Field evaluation of seven products to control cyanobacterial blooms in aquaculture

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Abstract

Harmful algal blooms negatively impact water quality in hypereutrophic systems that are common in aquaculture. However, few algaecides are approved for use in food-fish aquaculture. This study assessed the effectiveness of seven products, including hydrogen peroxide (as a concentrated liquid or in granular form (PAK-27)), peracetic acid (as VigorOx SP-15 and Peraclean), copper (as copper sulfate in unchelated (powder) or chelated (Captain) forms), and a clay-based product (as Phoslock) on phytoplankton (including cyanobacteria) and zooplankton biomass. Each product was tested in a 14-day laboratory and 35-day field experiment to assess their short- and long-term performance. Although some products (i.e., copper-based and liquid hydrogen peroxide) quickly reduced phytoplankton, effects were short-lived given that chlorophyll concentrations returned to starting concentrations within 21 days. In contrast, all but one product (i.e., concentrated liquid hydrogen peroxide) maintained low phycocyanin concentrations for 35 days. Zooplankton biomass trends showed large, negative effects for most algaecides; however zooplankton rebounded for most treatments except for copper-based products. In general, copper-based products remain the most efficient and cheapest choice to reduce total phytoplankton biomass in aquaculture systems. However, peracetic acid-based products effectively and quickly reduced cyanobacteria while having marginal effects on beneficial algae and zooplankton. Such algaecides could be effective alternatives to copper-based products for aquaculture farmers.

Declarations

Ethics approval and consent to participate: This research followed the guidelines provided by Auburn University for ethical research. Consent to participate was not applicable for this study.

Consent for publication: N/A

Availability of data and materials: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests: The two peracetic acid-based products used in this study were provided by Evonik (Peraclean®) and PeroxyChem (VigorOx®). Partial financial support in the form of an unrestricted gift was provided by SePRO prior to the start of this study.

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Introduction

Harmful algal blooms negatively impact water quality in freshwater, estuarine, and marine systems around the world (Chislock et al. 2013a and b). Such events are more common, extreme, and persistent in nutrient-rich systems like those found in aquaculture (Schrader et al. 2018; Tucker et al. 2020). Algal blooms often create anoxic or hypoxic conditions under periods of low light or as cells decay associated with microbial degradation. In intensive aquaculture systems, daily pond aeration is often required to maintain safe dissolved oxygen concentrations, which increases production costs. Secondary metabolites of toxigenic phytoplankton, such as microcystin which is a class of hepatotoxins produced by some genera of cyanobacteria (blue-green algae), may affect the liver, spleen, and kidneys facilitating sub-chronic issues (e.g., reduced growth and feeding, deformities, increased cortisol levels; Malbrouck and Kestemont 2006), or, in extreme situations, induce acute die-offs (Zimba et al. 2000). Moreover, some cyanobacterial genera can produce off-flavor compounds (e.g., 2-methylisoborneol (MIB), geosmin), which are non-harmful (Dionigi et al. 1993) but generate unwanted taint in fish fillets. This issue costs the U.S. catfish aquaculture industry an estimated $23 million annually due to lower market prices, prolonged holding times, and extended feeding (Hanson 2003).

To combat the issues generated by cyanobacterial blooms, aquaculture relies primarily on the use of chemical controls due to their effectiveness in rapidly reducing phytoplankton biomass (Bosma and Verdegem 2011; Schrader et al. 2005; Viriyatum and Boyd 2016). Past research has shown that a number of algaecide types can control nuisance algal blooms in environments similar to that of farm-pond aquaculture (Sinha et al. 2018; Schrader et al. 2005; Barrington et al. 2013; Bishop and Richardson 2018). For example, copper sulfate ($CuSO_4$) can

