Environmental Pollution 240 (2018) 590–598

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Hydrogen peroxide treatment promotes chlorophytes over toxic cyanobacteria in a hyper-eutrophic aquaculture pond☆

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ARTICLE INFO

Article history:
Received 15 December 2017
Received in revised form
2 May 2018
Accepted 3 May 2018

Keywords:
Algaecide
Diversity
Green algae
Microcystin
Phytoplankton
Zooplankton

ABSTRACT

Controlling blooms of toxigenic phytoplankton, including cyanobacteria, is a high priority for managers of aquatic systems that are used for drinking water, recreation, and aquaculture production. Although a variety of treatment approaches exist, hydrogen peroxide (H_2O_2) has the potential to be an effective and ecofriendly algaecide given that this compound may select against cyanobacteria while not producing harmful residues. To broadly evaluate the effectiveness of H_2O_2 on toxigenic phytoplankton, we tested multiple concentrations of H_2O_2 on (1) four cyanobacterial cultures, including filamentous *Anabaena*, *Cylindrospermopsis*, and *Planktothrix*, and unicellular *Microcystis*, in a 5-day laboratory experiment and (2) a dense cyanobacterial bloom in a 7-day field experiment conducted in a nutrient-rich aquaculture pond. In the laboratory experiment, half-maximal effective concentrations (EC_{50}) were similar for *Anabaena*, *Cylindrospermopsis*, and *Planktothrix* (average $EC_{50} = 0.41\ mg\ L^{-1}$) but were ~10x lower than observed for *Microcystis* ($EC_{50} = 5.06\ mg\ L^{-1}$). Results from a field experiment in an aquaculture pond showed that ≥ 1.3 and $\geq 6.7\ mg\ L^{-1}$ of H_2O_2 effectively eliminated *Planktothrix* and *Microcystis*, respectively. Moreover, $6.7\ mg\ L^{-1}$ of H_2O_2 reduced microcystin and enhanced phytoplankton diversity, while causing relatively small negative effects on zooplankton abundance. In contrast, $20\ mg\ L^{-1}$ of H_2O_2 showed the greatest negative effect on zooplankton. Our results demonstrate that H_2O_2 can be an effective, rapid algaecide for controlling toxigenic cyanobacteria when properly dosed.

1. Introduction

Eutrophication of freshwater systems is a global predicament that has important ramifications for the health of aquatic food webs, animals, and people through the promotion of cyanobacterial blooms, including taxa known to produce toxic secondary metabolites such as microcystins and saxitoxins (Neilan et al., 2013; Ibelings et al., 2014). Moreover, some cyanobacteria produce taste and odor compounds, such as geosmin and 2-Methylisoborneol (MIB), that have no known negative human health consequences but impart unpleasant musty flavors and odors in drinking water and aquaculture products (Zhang et al., 2011; Olsen et al., 2016).

Undoubtedly, controlling nutrient concentrations in aquatic systems is necessary for the long-term elimination of harmful cyanobacterial blooms; however, minimizing runoff inputs and managing both sedimentation and internal loading is challenging. Nutrient control in aquaculture is especially difficult given the need to regularly feed farmed fish. Although most water resource managers understand that reducing cyanobacterial blooms should be a long-term goal, most are keen to find solutions that create quick and noticeable improvements in water quality. Consequently, multiple methods have been developed aimed at reducing phytoplankton density or inhibiting their growth, including ultrasonication (Ahn et al., 2007a; Lürling et al., 2014), modified clays (Copetti et al., 2016), and bacterial or chemical agents (Cornish et al., 2000; Marsalek et al., 2012; Iredale et al., 2012; Greenfield et al., 2014). Although these techniques can be effective, some can harm non-target organisms or lead to chemical residual accumulation in treated ecosystems while others only produce short-lived effects (Matthijs et al., 2016). These drawbacks have

☆ This paper has been recommended for acceptance by Sarah Harmon.
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https://doi.org/10.1016/j.envpol.2018.05.012
0269-7491/

prevented their popularization as control methods for cyanobacterial blooms.

Hydrogen peroxide (H_2O_2) is a strong oxidant that is widely used for disinfection in water treatment and is on the U.S. Food and Drug Administration (FDA) approved aquaculture drugs list for aquaculture (U.S. FDA Approved Aquaculture Drugs, assessed 11 November 2017). Since H_2O_2 rapidly decomposes to H_2O and O_2 via biological, chemical, and photochemical mechanisms during oxidation, it does not leave harmful residues in the environment. Its strong oxidizing ability also promotes algal cell mortality by producing hydroxyl radicals under light exposure, which destroys proteins, lipids, and DNA (Latifi et al., 2009). More importantly, cyanobacteria are more sensitive than other phototrophs to H_2O_2 because of their unique cellular structure (Drábková et al., 2007). Thus, H_2O_2 is expected to be a selective algaecidal compound to control cyanobacterial blooms and may be a sensible alternative to controlling cyanobacterial blooms.

Prior studies have highlighted the potential of H_2O_2 for controlling cyanobacteria while also promoting non-toxic phytoplankton. For example, Barroin and Feuillade (1986) found the toxicity threshold of *Oscillatoria* (renamed to *Planktothrix*) under laboratory conditions to be 1.75 mg L^{-1} H_2O_2, whereas the dominant chlorophyte, *Pandorina* sp, was unaffected at a 10x higher H_2O_2 dose. Moreover, Drábková et al. (2007) also showed that cyanobacteria were negatively affected by H_2O_2 at concentrations 10 times less than that of green algae and diatoms, and that high light enhanced this effect across phytoplankton taxa. Lastly, Matthijs et al. (2012) performed an unreplicated experiment in a mesotrophic lake to test if H_2O_2 can be used to selectively suppress cyanobacteria in natural waters without affecting other organisms. In that study, *Planktothrix* was reduced by 99% only 3 d after the addition of 2 mg L^{-1} of H_2O_2, while eukaryotic phytoplankton and zooplankton remained largely unaffected. More recently, a field experiment confirmed that H_2O_2, used as sodium carbonate peroxyhydrate (a granular source of H_2O_2), caused a decline of phycocyanin concentrations and cell densities but did not affect chlorophyll *a* concentrations (Geer et al., 2017). Clearly, hydrogen peroxide has potential for controlling cyanobacteria in diverse systems.

Since freshwater algal blooms can be dominated by one or many cyanobacterial genera, including *Anabaena*, *Aphanizomenon*, *Cylindrospermopsis, Microcystis,* and *Planktothrix*, it would be useful to know if interspecific variation exists in H_2O_2 toxicity thresholds across important bloom-forming genera. Past efforts aimed at using H_2O_2 to control cyanobacterial blooms have focused on *Planktothrix* and *Microcystis*. Few studies exist documenting the effect of H_2O_2 on *Anabaena*, *Aphanizomenon*, and *Cylindrospermopsis*. Across these studies, H_2O_2 toxicity thresholds span several orders of magnitude from 0.33 to 60 mg L^{-1} (Barrington et al., 2013; Bauza et al., 2014; Wang et al., 2012).

