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Responses of Aquatic Organisms to Exposures of Sodium Carbonate Peroxyhydrate

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RESPONSES OF AQUATIC ORGANISMS TO EXPOSURES OF SODIUM CARBONATE PEROXYHYDRATE

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ABSTRACT

Sodium carbonate peroxyhydrate (SCP), a granular algaecide containing hydrogen peroxide (H_2O_2), is used to mitigate risks associated with noxious algae. Episodic exposures of SCP algaecides in aquatic systems prompt the need for a fundamental understanding of exposure-response relationships for aquatic organisms, both target and non-target, exposed to H_2O_2 as SCP. In the first experiment, influences of organic carbon on exposures of H_2O_2 as SCP and consequent responses of a frequent problematic alga (cyanobacterium *Microcystis aeruginosa*) were measured. Results indicate that the exposure of H_2O_2 as SCP necessary to control growth of a noxious alga is proportional to the density of the population. Using both density and the concentration of algal derived DOC to scale laboratory results to an *in situ* treatment could improve exposure predictions, which could decrease the chance of applying an ineffective concentration and maintain margins of safety for non-target organisms. In the second experiment, innate sensitivities of *M. aeruginosa* and non-target organisms including a eukaryotic alga (chlorophyte *Pseudokirchneriella subcapitata*), a microcrustacean (*Ceriodaphnia dubia*), a benthic amphipod (*Hyalella azteca*), and a fathead minnow (*Pimephales promelas*) were measured in relatively unconfounded, 96-h laboratory exposures of H_2O_2 as SCP. Results were used to interpret potential risks from SCP applications in aquatic environments. In terms of sensitivities, *M. aeruginosa* ≈ *C. dubia* > *H. azteca* > *P. subcapitata* > *P. promelas* to exposures of H_2O_2 as SCP. These results can be used to predict the distribution of responses likely to occur *in situ*, and indicate that SCP could mitigate risks associated with noxious cyanobacterial growths (e.g. *M.*

aeruginosa) while providing a margin of safety for non-target species. In the final experiment, experiments were conducted in the laboratory to physically model a site-specific exposure-response relationship and predict H_2O_2 exposures and target algal responses prior to the application of an SCP algaecide in a southeastern U.S. reservoir. Portions of the Six and Twenty Creek cove of Hartwell Lake, a man made reservoir bordering South Carolina and Georgia, were treated with Phycomycin® SCP (27% H_2O_2) to control an algal assemblage producing taste and odor. By utilizing algae and water from the study site in both laboratory and field experiments, differences in potential exposure modifying factors (i.e. water characteristics and specific algal sensitivity) were minimized, resulting in exposures and responses measured after the *in situ* application of SCP that were comparable to laboratory predictions. Exposures of H_2O_2 as SCP were labile and dynamic, thus an indirect comparison of laboratory and field experiments (i.e. responses observed *in situ* were used to infer the causative exposure based on results from the laboratory model) was necessary to corroborate the direct comparison of experiments (i.e. exposures eliciting equivalent responses were compared). Data from the experiments in this thesis increase our fundamental understanding of exposure-response relationships from SCP algaecides, and provide information supporting the effective and ecologically sound use of SCP algaecide in water resource management.

DEDICATION

I dedicate this thesis to the friends and family members who have never stopped supporting me, and to the memory of my grandmother, Violet Sykes.

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CHAPTER ONE

INTRODUCTION

Despite occupying a critical niche in aquatic systems, some algae can grow to densities that are problematic or "noxious," or produce secondary compounds such as toxins that interfere with use of water. Problematic algae are operationally defined here as a population or growth of algae that has disrupted or impeded the use(s) of a critical water resource such as drinking water, irrigation, and recreation (Pearl, 1988; Hoagland et al., 2002; Landsberg, 2002; Briand et al., 2003). Some algal genera (e.g. *Anabaena, Aphanizomeneon, Cylindrospermopsis, Euglena, Hapalosiphon, Lyngbya, Microcystis, Nodularia, Nostoc, Oscillatoria, Prymnesium*) produce toxins, which can pose risks for humans, domestic pets, livestock, and wildlife associated with fresh water resources (WHO, 2003; Falconer, 1999; Carmichael et al., 2001; Zurawell et al., 2005). Chemical algaecides are often used by water resource managers as an efficacious, time efficient, economically viable, and environmentally sound management technique to mitigate problems associated with algal growths (Mastin et al., 2002), particularly when immediate action is required.

Sodium carbonate peroxyhydrate (SCP) is an unstudied compound used as an algaecide to control growth of problematic algae. SCP is a granular compound that releases H_2O_2, an oxidant, when added to water. Exposures of H_2O_2 as SCP cause intercellular and extracellular damage that can adversely affect algal cells (Samuilov et al., 2001; Bandala et al., 2004; Qiao et al., 2005; Drabkova et al., 2007a,b; Finnegan et al., 2010; Mikula et al., 2012). A treatment goal for using an SCP algaecide is to

maximize efficacy for target algal species while minimizing risks for non-target species. To provide defensible evidence that will facilitate decisions made to achieve this goal, the experiments in this thesis were designed to evaluate *in situ* factors that could affect exposures and consequent responses, provide a comparison of the relative sensitivity of an array of target and non-target organisms, and demonstrate the predictive capabilities of laboratory studies for *in situ* SCP treatments.

Conceptually, responses of an algal cell or population at a specific site should be a function of the amount of active ingredient in or on each algal cell that will achieve control (Murray-Gulde et al., 2002). If this exposure per cell is estimated, then the total amount of algaecide required to obtain control of an algal population can be estimated. However, because H_2O_2 is an oxidant (Mallick and Mohn, 2000), it may react with organic carbon as well as target and non-target algae, fundamentally changing the exposure of SCP reaching each algal cell. In Chapter 2, "Influence of Dissolved and Particulate Fractions of Organic Carbon on Exposures of a Sodium Carbonate Peroxyhydrate Algaecide and Consequent Responses of *Microcystis aeruginosa*," the influence of organic carbon (particulate and dissolved) on exposures of an SCP algaecide and consequent responses of *M. aeruginosa* were measured. Estimating the "critical burden" for the targeted algae can be used to more accurately predict effective SCP algaecide exposures.

In "Comparative Toxicity of Sodium Carbonate Peroxyhydrate to Freshwater Organisms," responses were measured and compared for a target noxious alga (cyanobacterium *Microcystis aeruginosa*) and non-target organisms including a

eukaryotic alga (chlorophyte *Pseudokirchneriella subcapitata*), a microcrustacean (*Ceriodaphnia dubia*), a benthic amphipod (*Hyalella azteca*), and a fathead minnow (*Pimephales promelas*) exposed to H_2O_2 as SCP. To use SCP effectively and efficiently for controlling noxious algal growths in aquatic systems, comparative toxicity data are needed for both target and non-target species, which are anticipated to have different sensitivities. Laboratory experiments involve exposing organisms in relatively unconfounded situations in order to discern innate sensitivities. Ranking organisms in terms of their sensitivity to SCP provides information about types of algae (e.g. prokaryotic versus eukaryotic) that are relatively sensitive to SCP exposures, and can be used to calculate potential margins of safety for non-target organisms. Additionally, exposure-response relationships derived from laboratory experiments can be used to estimate and contrast the potency of SCP to different organisms. After relative sensitivity information is gained, comparisons of toxicity data for SCP and other algaecides can provide context for the relative toxicity of different active ingredients that are available for use (i.e. H_2O_2, copper formulations, endothal, and diquat dibromide). These comparative toxicity data provide information necessary for making scientifically defensible algal management decisions (Fitzgerald, 1964; Fitzgerald and Jackson, 1979; Mastin et al., 2002; Osgood, 2007).

A primary goal of toxicity testing is to predict responses of organisms *in situ*. In Chapter 3, "Predicting *In Situ* Responses of Taste and Odor Producing Algae in a Southeastern U.S. Reservoir to a Sodium Carbonate Peroxyhydrate Algaecide Using a Laboratory Exposure-Response Model," the use of an SCP algaecide to mitigate a

benthic algal assemblage producing taste and odor compounds in the Six and Twenty Creek cove of Hartwell Lake provided an opportunity to test hypotheses regarding potential convergence of exposures and responses measured in the laboratory and the field.

Objectives

"Influence of Dissolved and Particulate Fractions of Organic Carbon on Exposures of a Sodium Carbonate Peroxyhydrate Algaecide and Consequent Responses of *Microcystis aeruginosa*"

To further develop and expand information on responses of target species to exposures of H_2O_2, the overall objective of this experiment was to measure the collective influence of algal-derived organic carbon (particulate [POC] and dissolved [DOC]) on exposures of an SCP algaecide and consequent responses of *M. aeruginosa*. Specific objectives were to: i) measure the relationship between initial cell density and responses of *M. aeruginosa* to 96-h exposures of H_2O_2 as SCP (in terms of median effect concentrations [EC_{50}] for cell densities); ii) measure relationships between dissolved organic carbon concentration and responses of *M. aeruginosa* to 96-h exposures of H_2O_2 as SCP (in terms of 96-h EC_{50} for cell densities); and iii) compare the relative influences of POC and DOC on exposures of H_2O_2 as SCP achieving the 96-h EC_{50} of *M. aeruginosa*.

"Comparative Toxicity of Sodium Carbonate Peroxyhydrate to Freshwater Organisms"

The overall objective of this study was to compare responses of an array of freshwater organisms following exposures to H_2O_2 as SCP in laboratory formulated water. The specific objectives were to (i) measure and compare responses of a prokaryotic alga (*M. aeruginosa*) and a eukaryotic alga (*P. subcapitata*) in terms of cell density and chlorophyll *a* concentrations to 96-hr exposures of of H_2O_2 as SCP, (ii) measure and compare responses of a vertebrate (*P. promelas*) and invertebrates (*C. dubia* and *H. azteca*) in terms of mortality to 96-hr exposures of H_2O_2 as SCP, (iii) confirm exposures of H_2O_2 resulting from additions of SCP, and (iv) compare measured toxicity of SCP to vertebrates, invertebrates, and algae with published toxicity data for copper algaecide formulations, endothall, and diquat dibromide.

"Predicting *In Situ* Responses of Taste and Odor Producing Algae in a Southeastern U.S. Reservoir to a Sodium Carbonate Peroxyhydrate Algaecide Using a Laboratory Exposure-Response Model"

The overall objective of this study was to evaluate responses of a problematic algal assemblage to laboratory exposures of an SCP algaecide and compare responses with exposures and responses measured *in situ*. Specific objectives were to i) measure responses (in terms of chlorophyll *a* concentrations, phycocyanin concentrations, and cell densities) of a benthic algal assemblage from Hartwell Lake to 7-d exposures of H_2O_2 (as Phycomycin® SCP) in the laboratory, ii) to measure the exposure of H_2O_2 introduced to

Hartwell Lake (as Phycomycin® SCP) and consequent responses of the algal assemblage (in terms of chlorophyll *a* concentrations, phycocyanin concentrations, and cell densities), and iii) compare exposures and responses measured in the laboratory and *in situ*.

Organization of Thesis

This thesis is arranged in subsequent chapters intended for publication in peer-reviewed journals. Therefore, chapters two through four are written and formatted for a specific journal, and some of the introductory information and materials and methods were repeated. Chapter one is in press in the *Journal of Ecotoxicology and Environmental Safety*; chapters two and three are targeted for submission to the *Journal of Ecotoxicology and Environmental Safety*.

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CHAPTER TWO

INFLUENCE OF DISSOLVED AND PARTICULATE ORGANIC CARBON ON EXPOSURES OF A SODIUM CARBONATE PEROXYHYDRATE ALGAECIDE AND CONSEQUENT RESPONSES OF *MICROCYSTIS AERUGINOSA*.

Abstract

Algaecides formulated with sodium carbonate peroxyhydrate (SCP), a granular form of the oxidant hydrogen peroxide (H_2O_2), are used to control the growth of problematic algae and restore uses of critical water resources. The amount of H_2O_2 per cell required to control problematic algae (i.e. dose) can be used to scale exposures for densities of algae encountered *in situ*. However, oxidizable constituents in a water resource (i.e. dissolved and particulate organic carbon) can alter the dose of H_2O_2 reaching each algal cell, increasing the concentration of H_2O_2 necessary to achieve a desired response endpoint (e.g. EC_{50}). The overall objective of this study was to measure the influence of organic carbon (particulate [POC] and dissolved [DOC]) on exposures of an SCP algaecide and consequent responses of a frequent problematic alga (cyanobacterium *Microcystis aeruginosa*). To achieve this overall objective, 96-h median effects concentrations (96-h EC_{50} values) for a series of cell densities of *M. aeruginosa* exposed to H_2O_2 as SCP were measured and compared. As the density of algae increased from 9.72×10^5 to 2.31×10^7 cells mL^{-1}, measured 96-h EC_{50} values for *M. aeruginosa* in terms of cell density increased from 0.9 mg H_2O_2/L to 30.9 mg H_2O_2/L. The calculated dose of H_2O_2 as SCP achieving these EC_{50} values increased concomitantly with cell density from 8.79×10^{-10} mg H_2O_2/cell to 1.34×10^{-9} mg H_2O_2/cell. Calculated doses

likely increased due to competitive reactions between H_2O_2 and algal-related DOC, as increases in cell density and the dose achieving EC_{50} values were coupled with an increase in DOC from 4±1 mg/L to 24±1 mg/L. When designing *in situ* treatments for problematic algae using an SCP algaecide, both the density of algae and DOC can be used to scale exposures of SCP. Based on the mass of SCP that can be applied to a water resource, it is conceivable that algae *in situ* could achieve a density that is essentially unmanageable with a single application. Implementing a treatment before this density is achieved increases the likelihood of success with a single treatment and decreases the amount of product required, decreasing costs associated with treatment and potential risks for non-target organisms. Incorporating algal density and DOC concentrations into predictions of effective algaecide applications could decrease the possibility of applying an ineffective concentration and maintain margins of safety for non-target organisms.

Introduction

Algaecides are commonly used to restore uses of critical water resources impeded by growths of problematic algae. Exposures of algaecides and responses of target algae can be influenced by site-specific environmental characteristics (Fitzgerald et al., 1964; Breault et al., 1996; Deaver and Rodgers, 1996; Borgmann et al., 2005). Sodium carbonate peroxyhydrate (SCP) is a relatively unstudied algaecide containing hydrogen peroxide (H_2O_2; USEPA, 2002) that can be used to selectively control cyanobacteria (Drabkova et al., 2007; Geer et al., in press). Exposures of H_2O_2 from applications of an SCP algaecide oxidize algal cells (Drabkova et al., 2007a,b) causing intracellular and cell membrane damage that leads to cell death (Mallick and Mohn, 2000; Finnegan et al., 2010). As an oxidant, however, H_2O_2 may react with organic carbon apart from target/non-target algal cells. Therefore, when SCP is applied to an aquatic system as a means to control target algae, oxidizable constituents in the system (i.e. density of targeted/non-target algae and the concentration of dissolved organic carbon [DOC]) may influence the actual exposure of H_2O_2 as SCP reaching each algal cell (i.e. the dose). The present study focuses on laboratory experiments to measure the influence of cell density and DOC on concentrations of an SCP algaecide required to elicit mortality.

Algal density can influence the performance of copper algaecides (Fitzgerald, 1964; Nielsen and Kamp-Nielsen, 1970; Moreno-Garrido, 2000; Franklin et al., 2002; Murray-Gulde et al., 2002), and may similarly influence the effectiveness of an SCP application. Considering potential effects of cell density on an SCP algaecide, the exposure of H_2O_2 as SCP required to control a nuisance algal population could

theoretically be calculated as the product of the dose gaining control for one cell and the total density of cells: $X*Y=Z$, where X equals the mass of H_2O_2 that achieves control for one cell (mg H_2O_2 $cell^{-1}$) , Y equals cell density (cells mL^{-1}), and Z equals the concentration (mg H_2O_2 L^{-1}) achieving control of the population or assemblage of target algae. If algal density increases, then the exposure required to gain control of the population should similarly increase in a predictable way. For example, if the density were increased by an order of magnitude, e.g. from 10^5 cells mL^{-1} to 10^6 cells mL^{-1}, then the total exposure to control the population would necessarily increase by a factor of 10.

The density-dependent exposure-response model implies a linear relationship between cell density and the H_2O_2 exposure required to elicit mortality of the algal population. The assumption is that the mass of active ingredient achieving control for one cell is consistent at all cell densities. However, environmental factors competing for the oxidant (H_2O_2) could affect the mass of H_2O_2 available to achieve control for one cell. DOC, the organic fraction that passes through a 0.45-µm filter (Wetzel, 2001), may be subject to oxidation by H_2O_2 as SCP, potentially decreasing the concentration reaching each individual algal cell. Algae are a source of DOC (Wetzel, 2001; Nguyen et al., 2005; Lee 2008, Mostofa et al., 2012), which is released either by metabolic excretion or when cells die and their internal contents are released (Henderson et al., 2008). Furthermore, terrestrial organic matter inputs add to concentrations of algal-derived DOC in receiving source waters. Typically, dissolved fractions are present at greater concentrations than particulate fractions (POC, fraction of organic carbon retained by a 0.45-µm filter [Wetzel, 2001]). In lakes across the United States, DOC concentrations range from 2 to

10 mg L^{-1} (Wetzel, 2001), while POC concentrations range from 0.2 to 1.7 mg L^{-1} (Wetzel 2001). However, the density of a problematic algal population increases POC, potentially in excess of DOC. The total concentration of organic carbon (TOC = DOC + POC) associated with a problematic algal population is usually not uniformly distributed within a water resource. To control problematic algae with an SCP algaecide, the exposure must contact the target algae. Therefore, it follows that algal TOC could have the greatest effect on exposures of SCP and consequent target algal responses.

To test hypotheses regarding density and DOC dependent effects on the performance of an SCP algaecide, laboratory experiments were conducted using the prokaryotic cyanobacterium *Microcystis aeruginosa*, a common problematic alga (WHO, 1993; Falconer, 1999; Carmichael et al., 2001; Zurawell et al., 2005) that is sensitive to H_2O_2 (Drabkova et al., 2007) from SCP exposures (Geer et al., in press). The overall objective of this experiment was to measure the collective influence of algal-derived organic carbon (particulate and dissolved) on exposures of an SCP algaecide and consequent responses of *M. aeruginosa*. Specific objectives were to i) measure the relationship between initial cell density and responses of *M. aeruginosa* to 96-h exposures of H_2O_2 as SCP (in terms of 96-h median effect concentrations [EC_{50}] for cell densities); ii) measure relationships between dissolved organic carbon concentration and responses of *M. aeruginosa* to 96-h exposures of H_2O_2 as SCP (in terms of 96-h EC_{50} for cell densities); and iii) compare the relative influences of POC and DOC on exposures of H_2O_2 as SCP achieving the 96-h EC_{50} of *M. aeruginosa*.

Materials and Methods

Toxicity Testing Procedure

Unicellular *M. aeruginosa* was obtained from the Canadian Phycological Culture Center (CPCC 300) at the University of Waterloo in Ontario, Canada and cultured in COMBO medium (Kilham et al., 1998) at 23±2°C with an 18:6-h light:dark photoperiod, illuminated by cool-white fluorescent bulbs (Residential Ecolux 40 W, GE) at 2660 LUX. The algaecide Phycomycin® SCP (Arch Chemicals a Lonza Business, Applied Biochemists, Alpharetta, GA), was used as the source of SCP. Exposures were prepared by dissolving SCP granules (active ingredient 27.6% H_2O_2; Table 1) in algal growth medium. Toxicity experiments used static, non-renewal exposures in which 200 mL of COMBO medium containing *M. aeruginosa* were exposed in 250 mL borosilicate beakers to a series of concentrations of H_2O_2 as SCP, sufficient to capture a 96-h median effects concentration [96-h EC_{50}; concentration at which 50% of the population responded (in terms of cell densities)]. Toxicity experiments were initiated with targeted cell densities between $5x10^5$ and $5x10^7$ cells mL^{-1}. H_2O_2 concentrations were measured spectrophotometrically immediately after dissolution of SCP using the I_3^- method (MDL=0.1 mg H_2O_2 L^{-1}, CV= 1.5%) with a 1 cm cuvette and SpectraMax®M2 Microplate Reader (Molecular Devices Corp. Sunnyvale, CA 94089; Klassen et al., 1994; Kinley et al., 2015). Responses of algae to exposures (i.e. cell densities) were measured initially and after 96-h. Cell densities were measured using light microscopy and an Improved Neubauer hemocytometer (Hausser Scientific Co. Horsham, PA 19044). Water characteristics in exposures were measured at test initiation and completion. Dissolved

oxygen, pH, and conductivity were measured using a YSI® Model 52 dissolved oxygen meter, Orion® Model 250A pH meter and Triode® electrode, and Orion® Model 142 conductivity meter, respectively. Hardness and alkalinity of samples were measured according to *Standard Methods for Examination of Water and Wastewater* (APHA, 2005).