reduce excessive algal growth in ponds with moderate risk to the farmed fish when used appropriately, such as testing ambient alkalinity prior to treatment (Viriyatum and Boyd 2016). Despite this, there is concern that chemicals, such as heavy metals like copper, may persist in the environment for extended durations, have negative effects on non-target organisms, and may require repeated applications to prevent bloom resurgences, thus increasing water quality management costs and toxicity risks (Viriyatum and Boyd 2016). Only two algaecides are approved for algal bloom control in aquaculture (i.e., $CuSO_4*H_2O$ and Diuron (phenylurea-based herbicide-turned-algaecide product; to be used specifically for the control of cyanobacteria that produce MIB); EPA 2003). Such a limited variety of chemical controls is possibly due to the requirements needed to receive the U.S. Environmental Protect Agency's (EPA) approval under the Federal Insecticide Fungicide and Rodenticide Act (FIFRA; Laughinghouse et al. 2020). Further, copper sulfate is a cost-effective, low volume method to reduce cyanobacteria, and issues of toxicity can be mitigated by utilizing lower concentration, repeated doses (Tucker et al. 2005).

Despite the limited number of approved algaecides, recent research has identified numerous chemicals that can effectively reduce cyanobacterial biomass, including chelated copper (Bishop et al. 2017), granular (sodium carbonate peroxyhydrate) and liquid hydrogen peroxide (Sinha et al. 2018; Yang et al. 2018), and peracetic acid (Enviro Tech 2003). Clay compounds have also been identified as means to bind to cyanobacteria for removal (Lu et al. 2017) and/or by binding to phosphorus to reduce nutrient availability to blooms (Bishop and Richardson 2018). Despite a large amount of literature on the subject, research on the effectiveness of a specific algaecide is often context-specific considering each study is conducted under disparate conditions with varying cyanobacterial genera dominating the

system, thus leading to a dissonance in findings between studies. Such variation in results is more pronounced when experiments compare results across algaecides (Sinha et al. 2018) or attempt to extend results from the lab to the field (Yang et al. 2018). For example, Yang et al. (2018) observed that hydrogen peroxide (H_2O_2) in liquid form under uniform laboratory conditions was effective at eliminating *Dolichospermum* (earlier known as *Anabaena)*, *Cylindrospermopsis*, and *Planktothrix*, but was less effective at reducing *Microcystis*. Furthermore, the prolonged effectiveness of a treatment is questionable as many are assessed for short durations (<7 days; Barrington et al. 2013; Greenfield et al. 2014). Such differences in experimental design between published studies may lead to varying outcomes and subsequent inaccurate perceptions of the effectiveness of a product to reduce nuisance cyanobacterial blooms.

In general, few studies have tested multiple algaecides in a single study under uniform conditions (refer to Sinha et al. 2018). The purpose of this study was to compare the effectiveness of $CuSO_4$, as it is the only fully EPA approved algaecide for use in food-fish aquaculture to six other algaecides to control blooms of phytoplankton, specifically cyanobacteria, in the field. This study assessed the effectiveness of seven algaecides including, $CuSO_4$, Captain® (chelated copper), PAK-27® (sodium carbonate peroxyhydrate, H_2O_2 - based), liquid H_2O_2, VigorOx SP-15® (peracetic acid), Peraclean® (peracetic acid), and Phoslock® (modified clay for phosphorus binding, not an algaecide as the others, but hereby referred to as an 'algaecide' or 'product' to maintain uniformity) (online resource Table 1). The products were initially tested across a broad range of concentrations in a 14-day laboratory-based microcosm experiment to identify target concentrations for each algaecide in a

123 subsequent 35-day field mesocosm experiment where effects on phytoplankton and
124 zooplankton biomass were assessed.
125

