to be discerned.

Correlation coefficients were measured and used to quantify the strength of the linear relationship between copper concentration for each formulation and response within legal label concentrations. Correlation coefficients in terms of percent damaged trichomes were 0.99 (Clearigate®, 0.1 to 0.7 mg Cu/L), 0.19 (Cutrine® Ultra, 0.1 to 1.0 mg Cu/L), 0.57 (Cutrine-Plus®, 0.1 to 1.0 mg Cu/L) and 0.15 (Algimycin® PWF, 0.1 to 1.0 mg Cu/L). For percent difference in mass, correlation coefficients were 0.99 (Clearigate®, 0.1 to 0.7 mg Cu/L), 0.27 (Cutrine® Ultra, 0.1 to 1.0 mg Cu/L), 0.43 (Cutrine-Plus®, 0.1 to 1.0 mg Cu/L) and 0.19 (Algimycin® PWF, 0.1 to 1.0 mg Cu/L). Based on the magnitude of cyanobacterial response and correlation coefficient, the ethanolamine chelated algaecide formulated with D-limonene, Clearigate®, was used for subsequent experiments. Since exposures of Clearigate® resulted in a series of increasing responses of the cyanobacterium within legal label concentrations, this algaecide could be used to develop the *L. wollei* CET model.

***L. wollei* CET Model**

In terms of percent damaged trichomes, exposures of 0.4 and 0.7 mg Cu/L for a 24-hour duration, exposures of 0.4, 0.7 and 1.0 mg Cu/L for 8 hours, and exposures of 0.7 and 1.0 mg Cu/L for 1 hour resulted in statistically similar algal responses (87 – 100 % response) as the maximum exposure evaluated (1.0 mg Cu/L for 24 hours = 94 % response) (Figure 3.3A, $p \geq 0.05$). Responses of *L. wollei* in untreated controls had 13 $\pm$ 14% damaged trichomes at completion of the 7-day experiment. For percent differences in wet weight, the exposures of 0.1 mg Cu/L for 24 hours and exposures of 0.4 mg Cu/L and 1.0 mg Cu/L for 0.25 hours were also similar (13 – 43% response) to responses to the maximum exposure concentration and duration evaluated (39% response; Figure 3.3B). To decrease variance associated with responses of *L. wollei* in terms of percent difference in wet weight, responses throughout the remainder of this experiment were also measured in terms of dry weight (dried for 24 h at 100˚C). Responses of *L. wollei* in terms of dry weight corroborated the results in terms of percent damaged trichomes for exposures that resulted in responses not significantly different than responses to the maximum exposure (Figures 3.3A and 3.3C). Exposures consisting of concentrations and exposure durations less than those reported above would result in significantly less responses than can be achieved using the maximum exposure evaluated.

Influence of Biomass on the L. wollei CET Model

To discern the influence of initial cyanobacterial biomass on measured responses of *L. wollei* to copper algaecide exposures, different initial cyanobacterial biomasses were exposed to the greatest copper concentration and exposure duration from the *L.*

wollei CET model. Percent damaged trichomes ranged from 62 – 97% 7 days post-exposure initiation for initial *L. wollei* biomasses from 13 g WW/m^2 to 519 g WW/m^2, (Figure 3.4A) and were similar to responses measured using the maximum exposure from the *L. wollei* CET model (initial biomass = 52 g WW/m^2, copper concentration = 1 mg Cu/L and exposure duration = 24 hours, response = 81 %). For the initial cyanobacterial biomass of 1,558 g WW/m^2, damaged trichomes 7 days post-exposure initiation decreased to 23 $\pm$ 6%. Responses of *L. wollei* at 1,558 g WW/m^2 were significantly less than the response of *L. wollei* (81 % response, $p < 0.0016$, α=0.05) measured with an initial biomass of 52 g WW/m^2 used in the CET model exposed to the same copper concentration (1 mg Cu/L) for the same duration (24 hours). Percent damaged trichomes 7 days post-exposure initiation for 1,558 g WW/m^2 were not significantly different than the response for untreated controls (12 $\pm$ 11%, $p = 0.2161$, $\alpha = 0.05$).

In terms of percent difference in mass, significantly less cyanobacterial response (i.e. percent difference in wet and dry weight) resulted from an exposure of 1 mg Cu/L for 24 hours for the initial cyanobacterial biomass of 130 g WW/m^2 ($p < 0.0023$ wet weight and $p < 0.001$ dry weight, α=0.05) relative to the *L. wollei* biomass used in the CET model (Figure 3.4B). At the initial biomass of 130 g WW/m^2, responses of *L. wollei* decreased from 52$\pm$6% (CET model exposure) to 15$\pm$8% as wet weight and 58$\pm$1% (CET model exposure) to 39$\pm$2% as dry weight. Responses of *L. wollei* continued to decrease in terms of percent difference in mass with an initial biomass of 1,558 g WW/m^2 resulting in no measureable difference relative to the untreated control in terms of wet weight (0%) and 7% for dry weight.

For the experimental conditions of this study, initial cyanobacterial biomasses less than 52 g WW/m^2 were overexposed meaning that less copper exposure (i.e. duration, concentration or both) would result in the same response. Alternatively, for this accession of *L. wollei*, initial biomasses of 130 g WW/m^2 and larger would require a greater copper exposure to result in the same response as that predicted from the *L. wollei* CET model. Since the concentration applied is the maximum legal label concentration (1.0 mg Cu/L) and an initial biomass greater than and equal to 130 g WW/m^2 would require a copper exposure in exceedance of the maximum label concentration, responses of *L. wollei* comparable to those predicted from the CET model cannot be achieved with one algaecide application. This emphasizes the importance of treating prior to peak cyanobacterial biomass or early in a *L. wollei* infestation so that the greatest response can be achieved with one application. If the *L. wollei* biomass is equivalent to or greater than 130 g WW/m^2 for this site, several algaecide treatments may be required to incrementally decreased the biomass.

For this *L. wollei* from a 44,500 m^2 pond in Spartanburg County, SC, cyanobacterial responses can range from the predicted response (CET model) to no measurable response (relative to the untreated control) as a function of initial cyanobacterial biomass. Because of the range of cyanobacterial responses elicited using the same exposure parameters (i.e. copper concentration and exposure duration), the results from this experiment demonstrate that initial *L. wollei* biomass is an important exposure parameter driving responses of *L. wollei*. For this accession of *L. wollei*, the initial cyanobacterial biomasses that this CET model is applicable for is 52 g WW/m^2. To

expand predictions beyond a single initial biomass, a new model is necessary where initial cyanobacterial biomass is a significant contributor to the performance of Clearigate® for *L. wollei*.

Development of a New Model for Responses of L. wollei to a Copper Algaecide

The new model (BDC) for responses of *L. wollei* to Clearigate® has exposures bounded by previous results in this experiment. The exposure duration (from objective 2) of 0.25 hour did not result in $\geq$ 90% responses of *L. wollei* under the conditions of this experiment. Since for the BDC model exposures are arrayed to capture $\geq$ 90% responses, the exposure duration of 0.25 hour was excluded from the model. In terms of initial cyanobacterial biomass, responses of *L. wollei* with an initial biomass of 0.25 g WW/m^2 could not be detected because the mass at completion of the experiment was below the method detection limit (< 0.15 g WW/m^2). This initial biomass (0.25 g WW/m^2) was therefore not included in the BDC model and the lowest biomass included was 13 g WW/m^2, the second lowest biomass evaluated in this experiment. Based on the results of the third objective, the initial cyanobacterial biomass of 130 g WW/m^2 did not result in $\geq$ 90% responses of *L. wollei* to an exposure of 1.0 mg Cu/L for 24 hours. Therefore, there may be an initial cyanobacterial biomass between 52 g WW/m^2 and 130 g WW/m^2 that can be treated with 1.0 mg Cu/L for 24 hours and result in $\geq$ 90% responses of *L. wollei*. An additional biomass of 104 g WW/m^2, was therefore evaluated for the BDC model.