Initial DOC concentrations were measured in each experiment using high-temperature combustion (MDL=1.0 mg L^{-1}, CV= ±1%), according to standard method 5310B (APHA 2005). Aliquots of *M. aeruginosa* were collected prior to exposure to SCP and filtered through a Millipore® 0.45µm nitrocellulose filter to remove algal cells. Calibration curves were prepared by dissolving potassium hydrogen phthalate (KHP; ACS grade; ACROS Organics) in NANOpure® water. Standards and samples were maintained at 4°C prior to analysis (APHA, 2005) and analyzed with a Shimadzu model TOC-V total organic carbon/total nitrogen analyzer (Shimadzu Scientific Instruments Columbia, MD 21046).

Statistical Analysis

For each experiment, 96-h EC_{50} values for *M. aeruginosa* were calculated from exposure-response relationships in terms of cell densities using non-linear regression with a sigmoid and 4P logistic fit function. Inflection points calculated were used as EC_{50} values. Calculated EC_{50} values were compared among different initial cell densities and among different initial DOC concentrations. All data were analyzed using JMP v. 11.2.1 (2013; $\alpha = 0.05$).

Results and discussion

Relationship Between 96-h EC_{50} Values and Initial Cell Density

Measured 96-h EC_{50} values for *M. aeruginosa* exposed to H_2O_2 as SCP increased with increasing algal density. As initial density of *M. aeruginosa* increased from $9.7x10^5$ to $2.3x10^7$ cells mL^{-1}, measured 96-h EC_{50} values (in terms of cell density) for *M. aeruginosa* increased from 0.9 mg H_2O_2 L^{-1} to 30.9 mg H_2O_2 L^{-1} (Table 2, Figure 1). EC_{50} values were linearly related to initial algal density, increasing by 1.4 mg H_2O_2 L^{-1} for each incremental increase in density of $1.0x10^6$ cells mL^{-1} (R^2=0.999; Figure 2).

These results support those of Fitzgerald (1964), Murray-Gulde et al. (2002), and Bishop and Rodgers (2012), demonstrating that the amount of algaecide controlling a problematic algal population is proportional to the amount of algae present. By inverse prediction, the relationship can be used to estimate the densities of algae that may be treated within an algaecide's recommended label concentrations. For example, 10.2 mg H_2O_2 L^{-1} is, at present, the maximum recommended application concentration for the SCP algaecide used in this experiment (Table 1). For laboratory cultured *M. aeruginosa* in COMBO medium, the estimated density with an EC_{50} of 10.2 mg H_2O_2 L^{-1} was $8.1x10^6$ cells mL^{-1}. In the context of an *in situ* SCP application, multiple treatments could increase costs and increase risks for non-target organisms. Risks for non-target organisms from exposures to SCP can be estimated by margins of safety, which are a measure of the difference between the concentration of SCP used to control a target alga and the concentration eliciting toxicity to the non-target organism (Murray-Gulde et al., 2002). As demonstrated in the present laboratory experiment, algaecide concentrations necessary

to achieve an EC_{50} were positively related to initial cell density. Therefore, as initial density of algae *in situ* decreases, the amount of algaecide required to achieve control will likely decrease proportionally, increasing the potential for margins of safety for non-target organisms.

Relationship Between DOC Concentrations and 96-h EC_{50} Values

Measured concentrations of dissolved organic carbon associated with each algal cell density increased from 4±1 mg L^{-1} to 24±1 mg L^{-1} as algal density increased from $9.7x10^5$ cells mL^{-1} to $2.3x10^7$ cells mL^{-1} (Table 2). There was a linear relationship between increasing dissolved organic matter and 96-h EC_{50} values of *M. aeruginosa*; 96-h EC_{50} values increased by 1.6 mg H_2O_2 L^{-1} for each mg L^{-1} increase in DOC (R^2=0.994, Figure 2).

Murray-Gulde et al. (2002) and Bishop and Rodgers (2012) concluded that the relationship between algal density and algaecide concentrations achieving control could be used to predict the exposure required to control any density of algae. For this conclusion to be valid in the present study, the critical burden (i.e. concentration of H_2O_2 per cell achieving 96-h EC_{50}; Murray-Gulde et al., 2002; Bishop and Rodgers, 2011) would need to be constant and independent of population density. Critical burdens were calculated mathematically by dividing 96-h EC_{50} values by the associated cell density. As initial densities of *M. aeruginosa* increased from $9.7x10^5$ cells mL^{-1} to $2.3x10^7$ cells mL^{-1}, calculated critical burdens increased from $8.8\ x10^{-10}$ mg H_2O_2 $cell^{-1}$ to $1.3x10^{-9}$ mg H_2O_2 $cell^{-1}$ (Table 2). To determine the relative significance of differences between calculated critical burdens, each calculated critical burden was used to predict 96-h EC_{50} values for

the remaining two initial algal densities, and predicted 96-h EC_{50} values were compared with measured 96-h EC_{50} values. Predicted 96-h EC_{50} values were on average 26.8±19.1% different from measured values (Table 3).

Because calculated critical burdens achieving comparable response endpoints increased as initial densities of *M. aeruginosa* increased, and increasing initial densities were coupled with increasing DOC concentrations, calculated critical burdens likely differed because of H_2O_2 reactions with algal DOC. Critical burdens (mg H_2O_2 $cell^{-1}$) for each initial algal density were derived mathematically, and therefore include concentrations of H_2O_2 reacting with each algal cell and additional H_2O_2 likely reacting with DOC. While calculated critical burdens of both $9.7x10^5$ and $3.1x10^6$ cells mL^{-1} underestimated the measured EC_{50} at $2.3x10^7$ cells mL^{-1}, the percent error was greatest when using the critical burden for $9.7x10^5$ cells mL^{-1} as the predictor. Since there was a greater concentration of DOC present at the highest initial cell density ($2.3x10^7$ cells mL^{-1}), the concentration of H_2O_2 reacting with each cell was likely decreased to a greater extent by reactions with DOC at $2.3x10^7$ cells mL^{-1} than at lower cell densities of $9.7x10^5$ or $3.1x10^6$ cells mL^{-1}.

Relative Influence of Cell Density and DOC on 96-h EC_{50} Values

Results from this study demonstrate that both density of algae and DOC concentrations affect exposures of H_2O_2 as SCP necessary to achieve a response endpoint (i.e. 96-h EC_{50}) for *M. aeruginosa*. Both density and DOC could be used to predict the 96-h EC_{50}, however, there was also a correlation between cell density and DOC (Figure 2). This observation was expected, as algae produce and excrete organic matter

(Myklestad, 1995; Henderson et al., 2008; Qu et al., 2012). Because increasing production and excretion of organic matter is a function of an increasing number of algal cells, measured 96-h EC_{50} values were influenced primarily by cell density, rather than dissolved organic carbon.

Although density was a stronger predictor of responses of *M. aeruginosa* to SCP exposures, the effect of DOC can be significant. Predicted EC_{50} values differed from measured EC_{50} values by an average of 26.8 ± 19.1%, or 3.7 ± 5.0 mg H_2O_2/L (Table 3). Small changes in peroxide concentrations (i.e. 3 mg/L) could affect algaecide performance if potency slopes are sufficiently steep such that (1) responses are not consistent with the desired level of target algal control, or (2) margins of safety for non-target organisms decrease without additional control of the target algae.

Conclusions

Impacts of algal density and algal DOC on concentrations of H_2O_2 as SCP required to achieve the 96-h EC_{50} of *M. aeruginosa* were evaluated. As algal density and DOC increased, 96-h EC_{50} values for *M. aeruginosa* exposed to H_2O_2 as SCP increased predictably. The relationship between algal density and concentration of H_2O_2 achieving the EC_{50} could be used to predict the maximum density of algae that could be controlled with a single application. Implementing a treatment before prolific algal growth increases the likelihood of success with a single treatment and decreases the amount of product required, decreasing costs associated with treatment and potential risks for non-target organisms. The maximum density of cells controllable within recommended label concentrations should also be predictable by the concentrations of H_2O_2 per cell achieving the EC_{50}. Estimated critical burdens were not equivalent, and instead were concluded to be different due to reactions between H_2O_2 and algal derived DOC. When scaling laboratory results to an *in situ* treatment with an SCP algaecide, predictions of the exposure necessary to achieve control could be enhanced if DOC and algal density are known, which could decrease the chance of applying an ineffective concentration and maintain margins of safety for non-target organisms.

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TABLES AND FIGURES

Table 2.1: Physical and chemical properties of Phycomycin® SCP.

CAS number	497-19-8[a]
Formulation	SCP and inert ingredients
Active ingredient	85% SCP
Maximum application Concentration	36.9 mg L^{-1} (10.2 mg L^{-1} H_2O_2)[a]
Physical state	Coarse white grains[a]
Water solubility	140g/L at 24ºC[a]
pH	10.4-10.6 s.u. (1% solution)[a]
Boiling Point	Not applicable[a] (SCP decomposes when heated)[b]
Melting point	Not applicable (SCP decomposes when heated)[c]
Partition coefficient n-octanol/water	Not applicable (sodium carbonate peroxyhydrate is an organic salt)[b]

[a]AB (2007)
[b]OECD (2006)
[c]HERA (2002)

Table 2.2: Mean measured DOC concentrations, 96-h EC_{50} values (in terms of cell density), and calculated critical burdens (mg H_2O_2 $cell^{-1}$) for three densities of *M. aeruginosa* exposed to H_2O_2 as SCP. Critical burdens were calculated as 96-h EC_{50} value divided by initial cell density.

Initial Cell Density (cells mL^{-1})	DOC (mg/L)	95% CI	96-h EC_{50} (mg H_2O_2 L^{-1})	95% CI	Calculated Critical Burden (mg H_2O_2 $cell^{-1}$)
$9.7x10^5$	4	3-5	0.9	0.7-1.0	$8.8x10^{-10}$
$3.1x10^6$	7	6-8	2.9	2.5-3.4	$9.3x10^{-10}$
$2.3x10^7$	24	23-25	30.9	22.7-39.0	$1.3x10^{-9}$

Table 2.3: Comparison of predicted and measured 96-h EC_{50} values for *M. aeruginosa* exposed to H_2O_2 as SCP. Predicted 96-h EC_{50} values were determined mathematically by multiplying calculated critical burden (i.e. H_2O_2 concentration achieving 96-h EC_{50}) by initial density of algae. Percent error calculated as: (measured 96-h EC_{50} – predicted 96-h EC_{50}) / measured 96-h EC_{50}.

Density Used to Calculate Critical Burden (cells mL^{-1})	Calculated Critical Burden (mg H_2O_2 $cell^{-1}$)	Initial Density of Algae Predicted (cells mL^{-1})	Predicted 96-h EC_{50} (mg H_2O_2 L^{-1})	Measured 96-h EC_{50} (mg H_2O_2 L^{-1})	Measured 96-h EC_{50} – Predicted 96-h EC_{50}	Percent Error
$9.7x10^5$	$8.8x10^{-10}$	$3.1x10^6$	2.7	2.9	0.2	6.9
$9.7x10^5$	$8.8x10^{-10}$	$2.3x10^7$	20.3	30.9	10.6	34.3
$3.1x10^6$	$9.3x10^{-10}$	$9.7x10^5$	0.9	0.9	0.0	0.0
$3.1x10^6$	$9.3x10^{-10}$	$2.3x10^7$	21.5	30.9	9.4	30.4
$2.3x10^7$	$1.3x10^{-9}$	$9.7x10^5$	1.3	0.9	-0.4	44.4
$2.3x10^7$	$1.3x10^{-9}$	$3.1x10^6$	4.2	2.9	-1.3	44.8

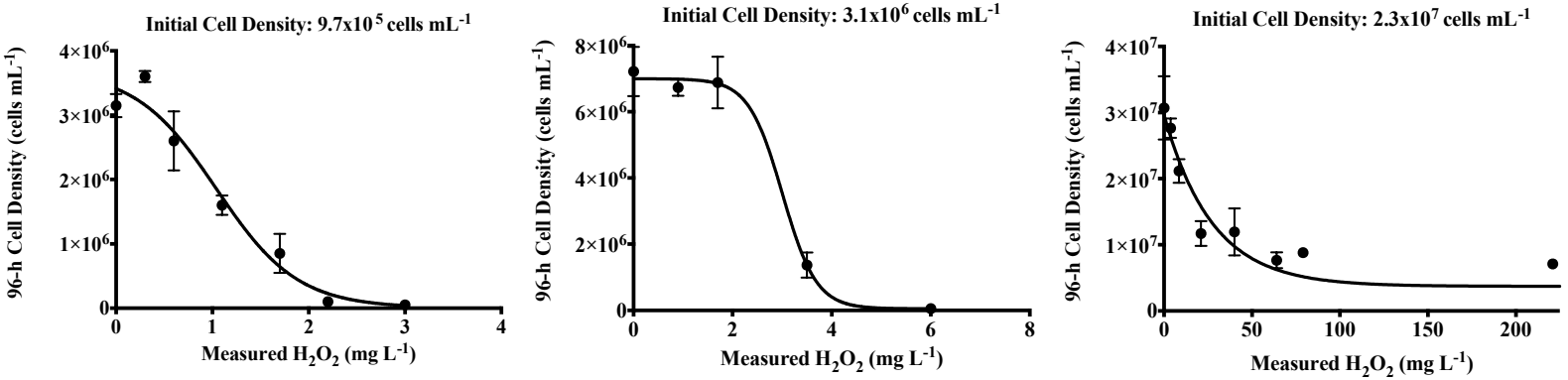

Figure 2.1: Mean responses of *M. aeruginosa* to measured exposures of H_2O_2 as SCP at initial densities of $9.7x10^5$ cells mL^{-1}, $3.1x10^6$ cells mL^{-1}, and $2.3x10^7$ cells mL^{-1} (n=3). Error bars represent ±1 standard deviation.

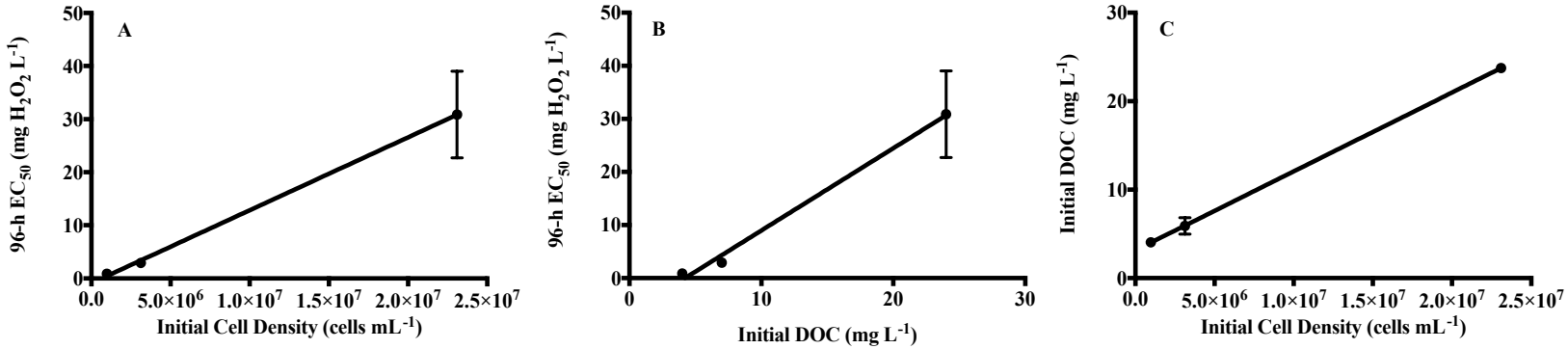

Figure 2.2: (A) Relationship between initial cell densities (n=3) and 96-h EC_{50} values, (B) correlation between initial concentrations of DOC (n=3) and 96-h EC_{50} values, and (C) correlation between initial cell densities (n=3) and initial concentrations of DOC (n=3). 96-h EC_{50} values were measured for *M. aeruginosa* exposed to H_2O_2 as SCP. Error bars represent 95% CI.

CHAPTER THREE

COMPARATIVE TOXICITY OF SODIUM CARBONATE PEROXYHYDRATE TO FRESHWATER ORGANISMS

Abstract

Sodium carbonate peroxyhydrate (SCP) is a granular algaecide containing H_2O_2 as an active ingredient to control growth of noxious algae. Measurements of sensitivities of target and non-target species to hydrogen peroxide (H_2O_2) are necessary for water resource managers to make informed decisions and minimize risks for non-target species when treating noxious algae. The objective of this study was to measure and compare responses among a target noxious alga (cyanobacterium *Microcystis aeruginosa*) and non-target organisms including a eukaryotic alga (chlorophyte *Pseudokirchneriella subcapitata*), a microcrustacean (*Ceriodaphnia dubia*), a benthic amphipod (*Hyalella azteca*), and a fathead minnow (*Pimephales promelas*) to exposures of H_2O_2 as SCP. H_2O_2 exposures were confirmed using the I_3^- method. SCP margins of safety for these organisms were compared with published toxicity data to provide context for other commonly used algaecides and herbicides (e.g. copper formulations, endothall, and diquat dibromide). Algal responses (cell density and chlorophyll *a* concentrations) and animal mortality were measured after 96-h aqueous exposures to SCP in laboratory-formulated water to estimate EC_{50} and LC_{50} values, as well as potency slopes. Despite a shorter test duration, *M. aeruginosa* was more sensitive to H_2O_2 as SCP (96-h EC_{50}: 0.9-1.0mg L^{-1} H_2O_2) than the eukaryotic alga *P. subcapitata* (7-d EC_{50}: 5.2-9.2mg L^{-1} H_2O_2),

indicating potential for selective control of prokaryotic algae. For the three non-target animals evaluated, measured 96-h LC_{50} values ranged from 1.0 to 19.7mg L^{-1} H_2O_2. *C. dubia* was the most sensitive species, and the least sensitive species was *P. promelas,* which is not likely to be affected by concentrations of H_2O_2 as SCP that would be used to control noxious algae (e.g. *M. aeruginosa*). Based on information from peer-reviewed literature, other algaecides could be similarly selective for cyanobacteria. Of the algaecides compared, SCP can selectively mitigate risks associated with noxious cyanobacterial growths (e.g. *M. aeruginosa*) while providing a margin of safety for non-target species (e.g. *P. promelas*).

Introduction

Sodium carbonate peroxyhydrate (SCP) is a relatively new, hydrogen peroxide (H_2O_2)-based active ingredient (USEPA, 2004) used by water resource managers in algaecide formulations to control growths of noxious algae that interfere with critical uses of water resources (Gettys et al., 2014). As a granular algaecide, SCP is applied by broadcast over a treatment area from a boat or the shore (Bishop and Rodgers, 2011), dissolved in site water and sprayed, or mixed and injected into the water column, depending on the specific location and distribution of target algae. A treatment goal for using an algaecide to control noxious algal growth is to maximize efficacy for target algal species while minimizing risks for non-target species. To use SCP effectively and efficiently for controlling noxious algal growths in aquatic systems, comparative toxicity data are needed for both target and non-target species, which are anticipated to have different sensitivities. Comparative toxicity studies often evaluate the toxicity of a single constituent to an array of organisms or contrast the toxicity of an array of constituents to a select organism(s). Laboratory experiments involve exposing organisms in relatively unconfounded situations in order to discern innate sensitivity. Ranking organisms in terms of their sensitivity to SCP provides information about types of algae (e.g. prokaryotic versus eukaryotic) that can be effectively controlled by SCP, and can be used to calculate potential margins of safety for non-target organisms. After sensitives are determined for a range of target and non-target organisms exposed to SCP, comparisons of toxicity data for SCP and other algaecides can provide context for the relative toxicity of different active ingredients that are available for use (i.e. H_2O_2, copper formulations,

endothal, and diquat dibromide). These comparative toxicity data provide information necessary for making scientifically defensible algal management decisions (Fitzgerald 1964; Fitzgerald and Jackson 1979; Mastin et al., 2002; Osgood 2007).