In this study, we assessed the toxicity of H_2O_2 on three filamentous and one currently unicellular but originally colonial cyanobacterial genera under laboratory conditions. Based on results from the laboratory study, we conducted a replicated, field enclosure experiment in a hyper-eutrophic aquaculture pond to investigate the effect of four different H_2O_2 concentrations on the plankton community, which included a dense cyanobacterial bloom dominated by toxic, filamentous *Planktothrix* and colonial *Microcystis,* as well as the associated zooplankton community.

2. Materials and methods

2.1. Laboratory experiment

The unicellular *Microcystis aeruginosa* (UTEX 2667) used for the laboratory experiment study was obtained from the University of Texas at Austin culture collection. Three filamentous cyanobacterial cultures, including *Anabaena flos-aquae* (also called *Dolichospermum flos-aquae*) clone R5 (AU pond R5; isolated 15 August 2010), *Planktothrix agardhii* clone G24 (AU pond E24; isolated 15 August 2010), and *Cylindrospermopsis raciborskii* clone R2 (AU pond R2; isolated 29 August 2010), were originally isolated from aquaculture ponds located at the E. W. Shell Fisheries Center of Auburn University in Auburn, Alabama. Axenic batch cultures of each strain were diluted with 60 ml of BG11 medium to achieve similar initial phycocyanin concentrations (filter blank corrected; data reported as raw fluorescence units (RFU); ~3000 RFU) with the CyanoFluor (Turner Designs, CA, USA) and added to 100 ml glass flasks at 25 °C on a12:12 h light:dark cycle at 40 μmol m^{-2} s^{-1} photosynthetically active radiation. Hydrogen peroxide (Baker Analyzed, 30%) was added into each flask to achieve the follow concentrations: 0 (control), 0.3, 0.9, 2.7, 8.1, and 24.3 mg L^{-1}. Each treatment was replicated three times. Photosynthetic changes associated with H_2O_2 additions, including photosynthetic activity and phycocyanin pigments, were monitored before the treatments as well as 1, 2, and 5 d after H_2O_2 was added to each flask. Due to sample volume constraints, samples were collected from each flask using sterile pipet tips and analyzed for quantum efficiency (measured as F_v/F_m, where F_v is the maximal variable fluorescence and F_m is the maximal fluorescence intensity) using the Aquapen C100 (Photon Systems Instruments, Brno, Czech Republic) after placing the samples in the dark for 5 min at room temperature. Flask subsamples were also analyzed for phycocyanin concentration with the CyanoFluor.

2.2. Field experiment

The mesocosm experiment was conducted in a small (1.5 hectare), shallow (maximum depth = 2.7m), hyper-productive (~1400 μg L^{-1} total nitrogen and 122 μg L^{-1} total phosphorus) catfish aquaculture pond (S9; Boyd and Shelton, 1984) located at the E. W. Shell Fisheries Center of Auburn University during May 2017. At the start of the experiment, there was a toxic cyanobacterial bloom dominated by *Planktothrix* and *Microcystis*. Twelve greenhouse plastic limnocorrals (1500 L) that were sealed at the bottom and open at the top were suspended from a floating PVC frame in the pond and filled by pumping pond water through a sieve (500 μm) to remove small fish. To increase the biomass of *Microcystis* colonies in the enclosures, we collected plankton with a 100 μm net and then added similar volumes of the concentrated plankton homogenate into each enclosure. All enclosures were fertilized with KNO_3 and K_2HPO_4 to reach the target nutrient levels (4 mg L^{-1} for total nitrogen and 0.2 mg L^{-1} for total phosphorus) and sampled at 9:00 am on day 0 before establishing the four H_2O_2 treatments (0, 1.3, 6.7, and 20 mg L^{-1} H_2O_2, single application; three replicates per treatment). One enclosure was damaged during the experiment and data collected from this enclosure was not used for later analyses. Each enclosure was mixed thoroughly prior to collecting integrated samples (surface to 1 m depth) with a rigid tube sampler at 9:00 am 1, 3, 5, and 7 days after the H_2O_2 addition. Samples were returned to the lab and processed for phytoplankton and zooplankton diversity and abundance, two algal pigments (chlorophyll *a* and phycocyanin), and the hepatotoxin microcystin (both intracellular (particulate) and extracellular (dissolved)).

Phytoplankton samples were preserved with 1% Lugol's iodine solution before settling a small volume (180 μl) in a Palmer chamber and enumerating all phytoplankton taxa observed in 10 fields under 100x magnification (Olsen et al., 2016). Nikon image software was used to estimate the biovolume for each taxon. Zooplankton were collected using a 60 μm filter and preserved in

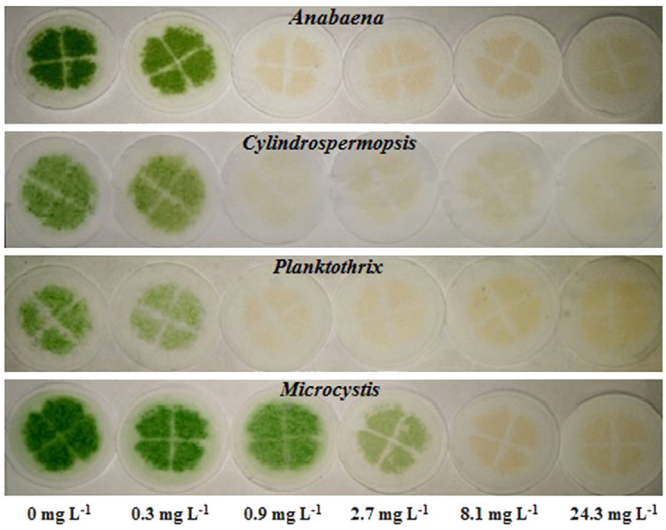

Fig. 1. The effect of six different concentrations of hydrogen peroxide (0, 0.3, 0.9, 2.7, 8.1, and 24.3 mg L^{-1}) on four cyanobacterial cultures (*Anabaena*, *Cylindrospermopsis*, *Planktothrix*, and *Microcystis*) under laboratory conditions. Filters show variation in algal abundance across treatments (50 ml of sample collected on each filter).

