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Field Demonstration of a Peroxide-Based Algaecide for Harmful Algal Bloom Control in Lake Okeechobee

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Environmental Laboratory

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Abstract

Large-scale cyanobacterial harmful algal blooms (cHABs) in Lake Okeechobee, Florida, and connected waterways routinely impair water resources. This study conducted a field demonstration of a peroxide-based algaecide in 2020 in the Pahokee Marina on Lake Okeechobee to evaluate the algaecide's suitability for near-future operational implementation. Within minutes of treatment, rapid oxidation of cHAB cells occurred in the form of bleaching and cell lysis. On average, levels in the treatment area decreased by 4 hours after treatment (HAT) and remained low out to 24 HAT: chlorophyll decreased 87%, phycocyanin decreased 85%, total microcystin levels decreased from 50 μg L^{-1} to 4 μg L^{-1} at 4 HAT and then increased to 11 μg L^{-1} by 24 HAT, hydrogen peroxide concentrations averaged 6.1 mg L^{-1} 0.5 HAT and then dropped below detection limits by 24 HAT, and *Microcystis* spp. cell densities decreased at 4 HAT in all but four sampling sites. However, inflows of cHAB-infested lake water in some portions of the treatment area resulted in lack of control at these sites. Because of their vulnerability to influxes of cHABs from surrounding nontreated waters via water-exchange processes driven by wind-induced surface currents, future applications must therefore consider treatment area size.

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Preface

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COL Christian Patterson was commander of ERDC, and the director was Dr. David W. Pittman.

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1 Introduction

1.1 Background

In recent years, the Lake Okeechobee Waterway (LOW) has received significant attention regarding chronic and widespread cyanobacterial harmful algal blooms (cHABS). Although cHABS on the LOW have occurred for decades, the intensity of bloom events in recent years has prompted states of emergency* in many surrounding counties (Havens, Hanlon, and James 1994; Kramer et al. 2018). Furthermore, bloom intensity, duration, and associated toxicity will likely increase with alterations in environmental conditions (for example, temperature, CO_2, eutrophication)† and water-use dynamics (for example, dynamic rainfall, residence time) (Davis et al. 2009; Gilbert, Dziallas, and Grossart 2011; Paerl and Huisman 2008). cHABs have impaired designated water-resource uses, including wildlife and fish habitat, recreation, tourism, residential and commercial developments, and property values (Alvarez et al. 2019; Dodds et al. 2009; Wolf and Klaiber 2017). Environmental impacts from cHABs can include hypoxic zones, depressed fish health, production and release of toxins, and impaired food-web integrity (Lampert 1982; Liu et al. 2014; Paerl 1988; Zhao et al. 2012). Consequently, increased public awareness of and concerns over cHABs support investigation into potential large-scale mitigation measures to preserve water-resource uses (Anderson, Cembella, and Hallegraeff 2012).

Blooms in the LOW can consist of many different cyanobacterial species and strains that cycle throughout different times of the year (Rosen et al. 2017). As a result, multiple types of toxins have been documented from cHABs, including anatoxin, cylindrospermopsin, microcystin, nodularin, and BMAA (β-N-methylamino-l-alanine), which can directly affect the health of humans and wildlife associated with or exposed to the water resource (Paerl and Huisman 2008). Additionally, numerous exposure routes for these toxins have been documented, including drinking water,

* Fla. Exec. Order No. 18-191 (July 9, 2018). https://www.flgov.com/wp-content/uploads/2018/07/EO-18-191.pdf.

† For a full list of the spelled-out forms of the chemical elements used in this document, please refer to *US Government Publishing Office Style Manual*, 31st ed. (Washington, DC: US Government Publishing Office, 2016), 265, https://www.govinfo.gov/content/pkg/GPO-STYLEMANUAL-2016/pdf/GPO-STYLEMANUAL-2016.pdf.

aerosolization, food-chain accumulation, and recreation (Brand et al. 2010; Falconer 1999; Fleming et al. 2002; May et al. 2018; Stommel, Field, and Caller 2013). Therefore, multiple regulatory agencies have suggested human health guidelines for some of these toxins, as exposure has been linked to illnesses such as liver cancer, Parkinson's disease, and ALS (amyotrophic lateral sclerosis) (Banack et al. 2015; Bell and Codd 1994; Ueno et al. 1996; EPA 2015; Chorus and Bartram 1999).

Acknowledging the health risks associated with cHABs has supported numerous prevention and control strategies. Significant attention has been placed specifically on nutrient-mitigation measures; however, nonpoint sources of nutrients such as storm water runoff, septic intrusion, watershed accumulations, and atmospheric deposition can be difficult to document and curtail (Badruzzaman et al. 2012; DeBusk, Hunt, and Sydorovych 2010; Dunne et al. 2011; Nürnberg and LaZerte 2016; Paerl 1997; Wetzel 2001). Likewise, nutrient accumulation in a watershed is an ongoing fuel source for cHABs (Jarvie et al. 2013; Schindler 2012; Søndergaard, Jensen, and Jeppesen 2001). In the LOW watershed in particular, introductions of 500 t* of phosphorus per year have been reported and are expected to continue for decades (Reddy et al. 2011). Furthermore, the LOW is shallow and lacks a thermocline, which results in water-column mixing, nutrient resuspension from sediments, and cycling of legacy phosphorus, and continuous introduction and accumulation of nutrients in LOW is expected to support cHABs for the foreseeable future (Missimer, Thomas, and Rosen 2021). Though proactive strategies like nutrient management are critical, economics and the scale and scope of operational feasibility may limit the effectiveness in this system and may take decades to effectually change bloom dynamics in situ.