Efficacy for target algal populations is central to a successful algaecide treatment design. Drabkova et al. (2007) suggested that prokaryotic cyanobacteria were more sensitive than eukaryotic green algae to exposures of H_2O_2. This indicates a potential benefit of SCP as a selective algaecide for treating target cyanobacteria, while minimizing effects for non-target eukaryotic algal species. Exposure to a series of H_2O_2 concentrations from SCP can identify sensitivities (i.e. EC_{50s}) of and potency (i.e. potency slopes) to *Microcystis aeruginosa* Kützing, a prokaryotic cyanobacterium that can produce toxins (e.g. microcystins and nodularins; WHO 1993, Falconer 1999, Carmichael et al., 2001; Zurawell et al., 2005), and *Pseudokirchneriella subcapitata* Gomont, a eukaryotic green alga that can benefit some water resources as a source of food for aquatic animals (USEPA, 2002). Management decisions to minimize risks for non-target organisms can be supported by comparative toxicity information (i.e. LC_{50} values and potency slopes) for a taxonomic range of animals (i.e. invertebrates and vertebrates). *Ceriodaphnia dubia* Richard (micro crustacean) and *Hyalella azteca* Saussure (amphipod) are invertebrates that inhabit water columns and sediment-water interfaces of North American water bodies, respectively (USEPA 2000; USEPA 2002; APHA 2005). *Pimephales promelas* Rafinesque (fathead minnow) inhabit waters of North America and provide a means for contrasting the responses of a vertebrate with responses of invertebrates test species (USEPA 2002; APHA 2005) in the present context

of exposures to an SCP algaecide. The non-target animal species included in this study are common test organisms for evaluating the potencies of pesticides and other toxic materials (USEPA 2000; USEPA 2002; APHA 2005). This taxonomic range of target and non-target species improves confidence with which toxicity data for SCP can be used for risk assessments.

Algaecide exposure concentrations must be confirmed to evaluate toxicity to target and non-target organisms (USEPA, 2002). The analytical method used to confirm H_2O_2 from SCP exposures must be sensitive enough to measure concentrations of H_2O_2 within the manufacturer's recommended application rates. Klassen et al. (1994) demonstrated a simple and sensitive as well as accurate method for measuring H_2O_2 concentrations as low as ~0.1mg L^{-1} by reacting samples with acidified potassium iodide (KI) and using visible wavelength spectrometry to measure the optical absorbance of the triiodide (I_3) formed. Kinley et al. (2015) indicated that the attributes of the I_3^- method (i.e. detection limit and storage stability) are sufficient to confirm concentrations of H_2O_2 from SCP additions in laboratory toxicity tests.

The overall objective of this study was to compare responses of an array of freshwater organisms following exposures to H_2O_2 as SCP in laboratory formulated water. To achieve this overall objective, specific objectives were to (i) measure and compare responses of a prokaryotic alga (*M. aeruginosa*) and a eukaryotic alga (*P. subcapitata*) in terms of cell density and chlorophyll *a* concentrations to 96-hr exposures of H_2O_2 as SCP, (ii) measure and compare responses of a vertebrate (*P. promelas*) and invertebrates (*C. dubia* and *H. azteca*) in terms of mortality to 96-hr exposures of H_2O_2 as

SCP, (iii) confirm exposures of H_2O_2 resulting from additions of SCP, and (iv) compare measured toxicity of SCP to vertebrates, invertebrates, and algae with published toxicity data for copper algaecide formulations, endothall, and diquat dibromide.

Materials and Methods

Preparation of SCP exposures

The algaecide Phycomycin® SCP (Arch Chemicals a Lonza Business, Applied Biochemists, Alpharetta, GA; Table 1), was used as the source of SCP. Exposures were accomplished by dissolving SCP granules (27.6% H_2O_2; AB, 2007) in moderately hard water or algal growth medium. H_2O_2 concentrations were measured spectrophotometrically immediately after complete dissolution of SCP using the I_3^- method (MDL=0.1 mg H_2O_2 L^{-1}, CV= 1.5%) with a 1 cm cuvette and SpectraMax®M2 Microplate Reader (Molecular Devices Corp. Sunnyvale, CA 94089; Klassen et al., 1994; Kinley et al., 2015).

Toxicity Testing Procedures

Static, non-renewal exposures were conducted in 250 mL borosilicate beakers (USEPA 1996a, 1996b). All organisms were exposed to a series of H_2O_2 concentrations as SCP to elicit responses ranging from no response to complete animal mortality or inhibition of algal growth.

P. subcapitata was obtained from the University of Texas culture collection (UTEX 1648, Austin, TX), and *M. aeruginosa* from the Canadian Phycological Culture Center (CPCC 300) at the University of Waterloo in Ontario, Canada. Prior to testing, both algae were grown in COMBO medium (Kilham et al., 1998) with an 18:6-h light:dark photoperiod at 23±2°C, illuminated by cool-white fluorescent bulbs (Residential Ecolux 40 W, GE) at 2660 LUX. Algae were exposed in 250 mL beakers containing 200 mL of COMBO medium with a cell density of ~10^6 cells mL^{-1}. Responses

of algae to exposures (i.e. cell densities and chlorophyll *a* concentrations) were measured initially and after 96-h. Cell densities were measured using light microscopy and an improved Neubauer hemocytometer (with a gridded sample chamber, MDL=5x10^4 cells mL^{-1}; Hausser Scientific Co. Horsham, PA 19044) and chlorophyll *a* concentrations were measured fluorometrically (MDL=10µg L^{-1}, CV=±10%) using a SpectraMax®M2 Microplate Reader (Molecular Devices Corp. Sunnyvale, CA 94089; APHA, 2005).

Freshwater animals (*P. promelas, C. dubia,* and *H. azteca*) were cultured at Clemson University's Aquatic Animal Research Laboratory (AARL) according to methods of the United States Environmental Protection Agency (USEPA, 2002), and in compliance with Clemson University's Institutional Animal Care and Use Committee (IACUC) protocols. All toxicity tests for *P. promelas* were conducted by exposing 30 organisms (<24-h old) per concentration (10 organisms per replicate for 3 replicates) in 250 mL borosilicate beakers. Toxicity tests for *C. dubia* were conducted by exposing 20 organisms (<24-h old) per concentration (5 organisms per replicate for 4 replicates) in 15 mL borosilicate vials. During exposures, *C. dubia* were fed once daily with 200 µL of a 1:1 mixture of *P. subcapitata* and YCT (yeast, cerophyll, trout chow). Toxicity tests for *H. azteca* were conducted by exposing 30 organisms (2-3 weeks old) per concentration (10 organisms per replicate for 3 replicates) in 250 mL borosilicate beakers. Amphipods were fed at test initiation with 2-3 7mm maple leaf disks. At least five exposure concentrations were used for each experiment, and untreated controls were moderately hard water only. Toxicity tests were conducted with an 18:6-h light:dark photoperiod at

23±2°C. After 96-h, the number of live organisms for each exposure concentration were counted (ASTM, 2014).

For quality assurance and quality control confirming the health of test organisms and ensuring precision through time, reference toxicity tests were conducted concurrently with SCP tests for all test species using a recommended reference toxicant, copper sulfate ($CuSO_4 \bullet 5H_2O$; Fisher Scientific; Jop et al., 1986; USEPA, 1991). Exposures as acid soluble copper concentrations were confirmed using flame atomic absorption spectroscopy and graphite atomic absorption spectroscopy (Agilent PSD 120 atomic absorption spectrometer; APHA, 2005).

Water characteristics in exposures were measured at test initiation and completion. Dissolved oxygen, pH, and conductivity were measured using a YSI® Model 52 dissolved oxygen meter (±0.1 mg/L), Orion® 4-Star pH meter and Triode® electrode (±0.01 SU), and YSI® 30 conductivity meter (±1 $\mu S/cm^2$), respectively. Hardness (±2 mg/L as $CaCO_3$) and alkalinity (±2 mg/L as $CaCO_3$) of samples were measured according to *Standard Methods for Examination of Water and Wastewater* (APHA, 2005).

Statistical analyses

Exposure-response relationships were calculated for each organism. For algal species, cell density and chlorophyll *a* concentrations were analyzed using non-linear regression with a sigmoid and 4-parameter logistic fit function. Inflection points calculated are synonymous with EC_{50} values. For animal species, 96-h median lethal effect concentrations (96-h LC_{50}) were calculated using Probit analysis. Potency slopes for all organisms were calculated using regression analyses following the logistic (algae)

or Probit (animals) procedures. The linear portion of each potency curve was used to derive regression equations estimating the potency slopes (Fuentes et al., 2011). Calculated median effect concentrations and slopes were used to compare responses of target and non-target organisms. All data were analyzed using JMP v. 11.2.1 (2013; α = 0.05).

Comparisons with other algaecides

To provide context for these data and data for other algaecides, a strategic literature review was performed to obtain target and non-target toxicity data (i.e. EC/LC_{50} values) for endothall, diquat dibromide, and formulations of copper based-algaecides. Criteria for inclusion of published toxicity data were (i) data were peer-reviewed, (ii) relevant response endpoints were measured, and (iii) sufficient information was provided for accurate interpretation of data [i.e. exposure duration, product versus active ingredient (i.e. diquat dibromide v. diquat cation, endothall salt v. endothall acid), water characteristics, age of test organisms]. Median effect concentrations were used to compare potencies of each algaecide for target organisms (i.e cyanobacteria). To compare toxicity of SCP and other algaecides to non-target organisms, margins of safety (MOS) were calculated. A margin of safety is the concentration at which control of target algae was obtained compared to concentrations causing adverse effects on non-target species.

Results and Discussion

Responses of target and non-target algae

At an initial cell density of $9.7x10^5$ cells mL^{-1}, 96-h EC_{50} values for *M. aeruginosa* in terms of chlorophyll *a* and cell density were 1.0 and 0.9 mg L^{-1} H_2O_2, respectively (Table 2). 96-h potency slopes for *M. aeruginosa* in terms of chlorophyll *a* and cell density were 50.6 percent response/mg H_2O_2 L^{-1} and 49.0 percent response/mg H_2O_2 L^{-1}, respectively. Densities of both *M. aeruginosa* and *P. subcapitata* increased in untreated controls throughout the course of the experiment.

The non-target green alga *P. subcapitata* was less sensitive by an order of magnitude (in terms of 96-h and 7-d EC_{50s}) than the target cyanobacterium *M. aeruginosa* at initial cell densities of 7.92×10^5 cells mL^{-1} and 9.72×10^5 cells mL^{-1}, respectively. Responses of *P. subcapitata* (chlorophyll *a* and cell densities) manifested in 96-h from exposures less than 276 mg L^{-1} H_2O_2 were insufficient to calculate EC_{50s}. Therefore, the experiment duration was extended to 7-d, and the range of responses manifested after this duration was used to calculate median effects concentrations. 7-d EC_{50} values in terms of chlorophyll *a* and cell density were 5.2 and 9.2 mg L^{-1} H_2O_2, respectively (Table 2). Furthermore, *P. subcapitata* was less sensitive than *M. aeruginosa* in terms of potency slopes; 7-d potency slopes of *P. subcapitata* in terms of chlorophyll *a* and cell density were 7.2 percent response/mg H_2O_2 L^{-1} and 7.4 percent response/mg H_2O_2 L^{-1}, respectively.

Often, a goal of an algaecide application is to selectively control a noxious alga or assemblage of algae. Supporting results from this study, other studies comparing

responses of cyanobacteria and eukaryotic algae reported cyanobacteria were more sensitive to H_2O_2 (Barroin and Feuillade, 1986; Drabkova et al., 2007a, 2007b; Barrington and Ghadouani, 2008; Matthijs et al., 2012; Barrington et al., 2013; Burson et al., 2014). Wide ranging responses of algae to algaecide exposures have been attributed to characteristics of the algae (e.g. structure, Fitzgerald, 1964; Fattom and Shilo, 1984; Speziale et al., 1991; Dyck, 1994). Cyanobacteria contain photosynthetic apparatuses in the thylakoid membrane and inter membrane space, in proximity to the plasma membrane (Barroin and Feuillade, 1986; Baulina, 2012), whereas eukaryotic algae have internalized, discrete, membrane bound organelles (i.e. chloroplasts; Barroin and Feuillade, 1986). Toxicity of H_2O_2 has been attributed to formation of hydroxyl radicals and resulting general oxidation of biomolecules (Russel et al., 2003; Drabkova et al., 2007; Finnegan et al., 2010). Targeted biomolecules include photosynthetic apparatuses as well as photosynthetic pigments (Drabkova et al., 2007). Internal, membrane-bound organelles may offer additional protection for photosynthetic apparatuses along with the cell's plasma membrane and cell wall when algae are exposed to H_2O_2.

Responses of non-target animals

In terms of both median lethal effect concentrations and potency slopes, invertebrates were more sensitive to 96-h exposures of H_2O_2 than the vertebrate *P. promelas* (Figure 3). The most sensitive invertebrate in this study was *C. dubia*: the 96-h LC_{50} for *C. dubia* was 1.0 mg L^{-1} H_2O_2 (Table 2) and the potency slope was 28.4 percentage mortality/mg H_2O_2 L^{-1}. The amphipod *H. azteca* was less sensitive than *C. dubia*: the 96-h LC_{50} was 3.6 mg L^{-1} H_2O_2 and the potency slope was 21.2 percentage

mortality/mg H_2O_2 L^{-1}. The vertebrate *P. promelas* was less sensitive than both invertebrates used in this study: the 96-h LC_{50} was 19.7 mg L^{-1} H_2O_2 and the potency slope was 5.8 percentage mortality/mg H_2O_2 L^{-1}.

Differences in toxicity of H_2O_2 as SCP to invertebrates and vertebrates are likely due to the mechanism of action, as well as organism sizes (Rand, 1995; Wright and Welbourn, 2002). The toxicity of H_2O_2 is attributed to general oxidation of biomolecules, including nucleic acids, proteins, lipids, and large polymers (Wright and Welbourn, 2002; Russel et al., 2003; Drabkova et al. 2007, Finnegan et al., 2010). Toxicity is likely a function of the quantity of H_2O_2 molecules reaching active sites. Larger organisms (i.e. *P. promelas*) would need to be exposed to a greater concentration compared to a smaller organism in order to involve the same concentration per unit weight and manifest similar responses (Wright and Welbourn, 2002).

Water characteristics

Water characteristics (pH, dissolved oxygen, conductivity, alkalinity, and hardness) measured at test initiation and completion were within ranges for tolerances of organisms (Table 5; ASTM, 2014). There was a trend of increasing alkalinity, conductivity, and pH in comparison with the untreated control with increasing concentrations of SCP (Table 3), likely due to adding mg L^{-1} level concentrations of SCP and initial alkalinity of test waters.

Reference toxicant exposures and responses

For reference toxicity tests, measured acid soluble copper concentrations from additions of copper sulfate were 98% ±8.7% of targeted exposures (Table 5). Water

characteristics did not change from test initiation to test conclusion, and were within tolerance limits for the organisms tested (Table 3; ASTM, 2014).

At an initial density of 4.73 x 10^6 cells mL^{-1} the 96-h EC_{50} values for *M. aeruginosa* in terms of chlorophyll *a* and cell density were 0.245 and 0.086 mg L^{-1} Cu as copper sulfate, respectively (Table 4). At an initial density of 8.0 x 10^5 cells mL^{-1} the 96-h EC_{50} values for *P. subcapitata* in terms of chlorophyll *a* and cell density were 1.65 mg L^{-1} Cu as copper sulfate and 0.692 mg L^{-1} Cu as copper sulfate, respectively. Potency slopes for *M. aeruginosa* in terms of chlorophyll *a* and cell density were 149.7 percent response/mg Cu L^{-1} and 77.8 percent response/mg Cu L^{-1}, respectively. Potency slopes for *P. subcapitata* in terms of chlorophyll *a* and cell density were 12.4 percent response/mg Cu L^{-1} and 12.4 percent response/mg Cu L^{-1} , respectively. *M. aeruginosa* and *P. subcapitata* densities in untreated controls increased throughout the course of the experiment. Responses of *M. aeruginosa* and *P. subcapitata* were consistent with reported inter- and intra-laboratory toxicity data (Calomeni et al., 2014; Hadjoudja et al., 2014). Chlorophyll *a* and cell density were used to measure algal responses in the present study. Chlorophyll *a* is a reliable aggregate measure of cell viability for an axenic algal culture, but assumes that chlorophyll *a* within non-viable cells breaks down and is not solubilized by acetone during extraction. Thus cell density, an individual measure allowing for evaluation of cell viability on a cell-by-cell basis, can be a more sensitive response measure if chlorophyll *a* does not degrade within the toxicity test duration (Calomeni et al., 2014).

The 96-h LC_{50} for *C. dubia* was 0.053 mg L^{-1} Cu as copper sulfate (Table 4). For *H. azteca*, the 96-h LC_{50} was 0.489 mg L^{-1} Cu as copper sulfate. The 96-h LC_{50} for *P. promelas* was 0.408 mg L^{-1} Cu as copper sulfate. *C. dubia* showed the greatest change in response with increasing copper concentrations; the potency slope of *C. dubia* was 740.9 percentage mortality/mg Cu L^{-1}, approximately 2.6 times greater than potency slope of *H. azteca* (283.3 percentage mortality/mg Cu L^{-1}) and approximately 3.3 times greater than potency slope of *P. promelas* (223.7 percentage mortality/mg Cu L^{-1}). Copper sulfate reference toxicity data in the present study were consistent with reported inter- and intra-laboratory toxicity data (Suedel et al., 1996; Deaver and Rodgers, 1996; Mastin and Rodgers, 2000; Murray-Gulde et al., 2002; Closson and Paul, 2014).

Comparison of target and non-target organism responses to SCP exposures

In the present study, median effect concentrations for three non-target species (*H. azteca, P. subcapitata, and P. promelas*) were greater than the median effect concentration for the target cyanobacterium, *M. aeruginosa*. Based on current registered application rates of SCP (0.2 to 10.2 mg L-1 H_2O_2), *P. promelas* is unlikely to be adversely affected by an SCP application; the 96-h LC_{50} for *P. promelas* and H_2O_2 as SCP was approximately 1.9 times greater than the maximum registered application rate for Phycomycin® SCP. Although results of the present study indicate that a margin of safety is not expected for *C. dubia*, as the 96-h LC_{50} for *C. dubia* was nearly equivalent to the 96-h EC_{50} for *M. aeruginosa*, exposures in this study were more conservative than field exposures. Organisms were confined within test chambers to ensure contact with exposures, while in aquatic systems, mobile organisms may avoid exposures. Naïve

organisms were tested (i.e. *P.promelas* and *C. dubia* were < 24-h old, *H. azteca* were 2-3 weeks old), and additional testing would be necessary to understand if all life stages respond equally to concentrations of SCP controlling *M. aeruginosa*. A margin of safety could exist for *C. dubia*, depending on site-specific factors, including target algae as an active site for oxidation by H_2O_2, initial concentration of SCP applied, actual exposure concentration of H_2O_2 achieved, proximity of the organisms to the exposure, and age and condition of the organism(s).

Confirmation of H_2O_2 *exposures from SCP*

Measured exposures were 86% ±16% of targeted exposures (Table 5). Detection limits of the I_3^- method were sufficient to measure concentrations of H_2O_2 as SCP within the recommended application range of SCP. Based on recommendations of Kinley et al (2015), exposure concentrations above detection limits and above the recommended application range were diluted prior to measurement. To improve accuracy and minimize potential interferences of algae, samples were filtered with a 0.45µm cellulose filter paper prior to measurement (Kinley et al., 2015). H_2O_2 measurements made using the I_3^- method (Klassen et al., 1994) were useful to confirm exposures that elicited responses of organisms as a function of increasing H_2O_2 concentrations.

Comparison of SCP with other algaecides

Using potency data for target organisms and margins of safety for non-target organisms, relative toxicities of sodium carbonate peroxyhydrate, copper (as copper sulfate and chelated copper formulations), endothall, and diquat dibromide were contrasted to provide context for the use of these algaecides in the field. In the United

States, the Environmental Protection Agency (USEPA) registers products with these active ingredients for control or suppression of algae (Gettys et al., 2014). In terms of effectiveness against target species, responses of target algae (i.e. cyanobacteria) to SCP exposures occurred at mg L^{-1} level concentrations, whereas target and non-target organisms responded to concentrations of copper, endothall, and diquat dibromide that were three orders of magnitude lower (i.e. µg L^{-1}).