95% aqueous ethanol. One milliliter of each zooplankton sample was settled in a Sedgwick Rafter chamber and all copepods, cladocera, and rotifers were enumerated under 40x magnification with a Nikon microscope. Filters (Pall A/E) for chlorophyll *a* and phycocyanin concentrations were collected from well-mixed samples and stored frozen in the dark until being analyzed using a Turner Designs Trilogy fluorometer with pigment specific modules (Kasinak et al., 2015). Chlorophyll *a* samples were measured fluorometrically after a 24 h extraction in 90% aqueous ethanol in darkness at 4 °C (Kasinak et al., 2015). Phycocyanin samples were measured fluorometrically after grinding, extracting each filter in a 50 mM phosphate buffer in darkness at 4 °C, and filtering (<0.2 μm) each sample (Kasinak et al., 2015). Filters for intracellular microcystin were also collected from well-mixed samples and stored frozen in the dark until being extracted with 75% acidic aqueous methanol and analyzed with a BioTek 96-well plate readers using enzyme-linked immunosorbent assay (ELISA; Chislock et al., 2014). Sample filtrate (<1 μm) was also analyzed using ELISA for extracellular microcystin.

2.3. Statistical analyses

The half-maximal effective concentration (EC_{50}; also called the

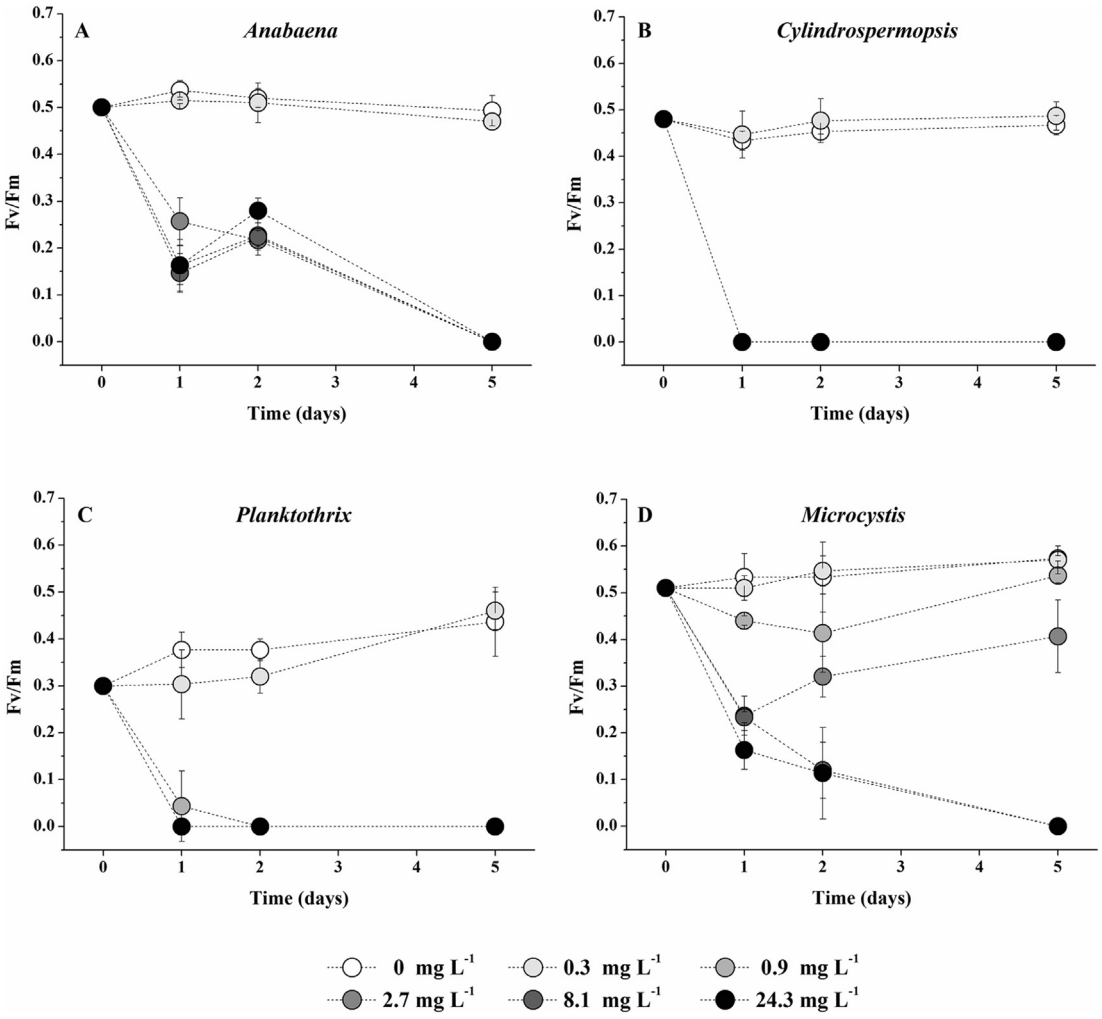

Fig. 2. The effect of six different concentrations of hydrogen peroxide (0, 0.3, 0.9, 2.7, 8.1, and 24.3 mg L^{-1}) on photosynthetic activity (F_v/F_m) of four cyanobacterial cultures, including (A) *Anabaena*, (B) *Cylindrospermopsis*, (C) *Planktothrix*, and (D) *Microcystis* under laboratory conditions over five days. F_v is the maximal variable fluorescence and F_m is the maximal fluorescence intensity. Results are expressed as the mean ± SD (standard deviation).

median inhibitory concentration) was used to evaluate the toxicity of H_2O_2 on four different cyanobacterial taxa. The EC_{50} value, based on the phycocyanin data measured on the 2 days after the H_2O_2 addition, was calculated for each cyanobacterium using Probit analysis. The EC_{50} values were analyzed by one-way analysis of variance (ANOVA). Differences of the H_2O_2 treatments in the laboratory and field experiments were compared using ANOVA followed by a Tukey's test. The Shannon–Wiener diversity index (Shannon and Weaver, 1949) and Simpson–Dominance index (Simpson, 1949) were used to evaluate the effect of H_2O_2 on phytoplankton diversity. All statistical analyses were performed with SPSS 13.0.