Direct control tools for cHABs such as algaecides can be an important component of an integrated management response. Compared to other known mitigation strategies, algaecides can provide immediate short-term control of cHABs, thus providing a rapid-response solution to restore water uses, protect public health, and minimize negative impacts to ecological processes. Likewise, algaecides could decrease the spread of

* For a full list of the spelled-out forms of the units of measure used in this document and their conversions, please refer to *US Government Publishing Office Style Manual*, 31st ed. (Washington, DC: US Government Publishing Office, 2016), 248–52 and 345–47, https://www.govinfo.gov/content/pkg/GPO-STYLEMANUAL-2016/pdf/GPO-STYLEMANUAL-2016.pdf.

cHABs and associated toxins. Copper-based algaecides (copper sulfate and chelated copper products) as well as the amine salt of endothall can be effective for cHAB control in certain situations; however, repeated use of these chemistries has raised concerns about impacts to nontarget organisms (Bishop, Johnson, and Rodgers 2014; Lembi 2014). Because of these concerns, the state of Florida does not permit the use of copper-based aquatic algaecides on public bodies of water. As a result, peroxide-based algaecides with documented cHAB efficacy have received interest as an alternative management tool (Lembi 2014; Matthijs et al. 2012; Netherland 2014; Crafton et al. 2019). Peroxide-based algaecides pose a negligible nontarget species toxicity threat; can be selective against cHABs, restoring beneficial phytoplankton populations; require short-exposure duration for control; and rapidly dissipate with no water-use restrictions (for example, drinking, recreation, irrigation, livestock watering) (Barrington, Reichwaldt, and Ghadouani 2013; Bauza et al. 2014; Drábková, Admiraal, and Maršálek 2006; Drábková et al. 2007; Lusty and Gobler 2020; Reichwaldt et al. 2012; SePRO 2018; Weenink et al. 2015).

Early-detection and rapid-response strategies, such as algaecides, are critical for the management of cHABs in the LOW, because cHABS can be highly mobile, intermittent over the course of the growing season, and can rapidly increase in density. Furthermore, algaecides approved for use to control cHABs on the LOW must adhere to stringent environmental regulations because of multiple water uses and potential interface with sensitive species such as the Florida manatee (*Trichechus manatus latirostris*) and the Everglades snail kite (*Rostrhamus sociabilis*). Although peroxide-based algaecide treatments have been effective at controlling cHABs in small <10 ac (<4 ha) enclosed or partial systems (Bishop and Rodgers 2011; Geer et al. 2017; Pokrzywinski et al. 2022; Sinha, Eggleton, and Lochmann 2018), the operational use of these products in large, hydrologically complex systems such as the LOW is still in the formative stages of field development and implementation. Therefore, this work evaluated the performance of an environmentally compatible peroxide-based algaecide to control cyanobacterial blooms in the LOW with respect to (a) effectiveness of bloom control, (b) documentation of aqueous toxin levels before and after treatment, and (c) guidance on best-use practices for how and where to deploy peroxide-based algaecides—particularly as a rapid-response tool.

1.2 Objective

This work evaluated the performance of an operational-scale, peroxide-based algaecide treatment to control cHABs in the LOW.

Specific objectives included the following:

1. Evaluate the effectiveness of PAK 27 (SePRO, Carmel, Indiana), a peroxide-based algaecide to control cHABs.
2. Monitor for changes to aqueous toxin levels pre- and posttreatment.
3. Provide guidance and best management practices for operational applications of PAK 27 for cHAB control.

1.3 Approach

To provide guidance on direct cHAB control, a field demonstration evaluated an operational-scale peroxide-based algaecide application at the Pahokee Marina in Lake Okeechobee, Florida.

2 Materials and Methods

2.1 Site description and monitoring

In 2020, several potential study sites across the lake were continually monitored via lock-keeper reports, satellite imagery, and cellular cameras. The city of Pahokee marina (N 26°49′34.579″, W 80°39′57.142″) was selected as the demonstration site. The Pahokee Marina is a 3.5 ha marina with an average depth of 2.4 m, located along the southeastern shoreline of Lake Okeechobee. It is owned and operated by the city of Pahokee, Florida, and cHABs have been documented to commonly occur within and around the marina. Furthermore, it was easily accessible, of sufficient size to represent an operational-scale treatment, and is protected on three sides to limit water exchange from wind and water currents, making it an ideal demonstration site. The marina is almost completely surrounded by a concrete jetty, with the exception of an approximately 35 m boat passage and two small gaps in the in the jetty that allow water to flow through (Figure 1). The marina also supports a boat ramp, multiple piers, and boat slips. In early July, a large, sustained bloom that mirrored bloom conditions observed on the eastern portions of the main lake was detected in the marina, which instigated rapid-response planning for the treatment demonstration.

2.2 Pretreatment preparation, sampling design, and data collection

Team members arrived at the Pahokee marina the afternoon of 13 July 2020 to confirm cellular-camera and satellite-imagery findings that indicated cHAB conditions. On-site observations at Pahokee Marina confirmed cHAB conditions existed in the marina, warranting treatment the following day (Figure 2). At this time, four limnocorrals made from 6 mm polyethylene tubing with a 61 cm diameter and 1.7 m height (total volume approximately 1.34 m^3) were deployed (Figure 3; Pokrzywinski et al. 2022). Tubing was sealed at the bottom and held approximately 10 cm above the water surface with foam-filled corrugated pipe to provide a closed system similar to Tucci and MSE Technology Applications (2007)* (Figure 3).