In addition to potencies, margins of safety for non-target species are an important factor that should be considered when comparing different algaecides. Margins of safety were calculated as the quotient of the maximum recommended application rate for each algaecide divided by the toxicity value (i.e. EC/LC_{50}) obtained from the literature review. The maximum application rate is the highest concentration registered for application to control noxious algae in water of the United States, as determined by the USEPA. Thus, these margins of safety are meant to be conservative; the actual concentration at which control of algae is obtained may be less than the maximum application rate. The primary utility of these margins of safety is for comparison of algaecides that elicit harmful effects for non-target organisms at different concentration levels (i.e. mg L^{-1} vs. µg L^{-1}). Values greater than one indicate a clear margin of safety; the concentration eliciting adverse effects to non-target species is greater than the highest concentration that could be applied to control or suppress a target alga. Values less than one do not indicate a lack of a margin of safety, rather that a margin of safety may be minimal, and site-specific information is required for accurate evaluation (i.e. site-specific organism sensitivity, water characteristics, and algaecide application rate). Margins of safety for non-target

organisms exposed to SCP ranged from 0.5 to 2.2 (Table 6). Margins of safety for non-target organisms exposed to copper-based algaecides ranged from <0.01 to 1.3. For diquat dibromide, margins of safety ranged from 0.13 to 37.8 (Table 7), while for endothall margins of safety ranged from 0.01 to greater than 0.05.

Median effect concentrations of non-target eukaryotic algae were greater than those for cyanobacteria for all four active ingredients reviewed. Additionally, margins of safety for non-target eukaryotic algae were greater than one for SCP (Table 6; Schrader et al., 1998) and two copper-based algaecide formulations (i.e. copper sulfate and Cutrine®-Ultra; present study; Schrader et al., 1998; Calomeni et al., 2014). Thus, these four active ingredients could be used selectively against target cyanobacteria (i.e *M. aeruginosa*). *C. dubia* was the most sensitive animal for both SCP and copper algaecides: margins of safety for *C. dubia* and copper-based algaecide exposures ranged from <0.01 to 0.1, while the margin of safety for *C. dubia* and SCP exposures was 0.13. Diquat dibromide may be more potent to *H. azteca* than both copper and SCP, as the margin of safety (0.13) was less than for copper (0.08-0.4) and SCP (0.4). The margin of safety for *P. promelas* exposed to SCP was 2.2, indicating that the LC_{50} was well above the highest concentration of SCP that could be used to control noxious algae. Similarly, the margin of safety for diquat dibromide was greater than one for fish *P. promelas* (20.4) and *L. macrochirus* (32.9-37.8), indicating that diquat dibromide may also be protective of fish species. It should be noted, however, that current allowable application concentrations for diquat dibromide are intended for suppression of algae only. To achieve control of algae, diquat dibromide must be used in conjunction with other registered algaecides (Syngenta

Crop Protection, LLC, Greensboro, NC). Copper-based algaecides and endothall products may have limited margins of safety for vertebrate fish. Seven of the eight published toxicity values included in this study for *P. promelas* exposed to copper-based algaecides resulted in margins of safety below one (0.1-0.7). While no toxicity information for vertebrate fish exposed to endothall fit criteria for inclusion in this study, endothall product labels include language that urges caution when applying concentrations above 0.3 mg L^{-1} due to risk of adverse effects to fish (United Phosphorous, Inc., King of Prussia, PA 19406). Compared to copper-based algaecides, diquat dibromide, and endothall, use of SCP could provide control of target algae with an enhanced margin of safety for non-target fish.

Considerations for Use of Laboratory Data in the Field

Results of the present comparative toxicity study can be used to predict the distribution of responses likely to occur in the field; however, they are not intended for direct translation to field situations. Organism responses in both the laboratory and field are a function of exposure, which has components of concentration, duration, form, and the frequency at which organisms are exposed (Rand, 1995). Environmental factors may influence one of more of these exposure parameters, fundamentally changing the exposure. The duration of exposure in a lacustrine environment may be influenced by dispersion and dilution. For H_2O_2 exposures specifically, oxidizable material in site water may consume the activity of H_2O_2, altering exposures for target and non-target species. Organisms may actively avoid an exposure, as has been extensively studied with fish and copper exposures (Sprague, 1964; Svecevicius, 2012). Further, a surficial algaecide

application to control noxious planktonic (free-floating) algae may not achieve sufficient exposure in the benthic region to adversely affect sediment-dwelling invertebrates. Likewise, exposures of algaecides in benthic areas of aquatic systems may not achieve sufficient exposures to adversely affect organisms near the surface.

In the present laboratory study, factors altering exposures were limited to evaluate responses of organisms to unconfounded exposures of H_2O_2 as SCP. To ensure maximum exposure amplitude and duration, naïve laboratory cultured organisms at their most sensitive life stage (as opposed to field-collected organisms) were confined within test chambers. By controlling toxicity test conditions to limit confounding factors, relative sensitivities of a range of organisms could be discerned, supporting predictions of the distribution of responses likely to occur in the field. Results of the present study indicate that in a field treatment, a noxious cyanobacterium (i.e. *M. aeruginosa*) is likely to respond to an exposure of H_2O_2 from SCP that is significantly lower than the exposure required to adversely affect fish (e.g. *P. promelas*). Within the constraints of conservative laboratory toxicity testing, incorporating site characteristics would provide more site-specific conditions for predicting exposures and subsequent responses in field situations (Fitzgerald and Jackson, 1979; Bishop and Rodgers, 2011; Calomeni et al., 2015).

Conclusions

In this comparative toxicity study, a cyanobacterium and two invertebrates were more sensitive than a eukaryotic alga and a vertebrate to exposures of H_2O_2 from an SCP algaecide. 96-h EC_{50} values ranged from 1.0 to 19.7 mg L^{-1} H_2O_2 for animals, while the 96-h EC_{50} for the cyanobacterium *M. aeruginosa* was 0.9-1.0 mg L^{-1} H_2O_2. The 7-d EC_{50} for the eukaryotic algae *P. subcapitata* was 5.2-9.2 mg L^{-1} H_2O_2, as responses to environmentally relevant concentrations of SCP were not manifested in 96-h, while the fish *P. promelas* was not sensitive to exposures within the recommended range of application concentrations for the source of SCP used in this study (Phycomycin® SCP; 0.2 to 10.2 mg L^{-1} H_2O_2). SCP is comparable to other algaecides (i.e. copper-based algaecides, endothall, and diquat dibromide) and could be used selectively for prokaryotic algae. However, SCP may be less potent than diquat dibromide to *H. azteca*, and less potent than copper algaecides to *C. dubia*, enhancing margins of safety for these species. Estimated margins of safety for organisms exposed to SCP in this study are conservative, as naïve organisms were used and site-specific factors that could influence exposures (e.g. dispersion, dilution, and water characteristics) were constrained in order to discern differences in responses of the organisms evaluated. While not directly predictive of specific concentrations to which organisms will respond in the field, results of this study can be used to predict the distribution of responses likely to occur. Results indicate that SCP could mitigate risks associated with noxious cyanobacterial growths (e.g. *M. aeruginosa*) while providing a margin of safety for non-target species.

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Table 3.1: Physical properties and fate characteristics of Phycomycin® SCP.

Active ingredient	85% SCP
Maximum application	36.9 mg L^{-1} (10.2 mg L^{-1} H_2O_2)[a]
Formulation	SCP and inert ingredients
Physical state	Coarse white grains[a]
Water solubility	140g/L at 24ºC[a]
Boiling Point	Not applicable[a]
pH	10.4-10.6 s.u. (1% solution)[a]
CAS number	497-19-8[a]

[a]AB (2007)

Table 3.2: 96-h and 7-d EC_{50} values (mg H_2O_2 L^{-1}) and potency slopes (percent response/mg H_2O_2 L^{-1}) for *M. aeruginosa* and *P. subcapitata*, respectively; 96-h LC_{50} values and potency slopes (percent mortality/mg H_2O_2 L^{-1}) for *C. dubia*, *P. promelas*, *H. azteca*, exposed to H_2O_2 as SCP.

Species	Initial Density	EC_{50} (95% CI)	Slope
M. aeruginosa	9.72×10^5 cells mL^{-1}		
Cell Density		0.9 (0.7-1.0)	49.0
Chlorophyll *a*		1.0 (0.9-1.0)	50.6
P. subcapitata	7.92×10^5 cells mL^{-1}		
Cell Density		9.2 (7.6-10.8)	7.4
Chlorophyll *a*		5.2 (3.2-7.2)	7.2
Species	Age	LC_{50} (95% CI)	Slope
C. dubia	< 24-h	1.0 (0.2-1.7)	28.4
H. azteca	2-3 weeks	3.6 (1.0-6.5)	21.2
P. promelas	< 24-h	19.7 (14.5-31.2)	5.8

Table 3.3: Ranges of water characteristics at test initiation and completion for toxicity tests. One replicate measured per exposure.

Organism	Time	Exposure	pH[1] (S.U)	D.O.[2] (mg/L)	Conductivity[3] (µS/cm)	Alkalinity[4] (mg/L as $CaCO_3$)	Hardness[4] (mg/L as $CaCO_3$)
C. dubia	Initial	SCP	8.22-8.82	7.32-8.54	382.3-394.6	70-80	80-90
		$CuSO_4$	8.10-8.25	7.11-8.11	378.0-406.4	70-75	80-100
	Final	SCP	8.17-8.76	8.38-9.34	369.5-402.1	70-80	80-90
		$CuSO_4$	8.08-8.23	8.22-8.86	378.0-406.4	70-75	80-100
P. promelas	Initial	SCP	8.20-9.94	8.37-8.65	367.0-526.1	70-160	80-85
		$CuSO_4$	8.08-8.32	7.89-9.23	318.2-328.3	50-65	90-125
	Final	SCP	8.15-9.85	8.09-8.59	350.2-518.0	70-160	80-85
		$CuSO_4$	8.14-8.30	7.02-8.44	405.0-459.0	50-65	90-110
H. azteca	Initial	SCP	8.15-9.20	7.65-8.20	385.0-415.0	70-90	80-85
		$CuSO_4$	7.85-8.27	7.32-8.02	350.0-370.0	65-90	100-125
	Final	SCP	8.08-9.22	8.26-8.64	366.0-430.3	70-90	80-85
		$CuSO_4$	7.89-8.17	8.05-8.26	390.0-410.0	65-75	68-88
P. subcapitata	Initial	SCP	7.94-10.0	8.32-9.07	417.1-714.0	60-185	72-80
		$CuSO_4$	7.46-7.94	9.07-10.4	417.1-420.7	60-80	72-76
	Final	SCP	7.97-9.23	6.93-8.61	472.0-1471	80-500	65-70
		$CuSO_4$	8.16-8.39	6.87-8.04	443.0-470.0	60-70	60-70
M. aeruginosa	Initial	SCP	8.54-8.84	10.7-11.5	389.8-416.1	70-110	50-55
		$CuSO_4$	8.24-8.33	8.00-9.11	440.1-514.6	60-70	60-80
	Final	SCP	7.95-9.60	8.08-12.6	333.2-397.2	80-115	45-55
		$CuSO_4$	8.15-8.40	7.48-8.70	488.0-524.0	60-75	60-80

[1]pH was measured using an Orion® 4-Star pH meter and Triode® electrode (±0.01 SU)

[2]Dissolved oxygen was measured using a YSI® Model 52 dissolved oxygen meter (±0.1 mg/L)

[3]Conductivity was measured using YSI® 30 conductivity meter (±1 $\mu S/cm^2$)

[4]Alkalinity and hardness were measured according to standard methods 2320 (±2 mg/L as $CaCO_3$) and 2340 (±2 mg/L as $CaCO_3$), respectively (APHA, 2005)

Table 3.4: 96-h and 7-d EC_{50} values (mg Cu L^{-1}) and potency slopes (percent response/mg Cu L^{-1}) for *M. aeruginosa* and *P. subcapitata*, respectively, and 96-h LC_{50} values and potency slopes (percent mortality/mg Cu L^{-1}) for *C. dubia*, *P. promelas*, *H. azteca*, exposed to copper as $CuSO_4$ in reference toxicity tests

Species	Initial Density	EC_{50} (95% CI)	Slope
M. aeruginosa	4.73×10^6 cells mL^{-1}		
Cell Density		0.245 (0.108-0.382)	77.8
Chlorophyll *a*		0.086 (0.00-0.219)	149.7
P. subcapitata	8.00×10^5 cells mL^{-1}		
Cell Density		1.65 (0.774-2.53)	12.4
Chlorophyll *a*		0.692 (0.577-0.808)	12.4
Species	Age	LC_{50} (95% CI)	Slope
C. dubia	< 24-h	0.053 (0.009-0.180)	740.9
H. azteca	2-3 weeks	0.489 (0.276-0.664)	283.3
P. promelas	< 24-h	0.408 (0.272-0.669)	223.7

Table 3.5: Targeted and mean measured H_2O_2 (as SCP) and Cu (as $CuSO_4$) concentrations in exposures (n=3).

Species	Targeted H_2O_2 Concentration (mg/L)	Measured H_2O_2 Concentration (mg/L)	Percent Error	Targeted Copper Concentration (mg/L)	Measured Copper Concentration (mg/L)	Percent Error
M. aeruginosa	0.0	< 0.2	-	0.000	0.061	-
	0.3	0.3	0.07	0.100	0.101	0.01
	0.6	0.4	0.27	0.200	0.212	0.06
	1.1	0.9	0.18	0.400	0.407	0.02
	1.7	1.1	0.34	0.800	0.836	0.04
	2.2	2.1	0.05	1.500	1.520	0.01
	3.0	3.4	0.12	-	-	-
P. subcapitata	0.0	< 0.2	-	0.000	0.020	-
	1.4	1.9	0.36	0.250	0.198	0.21
	2.8	2.5	0.11	0.500	0.514	0.03
	5.5	3.5	0.36	1.000	0.929	0.07
	9.9	8.7	0.12	3.000	2.730	0.09
	13.8	11.3	0.18	7.000	6.280	0.10
	27.6	19.8	0.28	10.00	10.56	0.06
C. dubia	0	< 0.2	-	0.000	0.008	-
	0.8	0.7	0.13	0.010	0.010	0.00
	1.7	1.2	0.29	0.030	0.024	0.20
	2.5	1.5	0.40	0.050	0.045	0.10
	3.3	3.0	0.09	0.070	0.063	0.10
	4.1	3.5	0.15	0.100	0.116	0.16
	-	-	-	0.150	0.138	0.08
H. azteca	0	< 0.2	-	0.000	< 0.005	-
	2.8	2.3	0.18	0.300	0.346	0.15
	4.1	3.5	0.15	0.400	0.401	0.00
	4.8	4.0	0.17	0.600	0.559	0.07

Table 2.5 continued						
H. azteca	5.5	4.6	0.16	0.700	0.674	0.04
	8.3	6.5	0.22	0.800	0.781	0.02
P. promelas	0.0	< 0.2	-	0.000	0.036	-
	5.5	5.0	0.09	0.150	0.150	-
	11.0	11.0	0.00	0.250	0.250	-
	16.6	15.4	0.07	0.350	0.396	0.13
	20.7	18.9	0.09	0.450	0.430	0.04
	24.8	20.7	0.17	0.600	0.618	0.03
	29	24.2	0.17	-	-	-
	35.9	28.7	0.20	-	-	-

Table 3.6: Comparison of measured and reported toxicity values and calculated margins of safety for SCP and copper-based algaecides

Test Species	Toxicant	Test Species Age/Density	Exposure Duration	Endpoint	Initial pH[1]	Toxicity Value (mg L^{-1} SCP)		Margin of Safety[1]	Reference
M. aeruginosa	Phycomycin® SCP	9.72 x 10^5 cells mL^{-1}	96-h	Chlorophyll *a*	8.54	EC_{50}	3.1	-	Present Study
M. aeruginosa	Phycomycin® SCP	9.72 x 10^5 cells mL^{-1}	96-h	Cell density	8.54	EC_{50}	3.5	-	Present Study
O. cf. chalybea	SCP (Aldrich)	0.18-0.27 A	96-h	Cell density (absorbance)	7.60-9.00	IC_{50}	8.6	-	Schrader et al., 1998
P. subcapitata	Phycomycin® SCP	0.80 x 10^6 cells mL^{-1}	7-d	Chlorophyll *a*	7.94	EC_{50}	18.9	0.51	Present Study
P. subcapitata	Phycomycin® SCP	0.80 x 10^6 cells mL^{-1}	7-d	Cell density	7.94	EC_{50}	33.8	0.92	Present Study
S. capricomutum	SCP (Aldrich)	0.19-0.26 A	96-h	Cell density (absorbance)	7.60-9.00	IC50	68.0	1.84	Schrader et al., 1998
C. dubia	Phycomycin® SCP	< 24-h	96-h	Mortality	8.22	LC_{50}	4.7	0.13	Present Study
Moina sp.	H_2O_2 (Sigma-Aldrich)	adult	48-h	Mortality	n/a	LC_{50}	2.0 (2)	0.20 (2)	Reichwaldt et al., 2011
H. azteca	Phycomycin® SCP	2-3 weeks	96-h	Mortality	8.15	LC_{50}	14.5	0.40	Present Study
Daphnia carinata	H_2O_2 (Sigma-Aldrich)	adult	48-h	Mortality	n/a	LC_{50}	5.6 (2)	0.55 (2)	Reichwaldt et al., 2011
P. promelas	Phycomycin® SCP	< 24-h	96-h	Mortality	8.20	LC_{50}	80.8	2.21	Present Study
Test Species	**Toxicant**	**Test Species Age/Density**	**Exposure Duration**	**Endpoint**	**Initial pH[1]**	**Toxicity Value (mg L^{-1} Cu)**		**Margin of Safety[1]**	**Reference**
M. aeruginosa	$CuSO_4$	10^6 cells mL^{-1}	48-h	Chlorophyll a	6.80	EC_{50}	0.050	-	Hadjoudja et al. 2009
M. aeruginosa	$CuSO_4$	4.73 x 10^6 cells mL^{-1}	96-h	Chlorophyll *a*	8.15	EC_{50}	0.086	-	Present Study
P. agardhi	Cutrine®-Ultra	2.21 x 10^6 cells mL^{-1}	96-h	Cell density	n/a	EC_{50}	0.100	-	Calomeni et al. 2014
P. agardhi	$CuSO_4$	1.27 x 10^6 cells mL^{-1}	96-h	Cell density	n/a	EC_{50}	0.180	-	Calomeni et al. 2014
M. aeruginosa	$CuSO_4$	4.73 x 10^6 cells mL^{-1}	96-h	Cell density	8.15	EC_{50}	0.245	-	Present Study
P. subcapitata	$CuSO_4$	8.00 x 10^5 cells mL^{-1}	96-h	Chlorophyll *a*	7.46	EC_{50}	0.692	0.35	Present Study
P. subcapitata	Cutrine®-Ultra	3.02 x 10^6 cells mL^{-1}	96-h	Cell density	n/a	EC_{50}	1.180	1.20	Calomeni et al. 2014
P. subcapitata	$CuSO_4$	8.00 x 10^5 cells mL^{-1}	96-h	Cell density	7.46	EC_{50}	1.650	0.83	Present Study
P. subcapitata	$CuSO_4$	3.22 x 10^6 cells mL^{-1}	96-h	Cell density	n/a	EC_{50}	3.000	0.15	Calomeni et al. 2014
D. magna	Algimycin®-PWF	<4h	96-h	Mortality	7.70	LC_{50}	0.005	<0.01	Johnson et al., 2008