As expected from the results of the objective evaluating the influence of initial cyanobacterial biomasses on responses, the initial cyanobacterial biomasses of 13 g WW/m^2 and 26 g WW/m^2 were overexposed and required a lower copper concentration

than 1 mg Cu/L to achieve the maximum response. For 13 g WW/m^2 these exposures were 0.3 mg Cu/L for a 1 hour exposure duration and 0.07 mg Cu/L for 8 and 24 hours (Fig. 5). A concentration of 0.4 mg Cu/L for a 1 hour exposure duration and 0.2 mg Cu/L for 8 and 24 hours resulted in the maximum response for the initial cyanobacterial biomass of 26 g WW/m^2.

Results from the BDC model demonstrate that as initial cyanobacterial biomass increases the copper concentration necessary to result in $\geq$ 90% response increases (Fig. 5). On a theoretical basis, this result is anticipated. In theory, there is a mass of copper per exposed algal population required to result in a specific response (De Schamphelaere et al. 2005). An exaggerated effect for benthic algae relative to planktonic algae is that as the mass of the cyanobacterial population increases, a greater copper concentration would then be necessary to result in a comparable response (Geer 2016, Kinley et al. 2017). The BDC model also indicated that as exposure duration increases a lower copper concentration is needed to result in $\geq$ 90% response of *L. wollei*. This has also been demonstrated in CET models for exposed vascular plants (Netherland 1991) and for the CET model in the current experiment.

Similar to CET models, the BDC model is specific to couplets of the exposed organism and algaecide. Conditions that may require careful consideration prior to use of the specific BDC model include different water characteristics (e.g. pH, hardness, alkalinity, conductivity, particulate and dissolved organic matter) and different algae and cyanobacteria (e.g. genera, species, accessions). This experiment demonstrated an

approach for development of BDC models that can be used for additional couplets of algaecides to exposed algae and cyanobacteria.

Conclusions

The purpose of this experiment was to determine the influence of initial cyanobacterial biomass on responses of the commonly problematic cyanobacterium, *L. wollei* predicted from a specific CET model developed for a copper-based algaecide. Of the copper based algaecides evaluated, Clearigate®, a formulation with D-limonene and chelated with ethanolamine had sufficient potency and captured a range of cyanobacterial responses within legal label concentrations to be used to develop the CET model. Using the *L. wollei* CET model, exposures of 0.4, 0.7 and 1.0 mg Cu/L for 24-hours, exposures of 0.4, 0.7 and 1.0 mg Cu/L for 8 hours, and exposures of 0.7 and 1.0 mg Cu/L for 1 hour resulted in maximum cyanobacterial responses measured at completion of the 7-day experiment (87-100% percent damaged trichomes, 13 – 43% wet weight and 47 – 63% dry weight). The initial cyanobacterial biomass used in the CET model was 52 g WW/m^2 and initial biomasses exceeding this value resulted in responses less than those predicted from the CET model. At the initial cyanobacterial biomass of 1,558 g WW/m^2, cyanobacterial responses to the same copper concentration and duration resulting in maximum cyanobacterial response based on the CET model were non-detect (percent damaged trichomes and wet weight) to 7% (dry weight) relative to untreated controls. Initial cyanobacterial biomass was subsequently captured in a new model for *L. wollei* exposed to Clearigate®, the BDC model. A useful feature of the BDC model relative to the CET model for Clearigate® exposed to *L. wollei* is that it expands the range of initial cyanobacterial biomasses (13 g WW/m^2 to 104 g WW/m^2) for which the model is predictive of performance.

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Table 3.1 Physical and chemical properties of copper-based algaecides.

Product	Clearigate®	Cutrine® Ultra	Cutrine-Plus®	Algimycin® PWF
Composition	D-limonene Triethanolamine Ethanolamine Basic copper carbonate	D-limonene Triethanolamine Ethanolamine Basic copper carbonate	Triethanolamine Ethanolamine Basic copper carbonate	Copper gluconate Copper citrate
Active ingredient	3.8 % Cu	9 % Cu	9 % Cu	9 % Cu
Minimum application concentration	0.1 mg Cu/L	0.1 mg Cu/L	0.1 mg Cu/L	0.1 mg Cu/L
Maximum application concentration	1.0 mg Cu/L	1.0 mg Cu/L	1.0 mg Cu/L	1.0 mg Cu/L
Physical description	Viscous blue liquid	Viscous blue liquid	Blue liquid	Blue liquid
pH	9.7-10.0	10.2-10.3	10.3-10.5	1.5-2.5

Table 3.2 Targeted and measured acid soluble and soluble copper concentrations (n=1) for experiments.

Objective	Targeted Copper Concentration (mg Cu/L)		Acid Soluble Copper Concentration (mg Cu/L)	Soluble Copper Concentration (mg Cu/L)
1. Responses of *L. wollei* to different copper algaecides	**Algaecide**	**Concertation**		
	Clearigate®	Untreated control	0.010	0.007
		0.1	0.117	0.089
		0.4	0.488	0.267
		0.7	0.793	0.555
		1.0	1.094	0.769
	Cutrine® Ultra	Untreated control	0.010	0.007
		0.1	0.118	0.083
		0.4	0.445	0.277
		0.7	0.703	0.567
		1.0	1.047	0.716
	Cutrine-Plus®	Untreated control	0.010	0.007
		0.1	0.090	0.073
		0.4	0.406	0.259
		0.7	0.763	0.532
		1.0	1.114	0.827
	Algimycin® PWF	Untreated control	0.010	0.007
		0.1	0.102	0.084
		0.4	0.322	0.260
		0.7	0.636	0.559
		1.0	0.919	0.829
2. CET model [a]		Untreated control	ND	ND
		0.1	0.092	0.075
		0.4	0.323	0.246
		0.7	0.604	0.436
		1.0	0.798	0.666
		Untreated control	0.018	0.020

3. Responses of *L. wollei* to copper exposures with different initial biomasses [b]		1.0		0.980	0.857
4. BDC model[c]	Initial algal Biomass (g wet weight/m^2)	Exposure Duration (hour)	Targeted Copper Concentration (mg Cu/L)		
	Untreated control	-	-	ND	ND
	13	1	0.3	0.303	0.204
		8	0.07	0.079	0.058
		24	0.07	0.079	0.058
	26	1	0.4	0.517	0.195
		8	0.2	0.243	0.088
		24	0.2	0.243	0.088
	52	1	1.0	1.330	0.505
		8	0.7	0.916	0.390
		24	0.4	0.517	0.195
	104	1	1.0	0.711	0.370
		8	0.7	0.503	0.284
		24	0.7	0.503	0.284

[a] Following completion of the exposure duration and replacement of the exposure with unamended site water, soluble copper concentrations ranged from ND (<0.005 mg Cu/L) to 0.020 mg Cu/L.

[b] Following completion of the exposure duration and replacement of the exposure with unamended site water, soluble copper concentrations ranged from 0.011 mg Cu/L (initial algal biomass = 26 g wet weight/m^2) to 0.061 mg Cu/L (initial algal biomass = 1,558 g wet weight/m^2).

[c] Following completion of the exposure duration and replacement of the exposure with unamended site water, soluble copper concentrations were non-detect (<0.005 mg Cu/L).

