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The Effects of Three Chemical Algaecides on Cell Numbers and Toxin Content of the Cyanobacteria *Microcystis aeruginosa* and *Anabaenopsis* sp.

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Abstract Toxic cyanobacteria blooms are a growing concern for public health and safety, due in part to the production of the hepatotoxin microcystin by certain species, including *Microcystis aeruginosa*. Management strategies for controlling cyanobacteria blooms include algaecide treatments, often with copper sulfate, and more recently oxidizers such as sodium percarbonate that produce hydrogen peroxide. This study assessed the effects of two copper-containing algaecides and one sodium percarbonate-containing algaecide on mitigating cell numbers and toxin content of cultured *M. aeruginosa* and summer (July) bloom samples of *Anabaenopsis* sp. in a brackish stormwater detention pond. Monitoring of the bloom revealed that *Anabaenopsis* sp. was associated with elevated levels of orthophosphate compared to nitrogen (dissolved inorganic nitrogen to phosphorus ratios were 0.19–1.80), and the bloom decline (September–October) was likely due to lower autumn water temperatures combined with potential grazing by the dinoflagellate *Protoperidinium quinquecorne*. Laboratory-based algaecide experiments included three dose levels, and cyanobacteria cell numbers and microcystin concentrations (particulate and dissolved) were evaluated over 7 d. Following exposure, copper-containing treatments generally had lower cell numbers than either sodium percarbonate-containing or control (no algaecide) treatments. Addition of algaecides did not reduce overall microcystin levels, and a release of toxin from the particulate to dissolved phase was observed in most treatments. These findings indicate that algaecide applications may visibly control cyanobacteria bloom densities, but not necessarily toxin concentrations, and have implications for public health and safety.

Keywords Cyanobacteria · Algaecide · Microcystin · Harmful algal blooms · Detention ponds

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Introduction

Harmful algal blooms (HABs) include representatives from a wide range of phytoplankton taxa and are a focus of marine and coastal research globally because of their negative impacts on human health and aquatic ecosystems. Examples of negative impacts associated with HABs include shellfish poisoning (Anderson 1997), bivalve and marine mammal mortalities (Scholin et al. 2000; Greenfield and Lonsdale 2002), and ecosystem disruption (Edvardsen and Paasche 1998). The annual frequency and

intensity of HABs have increased (Hallegraeff 1993; Anderson et al. 2008). Cyanobacteria in particular are responsible for the largest number of HABs in freshwater environments worldwide (Chorus and Bartram 1999; Huisman and Visser 2005), but they are also common in brackish and coastal waterbodies (Paerl 1988; Lewitus et al. 2008). Cyanobacteria HABs are primarily associated with certain genera such as *Microcystis*, *Anabaenopsis*, *Oscillatoria*, *Nostoc*, and *Planktothrix* that produce microcystins, a group of hepatotoxins that include ~90 cyclic congeners (Sivonen and Jones 1999). Microcystins represent a serious health concern because they can induce severe gastroenteritis, liver failure, and even death (Pouria et al. 1998; Falconer 2005). Blooms of microcystin-producing cyanobacteria are common in a wide range of aquatic ecosystems, particularly lakes and ponds associated with human residential and recreational activity (Gobler et al. 2007; Lewitus et al. 2008), presenting a considerable public health risk.

Cyanobacteria blooms are caused by a variety of physical, chemical, and biological factors. Warm, stagnant conditions, such as those typical of mid to late summer, are often associated with cyanobacteria bloom formation (Paerl 1988; Huisman et al. 2005; Lewitus et al. 2008; Elliot 2010). Additionally, numerous studies have shown that enrichment of nitrogen and phosphorus associated with runoff from urban, suburban, and agricultural lands, fertilization, and other anthropogenic activities promote the development of HABs, especially those caused by cyanobacteria (reviewed in Anderson et al. 2008; Heisler et al. 2008). Studies have shown that biological control of cyanobacteria blooms includes grazing pressure by microzooplankton (Gobler et al. 2007; Davis et al. 2012), but it has also been suggested that cyanobacteria are not always the preferred prey due to their chemical defenses and reduced nutritional quality (Wilson et al. 2006; Paerl and Paul 2012), so the extent to which top-down processes mitigate a bloom remains uncertain. The physical and chemical factors that cause cyanobacteria blooms are predicted to become enhanced under future climate conditions (Paerl and Huisman 2009; Elliot 2010; Paerl and Paul 2012), so it is reasonable to suggest that incidences of HABs caused by cyanobacteria will also increase. This is compounded by the tendency for cyanobacteria HABs to occur in waters with a high likelihood of human contact. For example, shallow brackish detention ponds are routinely built within residential and recreational (golf course) developments along the southeastern coast of the US to mitigate stormwater (Drescher et al. 2011). Although many detention ponds, such as the site considered here, are either directly or daisy-chain linked to tidal creeks and thus exchange water with receiving estuaries, long residence times make them prone to frequent stagnation during warmer months such that they accumulate fertilizers and other runoff, making them ideal settings for cyanobacteria HABs (Lewitus and Holland 2003; Serrano and DeLorenzo 2008; Lewitus et al. 2008). Given the ecological and public health threats of toxic cyanobacteria, combined with the likelihood of increased bloom occurrences, safe and effective bloom management is of paramount importance.