Table 3.6 continued

C. dubia	$CuSO_4$	<4h	96-h	Mortality	8.00	LC_{50}	0.004	<0.01	Johnson et al. 2008
C. dubia	Algimycin®-PWF	<4h	96-h	Mortality	8.00	LC_{50}	0.048	0.05	Johnson et al., 2008
D. magna	$CuSO_4$	<4h	96-h	Mortality	7.70	LC_{50}	0.005	<0.01	Johnson et al., 2008
C. dubia	Clearigate®	< 24-h	96-h	Mortality	7.20-8.00	LC_{50}	0.056	0.06	Murray-Gulde et al., 2002
C. dubia	$CuSO_4$	< 24-h	96-h	Mortality	7.20-8.00	LC_{50}	0.06	0.03	Murray-Gulde et al., 2002
C. dubia	$CuSO_4$	< 24-h	96-h	Mortality	8.10	LC_{50}	0.061	0.03	Present Study
C. dubia	Cutrine®-Plus	< 24-h	96-h	Mortality	7.20-8.00	LC_{50}	0.092	0.10	Murray-Gulde et al., 2002
H. azteca	$CuSO_4$	2-3 weeks	48-h	Mortality	6.50-8.20	LC_{50}	0.158	0.08	Mastin and Rodgers 2000
P. promelas	$CuSO_4$	<24-h	96-h	Mortality	7.70	LC_{50}	0.230	0.10	Johnson et al., 2008
P. promelas	Captain® XTR	45d	96-h	Mortality	8.10	LC_{50}	0.240	0.24	Closson and Paul 2014
H. azteca	Cutrine®-Plus	2-3 weeks	48-h	Mortality	6.40-8.00	LC_{50}	0.248	0.25	Mastin and Rodgers 2000
P. promelas	Algimycin®-PWF	<24-h	96-h	Mortality	7.70	LC_{50}	0.250	0.25	Johnson et al., 2008
P. promelas	Cutrine®-Plus	< 24-h	48-h	Mortality	6.40-8.00	LC_{50}	0.255	0.26	Mastin and Rodgers 2000
P. promelas	$CuSO_4$	45d	96-h	Mortality	8.10	LC_{50}	0.280	0.14	Closson and Paul 2014
H. azteca	Algimycin®-PWF	<4h	96-h	Mortality	7.80	LC_{50}	0.390	0.40	Johnson et al., 2008
H. azteca	$CuSO_6$	<4h	96-h	Mortality	7.80	LC_{50}	0.400	0.20	Johnson et al., 2008
H. azteca	Clearigate®	2-3 weeks	48-h	Mortality	7.30-8.00	LC_{50}	0.434	0.40	Mastin and Rodgers 2000
P. promelas	$CuSO_4$	< 24-h	48-h	Mortality	6.50-8.20	LC_{50}	0.480	0.24	Mastin and Rodgers 2000
P. promelas	Clearigate®	< 24-h	48-h	Mortality	7.30-8.00	LC_{50}	0.480	0.48	Mastin and Rodgers 2000
P. promelas	Clearigate®	< 24-h	96-h	Mortality	7.20-8.00	LC_{50}	0.481	0.48	Murray-Gulde et al., 2002
H. azteca	$CuSO_4$	2-3 weeks	96-h	Mortality	7.85	LC_{50}	0.508	0.25	Present Study
P. promelas	$CuSO_4$	< 24-h	96-h	Mortality	7.46	LC_{50}	0.519	0.26	Present Study
P. promelas	$CuSO_4$	< 24-h	96-h	Mortality	7.20-8.00	LC_{50}	0.675	0.34	Murray-Gulde et al., 2002
P. promelas	Captain®	45d	96-h	Mortality	8.10	LC_{50}	0.690	0.70	Closson and Paul 2014
P. promelas	Cutrine®-Plus	< 24-h	96-h	Mortality	7.20-8.00	LC_{50}	1.115	1.10	Murray-Gulde et al., 2002
L. macrochirus (Blucgill)	$CuSO_4$	10-12 days	96-h	Mortality	8.20	LC_{50}	2.640	1.30	Johnson et al., 2008

[1]Margin of safety= toxicity value / maximum recommended application concentration for the control of algae [SCP = 100 lbs SCP per acre-ft, or 36.9 mg L^{-1} SCP (Applied Biochemists Inc., Germantown, WI 53022); chelated copper-based algaecides = 1.0 mg L^{-1} Cu (Arch Chemicals a Lonza Business, Applied Biochemists, Alpharetta, GA); copper sulfate = 2.0 mg L^{-1} Cu (Arch Chemicals a Lonza Business, Applied Biochemists, Alpharetta, GA)]

[2]Toxicity values from Reichwaldt et al. (2012) have units of mg H_2O_2 L^{-1}, because liquid H_2O_2 rather than granular SCP was used to create exposures. Margins of safety were calculated with the concentration of H_2O_2 equivalent to the maximum concentration of SCP recommended for control of algae [i.e. 36.9 mg L^{-1} SCP, equivalent to 10.2 mg L^{-1} H_2O_2 (Arch Chemicals a Lonza Business, Applied Biochemists, Alpharetta, GA)]

Table 3.7: Comparison of reported toxicity values and calculated margins of safety for diquat dibromide and endothall.

Test Species	Toxicant	Test Species Age/Density	Exposure Duration	Endpoint	Initial pH[1]	Toxicity Value (mg L^{-1})		Correction Factor[1]	Corrected Toxicity Value (mg L^{-1} cation)[1]	Margin of Safety[2]	Referenc
O. cf. chalybea	Diquat dibromide, technical (Chem Service)	0.18-0.27 A	96-h	Cell density (absorbance)	7.60-9.00	IC_{50}	0.0122	0.536	0.01	--	Schrader et al., 1998
M. aeruginosa	Diquat dibromide	117 x 10^4 cells mL^{-1}	96-h	Carbon uptake	n/a	EC_{50}	0.098	0.536	0.05	--	Peterson et al., 1997
H. azteca	Diquat dibromide, technical (Chem Service)	4-8 mm	96-h	Inhibition	6.80-7.00	TL_m	0.048	None	0.05	0.13	Wilson 1969
S. capricornutum	Diquat dibromide	63 x 10^4 cells mL^{-1}	96-h	Carbon uptake	n/a	EC_{50}	0.492	0.536	0.26	0.72	Peterson et al., 1997
D. magna	n/a	First instar	48-h	Inhibition	n/a	EC_{50}	0.77	None	0.77	2.10	USEPA 1995
S. capricornutum	Diquat dibromide, technical (Chem Service)	0.19-0.26 A	96-h	Cell density (absorbance)	7.60-9.00	IC_{50}	1.73	0.536	0.93	2.50	Schrader et al., 1998
D. magna	n/a	First instar	48-h	Inhibition	n/a	EC_{50}	1.03	None	1.03	2.80	USEPA 1995
D. magna	n/a	First instar	48-h	Inhibition	n/a	EC_{50}	1.19	None	1.19	3.20	USEPA 1995
P. promelas	Diquat dibromide (Ortho Chemical Company)	n/a	96-h	Survival	7.10-7.40	TL_m	14.0	0.536	7.50	20.4	Surber and Pickering 1962
L. macrochirus (Blucgill)	n/a	n/a	72-h	Mortality	n/a	LC_{50}	12.1	None	12.10	32.9	USEPA 1995
L. macrochirus (Blucgill)	n/a	n/a	96-h	Mortality	n/a	LC_{50}	13.9	None	13.90	37.8	USEPA 1995
C. idella (Grass carp)	Diquat dibromide (Ortho Chemical Company)	n/a	96-h	Mortality	7.00-7.60	LC_{50}	53.0	0.536	28.41	77.2	El-Deen and Rogers 1992

Test Species	Toxicant	Test Species Age/Density	Exposure Duration	Endpoint	Initial pH[1]	Toxicity Value (mg/L)		Correction Factor[1]	Corrected Toxicity Value (mg L^{-1} endothall acid)[1]	Margin of Safety[2]	Reference
M. aeruginosa	Hydrothol® 191 (Elf AtoChem)	1.5 x 10^6 cells mL^{-1}	96-h	Cell density	n/a	EC_{50}	0.065	0.2336	0.015	0.01	Ruzycki et al., 1998
P. inundaium	Hydrothol® 191 (Elf AtoChem)	2.2 x 10^5 cells mL^{-1}	96-h	Cell density	n/a	EC_{50}	0.099	0.2336	0.023	0.01	Ruzycki et al., 1998
C. meneghiana	Hydrothol® 191 (Elf AtoChem)	5.3 x 10^5 cells mL^{-1}	96-h	Cell density	n/a	EC_{50}	0.232	0.2336	0.054	0.02	Ruzycki et al., 1998
S. acuminatus	Hydrothol® 191 (Elf AtoChem)	1.05 x 10^5 cells mL^{-1}	96-h	Cell density	n/a	EC_{50}	0.417	0.2336	0.097	0.03	Ruzycki et al., 1998

Table 3.7 continued

C. vulgaris	Hydrothol® 191 (Elf AtoChem)	3x 10^5 cells mL^{-1}	96-h	Cell density	n/a	EC_{50}	>0.6	0.2336	>0.14	>0.05	Ruzycki et al., 1998

[1]Correction factor used to express toxicity value in terms of active ingredient [diquat cation and 7-oxabicyclo [2.2.1] heptane-2,3-dicarboxylic acid (i.e. endothall acid) for diquat and endothall, respectively]. Assumption is 3.73 lbs of diquat dibromide is equivalent to 2 lbs of diquat cation (Syngenta Crop Protection, LLC, Greensboro, NC 27419-8300) and the acid equivalence of Hydrothol® 191 is 23.36% (United Phosphorous, Inc., King of Prussia, PA 19406)

[2]Margin of safety = toxicity value / maximum recommended application rate for suppression (diquat dibromide) or control (endothall) of algae [diquat dibromide = 0.5 gallons of product per surface acre for 1 ft depth, equivalent to 0.368mg L^{-1} diquat cation (Syngenta Crop Protection, LLC, Greensboro, NC 27419-8300); endothall = 3mg L^{-1} endothall acid (based on label for Hydrothol® Granular; United Phosphorous, Inc., King of Prussia, PA 19406)]

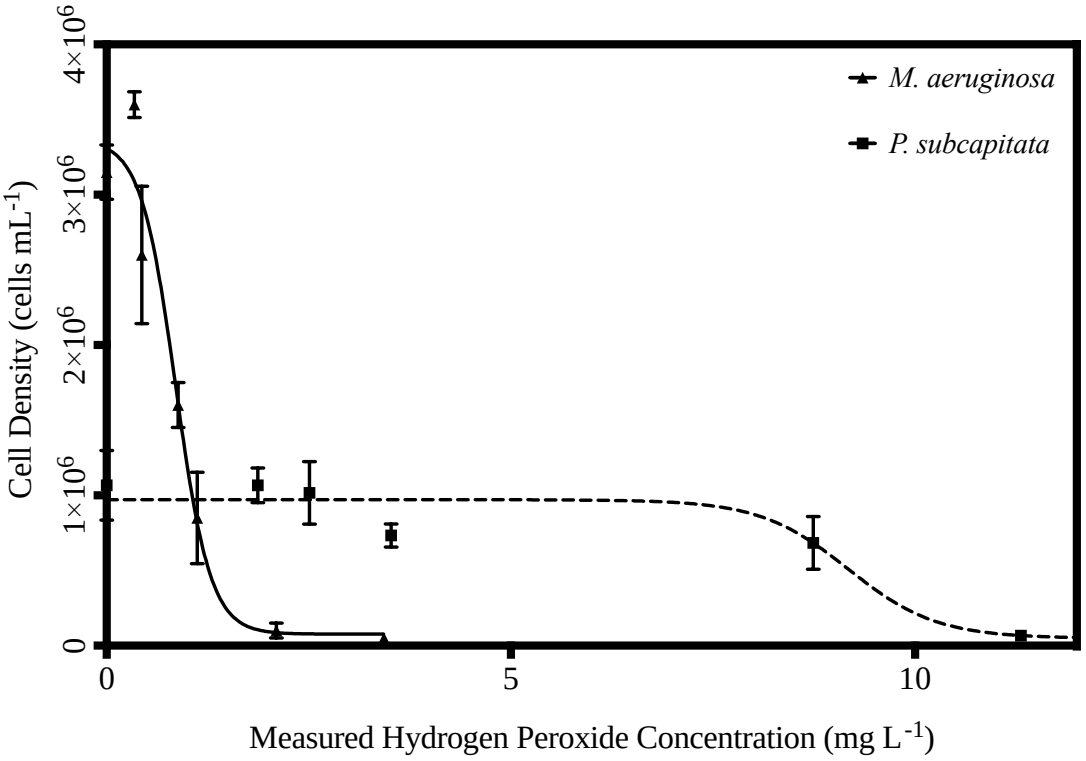

Figure 3.1: Mean responses of *M. aeruginosa* and *P. subcapitata* measured by cell density to 96-h and 7-d exposures of H_2O_2 as SCP, respectively (n=3). Error bars represent ±1 standard deviation. Initial densities of *M. aeruginosa* and *P. subcapitata* were 9.7×10^5 cells mL^{-1} and 7.9×10^5 cells mL^{-1}, respectively.

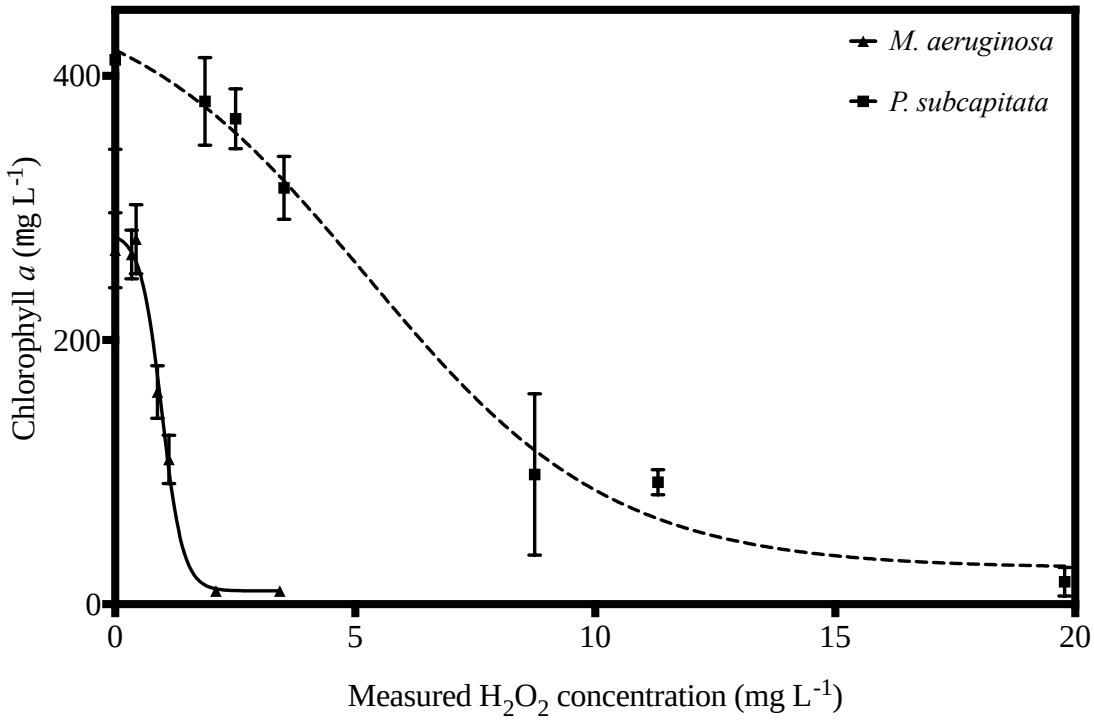

Figure 3.2: Mean responses of *M. aeruginosa* and *P. subcapitata* measured by chlorophyll *a* to 96-h and 7-d exposures of H_2O_2 as SCP, respectively (n=3). Error bars represent ±1 standard deviation. Initial densities of *M. aeruginosa* and *P. subcapitata* were 9.7×10^5 cells mL^{-1} and 7.9×10^5 cells mL^{-1}, respectively.

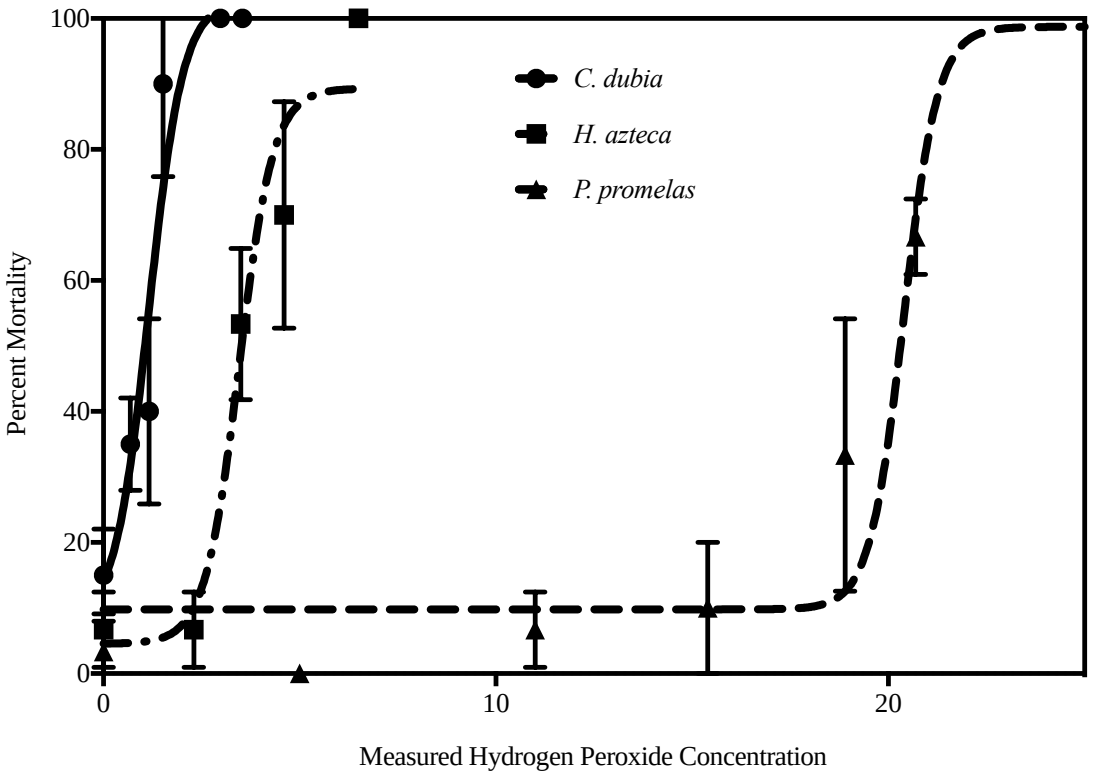

Figure 3.3: Mean responses of *P. promelas*, *C. dubia*, and *H. azteca* in terms of mortality to 96-h exposures of H_2O_2 as SCP (n=3). Error bars represent ±1 standard deviation.

CHAPTER FOUR

PREDICTING *IN SITU* RESPONSES OF TASTE AND ODOR PRODUCING ALGAE IN A SOUTHEASTERN U.S. RESERVOIR TO A SODIUM CARBONATE PEROXYHYDRATE ALGAECIDE USING A LABORATORY EXPOSURE-RESPONSE MODEL

Abstract

Efficacy of an *in situ* algaecide treatment can be predicted prior to application by physically modeling exposures and responses with laboratory experiments. Use of a sodium carbonate peroxyhydrate (SCP) algaecide to control a benthic algal assemblage producing taste and odor compounds in a drinking water reservoir (Hartwell Lake, Anderson, SC) provided an opportunity to test hypotheses regarding potential convergence of laboratory and *in situ* exposures and responses. Objectives of this study were to 1) measure responses (in terms of chlorophyll *a* concentrations, phycocyanin concentrations, and cell densities) of a benthic algal assemblage from Hartwell Lake to 7-d exposures of hydrogen peroxide (H_2O_2; as Phycomycin® SCP) in the laboratory, 2) to measure the exposure of H_2O_2 introduced to Hartwell Lake (as Phycomycin® SCP) and consequent responses of the algal assemblage (in terms of chlorophyll *a* concentrations, phycocyanin concentrations, and cell densities), and 3) compare exposures and responses measured in the laboratory and *in situ*. Laboratory exposures dissipated within 48 hours, with an overall first order rate coefficient of 0.08 h^{-1}, and an overall half-life of 8.6 h. Although chlorophyll *a* measurements did not indicate a response, significant responses of the algal assemblage in terms of phycocyanin and cell density were measured 4 days

after treatment (4-DAT) following exposures of 453, 615, and 812 mg H_2O_2 m^{-2}. The initial exposure of H_2O_2 (619±428 mg H_2O_2 m^{-2}) measured *in situ* within 0.3m (1 ft.) of the sediment-water interface dissipated within 30 hours, with a first order rate coefficient of 0.061 h^{-1}, and a half-life of 11.4 h. Chlorophyll *a* concentrations indicated a differential response of target eukaryotic algae compared to target prokaryotes; however, significant responses in terms of phycocyanin concentrations, target algal densities and decreases in concentrations of 2-methylisoborneol (MIB) and geosmin at the intake of the drinking water treatment facility indicated that, relative to an untreated control site, the algaecide application provided a beneficial alternative to a no-treatment decision. Exposures of H_2O_2 from SCP are labile and dynamic; the initial *in situ* exposure had a large deviation (i.e. ±428 mg H_2O_2 m^{-2}) and was an order of magnitude less than the targeted exposure. It is likely SCP granules do not settle uniformly, and that some H_2O_2 activity was lost due to dissipation as granules settled through the water column. In such situations, measured responses can be compared to infer comparable exposures and confirm accuracy of the laboratory model. In the present study, inferential evidence corroborated the direct comparison of exposures: significant responses measured *in situ* in terms of phycocyanin concentrations and target algal densities were comparable to responses obtained from effective laboratory exposures (i.e. 453-812 mg H_2O_2 m^{-2}), corroborating exposures directly comparable in terms of initial exposure and exposure duration (i.e. half-life). Data derived from this experiment provide evidence for the design and use of a physical laboratory model in predicting responses of algae in the field.