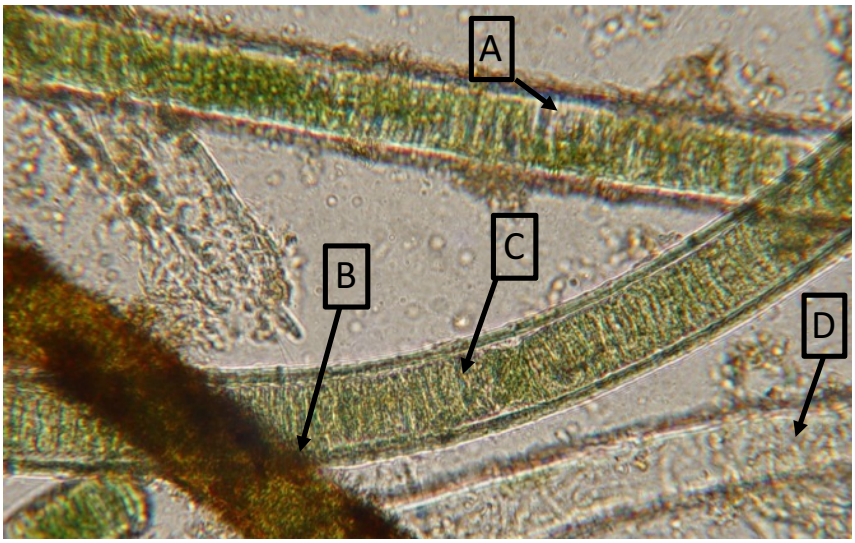

Figure 3.1 *Lyngbya wollei from a 44,500 m^2 pond in Spartanburg County, SC. A indicates chlorotic cells, B indicates brown cells, C indicates viable cells within trichomes segments and D indicates a mucilaginous sheath with cells absent.*

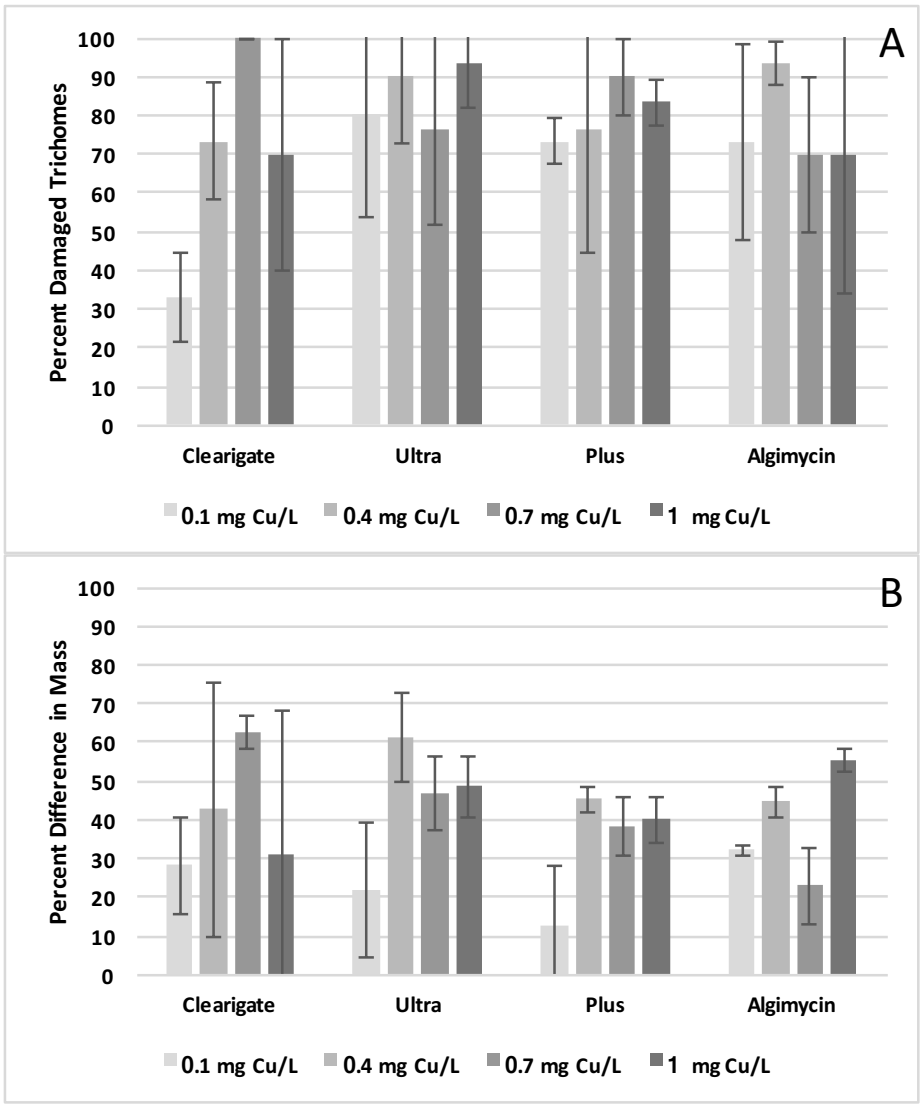

Figure 3.2 Average responses (n=3) of *Lyngbya wollei* in terms of percent damaged trichomes (A) and percent difference in wet weight (B) 7 days after addition of Clearigate®, Cutrine® Ultra, Cutrine-Plus® and Algimycin® PWF at 0.1, 0.4, 0.7 and 1.0 mg Cu/L with a 7-day exposure duration. Error bars are $\pm$ 1 standard deviation.

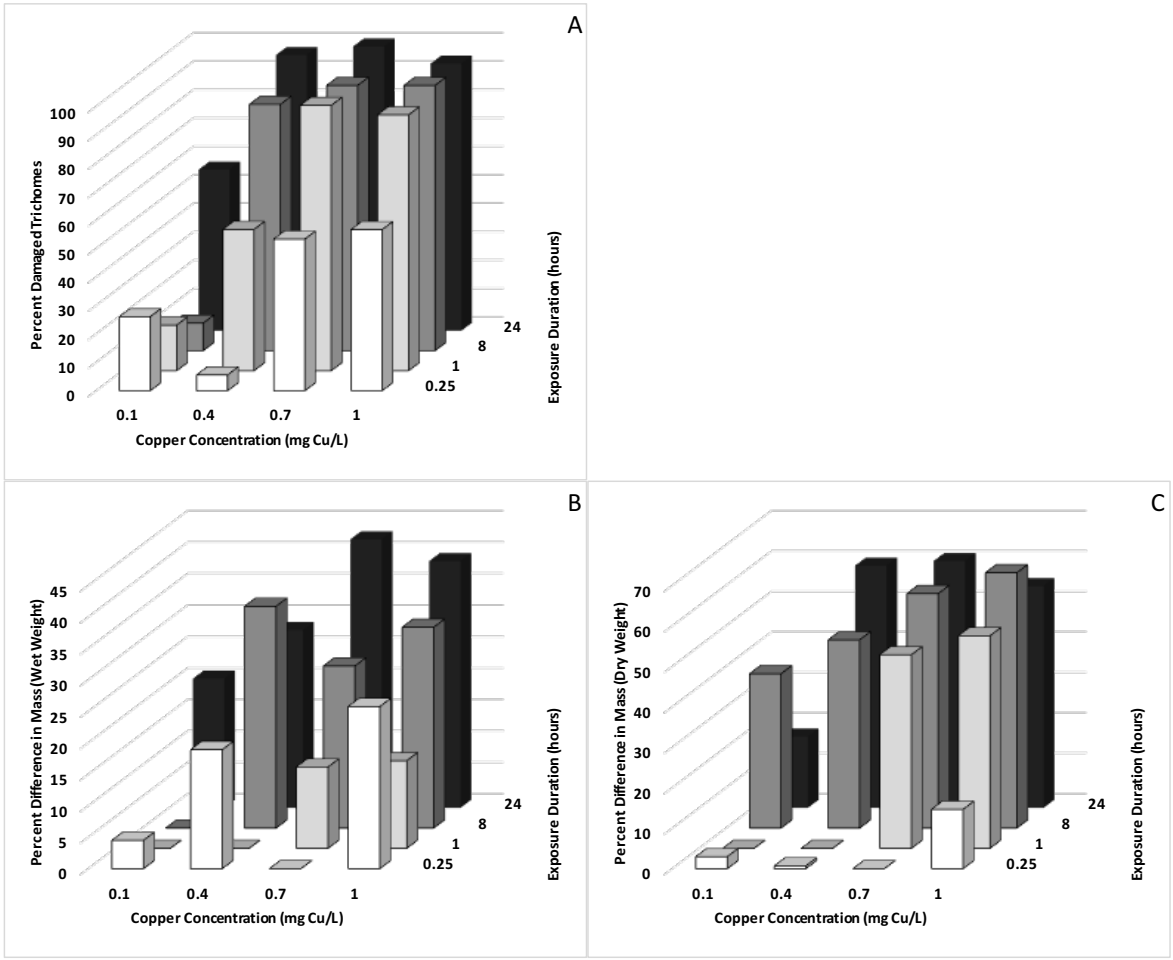

Figure 3.3 Average responses (n=3) of *Lyngbya wollei* in terms of percent damaged trichomes (A), percent difference in wet weight (B) and percent difference in dry weight (C) 7 days after exposure initiation for a series of copper concentrations as Clearigate® and exposure durations for the concentration-exposure time model.

