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Lyngbya wollei responses to copper algaecide exposures predicted using a concentration–exposure time (CET) model: Influence of initial biomass

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ABSTRACT

Concentration–exposure time models (CET) are used to predict responses of aquatic vascular plants to herbicides and could be applicable for cyanobacterial responses to algaecides if the model captures appropriate variables. For the cyanobacterium *Lyngbya wollei*, initial biomass upon application may be an important parameter driving responses to copper algaecides. Objectives were to 1) discern an algaecide with sufficient potency and relationship between copper concentration and response for a *L. wollei* CET model, 2) develop a CET model for the algaecide and *L. wollei*, 3) determine the influence of initial biomass on measured responses, and 4) develop a new model to predict *L wollei* responses with biomass as a variable. Emulsified 3.8% copper ethanolamine had sufficient potency ($\geq$ 90% response), and increasing copper concentrations resulted in increasing responses ($R^2 = 0.99$). Exposures of 0.4, 0.7, and 1.0 mg Cu L^{-1} for 24 h, exposures of 0.4, 0.7, and 1.0 mg Cu L^{-1} for 8 h, and exposures of 0.7 and 1.0 mg Cu L^{-1} for 1 h resulted in 87 to 100% response of *L. wollei* (percent damaged trichomes). Initial biomasses greater than those used for the CET model (52 g wet weight [WW] m^{-2}) decreased responses to nondetect (1,558 g WW m^{-2}). Because initial biomass influenced responses, a new model was developed with biomass as a variable (biomass, duration, and concentration [BDC] model). The BDC model increased the range of initial biomasses (13 to 104 g WW m^{-2}) in which performance of the copper algaecide for controlling the growth of *L. wollei* could be predicted.

Key words: algae, chelated copper, cyanobacteria, efficacy.

INTRODUCTION

Before treatment of problematic algae or cyanobacteria in water resources with algaecides, predictions of effective exposures are necessary to guide management decisions. This is because characteristics of the infested site (e.g., pH, conductivity, alkalinity, hardness, dissolved and particulate organic matter, water mixing) and innate sensitivity of the target algal population(s) can alter the concentration of algaecide needed to achieve control (Rodgers et al. 2010, Bishop and Rodgers 2011, Isaacs et al. 2013, Greenfield et al. 2014, Calomeni et al. 2015). Herbicide treatments for vascular plants are generally analogous situations to algaecide treatments for algae and cyanobacteria. For specific couplets of a herbicide and vascular plant, concentration–exposure time (CET) models have been useful for prediction of effective herbicide exposures (Van and Conant 1988, Getsinger 1991, Getsinger and Netherland 1997). The fundamental basis for CET models is the theory that sufficient contact time is necessary to achieve a critical concentration of herbicide within the target vascular plant. This concept applies to herbicides that have exposure durations altered by site characteristics (e.g., lotic systems, large aquatic systems with relatively small treatment areas, etc.) (Van and Conant 1988, Netherland 1991). CET models may also be applicable for algaecide treatments for problematic algae and cyanobacteria. To test this hypothesis, a CET model was developed for the problematic cyanobacterium *Lyngbya wollei* and exposures of a copper-based algaecide. For cyanobacteria, an additional independent variable, the influence of initial biomass, may be necessary to predict responses to algaecide exposures accurately. As an extension of the theory driving the CET model, the biomass of a cyanobacterial population can alter the concentration of algaecide within an alga, influencing the response. If initial biomass is a driving exposure characteristic for accurate prediction of cyanobacterial responses to algaecide exposures, a different model that includes initial biomass as a variable will be needed.

Similar to vascular plant CET models (Getsinger and Netherland 1997), unique properties of a cyanobacterium (e.g., sensitivity) and an algaecide (e.g., potency, mechanisms of action, formulation) necessitate a specific CET model for each cyanobacterium–algaecide combination. Initially, CET model development for cyanobacteria should logically be prioritized for common and ubiquitous problematic cyanobacteria and frequently used algaecides. *Lyngbya wollei* is a problematic cyanobacterium that interferes with designated water resource uses throughout North America from Florida (Foss et al. 2012) to Canada (Vis et al. 2008). This cyanobacterium forms aesthetically objectionable growths, can produce paralytic shellfish poisons (Carmichael et al.

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Table 1. Physical and chemical properties of copper-based algaecides.

Product	Copper (3.8%) Ethanolamine with D-limonene	Copper (9%) Ethanolamine with D-limonene	Copper Ethanolamine	Copper Citrate and Gluconate
Abbreviated name[1]	Emulsified 3.8% copper EA	Emulsified 9% copper EA	Copper EA	Copper CG
Composition	D-Limonene, triethanolamine, ethanolamine, basic copper carbonate	d-Limonene, triethanolamine, ethanolamine, basic copper carbonate	Triethanolamine, ethanolamine, basic copper carbonate	Copper gluconate, copper citrate
Active ingredient	3.8% Cu	9% Cu	9% Cu	9% Cu
Minimum application concentration	0.1 mg Cu L^{-1}	0.1 mg Cu L^{-1}	0.1 mg Cu L^{-1}	0.1 mg Cu L^{-1}
Maximum application concentration	1.0 mg Cu L^{-1}	1.0 mg Cu L^{-1}	1.0 mg Cu L^{-1}	1.0 mg Cu L^{-1}
Physical description	Viscous blue liquid	Viscous blue liquid	Blue liquid	Blue liquid
pH	9.7–10.0	10.2–10.3	10.3–10.5	1.5–2.5

[1]Abbreviated name used throughout manuscript.

1997, Foss et al. 2012, Lajeunese et al. 2012), and extirpates native species within infested areas (invertebrates and fish, Mastin et al. 2002). In the current experiment, *L. wollei* that was impeding recreational uses (i.e., fishing, swimming, boating) was collected from a 44,500-m^2 pond in Spartanburg, SC and exposed to copper-based algaecides. Copper-based algaecides have been used to treat algae and cyanobacteria, producing taste and odor compounds in potable water for more than a century (Moore and Kellerman 1904), and continue to be used in irrigation canals, ponds, lakes, and reservoirs (Netherland 2014). Exposures of copper-based algaecides result in aqueous copper concentrations exceeding pretreatment concentrations for minutes to days after application (Button et al. 1977, Anderson 1999, McNevin and Boyd 2004, Liu et al. 2006, Calomeni et al. 2017), depending on site characteristics. Wide-ranging copper exposure durations (i.e., minutes to days) are anticipated to have a similarly wide-ranging influence on responses. Ranging exposure durations support the development of a unique cyanobacterial CET model specifically for copper-based algaecides.