Methods

Laboratory experiment

Shoreline pond water samples were collected in the morning using buckets from three active catfish aquaculture ponds experiencing cyanobacterial blooms (dominated by *Microcystis*) on the E.W. Shell Fisheries Center of Auburn University, AL during May 2019. The pond water was combined in equal parts, returned to the lab, filtered through a 500 µm mesh to remove large debris, and placed into an acid-washed bucket. To supplement phytoplankton densities, BG-11 media (Rippka et al. 1979) was stirred into the combined pond water such that the media comprised 10% of the total volume. The tested phytoplankton community consisted mostly of cyanobacteria (91.5% total biovolume that included *Microcystis* (79.1%), *Raphidiopsis* (11.3%), and *Oscillatoria* (5.9%)) but also contained green algae (7.5%) and diatoms (1%). The mixture was then distributed to 87, 500 mL glass jars to a volume of 435 mL. Jars were capped and mixed before collecting A/E filtered samples for two algal pigments, chlorophyll (i.e., chlorophyll-*a*; a measure of total phytoplankton abundance) and phycocyanin content (measure of cyanobacterial abundance), that were measured using fluorometry (Turner Designs Trilogy®). Chlorophyll was determined by extracting filters in 90% ethanol for 24 hours at 4 ºC (20 mL pond water; Sartory and Grobbelaar 1984). Phycocyanin was measured by extracting filters in a 50 mM phosphate buffer (Ricca Chemical ®) for four hours in the dark (20 mL pond water; Kasinak et al. 2014). After collecting initial algal pigment samples, 395 mL of pond water remained in each jar.

Jars were then dosed with one of seven products (Table 1; online resource Table 1). Each product was tested at four different treatment concentrations with three replicates for each concentration. Control jars that received no chemical additions were also included. Secondary

149 stocks of each chemical were made with DI water at a concentration such that each jar received a
150 5 mL addition of the secondary stock to achieve the required chemical dosage (total jar volume
151 now 400 mL). Control jars received 5 mL of DI water containing no chemicals. Phoslock
152 treatments were based on the amount of total phosphorus present within a water body. As such,
153 total phosphorus was measured for the pond water and BG-11 mixture before the treatment using
154 a colorimetric assay spectrophotometry (Gross and Boyd 1998) and found to be 2.2 mg/L. After
155 the 5 mL of the secondary stocks were added, jars were then inverted three times, their caps
156 loosened, and incubated at 30 °C on an 8 hr light: 16 hr dark schedule (fluorescent lighting;
157 intensity = 80 μmol/m^2/s).
158 The laboratory experiment lasted for 14 days. Jars were mixed by inverting three times
159 and rotated within the incubator (Percival® model I-36VL) daily to minimize light variation
160 across jars. Algal pigment measurements were collected via pipette on days 0, 1, 3, 5, and 7 (20
161 mL for both chlorophyll and phycocyanin). A repeated-measures analysis of variance (RM-
162 ANOVA) using a restricted maximum log-likelihood (REML) method was used to assess
163 differences in total phytoplankton (chlorophyll) and cyanobacterial (phycocyanin) densities over
164 time. Tukey's multiple comparison tests were used to compare mean effects among treatments.
165 The analysis was performed using the *nlme* package in R (Pinheiro et al. 2020). The lowest
166 concentration of each product that clearly and effectively reduced total phytoplankton (using
167 chlorophyll values) and specifically cyanobacterial biomass (using phycocyanin values) to that
168 of the control was selected for use in the field experiment.
169
170 *Field experiment*