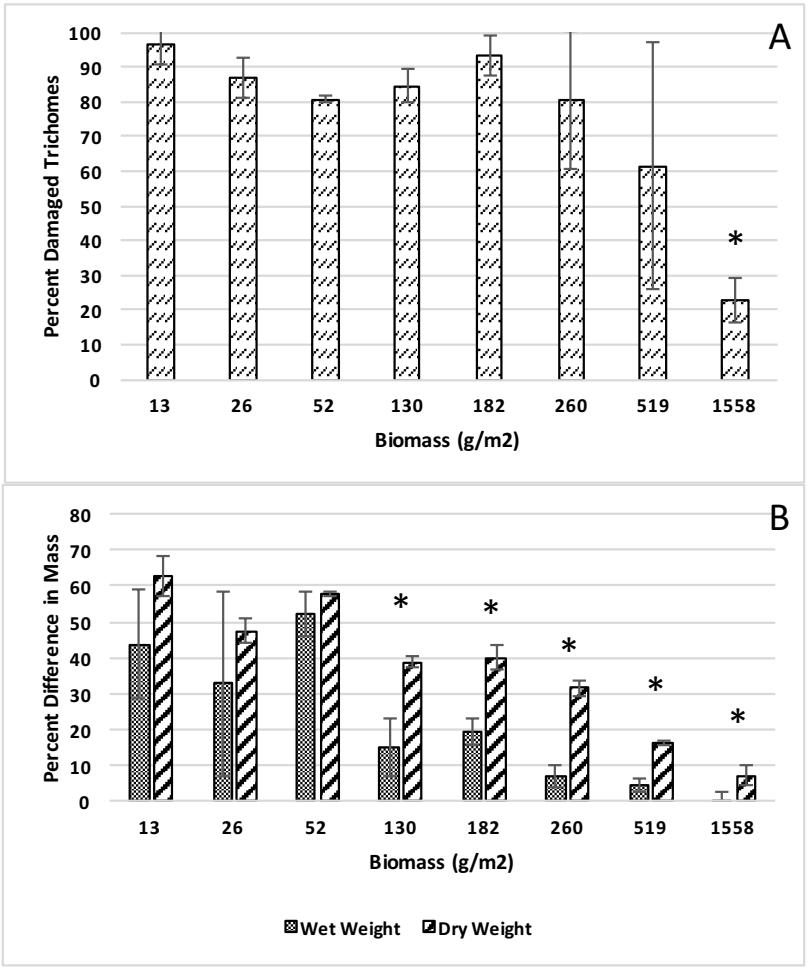

Figure 3.4 Average responses (n=3) of *Lyngbya wollei* with a series of biomasses at exposure initiation in terms of percent damaged trichomes (A) and percent difference in (wet and dry) mass (B) 7 days after exposure initiation of Clearigate® at 1.0 mg Cu/L for a 24-hour exposure duration. Error bars are $\pm$ 1 standard deviation. Asterisks are used to indicate significantly different responses relative to responses of the initial algal biomass used for the concentration-exposure time model (52 g wet weight[WW]/m^2). At the initial cyanobacterial biomass of 0.25 g WW/m^2, the final biomass was non-detect (< 0.15 g WW/m^2) 7 days post-exposure initiation and the datum was excluded.

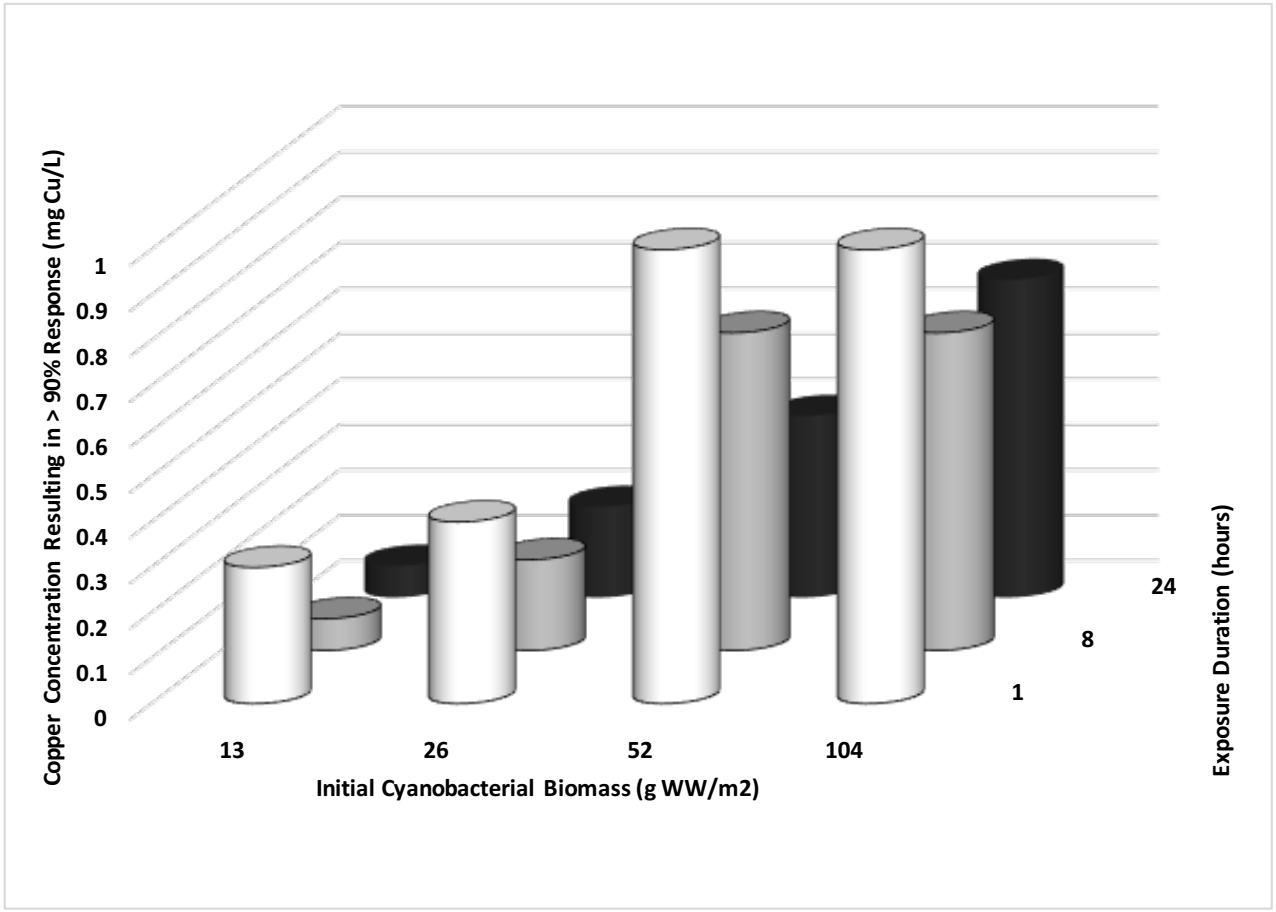

Figure 3.5 Copper concentrations from applications of Clearigate® resulting in ≥ 90% response of *Lyngbya wollei* 7 days after treatment for a series of initial biomasses and exposure durations for the biomass-duration-concentration model (BDC).