Introduction

Benthic algae and periphyton can produce earthy, musty taste and odor compounds such as 2-methylisoborneol (MIB) and geosmin (Utkilen and Frøshaug, 1992; Watson and Ridal, 2004; Vilalta et al., 2004; Ridal et al., 2007). These compounds can impair water resources intended for drinking water when concentrations render finished water undesirable for consumption. Algaecides can rapidly restore usages when problematic algae impair critical water resources and immediate response is required. Sodium carbonate peroxyhydrate (SCP) is a relatively new, hydrogen peroxide (H_2O_2)-based active ingredient (USEPA, 2004) used by water resource managers in algaecide formulations to control growths of noxious algae (Gettys et al., 2014), and is certified by the National Sanitation Foundation (NSF) for use in drinking water reservoirs. H_2O_2 released by SCP oxidizes algal cells (Drabkova et al. 2007a,b; Finnegan et al., 2010). Laboratory experiments can be conducted to measure a site-specific exposure-response relationship for SCP and target algae (Rodgers et al., 2010; Bishop and Rodgers, 2011), and can predict responses of target algae to an *in situ* SCP algaecide application, decreasing uncertainty about the potential outcome. Confirming laboratory predictions with *in situ* data measured post-application can further decrease uncertainty. Use of an SCP algaecide to mitigate a benthic algal assemblage producing taste and odor compounds in the Six-and-Twenty Creek cove of Hartwell Lake (Anderson, SC) provided an opportunity to test hypotheses regarding potential convergence of laboratory

and *in situ* exposures and responses. MIB and geosmin were identified as the problematic taste and odor compounds, and benthic algae known to produce taste and odor compounds, including cyanobacteria (*Oscillatoria*, *Anabaena*, and *Planktothrix*) and diatoms (*Tabellaria* and *Fragilaria*) (Palmer, 1960), were identified as putative sources of taste and odor production.

Efficacy of an *in situ* algaecide treatment can be predicted prior to application by physically modeling exposures and responses with preliminary laboratory evaluations (Rodgers et al., 2010; Bishop and Rodgers, 2011; Matthijs et al., 2012; Barrington et al., 2013; Burson et al., 2014). Laboratory toxicity tests can be designed to capture the range of responses of organisms by using a series of exposure concentrations (Rand, 1995). The relationship between exposures and responses is typically described by a sigmoidal curve, which has distinct features: first, there is a minimum exposure below which no response occurs or can be measured (i.e. lower threshold). Second, there is a maximum response at which further increases in exposure will not result in increased responses (i.e. upper threshold). Third, from the lower threshold to the upper threshold responses typically increase proportionally with increases in exposure (i.e. potency slope). In the present context, by bounding laboratory SCP exposures within the range of concentrations that would be applied *in situ* (i.e. label recommended range of concentrations), the potency slope and upper threshold of the resulting exposure-response curve can be used to predict the extent of control that can be achieved from an *in situ* algaecide application (Fitzgerald and Jackson, 1979; Rodgers et al., 2010; Bishop and Rodgers, 2011). Additionally, the relationship can be used to interpret exposures achieved *in situ*.

To accurately predict *in situ* responses, target organisms are exposed in the laboratory in a manner comparable to an *in situ* application. Factors that influence organism responses to constituents include both innate organism sensitivity and site characteristics (USEPA, 2002). When dissolved in water, SCP dissociates into H_2O_2, which is a reactive oxygen species (Mittler, 2002) and an oxidant (Mallick and Mohn 2000). Therefore, when SCP is used to control problematic algae, oxidizable constituents inherent in the system, such as the density of target algae and the concentration of dissolved organic carbon, may influence the exposure of target algae to SCP. Incorporating site water and algae in laboratory experiments captures sufficient characteristics of the treatment site that exposures and responses comparable to and predictive of an *in situ* SCP application can be obtained.

Inaccurate translation of the laboratory model can arise from the field site's increased scale and complexity. The accuracy of laboratory predictions can be assessed by measuring and comparing exposures and responses from *in situ* algaecide applications. Because of the fundamental relationship between exposures and responses, *in situ* results can be compared to those from the laboratory model directly, through comparable exposures (eliciting comparable responses), and indirectly, by observing responses in the field and inferring the causative exposure from the laboratory model.

The overall objective of this study was to evaluate responses of a problematic algal assemblage to laboratory exposures of an SCP algaecide and compare responses with exposures and responses measured *in situ*. Specific objectives were to 1) measure responses (in terms of chlorophyll *a* concentrations, phycocyanin concentrations, and cell

densities) of a benthic algal assemblage from Hartwell Lake to 7-d exposures of H_2O_2 (as Phycomycin® SCP) in the laboratory, 2) to measure the exposure of H_2O_2 introduced to Hartwell Lake (as Phycomycin® SCP) and consequent responses of the algal assemblage (in terms of chlorophyll *a* concentrations, phycocyanin concentrations, and cell densities), and 3) compare exposures and responses measured in the laboratory and *in situ*.

Materials and Methods

Study Site

Hartwell Lake is a 22,662.4-hectare (56,000 acres) reservoir in the southeast United States, bordering South Carolina and Georgia. The reservoir is managed by the U.S. Army Corps of Engineers for hydropower, flood control, navigation, recreation, water quality, drinking water supply, and fish and wildlife management (USACE, 1992). Within the Six-and-Twenty Creek cove of Hartwell Lake, the SCP algaecide Phycomycin® SCP (Arch Chemicals a Lonza Business, Applied Biochemists, Alpharetta, GA) was applied in multiple coves east of a drinking water intake structure to control the algal assemblage producing taste and odor. The study site (34°33'33.91"N 82°44'0.30"W) was a cove of approximately 2.43 hectares (6 acres) treated with Phycomycin® SCP in the fall of 2015.

Laboratory Toxicity Testing

Samples of the algal assemblage were collected from multiple locations within the study site. Samples were gently rinsed from substrates (e.g. submerged rocks) into a 1 L high-density polyethylene (HPDE) Nalgene bottle. Site water was collected at the sediment water interface using a Van Dorn bottle and transported to the laboratory. Site algae and water were allowed to acclimate to laboratory conditions of light and temperature for 24-h prior to testing (Bishop and Rodgers, 2011). Static, non-renewal exposures were conducted in 250 mL borosilicate beakers (USEPA, 1996a,b) by exposing 0.5 g of site algae to Phycomycin® SCP in site water. Nine replicates per concentration and nine replicates of untreated controls were tested. Tests were conducted

at 23±2°C and were illuminated on a 18:6-h light:dark photoperiod by cool white fluorescence lighting at an intensity of 3100 ± 100 lux.

Laboratory experiments were designed to be a scaled representation of an *in situ* application (Bishop and Rodgers, 2011). The series of SCP concentrations used in this experiment encompassed the range of label recommended concentrations. Concentrations were scaled to the field with the assumption that the bottom 0.61m (2 ft) of the water column from the sediment-water interface would be treated in order to maximally expose the target benthic algae. Exposures were accomplished by dissolving granules of Phycomycin® SCP (Table 1) in site water. Granules of SCP were weighed for each exposure using a calibrated A&D GR-202 dual range (0.0001 g) balance (A&D Engineering, Inc., San Jose, CA 95131). Targeted exposures ranged from 37 to 1234 mg H_2O_2 m^{-2} (representing 0.3-10.2 mg H_2O_2 L^{-1} *in situ;* 10.2 mg H_2O_2 L^{-1} is the maximum recommended label concentration). H_2O_2 concentrations were measured spectrophotometrically immediately after dissolution of SCP using the I_3^- method (MDL=0.2 mg H_2O_2 L^{-1}, CV= ±1.5%; Klassen et al., 1994; Kinley et al., 2015) with a 1 cm cuvette and SpectraMax®M2 Microplate Reader (Molecular Devices Corp. Sunnyvale, CA 94089). Exposure durations were quantified by measuring exposures five times within 48-h of initial exposure and then using analytically informed sampling until exposure measurements were less than the detection limit (i.e. less than 0.2 mg H_2O_2 L^{-1}) Three-milliliter aliquots were collected from 3 randomly selected replicates of each exposure, mixed with reagents for H_2O_2 analysis, and measured. Based on a storage stability study by Kinley et al. (2015), any samples not measured immediately were

stored in the dark at 2 ± 1ºC for less than 96-h before analysis. Water characteristics in exposures were measured at test initiation and completion. Dissolved oxygen, pH, and conductivity were measured using a YSI® Model 52 dissolved oxygen meter (±0.1 mg/L), an Orion® 4-Star pH meter with a Triode® electrode (±0.01 SU), and a YSI® 30 conductivity meter (±1 µS/cm^2), respectively. Hardness (±2 mg/L as $CaCO_3$) and alkalinity (±2 mg/L as $CaCO_3$) of samples were measured according to *Standard Methods for Examination of Water and Wastewater* (APHA, 2005).

Multiple measures were utilized to discern responses of the algal assemblage to laboratory exposures of SCP. Chlorophyll *a* concentrations, phycocyanin concentrations, and cell densities were measured in triplicate prior to treatment, 4 days after treatment (4-DAT), and 7-DAT. Chlorophyll *a* concentrations were measured fluorometrically following standard methods with a SpectraMax® M2 Microplate Reader (MDL=10µg L^{-1}, CV= ±4.5%; APHA, 2005), and expressed as the mass of chlorophyll *a* extracted per square meter of substrate (i.e. µg m^{-2}). Phycocyanin concentrations were analyzed according to Lawrenz et al (2011; MDL=10µg L^{-1}, CV= ±5.0%), and also expressed as the mass extracted per square meter of substrate (i.e. µg m^{-2}). Cell densities, defined in the present context as the number of target algal cells (i.e. cyanobacteria genera *Oscillatoria*, *Anabaena*, and *Planktothrix* and diatom genera *Tabellaria* and *Fragilaria*) per square meter of substrate (i.e. cells m^{-2}), were determined using light microscopy (Leitz Dialux 20, Leitz US Scopes, Paramount, California) and a Sedgwick-Rafter counting cell, according to standard method 10300C (MDL=1.0x10^3 cells m^{-2}) APHA, 2005).

Analysis of Field Exposures

SCP was applied *in situ* at a concentration of 37 mg L^{-1} (10.2 mg H_2O_2 L^{-1}) as a granular, surface application. As SCP is denser than water (Table 1), granules settled to the sediment water interface after SCP was broadcast from a boat. To measure initial H_2O_2 exposures, water samples were collected in triplicate at three locations in the study site using a Van Dorn bottle, immediately after the application (i.e. within minutes of application). Samples were collected in close proximity to the sediment-water interface, at a depth of approximately 3.05m (10 ft). To quantify the duration of the field exposure, analytically informed samples were collected in triplicate from the three sampling locations until concentrations were less than the detection limit. To determine the detection limit, samples were collected synoptically from an untreated area of Hartwell Lake. Each 50 mL sample was collected in a polyethylene terephthalate centrifuge tube and stored on ice away from sunlight. Samples were mixed with analytical reagents at the site and prior to transport to Clemson University. Laboratory analysis of H_2O_2 concentrations followed the methods outlined in section 2.2. Samples not immediately analyzed were stored in the dark at 2 ± 1ºC for less than 96-h before analysis, based on the storage stability of the I_3^- method (Kinley et al., 2015).

Responses of Algae to Field Applications

Algal samples were collected before treatment, 4 days after treatment (4-DAT), and 7-DAT to determine the effectiveness of Phycomycin® SCP exposures and to compare with the laboratory model. An untreated area of Hartwell Lake was sampled during this period for use as an untreated control. Three composite algal samples were

collected from three sites within the treated study site and three sites at the untreated area. Samples were gently rinsed from substrates (e.g. submerged rocks) into 50 mL polyethylene terephthalate centrifuge tubes and stored on ice for transport to the laboratory. Responses of the algal assemblage (i.e. chlorophyll *a* concentrations, phycocyanin concentrations, and cell densities) were analyzed less than 24-h after sample collection. Responses were measured following methods outlined in section 2.2.

In situ concentrations of MIB and geosmin were quantified as an additional response parameter. Samples were collected from two locations downstream of the treatment area and one location upstream in an unexposed area. Analysis was performed by Regional Water Authority (New Haven, CT 06511) according to standard method 6040D (APHA, 2005).

Water characteristics in treated and untreated areas were measured prior to application, 4-DAT, and 7-DAT. Dissolved oxygen, pH, conductivity, hardness, and alkalinity were measured as outlined in section 2.2.

Statistical Analysis

Differences in algal responses to laboratory treatments were discerned using one-way analysis of variance (ANOVA) with specific differences identified through multiple comparisons testing (Tukey's). A paired t-test procedure was used to determine statistically significant differences in algal response measurements between the laboratory and *in situ* treatments 4-DAT and 7-DAT. Exposure data analysis was performed using ANOVA followed by Tukey's multiple comparisons testing to identify differences between treatments. Regression analysis was performed to determine the rate

of decline and half-life of H_2O_2 concentrations. A paired t-test was used to determine statistically significant differences in exposure measurements between the laboratory and *in situ* experiments. All data were analyzed using JMP® Pro 12.0.1 (2015; $\alpha = 0.05$).

Results and Discussion

Measured Laboratory Exposures and Consequent Algal Responses

Laboratory Exposures

Measured initial exposures of H_2O_2 as SCP were between 66% ± 1.5% and 96% ± 3.5% of targeted exposures (average = 76% ± 11%; Table 4), therefore all results are reported as measured exposures. Measured initial exposures of 238, 453, 615, and 812 mg H_2O_2 m^{-2} were significantly different from targeted exposures.

Within 48 hours, all exposures of H_2O_2 declined to less than the detection limit (0.2 mg H_2O_2 L^{-1}) and were not significantly different from untreated controls (Figure 2). Changes in peroxide concentration over time fit a first order rate model for all five exposures; there was a linear relationship between the natural log of the H_2O_2 concentration ($\ln[H_2O_2]$) and time (R^2=0.67; Figure 2). Rates of H_2O_2 dissipation were similar for all five exposures, (p=0.5267; α=0.05; Figure 2), with an overall first order rate coefficient of H_2O_2 as SCP of 0.08 h^{-1}, and an overall half-life of 8.6 h.

Algal Responses to Laboratory Exposures

Densities of target algae measured 4-DAT were significantly different ($p<0.05$) from pretreatment at exposures of 812, 615, 453, and 238 mg H_2O_2 m^{-2}, decreasing 91.1±1.1%, 77.3±2.8%, 71.3±14.0%, and 37.6±13.8%, respectively (Figure 5). Densities measured 7-DAT remained significantly different from pretreatment at each of these exposures, but were not significantly different from densities measured 4-DAT ($p>0.05$; Figure 5). In untreated controls, no change was measured 4-DAT or 7-DAT. Phycocyanin concentrations measured 4-DAT were significantly different from pretreatment at

exposures of 812, 615, and 453 mg H_2O_2 m^{-2}, decreasing 85.7±5.4%, 66.7±4.2%, and 67.6±7.8%, respectively (Figure 5). Phycocyanin concentrations measured 7-DAT remained significantly different from pretreatment and were not significantly different from 4-DAT, except at exposures of 238 mg H_2O_2 m^{-2} and 615 mg H_2O_2 m^{-2}. In untreated controls, there was no change in phycocyanin concntrations measured 4-DAT or 7-DAT. Chlorophyll *a* concentrations measured 4-DAT and 7-DAT did not change significantly from pretreatment in any of the five exposures of H_2O_2 as SCP or in untreated controls (Figure 3).

Although significant responses in the laboratory were measured in terms of phycocyanin concentrations and target algal densities, chlorophyll *a* concentrations did not change significantly from pretreatment in any of the five H_2O_2 exposures or the untreated controls. For the targeted algae, this could be explained by a differential sensitivity of cyanobacteria compared to diatoms (eukaryotic algae), which has been measured for H_2O_2 exposures (Barroin and Feuillade, 1986; Drabkova et al., 2007a,b; Barrington and Ghadouani, 2008; Matthijs et al., 2012; Barrington et al., 2013; Burson et al., 2014) and exposures of H_2O_2 as SCP (Geer et al., in press). Chlorophyll *a* is an aggregate measure of cell viability, and is not always useful for measuring the responses of algal assemblages to algaecide exposures (Calomeni and Rodgers, 2015). Phycocyanin is a photosynthetic pigment specific to prokaryotic algae, and concentrations of phycocyanin coupled with declining densities of target algae suggests that prokaryotic organisms were affected by exposures of H_2O_2 as SCP.

Responses of the algal assemblage to laboratory exposures of H_2O_2 in terms of phycocyanin and target algal densities indicates that control of an algal assemblage can be achieved with an *in situ* application of SCP. In contrast, phycocyanin concentrations and target algal densities of untreated controls did not change significantly from pretreatment, indicating that the algal assemblage would remain viable if the decision was made not to implement a treatment. These results can be used to select an appropriate concentration for application *in situ*. Although the magnitude of responses (i.e. percent decrease of phycocyanin concentrations and target algal densities 4-DAT from pretredsatment) appeared to increase with increasing exposures of H_2O_2 (R^2=0.70, 0.79 respectively; Figure 6), significant differences between treatments were masked by the variance of algal responses. Based on significant changes from pretreatment in phycocyanin concentrations and densities of target algae measured 4-DAT and 7-DAT, an exposure greater than 453 mg H_2O_2 m^{-2} was predicted to be effective. In addition to selecting an appropriate concentration for use in the field, preliminary laboratory experiments can be used to guide post treatment monitoring of algal responses. Our results indicate that significant responses of the algal assemblage in terms of phycocyanin and cell density will be manifested 4-DAT, as additional decline 7-DAT was not discernable.

Measured In Situ *Exposure and Consequent Algal Responses*

Measured In Situ Exposure

The *in situ* SCP application was designed to expose the targeted algae to 22,500 mg H_2O_2 m^{-2}, scaled for a depth of 1.83m (6 ft) at the sediment-water interface

(equivalent to a concentration of 10.2 mg L^{-1} H_2O_2 as Phycomycin SCP). The maximum exposure of H_2O_2, measured immediately after application at a depth of approximately 3.05m (10ft) and within 0.3m (1ft) of the sediment-water interface, was 996 mg H_2O_2 m^{-2}, approximately 4.4% of the targeted initial exposure. Within 30 hours, concentrations of H_2O_2 were below the detection limit (i.e. were not significantly different from untreated controls; Figure 3). The change in the natural log of the H_2O_2 concentration ($\ln[H_2O_2]$) with time fit a linear regression model (R^2=0.67). The slope of the linear regression yielded an *in situ* first order rate coefficient for the *in situ* dissipation of H_2O_2 as SCP of 0.061 h^{-1} and a first order half-life of 11.4 h (Table 4).

H_2O_2 from an *in situ* application of an SCP algaecide can be challenging to measure. Due to the instability of H_2O_2, Matthijs et al. (2012) could not use the laboratory catalase method to measure *in situ* exposures. Matthijs et al. (2012) as well as Burson et al. (2014) were able to estimate *in situ* H_2O_2 concentrations using Quantofix test sticks, which are intended for measurement of distinct H_2O_2 concentrations (i.e. 1, 10, 30, and 100 mg L^{-1}). In the present study, the triiodide method was useful for measuring *in situ* H_2O_2 concentrations because samples could be fixed for analysis at the field site, without need for additional laboratory equipment until analysis with a spectrophotometer. Due to the storage stability of fixed samples and the sensitivity of the method, our results demonstrate the utility of the triiodide method for measuring *in situ* H_2O_2 exposures after an SCP application.