To develop the *L. wollei* CET model, laboratory experiments were conducted sequentially. Laboratory-scale experiments are appropriate for discerning the influences of specific exposure characteristics (i.e., concentration, exposure duration, and initial biomass) on responses (Giesy and Odum 1980). Initial experiments were designed to determine an effective algaecide for controlling the growth of this accession of *L. wollei*. Several copper-based products are available for use as algaecides and responses of *L. wollei* to copper-based algaecides range as a function of the formulation (Calomeni et al. 2015). Chelated copper formulations were evaluated because of the robust structure of *L. wollei* (i.e., mucilaginous sheath) and the theory that algae are more sensitive to chelated copper formulations relative to nonchelated (e.g., copper sulfate pentahydrate) (Bishop and Rodgers 2011, Rodgers et al. 2010, Calomeni et al. 2014). Chelated copper algaecide formulations include 1) emulsified copper ethanolamine (EA) (e.g., 3.8%[1] and 9%[2] copper), 2) copper EA,[3] and 3) copper citrate and gluconate (CG)[4] (Table 1). On the basis of decisions made during their registration with the U.S. Environmental Protection Agency, algaecides have restrictions regarding the concentration of active ingredient applied to an aquatic system during treatment. These restrictions are specified on the product label and are termed "legal label concentration" throughout this manuscript. To develop the CET model, a sufficiently potent copper algaecide formulation is needed to discern differences in cyanobacterial responses due to exposure duration. "Sufficiently" potent is defined in this manuscript as an algaecide that results in 1) significant (i.e., $\geq 90\%$) responses and 2) increasing cyanobacterial responses as copper concentrations increase within legal label concentrations (0.1 to 1.0 mg Cu L^{-1}).

Once questions regarding a sufficiently potent algaecide formulation are resolved, exposure duration is the next critical independent variable to evaluate. Similar to vascular plants (Getsinger and Netherland 1997), exposure duration is likely positively correlated with cyanobacterial responses until exposure durations exceed a threshold duration. Once exceeded, there is no relationship between time and cyanobacterial response. Exposure duration is anticipated to be an independent variable that drives predicted *L. wollei* responses to copper algaecides.

Lyngbya wollei biomasses can differ as a function of location and season (i.e., winter to summer) (Beer et al. 1986, Bridgeman et al. 2012). The average *L. wollei* biomass observed *in situ* is 307 g dry weight (DW) m^{-2} ($n = 61$). Minimum and maximum biomasses range from 0.25 g DW m^{-2} to 1, 508 g DW m^{-2} in aquatic systems (Beer et al. 1986, Speziale et al. 1991, Cowell and Botts 1994, Macbeth 2004, Vis et al. 2008, Bridgeman et al. 2012, Lévesque et al. 2012, Panek 2012). By altering the biomass of *L. wollei* at initiation of a copper exposure (termed "initial biomass" throughout this manuscript), the dose or the mass of copper per mass of the cyanobacterium (Kinley et al. 2017) changes. Theoretically, the consequent change in dose from an alteration in initial biomass can result in a difference in responses of *L. wollei*. If measured *L. wollei* responses differ for a range of initial biomasses observed in aquatic systems, then a new model will be needed with biomass as an independent variable.

The overall objective of this experiment was to discern if initial biomass is a driver for *L. wollei* responses, necessitating a new model for response prediction. Specific objectives were to 1) discern a sufficiently potent algaecide for the *L. wollei* CET model, 2) develop the *L. wollei* CET model by measuring cyanobacterial responses to a series of exposure

durations and concentrations of the sufficiently potent algaecide, 3) discern the influence of initial biomass on *L. wollei* responses by comparing the effects predicted by the CET model with responses measured with a series of initial biomasses, and 4) develop a new model for *L. wollei* if initial biomass alters *L. wollei* responses.

MATERIALS AND METHODS

Identification of a sufficiently potent algaecide

Lyngbya wollei and water samples were collected during the summer of 2017 from a 44,500-m^2 pond in Spartanburg, SC. *Lyngbya wollei* samples were collected using a rake and water samples were collected using a 20-L high-density polyethylene container. The pond infested with the cyanobacterium was originally used for recreation, including fishing, swimming, and boating until recent growths of *L. wollei* impeded these uses. Once transported to the laboratory, cyanobacterial and water samples (mean [$n = 6$] ± SD; pH = 6.9 ± 0.4 standard units, conductivity = 76.2 ± 4.8 μS cm^{-1}, alkalinity = 35 ± 7 mg L^{-1} as $CaCO_3$, hardness = 29 ± 5 mg L^{-1} as $CaCO_3$, dissolved oxygen = 9 ± 2 mg O_2 L^{-1}) were maintained at 22 ± 1 C with an 18 : 6-h light : dark cycle provided by cool-white fluorescent bulbs[5] at 2,660 lux before and during experiments.

To determine a copper-based algaecide to use to develop a *L. wollei* CET model, the cyanobacterium was exposed to a series of copper concentrations from different copper-based algaecides (Table 1). *Lyngbya wollei* was exposed in 250-ml borosilicate glass beakers by adding appropriate volumes of 1,000 mg Cu L^{-1} stock solutions of each algaecide separately to 200 ml of site water containing the cyanobacterium. A series of copper concentrations (0.1, 0.4, 0.7, and 1.0 mg Cu L^{-1}) for each algaecide was arrayed, with three replicates per concentration. Copper concentrations were confirmed immediately after addition of copper algaecide. Soluble (USEPA 1992) and acid-soluble (USEPA 1991) copper concentrations were measured with a Perkin Elmer (Waltham, MA) Optima 3100RL inductively coupled plasma–optical emission spectrometer (method detection limit = 0.050 mg Cu L^{-1}) and graphite furnace atomic absorption spectrometry (Varian Inc. AA 280FS fast sequential atomic absorption spectrometer, method detection limit = 0.005 mg Cu L^{-1}) (APHA 2012). Quality assurance and control included replicate samples, standards, and blank matrix spike recovery.

Percent damaged trichomes and percent difference in *L. wollei* weight (wet and dry) were used to measure cyanobacterial responses. Percent damaged trichomes indicates the viability of cells after exposure on the basis of microscopic observations of cellular structure and pigment. Percent damaged trichomes is a sensitive measurement of algal responses because individual cells within trichomes respond to exposures rapidly (Calomeni et al. 2014, Calomeni and Rodgers 2015). To discern differences in mass, cells have to be nonviable and also decompose, requiring more time. Multiple response measures were used for responses of *L. wollei* to copper concentrations because of the complexity of response measurements (Calomeni and Rodgers 2015). Multiple response measurements establish lines of evidence for an effective treatment (Calomeni et al. 2015). Responses to copper concentrations were measured 7 d postinitiation of exposures. On the basis of preliminary experiments, this was sufficient time for responses of *L. wollei* to be manifested.