CHAPTER FOUR

RELATIONSHIP AMONG AQUEOUS COPPER HALF-LIVES AND RESPONSES OF *PIMEPHALES PROMELAS* TO A SERIES OF COPPER SULFATE PENTAHYDRATE CONCENTRATIONS

Abstract

Copper algaecide exposures in situ are often of shorter duration than exposures in static toxicity experiments. Consequently, responses of organisms to copper exposures in static experiments may overestimate effects following in situ copper exposures. To incorporate exposure duration in predictions of organism responses, *Pimephales promelas* responses to $CuSO_4 \bullet 5H_2O$ were measured in static (96 hours) and pulse (1.5, 4, 8 and 15 hour half-lives) toxicity experiments. Copper concentrations sorbed to fry were used to indicate a consequence of different exposures. Responses of *P. promelas* to $CuSO_4 \bullet 5H_2O$ in the static experiment resulted in 96 hour LC_{50s} of 166 μg Cu/L (95% confidence interval [CI], 142-189 μg Cu/L) as soluble copper and 162 μg Cu/L (CI, 140 – 183 μg Cu/L) as acid soluble copper. Relative to static 96 hour LC_{50s}, the 1.5 hour pulse experiment resulted in a factor of 10 increase in LC_{50s} calculated from responses measured 96 hours following exposure initiation, while a half-life of 15 hours did not result in different LC_{50s}. Sorbed copper extracted from fry in the 1.5, 4 and 8 hour half-life experiments was less than the static experiment. However, copper sorbed to fry in the 15 hour half-life experiment was not different than the static experiment. The relationship between 96 hour LC_{50s} and 1/half-life was expressed using the equations $y=116+1{,}360x$ ($R^2 = 0.97$) for soluble copper and $y=147+1{,}620x$ ($R^2 = 0.98$) for acid soluble copper. Incorporation of exposure duration for predictions of *P. promelas*

responses to copper pulse exposures can increase the accuracy of predictions by more than an order of magnitude.

Introduction

Often laboratory toxicity experiments are conducted to measure organism responses and predict responses in aquatic systems (e.g. lakes, ponds and reservoirs). The predictive capacity of laboratory toxicity experiments is based on the fundamental concept that comparable exposures elicit similar responses for a given species and set of environmental conditions. Laboratory exposures of constituents that are relatively conservative (i.e. remain in solution and do not degrade) in "clean" laboratory waters would not be directly comparable to exposures that dissipate in situ (i.e. pulse exposures). Copper-based algaecide exposures are an example for which there are disconnects between fate processes that are captured in an enclosed vessel (i.e. laboratory experiment) and in situ. Copper from algaecide applications result in pulse (i.e. rapidly dissipating) exposures (Murray-Gulde et al. 2002, Calomeni et al. 2017) as a result of fate processes (Calomeni et al. 2017). By design, static laboratory toxicity experiments hinder dissipation of copper from the aqueous phase as dilution is limited and environmentally relevant masses of ligands (i.e. sediments or algae) are lacking. Thereby, laboratory toxicity experiments conducted with constant copper concentrations likely represent ultraconservative exposures relative to copper-based algaecide exposures in situ and consequently overestimate organism responses.

Fundamentally, organism responses are likely a function of sorbed copper (Di Toro et al. 2001, Escher and Hermens 2002) and exposure duration should result in different sorbed copper concentrations under non-equilibrium conditions. Sorption can be modeled for a constant concentration using a modified first order rate equation

incorporating rates for partitioning to and from the organism (equation 1, Hickie et al. 1995).

$$C_f = C_i \frac{k1}{k2}(1 - e^{-k2*t}) \text{ [Equation 1]}$$

Where: C_f = sorbed copper concentration
C_i = initial aqueous copper concentration
k1 = net partitioning rate to the organism
k2 = net partitioning rate from the organism
t = time

Theoretically, an exposure scenario with constant copper concentration would result in a quasi-equilibrium between the aqueous phase and sorbed copper by exposed organisms. The quasi-equilibrium would elicit a plateau in sorbed copper. In a pulse exposure scenario, a first order dissipation equation is used to model the aqueous copper concentration (C_w) yielding equation 2.

$$C_f = (C_w * e^{-k3*t}) * \frac{k1}{k2}(1 - e^{-k2*t}) \text{ [Equation 2]}$$

Where: *k3* = copper dissipation rate
C_w = aqueous copper concentration

The following potential outcomes are possible for comparing pulse and static exposures with the same initial copper concentration: 1) copper dissipates prior to achieving quasi-equilibrium resulting in less sorbed copper by biota relative to a static exposure; or 2) copper dissipates at a rate in which quasi-equilibrium can be achieved resulting in the same mass of sorbed copper by biota relative to a static exposure. Under the first pulse exposure scenario, a greater initial copper concentration would be required to elicit the same organism response as a constant copper exposure. For the second pulse exposure

scenario, both a pulse exposure and constant copper exposure would result in the same organism response and theoretically the same sorbed copper concentration. The overall objective of this experiment is to define the relationship between half-life of aqueous copper and organism responses encompassing these two scenarios.

In the present experiment, *Pimephales promelas* responses to different copper sulfate pentahydrate ($CuSO_4$•5 H_2O) exposures as a function of concentration and half-life were compared. Aqueous copper half-lives following algaecide applications are influenced by characteristics of the application and site (Calomeni et al. 2017). Reported copper half-lives following algaecide applications range from approximately 0.7 hour to several days (Murray-Gulde et al. 2002, Calomeni et al. 2017). Comparisons of measured toxicity data for different half-lives of copper exposures and for a static exposure, provide information on the relative conservatism of standard laboratory toxicity experiments. Further, these toxicity data can be used to determine the pulse exposure duration that results in the same organism response as a static toxicity experiment.

Copper sulfate pentahydrate ($CuSO_4$•5 H_2O) was used as the copper-based algaecide in this experiment because it has been used to decrease problematic algal growths for over a century (Moore and Kellerman 1904) and there are extensive data regarding *P. promelas* responses to $CuSO_4$•5 H_2O. Less than 24-hour old *P. promelas* are relatively sensitive to $CuSO_4$•5 H_2O exposures (US EPA 2007), therefore differences in responses to incremental exposures can be measured.

For copper-based algaecides, dissipating aqueous concentrations could result in measurable alterations in organism responses relative to predicted responses if the initial copper concentration was the only exposure characteristic considered (Bailey et al. 1985). The relationship between exposure half-life and organism response can provide predictions of the anticipated organism responses if the half-life and initial copper concentration are measured in situ. Specific objectives of this research are to 1) measure responses of < 24 hour old *P. promelas* in terms of survival exposed to a series of copper concentrations as copper sulfate pentahydrate in toxicity experiments with four different half-lives and to static exposures, 2) measure sorbed copper to *P. promelas* at completion of the toxicity experiment (i.e. 96 hours after exposure initiation) as a parameter to indicate the consequence of different exposure conditions (i.e. dissipating and static) and 3) calculate the relationship between 1/half-lives and responses of *P. promelas* to copper sulfate pentahydrate concentrations.

Materials and Methods

Copper Exposures

Exposures of *P. promelas* were conducted in laboratory formulated moderately hard water prepared using NANOpure® water and reagent grade chemicals based on recommended culture methods (US EPA 2002). Water characteristics were measured (Table 4.1) at experiment initiation and completion (i.e. 96 hour after initiation of exposures), to ensure consistency in terms of appropriate conditions to meet the environmental tolerances and requirements of *P. promelas* and to provide explanatory parameters for exposures; since water characteristics influence copper exposures and consequent organism responses (Di Toro et al. 2001; Cu Criteria Doc).

A 1,000 mg Cu/L stock copper solution was prepared by adding 0.393 g copper sulfate pentahydrate (Fisher Scientific; Pittsburgh, PA) crystals to 100 mL NanoPure® water and inverting until dissolution. Appropriate volumes of the stock copper solution were then added to prepare a series of copper exposures (Table 4.2). Based on preliminary toxicity experiments, these exposures were used because they bound anticipated 96 hour LC_{50s}. Pulse exposures were simulated using 1.5, 4, 8 and 15-hour half-lives by removing half of the exposure solution every 1.5, 4, 8 and 15 hours, respectively, and adding un-amended laboratory formulated water. These half-lives were selected based on preliminary experiments to capture pulse exposure scenarios that result in different responses relative to static exposures, as well as, to capture pulse exposures that result in the same organism responses as static experiments. Static toxicity experiments were conducted as 96 hour non-renewal exposures. Results from the static

toxicity experiment were also used as responses to a reference toxicant for inter-laboratory comparisons of *P. promelas* sensitivities to copper sulfate.

