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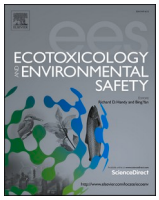

Laboratory-scale evaluation of algaecide effectiveness for control of microcystin-producing cyanobacteria from Lake Okeechobee, Florida (USA)

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ABSTRACT

Growth of microcystin-producing cyanobacteria in Lake Okeechobee (Florida, USA) and surrounding waters has resulted in adverse health impacts for humans and endangered species, as well as significant economic losses. As these issues worsen, there is growing pressure for efficacious solutions to rapidly mitigate harmful algal blooms (HABs) and protect critical freshwater resources. Applications of USEPA-registered algaecides as management tactics meet many decision-making criteria often required by water resource managers (e.g., effective, scalable, selective), but have not yet been evaluated on a large scale within the Lake Okeechobee waterway. This study was conducted to bolster the peer-reviewed database for available management tactics against microcystin-producing cyanobacteria in waters of this region. Laboratory-scale experiments can be conducted first to minimize uncertainty at larger scales and improve confidence in decision-making. In this study, samples containing microcystin-producing cyanobacteria collected from Lake Okeechobee were exposed to several USEPA-registered algaecides in laboratory toxicity experiments. Responses of target cyanobacteria were measured 3 days after treatment (DAT) in terms of cell density, chlorophyll-*a* concentrations, and phycocyanin concentrations. Based on responses of the cyanobacteria, minimum effective exposure concentrations were identified for each algaecide. Microcystin release (i.e. proportion of total microcystins in the aqueous phase) was measured and compared 1 DAT among effective exposures. Total microcystin concentrations were measured in effective treatments at 1, 4, and 9 DAT to discern potential for microcystin persistence following exposures to the effective formulations and exposure concentrations. Overall, several formulations including GreenClean Liquid® 5.0, GreenClean Liquid® 5.0 combined with Hydrothol® 191, and the copper-based algaecides evaluated (Algimycin® PWF, Argos, Captain® XTR, Cutrine® Ultra, and SeClear®) achieved significant and similar effects on target cyanobacteria. The chelated copper-based formulations (Algimycin® PWF, Argos, Captain® XTR, and Cutrine® Ultra) resulted in relatively less microcystin release 1 DAT and lesser total microcystin concentrations 4 DAT. At 9 DAT, total microcystin concentrations were significantly lower than in untreated controls in all treatments evaluated. These results provide the necessary comparative performance data for preliminary decision-making and designing additional studies at larger scales. Importantly, the comparative toxicity data and approach provided in this study demonstrate the initial steps for development of site-specific management strategies for Lake Okeechobee and other areas impacted by harmful algal blooms with large spatial and temporal scales.

1. Introduction

Noxious algal growths in Florida's inland waters and along the coast have been a problem for decades. In recent years, dense blooms of toxin-producing cyanobacteria (blue-green algae) have plagued Lake Okeechobee and the surrounding waterways, resulting in measurable adverse health effects for people and endangered species, as well as significant losses in revenue generated by tourism, recreation, and property values. Millions of dollars in income have been lost among local businesses near the St. Lucie and Caloosahatchee Rivers and

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Estuaries (Alvarez et al., 2019). In response to these harmful algal blooms (HABs), the governor of Florida declared a state of emergency in Martin and St. Lucie counties in 2016 (Scott, 2016) and in Palm Beach, Martin, St. Lucie, Glades, Hendry, Lee and Okeechobee counties in 2018 (Scott, 2018). Following the 2018 declaration, the Center for Disease Control and Prevention (CDC) was awarded 1 million dollars to respond to communities facing health issues due to exposures to cyanobacteria.

Lake Okeechobee is the largest freshwater lake in Florida occupying about 1900 km^2 (~730 square miles) with an average depth of about 2.7 m, located in Glades, Okeechobee, Martin, Palm Beach, and Hendry counties. Primarily fed by the Kissimmee River, Fisheating Creek, Lake Istokpoga, and smaller sources, Lake Okeechobee drains an area of 12,000 km^2 (SFWMD, 1989). This drainage area extends from North of Orlando through the Everglades to Florida Bay, with agricultural, suburban, and urban land uses in the watershed (Reddy et al., 1996). Agricultural land in the watershed is used predominantly for beef cattle, dairy, sugarcane, citrus, and other crops. Lake Okeechobee is part of a complex waterway managed by the United States Army Corps of Engineers (USACE) with input from the South Florida Water Management District (SFWMD), the Florida Department of Environmental Protection (DEP) and the Florida Fish and Wildlife Conservation Commission (FWC). Authorized uses of the lake include flood and storm risk management, navigation, water supply, enhancement of fish and wildlife, and enhancement of recreation. The Lake Okeechobee Regulation Schedule (LORS) is the current water control plan for the lake and the Everglades Agricultural Area (EAA) that sets seasonal targets for stage and water releases from this shallow sub-tropical lake to avoid jeopardizing the integrity of the Herbert Hoover Dike, and ultimately to prevent water from inundating the surrounding populated areas. Normally, water is discharged from Lake Okeechobee south through a series of canals into the EAA for water supply and storage, which then flows into the Water Conservation Areas (WCA) and the Stormwater Treatment Areas (STA) of the SFWMD. However, due to topography and storage limitations south of the lake, excess water from Lake Okeechobee can sometimes be released to the Atlantic Ocean and Gulf of Mexico through dredged canals connecting to coastal rivers, such as the St. Lucie Canal to the St. Lucie River and the Caloosahatchee Canal and Lake Hicpochee to the Caloosahatchee River. Releases can result in problems associated with rapid fluctuations in water characteristics (e.g., salinity) and downstream transport of nutrients and cyanobacteria into the adjacent estuaries.

To date, no substantive plan is currently in place that can provide effective short-term or long-term solutions for cyanobacterial blooms in Lake Okeechobee or surrounding waterways. As awareness of the risks and damages associated with toxin producing-cyanobacteria rises, "no action" is not an acceptable management decision. Scientifically defensible solutions are crucial for accomplishing effective and efficient mitigation and control of target cyanobacteria, especially in large public water resources. Long-term solutions (e.g. nutrient control in watershed) are attractive and desirable, but may require years to decades to see results, if at all (Havens and James, 2005; Canfield et al., 2018). For example, a bloom of cyanobacteria covering over 259 km^2 of Lake Okeechobee in 1986 initially prompted Florida legislature to pass the Surface Water Improvement and Management Act (SWIM Act), in an effort to manage nutrient inputs (with a focus on non-point sources) to the lake. Since implementation of the SWIM Act over 30 years ago, annual inputs of total phosphorus to Lake Okeechobee have not significantly declined, nor have average chlorophyll-*a* concentrations (Canfield et al., 2018). The long-term tactic of nutrient control requires essentially eliminating nutrient flow into Lake Okeechobee from the watershed, as well as sequestering or removing all sediment-associated nutrients to cease internal recycling. Further, there are factors contributing to proliferation of cyanobacteria blooms other than nutrient inputs and cycling, including warming waters, tropical storms, and intense precipitation events. Though evaluation and proper management of nutrient loading from the watershed is important, it is often viewed as the sole line of defense for mitigating HABs. Both long-term and short-term approaches have a place in management but must be recognized as tactics with different goals and possible outcomes.