Management strategies for mitigating cyanobacteria blooms include nutrient reduction, aeration, flocculation, dilution, grazing, and a variety of algaecides (reviewed in Chorus and Bartram 1999; Huisman et al. 2005). Copper sulfate ($CuSO_4$) in particular is a common ingredient of commercial algaecides because it is inexpensive and cyanobacteria are more sensitive to high concentrations of copper than other phytoplankton taxa (Brand et al. 1986; Chorus and Bartram 1999; Le Jeune et al. 2006). Copper (Cu) toxicity acts on cyanobacteria through multiple routes, including substitution for magnesium in chlorophyll (Jančula and Maršálek 2011) and associated thylakoid disruption combined with inhibition of electron transport in photosystem II (Verhoeven and Eloff 1979; Baron et al. 1995; Qian et al. 2010). In addition to cyanobacteria, elevated copper levels pose serious physiological and ecological threats to secondary and tertiary consumers, such as fish and invertebrates (Wilson and Taylor 1993; Grosell et al. 2007; Korosi and Smol 2012; Al-Bairuti et al. 2013), and consequently they can have detrimental long-term consequences for aquatic food webs (Korosi and Smol 2012). Thus, chemical alternatives to copper-based algaecides have been proposed, particularly hydrogen peroxide (H_2O_2) (Barroin and Feuillade 1986; Drábková et al. 2007; Barrington and Ghadouani 2008). Similar to copper sulfate, hydrogen peroxide inhibits electron transport and photosynthetic activity in cyanobacteria, rendering photosystem II inactive (Samuilov et al. 2004; Barrington and Ghadouani 2008). Research has shown that cyanobacteria tend to be more sensitive to hydrogen peroxide than other phytoplankton taxa (Barroin and Feuillade 1986; Barrington and Ghadouani 2008; Matthijs et al. 2012). As a result, hydrogen peroxide has been applied to control cyanobacteria in several systems, including waste stabilization ponds (Barrington et al. 2011, 2013) and a lake (Matthijs et al. 2012). Safety concerns prevent adding large volumes of concentrated hydrogen peroxide directly to water bodies. However, it can be produced through oxidation, such as from sodium percarbonate, so algaecides containing oxidizers are available commercially.

Both copper sulfate and hydrogen peroxide disrupt cyanobacteria cells, but the extent to which algaecides using these reactive compounds mitigate toxin content is less understood, representing an important gap in our understanding of bloom management. The objective of this

study was to investigate the relative abilities of three commercially available algaecides (two containing copper sulfate and one containing sodium percarbonate) for controlling cyanobacteria cell concentrations and toxin content (as microcystin levels). Specifically, we conducted laboratory experiments using cultured and bloom cyanobacteria to evaluate the efficacy of each algaecide for controlling (1) bloom levels of cyanobacteria and (2) the partitioning of microcystin in both the particulate (intracellular) and dissolved (extracellular) fractions. Each experiment was conducted at three treatment levels to assess the effects of dose and chemical nature of algaecides on microcystin levels and cell densities.

Materials and Methods

Phytoplankton Culturing and Enumeration

Microcystis aeruginosa (SC isolate) was grown under a 12:12 light:dark cycle at 25 °C (temperatures typical during mid-summer in coastal SC ponds) and 140 μE m^{-2} s^{-1}. This irradiance level is within range of those associated with maximum *Microcystis* growth and cellular microcystin level (Wiedner et al. 2003) but below irradiances that cause severe oxidative stress (Drábková et al. 2007). *M. aeruginosa* was grown in sterile 0.2-μm filtered *f*/2 medium (Guillard and Ryther 1962) without silica using water collected from the South Atlantic Bight that was diluted with sterile distilled water to a final salinity of 5 psu. *M. aeruginosa* was counted by sampling triplicate 1 mL aliquots of late log-phase culture, staining each aliquot with a drop of Lugol's iodine solution, and enumerating individual cells by light microscopy using a 0.1 mm haemocytometer. *Anabaenopsis* sp. was sampled from bloom water by removing triplicate 1 mL aliquots, staining each aliquot with a drop of Lugol's iodine solution, then enumerating as for *M. aeruginosa*.

Sampling Location

Kiawah Island is a barrier island located ~45 km southwest of Charleston, South Carolina (SC), USA (Fig. 1). It is characterized by extensive landscaping, golf course construction, and networks of detention ponds as catchments for stormwater runoff (Lewitus et al. 2003, 2008; Holland et al. 2004). There are 136 detention ponds on Kiawah Island within ~335 acres (1.356 km^2), and the majority of these ponds are shallow (1–3 m depth), brackish to marine systems (Lewitus et al. 2003). Monitoring and research of water quality, nutrient loading, and phytoplankton in Kiawah Island detention ponds has been ongoing since 2001. These and related efforts have shown that due to low flushing, SC detention ponds often accumulate nutrient runoff from fertilizers, and this enrichment makes them susceptible to HABs (Lewitus and Holland 2003; Lewitus et al. 2003, 2008; Drescher et al. 2011). Blooms caused by cyanobacteria, including HAB species, commonly recur in Kiawah Island detention ponds, with *Microcystis*, *Anabaenopsis*, *Oscillatoria*, *Cylindrospermopsis*, and *Aphanizomenon* being among the most common genera (Brock 2006; Lewitus et al. 2008; Siegel et al. 2011).

The site used for this study, Kiawah Island pond number 093 (henceforth K093), is a shallow (~1.5 m maximum depth) mesohaline (typical salinity range 15–25 psu) system located on the northeastern end of Kiawah Island that covers ~13 acres (0.053 km^2) of surface area (Fig. 1). During summer (June–September), water temperatures are usually 24–32 °C. K093 is surrounded by residential housing, a golf course, salt marsh, and road, and it is connected to a tidal creek by stormwater pipes, such that tidal exchange occurs between the pond and the creek. K093 is used for recreational shrimping, crabbing, and fishing.

Field Sampling and Analyses

Triplicate 1 L surface (0.3 m depth) water samples were collected from the K093 dock (Fig. 1) at mid-ebb tide using opaque Nalgene bottles that were previously acid-washed (immersion in 10 % hydrochloric acid, HCl, for 4 h followed by rinsing three times with distilled water), placed in the dark inside a cooler, then immediately transported to the laboratory for processing. Sample intervals were frequent (2–3 times wk^{-1}) following initial observation of the *Anabaenopsis* sp. bloom (20 July, 2009), then every 1–2 wks while the bloom persisted (through 1 October, 2009). During each sampling event, standard water quality parameters (temperature, salinity, and dissolved oxygen) were recorded from surface depths using a hand-held YSI 85 unit (YSI Incorporated, Yellow Springs, OH, USA), and pH was measured using a hand-held pH meter.