* Nicholas J. Tucci and MSE Technology Applications, "Mine Waste Technology Program Activity IV, Project 30 Final Report—Algal Bioremediation of the Berkeley Pit Lake System: An In Situ Test Using Limnocorrals," MWTP-275, US Department of Energy Contract No. DE-AC09-96EW96405 (Cincinnati, OH: Environmental Protection Agency–National Risk Management Research Laboratory, 2007).

Additionally, three PVC pipe frames 1.3 cm^2 were attached to limnocorrals to act as support ribs and prevent limnocorral collapse. Limnocorrals were deployed inside the marina along the northeast wall (Figure 1) to capture nontreated algae growth within the treatment area. Limnocorrals were tied to dock moorings, and a 16 kg anchor was attached to hold limnocorrals in place during the study. After deployment, limnocorrals were filled with surrounding water using a submersible pump. These mesocosms were not treated with algaecide and served as references. Pretreatment water samples were then collected from each limnocorral: 3 sites outside the marina and 22 sites inside the marina using the sampling design in Figure 1. There were 18 sample sites within the treated area (inside the marina); however, sample sites were grouped in pairs and mixed prior to analysis, as shown by the *white ovals* in Figure 1.

Figure 1. Map of sample sites at the Pahokee Marina, Florida, demonstration site in July 2020. *Blue points* are open-water sample points, and *gold ovals* are nontreated reference mesocosms. *Large white ovals* designate samples that were bulked for analysis representing one data point.

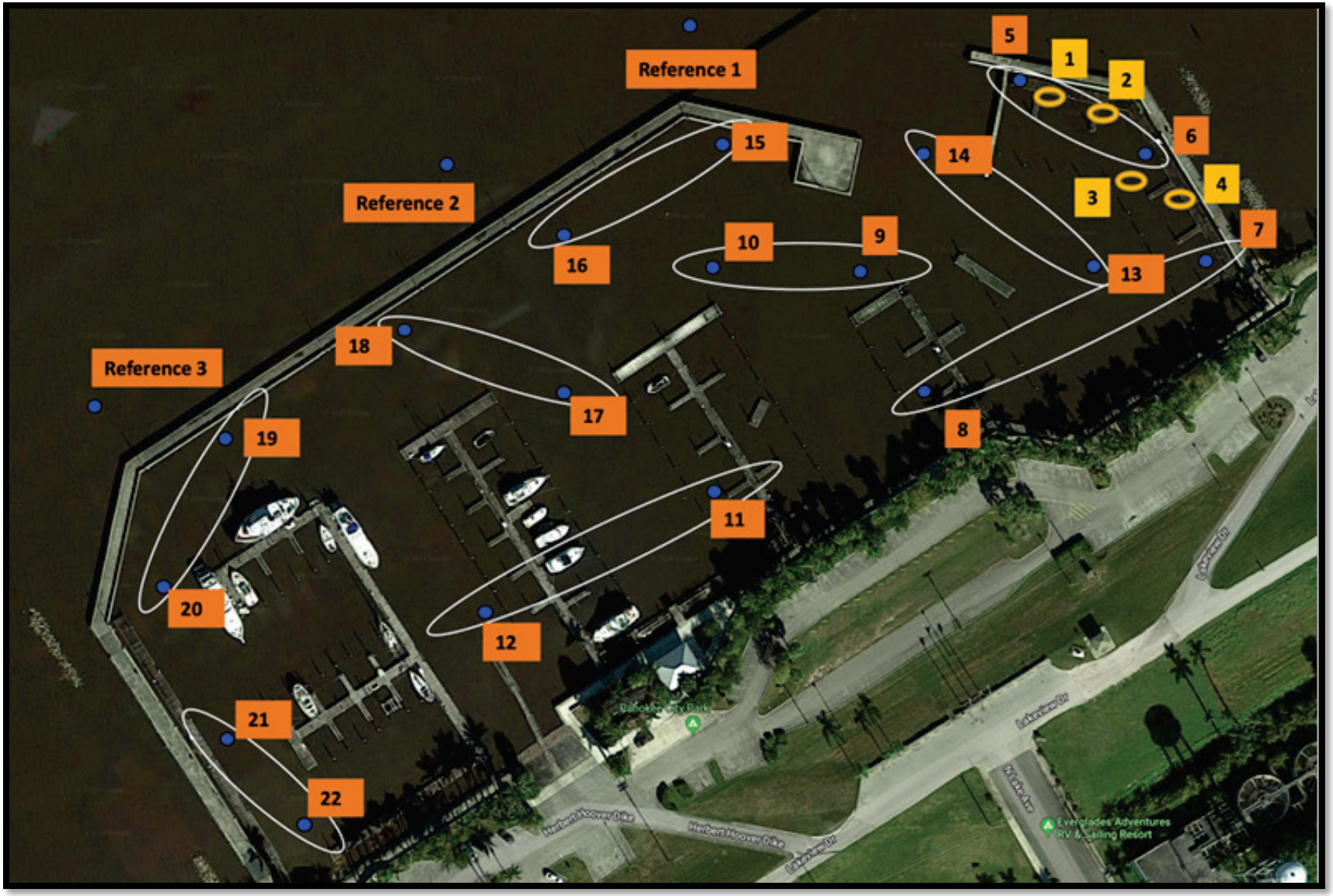

Figure 2. Pretreatment bloom conditions at Pahokee Marina, Florida, 13 July 2020.

Figure 3. Nontreated reference limnocorral design and deployment in Pahokee Marina, Florida, 13 July 2020.