Short-term solutions are important for mitigating immediate risks for residents, visitors, pets, wildlife, and livestock, and restoring beneficial services (i.e., uses) of impacted waters, and should be evaluated. Management objectives for Lake Okeechobee and associated waters must include management of toxin production by several cyanobacteria (e.g. *Aphanizomenon*, *Chrysosporum*, *Cuspidothrix*, *Dolichospermum*, *Microcystis*, and *Raphidiopsis*). The use of USEPA-registered chemical algaecides for management of cyanobacteria is a short-term solution for which there are extensive peer-reviewed data to support effectiveness against target species (Peterson et al., 1997; Ruzycki et al., 1998; Hadjoudja et al., 2009; Matthijs et al., 2012; Calomeni et al., 2014; Geer et al., 2016), margins of safety for non-target species (Surber and Pickering, 1962; Wilson and Bond, 1969; Murray-Gulde et al., 2002; Closson et al., 2014; Calomeni et al., 2015; Geer et al., 2016), microcystin release and dissipation following exposures (Iwinski et al., 2016a, 2017; Kinley et al., 2017, 2018), scalability (Bishop and Rodgers, 2011; Huddleston et al., 2015; Geer et al., 2017), and rapid but temporary results (Isaacs et al., 2013).

Use of USEPA-registered algaecides for management of toxin-producing cyanobacteria is a promising tactic that meets many common decision-making criteria that water resource managers often require (i.e. effectiveness, selectivity, durability, scalability, availability, cost, ease of application, and ease of transport and storage), and there are peer-reviewed data to support these criteria being met (see citations in prior paragraph). However, to date, this tactic has not been used at a large scale for control of cyanobacteria in Lake Okeechobee and surrounding waters, thus, there are no peer-reviewed data available regarding performance in these aquatic systems. Due to the range of sensitivities of cyanobacterial species (and strains of species) from site to site and the variability of water characteristics (e.g., pH, dissolved organic carbon, and hardness) that can alter exposures (and ultimately performance) of algaecides, laboratory toxicity studies can be conducted prior to full-scale applications to discern relatively effective formulations and exposure concentrations for a given site. At the laboratory scale, concepts regarding toxicity thresholds, potencies, and comparative effectiveness among available algaecide formulations can be tested using site-collected cyanobacteria in site water, while minimizing confounding factors. Analytical confirmation of active ingredient exposure concentrations and multiple lines of evidence of cyanobacterial responses to those exposures are necessary to test these hypotheses (Calomeni et al., 2018a). These lines of evidence include individual-level parameters (e.g., enumeration of viable cells) and assemblage-level parameters (e.g., concentrations of photosynthetic pigments, such as chlorophyll-*a* and phycocyanin). Results from these studies confirm the effective concentration of algaecide required to control the growth of target cyanobacteria for a specific site and at a specific cell density, thereby decreasing uncertainty regarding the outcome of an algaecide treatment and providing comparative toxicity data for decision making.

Toxin release and persistence following algaecide exposures can also be evaluated and compared among formulations at the laboratory scale to provide preliminary data for management scenarios in which those aspects of a treatment are important. Toxin release by cyanobacteria following algaecide exposures is a common concern among water resource managers based on the perception that any exposure of an algaecide will result in complete intracellular toxin release from cyanobacteria. This perception is more commonly focused on copper-based algaecides due to earlier studies that reported relatively high or complete microcystin release after using unspecified or illegal (in the United States) concentrations of copper in treatments (Jones and Orr, 1994; Touchette et al., 2008). More recent research regarding this topic has shown that microcystin release depends on exposure concentration of copper-based algaecides, and that cyanobacteria can be adversely affected to the point of senescence while minimizing toxin release

(Iwinski et al., 2016; Kinley et al., 2017). It is also important to recognize that human recreation and drinking water guidelines for endotoxins are based on total toxin concentration (WHO, 2003; USEPA, 2019). Thus, in waters used for recreation by people and pets, and by organisms that inhabit or use the water, risk arises from the sum of both intracellular and dissolved (i.e. aqueous) forms. However, when applying algaecides near intake structures for drinking water treatment plants, it is logical to prioritize minimal toxin release because in-plant processes that can physically remove cyanobacteria cells (e.g. flocculation and sedimentation, dissolved air flotation, and filtration) are generally more effective and efficient for removing endotoxins (via removing the cells from the water) than conventional oxidation and sorption processes targeted for removal of dissolved organics (Svrcek and Smith, 2004). Therefore, when treating in potable source waters, it is beneficial to understand the relationship between algaecide exposure concentration and extent of toxin release before and after an algaecide is applied, and the consequences of timing a treatment.

Since toxin persistence following algaecide exposures is an additional concern from a risk perspective, relative rate and extent of toxin dissipation following algaecide exposures is another subject that can be studied initially in laboratory-scale experiments. Measurements of total microcystin concentrations with time after algaecide exposures provide preliminary information for potential effects of algaecide exposures on microcystin-degrading microbial populations, since these effects should manifest in altered rates of microcystin dissipation. Scaled-up experiments will ultimately be necessary to test hypotheses of microcystin dissipation following algaecide exposures, since fate processes including dilution and dispersion could be dominant at a site, and sediment microbial populations could be more robust. For the purposes of this study, microcystin-producing cyanobacteria collected from Lake Okeechobee were exposed and evaluated. Since microcystins are more commonly monitored and used as a metric for risks in the lake and surrounding waters, the focus of potential toxin release and persistence in this study was therefore on microcystins.

This study was conducted to bolster the peer-reviewed database for available management strategies targeting microcystin-producing cyanobacteria in Lake Okeechobee and surrounding waters. A laboratory-scale evaluation can be utilized as a first step in this process to minimize uncertainty at larger scales and provide confidence in decision-making. The overall objective of this study was to measure and compare effects of several EPA-registered algaecide formulations on microcystin-producing cyanobacteria collected from Lake Okeechobee. Specific objectives were to 1) collect samples containing microcystin-producing cyanobacteria from Lake Okeechobee, 2) measure responses of site-collected cyanobacteria to exposures of USEPA-registered algaecide formulations in terms of cell density, chlorophyll-*a* concentrations, and phycocyanin concentrations 3 DAT, 3) discern minimum effective exposure concentrations for each formulation, 4) measure and compare the extent of microcystin release at 1 DAT in minimum effective exposures, and 5) measure and compare the extent of total microcystin dissipation within 9 DAT in minimum effective exposures.

2. Materials and methods

2.1. Site collection and water characteristics

Site water and associated cyanobacteria were collected from a site on the southern end of Lake Okeechobee (26°42′15.7″ N 80°42′56.3″ W) on August 7, 2019 (map included with supplementary material). The cyanobacteria and site water were stored in 19-L high-density polyethylene containers during transport to the University of Florida, Ft. Lauderdale Research and Education Center in Davie, FL. At the laboratory, water characteristics including dissolved oxygen, temperature, specific conductance, and pH were measured using a YSI Multiparameter Sonde (Model EXO3; YSI, Inc.). Alkalinity and hardness were measured using titrations (Standard Methods 2320 B and 2340 B, respectively; APHA, 2017). Water characteristics measured at initiation of the experiment are presented with the supplementary material for this study.

2.2. Preparation of experimental treatments

Laboratory toxicity experiments were conducted using fundamental design principles and protocols described by Calomeni et al. (2018a). The site-collected cyanobacterial assemblage was quantified using a Bürker-Türk counting chamber and contained a total cell density of 6.14 $\times$ 10^6 cells/mL, with 71% of the total density consisting of *Microcystis aeruginosa* (Kützing) Kützing and 29% consisting of *Pseudanabaena mucicola* (Naumann & Huber-Pestalozzi) Schwabe. Experimental treatments were prepared by adding 200 mL of homogenized site water and the cyanobacteria assemblage (at the above stated cell density) to 400 mL borosilicate beakers. Ten individual algaecide formulations and one combination of algaecide formulations were evaluated in this study (physical and chemical properties of algaecides included in supplementary material), at 4 exposure concentrations (n = 3) within the legal (i.e. label) application range for each formulation (Table 1). The evaluated formulations included 5 copper-based algaecides (Algimycin® PWF, Argos, Captain® XTR, Cutrine® Ultra, and SeClear®), 4 hydrogen peroxide-based algaecides (GreenClean® Pro, GreenClean® Liquid 5.0, PAK® 27, and Phycomycin® SCP), and 1 endothall-based algaecide (Hydrothol® 191). The combination evaluated included GreenClean® Liquid 5.0 and Hydrothol® 191, with one concentration of Hydrothol® 191 and a range of concentrations of GreenClean® Liquid 5.0 (Table 1). The formulations evaluated in this study were selected to represent the types of formulations available among USEPA-registered algaecides. Untreated controls consisting of site water and cyanobacteria were also prepared in the same volume at the same initial cell density (n = 3). Target exposure concentrations were prepared via addition of appropriate volumes from stock solutions to site water in beakers for all formulations except for the solid peroxide formulations (GreenClean® Pro, PAK® 27, and Phycomycin® SCP). For these formulations, the appropriate mass of product was weighed and added directly to the site water containing cyanobacteria. Solutions with amended granular algaecides were then inverted until the granules completely dissolved. For the duration of the experiment, treatment vessels were maintained at 21–23 °C and illuminated with cool white fluorescent bulbs (6800 K) at

Table 1
Algaecide formulations and exposure concentrations evaluated in this study.