Copper concentrations were confirmed at exposure initiation and following each simulated half-life for the pulse exposure experiments. For the static toxicity experiment, copper concentrations were initially confirmed and were also measured at experiment completion. Concentrations were measured as soluble and acid soluble copper using a graphite furnace atomic absorption spectrophotometer (GFAAS, method detection limit 5 μg Cu/L) and an inductively coupled plasma optical emission spectrometer (ICP-OES, method detection limit 20 μg/L). Quality assurance and control for analytical methods included comparisons to standard curves, replicate analysis, and blank spike recovery.

***P. promelas* Toxicity Experiments**

P. promelas Rafinesque were obtained from cultures at Clemson University Aquatic Animal Research Laboratory (AARL) maintained according to methods outlined in US EPA (1987). Less than 24-hour old fry were exposed in 250 mL borosilicate glass beakers filled with 200 mL exposure water. The United States Environmental Protection Agency (US EPA) freshwater toxicity testing protocol was modified (i.e. half-life) for the current experiment (US EPA 2002, Method 2000.0). Thirty *P. promelas* were exposed per concentration (3 experimental chambers per concentration, 10 organisms per experimental chamber). Organism responses were recorded 96 hours following exposure initiation. Surviving fry were recorded if they exhibited movement when water flowed over them via pipette.

To compare organism responses, percent survival following exposures to copper sulfate pentahydrate were fit to a 3P logistic regression (JMP Pro 12). Data were evaluated for normal distribution of residuals, and homogeneity of variance. All data met these assumptions and 96 hour LC_{50s} were calculated. Potency slopes were calculated using the linear portion of the exposure-response curve (i.e. 88% – 12% survival) by linear regression models. A general linear model was used to test the homogeneity of potency slopes (i.e. interaction between each toxicity experiment [static, 1.5, 4, 8 and 15-hour half-lives] and copper concentration).

Measurement of Sorbed Copper Concentrations to P. promelas

Following toxicity experiment completion, all remaining fry were removed from each exposure and replicate. Fry were frozen at - 18º C for approximately 12 hours then thawed. Copper was extracted from fish tissue using 500 μL trace metal grade concentrated nitric acid (Fisher Scientific Company; Pittsburgh, PA) for 24 hr. NanoPure® water (4.5 mL) was then added and copper was measured using a GFAAS. Differences in sorbed copper for the highest exposure in each pulse toxicity experiment (resulting in 0% -30% survival) relative to the static toxicity experiment were discerned using Student's *t*-tests (Prob < t). The highest exposure from each toxicity experiment was used to ensure measureable sorbed copper concentrations at toxicity experiment completion.

Relationship Between Half-lives and Responses of P. promelas to Copper Sulfate Pentahydrate Concentrations

The relationship between LC_{50} and exposure time often follows a hyperbolic relationship (Green 1965, Sprague 1969, Verhaar et al. 1999, Lee et al. 2002, Sanchez-Bayo and Goka 2007, Sanchez-Bayo 2009). A hyperbolic relationship is often observed because in theory the relationship between LC_{50} and exposure time should approach two asymptotes (i.e. vertical and horizontal asymptotes). As exposure time approaches zero, the copper concentration resulting in an LC_{50} follows a vertical asymptote. As exposure time increases, quasi-equilibrium can be achieved in terms of the mass of sorbed copper resulting in the same response regardless of exposure duration (i.e. horizontal asymptote). The hyperbolic relationship between LC_{50s} and exposure time can be linearized using the following equation (3) (Green et al. 1965). Linearization of this relationship results in an equation that can be used to interpolate LC_{50s} from half-lives.

$$LC_{50} = a + b(t^{-1}) \text{ [Equation 3]}$$

Where: LC_{50} = the lethal concentration for 50% of exposed organisms
a = the LC_{50} when the relationship between LC_{50} and half-life approaches a horizontal asymptote
b = slope $\frac{\Delta LC_{50}}{\Delta 1/t}$
t = half-life (hours)

Results and Discussion

Responses of P. promelas (<24 hour old) to Copper Sulfate Pentahydrate Exposures in Static and Pulse Toxicity Experiments

Water characteristics were comparable among exposures (Table 4.3) and met the environmental tolerances of *P. promelas* (US EPA 1994, US EPA 1987). Ninety-six-hour static toxicity experiments resulted in a LC_{50} of 166 μg Cu/L (95% confidence interval [CI], 142-189 μg Cu/L) as soluble copper and an LC_{50} of 162 μg Cu/L (CI, 140 – 183 μg Cu/L) as acid soluble copper. Responses (96 hour LC_{50s}) of *P. promelas* to exposures of copper sulfate pentahydrate in the current static toxicity experiment were within the range of LC_{50s} reported for static toxicity experiments with similar conditions (Table 4). These results demonstrate that the sensitivity of *P. promelas* to exposures of copper sulfate pentahydrate in the current experiment was comparable to the sensitivity of *P. promelas* in experiments previously published.

Initial copper concentrations and responses measured 96 hours following exposure initiation were used to calculate LC_{50s}. This concentration was used to serve as a point of comparison with the 96 hour LC_{50} from the current static toxicity experiment. Exposures with a half-life of 1.5 hours resulted in approximately an order of magnitude increase in LC_{50s} calculated from measurements 96 hours following exposure initiation relative to the static toxicity experiment (Table 5). *P. promelas* responses to copper exposures with 4 and 8 hour half-lives resulted in LC_{50s} approximately 2 (soluble) to 3 (acid soluble) times greater than the LC_{50} for the 96 hour static toxicity experiment. Significant differences in 96 hour LC_{50s} were not discerned between the 15 hour half-life

toxicity experiment and the static toxicity experiment (Table 5). There were no statistically significant differences in potency slopes ($\alpha = 0.05$, p = 0.2013 for soluble copper and p = 0.1325 for acid soluble copper) among toxicity experiments (i.e. static and pulse exposures).

Results from static toxicity experiments are used in risk assessments for copper-based algaecides in situ with the qualification that they are conservative (US EPA 2006). One potential source for this conservatism is the difference in exposure duration. Copper exposures in beakers remain relatively stable under static conditions. In the current static toxicity experiment, copper concentrations measured at toxicity experiment completion were 74 $\pm$ 2% (soluble) and 93 $\pm$ 7% (acid soluble) of the initial concentration. Based on the results of the current experiment, static toxicity experiments could overestimate the relative sensitivity of *P. promelas* to copper sulfate pentahydrate by an order of magnitude (relative to the 96 hour LC_{50} for the 1.5-hour half-life toxicity experiment). Alternatively, if copper dissipation half-lives are approximately 15 hours or greater, static toxicity experiments will be predictive of organism responses under similar environmental conditions.

Sorbed Copper Concentrations to P. promelas

Based on the hypothesis that sorbed copper to an organism is driven by exposures (i.e. concentration and duration), differences in exposure duration (static and pulse) will be evident by differences in measured copper sorbed to fish. For the 96 hour static exposures, sorbed copper generally increased as a function of initial copper concentration (coefficient of correlation [R^2] = 0.66). The sorbed copper extracted from fry in the static

toxicity experiment increased from 17 ± 11 ng Cu/fry resulting in 70% survival to 284 ± 22 ng Cu/fry resulting in 0% survival. Sorbed copper to *P. promelas* that were exposed to the highest concentration tested (resulting in 0 - 30 % survival) for each toxicity experiment were compared. Measured copper concentrations sorbed to fry were 44 ± 21 ng Cu/fry (1.5 hour half-life), 19 ± 2 ng Cu/fry (4 hour half-life), 15 ± 7 ng Cu/fry (8 hour half-life) and 251 ± 308 ng Cu/fry (15 hour half-life) for pulse toxicity experiments. Sorbed copper extracted from fry in the 1.5 ($p < 0.0001$), 4 ($p = 0.0009$), and 8 ($p = 0.4349$) hour half-life toxicity experiments were significantly different (*t*-test; $\propto = 0.05$) than the sorbed copper from fry in the static toxicity experiment. Sorbed copper extracted from fry in the 15-hour half-life experiment was not significantly different from sorbed copper extracted from fry in the static toxicity experiment (*t*-test; $\propto = 0.05$, $p = 0.4349$).