2.3 Algaecide treatment and posttreatment data collection

On 14 July 2020, a peroxide-based algaecide (PAK 27 Algaecide; EPA Reg. No. 68660-9-67690, 2013; SePRO Corporation, Carmel, IN) was applied at Pahokee Marina via two airboats (application start: 0700, end: 0840) equipped with centrifugal spreaders (Figure 4). Algaecide was applied to the marina at the required rate to achieve a hydrogen peroxide concentration of 10.2 mg L^{-1} (maximum label rate; 365 kg of product per hectare-meter). Weather conditions in the marina during treatment were clear (0% cloud cover), sunny, and calm (winds <1.6 kph, minimal fetch). All reference mesocosms were covered with polyethylene tarps during treatment to ensure no algaecide entered the water and then removed immediately after product application.

Water samples were collected using an integrated water sampler made from PVC pipe (3.8 cm diameter) fitted with a rubber stopper to a 1 m depth (Pokrzywinski et al. 2022). Water samples were taken at each sampling point at pretreatment, 4, and 24 hours after treatment (HAT). As previously mentioned, samples were bulked in pairs of adjacent samples sites, resulting in 9 total data points for the treatment area. Simultaneously, readings of optical dissolved oxygen (DO) and pH were recorded with a handheld multiparameter meter (YSI, Yellow Springs, Ohio) at a 0.5 m depth. Once collected, water samples were immediately placed into coolers on ice (0°C) until they were shipped overnight to the phycology laboratory at the US Army Engineer Research and Development Center (ERDC) for chlorophyll, phycocyanin, free microcystin, and total microcystin analyses. Likewise, an aliquot sample was preserved with formaldehyde and sent to the University of Florida phycology laboratory in Fort Lauderdale, Florida, for species identification and quantification.

Hydrogen peroxide (H_2O_2) concentrations were also quantified from water samples at pretreatment, 0.5, 4, and 24 HAT using the triiodide (I_3) method, which has been used to quantify hydrogen peroxide under field conditions (Geer et al. 2017; Kinley et al. 2015; Klassen, Marchington, and McGowan 1994; Pokrzywinski et al. 2022). A standard curve was prepared with 30% hydrogen peroxide (Fisher Scientific, Waltham, Massachusetts) at a detection limit of 0.3 mg L^{-1} using a spectrometer (Pasco PS-2600, Pasco Scientific, Roseville, California) following acidification reactions. Analyses were conducted on-site within 1 h of sample collection.

Figure 4. Algaecide treatment application via airboat at Pahokee Marina, Florida, 14 July 2020.

2.4 Laboratory processing and data analysis

Extracted chlorophyl was quantified using Environmental Protection Agency (EPA) method 446 in 90% acetone (Arar 1997). Modifications included homogenization of filters by bead beating using 0.1 mm silica beads at 4.5 $m \cdot s^{-1}$ (~500 rpm) for 1 min using a Fast Prep 24 Homogenizer (MP Biomedicals, Santa Ana, California). A total volume of 6 mL was used for pigment extractions. Absorbance was measured via UV-Vis (ultraviolet-visible) 1800 spectrophotometer (Shimadzu, Kyoto, Japan) at 750 nm (turbidity) and 664 nm (chlorophyll a, Chl a) determination as reported in EPA method 446 (Arar 1997).

Phycocyanin was quantified after extraction using methods described by Yéprémian et al. (2016). Once samples were filtered, filters were added to lysing matrix in B tubers (MP Biomedicals, Santa Ana, California) with saline buffer solution. Samples were then homogenized using a Fast Prep 24 homogenizer at 4.5 m s^{-1} (approximately 500 rpm) for 1 min. Following homogenization, tubes were left to steep in the dark for 16 to 24 h at 4°C. During the steeping process, tubes were inverted one to two times to ensure proper extraction. At the end of the steeping period, samples were spun in a centrifuge, and supernatant was analyzed for phycocyanin levels using a UV-Vis 1800 spectrophotometer (Shimadzu, Kyoto, Japan) at 750, 620, and 650 nm.

Total and free microcystin were quantified using enzyme-linked immunosorbent assay (ELISA) according to manufacturer's instructions (Eurofins Abraxis, Waminster, Pennsylvania) with raw sample water and homogenization, respectively. Homogenization was conducted via bead beating (0.1 mm silica beads) at 4.0 $m \cdot s^{-1}$ for 1 min using a Fast Prep 24 Homogenizer. Aliquots from homogenate were then used for ELISA under EPA method 546 with a limit of detection of 0.01 μg L^{-1} (Zaffiro, Rosenblum, and Wendelken 2016).

Identification and quantification of *Microcystis* spp. were conducted at the University of Florida phycology laboratory in Fort Lauderdale, Florida. Samples for taxonomic identification and quantification were preserved with 4% formaldehyde until arriving at the laboratory. Cells were sedimented in 20 mL Utermöhl chambers using Lugol's iodine solution and classified using methods described by Guiry and Guiry (2019) (Utermöhl 1958). Cells were counted 24 h after sedimentation on the basis

of 20 field of views using an inverted microscope (Olympus CKX41, Olympus Corporation, Shinjuku City, Japan). Dilution factors, field of views, and chamber height were then used to calculate cell concentrations.

Data were analyzed using R software version 3.6.1 (The R Foundation 2019) to calculate means and descriptive statistics. Because of changes in water-exchange processes observed in the field shortly after treatment and in preliminary data interpretation, data from spatially similar sample sites were grouped into three categories: mesocosm reference, treated (all sites inside the marina), and exterior reference sites (Figure 1). Sites 5 and 6, 7 and 8, and 21 and 22 were broken out of the data set for 4 and 24 HAT data because of distinct differences from nontreated water inflows in response variables at these sites. Therefore, pretreatment data in the treated area reflects all sample points inside the marina; however, at 4 and 24 HAT, the broken-out sample sites were excluded from the main treated area mean.