3. Results

The four cyanobacterial genera showed large variation in their resistance to H_2O_2 (Fig. 1). For example, H_2O_2 doses of $\geq$0.9 mg L^{-1} significantly decreased the pigment concentration (Fig. 1) and F_v/F_m value in three filamentous cyanobacteria (Fig. 2; *Anabaena*: $P < 0.001$; *Cylindrospermopsis*: $P < 0.00001$; *Planktothrix*: $P < 0.0001$) after only 1 day and was maintained at low levels for each filamentous cyanobacterial taxa until day 5 ($P < 0.01$). In contrast, a higher H_2O_2 dose ($\geq$2.7 mg L^{-1}) was needed to significantly reduce F_v/F_m for *Microcystis* after 1 d ($P < 0.00001$). After 5 d of the H_2O_2 addition, photosynthetic activity was only detected in the treatments $\leq$2.7 mg L^{-1} H_2O_2 (Fig. 2D). No significant effect in F_v/F_m was found for *Microcystis* treated with H_2O_2 doses $\leq$0.9 mg L^{-1} after 5 days (Fig. 2; $P = 0.78$). Phycocyanin concentrations (reported as CyanFluor RFUs) showed similar patterns observed for photosynthetic activity (Fig. 3). For example, the phycocyanin concentration for each filamentous cyanobacterium decreased from 3000 to near 0 after 1 day of H_2O_2 treatments $\geq$0.9 mg L^{-1} and was maintained near 0 for the remainder of the 5-day experiment (Fig. 3A–C). In contrast, only H_2O_2 doses $\geq$8.1 mg L^{-1} produced the same inhibitory effect in *Microcystis* over five days (Fig. 3D). The phycocyanin concentration of *Microcystis* treated with 0.9 and 2.7 mg L^{-1} of H_2O_2 remained at ~17,000 or ~3,000, respectively, despite being lower than the controls (~27,000; Fig. 3D) by the end of the experiment. The H_2O_2 EC_{50} values were similar for the three filamentous cyanobacteria (~0.41) but an order of magnitude lower than the EC_{50} observed for *Microcystis* (5.06; Table 1).

Observations made during the field experiment were generally similar to those observed for the laboratory-based experiment. For example, after 1 day, H_2O_2 addition caused significant decreases in chlorophyll *a* (Fig. 4A; $P = 0.022$) and phycocyanin (Fig. 4B; $P < 0.0001$) concentrations. However, chlorophyll *a* concentrations in all treatments recovered and were not different compared with the control after 7 days (Fig. 4A; $P = 0.20$). For phycocyanin, only the 1.3 mg L^{-1} treatments returned to the same concentration as the

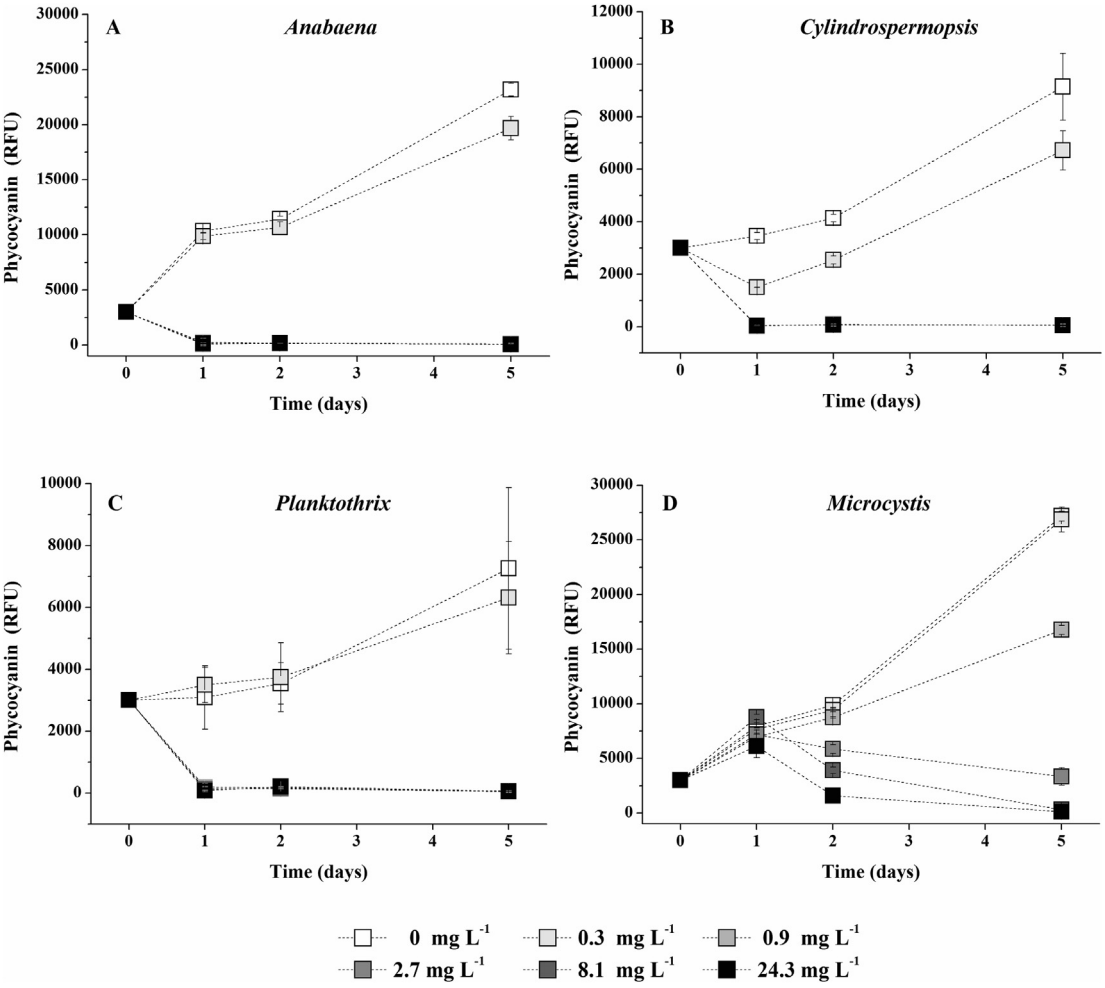

Fig. 3. The effect of six different concentrations of hydrogen peroxide (0, 0.3, 0.9, 2.7, 8.1, and 24.3 mg L^{-1}) on phycocyanin concentration (measured as raw fluorescence units (RFU)) of four cyanobacterial cultures, including (A) *Anabaena*, (B) *Cylindrospermopsis*, (C) *Planktothrix*, and (D) *Microcystis* under laboratory conditions over five days. Results are expressed as the mean ± SD.

Table 1
The half-maximal effective concentration ($EC_{50;}$ mg L^{-1}) of hydrogen peroxide on four cyanobacterial cultures (*Anabaena*, *Cylindrospermopsis*, *Planktothrix*, and *Microcystis*) under laboratory conditions. Results are expressed as the mean ± SD.

Cyanobacterial genera	EC_{50}(mg L^{-1})
Anabaena	0.50 (±0.04)[a]
Cylindrospermopsis	0.32 (±0.02)[a]
Planktothrix	0.42 (±0.04)[a]
Microcystis	5.06 (±0.24)

[a] Indicate a significant difference with *Microcystis* ($P < 0.01$).