Upon return to the laboratory, whole water samples (up to 40 mL) from each replicate were filtered through a 0.7 μm glass fiber filter (GF/F) for total chlorophyll *a* (a common proxy for phytoplankton biomass). Sampling processing, evaluations of pigment standards, and quality assurance/quality control (QA/QC) procedures followed well-established methods (non-acidification version of EPA method 445.0; Arar and Collins 1992). Briefly, filters containing sample were placed into acid-washed (using 10 % HCl as described above) 25 mL scintillation vials, then 1 mL of saturated magnesium carbonate ($MgCO_3$) was added to ensure that cell membranes ruptured

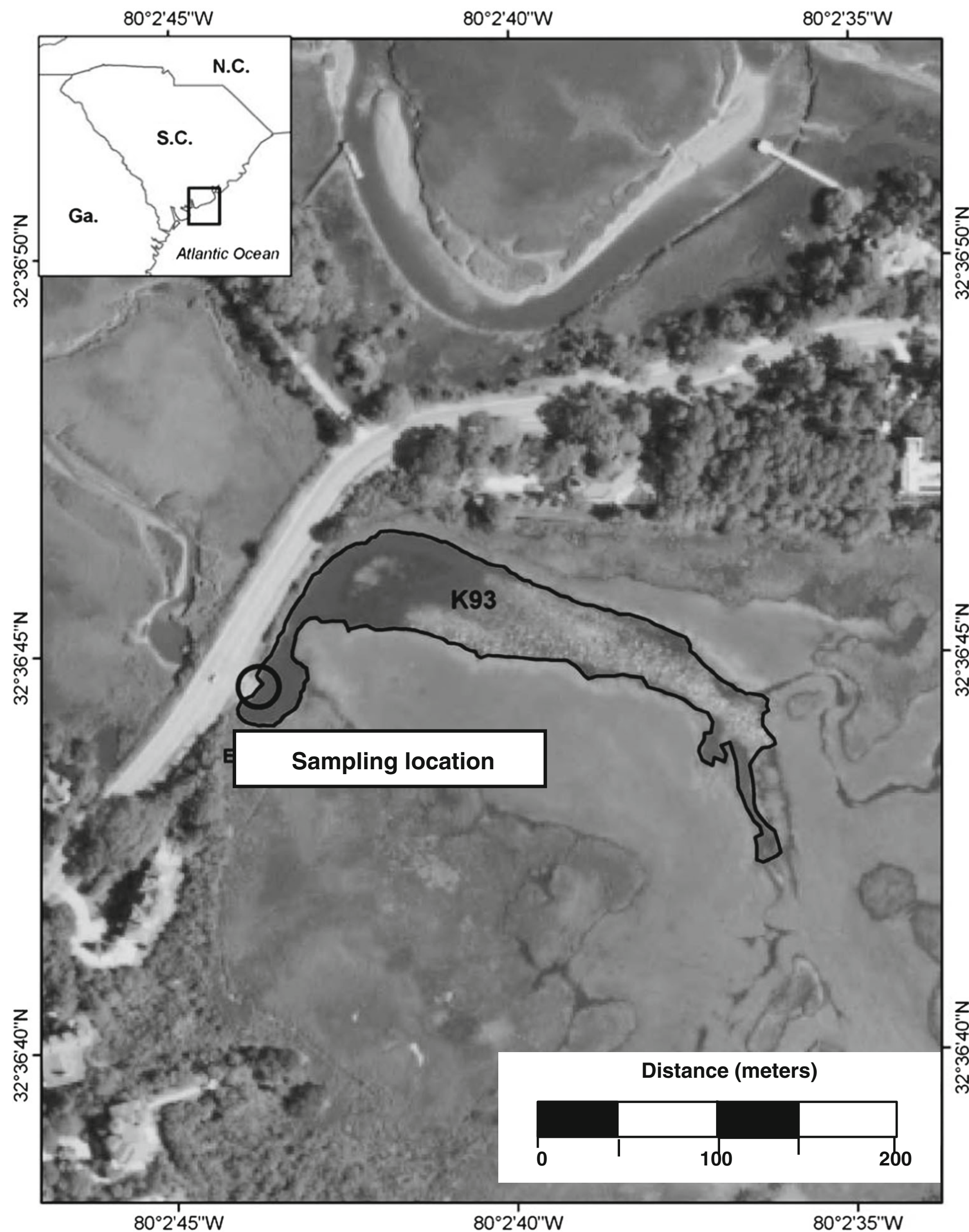

Fig. 1 Location of the 2009 *Anabaenopsis* sp. bloom event. *Inset* depicts southeastern coast of the US, highlighting region of Kiawah Island, SC. Sampling site denoted by *circle*, and *black outline* depicts area of pond K093 that is >0.5 m deep year-round

completely, and samples were frozen (−20 °C) until analysis. To evaluate pigment levels, 9 mL of high-performance liquid chromatography (HPLC) grade acetone (90 %) were added to each replicate, then chlorophyll *a* was extracted (−20 °C for 36 h). Following extraction, concentrations (μg L^{-1}) of chlorophyll *a* were evaluated using a Turner Design 700 fluorometer according to manufacturer specifications (Arar and Collins 1992).