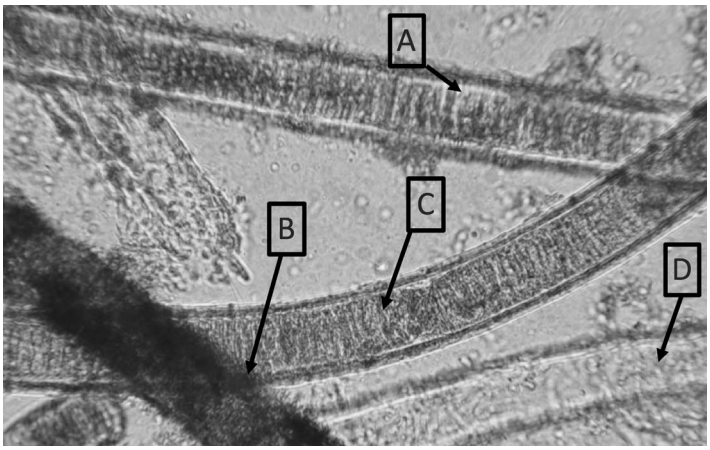

Figure. 1. *Lyngbya wollei* from a 44,500=m^2 pond in Spartanburg, SC. A indicates chlorotic cells, B indicates a brown mucilaginous sheath with cells absent, C indicates viable cells within trichome segments, and D indicates a mucilaginous sheath with cells absent. A, B, and D were identified as damaged.

To discern percent damaged trichomes, cells within trichomes were evaluated at ×400 magnification with a Leica ATC2000 binocular compound microscope. A fraction of cells within a trichome (even in untreated controls) can be damaged, and the designation of damaged and not-damaged is a binary parameter. A criterion, therefore, was established for identifying a damaged trichome. The criterion is, if more than 50% of cells within the trichome segment for a field of view (×400 magnification) were damaged, the trichome was recorded as such. Trichome segments were damaged if cells were absent and chlorotic and if the mucilaginous sheath was brown with cells absent (Figure 1). Ten trichome segments were enumerated per replicate for a total of approximately 530 cells (1,590 cells per exposure). To measure percent difference in *L. wollei* mass as wet weight (WW), at the completion of the 7-d experiment the remaining mass of cyanobacterium was removed from beakers and blotted dry with Kimwipes[6] immediately before weighing (Wong et al. 2009, Saunders et al. 2012, Burns et al. 2015). Percent difference in *L. wollei* mass as DW was measured by drying the cyanobacterial mass for 24 h at 100 C before weighing. Responses of *L. wollei* were expressed as percentages using the following equations (Equations 1 and 2). The copper-based algaecide that resulted in increasing responses of *L. wollei* to ≥ 90 % within legal label concentrations (Table 1) was used in subsequent experiments. Linear regression and correlation coefficients (R^2) were used to determine the strength of the relationship between copper concentration and response.

$$P_{\text{trichome}} = \frac{T_{\text{exposure}}}{10} \times 100\% \quad [1]$$

where $P_{\text{trichrome}}$ = percent damaged trichomes and T_{exposure}

= number of damaged trichome segments out of 10 trichome segments discerned 7 d after experiment initiation.

$$P_{\text{mass}} = \frac{(M_{\text{control}} - M_{\text{exposure}})}{M_{\text{control}}} \times 100\% \quad [2]$$

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

Lyngbya wollei CET model

Lyngbya wollei was exposed to a series of copper concentrations and durations of exposure for the CET model. Exposure durations were arrayed to capture durations likely to result from copper algaecide applications *in situ* (i.e., minutes to days) and those durations producing a range of cyanobacterial responses on the basis of preliminary experiments. The copper-based algaecide concentrations applied were 0.1, 0.4, 0.7, and 1.0 mg Cu L^{-1} for 0.25 h, 1 h, 8 h, and 24 h in separate experimental chambers with three replicates per exposure (i.e., concentration and exposure duration). Beakers containing algae and site water without copper treatment were included as untreated controls. At the end of each exposure duration, all beakers, including the untreated controls, were drained. Site water (untreated) was then used to rinse the beaker and the *L. wollei* mass three times, and the beakers were refilled with untreated site water. Copper concentrations were measured immediately after the addition of algaecide to site water containing algae and at the end of the exposure duration using methods detailed previously. Again, cyanobacterial responses were measured 7 d after exposure initiation to allow sufficient time for responses to manifest. Cyanobacterial responses were compared using analysis of variance (ANOVA) and linear contrasts (JMP Pro V.12).

Influence of initial biomass on the *L. wollei* CET model

The initial biomass used for the *L. wollei* CET model was 52 g WW m^{-2}. To discern the influence of initial biomass on measured responses of *L. wollei*, different initial biomasses were exposed to the same copper concentration and duration (1 mg Cu L^{-1} for 24 h). Nine initial *L. wollei* biomasses (three replicates per biomass) were arrayed (0.25, 13, 26, 52, 130, 182, 260, 519, and 1,558 g WW m^{-2}), capturing the four orders-of-magnitude difference in *L. wollei* biomasses observed *in situ*. Copper concentrations were confirmed, and responses of *L. wollei* were measured as previously described. Responses of *L. wollei* with different initial biomasses were compared with responses measured using the maximum exposure for the *L. wollei* CET model (initial biomass = 52 g WW m^{-2}, copper concentration = 1.0 mg Cu L^{-1} and 24-h exposure duration) using ANOVA and Student's *t* test.

Development of a new model for responses of *L. wollei* to a copper-algaecide

If initial biomass significantly influences responses of *L. wollei*, a new model is needed. This new model is termed the biomass, duration, and concentration model, or BDC. To incorporate initial biomass as a variable, a model with two independent variables (i.e., CET, exposure duration and concentration) and one dependent variable (i.e., cyanobacterial response) would be expanded to include three independent variables (i.e., BDC, exposure duration, concentration and initial biomass as well as the dependent variable, cyanobacterial response). As a means to simplify the BDC model, the copper concentration that results in the intended cyanobacterial response ($\geq 90\%$) after an algaecide application is the focus. Site characteristics, specifically exposure duration and initial biomass, are then independent variables. The copper concentrations resulting in $\geq 90\%$ cyanobacterial response is the dependent variable.