Trade Name	Range of Concentrations Evaluated as Active Ingredient	Concentrations in terms of application concentration of product
Algimycin® PWF	0.3, 0.5, 0.7, 1.0 mg Cu/L	1.59, 2.66, 3.72, 5.31 gallons/acre-ft Algimycin® PWF
Argos	0.3, 0.5, 0.7, 1.0 mg Cu/L	0.9, 1.5, 2.1, 3 gallons/acre-ft Argos
Captain® XTR	0.3, 0.5, 0.7, 1.0 mg Cu/L	0.9, 1.5, 2.1, 3 gallons/acre-ft Captain® XTR
Cutrine® Ultra	0.3, 0.5, 0.7, 1.0 mg Cu/L	0.9, 1.5, 2.1, 3 gallons/acre-ft Cutrine® Ultra
SeClear®	0.3, 0.5, 0.7, 1.0 mg Cu/L	1.95, 3.25, 4.55, 6.5 gallons/acre-ft SeClear®
GreenClean® Pro	2, 5, 7, 10 mg H_2O_2/L	20, 49, 69, 100 lbs/acre-ft GreenClean® Pro
PAK® 27	2, 5, 7, 10 mg H_2O_2/L	20, 49, 69, 100 lbs/acre-ft PAK® 27
Phycomycin® SCP	2, 5, 7, 10 mg H_2O_2/L	20, 49, 69, 100 lbs/acre-ft Phycomycin® SCP
GreenClean® Liquid 5.0	4, 10, 15, 22 mg H_2O_2/L	5, 13, 20, 28.5 gallons/acre-ft GreenClean® Liquid 5.0
Hydrothol® 191	0.15, 0.3, 0.5, and 1.0 mg endothall acid/L	0.225, 0.45, 0.75, 1.5 gallons/acre-ft Hydrothol® 191
GreenClean Liquid 5.0 + Hydrothol® 191	4, 10, 15, 22 mg H_2O_2/L each mixed with 0.3 mg endothall acid/L	5, 13, 20, 28.5 gallons/acre-ft GreenClean Liquid 5.0 and 0.45 gallons/acre-ft Hydrothol® 191

1.9–2.0 k LUX for a 12:12-h light: dark photoperiod (to provide suitable and realistic growing conditions for the site collected cyanobacteria), and were loosely covered with colorless, transparent plastic film to decrease evaporation.

Water samples were collected immediately following exposure initiation to confirm concentrations of active ingredients (copper, hydrogen peroxide, and endothall) in each treatment replicate (where those active ingredients were relevant to measure) and untreated control replicate. Copper concentrations from exposures of the copper-based algaecides were measured by analysis of acid soluble copper using inductively coupled plasma optical emission spectrometry (ICP-OES; PerkinElmer Avio™ 200) according to USEPA Method 200.7 (USEPA, 2001). Hydrogen peroxide concentrations were measured using a colorimetric method (Klassen et al., 1994; Kinley et al., 2015). Endothall concentrations were measured using enzyme-linked immunosorbent assay (ELISA) (Hunt et al., 2015).

2.3. Evaluation of cyanobacteria responses to algaecide exposures

Cyanobacterial responses were measured at 72-h following exposure initiations (3 days after treatment [DAT]). For these measurements, samples were collected from the center of homogenized treatment chambers. Responses were measured in terms of cell densities and pigment concentrations (chlorophyll-*a* and phycocyanin) for all experimental treatments and untreated controls. Cell densities were enumerated using a Bürker-Turk counting chamber with spring clips at 400× magnification on a compound epi-fluorescent microscope (Amscope XYL-606). Pigments (chlorophyll-*a* and phycocyanin) were extracted from cyanobacteria cells according to Yepremian et al. (2017a, 2017b). Pigment concentrations were measured spectrophotometrically (Biomate 3, Thermo Electron spectrophotometer) at 664 nm and 620 nm for chlorophyll-*a* and phycocyanin, respectively. Linear regressions of a series of concentrations of chlorophyll-*a* and phycocyanin standard (Millipore Sigma) solutions were used to calculate pigment concentrations from measured absorbances.

2.4. Microcystin release and dissipation

Total and aqueous microcystin concentrations were measured using ELISA following the manufacturer's protocol (Abraxis®, Warminster, PA, USA). Total microcystins are defined here as the microcystin concentration measured following 3 freeze-thaw cycles without filtration (sum of intra- and extracellular microcystin concentrations). Aqueous microcystins were measured from the filtrate of a 0.45 μm polyethersulfone (PES) filter without a freeze-thaw cycle. To confirm that microcystins were not adsorbed by the filters used, an analytical standard containing 5 μg/L microcystin was filtered twice through a PES filter. The standard error, calculated as a comparison of the unfiltered standard to the twice-filtered standard, was 4% which is within the percent error anticipated for measurements using ELISA.

Total and aqueous microcystin concentrations were first measured in the initial homogenized batch of site water and cyanobacteria (that was distributed among experimental treatments) before the exposures were initiated. At 1 DAT, total and aqueous microcystin concentrations were measured from homogenized treatment vessels to provide data on comparative microcystin release among algaecide formulations. This time point was selected for these measurements since in prior experiments, the greatest extent of microcystin release has been observed at 24-h after algaecide treatments (Iwinski et al., 2016; Kinley et al., 2017). Cellular microcystin concentrations were calculated by subtracting the aqueous microcystin from the total microcystin concentration. At 3 DAT, percent saturation of dissolved oxygen in experimental chambers decreased to less than 40% saturation, or less than 4 mg O_2/L. Aeration was added to the effective treatments for which microcystins continued to be measured, to reflect natural settings and promote conditions suitable for aerobic microcystin-degrading bacteria. Total and aqueous microcystin concentrations were measured in these treatments at 4 and 9 DAT to evaluate the relative dissipation of microcystins among the treatments, based on half-lives that have previously been reported in peer-reviewed studies.

2.5. Statistical analyses

All data were analyzed using JMP® Pro 12.0.1 (SAS Institute, Cary, NC; $\alpha = 0.05$). To discern the minimum effective exposure concentration for each formulation or combination of formulations, responses in terms of 3 DAT cell density, chlorophyll-*a* concentrations, and phycocyanin concentrations were compared using least squares regression analysis. The minimum effective exposure concentration for each formulation is defined here as the lowest concentration at which no statistically significant increases in response are achieved with increase in concentration. This is the lowest exposure concentration with the maximum measurable effects to target cyanobacteria based on the 3 response parameters evaluated.

Analysis of variance (ANOVA) was used to analyze for differences among the untreated control and exposure concentrations within each formulation, with specific differences identified through multiple comparisons testing (Tukey's test). Linear contrasts were used to gain statistical power for comparisons of specific pairs where further clarification was necessary (i.e. difference between two highest exposure concentrations). If there were conflicting results among the 3 response parameters in terms of the minimum effective concentration, both concentrations were reported.