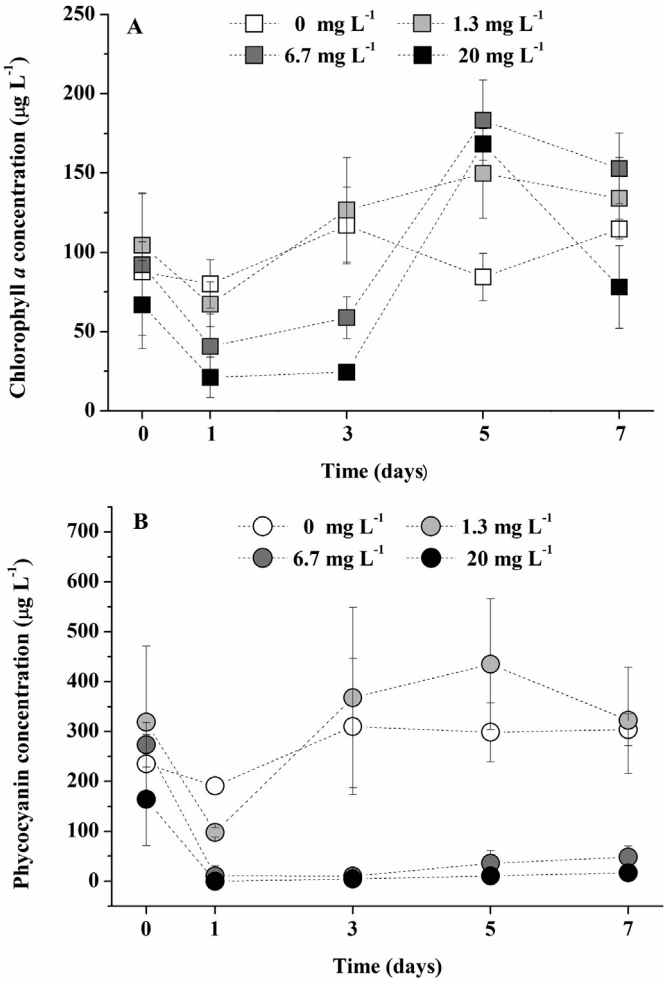

Fig. 4. The effect of four different concentrations of hydrogen peroxide (0, 1.3, 6.7, and 20 mg L^{-1}) on (A) chlorophyll *a* and (B) phycocyanin concentrations (μg L^{-1}) during a 7-day field experiment. Results are expressed as the mean ± SD.

control (Fig. 4B; $P = 0.981$) after 7 days, while the 6.7 and 20 mg L^{-1} addition enclosures remained at very low concentrations (Fig. 4B) throughout the experiment.

Planktothrix and *Microcystis* dominated all of the enclosures at the start of the experiment (Fig. 5; Table 2). The diameter of *Microcystis* colonies in our enclosures ranged from 16 to 280 μm (72 μm mean diameter). Of these two cyanobacterial taxa, the lowest H_2O_2 dose (1.3 mg L^{-1}) proved to be most effective against *Planktothrix* (Fig. 5A). *Planktothrix* biovolume gradually decreased from 1.03 - 1.39 × 10^8 μm^3 mL^{-1} to 0.04 - 0.08 × 10^8 μm^3 mL^{-1} in all treated enclosures after 7 days (Fig. 5A). In contrast, *Microcystis* showed a higher resistance to the 1.3 mg L^{-1} H_2O_2 dose after 7 days

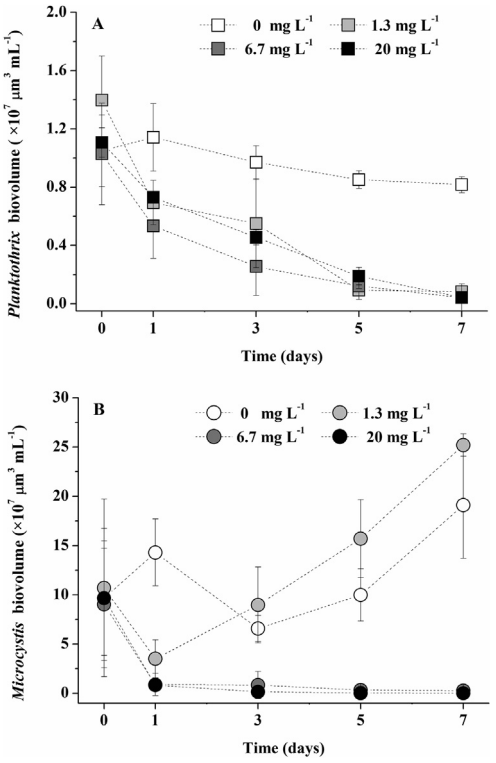

Fig. 5. The effect of four different concentrations of hydrogen peroxide (0, 1.3, 6.7, and 20 mg L^{-1}) on (A) *Planktothrix* and (B) *Microcystis* biovolume (μm^3 mL^{-1}) during a 7-day field experiment. Results are expressed as the mean ± SD.

($P = 0.133$ compared to control). Higher doses of H_2O_2 (6.7 and 20 mg L^{-1}) caused rapid and sustained reductions of *Microcystis* relative to the control (Fig. 5B; $P < 0.001$).

The effects of H_2O_2 treatments on microcystin were interesting and concentration dependent. For example, intracellular microcystin was not affected by the 1.3 mg L^{-1} H_2O_2 dose ($P = 0.27$; compared to control) after 7 days (Fig. 6A) but rapidly declined after 1 day in the 6.7 and 20 mg L^{-1} H_2O_2 treatments, from 3.74 to 2.99 μg L^{-1} to 0.13 and 0.27 μg L^{-1}, respectively. Intracellular microcystin was maintained at low concentrations until the completion of the experiment in these two higher H_2O_2 treatments (Fig. 6A; $P \leq 0.058$ compared to control). Extracellular microcystin increased quickly in all H_2O_2 treatments 1 day after treatment due to the release of intracellular toxins, but then significantly declined to low concentrations similar to those in the control enclosures by day 7 (Fig. 6B; $P > 0.06$).

Plankton diversity was affected by H_2O_2 treatments. For instance, 6.7 and 20 mg L^{-1} of H_2O_2 enhanced phytoplankton diversity (Table 2). Compared with their initial values, 6.7 and 20 mg L^{-1} treatments more than doubled the Shannon–Wiener diversity index after 7 days, while a significant diversity increase was also observed for the Simpson–Dominance index for 6.7 mg L^{-1} treatment. Regarding zooplankton, repeated measure analysis showed that the 1.3 and 6.7 mg L^{-1} H_2O_2 treatment led to similar total zooplankton density (including copepods, cladocera, and rotifers) as the control ($P > 0.05$; Fig. 7A). Significant inhibitory effects of H_2O_2 on zooplankton were observed in the enclosures treated with 20 mg L^{-1} ($P < 0.05$; Fig. 7A).