Results from the previous objectives were used to bound exposures for the BDC model. Specifically, a series of exposure durations (objective 2) and initial biomasses (objective 3) were arrayed, which resulted in $\geq 90\%$ response for legal label copper concentrations. *Lyngbya wollei* was exposed to a series of copper concentrations (i.e., bounded by the maximum legal label concentration of copper) for each combination of exposure duration and biomass. Responses of *L. wollei* for each copper concentration, exposure duration, and biomass were compared statistically (ANOVA and linear contrasts) with maximum responses of *L. wollei* (initial biomass = 52 g WW m^{-2}, copper concentration = 1.0 mg Cu L^{-1}, and 24-h exposure duration). The lowest copper concentration for each combination of biomass and exposure duration that resulted in a comparable response relative to this maximum response of *L. wollei* was used for the BDC model.

RESULTS AND DISCUSSION

Confirmation of copper exposure concentrations

Copper concentrations (acid soluble and soluble) measured in untreated site water (pretreatment copper concentrations) ranged from nondetect (< 0.005 mg Cu L^{-1}) to 0.020 mg Cu L^{-1} (Table 2). After addition of copper-based algaecide, average percent differences relative to targeted copper concentrations were 3% ± 17% (acid soluble) and 31% ± 15% (soluble) (Table 2). Because measured acid-soluble copper concentrations were comparable with targeted copper concentrations, the targeted copper concentration was used for comparisons throughout this experiment. For those experiments in which untreated site water replaced copper exposures after the completion of exposure durations, soluble copper concentrations decreased to between nondetect and 0.061 mg Cu L^{-1} (initial algal biomass = 1,558 g WW m^{-2}).

Table 2. Targeted and measured acid-soluble and soluble copper concentrations ($N = 1$) for experiments.

Objective	Targeted Copper Concentration (mg Cu L^{-1})			Acid-soluble Copper Concentration (mg Cu L^{-1})	Soluble Copper Concentration (mg Cu L^{-1})
1. Responses of *Lyngbya wollei* to different copper algaecides	Algaecide	Concentration			
	Emulsified 3.8% copper ethanolamine (EA)	Untreated control		0.010	0.007
		0.1		0.117	0.089
		0.4		0.488	0.267
		0.7		0.793	0.555
		1.0		1.094	0.769
	Emulsified 9% copper EA	Untreated control		0.010	0.007
		0.1		0.118	0.083
		0.4		0.445	0.277
		0.7		0.703	0.567
		1.0		1.047	0.716
	Copper EA	Untreated control		0.010	0.007
		0.1		0.090	0.073
		0.4		0.406	0.259
		0.7		0.763	0.532
		1.0		1.114	0.827
	Copper citrate and gluconate	Untreated control		0.010	0.007
		0.1		0.102	0.084
		0.4		0.322	0.260
		0.7		0.636	0.559
		1.0		0.919	0.829
2. CET model[1]	Untreated control			ND	ND
	0.1			0.092	0.075
	0.4			0.323	0.246
	0.7			0.604	0.436
	1.0			0.798	0.666
3.[2]	Untreated control			0.018	0.020
	1.0			0.980	0.857
4. BDC model[3]	Initial algal biomass (g wet weight m^{-2})	Exposure duration (hour)	Targeted copper concentration (mg Cu L^{-1})		
	Untreated control	—	—	ND	ND
	13	1	0.3	0.303	0.204
		8	0.07	0.079	0.058
		24	0.07	0.079	0.058
	26	1	0.4	0.517	0.195
		8	0.2	0.243	0.088
		24	0.2	0.243	0.088
	52	1	1.0	1.330	0.505
		8	0.7	0.916	0.390
		24	0.4	0.517	0.195
	104	1	1.0	0.711	0.370
		8	0.7	0.503	0.284
		24	0.7	0.503	0.284

[1]After completion of the exposure duration and replacement of the exposure with unamended site water, soluble copper concentrations ranged from not detected (ND) (< 0.005 mg Cu L^{-1}) to 0.020 mg Cu L^{-1}.
[2]After completion of the exposure duration and replacement of the exposure with unamended site water, soluble copper concentrations ranged from 0.011 mg Cu L^{-1} (initial algal biomass = 26 g wet weight m^{-2}) to 0.061 mg Cu L^{-1} (initial algal biomass = 1,558 g wet weight m^{-2}).
[3]After completion of the exposure duration and replacement of the exposure with unamended site water, soluble copper concentrations were nondetect (< 0.005 mg Cu L^{-1}).

Identification of a sufficiently potent algaecide

All algaecides evaluated resulted in ≥ 90% cyanobacterial response as percent damaged trichomes 7 d postaddition of algaecide (Figure 2A). Exposures of 0.7 mg Cu L^{-1} for emulsified 3.8% copper EA, 1.0 mg Cu L^{-1} for emulsified 9% copper EA, 0.7 mg Cu L^{-1} for copper EA, and 0.4 mg Cu L^{-1} for copper CG resulted in the greatest response achieved as percent damaged trichomes for each algaecide. *Lyngbya wollei* within untreated controls had 0% ± 0% damaged trichomes at the completion of the 7-d experiment. For percent difference in mass, the maximum measured response for *L. wollei* was 63% ± 4% after an exposure of emulsified 3.5% copper EA at 0.7 mg Cu L^{-1} (Figure 2B).

Correlation coefficients of percent damaged trichomes were 0.99 (emulsified 3.8% copper EA, 0.1 to 0.7 mg Cu L^{-1}), 0.19 (emulsified 9% copper EA, 0.1 to 1.0 mg Cu L^{-1}), 0.57 (copper EA, 0.1 to 1.0 mg Cu L^{-1}), and 0.15 (copper CG, 0.1 to 1.0 mg Cu L^{-1}). For percent difference in mass, correlation coefficients were 0.99 (emulsified 3.8% copper EA, 0.1 to 0.7 mg Cu L^{-1}), 0.27 (emulsified 9% copper EA, 0.1 to 1.0 mg Cu L^{-1}), 0.43 (copper EA, 0.1 to 1.0 mg Cu L^{-1}), and 0.19 (copper CG, 0.1 to 1.0 mg Cu L^{-1}). On the basis of