Measured Algal Responses

In the treated area, target algal densities measured 4-DAT declined significantly by 85.5±7.2% from pretreatment ($p=0.0072$), and remained significantly less than pretreatment 7-DAT ($p=0.0353$). In the untreated site densities measured 4-DAT and 7-DAT increased 40.0±154% and 1,030±727%, respectively, although increases were not significant. Phycocyanin concentrations measured in samples from the treated study site declined significantly from pretreatment ($p<0.0001$) by 72.2±12.7% 4-DAT, and remained significantly less than pretreatment 7-DAT ($p<0.0001$), paralleling target algal densities. In the untreated area, concentrations declined 4-DAT and 7-DAT, but variances associated with the changes were not sufficient to be significantly different from pretreatment. Chlorophyll *a* concentrations measured 4-DAT and 7-DAT did not change significantly from pretreatment in either the treated or untreated sites.

Samples collected from Hartwell Lake were analyzed for MIB and geosmin as additional evidence indicating a target algal response. Pretreatment concentrations of MIB were 39.2 ng L^{-1} at the water intake structure and 44.9 ng L^{-1} in a cove adjacent to the study site. MIB declined 7-DAT to 27.17 ng L^{-1} at the water intake and 33.2 ng L^{-1} near the study site. Concentrations were at or below the detection limit (1 ng L^{-1}) at both locations 21-DAT, and remained below detection through 37-DAT. Pretreatment geosmin concentrations were 4.53 ng L^{-1} at the water intake structure and 4.1 near the treated cove, and declined 7-DAT to 3 ng L^{-1} and 2.7 ng L^{-1} at the water intake and near the treated cove, respectively. Geosmin concentrations measured 21-DAT decreased further to 1.3 and 1.1 ng L^{-1} at the water intake and near the treated cove, respectively.

There was no addition decline measured 37-DAT after treatment. In the untreated area, pretreatment (i.e. prior to application of SCP in the treated area) concentrations of MIB and geosmin measured 15 days before treatment were 23.5 and 74.4 ng L^{-1}, respectively. Post-treatment concentrations were 5.6 and 66.2 ng L^{-1} measured 21-DAT, and 11.6 and 33.6 ng L^{-1} measured 37-DAT.

Significant responses in terms of phycocyanin concentrations and target algal densities confirm that some of the target algae comprising the benthic algal assemblage were affected by the application of H_2O_2 as SCP. The lack of a significant response in terms of chlorophyll *a* concentrations indicates that the exposure was not sufficient to affect all algae in the study site (i.e. eukaryotic target and non-target algae). As the algae were considered problematic due to their production of taste and odor compounds MIB and geosmin, measures of these tertiary alcohols provide lines of evidence necessary to discern the effect of the SCP exposure on the assemblage. Relative to the untreated site, decreases in MIB and geosmin at the intake of the water treatment facility corroborate significant responses (in terms of phycocyanin concentration and target algal densities) in the study site, indicating that the algaecide application provided a beneficial alternative to a no-treatment decision.

Comparison of Laboratory and Field Exposures and Responses

In situ algal responses were compared to the laboratory responses from an exposure of 812 mg H_2O_2 m^{-2}. In both the laboratory and *in situ* experiments, chlorophyll *a* concentrations measured post treatment (i.e. 4 and 7-DAT) did not change significantly from pretreatment concentrations. Cell density decreased to a greater extent in the

laboratory than *in situ* (91.1±1.1% compared to 85.5±7.2%; Figure 8), although the difference was not statistically significant. Phycocyanin concentrations were comparable between laboratory and *in situ* experiments (85.7±5.4% compared to 72.2±12.7%; Figure 8), paralleling target algal density similarities between laboratory and *in situ* experiments. However, due to the variance of *in situ* phycocyanin and target algal density responses, and lack of significant differences among laboratory exposures, responses measured 4-DAT *in situ* were also comparable to responses obtained 4-DAT from laboratory exposures of 615 and 453mg H_2O_2 m^{-2}. *In situ* responses measured 7-DAT were comparable to laboratory responses elicited from exposures of 615, 453, and 238 mg H_2O_2 m^{-2}. Because responses measured *in situ* 4-DAT and 7-DAT were consistent with the laboratory prediction (i.e. that an exposure greater than 453 mg H_2O_2 m^{-2} would yield significant decreases in target algal densities and phycocyanin concentrations relative to pretreatment), the exposure obtained *in situ* was inferred to be comparable to effective laboratory exposures (i.e. 453-812 mg H_2O_2 m^{-2}).

By indirect comparison, laboratory and *in situ* algal responses provide a line of evidence that the laboratory model provided an accurate prediction of the results of an *in situ* SCP application. Several other studies have similarly conducted laboratory experiments with site water and algae and found *in situ* responses to be comparable (Bishop and Rodgers, 2012; Matthijs et al., 2012; Barrington et al., 2013; Burson et al., 2014). In the present study, laboratory and *in situ* experiments were compared directly in terms of measured H_2O_2 exposures. *In situ* predictions were based on responses of the algal assemblage to a mean initial exposure of 812 mg H_2O_2 m^{-2} measured in the

laboratory, which was not significantly different from the mean initial exposure measured *in situ* (619 mg H_2O_2 m^{-2}). Due to the variance associated with the measured *in situ* exposure (i.e. standard deviation ±428 mg H_2O_2 m^{-2}), the exposure measured *in situ* was not significantly different than any of the laboratory exposures that produced significant responses (i.e. 453–812 mg H_2O_2 m^{-2}). By direct comparison of exposures, measured *in situ* exposures confirm that the laboratory exposure-response model was accurate in predicting exposures necessary to affect the target benthic algal assemblage.

Based on direct and indirect comparison, the application of SCP *in situ* obtained results consistent with laboratory predictions. Results were consistent because exposures obtained in the laboratory were comparable to exposures obtained in the field. Comparable half-lives of H_2O_2 as SCP (i.e. overall $t_{1/2}$ in the laboratory was 8.6 h compared to 11.4 h *in situ*; Table 4) suggest that processes affecting the dissipation of H_2O_2 over time were captured in the laboratory by the inclusion of site-collected water and algae in the experimental design. Although half-lives indicate that exposures were comparable in terms of exposure duration, initial exposures were more variable *in situ* than in the laboratory. Deviation of laboratory exposures ranged from 1.1 to 18.3 mg H_2O_2 m^{-2}, while the initial exposure *in situ* varied by ±428 mg H_2O_2 m^{-2} (Table 4). To achieve an exposure of H_2O_2 at the sediment water-interface, granules of SCP had to 1) settle through the water column and 2) dissociate into H_2O_2. The variability associated with the measured *in situ* exposure, and the order of magnitude difference between the measured exposure and targeted exposure, are likely the result of inconsistent settling rates of SCP granules, such that they do not arrive uniformly at the sediment-water

interface. Furthermore, since exposures were measured at a depth of approximately 3.05m (10 ft), some of the activity of H_2O_2 was likely lost due to concurrent settling and dissociation into H_2O_2. Therefore, when treating benthic algae with a surface broadcast of SCP granules, our results indicate that it is critical to consider the depth of the water column/volume of water being treated in order to accurately translate effective laboratory exposures and obtain a comparable exposure *in situ*. Barrington et al. (2013), in the context of a liquid H_2O_2 application, similarly noted that water volume is an important factor when determining the amount of H_2O_2 needed to control a noxious algal growth. In the present experiment, to ensure that an adequate exposure reached the target algae at the sediment-water interface, the *in situ* application was adjusted for a depth of 1.83m, rather than 0.61m as was used in the laboratory. As a result, sufficient SCP reached the sediment-water interface *in situ* such that the measured exposure was comparable to effective laboratory exposures.

4. Conclusions

Benthic algae collected from Hartwell Lake and exposed to H_2O_2 as SCP in laboratory experiments responded similarly (in terms of chlorophyll *a*, phycocyanin, and cell densities) to algae exposed *in situ*. An effective exposure of Phycomycin SCP (i.e. at least 453 ±4.3mg H_2O_2 m^{-2}) was identified to produce the desired response from the targeted algal species. By utilizing field-collected algae and water in both laboratory and field experiments, differences in potential exposure modifying factors (i.e. water characteristics and specific algal sensitivity) were minimized. As a result, *in situ* exposures were comparable to effective laboratory exposures, both by direct comparison of exposures (in terms of initial exposure achieved and exposure duration) and by indirect comparison of equivalent responses, inferring that exposures had to be comparable to achieve comparable responses. Our results are consistent with the results of previous studies in which preliminary laboratory experiments with field collected algae preceded *in situ* treatments, in an attempt to discern effective concentrations of H_2O_2 (Burson et al., 2014; Matthijs et al., 2012; Barrington et al., 2013) and H_2O_2 as SCP (Bishop and Rodgers, 2011) for use *in situ*. We contributed to this approach by using the triiodide method to measure laboratory and *in situ* H_2O_2 exposures, such that laboratory predictions confirmed by comparable algal responses were augmented with measures of equivalent H_2O_2 exposures. This experiment provides evidence for the use of a physical laboratory model in predicting responses of algae *in situ*.

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TABLES AND FIGURES

Table 4.1: Physical and chemical properties of Phycomycin® SCP.

CAS number	497-19-8[a]
Formulation	SCP and inert ingredients
Active ingredient	85% SCP
Maximum application Concentration	36.9 mg L^{-1} (10.2 mg L^{-1} H_2O_2)[a]
Physical state	Coarse white grains[a]
Water solubility	140g/L at 24ºC[a]
pH	10.4-10.6 s.u. (1% solution)[a]
Boiling Point	Not applicable[a] (SCP decomposes when heated)[b]
Melting point	Not applicable (SCP decomposes when heated)[c]
Partition coefficient n-octanol/water	Not applicable (sodium carbonate peroxyhydrate is an organic salt)[b]

[a]AB (2007)
[b]OECD (2006)
[c]HERA (2002)

Table 4.2: Water characteristics at test initiations and completions (7-DAT) for laboratory exposures of H_2O_2 as SCP in Hartwell Lake site water.

	Exposure											
	Control		**0.3**		**2.5**		**5.2**		**7.5**		**10.2**	
Parameter	Initial	7-DAT	Initial	7-DAT	Initial	7-DAT	Initial	7-DAT	Initial	7-DAT	Initial	7-DAT
pH[1] (S.U)[1]	7.29	7.02	6.95	7.3	7.02	7.5	7.25	7.87	7.38	8.04	7.42	8.17
Dissolved O_2 (mg/L)[2]	8.77	8.53	8.36	8.3	8.15	8.12	8.87	8.61	8.29	8.67	8.56	8.23
Conductivity (µS/cm)[3]	50	35	55	56	71	71	93	95	100	102	136	142
Alkalinity (mg/L as $CaCO_3$)[4]	20	15	20	20	25	30	35	30	45	35	60	70
Hardness (mg/L as $CaCO_3$)[4]	10	15	10	15	10	10	10	10	10	15	10	15

[1]pH was measured using an Orion® 4-Star pH meter and Triode® electrode (±0.01 SU)
[2]Dissolved oxygen was measured using a YSI® Model 52 dissolved oxygen meter (±0.1 mg/L)
[3]Conductivity was measured using YSI® 30 conductivity meter (±1 µS/cm[2])
[4]Alkalinity and hardness were measured according to standard methods 2320 (±2 mg/L as $CaCO_3$) and 2340 (±2 mg/L as $CaCO_3$), respectively (APHA, 2005)

Table 4.3: Water characteristics for Hartwell Lake pretreatment and 7-DAT.

Parameter	Water characteristics (average)		
	Initial	Control Site 7-DAT	Treated Site 7-DAT
pH (SU)[1]	7.8	7.5	7.4
Dissolved O_2 (mg L^{-1})[2]	8.5	8.6	8.2
Conductivity ($\mu S/cm^2$)[3]	57	59	51
Alkalinity (mg L^{-1} as $CaCO_3$)[4]	25	15	20
Hardness (mg L^{-1} as $CaCO_3$)[4]	15	15	15

[1]pH was measured using an Orion® 4-Star pH meter and Triode® electrode (±0.01 SU)
[2]Dissolved oxygen was measured using a YSI® Model 52 dissolved oxygen meter (±0.1 mg/L)
[3]Conductivity was measured using YSI® 30 conductivity meter (±1 $\mu S/cm^2$)
[4]Alkalinity and hardness were measured according to standard methods 2320 (±2 mg/L as $CaCO_3$) and 2340 (±2 mg/L as $CaCO_3$), respectively (APHA, 2005)

Table 4.4: Comparison of measured laboratory and *in situ* initial H_2O_2 exposures (n=3).

Experiment	Target Concentration (mg H_2O_2 L^{-1})	% of MLR	Depth of Water Column Treated (m)	Target Exposure (mg H_2O_2 m^{-2})	Measured Exposure (mg H_2O_2 m^{-2})	Deviation	% of Target Exposure	Rate of Dissipation (k)	Half-life
Laboratory	0.3	3	0.61	36.6	35	1.3	95.7	0.131	5.3
Laboratory	2.5	25	0.61	303	238	1.1	78.4	0.099	7.0
Laboratory	5.2	51	0.61	638	453	4.3	71.1	0.094	7.3
Laboratory	7.5	74	0.61	909	615	2.1	67.7	0.096	7.2
Laboratory	10.2	100	0.61	1234	812	18.3	65.9	0.105	6.6
In situ	10.2	100	1.83	22500	619	430	2.8	0.061	11.4

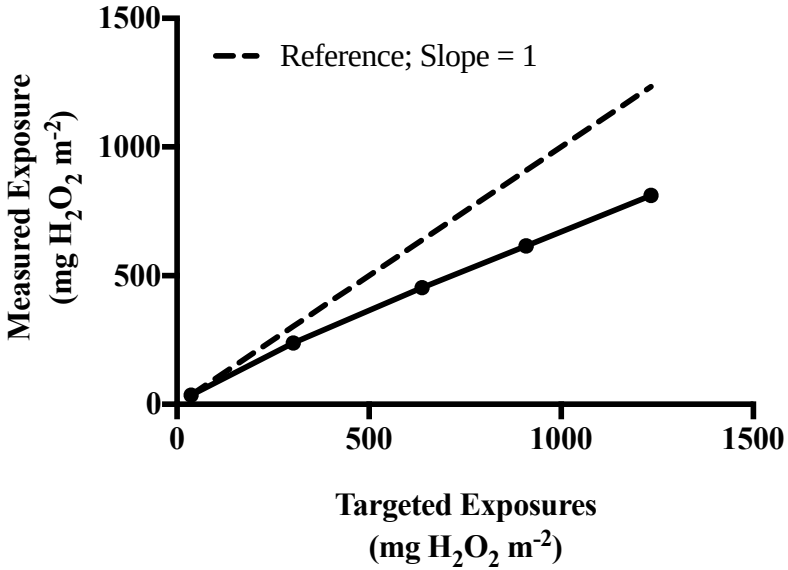

Figure 4.1: Comparison of targeted and measured initial laboratory exposures of H_2O_2 as SCP. Error bars indicate ± 1 standard deviation.

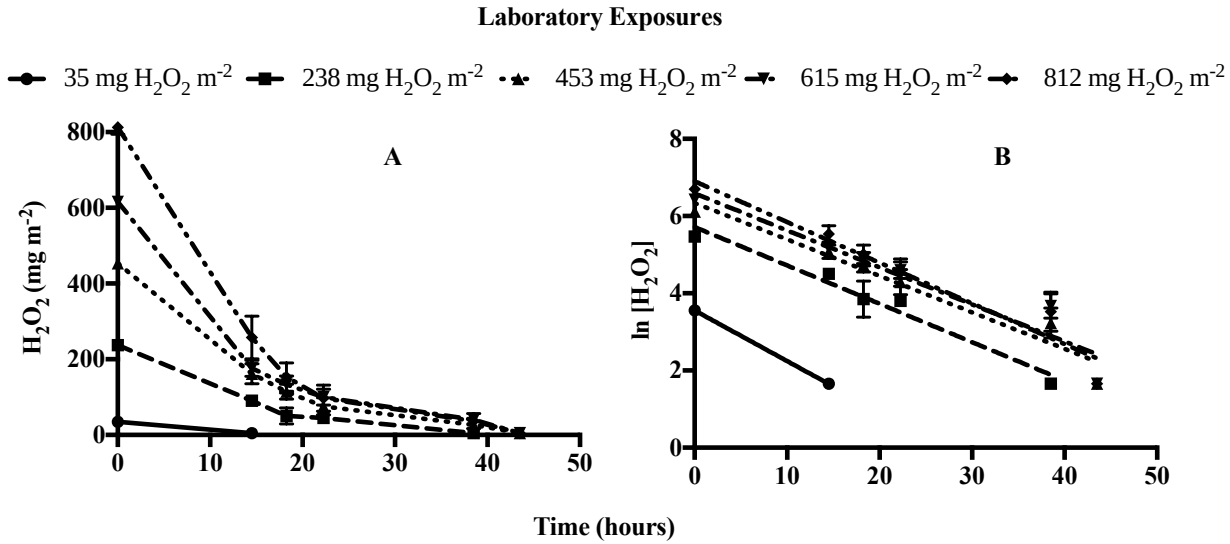

Figure 4.2: Change in (A) mean measured H_2O_2 exposure over time and (B) mean ln(H2O2) concentrations over time in laboratory exposures of H_2O_2 as SCP (n=3). Error bars represent ±1 standard deviation.

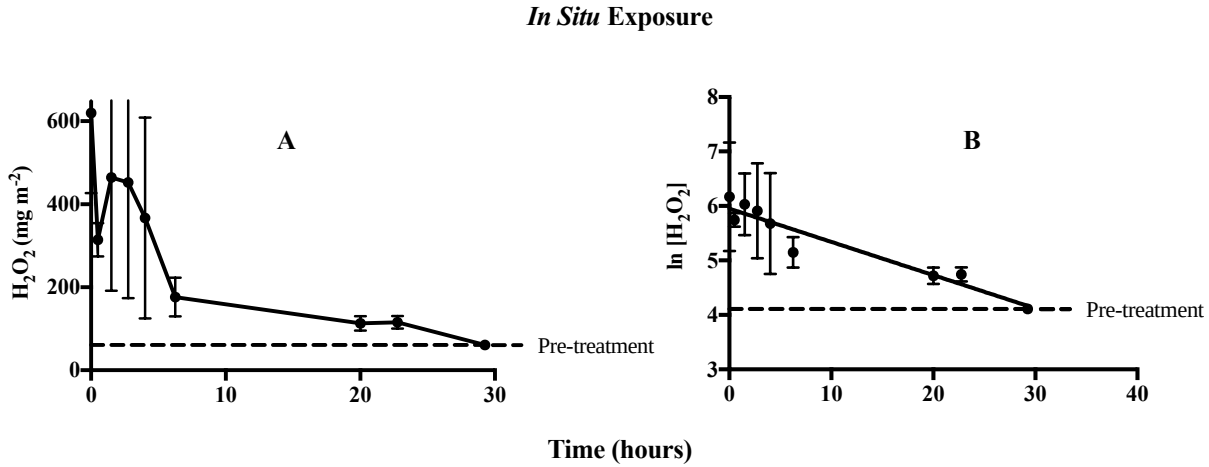

Figure 4.3: *In situ* change in (A) mean measured H_2O_2 concentration over time and (B) mean ln[H2O2] over time from exposures of H_2O_2 as SCP (n=3). Error bars represent ±1 standard deviation.