4. Discussion

Hydrogen peroxide was shown to be an effective treatment

Table 2
The effect of four different concentrations (0, 1.3, 6.7, and 20 mg L^{-1}) of hydrogen peroxide on phytoplankton taxa biovolume (μm^3 mL^{-1}) and two phytoplankton diversity indices (Shannon-Weiner Diversity and Simpson-Dominance Index) at the start (day 0) and end (day 7) of the field experiment.

	Day 0				Day 7			
	0 mg L^{-1}	1.3 mg L^{-1}	6.7 mg L^{-1}	20 mg L^{-1}	0 mg L^{-1}	1.3 mg L^{-1}	6.7 mg L^{-1}	20 mg L^{-1}
Cyanobacteria	**108,744,412**	**121,956,891**	**102,218,486**	**109,717,503**	**202,797,641**	**255,822,171**	**16,361,364**	**1,510,184**
Anabaena	1,298,605	1,056,328	1,347,061	1,763,777	3,130,220			
Arthrospira	160,687		160,687					
Chroococcus	124,210	180,014	125,967	183,615	831,667	2,603,009	13,236,464	1,071,986
Microcystis	96,658,914	106,752,104	90,300,590	96,703,365	190,669,103	252,396,979	2,704,346	
Planktothrix	10,501,996	13,968,444	10,284,181	11,066,746	8,166,651	822,183	420,555	438,198
Chlorophytes	**1,612,878**	**2,427,864**	**4,535,885**	**6,961,571**	**924,919**	**1,610,040**	**8,820,604**	**8,514,181**
Actinastrum						164,991	54,997	
Ankistrodesmus	1105	13,256	2209	3314	1105		134,885	1657
Chlamydomonas	15,444	57,916	19,305	81,082	19,305		37,779	23,166
Chlorella	2919	2919	6567	6567		164,166	299,148	
Closterium	42,730	3662	119,330	1831	2136		39,373	
Coelastrum	1,141,463	1,712,194	3,424,389	6,278,046	88,261	220,652	2,471,302	926,738
Cosmarium							75,784	
Golenkinia	26,045	26,045	31,275	23,457	109,464	31,275	838,672	6,016,406
Oocystis		56,850	48,095	46,129	7813	7813	24,562	186,599
Pandorina							186,690	258,929
Pediastrum	165,438	496,314	661,752	496,314	496,314	330,876	2,481,569	496,314
Scenedesmus	29,319	26,308	43,847	13,154	32,075	122,771	1,025,063	604,373
Schroederia				11,678			7785	
Selenastrum							14,784	
Staurastrum	188,416		156,376		168,446	544,755	1,128,211	
Tetraedron		24,304						
Treubaria		8097	22,740			22,740		
Diatoms	**17,114**	**3377**	**128,187**	**13,809**	**101,522**	**0**	**48,461**	**0**
Aulacoseira			91,506					
Cyclotella					101,522			
Fragilaria							22,806	
Navicula	17,114	3377	36,681	13,809			1126	
Synedra							24,529	
Others	**1,259,504**	**1,816,323**	**2,346,955**	**1,029,900**	**1,241,834**	**7,818,366**	**746,660**	**2,705,722**
Euglena	96,475	192,950	199,542	144,712	482,374	192,950		806,017
Peridiniopsis	283,341	366,676	258,257	125,003	83,336	6,928,361	565,089	650,017
Trachelomonas						571,386		
Cryptomonas	879,688	1,256,697	1,889,157	760,185	676,124	125,670	181,570	1,249,688
Shannon–Wiener Diversity Index	**0.54**	**0.60**	**0.75**	**0.69**	**0.37**	**0.29**	**1.77**	**1.85**
Simpson–Dominance Index	**0.23**	**0.17**	**0.35**	**0.35**	**0.14**	**0.10**	**0.59**	**0.25**

against toxigenic cyanobacteria in both laboratory- and field-based experiments. In the latter experiment, high quality and harmless chlorophytes were promoted by higher H_2O_2 treatments (>6.7 mg L^{-1}), which should improve trophic transfer efficiency in productive aquatic systems. The toxicity of H_2O_2 to phytoplankton is mainly attributed to the production of hydroxyl radicals that destroy cell membrane integrity (Mikula et al., 2012) and may damage the photosynthetic apparatus thus inhibiting photosynthetic electron transfer (Barrington and Ghadouani, 2008). Higher sensitivity of cyanobacteria to H_2O_2 than other associated phytoplankton was observed more than three decades ago (Barroin and Feuillade, 1986) and is likely due to the inability of cyanobacteria to eliminate reactive oxygen (Latifi et al., 2009) or that their photosynthetic apparatus is connected directly to the cellular plasma membrane (Grossman et al., 1995). These mechanisms could explain our laboratory-based observations with four cultures of cyanobacteria that showed a rapid decrease in the quantum efficiency (measured as F_v/F_m; Fig. 2) of the cultures immediately after the addition of H_2O_2. Moreover, the rapid release of microcystin in the two highest H_2O_2 treatments in the field experiments (Fig. 6B) suggests a H_2O_2-mediated disruption in the cyanobacterial cell membrane. However, cell membrane integrity was not evaluated in this study.

Although the toxicity of H_2O_2 to *Planktothrix* and *Microcystis* has been studied individually (Drábková et al., 2007; Matthijs et al., 2012; Wang et al., 2012; Bauza et al., 2014), this is the first time H_2O_2 toxicity has been tested on both *Anabaena* and *Cylindrospermopsis* concurrently with *Planktothrix* and *Microcystis* to compare its toxicity under similar conditions. Due to the high morphological diversity of cyanobacterial species included in our lab experiment, it was challenging to reach an equal initial biomass for each species by cell number or volume. Phycocyanin concentration has been generally used to estimate the cyanobacteria biomass in the field as it correlates to cyanobacterial biomass and is easy to measure (Ahn et al., 2007b; Chang et al., 2012; Bowling et al., 2016). For this reason, the same phycocyanin concentration was used to represent the approximately equal initial biomass levels for each species.