Results from static toxicity experiments suggest that sorbed copper concentrations are linearly correlated with organism responses and concentrations tested. In theory, this correlation is often observed because copper concentrations in static toxicity experiments achieve quasi-equilibrium between the organism and exposure water. It follows that sorbed copper concentrations would then be predictive of an organism response. The sorbed copper concentration resulting in an organism response is often referred to as a critical body burden, critical body residue, or conditional stability constant (Escher and Hermens 2002, Verhaar et al. 1999, US EPA 2007) and has been used to predict the response of an exposed organism. Alternatively, with non-equilibrium exposures typically resulting from copper-based algaecide applications, the sorbed copper

concentration resulting in a specific response under static conditions does not result in the same response under non-equilibrium conditions. Simply stated, critical body residues or burdens developed using static conditions may not be predictive of an organism response following a pulse or dissipating exposure from a copper-based algaecide.

Relationship Between Half-Lives and Responses of P. promelas to Copper Sulfate Pentahydrate Concentrations.

Predictions of the influence of different exposures elicited by rates of copper dissipation on measured responses are necessary for a wide range of half-lives because dissipation half-lives from copper-based algaecide treatments can range widely (i.e. minutes to days, Calomeni et al. 2017). Relationships between 96 hour LC_{50s} for static and pulse toxicity experiments were discerned using equation 4 and presented in Figure 4.6. Using this equation (4), the following relationships were calculated $y = 116 + 1{,}360x$ ($R^2 = 0.97$) for soluble copper concentrations and $y = 147 + 1{,}620x$ ($R^2 = 0.98$) for acid soluble copper concentrations (Figure 4.6).

Conclusions

For copper sulfate pentahydrate exposures that dissipated with a half-life of 1.5 hours, static toxicity experiments overestimated the responses of *P. promelas* by an order of magnitude. A half-life of 15 hours resulted in a comparable LC_{50} (calculated from responses measured 96 hours after exposure initiation) as was calculated for the static toxicity experiment. As a result of differences in exposure duration, copper concentrations sorbed to fry in the 1.5, 4 and 8 hour half-life toxicity experiments were dissimilar to sorbed copper concentrations to fry in static toxicity experiments. Alternatively, sorbed copper extracted from fry exposed to the highest concentration tested for the 15 hour half-life experiment was not significantly different than sorbed copper extracted from fry exposed to the highest concentration tested in the static toxicity experiment. The relationship between 1/half-life and 96 hour LC_{50} was expressed as a linear relationship (y = LC_{50} calculated from responses measured 96 hours after exposure initiation, x = 1/half-life) with the formula $y = 116 + 1{,}360x$ ($R^2 = 0.97$) for soluble copper concentrations and $y = 147 + 1{,}620x$ ($R^2 = 0.98$) for acid soluble copper concentrations. Results from this study can be used to predict the responses of *P. promelas* to copper sulfate pentahydrate exposures with different half-lives. The present experiments highlight the importance of exposure duration as an in situ exposure modifying factor for organism responses to copper algaecide exposures. Ultimately, measurement and incorporation of in situ copper dissipation rates could increase the accuracy of predictions of organism (*P. promelas*) responses to copper-based algaecides by greater than an order of magnitude. Specific predictions for organism responses as a

function of exposure duration can improve the reliability of risk assessments for pulse exposures of copper.

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Table 4.1 Analytical methods, citations and method detection limits for water characteristics (i.e. pH, temperature, dissolved oxygen, conductivity, alkalinity and hardness).

Parameter	Methods	Method Detection Limit/ Variance
pH	Electrometric method 4500-H^+ B: Orion model 420A (APHA 2012)	0.01 S.U.
Temperature	Direct measurement HOBO pendant® Data Logger	$\pm$ 0.53°C
Dissolved Oxygen	Direct measurement Hach® HQ30d Portable Meter with IntelliCAL™ LDO101 Standard Luminescent/Optical Dissolved Oxygen (LDO) Probe	$\pm$ 0.1 mg/L
Conductivity	Direct measurement 2510 B: YSI 30 (APHA 2012)	0.1 µS/cm
Alkalinity	Titration method 2320 B (APHA 2012)	2 mg/L as $CaCO_3$
Hardness	EDTA Titrimetric Method 2340 C (APHA 2012)	2 mg/L as $CaCO_3$

Table 4.2 Copper sulfate pentahydrate concentrations for static and pulse exposure toxicity experiments for *Pimephales promelas*.

Targeted Copper Concentrations (*µ*g Cu/L)	**Half-life**
Untreated Control, 100, 200, 300, 400, 500	NA[a]
Untreated Control, 600, 800, 1000, 1200, 1,500, 1,800	1.5 h
Untreated Control, 300, 500, 700, 900, 1,100	4 h
Untreated Control, 200, 300, 400, 500, 600	8 h
Untreated Control, 100, 200, 300, 400, 600	15 h

[a]NA=not applicable. Conducted as a 96 hour static non-renewal toxicity experiment.

Table 4.3 Average measured water characteristics (i.e. pH, conductivity, alkalinity, hardness, temperature and dissolved oxygen concentration) in exposures (n=37).

Parameter	Average ± Standard Deviation
pH (S.U.)	7.64 ± 0.43
Conductivity (μS/cm)	326 ± 26
Alkalinity (mg $CaCO_3$/L)	72 ± 9
Hardness (mg $CaCO_3$/L)	94 ± 15
Temperature (°C)	23.3 ± 0.8
Dissolved Oxygen (mg O_2/L)	8.4 ± 0.3

Table 4.4 Results from static toxicity experiments for *Pimephales promelas* exposed to copper sulfate pentahydrate[a].

Life stage	Method	Exposure duration	LC_{50} and 95% CI	Reference
<24 hour	Static non-renewal	168 hours	169 (89-284)	Kinley et al. 2016
<24 hour	Static non-renewal	96 hours	408 (272-669)	Geer et al. 2016
$\leq$24h	Static non-renewal	96 hours	230[b]	Johnson et al. 2008
Not specified	Static renewal with exposure water from experiment initiation	96 hours	656[b]	Murray-Gulde et al. 2002
<24h	Not specified	48 hours	19.2 ± 3.1[c]	Mastin and Rodgers 2000

[a] Exposures were elicited in moderately hard water (61 – 120 mg $CaCO_3$/L, Briggs and Ficke) with an alkalinity of 51 – 100 mg $CaCO_3$/L. Responses were measured as acid soluble copper concentrations resulting in 50% survival (LC_{50}).
[b] confidence interval not specified
[c] standard deviation (n=3).

Table 4.5 Calculated LC_{50s}, 95% confidence intervals, potency slopes and corresponding correlation coefficients (R^2) for *Pimephales promelas* exposed to copper sulfate pentahydrate in static non-renewal (96 hour) and pulse exposure (1.5, 4, 8 and 15 hour half-lives) toxicity experiments.

Exposure Duration		LC_{50} (*µ*g Cu/L)	95% Confidence Interval (*µ*g Cu/L)	Potency Slope (%survival/ *µ*g Cu/L)	R^2 for potency slope
Static 96 hour	Soluble	166	142-189	-0.274	0.99
	Acid Soluble	162	140-183	-0.284	0.99
1.5-hour half-life	Soluble	1,044	999-1,091	-0.152	0.98
	Acid Soluble	1,223	1,213-1,233	-0.112	0.89
4-hour half-life	Soluble	375	349-402	-0.200	0.89
	Acid Soluble	535	506-565	-0.159	0.86
8-hour half-life	Soluble	365	339-391	-0.276	0.88
	Acid Soluble	444	411-478	-0.208	0.86
15-hour half-life	Soluble	154	128-179	-0.226	0.98
	Acid Soluble	184	153-215	-0.197	0.99