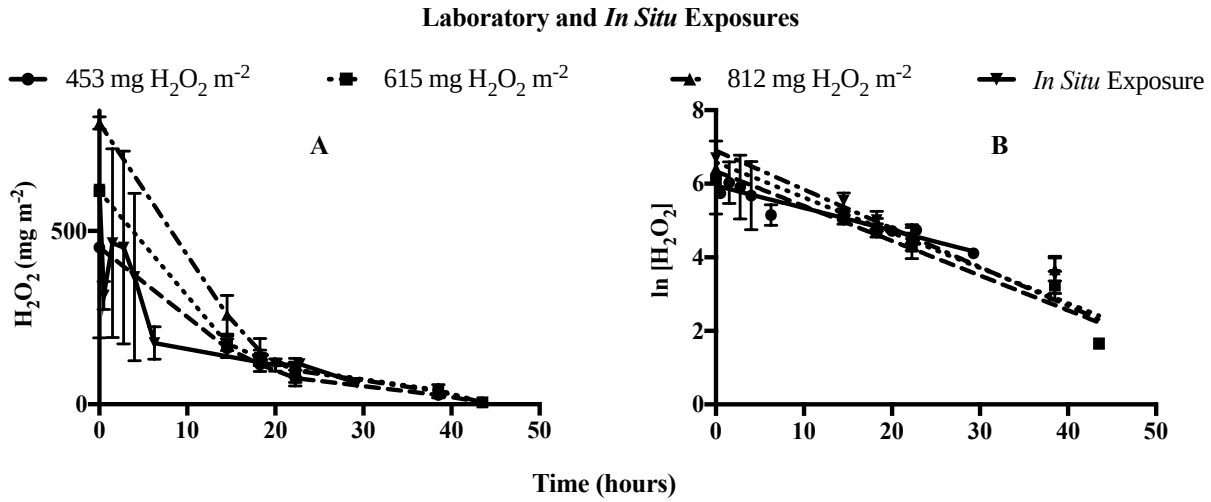

Figure 4.4: Comparison of the change in (A) mean H_2O_2 concentration over time and (B) mean ln(H2O2) concentrations over time from laboratory and *in situ* exposures of H_2O_2 as SCP (n=3). Error bars represent ±1 standard deviation.

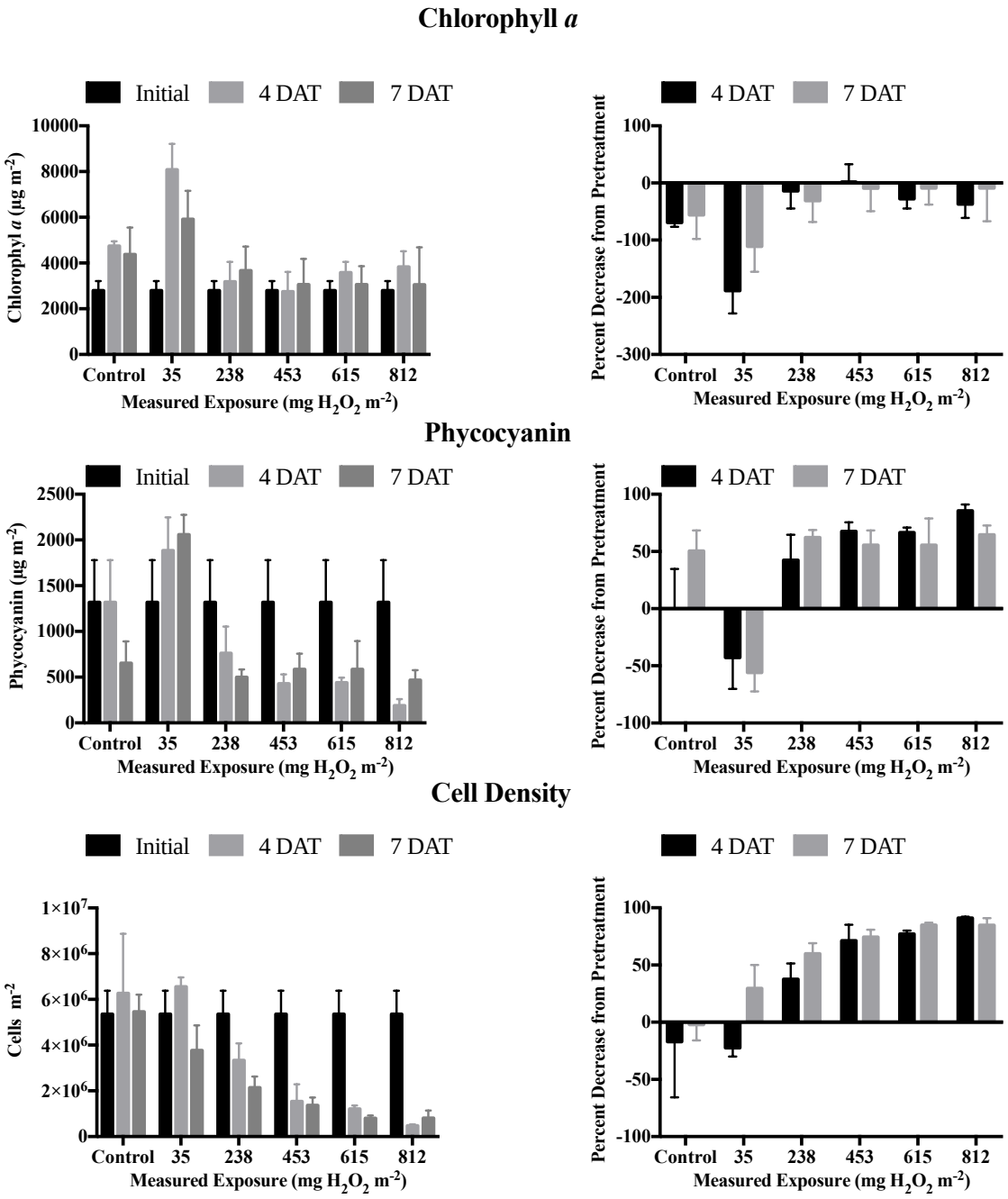

Figure 4.5: Mean responses of the benthic algal assemblage from Hartwell Lake (in terms of chlorophyll *a*, phycocyanin, and cell density, and the percent decrease of responses relative to pretreatment amounts) to laboratory exposures of H_2O_2 as SCP (*n*=3). Positive values indicate a decrease in response and negative values indicate an increase in response from pretreatment amounts. Error bars represent ±1 standard deviation

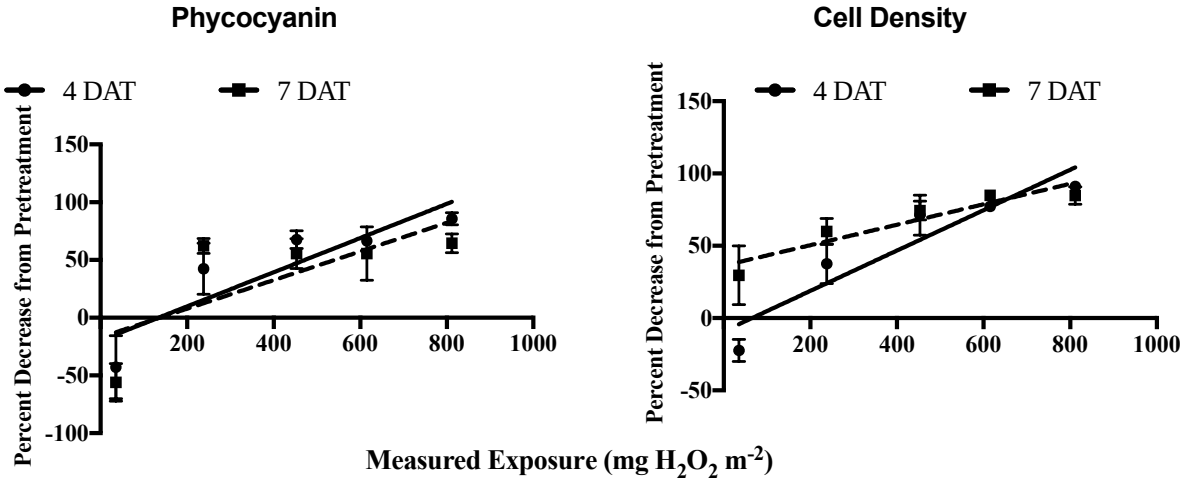

Figure 4.6: Linear relationship between the percent decrease of phycocyanin and cell density with increasing exposure concentrations of H_2O_2 as SCP. Error bars indicate ± 1 standard deviation.

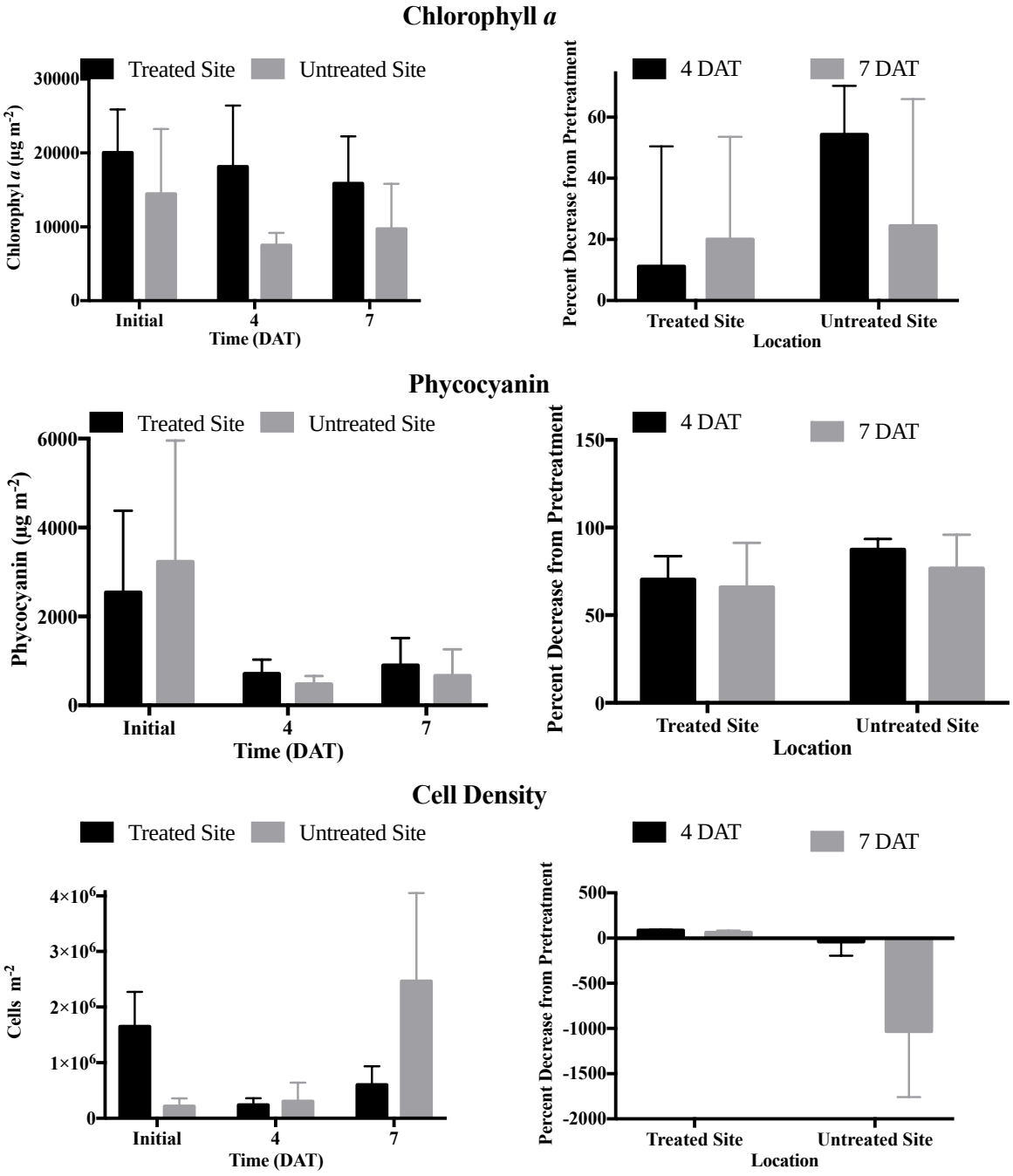

Figure 4.7: Mean *in situ* responses of the benthic algal assemblage from Hartwell Lake (in terms of chlorophyll *a*, phycocyanin, and cell density, and the percent decrease of responses relative to pretreatment amounts) to H_2O_2 from an application of SCP (n=3). Positive values indicate a decrease in response and negative values indicate an increase in response from pretreatment amounts. Error bars represent ±1 standard deviation.

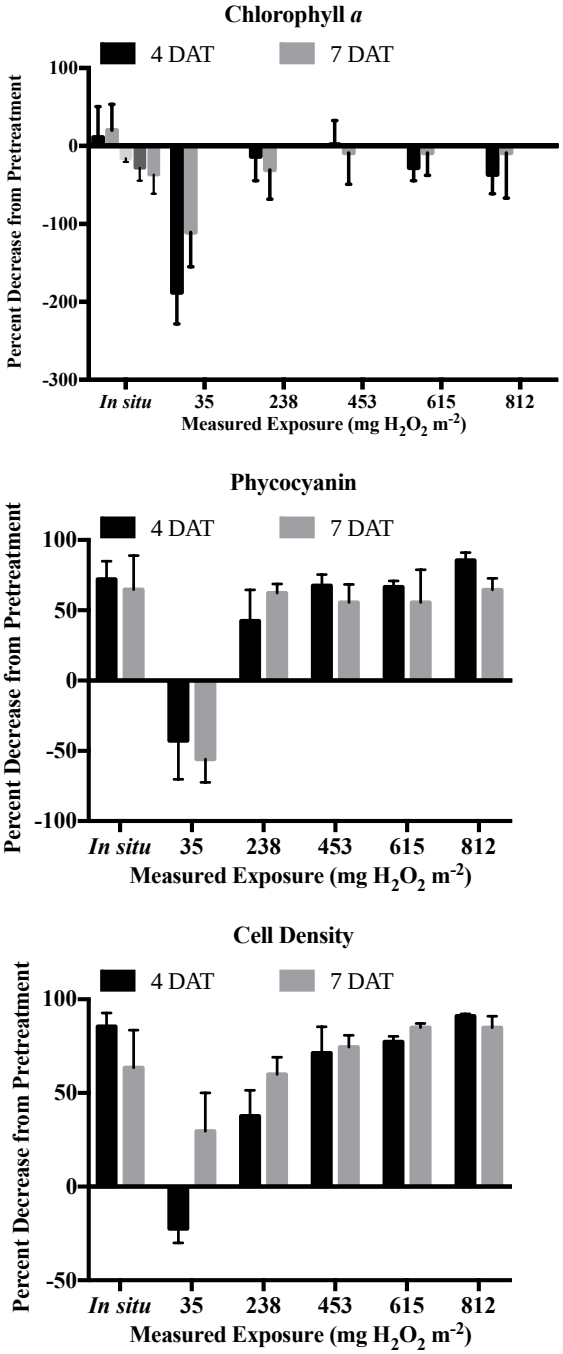

Figure 4.8: Comparison of mean responses of the benthic algal assemblage from Hartwell Lake (in terms of percent decrease relative to pretreatment amounts) between laboratory and *in situ* exposures of H_2O_2 as SCP (n=3). Positive values indicate a decrease in response and negative values indicate an increase in response from pretreatment amounts. Error bars represent ±1 standard deviation.

CHAPTER FIVE

SUMMARY AND CONCLUSIONS

The overall objective of this research was to evaluate exposures of an SCP algaecide to efficiently and effectively control problematic algal growth in a field situation while decreasing potential risks to non-target species. Experiments were conducted to 1) measure influences of particulate and dissolved forms of organic carbon on exposures of an SCP algaecide and consequent effects on a target alga; 2) measure and compare responses of an array of freshwater organisms following exposures to hydrogen peroxide (H_2O_2) as SCP in laboratory formulated water; and 3) evaluate responses of putative algal taste and odor producers to laboratory exposures of an SCP algaecide and compare results with exposures and responses measured *in situ*. This research as a whole enhances our understanding of SCP as an algaecide and its ability to mitigate risks associated with noxious algal growths in water resources.

In "Influence of Dissolved and Particulate Fractions of Organic Carbon on Exposures of a Sodium Carbonate Peroxyhydrate Algaecide and Consequent Responses of *Microcystis aeruginosa*." 96-h median effects concentrations (96-h EC50s) of a series of densities of *M. aeruginosa* exposed to H_2O_2 as SCP were measured and compared. As the density of algae increased from $9.7x10^5$ to $2.3x10^7$ cells/mL, measured 96-h EC_{50} values for *M. aeruginosa* in terms of cell density increased from 0.9 mg H_2O_2/L to 30.9 mg H_2O_2 L^{-1}. The exposure per cell of H_2O_2 as SCP achieving 96-h EC_{50} values increased concomitantly with cell density from $8.79x10^{-10}$ mg H_2O_2 /cell to $1.34x10^{-9}$ mg H_2O_2 /cell. Exposures likely increased due to competitive reactions between H_2O_2 and algal-

related dissolved organic carbon, as the increase in cell density was coupled with an increase in dissolved organic carbon from 4±1 mg/L to 24±1 mg/L. Based on results of the study, concentrations of H_2O_2 as SCP achieving control of *M. aeruginosa* are proportional to the density of algae and DOC concentration. Based on label limited concentrations of SCP that could be applied to a water resource, there is a limit to the density of algae that may be controlled with a single application. Implementing a treatment before prolific algal growth increases the likelihood of success with a single treatment and decreases the amount of product required, decreasing costs associated with treatment and potential risks for non-target organisms. Critical burdens were not equivalent, and instead were concluded to be different due to reactions between H_2O_2 and algal derived DOC. When scaling laboratory results to an *in situ* treatment with an SCP algaecide, predictions of exposures necessary to achieve control could be enhanced if DOC and algal density are known, which could decrease the chance of applying an ineffective concentration and maintain margins of safety for non-target organisms.

In "Comparative Toxicity of Sodium Carbonate Peroxyhydrate to Freshwater Organisms", a cyanobacterium and two invertebrates were more sensitive than a eukaryotic alga and a vertebrate to exposures of H_2O_2 from an SCP algaecide. 96-h EC_{50} values ranged from 1.0 to 19.7 mg L^{-1} H_2O_2 for animals, while the 96-h EC_{50} for the cyanobacterium *M. aeruginosa* was 0.9 mg L^{-1} H_2O_2. The 7-d EC_{50} for the eukaryotic algae *P. subcapitata* was 5.2 mg L^{-1} H_2O_2, as responses to environmentally relevant concentrations of SCP were not manifested in 96-h, while the fish *P. promelas* was not sensitive to exposures within the recommended range of application concentrations for

the source of SCP used in this study (Phycomycin® SCP; 0.2 to 10.2 mg L^{-1} H_2O_2). SCP is comparable to other algaecides (i.e. copper-based algaecides, endothall, and diquat dibromide) in that it could be used selectively for control of prokaryotic algae. However, SCP may be less potent than diquat dibromide to *H. azteca*, and less potent than copper algaecides to *C. dubia*, enhancing margins of safety for these species. While not directly predictive of specific concentrations at which organisms will respond to *in situ*, results of this study can be used to predict the distribution of responses likely to occur. Results indicate that SCP could mitigate risks associated with noxious cyanobacterial growths (e.g. *M. aeruginosa*) while providing a margin of safety for non-target species.

In "Predicting *In Situ* Responses of Taste and Odor Producing Algae in a Southeastern U.S Reservoir to a Sodium Carbonate Peroxyhydrate Algaecide Using a Laboratory Exposure-Response Model," the potential convergence of exposures and responses measured in a laboratory experiment and *in situ* was evaluated. By utilizing the same algae and water in both laboratory and field experiments, differences in potential exposure modifying factors (i.e. water characteristics and specific algal sensitivity) were minimized such that results obtained from the *in situ* application were comparable to the laboratory exposure-response relationship. Benthic algae were not uniformly affected by laboratory or *in situ* exposures of H_2O_2 from SCP, as indicated by chlorophyll *a* concentrations, while significant responses in both experiments in terms of phycocyanin and cell densities indicated that cyanobacteria within the benthic algal assemblage responded to the application of SCP. Additional measurements of MIB and geosmin concentrations *in situ* corroborated significant responses measured in terms of

phycocyanin and cell densities, indicating that the *in situ* application was successful in achieving the site-specific performance goals. These results are consistent with the results of previous studies in which preliminary laboratory experiments with field collected algae preceded *in situ* treatments, in an attempt to discern effective concentrations of H_2O_2 (liquid) and H_2O_2 as SCP (granular) for use *in situ*. We contributed to this approach by using the triiodide method to measure laboratory and *in situ* H_2O_2 exposures, such that laboratory predictions could be directly compared to the *in situ* exposure (i.e. compared by comparing exposures that elicited comparable responses). However, as exposures of H_2O_2 as SCP were labile and dynamic, an indirect comparison of laboratory and field experiments (i.e. responses observed *in situ* were used to infer the causative exposure from the laboratory model) was necessary to corroborate exposures directly comparable in terms of initial exposure and exposure duration (i.e. half-life).

The approach outlined in this research has applications universally, and can be used as a component of a water resource management plan to make site specific predictions that increase algaecide effectiveness when remediating critical water resources that have been impaired by problematic algal growth. As a whole, this research highlights important considerations when designing and implementing a management plan to control noxious algal growths with an SCP algaecide.