For nutrient analyses, aliquots (25 mL) of whole water from each sample replicate were filtered through precombusted (450 °C for 4 h) GF/F filters into acid-washed (using 10 % HCl as described above) scintillation vials. Subsequent sample processing followed well-established methods for orthophosphate (PO_4^{3-}) (Hach method 31-115-01-1-H), nitrite + nitrate combined ($NO_2^- + NO_3^-$) (Hach method 31-107-04-1-D), and ammonium (NH_4^+) (Hach method 31-107-06-1-B) using a Lachat Quick-Chem 8000 nutrient auto-analyzer with an ASX 500 autosampler (Grasshoff 1983; Johnson and Petty 1983; Zimmerman and Keefe 1991). QA/QC procedures followed manufacturer guidelines. Briefly, a calibration curve was generated at the beginning and end of each

analysis batch (up to 30 samples) using reference standards from the manufacturer (Hach Inc.). If $R^2 > 0.98$, the curve was considered valid. One blank (ultrapure distilled water) and one standard (mid-range concentration) sample was run at the beginning, end, and every 15 samples to assess potential instrument drift and recalibrated as warranted.

To determine qualitative phytoplankton community composition, an aliquot ($\sim$2–3 mL) of whole water from one sample replicate (chosen at random) was dispensed into a Lab-Tek II Chamber slide, settled for 5 min, and then viewed with a Nikon Eclipse TS100 inverted microscope. All observed phytoplankton were identified to the lowest taxonomic level possible. Bloom taxa were enumerated by preserving an aliquot (100 mL) from the same replicate with Lugol's iodine solution (3 % final preservative concentration), then individual cells (within colonies/chains and excluding heterocysts for *Anabaenopsis* sp.) were counted with a 0.1 mm haemocytometer until either a minimum of 300 cells or the entire chamber was counted, whichever occurred first.

Algaecide Exposure Experiments

Two copper-containing algaecides (Captain* and K-Tea^{TM}, trademarks of SePRO Corporation) and one non-copper-containing algaecide (Pak^{TM} 27, formerly by Solvay Chemicals Inc., currently manufactured by SePRO Corporation) were evaluated for controlling the growth and toxin release of cultured *M. aeruginosa* and *Anabaenopsis* sp. bloom water. Captain* is a chelated elemental copper compound containing 15.9 % active copper carbonate that targets filamentous and planktonic algae, including cyanobacteria. K-Tea^{TM} is also a chelated elemental copper compound, but it contains 8 % elemental copper derived from a copper–triethanolamine complex and copper hydroxide. K-Tea^{TM} targets a wide range of algae taxa, including cyanobacteria, chlorophytes, diatoms, and dinoflagellates. The active ingredient in Pak^{TM} 27 is sodium percarbonate (85 %), and it presents an alternative to copper-derived compounds, primarily targeting cyanobacteria. While each of the three products comes with a label describing specific instructions for use, application of algaecides to water bodies often varies with user. Therefore, three dose levels were tested: half the manufacturer's recommended dose level (0.5×), at the manufacturer's recommended dose (1×), and twice the manufacturer's recommended dose (2×). Manufacturer's recommended dosages were as follows: Captain (0.4 ppm), K-Tea^{TM} (0.5 ppm), and Pak^{TM} 27 (6.5 lbs acre-ft^{-1}). We recognize that the molar concentrations of active compounds varied between algaecides, but since the goal of this study was to evaluate algal responses to products as managers typically use them, we did not normalize to account for these differences. Control treatments contained no algaecide.

Two series of experiments were conducted. The first used log-phase cultured *M. aeruginosa* (culturing conditions described above), and the second used water from K093 during an *Anabaenopsis* sp. bloom (collected 21 July, 2009). To evaluate the efficacy of each algaecide for mitigating cyanobacteria, initial concentrations typical of those observed during blooms (target concentration $\sim 5.00 \times 10^5$ cells mL^{-1}) were used. For each experiment, triplicate algal treatments (1 L each) were incubated using a 12:12 light:dark cycle at 140 μE m^{-2} s^{-1} at 25 °C in 0.2-μm filtered sterile seawater at the same salinities used for cultured (5 psu for *M. aeruginosa*) and field collected (21 psu for *Anabaenopsis* sp.) algae using sterile glass flasks according to the above three dose levels.

To evaluate the effects of each algaecide on cyanobacteria cell concentrations, flasks were gently mixed, then a 1 mL aliquot was collected, placed in an opaque 1.5 mL microcentrifuge tube, and preserved with 1 drop of Lugol's iodine solution when algaecides were first added (t0), then at 4, 8, 24, 48, 72 h and 7 d following inoculation. This experimental duration was chosen because algaecide applications to ponds and lakes usually visibly reduce blooms in less than 1 week's time. All preserved samples were subsequently enumerated using light microscopy and a 0.1 mm haemocytometer as described above.

Microcystin concentrations were evaluated using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Abraxis microcystins/nodularins kit, product number 520011) according to the manufacturer's procedure and using reference standards included within the kits. When complete, optical density (450 nm) was analyzed using a Synergy HT microplate reader (Biotek). To evaluate particulate (intracellular) microcystin levels, flasks were gently mixed as above, then whole water (1 mL) was removed from each replicate, placed in a 1.5 mL microcentrifuge tube, and immediately frozen for subsequent analyses. To evaluate dissolved (extracellular) microcystin levels, additional aliquots ($\sim$5–10 mL) were removed from each replicate then gently syringe-filtered through pre-combusted GF/F filters (0.7 μm), and 1 mL of the filtrate was stored in a 1.5 mL microcentrifuge tube as above. All toxin analyses were performed within 14 d of sampling, and no samples were thawed more than once. Particulate (intracellular) microcystin was calculated as the difference between whole and dissolved (extracellular) fractions. Microcystin was evaluated for the following time points: 0, 4, 24 h and 7 d following addition of algaecide.