Microcystis showed a remarkably higher (10x) resistance to H_2O_2 than the filamentous cyanobacterial taxa tested both in our laboratory and field experiments (Figs. 1–3, 5; Table 1). Some mechanisms that have been used to explain the competitive dominance of *Microcystis* in diverse freshwater systems include its higher floating regulation, colonial morphology, and high-irradiance resistance (Huisman et al., 2005). However, these mechanisms cannot explain higher resistance of *Microcystis* to H_2O_2 in this study since the experiment was conducted in the laboratory with relatively low light compared to nature and that the

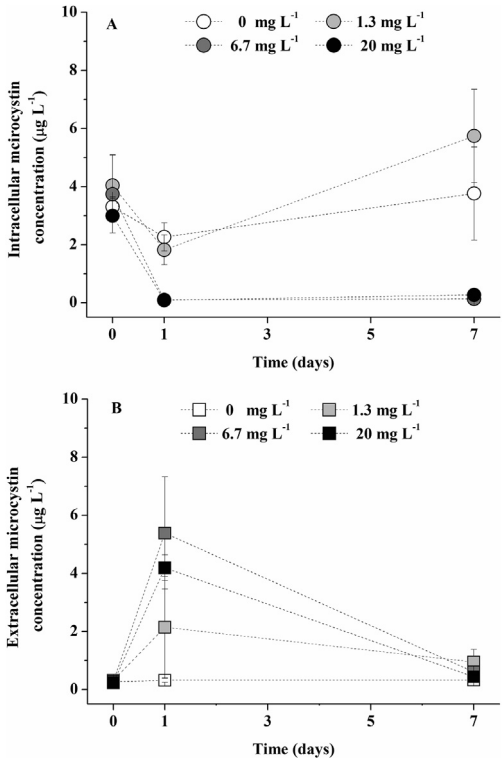

Fig. 6. The effect of four different concentrations of hydrogen peroxide (0, 1.3, 6.7, and 20 mg L^{-1}) on (A) intracellular and (B) extracellular microcystin concentrations (μg L^{-1}) during a 7-day field experiment. Results are expressed as the mean ± SD.

Microcystis culture used was unicellular. In addition, Dziallas and Grossart (2011) found that H_2O_2 toxicity to toxic *Microcystis* strains is significantly lower than non-toxic conspecifics because microcystins may play a role in stabilizing the photosynthetic apparatus and enhances the fitness of toxin producing strains under oxidative stress (Phelan and Downing, 2011; Zilliges et al., 2011). Whether differences in the ability to produce toxic secondary metabolites, like microcystin, of the cyanobacterial taxa used in this study is responsible for the variation in observed resistance to H_2O_2 is in need of further study.

Intracellular microcystin concentrations are dependent on the biomass of cyanobacterial cells as well as the internal toxin production and storage per unit cell. Consequently, in the field experiment, we found large (>90%) decreases in intracellular microcystin concentration in the two highest H_2O_2 treatments (6.7 and 20 mg L^{-1}; Fig. 6A) concomitant with large reductions (90%) in cyanobacterial abundance relative to 0 (control) and 1.3 mg L^{-1} H_2O_2 treatments (Fig. 4, Table 2). Toxin release after cell disruption is a key problem associated with the use of algaecides in the control of cyanobacteria. In the field experiment, extracellular microcystin concentration rose rapidly after the addition of H_2O_2 in all treatments (Fig. 5B). However, H_2O_2 can enhance the photocatalytic oxidation of microcystins, making them degrade into non-toxic by-products (Rodriguez et al., 2007; Lürling et al., 2014). Consistent with previous studies (Matthijs et al., 2012; Papadimitriou et al., 2016), the results of our field experiment study show that H_2O_2 degraded extracellular toxins and significantly reduced their levels by the end of the experiment (Fig. 6B).

Understanding the effect of H_2O_2 on non-target organisms should be assessed before its use in any aquatic ecosystem and especially commercial aquaculture ponds. Although phytoplankton were generally reduced after H_2O_2 additions (Fig. 4A), cyanobacteria were consistently repressed throughout the 7-day field experiment (Fig. 4B) while other phytoplankton, especially chlorophytes, quickly rebounded. Green algae biomass did not increase significantly in the 1.3 mg L^{-1} H_2O_2 treatment because *Microcystis* was not affected by the low dose of H_2O_2. These results are consistent with prior field experiments (Matthijs et al., 2012; Weenink et al., 2015). Although 6.7 mg L^{-1} is near the H_2O_2 toxicity threshold of specific non-cyanobacterial phytoplankton (Drábková et al., 2007), we contend that the reduction of competition from bloom-forming cyanobacteria creates a better habitat for other, harmless phytoplankton taxa thus increasing their abundance and diversity. Indeed, the enhancement of phytoplankton diversity after H_2O_2 treatment is also supported by the increase of both Shannon–Wiener diversity and Simpson–Dominance indices (Table 2). However, the 20 mg L^{-1} H_2O_2 treatment showed harmful effects to some non-cyanobacterial phytoplankton taxa, which reduced species numbers and consequently decreased the Simpson–Dominance index despite an increase in the Shannon–Wiener diversity index (Table 2).

Since H_2O_2 treatments could also impact zooplankton, which may influence phytoplankton species composition and abundance through grazing and nutrient recycling as well as fish production through planktivory, we were also interested in the effects of different H_2O_2 doses on the zooplankton community. Matthijs et al. (2012) found that the addition of 2 mg L^{-1} H_2O_2 did not cause harmful effect on zooplankton. In this field experiment, the two lower H_2O_2 treatments (0 and 1.3 mg L^{-1}) significantly increased total zooplankton biomass (especially rotifers and cladocera; Fig. 7) in response to small reductions in cyanobacteria (Fig. 4) or intracellular microcystin (Fig. 6). Total zooplankton density was marginally affected by the 6.7 mg L^{-1} H_2O_2 treatment. However, the highest H_2O_2 treatment (20 mg L^{-1}) quickly caused significant declines in total zooplankton abundance relative to the control that persisted during the 7-day field experiment (Fig. 7A). Thus, water resource managers should consider dosing needs when controlling cyanobacterial blooms given that higher doses could have negative consequences on non-target organisms, such as zooplankton.