The extent of microcystin release measured 1 DAT was compared using the same statistical procedures, where ANOVA was first used to analyze for differences between the fraction of total microcystins in the aqueous phase in the untreated control and all treatments, and specific differences between treatments were identified through multiple comparisons testing (Tukey's test). Total microcystin concentrations were compared between the untreated control and measurements collected at 1, 4, and 9 DAT for each treatment to discern differences from the untreated control at each time point.

3. Results

3.1. Exposure concentrations of active ingredients

The average percent errors between targeted and measured exposure concentrations of active ingredients were 6% ± 6% (1 standard deviation [SD]) for copper exposures, 38% ± 14% (1 SD) for peroxide exposures, and 41% ± 12% (1 SD) for endothall exposures applied individually (Supplementary Material). Average percent errors calculated for peroxide and endothall exposures applied in combination were 21% ± 3% (1 SD) for peroxide and 34% ± 7% (1 SD) for endothall (Supplementary Material). The relative percent errors were within the range for those anticipated based on the matrix (i.e., surface water and cyanobacteria) and analytical techniques utilized to measure copper (2%–20%; Calomeni et al., 2014; Calomeni et al., 2018b) and peroxide exposures (22%–36%; Geer et al., 2016; Geer et al., 2017). Measured concentrations for the two treatments containing endothall were consistently lower than the targeted concentrations (Supplementary Material). Since measured exposures were less than targeted exposures, all results are presented in terms of the measured concentrations.

3.2. Site-collected cyanobacteria responses to algaecide exposures in terms of cell density, chlorophyll-a concentrations, and phycocyanin concentrations and minimum effective exposure concentrations

Cell densities were measured before and after treatments as a line of evidence for cyanobacteria responses at the individual level. From exposure initiation to 3 DAT, cell densities in untreated controls significantly increased from 6.14×10^6 cells/mL to 8.06×10^6 cells/mL

($p = 0.0041$; $\alpha = 0.05$), indicating that cyanobacteria in untreated controls remained viable during the study duration.

Among the copper-based formulations, SeClear® achieved the greatest decline in average cell densities, with multiple exposure concentrations resulting in >90% decreases compared to the untreated control. Responses of cyanobacteria to the three highest concentrations of SeClear® evaluated (0.51–1.01 mg Cu/L) were significantly different from the untreated control and the lowest exposure evaluated, but similar to each other ($p = 0.9634$–0.9994; Fig. 1), thus, the minimum effective exposure concentration of SeClear® based on cell density was 0.51 mg Cu/L. For the chelated copper-based formulations (Algimycin® PWF, Argos, Captain® XTR, and Cutrine® Ultra), the second to highest concentration (nominal concentration = 0.7 mg Cu/L) was the minimum effective exposure concentration (Fig. 1), meaning responses between exposures targeting 0.7 and 1.0 mg Cu/L were statistically similar for each of these formulations. The measured exposure concentrations for these treatments were 0.63 mg Cu/L (Algimycin® PWF), 0.70 mg Cu/L (Argos), 0.74 mg Cu/L (Captain® XTR), and 0.71 mg Cu/L (Cutrine® Ultra). At these concentrations, declines in average cell densities relative to untreated controls ranged from 77% (Cutrine® Ultra) to 90% (Captain® XTR) (Fig. 1).

The two highest concentrations of GreenClean® Liquid 5.0 evaluated (11.4 mg H_2O_2/L and 17.0 mg H_2O_2/L) resulted in similar responses of the assemblage ($p = 0.9998$), with declines in cell densities > 95% relative to the untreated control (Fig. 1). Thus, the minimum effective exposure was 11.4 mg H_2O_2/L for GreenClean® Liquid 5.0 applied alone. For GreenClean® Liquid 5.0 and Hydrothol® 191 applied in combination, cell densities decreased by > 95% and were similar ($p = 0.9983$) at the two greatest concentrations of peroxide (11.8 mg H_2O_2/L and 17.3 mg H_2O_2/L). Thus, the lowest effective treatment for this combination was 11.8 mg H_2O_2/L mixed with 0.198 mg endothall acid/L.

Among the granular peroxide formulations, the minimum effective measured exposure concentrations were 2.9 mg/L H_2O_2 for Phycomycin® SCP, 3.9 mg/L H_2O_2 for PAK® 27, and 4.7 mg/L H_2O_2 for GreenClean® Pro. For Hydrothol® 191 applied individually, the minimum effective exposure was 0.139 mg endothall acid/L. However, since the maximum measured responses from these formulations were less than the other formulations in preceding paragraphs, the highest evaluated concentrations were treated as the minimum effective exposures for further analysis. The highest exposure concentrations evaluated for the solid hydrogen peroxide-based formulations (GreenClean® Pro, PAK® 27, and Phycomycin® SCP) and Hydrothol® 191 (applied as a single formulation) resulted in decreases ranging from 34 to 48% in cell density relative to the untreated control 3 DAT (Fig. 1).

Responses of cyanobacteria in terms of declines in chlorophyll-*a* concentrations were slightly less sensitive than cell densities, but similar patterns in exposure-response relationships were observed (Fig. 2). Among the copper-based formulations, 0.51 mg Cu/L was the minimum effective concentration of SeClear®, while 0.63 and 0.74 mg Cu/L were the minimum effective concentrations for Algimycin® PWF and Captain® XTR, respectively. Exposures of 0.99 and 0.96 mg Cu/L were the minimum effective concentrations for Argos and Cutrine® Ultra, respectively. Among these exposure concentrations, declines in average chlorophyll-*a* concentrations ranged from 70% (Algimycin® PWF) to 80% (Cutrine® Ultra) (Fig. 2).

GreenClean® Liquid 5.0 applied individually and in combination with Hydrothol® 191 at the two highest concentrations resulted in >90% declines in chlorophyll-*a* relative to the untreated control (Fig. 2). Responses were statistically similar between the second highest and highest concentrations for these treatments ($p = 1.000$ for GreenClean® Liquid 5.0 applied alone and $p = 0.4277$ for combination). Thus, for GreenClean® Liquid 5.0 applied alone, the minimum effective exposure concentration was 11.4 mg H_2O_2/L. For GreenClean® Liquid 5.0 and Hydrothol® 191 applied in combination, the minimum effective exposure concentration was 11.8 mg H_2O_2/L and 0.198 mg endothall acid/L.

The solid hydrogen peroxide-based algaecides resulted in between 6% (PAK® 27) and 18% (GreenClean® Pro) decreases in chlorophyll-*a* concentrations for the highest concentration evaluated (targeting 10 mg H_2O_2/L) relative to the untreated controls 3 DAT (Fig. 2). At the highest concentration of Hydrothol® 191 evaluated, there was a 0% decrease in average chlorophyll-*a* concentrations relative to the untreated control