All data are presented as means of triplicate measurements ($n = 3$) $\pm$ standard deviations (SD). Statistical comparisons were made using nonparametric analyses by

Table 1 Surface water quality over the duration of a bloom of *Anabaenopsis* sp. in Kiawah Pond K093 during July (Jul), August, (Aug), September (Sept), and October (Oct) of 2009

Date (2009)	*T*	*S*	DO	pH	NH_4^+	$NO_2^- + NO_3^-$	PO_4^{3-}	chl *a*	*Anabaenopsis* sp.
6-Jul	28.6	19.5	1.86	8.2	n.d.	n.d.	n.d.	88.8 (8.13)	+
20-Jul	27.8	20.5	1.65	8.6	n.d.	n.d.	n.d.	503.9 (24.0)	++
21-Jul	28.6	20.7	15.03	9.1	1.36 (1.12)	1.27 (0.55)	13.86 (0.36)	820.2 (86.7)	3.49×10^6
22-Jul	28.9	22.9	3.98	9.1	3.55 (1.94)	0.62 (0.09)	11.19 (1.19)	869.8 (97.3)	++
28-Jul	28.9	22.3	3.98	8.9	4.17 (1.06)	0.44 (0.13)	6.45 (0.26)	786.0 (34.3)	++
30-Jul	27.2	21.1	4.01	8.9	2.69 (0.42)	0.78 (0.07)	2.57 (0.41)	917.9 (75.1)	++
18-Aug	32.4	17.1	14.33	8.5	2.67 (1.13)	0.34 (0.06)	21.16 (0.43)	193.4 (22.6)	++
1-Sept	26.9	12.7	9.57	9.0	2.76 (0.72)	3.06 (0.23)	3.23 (0.08)	718.8 (33.8)	7.01×10^6
16-Sept	28.5	20.5	16.28	8.5	4.64 (2.59)	0.23 (0.02)	2.75 (0.16)	471.3 (13.9)	2.67×10^6
1-Oct	23.3	16.8	4.48	7.8	4.73 (1.19)	1.64 (0.30)	23.13 (0.31)	33.8 (5.0)	−

Environmental parameters include temperature (*T*, °C), salinity (*S*, psu), dissolved oxygen (DO, mg L^{-1}), pH; mean ($n = 3$) (SD) nutrients (μM) as ammonium (NH_4^+), nitrite + nitrate ($NO_2^-+NO_3^-$), orthophosphate (PO_4^{3-}); phytoplankton biomass as mean ($n = 3$) (SD) chlorophyll *a* (chl *a*, μg L^{-1}), and when available, counts (*Anabaenopsis* sp., cells mL^{-1})

+, organism was present as part of a mixed phytoplankton assemblage; ++, organism was numerically dominant; −, indicating organism was absent; n.d., no data

analysis of variance (ANOVA; $\alpha = 0.05$) (Sokal and Rohlf 1995) followed by a Dunnett's post hoc test.

Results

Field Sampling and Analyses

During the study period, surface water temperatures of K093 ranged from 23.3 to 32.4 °C, and salinities ranged from 12.7 to 22.9 psu (Table 1). Dissolved oxygen levels exhibited wide fluctuations during the sampling period with a minimum of 1.65 mg L^{-1} (hypoxia) and a maximum of 16.28 mg L^{-1} (super-saturation). Surface pH was fairly alkaline, ranging from 7.8 to 9.1. Dissolved inorganic nitrogen primarily consisted of ammonium, with mean levels ranging from 1.36 to 4.73 μM (Table 1). By comparison, nitrite + nitrate was typically <1 μM, with mean concentrations ranging from 0.34 to 1.64 μM. Mean concentrations of dissolved inorganic phosphorus, as orthophosphate, were often 1–2 orders of magnitude greater than total dissolved inorganic nitrogen (ammonium, nitrite, and nitrate combined), with levels of orthophosphate ranging from 2.57 to 23.13 μM.

Mean phytoplankton biomass (chlorophyll *a*) was greatest between 21 and 30 July, reaching a maximum of 917.9 μg L^{-1} compared to a minimum of 33.9 μg L^{-1} on 1 October (Table 1). This exceptionally high biomass was coincident with a dense bloom of the cyanobacteria *Anabaenopsis* sp. and cell concentrations exceeding 3.49×10^7 cells mL^{-1}. Other phytoplankton observed over the study period included cyanobacteria (*Oscillatoria* sp.), diatoms (the genera *Navicula*, *Amphiprora*, and the species *Cylindrotheca closterium*), a raphidophyte (*Chattonella subsalsa*), and dinoflagellates (*Prorocentrum minimum*, *Heterocapsa rotunda*, and *Amphidinium* sp.). The dinoflagellate *Protoperidinium quinquecorne* was the only species besides *Anabaenopsis* sp. to reach bloom concentrations at ~200 cells mL^{-1} on 16 September.

Algaecide Exposure Experiments

Algaecides tested in this study controlled the cell concentration, but not overall toxin levels, of cyanobacteria. For cultured *M. aeruginosa*, Pak™ 27 was the least effective algaecide in reducing cell concentrations in all treatments by day 7. Specifically, cell concentrations in Captain* and control treatments were not significantly different (ANOVA, $P > 0.05$) from each other at 0.5×, but cell concentrations were slightly more than double in the 0.5× Pak™ 27 treatment (Fig. 2a). Cell concentrations in Captain* and K-Tea™ treatments were significantly (ANOVA, $P < 0.05$) less than both Pak™ 27 and control treatments after 7 d for 1× and 2× (Fig. 2b, c). Experiments using bloom cyanobacteria (*Anabaenopsis* sp.) produced trends similar to *M. aeruginosa*, with cell concentrations in both Captain* and K-Tea™ treatments being significantly (ANOVA, $P < 0.05$) less than cell concentrations in either Pak™ 27 or control treatments for all dose levels by day 7 (Fig. 3).