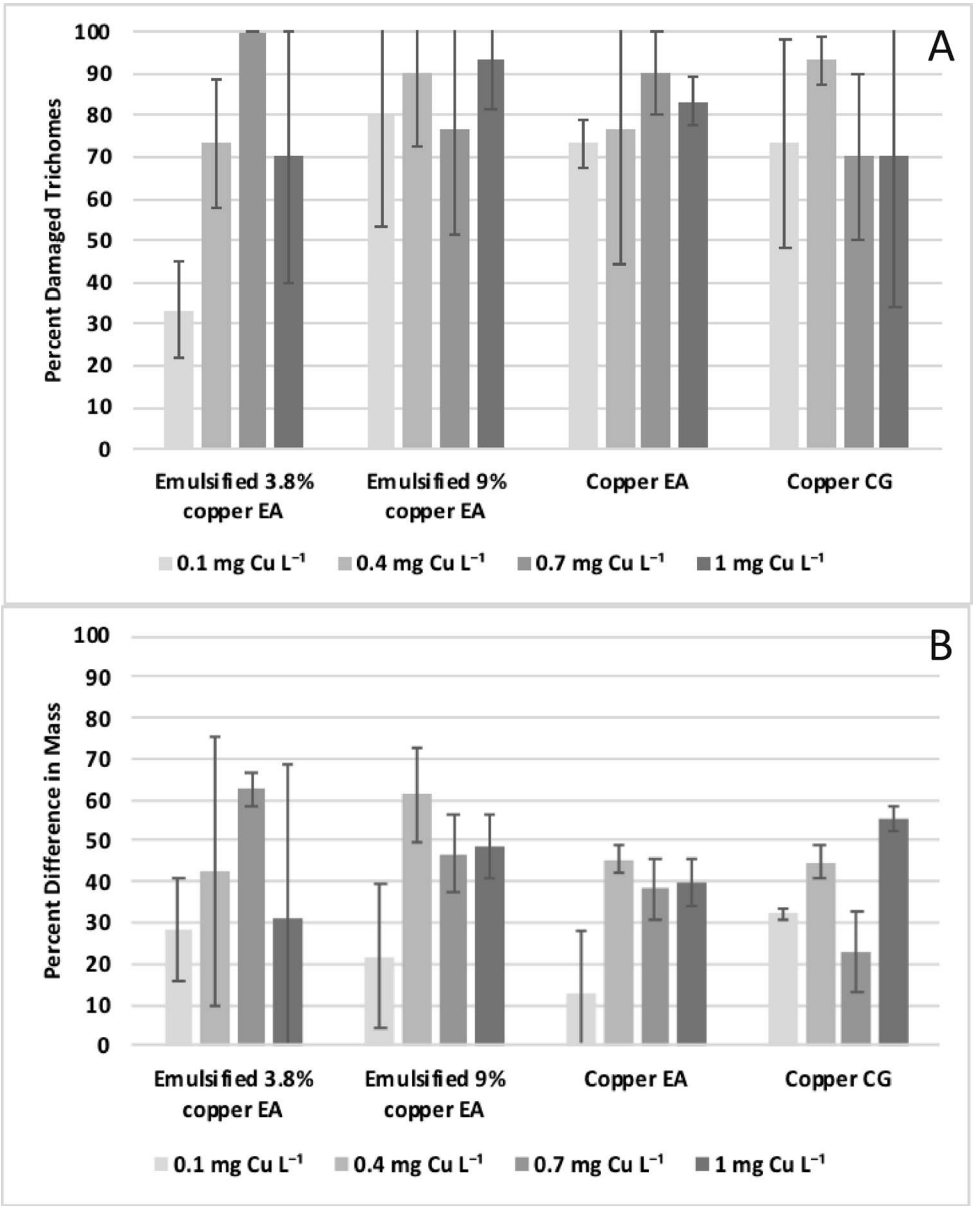

Figure 2. Average responses ($n = 3$) of *Lyngbya wollei* in terms of percent damaged trichomes (A) and percent difference in wet weight (B) measured 7 d after addition of the copper algaecides at 0.1, 0.4, 0.7, and 1.0 mg Cu L^{-1} with a 7-d exposure duration. Error bars are ± 1 SD.

the magnitude of cyanobacterial response and correlation coefficient, emulsified 3.8% copper EA was sufficiently potent and was used for subsequent experiments.

Lyngbya wollei CET model

Since CET models are intended to predict exposures (i.e., concentration and exposure duration) necessary to result in control, the following exposures eliciting the maximum measureable response (≥ 90% response) were identified. In terms of percent damaged trichomes and DW, exposures of 0.4 and 0.7 mg Cu L^{-1} for a 24-h duration, exposures of 0.4, 0.7, and 1.0 mg Cu L^{-1} for 8 h, and exposures of 0.7 and 1.0 mg Cu L^{-1} for 1 h resulted in statistically similar algal responses (87 to 100% response) as the maximum exposure evaluated (1.0 mg Cu L^{-1} for 24 h = 94% response) (Figure 3A and C, $P \geq 0.05$). Responses of *L. wollei* in untreated controls had 13% ± 14% damaged trichomes at completion of the 7-d experiment. For percent differences in WW, the exposures of 0.1 mg Cu L^{-1} for 24 h and exposures of 0.4 mg Cu L^{-1} and 1.0 mg Cu L^{-1} for 0.25 h were also similar (13 to 43% response) to responses to the maximum exposure concentration and duration evaluated (39% response; Figure 3B).

The CET model is bound by the specific concentrations and exposure durations used to develop the model. For the CET model to have utility for predicting outcomes *in situ*, these concentrations and exposure durations need to be realistic and practical. The bounds of the *L. wollei* CET model for copper concentrations are legal label concentra-