3 Results and Discussion

3.1 Visual observations

Immediately after treatment, oxidation was visible at the water surface and continued through early afternoon on 14 July 2020. This oxidation was visible in the form of bubble production, giving the water an effervescent appearance (Figure 5). Additionally, dense colonies of algae began to show symptoms of bleaching as a result of the oxidation (Figure 6). In nontreated mesocosms, algae remained unaffected by treatment, indicating the water was not exchanged between the interior of the mesocosm and surrounding treated water (Figure 6).

At 3 HAT, winds out of the northwest increased, and large cHAB bands suspended in the water in the main lake began approaching the marina (Figure 7). Consequently, cyanobacteria biomass became very dense along the exterior marina jetties with some infiltration into the treated marina through the main entrance and two flow throughs in the jetty wall (Figure 7). Despite the observed influx of nontreated, cHAB-infested lake water, cHAB control was persistent in the majority of the marina for several hours (Figure 8). Treatment to the internal areas of the marina, where dock slips were located, visually suggested that excellent cHAB control was sustained for 4 to 8 HAT, until new cHAB-infested waters entered the marina (Figure 9).

Figure 5. Algaecide activity observed shortly after application at Pahokee Marina, Florida, 14 July 2020.

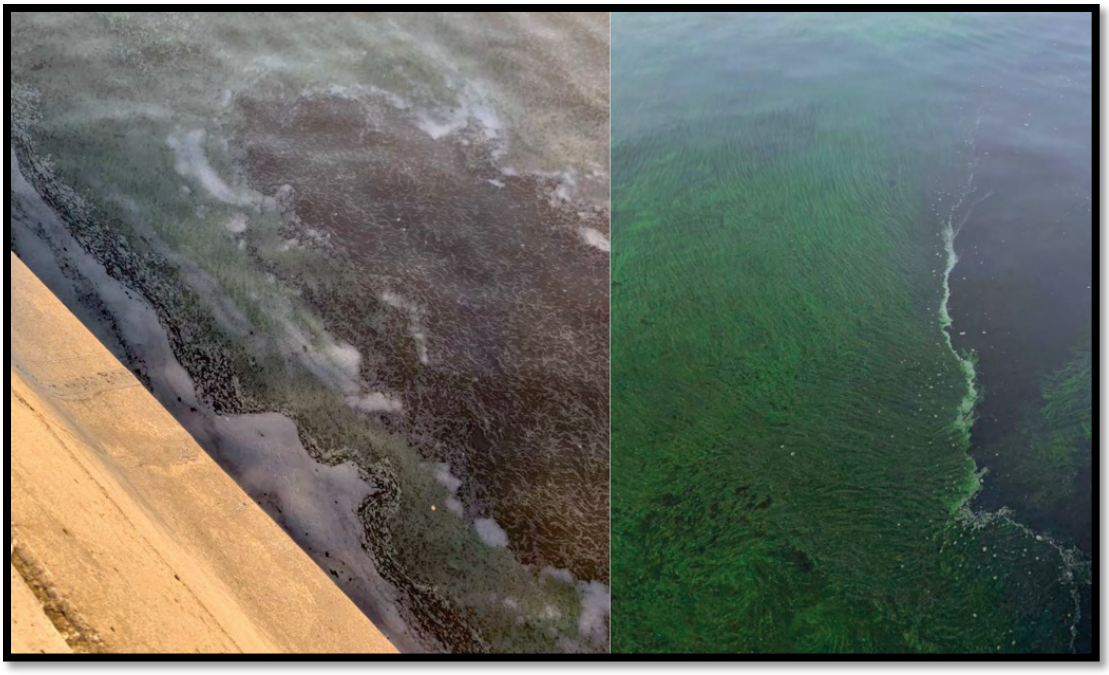

Figure 6. Algaecide activity around an unaffected reference mesocosm at Pahokee Marina, Florida, 14 July 2020.

Figure 7. Cyanobacterial harmful algal bloom (cHAB) conditions outside of Pahokee Marina, Florida, 4 hours after treatment (HAT) on 14 July 2020.

Figure 8. Bloom conditions inside and outside of Pahokee Marina, Florida, 5 HAT on 14 July 2020.

Figure 9. Time series of dock slips before, during, and after a peroxide-based algaecide treatment for cHAB control in a field demonstration on Lake Okeechobee, Florida, in July 2020.

3.2 Water quality and hydrogen peroxide monitoring

Average water pH prior to treatment in the marina was 8.2 and was slightly higher in mesocosms and exterior reference sites (8.4 and 8.5, respectively) (Table 1). At 4 HAT water pH in the treated area remained at 8.2; however, at sites 7 and 8, where cHAB density was high because of accumulation against the jetty water, pH was 7.4. By 24 HAT, water pH in all treated areas was approximately 8.6 and slightly more alkaline (8.8) at exterior reference sites.

DO was not measured prior to treatment because of delays in shipping water meters to the study site. However, at 4 HAT DO in treated areas ranged from 5.7 to 6.2 mg L^{-1}, whereas DO at exterior reference sites averaged 13.6 mg L^{-1} (Table 1). Unfortunately, because of limitations and artifacts of this demonstration study's design, these data do not indicate the cause of the lower DO levels at 4 HAT inside the marina compared to exterior reference sites. The slightly reduced DO and pH levels inside the marina shortly after treatment indicate signs of cHAB control, likely from cHAB lysis and increased free carbon dioxide levels compared to the productivity observed outside of the marina. Regardless, even the minimum DO levels recorded in the marina were above levels of concern to nontarget organisms. By 24 HAT DO in treated areas averaged around 9.2 mg L^{-1} and was slightly greater in exterior reference sites (10.5 mg L^{-1}).