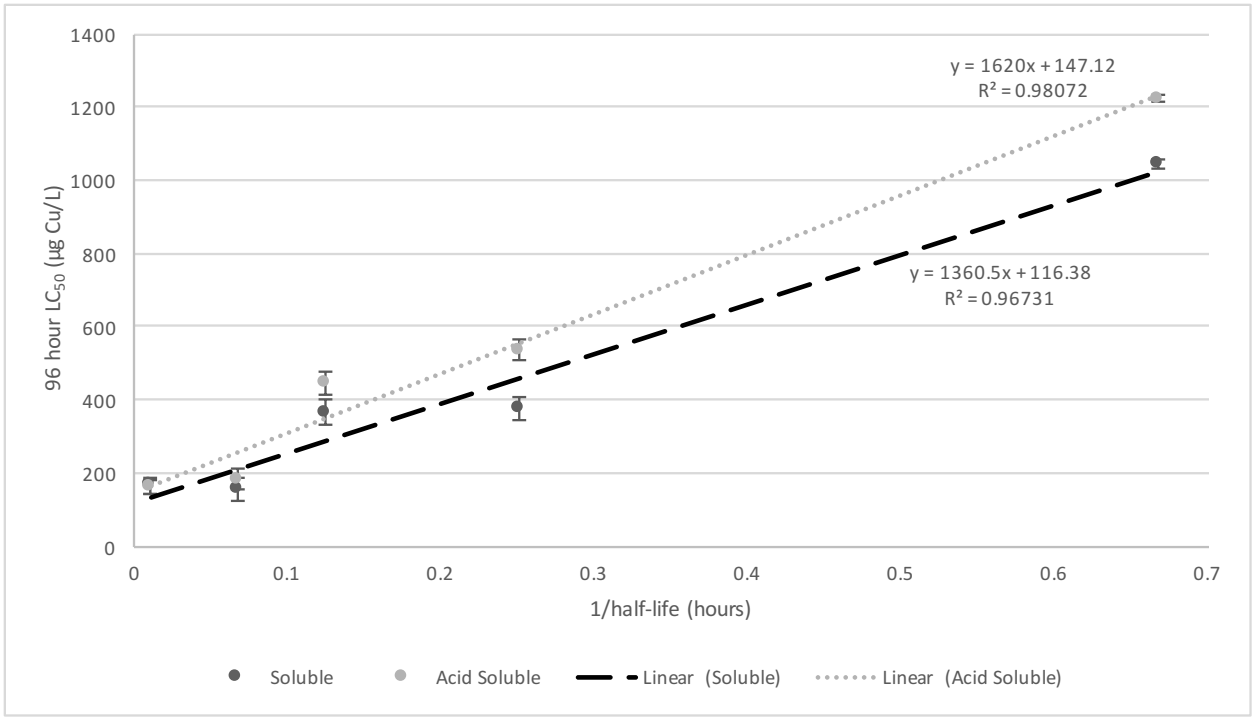

Figure 4.6 Relationships between 96 hour LC_{50s} for static and pulse toxicity experiments and 1/half-life.

CHAPTER FIVE

SUMMARY AND CONCLUSIONS

When copper-based algaecides are applied to aquatic systems, aqueous copper concentrations exceed pretreatment concentrations for minutes to days after the application. These copper exposures are often termed pulse exposures and result in altered responses of organisms as a function of exposure duration. Therefore, prediction of the duration of copper pulse exposures and relationships between exposure duration and consequent effects on organisms is necessary to characterize risks from copper-based algaecides. This research was conducted to: 1) characterize fate processes to model copper-pulse exposures in situ 2) evaluate effects of copper pulse exposures on responses of a target species (i.e. cyanobacterium, *Lyngbya wollei*) and 3) evaluate effects of copper pulse exposures on responses of a non-target species (i.e. the fish, *Pimephales promelas*) (Figure 1.1).

Characterization of Fate Processes to Model Copper-pulse Exposures In Situ

The overall objective of this experiment was to model copper dissipation for an in situ copper-based algaecide application. Scaled experiments (i.e. laboratory, mesocosm, and in situ) were used to measure individual and cumulative copper dissipation rates and subsequently rank dominant processes influencing copper fate. Dilution (half-life = 0.03 days) and sediment sorption (half-life = 3 days) were identified as dominant fate processes based on laboratory scale experiments. When these dissipation rates were incorporated in a mathematic (mass balance, half-life = 0.03 day) and a physical (mesocosm, half-life = 0.02 day) model, predicted copper dissipation rates approximated

dissipation in situ (half-life = 0.03 day). This experiment demonstrated the importance of using dominant fate processes (in this case dilution) to predict copper dissipation rates from pulse exposures.

Evaluate Effects of Copper Pulse Exposures on Responses of a Target Species (i.e. Cyanobacterium, Lyngbya wollei)

The purpose of this experiment was to discern the influence of *Lyngbya wollei* biomass at initiation of a copper algaecide exposure on responses predicted using concentration and exposure duration alone (concentration-exposure time model [CET model]). Of the chelated copper algaecides evaluated, Clearigate®, a formulation with D-limonene and chelated with ethanolamine had sufficient potency and captured a range of cyanobacterial responses within legal label concentrations to be used to develop the CET model. Copper concentrations at 0.4 mg Cu/L, 0.7 mg Cu/L and 1.0 mg Cu/L for 24-hour and 8-hour exposure durations as well as 0.7 mg Cu/L and 1.0 mg Cu/L for a 1 hour exposure duration resulted in maximum cyanobacterial responses (initial biomass of 52 g WW/m^2) measured at completion of the 7-day experiment (87-100% percent damaged trichomes, 13 – 43% wet weight and 47 – 63% dry weight). Based on the previous results, for an exposure of 1 mg Cu/L for 24 hours, maximum responses were predicted although at an initial cyanobacterial biomass of 1,558 g WW/m^2 no response was measured relative to untreated controls. This provided evidence that biomass is a variable driving *Lyngbya wollei* responses and was subsequently incorporated into a model that considers biomass, exposure duration and copper concentration (BDC model) as variables. This new model expanded the initial cyanobacterial biomasses from 52 g

WW/m^2 only (CET model) to a range from 13 g WW/m^2 to 104 g WW/m^2 (BDC model) in which the model is predictive of responses of *Lyngbya wollei*.

Evaluate Effects of Copper Pulse Exposures on Responses of a Non-target Species (i.e. fish Pimephales promelas)

The aim of this experiment was to measure the relationship between exposure duration of a copper-based algaecide, $CuSO_4$•5 H_2O and responses of the fish *P. promelas*. Exposure durations with a range of half-lives (1.5, 4, 8 and 15 hours) were simulated in the laboratory and compared to standard toxicity experiments with static, 96-hour exposures. Responses of *P. promelas* to $CuSO_4$•5H_2O to the static exposure resulted in 96 hour LC_{50s} of 166 μg Cu/L (95% confidence interval [CI], 142-189 μg Cu/L) as soluble copper and 162 μg Cu/L (CI, 140 – 183 μg Cu/L) as acid soluble copper. The relative sensitivity of *P. promelas* to $CuSO_4$•5 H_2O, as indicated by LC_{50s}, (calculated from responses measured 96 hours following exposure initiation) was an order of magnitude less with 1.5 hour half-lives and approximately 3 times less with 4 and 8 hour half-lives. Responses of *P. promelas* to a $CuSO_4$•5 H_2O exposure with a 15 hour half-life were comparable to *P. promelas* responses to the static 96 hour exposure. Results from this experiment demonstrated the relative conservatism of standard laboratory toxicity experiments when applied to pulse exposure scenarios of relatively short durations.

Overall Summary and Conclusions

Fundamentally, an understanding of exposure is needed to anticipate consequent organism responses. The three experiments presented in this dissertation focus on pulse exposures which differ from static exposures because of divergences in exposure

duration. Information is needed on what exposure durations require predictions based on pulse exposures and at what threshold will an exposure duration result in a comparable response as a static exposure. The experiments presented in this dissertation provided approaches to understand the limits and bounds of pulse exposures. Specifically, there are exposure durations (i.e. minutes) in which responses of organisms deviate greatly from responses predicted from static exposures. Alternatively, there are exposure durations in which responses can be accurately predicted with static exposures (i.e. > 0.5 day). For the noxious cyanobacterium *Lyngbya wollei*, this information can be used to target specific exposures to mitigate issues caused by this species. For the non-target species *P. promelas*, this information can be used to minimize or eliminate adverse effects. Ultimately, these experiments provided a characterization of copper pulse exposures from algaecide applications that can be used to inform more efficient use of products and accurate predictions of risk.