Evaluations of toxin levels focused on the *M. aeruginosa* culture because microcystin concentrations of bloom samples were <0.1 ppb and therefore not a public health concern. The initial range for total (particulate + dissolved) microcystin concentration at the start of the *M. aeruginosa* experiments was typically 11–30 ppb, a moderate toxin range. Accordingly, the ratio of particulate:dissolved

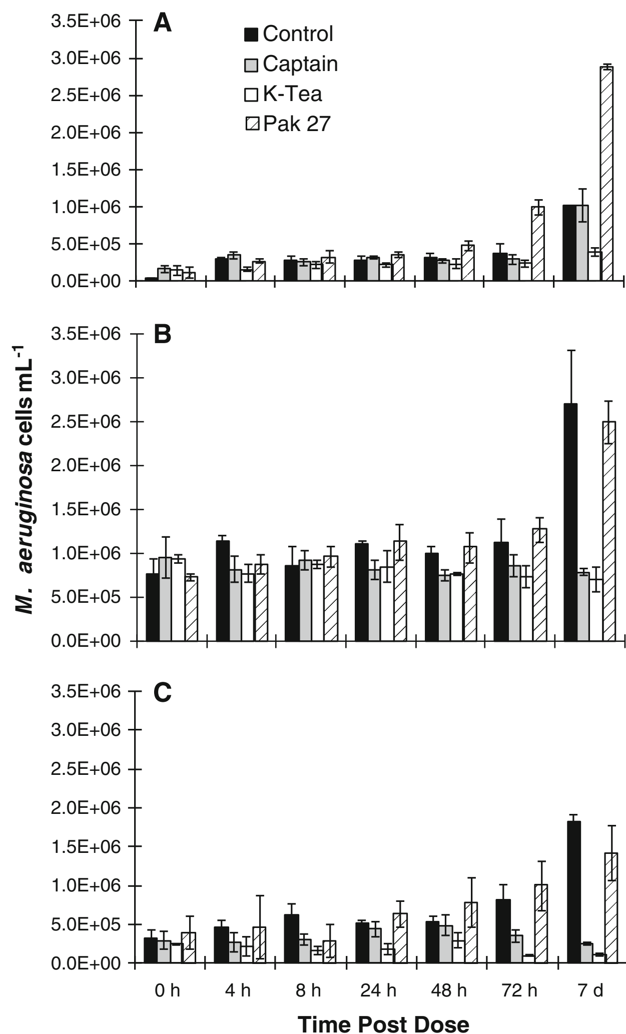

Fig. 2 Mean ($n = 3 \pm$ SD) cell concentrations of *Microcystis aeruginosa* exposed to commercially available algaecide mixtures at **a** half the manufacturer's recommended dose strength (0.5×), **b** at the manufacturer's recommended dose (1×), and **c** twice the manufacturer's recommended dose (2×). Control treatments received no algaecide

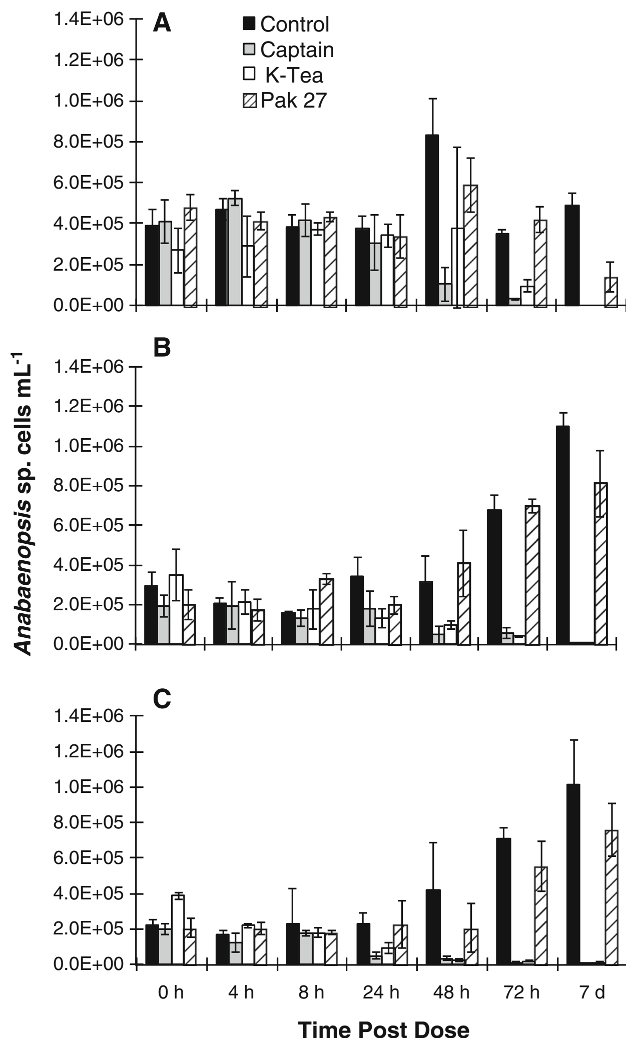

Fig. 3 Mean ($n = 3 \pm$ SD) cell concentrations of *Anabaenopsis* sp. exposed to commercially available algaecide mixtures at **a** half the manufacturer's recommended dose strength (0.5×), **b** at the manufacturer's recommended dose (1×), and **c** twice the manufacturer's recommended dose (2×). Control treatments received no algaecide

microcystin was typically >1, indicating that the majority of toxin was intracellular (Table 2). After 7 d, the ratio of particulate:dissolved microcystin shifted such that microcystin levels were comparatively higher in the dissolved phase, particularly for the 0.5× and 2× doses, although particulate microcystin remained comparable to or slightly greater than initial at 1×. Total microcystin levels generally increased over the duration of each experiment such that the final toxin content was often 2–10× greater than initial levels.