Hydrogen peroxide levels detected 0.5 HAT were below the target concentration of 10 mg L^{-1}, indicating rapid degradation to byproducts, including oxygen and water (Table 2). Average hydrogen peroxide in the treated area was 6.1 mg L^{-1} 0.5 HAT and was slightly lower (4.6 mg L^{-1}) at sites 7 and 8. As expected, by 4 HAT hydrogen peroxide levels continued to decrease at most sites but remained above 2.4 mg L^{-1} until 24 HAT, at which time concentrations were below detection limits.

Table 1. Water pH and dissolved oxygen before and after a peroxide-based algaecide treatment for cHAB control in a field demonstration on Lake Okeechobee, Florida, in 2020.

Site[a]	pH			Dissolved oxygen (mg L^{-1})		
	Pretreatment	4 HAT[b]	24 HAT	Pretreatment	4 HAT	24 HAT
Mesocosm reference	8.4 (0.1)[c]	8.5 (0.1)	8.2 (0.04)	13.8 (0.8)	6.8 (0.5)	5.7 (0.1)
Treated	8.2 (0.1)	8.2 (0.1)	8.6 (0.02)	9.2 (0.7)	6.2 (0.5)	9.6 (0.2)
5 and 6	n/a[d]	8.4	8.6	n/a	5.7	9.2
7 and 8	n/a	7.4	8.6	n/a	5.7	9.2
21 and 22	n/a	8.3	8.5	n/a	6.0	8.8
Exterior reference	8.5 (0.1)	8.9 (0.04)	8.8 (0.02)	7.2 (0.5)	13.6 (0.2)	10.5 (0.2)

[a] Mesocosm reference (*n* = 4), treated (*n* = 9), exterior reference (*n* = 3). See Figure 1 for sampling sites map.

[b] HAT—hours after treatment

[c] Standard error of the mean is shown in parentheses where appropriate.

[d] Data from these sites are contained in the treated site, comprising all sites. These sites are broken out of the treated mean for posttreatment data because of distinct water-exchange influences.

Table 2. Hydrogen peroxide concentrations before and after a peroxide-based algaecide treatment for cHAB control in a field demonstration on Lake Okeechobee, Florida, in July 2020.

Site[a]	Hydrogen peroxide (mg L^{-1})			
	Pretreatment	0.5 HAT[b]	4 HAT	24 HAT
Mesocosm reference	*[c]	*	*	*
Treated	*	6.1 (0.8)[d]	3.6 (0.6)	*
5 and 6	*	7.7	4.2	*
7 and 8	*	4.6	5.0	*
21 and 22	*	7.2	2.4	*
Exterior reference	*	*	*	*

[a] Mesocosm reference (*n* = 4), treated (*n* = 9), exterior reference (*n* = 3). See Figure 1 for sampling map.

[b] HAT—hours after treatment

[c] Asterisk (*) signifies hydrogen peroxide concentration <1 mg L^{-1} (limit of quantification).

[d] Standard error of the mean is shown in parentheses where appropriate.

3.3 Chlorophyll, phycocyanin, and microcystin levels

Pretreatment chlorophyll levels were greatest in mesocosms (967 μg L^{-1}), followed by the treatment area (377 μg L^{-1}) and exterior reference sites (65 μg L^{-1}) (Table 3). Prior to treatment, cHAB in the marina was dense in corners and areas protected from currents (Figure 2). Consequently, this protection is reflected in the chlorophyll data in mesocosms, since they were located and filled with surrounding water in the northeast corner of the marina (Figure 1). Furthermore, pretreatment chlorophyll levels in the marina were likely greater than the exterior reference sites because of wind direction and the sequestering of cHAB in protected areas of the marina. At 4 HAT, chlorophyll levels were reduced 87% and 67% from pretreatment levels in the treated area and sites 5 and 6, respectively. However, chlorophyll levels at sites 7 and 8 increased to 1,106 μg L^{-1} at 4 HAT, which reflected the intrusion of nontreated, cHAB-infested water that accumulated along the jetty from the main lake. Decreased chlorophyll levels were detected in reference mesocosms 4 HAT compared to pretreatment levels; however, exterior reference sites exhibited an increase to 645 μg L^{-1}, reflecting the wind shift and visual accumulation of cHAB against the jetty. By 24 HAT, chlorophyll levels in mesocosms and exterior reference sites rose to 5,381 and 1,574 μg L^{-1}, respectively. Sites 5, 6, 7, and 8, which were located within the treated area but were subject to inflows of nontreated water, documented similar chlorophyll levels of 3,800 μg L^{-1}. In contrast, chlorophyll levels in the protected treated area remained low, at 171 μg L^{-1}.

Phycocyanin levels closely resembled chlorophyl data trends (Table 3). Pretreatment phycocyanin levels were greatest in mesocosms, followed by the treatment area and the exterior reference sites. At 4 HAT, phycocyanin levels in mesocosms slightly decreased to 191 μg L^{-1}; however, in the main treated area, phycocyanin was reduced 29 μg L^{-1}. Sites 21 and 22 showed an increase in phycocyanin levels at 4 HAT, which was likely due to inflow from an adjacent jetty break. Likewise, phycocyanin levels at exterior reference sites increased to 233 μg L^{-1}, reflecting the shift in wind direction and water currents, accumulating cHAB at these sites. Similar to the chlorophyll concentrations, phycocyanin levels dramatically increased by 24 HAT in the reference mesocosms and exterior reference sites, whereas levels remained low (38 μg L^{-1}) in the main treated area. Kinley-Baird et al. (2021) reported no reductions in Chl a or phycocyanin

concentrations after treatment with the peroxide-based algaecide tested in the current study.