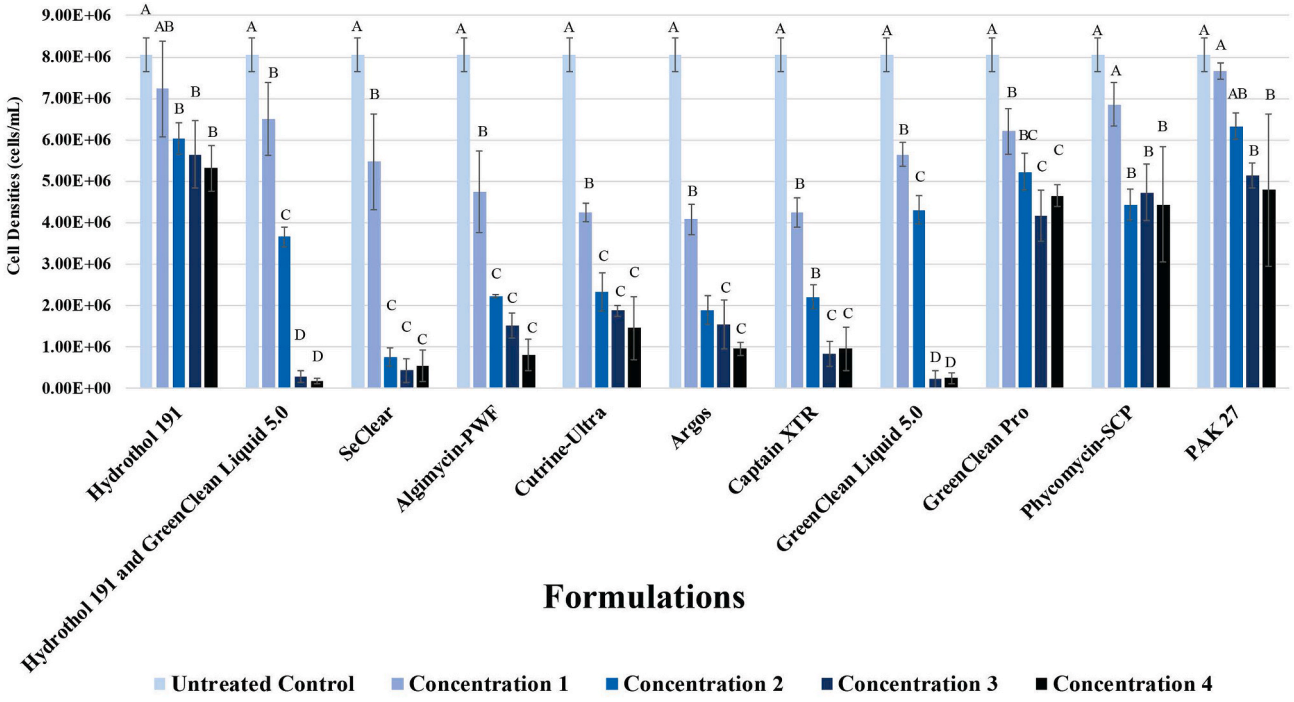

Fig. 1. Average cell densities 3 days after treatment (DAT) in untreated controls and experimental treatments (n = 3). Error bars represent ±1 standard deviation and different letters indicate significant differences among concentrations of a formulation. Concentrations 1 through 4 represent increasing concentrations of each formulation.

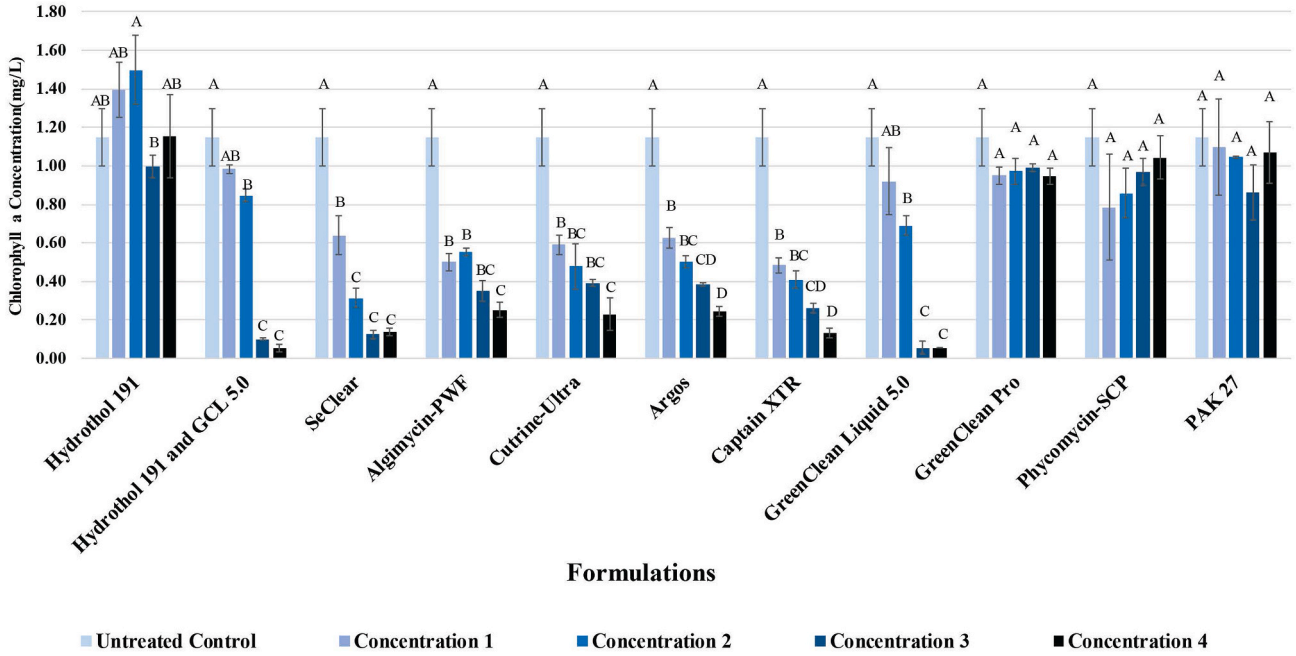

Fig. 2. Average chlorophyll-*a* concentrations 3 days after treatment (DAT) in untreated controls and experimental treatments (n = 3). Error bars represent ±1 standard deviation and different letters indicate significant differences among concentrations of a formulation. Concentrations 1 through 4 represent increasing concentrations of each formulation. GCL 5.0 = GreenClean® Liquid 5.0.

(Fig. 2).

In terms of phycocyanin concentrations, similar exposure-response relationships were observed as with cell densities and chlorophyll-*a* concentrations (Fig. 3). Among the copper-based formulations, 0.51 mg Cu/L was the minimum effective concentration of SeClear®, while 0.74 and 0.71 mg Cu/L were the minimum effective concentrations for Captain® XTR and Cutrine® Ultra, respectively. Exposures of 0.86 and 0.99 mg Cu/L were the minimum effective concentrations for Algimycin® PWF and Argos, respectively. Among these minimum effective exposure concentrations, declines in average phycocyanin concentrations ranged from 83% (Captain® XTR) to 96% (SeClear®) (Fig. 3).