Discussion

Cyanobacteria are responsible for more HABs worldwide than any other phytoplankton taxa, and incidences of toxic blooms are expected to continue to increase (Hallegraeff 1993; Chorus and Bartram 1999; Paerl and Huisman 2009; Paerl and Paul 2012). This growing environmental and public health concern makes effective and environmentally safe management a critical need. While others have investigated the individual effects of either copper sulfate or hydrogen peroxide on cyanobacteria (García-Villada et al. 2004; Samuilov et al. 2004; Le Jeune et al. 2006; Barrington and Ghadouani 2008), only a few published studies have compared the two (Qian et al. 2010; Zhou et al. 2013; Fan et al. 2014), and of these none have evaluated cultured and field-collected algae. This study assessed the short-term effects of three treatment concentrations of commercially available copper sulfate and sodium percarbonate (an oxidizing agent for hydrogen peroxide production)-based algaecides on the growth and

Table 2 Ratios of particulate to dissolved microcystin (ppb) as determined from mean ($n = 3$) (SD) toxin levels from *Microcystis aeruginosa* exposed to short-term algaecide treatments at 0.5×, 1×, and 2× the recommended manufacturer's dose levels

Dose level	Treatment				
	Post dose	Control	Captain*	K-Tea™	Pak™ 27
0.5×	0 h	4.89 (4.63)	6.45 (3.47)	7.25 (3.80)	7.11 (7.90)
	4 h	2.29 (0.80)	3.57 (1.66)	1.21 (0.66)	2.40 (1.50)
	24 h	0.53 (0.30)	2.96 (1.40)	1.37 (0.80)	0.90 (0.30)
	7 d	0.72 (0.25)	4.39 (1.22)	0.11 (0.07)	0.98 (0.45)
1×	0 h	1.99 (0.81)	1.95 (0.48)	0.86 (0.22)	0.80 (0.59)
	4 h	0.86 (0.90)	1.12 (0.53)	4.68 (1.40)	1.37 (0.89)
	24 h	0.84 (1.04)	2.15 (2.97)	1.60 (2.25)	4.65 (5.89)
	7 d	3.70 (0.00)	2.07 (1.13)	1.05 (0.41)	1.36 (1.39)
2×	0 h	3.08 (0.49)	3.32 (1.26)	3.45 (0.20)	2.05 (0.67)
	4 h	2.10 (0.15)	3.33 (0.41)	2.53 (0.76)	2.61 (0.28)
	24 h	1.91 (1.05)	3.98 (3.43)	1.29 (0.29)	1.68 (0.20)
	7 d	0.38 (0.30)	0.55 (0.18)	0.32 (0.30)	1.08 (0.05)

toxin content of cyanobacteria using cultures and field samples. All three algaecides reduced cell numbers of cyanobacteria relative to control treatments within a 7 d period. However, the two copper sulfate-based products (Captain* and K-Tea™) generally resulted in a greater reduction of cell numbers relative to treatments containing the sodium percarbonate-based compound (Pak™ 27). Despite reductions in cell numbers, none of the treatments mitigated the overall toxin levels.

Field monitoring during the *Anabaenopsis* sp. bloom at Kiawah Island pond K093 revealed environmental conditions typical of those found in SC detention ponds during mid to late summer. Surface temperatures were very warm, and salinity ranges were mesohaline, similar to what has been reported in other studies of coastal detention ponds (Lewitus et al. 2004, 2008; Siegel et al. 2011). The large variability in dissolved oxygen levels observed during the sampling period was possibly related to initial stagnation of the water column followed by production of oxygen during photosynthesis as the bloom expanded. Stagnation is well-known to facilitate cyanobacteria blooms (Chorus and Bartram 1999; Huisman et al. 2005; Anderson et al. 2008; Paerl and Huisman 2009; Elliot 2010). It is unlikely that sampling time (and therefore oxygen balance attributed to diel cycling of photosynthesis and respiration rates) played a major role as all samples were collected between 10:30 am and noon. Although organic nutrients were not measured during this study, evidence suggests that inorganic nutrient availability likely contributed to bloom development and persistence. Concentrations of orthophosphate were high during much of the sampling period, and ratios of dissolved inorganic nitrogen (nitrate, nitrite, and ammonium combined) to dissolved inorganic phosphorus (as orthophosphate) ranged between 0.19 and 1.80. These values are substantially below the optimal nitrogen:phosphorus ratio of 16:1 for marine phytoplankton (Redfield et al. 1963), suggesting an enrichment of phosphorus. Numerous studies have linked phosphorus loading with cyanobacteria blooms in freshwater systems (Paerl 1988; Chorus and Bartram 1999; Heisler et al. 2008; Davis et al. 2009), but few have reported this association for brackish systems. Moreover, since members of the genus *Anabaenopsis* can fix nitrogen (convert atmospheric nitrogen to ammonia), they probably had a competitive advantage over non-nitrogen fixers given the low nitrogen to phosphorus ratios and were consequently able to proliferate.

The termination of the *Anabaenopsis* sp. bloom was likely attributed to several factors. During September, water temperatures began to drop, and they were considerably lower by 1 October compared to the bloom peak (July–August). In addition, a co-occurring bloom of the heterotrophic dinoflagellate *P. quinquecorne* (~200 cells mL^{-1}) was observed on 16 September, so it is likely that this species exerted top-down grazing control on cyanobacteria. The extent to which *P. quinquecorne* grazing reduced *Anabaenopsis* sp. concentrations relative to the effects of lower water temperatures are not known, however, Davis et al. (2012) showed microzooplankton can graze on cyanobacteria populations in freshwater systems, and cyanobacteria blooms have been shown to persist in Kiawah Island detention ponds during January (Brock 2006).