Prior to treatment, free microcystin levels inside and outside the marina averaged 2.8 μg L^{-1} and 1.5 μg L^{-1}, respectively (Table 4). By 4 HAT, free microcystin levels increased 1.7 to 9.9-fold at sites 5 and 6, 7 and 8, and 21 and 22. In contrast, free microcystin levels in the whole treatment area, reference mesocosms, and external reference sites were similar at 4 HAT to pretreatment levels. By 24 HAT, free microcystin at all sampling sites increased. Compared to pretreatment levels, mesocosm reference and exterior reference sites experienced 40% and 1.27% increases in free microcystin at 24 HAT, respectively. However, extreme increases in free microcystin at 24 HAT were reported at sites 5 and 6 and 7 and 8, which both rose to 38 μg L^{-1}. This extreme increase in free microcystin levels at these particular sites was likely caused by wind-driven surface currents transporting new cHAB from the main lake, ultimately resulting in a dense accumulation of cHABs in the eastern corner of the marina.

Total microcystin data trended oppositely from free microcystin data (Table 4). Total microcystin levels at the exterior reference sites increased over time, ultimately reaching 573 μg L^{-1} at 24 HAT. Likewise, reference mesocosms exhibited an increase in average total microcystin of 157 μg L^{-1} at pretreatment to 1,064 μg L^{-1} by 24 HAT. In the main treatment area, total microcystin decreased from 50 μg L^{-1} at pretreatment to 4 μg L^{-1} at 4 HAT and sustained low levels by 24 HAT (11 μg L^{-1}). Treated sites 5 and 6 were reduced to 17 μg L^{-1} total microcystin at 4 HAT; however, total microcystins in these sites drastically increased to 889 μg L^{-1} by 24 HAT because of accumulated nontreated water. Similarly, total microcystin at treated sites 7 and 8 increased from pretreatment levels at 4 HAT and then increased further by 24 HAT because of inflows. Interestingly, sites 21 and 22 saw slight increases in total microcystin by 4 HAT but then reduced back down below pretreatment levels by 24 HAT. This result may have been due to shifts in water movement, where 24 HAT values reflected the main treatment area.

Release of some intracellular toxins to the extracellular or dissolved fraction following hydrogen peroxide exposure is common and has been documented in prior works, though not observed in the 4 HAT analyses in this demonstration (Barrington, Reichwaldt, and Ghadouani 2013; Greenfield et al. 2014; Lürling, Meng, and Faassen 2014; Matthijs et al.

2012). However, concomitant decreases in total microcystin have also been shown posttreatment, likely because of control of the toxin source, rapid dilution, biodegradation, and dissipation (Barrington, Reichwaldt, and Ghadouani 2013; Sinha, Eggleton, and Lochmann 2018). Under specific conditions with catalysts (for example, ultraviolet light), hydrogen peroxide can also directly oxidize microcystin (Cornish, Lawton, and Roberston 2000; Bandala et al. 2004). Dissolved toxins are not readily absorbed by humans in contact recreation nor can they highly concentrate in localized areas where intracellular toxins can accumulate in high-use areas (EPA 2015; Chorus and Bartram 1999). Strategic algaecide use can subsequently decrease human health exposure potential and restore or maintain toxin levels below risk guidelines. However, not implementing cHAB management is predicted to result in increased cHAB density, toxin production, and exposure-risk potential, including chronic exposure (White, Duivenvoorden, and Fabbro 2005; Lehman et al. 2013).

Table 3. Extracted chlorophyll and phycocyanin before and after a peroxide-based algaecide treatment for cHAB control in a field demonstration on Lake Okeechobee, Florida, in July 2020.

	Extracted chlorophyll (µ L^{-1})			Extracted phycocyanin (µ L^{-1})		
Site[a]	Pretreatment	4 HAT[b]	24 HAT	Pretreatment	4 HAT	24 HAT
Mesocosm reference	967 (149)[c]	709 (119)	5,381 (1,967)	689 (252)	191 (48)	6,372 (2,096)
Treated	377 (165)	49 (14)	171 (21)	197 (116)	29 (3)	38 (7)
5 and 6	n/a[d]	122	3,880	n/a	24	1,200
7 and 8	n/a	1,106	3,880	n/a	163	1,200
21 and 22	n/a	377	232	n/a	329	40
Exterior reference	65 (0.1)	645 (60)	1,574 (1,129)	36 (4)	233 (70)	1,170 (1,040)

[a] Mesocosm reference (*n* = 4), treated (*n* = 9), exterior reference (*n* = 3). See Figure 1 for sampling sites map.

[b] HAT—hours after treatment

[c] Standard error of the mean is shown in parentheses where appropriate.

[d] Data from these sites are contained in the treated site, comprising all sites. These sites are broken out of the treated mean for posttreatment data because of distinct water-exchange influences.

Table 4. Free and total microcystin before and after a peroxide-based algaecide treatment for cHAB control in a field demonstration on Lake Okeechobee, Florida, in July 2020.

Site[a]	Free microcystin (µ L^{-1})			Total microcystin (µ L^{-1})		
	Pretreatment	4 HAT[b]	24 HAT	Pretreatment	4 HAT	24 HAT
Mesocosm reference	2.5 (0.4)[c]	2.8 (1.2)	3.5 (0.6)	157 (25)	167 (50)	1,064 (412)
Treated	2.8 (1.0)	2.4 (0.5)	4.1 (0.7)	50 (28)	4 (2)	11 (1.2)
5 and 6	n/a[d]	4.9	38	n/a	17	889
7 and 8	n/a	27.6	38	n/a	208	889
21 and 22	n/a	8.7	4.4	n/a	131	14
Exterior reference	1.5 (0.2)	1.0 (0.03)	3.4 (1.0)	2 (0.4)	148 (26)	573 (508)

[a] Mesocosm reference (*n* = 4), treated (*n* = 9), exterior reference (*n* = 3). See Figure 1 for sampling sites map.