Decreases greater than 93% were achieved at the two highest

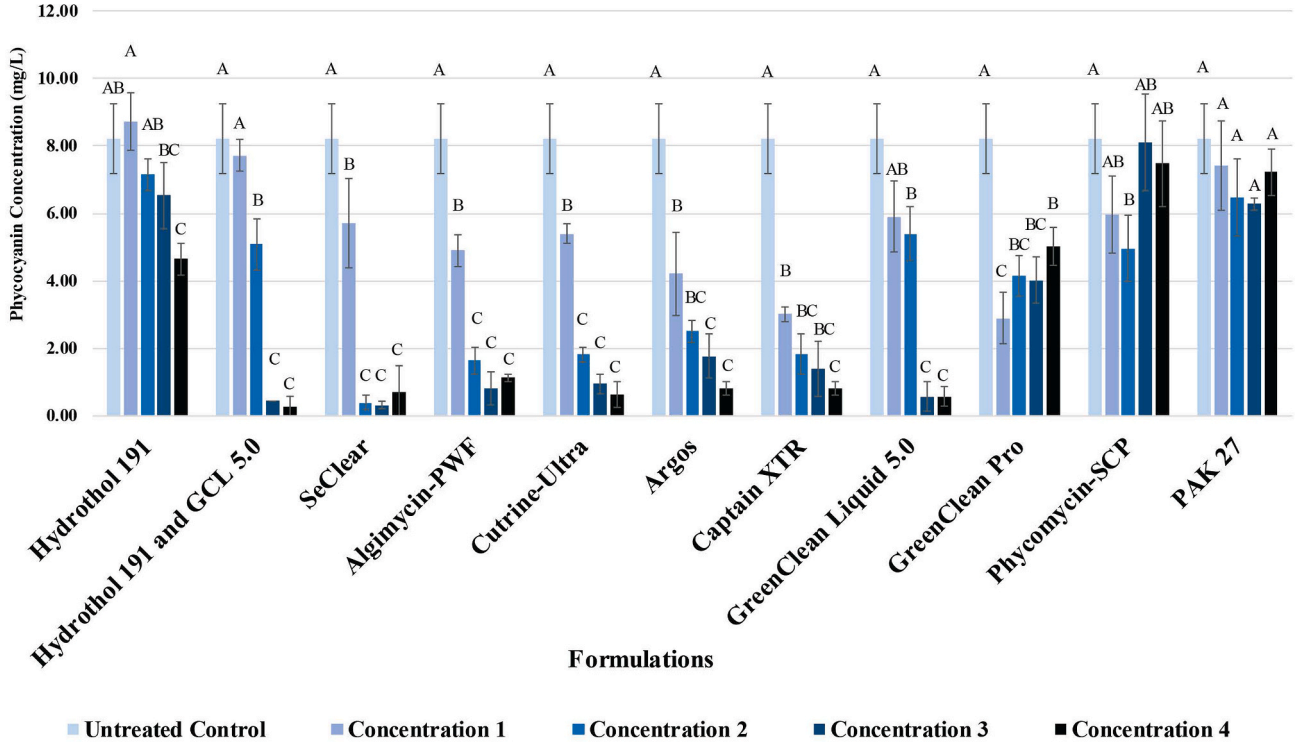

Fig. 3. Average phycocyanin concentrations 3 days after treatment (DAT) in untreated controls and experimental treatments (n = 3). Error bars represent ±1 standard deviation and different letters indicate significant differences among concentrations of a formulation. Concentrations 1 through 4 represent increasing concentrations of each formulation. GCL 5.0 = GreenClean® Liquid 5.0.

concentrations of GreenClean® Liquid 5.0 applied alone and in combination with Hydrothol® 191 (Fig. 3). Responses to the two highest exposure concentrations were similar for GreenClean® Liquid 5.0 applied alone ($p = 1.000$) and in combination with Hydrothol® 191 ($p = 0.7220$). Therefore, the minimum effective concentrations for these treatments were 11.4 mg H_2O_2/L for GreenClean® Liquid 5.0 applied alone and 11.8 mg H_2O_2/L with 0.198 mg endothall acid/L for the combination.

Decreases in average phycocyanin concentrations achieved by the highest concentration evaluated of the solid hydrogen peroxide-based algaecides were 9% (Phycomycin® SCP), 12% (PAK® 27), and 39% (GreenClean® Pro). The greatest percent decrease in phycocyanin concentrations elicited by Hydrothol® 191 was 44%, following an exposure concentration of 0.67 mg endothall acid/L (Fig. 3).

Based on the 3 response parameters evaluated, the minimum effective exposure concentrations for the evaluated formulations were as follows: Algimycin® PWF: 0.63 and 0.86 mg Cu/L; Argos: 0.7 and 0.99 mg Cu/L; Captain® XTR: 0.74 mg Cu/L; Cutrine® Ultra: 0.71 and 0.96 mg Cu/L; SeClear®: 0.51 mg Cu/L; GreenClean® Liquid 5.0: 11.4 mg H_2O_2/L; GreenClean® Liquid 5.0 + Hydrothol® 191: 11.8 mg H_2O_2/L + 0.198 mg endothall acid/L; GreenClean® Pro: 6 mg H_2O_2/L; PAK® 27: 7.6 mg H_2O_2/L; Phycomycin® SCP: 6.8 mg H_2O_2/L; Hydrothol® 191: 0.66 mg endothall acid/L. The more effective formulations overall included all the copper-based products, GreenClean® Liquid 5.0, and the combination of GreenClean® Liquid 5.0 with Hydrothol® 191. Thus, subsequent results regarding microcystin release and persistence are reported for these formulations and their minimum effective exposure concentrations.

3.3. Extent of microcystin release

To address concurrent questions about microcystin release and dissipation, exposures that were deemed effective based on the magnitude of cyanobacterial response (i.e., cell density, chlorophyll-*a*, and phycocyanin concentrations) were further evaluated. Total and aqueous microcystin concentrations in the untreated controls 1 DAT were 787 ± 49 µg/L and <30 µg/L, respectively. Average percent release of microcystin 1 DAT ranged among the effective algaecide formulations and concentrations evaluated. For GreenClean® Liquid 5.0 applied alone, 79% of the microcystin was in aqueous form, while the combination of Hydrothol® 191 and GreenClean® Liquid 5.0 resulted in 99% of total microcystins in the aqueous phase (Fig. 4). The copper sulfate-based formulation, SeClear®, resulted in 91% of total microcystins in the aqueous phase 1 DAT (Fig. 4). The exposures that resulted in lower proportions of microcystins in the aqueous phase 1 DAT were 0.63 mg Cu/L of Algimycin® PWF (10%), 0.71 mg Cu/L of Cutrine® Ultra (14%), 0.70 mg Cu/L of Argos (17%), and 0.74 mg Cu/L of Captain® XTR (23%). The highest evaluated exposure concentrations of Algimycin® PWF (0.86 mg Cu/L), Argos (0.99 mg Cu/L), and Cutrine® Ultra (0.96 mg Cu/L) resulted in 36%, 67%, and 54% of total microcystins in the aqueous phase at 1 DAT, respectively. Overall, in terms of percent microcystin release, the chelated copper formulations applied at a nominal concentration of 0.7 mg Cu/L were similar to the untreated control and significantly different from SeClear® and GreenClean® Liquid 5.0 applied individually and in combination with Hydrothol® 191 (Fig. 4).

3.4. Dissipation of total (sum of cellular and aqueous) microcystin

Average total microcystin concentrations increased in untreated controls from 491 ± 94 µg/L at experiment initiation to 787 ± 49 µg/L at 1 DAT and decreased slightly to 776 ± 51 µg/L at 4 DAT (Fig. 5). Total microcystin concentrations remained elevated at 9 DAT and averaged 547 ± 173 µg/L in untreated controls. At 1 DAT, total microcystin concentrations decreased significantly relative to untreated controls for the combination of Hydrothol® 191 and GreenClean® Liquid 5.0 as well as GreenClean® Liquid 5.0 and Captain® XTR applied individually (Fig. 5), to concentrations of 366, 427, and 584 µg/L, respectively. In all other exposures, total microcystin concentrations remained similar to untreated controls 1 DAT (Fig. 5).