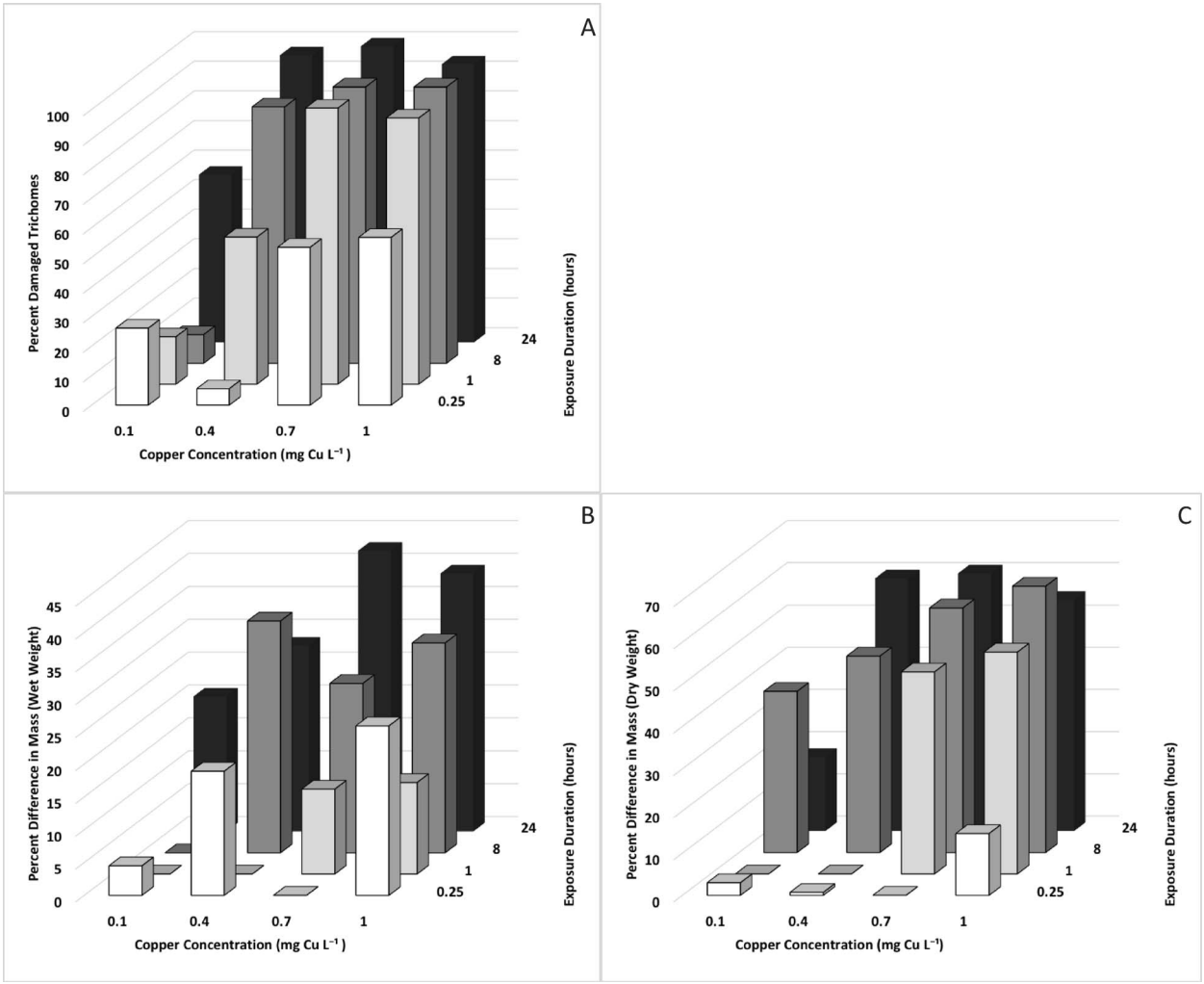

Figure 3. Average responses ($n = 3$) of *Lyngbya wollei* in terms of percent damaged trichomes (A), percent difference in wet weight (B), and percent difference in dry weight (C) measured 7 d after exposure initiation for a series of copper concentrations as emulsified 3.8% copper EA and exposure durations for the concentration–exposure time model.

tions. The bounds for exposure durations range from situations in which rapid copper dissipation rates can occur (e.g., rapid dilution due to water movement, wind movement) to situations with relatively slow copper dissipation rates (Calomeni et al. 2017). Also important, by using realistic and practical bounds for this model, the limits of cyanobacterial responses that can be achieved legally *in situ* are captured.

Considering the previous points, the lowest concentration evaluated (0.1 mg Cu L^{-1}) represents a situation in which the maximum response could not be achieved regardless of the duration of exposure. In contrast, the maximum concentration applied (1.0 mg Cu L^{-1}) represents the response anticipated for the maximum legal label concentration. For exposure duration, 0.25 h was insufficient to result in the maximum measurable response for any legal label concentration evaluated. This indicates that even at the maximum label concentration (1.0 mg Cu L^{-1}), control ($\geq$ 90% response) could not be achieved for exposures of 0.25 h. For the upper bound of exposure durations (8 and 24 h), responses for equivalent concentrations were comparable, demonstrating that exposures longer than 8 h would not result in any added cyanobacterial responses.

Influence of biomass on the *L. wollei* CET model

Percent damaged trichomes ranged from 62 to 97% measured 7 d postexposure initiation for initial *L. wollei* biomasses from 13 g WW m^{-2} to 519 g WW m^{-2} (Figure 4A), and were similar to responses predicted from the *L. wollei* CET model (initial biomass = 52 g WW m^{-2}, copper concentration = 1 mg Cu L^{-1}, and exposure duration = 24 h, response = 81%). For the initial biomass of 1,558 g WW m^{-2}, damaged trichomes measured 7 d postexposure initiation were 23% ± 6%. Responses of *L. wollei* at 1,558 g WW m^{-2} were significantly less than those predicted from the CET model (81% response, $P < 0.0016$, $\alpha = 0.05$) and were not significantly different from the response measured for untreated controls (12% ± 11%, $P = 0.2161$, $\alpha = 0.05$).

For percent difference in mass (wet and dry weights), significantly less cyanobacterial response occurred with an