[b] HAT—hours after treatment

[c] Standard error of the mean is shown in parentheses where appropriate.

[d] Data from these sites are contained in the treated site, comprising all sites. These sites are broken out of the treated mean for posttreatment data because of distinct water-exchange influences.

3.4 Species composition

Mean pretreatment *Microcystis* spp. cell density in the treated area was 896,111 cells mL^{-1} (Table 5). However, mean cell densities in the treated area were reduced 78% by 4 HAT, and 8 sample points contained nondetectable cell counts by 4 HAT. However, cell densities at sites 13 and 14 and 21 and 22 were not reduced, which is likely due to nontreated water intrusion. Conversely, cell densities at nontreated sites were above 1 million cells mL^{-1} at 4 HAT. Kinley-Baird et al. (2021) reported slight reductions in *Microcystis* spp. cell densities after treatments with peroxide-algaecides compared to other algaecides in a laboratory assay. These findings do not agree with our observations of the field evaluation of peroxide-based algaecide. One possible explanation is the level of solar irradiance exposure during treatment. Drábková, Admiraal, and Maršálek (2006) reported that *M. aeruginosa* was more sensitive to hydrogen peroxide with increasing irradiances. Moreover, light intensity has been shown to be a critical aspect to peroxide effectiveness on cyanobacteria and significantly alters responses, even at lower peroxide concentrations (Piel et al. 2020; Sandrini et al. 2020). The high solar irradiance experienced in Florida during July 2020 compared with laboratory fluorescent lighting could be one major factor to the differential responses

observed. Consequently, our field treatment, which was conducted under natural light conditions, likely aided in the *M. aeruginosa* sensitivity to hydrogen peroxide compared to laboratory evaluations.

Table 5. *Microsystis* spp. cell counts before and after a peroxide-based algaecide treatment for cHAB control in a field demonstration on Lake Okeechobee, Florida, in July 2020.

Site[a]	Pretreatment (cells mL^{-1})	4 HAT[b] (cells mL^{-1})
Mesocosm reference	7,550,000 (2,014,004)	5,220,000 (1,703,101)[c]
5 and 6	1,687,500	156,000
7 and 8	2,690,000	906,000
9 and 10	1,280,000	0
11 and 12	438,000	0
13 and 14	62,500	62,500
15 and 16	250,000	0
17 and 18	719,000	0
19 and 20	375,000	62,500
21 and 22	563,000	625,000
Exterior reference	93,800 (18,042)	1,350,000 (75,116)

[a] Mesocosm reference (n = 4), exterior reference (n = 3). See Figure 1 for sites sampling map.

[b] HAT—hours after treatment

[c] Standard error of the mean is shown in parentheses where appropriate.

4 Conclusions

This work evaluated an early-detection, rapid-response management strategy for control of cHABs with a peroxide-based algaecide, PAK 27. Using these data, the following conclusions can be drawn:

1. The PAK 27 peroxide-algaecide treatment provided effective short-term control (<24 h) of cHABs within the targeted treatment area.
2. Small treatment areas are vulnerable to influxes of cHABs from surrounding nontreated waters, primarily to water-exchange processes driven by wind-induced surface currents.
3. Strategic algaecide use can ultimately decrease cyanotoxin levels.
4. Risk of cHAB spread, influence on human and lake ecology health, and potential for discharge to sensitive estuaries and other receiving waters will likely occur without management.

This report recommends the following when using peroxide-based algaecide for the treatment and management of cHABs:

1. Minimize the influence of confounding factors such as wind-induced water-exchange processes that typically occur in large, shallow lake systems to increase the predictive ability of scaled testing for future research and operational-scale demonstrations.
2. Timely and effective initiation of rapid-response management techniques requires early detection of cHABs.

Future research should evaluate other management strategies for long-term cHAB control. Factors affecting cHAB recovery time after algaecide treatment in the absence of water exchange is of interest for future work as well. The current study evaluated cHAB treatment in a semiclosed system; however, further research is needed to document algaecide performance in other key areas such as locks, residential canals, and flowing water.

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14. ABSTRACT

Large-scale cyanobacterial harmful algal blooms (cHABs) in Lake Okeechobee, Florida, and connected waterways routinely impair water resources. This study conducted a field demonstration of a peroxide-based algaecide in 2020 in the Pahokee Marina on Lake Okeechobee to evaluate the algaecide's suitability for near-future operational implementation. Within minutes of treatment, rapid oxidation of cHAB cells occurred in the form of bleaching and cell lysis. On average, levels in the treatment area decreased by 4 hours after treatment (HAT) and remained low out to 24 HAT: chlorophyll decreased 87%, phycocyanin decreased 85%, total microcystin levels decreased from 50 (g L^{-1} to 4 (g L^{-1} at 4 HAT and then increased to 11 (g L^{-1} by 24 HAT, hydrogen peroxide concentrations averaged 6.1 mg L^{-1} 0.5 HAT and then dropped below detection limits by 24 HAT, and Microcystis spp. cell densities decreased at 4 HAT in all but four sampling sites. However, inflows of cHAB-infested lake water in some portions of the treatment area resulted in lack of control at these sites. Because of their vulnerability to influxes of cHABs from surrounding nontreated waters via water-exchange processes driven by wind-induced surface currents, future applications must therefore consider treatment area size.

15. SUBJECT TERMS

Algaecides--Evaluation; Cyanobacterial blooms--Control; Okeechobee, Lake (Fla.); Peroxides

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