By 4 DAT, all exposures except for SeClear® and Hydrothol® 191 + GreenClean® Liquid 5.0 applied in combination resulted in significant decreases in total microcystin concentrations relative to untreated controls (Fig. 5). For example, average total microcystin concentrations were 28 µg/L for Captain® XTR (0.74 mg Cu/L), 16 µg/L for Argos (0.70 mg Cu/L), and 13 µg/L and 11 µg/L for Algimycin® PWF (0.86 mg Cu/L and 0.63 mg Cu/L, respectively) 4 DAT. All exposures were significantly different from the untreated control by 9 DAT. At this time, total

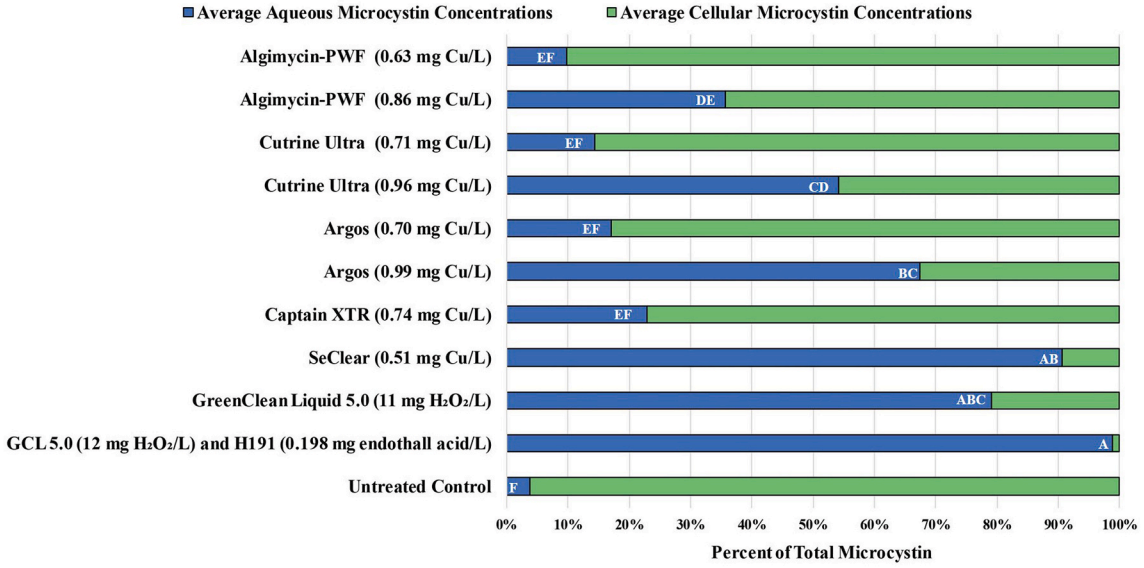

Fig. 4. Average percent aqueous and cellular microcystin measured 1 day after treatment (DAT) for each effective exposure (n = 3). Treatments not connected by the same letter are statistically different. GCL 5.0 = GreenClean® Liquid 5.0 and H191 = Hydrothol® 191.

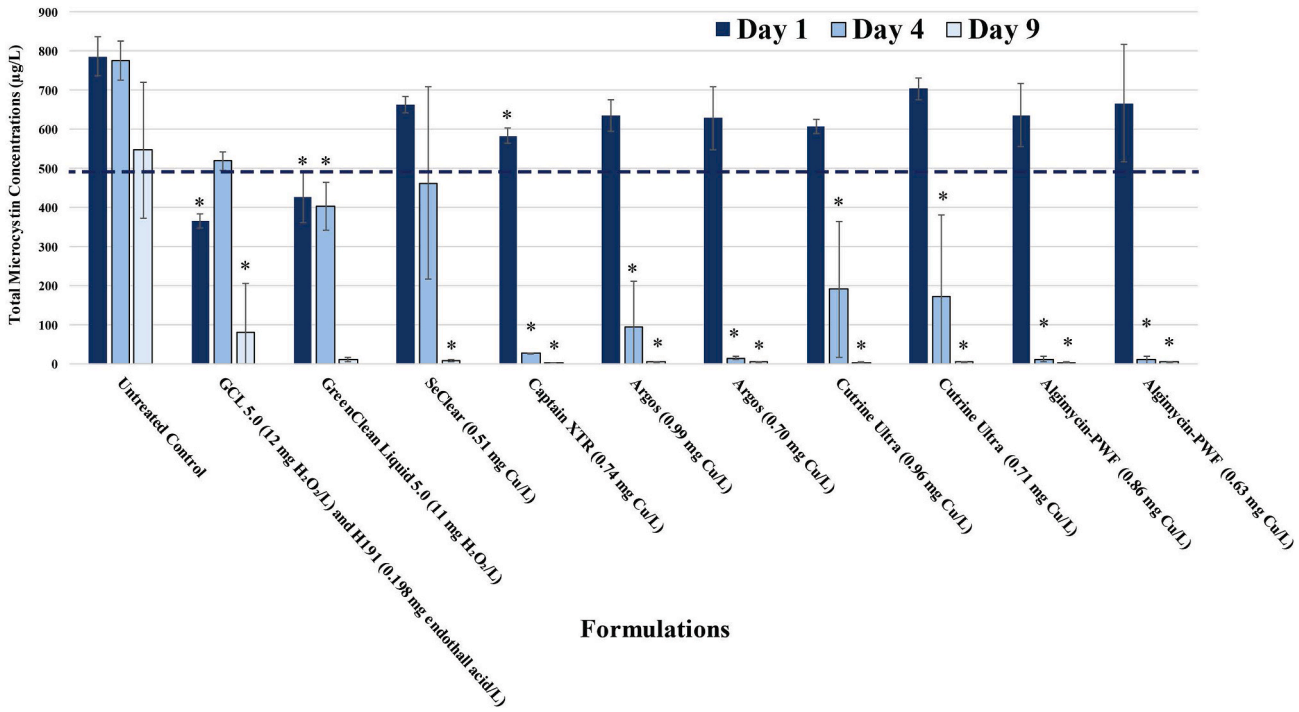

Fig. 5. Total microcystin concentrations measured on 1, 4 and 9 days after treatment (DAT) for each effective exposure (n = 3). Error bars represent ±1 standard deviation and asterisks indicate significant differences from the untreated control on the same day after exposure initiation. The dotted line indicates the average total microcystin concentration at experiment initiation (Day 0). GCL 5.0 = GreenClean® Liquid 5.0 and H191 = Hydrothol® 191.

microcystin concentrations decreased to less than 10 µg/L for all exposures except for GreenClean® Liquid 5.0 applied individually and in combination with Hydrothol® 191.

4. Discussion

4.1. Responses of site-collected cyanobacteria to algaecide exposures

The species *Microcystis aeruginosa* and *Pseudanabaena mucicola* identified in the cyanobacterial assemblage from Lake Okeechobee and used in this study are common and were previously identified in samples collected from the lake during the 2016 bloom (Rosen et al., 2017) and *Microcystis* was identified from the 2018 bloom (Krimsky et al., 2018). Differences in responses of microcystin-producing cyanobacteria to algaecide exposures were apparent in this study. All copper-based algaecides and the liquid hydrogen peroxide-based algaecide evaluated were more effective than the granular hydrogen peroxide-based algaecides and the endothall-based algaecide evaluated. These differences were likely due to intrinsic factors including relative sensitivities of the site-specific cyanobacteria as well as factors that modify the effectiveness among algaecide formulations such as pH, hardness, temperature, particulate and dissolved organic carbon, and cell density of the target assemblage (Fitzgerald, 1964). In these toxicity experiments, a relatively high cell density was evaluated (6×10^6 cells/mL); therefore, formulations with greater potencies applied at higher concentrations were necessary to measure responses in cyanobacteria (Kinley et al., 2017). This cell density was intentionally targeted to reflect conditions commonly observed in the field so that robust, effective products could be identified, and so that release and decline of microcystins could be accurately measured. At relatively lower cell densities (e.g., $\leq 1 \times 10^6$ cells/mL), it is likely that lower concentrations of the effective formulations would be needed to achieve comparable responses, and other apparently less effective formulations in this study could achieve greater cyanobacterial responses than what was observed. Results from these experiments do not warrant exclusion of certain formulations from further evaluation and all the formulations evaluated in this study have utility in different situations for both demonstration and full-scale treatments. Each demonstration and full-scale site is unique in terms of its designated uses, defined problem, and management goals, and those parameters must be considered to identify a specific formulation for management.

4.2. Extent of microcystin release following algaecide exposures

In prior studies (Iwinski et al., 2016; Kinley et al., 2017) and in the present study, the data show that the viability of cyanobacterial cells can significantly decrease following exposure to algaecides, while microcystin release is minimal. Among several of the effective algaecides and exposure concentrations, less than 20% of the total microcystin concentrations were in the aqueous phase 1 DAT. These results emphasize that laboratory experiments can be used to identify appropriate algaecides if minimizing toxin release is a management priority. Target sites for this approach would include areas near intake structures for drinking water treatment plants. However, at other sites, minimizing toxin release is not a priority, since total microcystins (i.e. sum of intracellular and dissolved forms) are the relevant source of risk in scenarios where protection of aquatic life or protection of human health is the management goal. It is important to emphasize that release of microcystins from cells does not increase potential for risk, contrary to what is commonly perceived and propagated.