Both copper-containing algaecides (Captain* and K-Tea™) used in this study reduced numbers of *Anabaenopsis* sp. and *M. aeruginosa* more effectively than the sodium percarbonate-containing product (Pak™ 27). These observations agree with prior studies showing that high levels of copper reduce cyanobacteria division rates (Brand et al. 1986; Le Jeune et al. 2006). By the end of the 7 d test period, cells of *Anabaenopsis* sp. were almost entirely disrupted for all dose levels of Captain* and K-Tea™ experiments. By comparison, *M. aeruginosa* tended to be more resilient to algaecide treatment than *Anabaenopsis* sp., and dose did exert an effect. For example, in Captain* experiments, *M. aeruginosa* cell numbers were greater at 7 d relative to cells numbers at t0 (immediately following algaecide treatment) for the 0.5× treatment whereas cell numbers at 7 d were lower relative to t0 levels for both the 1× and 2× treatments. A similar trend was found for K-Tea™ treatments, but cell numbers were generally lower at the end of the 7 d experiment relative to Captain* treatments. It is uncertain why algaecides seemed to be more effective at reducing cell numbers of *Anabaenopsis* sp. than *M. aeruginosa*. Organic carbon

levels affect the sensitivity of *M. aeruginosa* to copper (Zeng et al. 2010), but in this study cyanobacteria were in filtered water making organic carbon unlikely to be a major factor. Laboratory studies have shown that copper resistant mutants can emerge for *M. aeruginosa* (García-Villada et al. 2004), so this may be a possibility. For Pak^{TM} 27, dose level had a minimal effect on cell numbers; although cell concentrations were typically less than the control by 7 d, this difference was not always statistically significant. The exception is *M. aeruginosa* 0.5×, where cell concentrations were higher in the Pak^{TM} 27 treatments than control treatments by 7 d. Results from this study differ from Barrington and Ghadouani (2008) who reported a dose-dependent response for hydrogen peroxide at reducing cyanobacteria densities, although their experiments lasted only 48 h. Importantly, since this study tested an algaecide product rather than hydrogen peroxide directly, findings reported here do not necessarily mean that hydrogen peroxide is an ineffective management option for reducing cyanobacteria cell numbers. Sodium percarbonate is the active compound used to generate hydrogen peroxide in Pak^{TM} 27, so one possibility is that this oxidizer or its associated catalyst may be less effective at generating sufficient levels of hydrogen peroxide than other options. Another possibility is that since our field experiment used a mixed phytoplankton assemblage, species that co-occurred with *Anabaenopsis* sp. may have broken down hydrogen peroxide thus rendering Pak^{TM} 27 less effective on cyanobacteria.

Despite the reduction of cyanobacteria cell numbers in algaecide treatments, microcystin was detected throughout all *M. aeruginosa* experiments. While the actual ratios varied among experiments, the ratio of particulate (intracellular) to dissolved (extracellular) microcystin generally decreased over time. This suggests that as cells lysed, toxin was released into the water, where it persisted. These findings support Barrington et al. (2013), who observed an increase in dissolved microcystin relative to intracellular microcystin within 5 d of hydrogen peroxide application to cyanobacteria assemblages. A tendency for control treatments to also have lower particulate to dissolved ratios after 7 d suggests that leaching of microcystin from intact (non-lysed) cells also occurred. The detection of microcystin for up to 7 d following treatment in a laboratory setting contrasts work by Jones and Orr (1994) who showed that microcystin was detected in a confined area within a lake for less than 24 h following treatment with a copper-containing algaecide. However, their study also reported high levels of microcystin for over 9 d following application in a separate region of the same lake. Mesocosm studies have shown that microcystin can persist in the dissolved and particulate phases for up to 30 and 15 d, respectively, following a bloom (Lahti et al. 1997), but degradation rates follow first order kinetics and may be mediated by light and water temperature (Kenefick et al. 1993; Lahti et al. 1997). Both the current study and Jones and Orr (1994) suggest that algaecide applications do not fully mitigate cyanobacteria bloom toxin levels. Although it was not entirely clear why, the exception to these findings was the 1× treatment, where particulate:dissolved microcystin was generally not statistically different from t0 by 7 d.

From a management perspective, copper-containing algaecides generally controlled cyanobacteria populations more effectively than the sodium percarbonate-based compound, but lingering microcystin in the water itself may still pose a threat to public health and safety for a week or potentially longer following either application. While nutrient reduction strategies are likely the best long-term management solution for diminishing the likelihood of cyanobacteria HABs to develop in the first place, should algaecides be used it is important to consider the advantages and disadvantages of each strategy. Copper-based algaecides, such as Captain* and K-Tea^{TM}, have practical advantages over sodium percarbonate-based compounds, such as Pak^{TM} 27, because they are both effective at reducing cyanobacteria cell numbers and cost-efficient. However, numerous studies have shown that copper and copper-based applications are highly toxic to other aquatic taxa and have been linked to food web disruption (Wilson and Taylor 1993; de Oliveira et al. 2004; Grosell et al. 2007; Jančula and Maršálek 2011; Korosi and Smol 2012; Al-Bairuti et al. 2013). Hydrogen peroxide has also been shown to be toxic to certain daphnids (McHuron et al. 2008; Jančula and Maršálek 2011) as well as fish, although the level of sensitivity varies widely among fish species (Gaikowski et al. 1999; Jančula and Maršálek 2011). While hydrogen peroxide poses fewer detrimental consequences to aquatic food webs, it presents a safety hazard if handled improperly. Thus, managers should consider not only cost, but also the ecological consequences of various cyanobacteria bloom prevention and control approaches when determining a strategy for a particular waterbody.

In conclusion, the three algaecides assessed here, Captain*, K-Tea^{TM}, and Pak^{TM} 27, all reduced the numbers of cyanobacteria cells, with the two copper-based compounds (Captain* and K-Tea^{TM}) being generally more effective. However, none of the algaecides reduced overall microcystin levels, and a greater partitioning of toxin from the particulate to dissolved phase occurred over time. Field studies provided further insight as to the potential role of nutrients, notably inorganic phosphorus and nitrogen, for facilitating cyanobacteria blooms in brackish environments, as well as the potential role of grazing for mitigating blooms. Given the increased frequency of cyanobacteria blooms worldwide, combined with the close association of blooms with human activity, determining environmentally

safe management strategies that not only mitigate blooms visibly, but also their toxin content, will be crucial for protecting public health and safety.

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