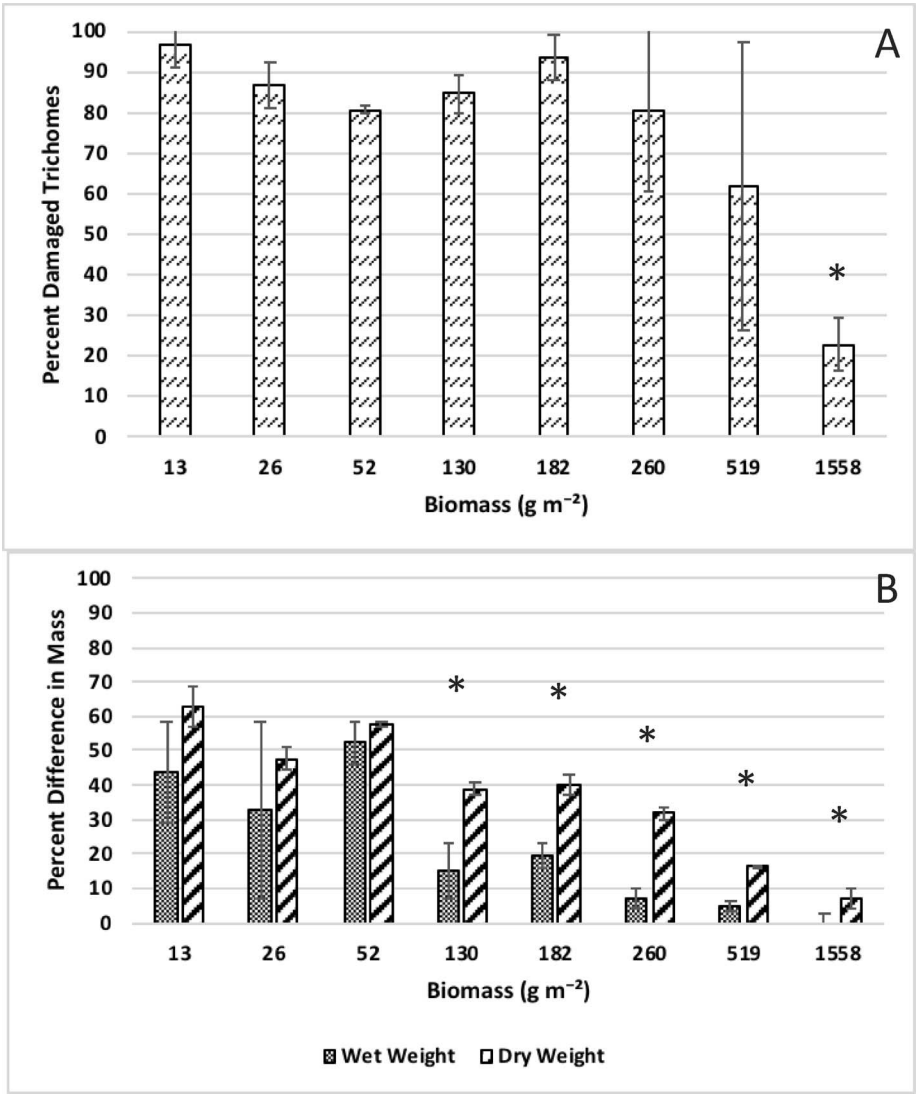

Figure 4. Average responses ($n = 3$) of *Lyngbya wollei* with a series of biomasses at exposure initiation in terms of percent damaged trichomes (A) and percent difference in (wet and dry) mass (B) measured 7 d after exposure initiation of emulsified 3.8% copper EA at 1.0 mg Cu L^{-1} for a 24-h exposure duration. Error bars are ± 1 SD. Asterisks are used to indicate significantly different responses relative to responses of the initial algal biomass used for the concentration–exposure time model (52 g wet weight [WW] m^{-2}). At the initial biomass of 0.25 g WW m^{-2}, the final biomass was nondetect (< 0.15 g WW m^{-2}) 7 d postexposure initiation and the datum was excluded.

initial biomass of 130 g WW m^{-2} ($P < 0.0023$ WW and $P < 0.001$ DW, $\alpha = 0.05$) relative to responses predicted by the CET model (Figure 4B). For the initial biomass of 130 g WW m^{-2}, responses of *L. wollei* were 15% ± 8% as WW and 39% ± 2% as DW. This is in comparison with approximately 55% response for wet and dry weights predicted from the CET model. Responses of *L. wollei* continued to decrease as initial biomass increased, with an initial biomass of 1,558 g WW m^{-2} resulting in no measureable difference relative to the untreated control as WW (0%) and a 7% difference for DW.

For the experimental conditions of this study, initial biomasses less than 52 g WW m^{-2} were likely overexposed, meaning that less copper exposure (i.e., duration, concentration, or both) would result in the same response. Alternatively, for this accession of *L. wollei*, initial biomasses of 130 g WW m^{-2} and larger would require a greater copper exposure to result in control. The concentration applied for this objective was the maximum legal label concentration (1.0 mg Cu L^{-1}). Therefore, control cannot be achieved with one algaecide application at this initial biomass. Additional applications would be necessary, emphasizing the importance of treating before peak cyanobacterial biomass or early in a *L. wollei* infestation so that the greatest response can be achieved with one application. If the *L. wollei* biomass is equivalent to or greater than 130 g WW m^{-2} for this site, several algaecide treatments may be required to incrementally decreased the biomass.

For this *L. wollei* from a 44,500-m^2 pond in Spartanburg, SC, cyanobacterial responses can range from the predicted response (CET model) to no measurable response (relative to the untreated control) as a function of initial biomass.