4.3. Dissipation of microcystins after algaecide exposures

By 4 DAT, total microcystin concentrations significantly declined (compared to untreated controls) in nearly all algaecide formulations evaluated, except for SeClear® and GreenClean® Liquid 5.0 applied individually and in combination with Hydrothol® 191. By 9 DAT, total microcystin concentrations declined to less than 10 µg/L in all treatments except for GreenClean® Liquid 5.0 applied individually and in combination with Hydrothol® 191. Ultimately, demonstration-scale studies will be necessary to measure rates of microcystin dissipation after algaecide treatments to test these hypotheses under more realistic

conditions. Dilution and dispersion are often dominant aqueous fate processes in aquatic systems that could drastically alter overall rates of microcystin dissipation. In addition, presence of sediments with abundant microbial communities can support more rapid breakdown of cyanobacteria cells and microcystins after treatments. Ultimately, each site is different and knowledge of the site characteristics, especially parameters like flow rate and water residence time, will be beneficial to predicting microcystin dissipation rate in the field. At treatment sites where water residence time is relatively longer (i.e. narrow, isolated homeowner canals) due to lower flow rates (meaning dilution and dispersion may not be the dominant fate processes), or at sites where there are known sensitive or listed species present, selection of formulations that demonstrate lesser impacts on microcystin dissipation (in the laboratory and field) can help minimize potential for risk. Demonstration-scale studies will help further refine identification and selection of specific products for different types of sites.

4.4. Comparative toxicity data to inform scaling

The goal of this study was to provide laboratory data that would be necessary for scaling algaecide treatments for demonstration-scale studies and full-scale applications. Since the context of this study was focused on management of microcystin-producing cyanobacteria in Lake Okeechobee and nearby waters, the laboratory experiments conducted were designed to provide information needed for those sites. Given the enormity of Lake Okeechobee, algaecides will not be applied to the entire surface area. In larger systems, priority areas can be designated as management units in which there are specific designated uses and management goals, and that information can guide evaluation and identification of effective algaecides. For example, the towns/cities of Pahokee, Belle Glade, Okeechobee, Clewiston, South Bay, and Bryant use surface water from Lake Okeechobee for potable water supply. If a management plan (using algaecides) was developed for these intake areas, the priority could be focused on identifying effective algaecides that result in minimal microcystin (or other toxin) release and persistence (Fig. 6). At sites where recreation (e.g., boating, fishing, and

Fig. 6. Conceptual model for identification of candidate algaecide formulations and concentrations for treatment of microcystin producing cyanobacteria in Lake Okeechobee and surrounding areas.

swimming) is prevalent, or water residence time is relatively longer, the priority could be focused on identifying effective algaecides that do not demonstrate lags in toxin dissipation after treatment (Fig. 6). Similarly, at sites where there are threatened or endangered aquatic species, a criterion of minimizing microcystin persistence could be established when identifying an effective formulation, since microcystins are a source of risk for aquatic life.

At all sites, certain characteristics will be crucial for scaling algaecide applications. Due to the size of Lake Okeechobee and surrounding areas, water characteristics that impact algaecide effectiveness are anticipated to vary from site to site. Similarly, the genera and species of cyanobacteria, and cell densities present at the time of treatment usually differ spatially and temporally. The data generated from this study for a broad range of USEPA-registered algaecides in addition to the breadth of literature available for USEPA-registered algaecides can be used to make informed decisions on use of specific algaecides depending on the combination of factors that may occur at a site (Fig. 6). Although these studies were targeted for the Lake Okeechobee area, the approach taken is widely applicable and can be used to identify effective formulations and exposure concentrations of algaecides for control of harmful or nuisance algae for a variety of aquatic systems when considering the appropriate site-specific parameters.

This study provides data and useful information to bolster the knowledge base on available management strategies for microcystin-producing cyanobacteria in and around Lake Okeechobee. As frequency, duration, and intensity of HABs increase in Florida and across the United States (and globally), and there is more public and political pressure for sound solutions, science-driven management is crucial. To justify expenses associated with management of HABs in freshwater resources, certain decision-making criteria must be met. These include effectiveness, durability, availability, scalability, selectivity, non-target species risks, and more. All available management tactics for HABs (physical, chemical, and biological) can be rigorously scrutinized against these criteria to support environmentally defensible, socially acceptable, and economically feasible management decisions.

5. Conclusions

In this study, the overall objective was to evaluate the comparative effectiveness of selected USEPA-registered algaecides against microcystin-producing cyanobacteria collected from Lake Okeechobee. The purpose of conducting these experiments was to bolster the database on available short-term management strategies for harmful cyanobacteria in freshwater resources of the Lake Okeechobee waterway. Responses of the site-collected cyanobacteria following these treatments were measured using several lines of evidence. Microcystin release and dissipation after algaecide exposures were also measured among the formulations to provide those comparative data at the laboratory scale.

Results from comparative toxicity experiments show that several of the evaluated formulations resulted in significant and environmentally relevant declines in cell densities and photosynthetic pigments of target cyanobacteria 3 DAT. These formulations included a liquid hydrogen peroxide-based algaecide, GreenClean® Liquid 5.0, applied individually and in combination with an endothall-based algaecide, Hydrothol® 191, as well as the copper-based products (Algimycin® PWF, Argos, Captain® XTR, Cutrine® Ultra, and SeClear®).

The chelated copper-based formulations (Algimycin® PWF, Argos, Captain® XTR, and Cutrine® Ultra) resulted in a lesser extent of microcystin release 1 DAT than the other evaluated formulations. The chelated copper-based formulations also resulted in relatively lower total microcystin concentrations by 4 DAT, and all evaluated formulations resulted in significantly lower total microcystin concentrations than the untreated control by 9 DAT. At 9 DAT, most of the treatment vessels contained <10 μg/L total microcystins as compared to an average initial concentration of 494 μg/L. Additional field studies (demonstration-scale and full-scale) will be necessary to test these hypotheses under more realistic conditions with relevant fate processes including dilution, dispersion, and microbial degradation at the sediment-water interface.

Laboratory-scale experiments can provide data and information necessary to identify effective formulations and application concentrations for implementation at larger scales. Since every management site differs in terms of water characteristics, target cyanobacterial species, cell densities of target assemblages, and designated uses of the water resource, laboratory-scale evaluations of algaecide performance can provide site-specific data, thereby minimizing uncertainties of outcomes for full-scale algaecide applications. Thus, this study demonstrates a physical framework for how those studies can be conducted for a specific site. Importantly, the comparative toxicity data and approach provided in this study demonstrate the initial steps for development of site-specific management strategies for Lake Okeechobee and other areas impacted by harmful algal blooms with large spatial and temporal scales.

Credit author statement

Ciera Kinley-Baird: Conceptualization, Funding acquisition, Investigation, Project administration, Formal analysis, Supervision, Validation, Writing (Writing - original draft, review, and editing), Alyssa Calomeni: Conceptualization, Project administration, Investigation, Methodology, Supervision, Formal analysis, Writing (Writing - original draft, review, and editing), David Berthold: Investigation, Resources, Forrest Lefler: Investigation, Resources, Max Barbosa: Investigation, Resources, John Rodgers: Conceptualization, Funding acquisition, writing (Writing - original draft), Dail Laughinghouse: Funding acquisition, Investigation, Methodology, Resources, writing (Writing - original draft, review, and editing)

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecoenv.2020.111233.

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