Although the toxicity of H_2O_2 to other filamentous cyanobacterial taxa (except for *Planktothrix*) was not evaluated in the field experiment, H_2O_2 may be an effective algaecide to control other filamentous cyanobacteria based on the results from this and past studies (Matthijs et al., 2012; Bauza et al., 2014). In these studies, lower H_2O_2 concentrations (e.g., ~1.3 mg L^{-1}) were sufficient to eliminate most filamentous cyanobacterial cells without large negative effects on non-target taxa. However, *Microcystis* appears to have a higher H_2O_2 toxicity threshold (Table 1). The 6.7 mg L^{-1} H_2O_2 treatment showed to be effective at quickly eliminating *Microcystis* colonies and microcystin during the enclosure experiment, while also having small negative effects on zooplankton density and positive effects on phytoplankton diversity. A suggested dose of 6.7 mg L^{-1} H_2O_2 is lower than similar experiments performed by Wang et al. (2012). One reason for this discrepancy could be due to *Microcystis* colonies in their experiment (diameter above 100 μm) being larger than those found in our enclosures (72 μm mean colony diameter). Previous studies have shown that bigger colony size increases tolerance of *Microcystis* to H_2O_2 toxicity (Liu et al., 2017). Another explanation for this discrepancy could be that the *Microcystis* colony density in this study was not as high as that of Wang et al. (2012). It may be that a dose higher than 6.7 mg L^{-1} H_2O_2 is needed to control *Microcystis* blooms with more dense or larger colonies. Considering the non-target effects of higher H_2O_2 doses on zooplankton, care is encouraged during treatment dosing. However, an appropriate dose of H_2O_2, such as 6.7 mg L^{-1}, should be considered for use in waterbodies with low *Microcystis* biomass or in the early stage of a *Microcystis* bloom dominated by small colonies (Zhu et al., 2016).

Ultraviolet radiation and high direct sunlight conditions could

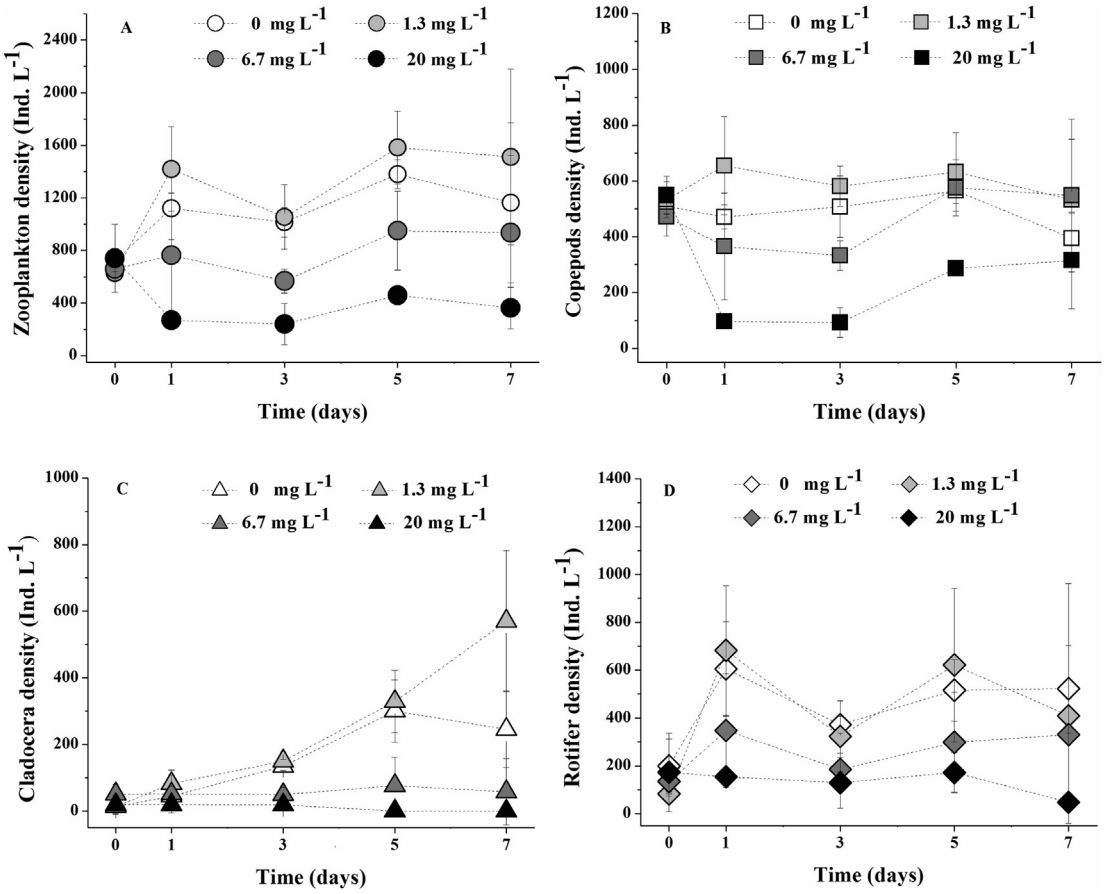

Fig. 7. The effect of four different concentrations of hydrogen peroxide (0, 1.3, 6.7, and 20 mg L^{-1}) on zooplankton density (individuals L^{-1}) during a 7-day field experiment. (A) Total zooplankton (Copepods + Cladocera + Rotifers), (B) Copepods, (C) Cladocera, and (D) Rotifers. Results are expressed as the mean ± SD.

promote the reaction of H_2O_2 and produce more hydroxyl radicals, a strong reactive oxygen species that can damage cyanobacterial cells by destroying their membrane integrity and photosynthetic apparatus (Mikula et al., 2012; Barrington and Ghadouani, 2008). According to results in Drábková et al. (2007), initial concentrations of H_2O_2 were decomposed to less than 4% after 3 h of exposure under high irradiance, but showed greater toxicity to algal cells than under the lower irradiance. Though H_2O_2 concentrations were not measured in this experiment, the H_2O_2 levels were expected to decrease quickly over time based on two large-scale field H_2O_2 manipulations (Matthijs et al., 2012; Burson et al., 2014). In these studies, H_2O_2 declined by ≥ 60% or ≥95% within one or two days after treatment, respectively. Consequently, we contend that for H_2O_2 to affect algal cells prior to breaking down, the treatment must be evenly, quickly, and thoroughly mixed. Future research should evaluate the effects of different H_2O_2 application concentrations under different light intensities and UV radiation.

5. Conclusions

Blooms of cyanobacteria pose serious threats to aquatic ecosystems around the world. Consequently, a variety of techniques to control cyanobacterial blooms have been developed and tested. We were interested in further testing the utility of H_2O_2 in a hypereutrophic aquaculture pond that regularly suffers from toxic cyanobacterial blooms. Using a gradient design field experiment, we found that H_2O_2 doses >1.3 mg L^{-1} where effective at significantly reducing cyanobacteria and the hepatotoxin, microcystin, while also promoting other phytoplankton taxa, including high quality chlorophytes. Negative effects on zooplankton at the highest H_2O_2 concentration (20 mg L^{-1}) highlight the need to conduct small-scale studies testing various H_2O_2 concentrations prior to whole system treatments.

Acknowledgments

This project was supported by the National Natural Science Foundation of China (grant # 31570457), the United States Department of Agriculture's National Institute of Food and Agriculture (grant # 2017-70007-27132), and the School of Fisheries, Aquaculture, and Aquatic Sciences and the College of Agriculture at Auburn University.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.envpol.2018.05.012.

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