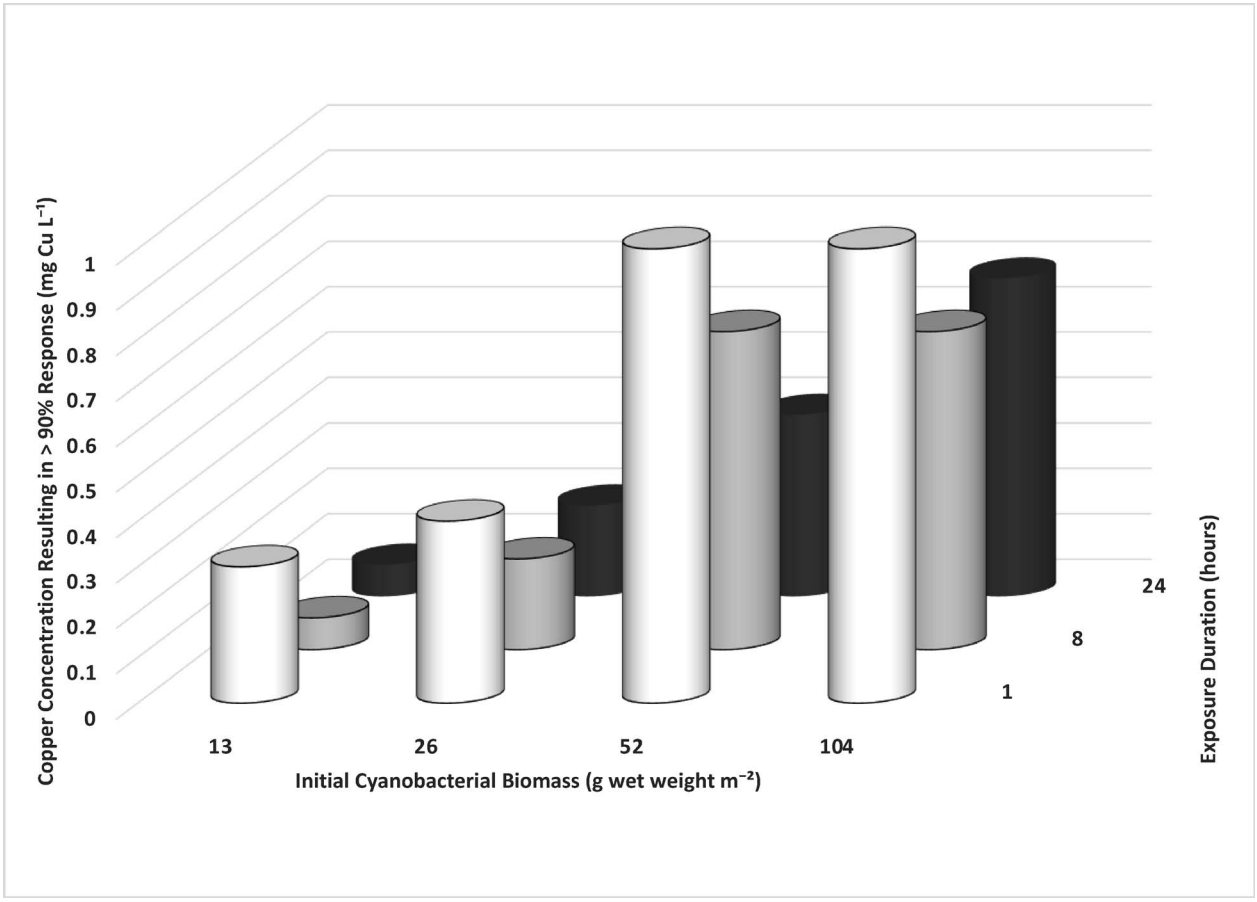

Figure 5. Copper concentrations from applications of emulsified 3.8% copper EA resulting in ≥ 90% response of *Lyngbya wollei* measured 7 d after treatment for a series of initial biomasses and exposure durations for the biomass–duration–concentration model (BDC).

Because of the range of cyanobacterial responses elicited using the same exposure parameters (i.e., copper concentration and exposure duration), the results from this objective demonstrate that initial *L. wollei* biomass is an important exposure parameter driving responses of *L. wollei*. The CET model developed for the current experiment predicts responses for *L. wollei* at an initial biomass of 52 g WW m^{-2}. To expand predictions beyond a single initial biomass, a new model is necessary.

Development of a new model for responses of *L. wollei* to a copper-algaecide

With the inclusion of initial biomass as a variable in the BDC model, prediction of effective algaecide treatments was expanded to capture an order-of-magnitude difference in initial biomasses (13 g WW m^{-2} to 104 g WW m^{-2}). As expected from the results of the objective evaluating the influence of initial biomasses on responses, the initial biomasses of 13 g WW m^{-2} and 26 g WW m^{-2} were overexposed and required a lower copper concentration than 1 mg Cu L^{-1} to achieve the maximum response. For 13 g WW m^{-2} these exposures were 0.3 mg Cu L^{-1} for a 1-h exposure duration and 0.07 mg Cu L^{-1} for 8 and 24 h (Figure 5). A concentration of 0.4 mg Cu L^{-1} for a 1-h exposure duration and 0.2 mg Cu L^{-1} for 8 and 24 h resulted in the maximum response for the initial biomass of 26 g WW m^{-2}.

The relatively low copper concentration (0.3 mg Cu L^{-1} and 0.07 mg Cu L^{-1}) needed to result in ≥ 90% response for biomasses of 13 g WW m^{-2} and 26 g WW m^{-2} emphasizes the importance of treating early. If *L. wollei* is treated expeditiously within a growing season and an infestation, an exposure of less than 1% to 30% of the maximum label rate will be needed for control. If time is allowed for the cyanobacterial biomass to increase to 52 and 104 g WW m^{-2}, the maximum legal label concentration will be necessary to achieve control (≥ 90% response, Figure 5). Regarding exposure duration, 8- and 24-h exposures required comparable copper concentrations to result in ≥ 90% response. This indicates that for *L. wollei* exposed to emulsified 3.8% copper EA, contact durations greater than 8 h will not result in measurable increases in algaecide effectiveness. For a contact duration of 1 h, exposures required between 1.4 and 4 times greater copper concentrations than 8 and 24 h to result in ≥ 90% response.

SUMMARY

As the CET model has been predictive of vascular plant responses to herbicides (Van and Conant 1988, Getsinger 1991, Getsinger and Netherland 1997), the CET model also

applies to responses of cyanobacteria to algaecides under certain conditions. The results from the current manuscript demonstrate that the CET model is predictive of responses for the initial biomass that was used to develop the model. This suggests that for treatment of *L. wollei in situ* with limited variability in initial biomass, CET models can be used. Limited variability in initial biomass would occur in aquatic systems in which 1) the cyanobacterial biomass is "topped out," 2) the cyanobacterial biomass is well controlled by prior algaecide applications, or 3) the algaecide treatment is scheduled to occur early in the "growing season."

For cyanobacterial infestations that do not meet these criteria, resulting in biomasses that range widely, the BDC model is more appropriate. The BDC model provides a tool to integrate information regarding initial biomass and duration of exposure to predict an effective treatment. This tool would be useful for sites in which cyanobacterial biomasses range throughout the season, annually, or within treatment areas in an aquatic system.

Similar to CET models, the BDC model is specific to couplets of the exposed organism and algaecide. Conditions that may require careful consideration before use of the specific CET and BDC models include different water characteristics (e.g., pH, hardness, alkalinity, conductivity, particulate and dissolved organic matter) and different algae and cyanobacteria (e.g., genera, species, accessions). Depending on the site and situation, a CET or BDC model can be used to predict an effective algaecide treatment before application.

SOURCES OF MATERIALS

[1]Clearigate® (emulsified 3.8% copper EA), Applied Biochemists, A Lonza Business, W175N11163 Stonewood Dr. Ste. 234, Germantown, WI 53022.

[2]Cutrine® Ultra (emulsified 9% copper EA), Applied Biochemists, A Lonza Business, W175N11163 Stonewood Dr. Ste. 234, Germantown, WI 53022.

[3]Cutrine-Plus® (copper EA), Applied Biochemists, A Lonza Business, W175N11163 Stonewood Dr. Ste. 234, Germantown, WI 53022.

[4]Algimycin® PWF (copper CG), Applied Biochemists, A Lonza Business, W175N11163 Stonewood Dr. Ste. 234, Germantown, WI 53022.

[5]Residential Ecolux 40 W, General Electric, 41 Farnesworth St., Boston, MA 02210.

[6]Kimwipes® delicate task wipers, Kimberly-Clark™Professional, 1400 Holcomb Bridge Rd., Roswell, GA 30076.

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