171 The field experiment was conducted during June 2019 in a 22-acre earthen aquaculture
172 pond containing hybrid catfish (blue x channel catfish; *Ictalurus punctatus* x *I. furcatus*) housed
173 within an in-pond raceway system at the E.W. Shell Fisheries Center of Auburn University, AL
174 (S1; Boyd and Sheldon 1984). Each product was tested in three, randomized replicate
175 mesocosms, and the control had four replicates (25 mesocosms in total). Mesocosms were
176 cylinder-shaped and made of greenhouse plastic (1310 L volume) that were sealed at the bottom
177 and open at the top and suspended to a floating dock positioned in the center of the pond (Fig. 1).
178 Mesocosms were filled with surrounding pond water after being sieved through 200 μm mesh to
179 exclude large debris but to include ambient zooplankton and phytoplankton. Prior to filling, the
180 pond was sampled for total nitrogen and phosphorus (both measured using persulfate digestion
181 and spectroscopy (Gross and Boyd 1998)). Based on these values, potassium phosphate
182 (K_2HPO_4) and potassium nitrate (KNO_3) were added to each mesocosm to reach concentrations
183 of 2.6 mg/L total nitrogen and 0.22 mg/L total phosphorus. Mesocosms were then left for 11
184 days to allow phytoplankton abundance to increase and stabilize.
185 On day 0 (11 days after filling and fertilizing), two integrated vertical water samples were
186 obtained using a rigid tube sampler (inside diameter = 51 mm) to a depth of 1 meter (4 L of
187 sample collected total). Samples were combined in a bucket and placed into a plastic cubitainer.
188 Water samples were returned to the lab to be processed for chlorophyll and phycocyanin
189 pigments, as well as for phytoplankton and zooplankton diversity and abundance. Phytoplankton
190 samples were preserved using 1% Lugol's iodine solution. The preserved samples were then
191 settled in a Hydro-bios® settling chamber and enumerated on an inverted microscope by
192 counting cells observed in 25 fields from 100-400x (Yang et al. 2018). Zooplankton from 2 L of
193 sample were collected on a 100 μm filter and preserved in 95% ethanol before enumeration in a

194 Sedgewick-Rafter chamber on a compound microscope by counting all zooplankton observed at
195 100x (Yang et al. 2018). Phytoplankton and zooplankton were identified using Edmondson
196 (1959). Phytoplankton were identified to the genus level. Zooplankton were identified to the sub-
197 order or genus. Dominant phytoplankton included green algae (*Staurastrum* and *Gloeocystis*) and
198 cyanobacteria (*Microcystis* and *Pseudanabaena*).
199 After sampling water quality for day 0 measurements, the mesocosms were either left
200 untreated (controls) or treated with a one of seven algaecides (Table 2). Mesocosms were
201 randomly assigned. Mesocosms were mixed with a tube sampler for 10 seconds after the
202 application of each product. Integrated water samples were then collected from each mesocosm
203 on days 1, 3, 7, 14, 21, 28, and 35. Chlorophyll and phycocyanin values were measured for all
204 sampled days. Phytoplankton and zooplankton samples were counted for day 0, 1, 7, and 35.
205 Products were assessed foremost on their ability to reduce cyanobacteria. Changes in the
206 total phytoplankton and zooplankton biomass were also assessed between product treatments. A
207 repeated-measures analysis of variance (RM-ANOVA) using a restricted maximum log-
208 likelihood (REML) method was used to assess these differences in total phytoplankton
209 (measured as chlorophyll and phytoplankton biovolume), cyanobacterial density (phycocyanin),
210 and zooplankton density between product treatments over time. Tukey's multiple comparison
211 tests were used to compare mean effects among treatments. The analysis was performed using
212 the *nlme* package in R (Pinheiro et al. 2020).
213

Results

Laboratory experiment

Seven algaecides were tested at four treatment concentrations over the 14-day laboratory experiment by measuring changes in phytoplankton (measured as chlorophyll; Fig. 2a and c) and cyanobacterial (measured as phycocyanin; Fig. 2b and d) abundances over time. Briefly, across all products, there were large effects of treatment ($p < 0.000001$), time ($p \leq 0.021$), and the treatment x time interaction ($p < 0.000001$) on chlorophyll and phycocyanin concentrations (RM-ANOVA). The copper-based products, $CuSO_4$ and Captain, significantly reduced phytoplankton and cyanobacteria with concentrations ≥0.2 mg/L as Cu ($p \leq 0.05$; Online Resource Figs. 1 and 5). At these concentrations, cyanobacteria were fully removed from the jars with both products by day three, while total phytoplankton biomass quickly declined and largely remained <200 µg/L (compared to starting chlorophyll concentrations ~500 µg/L) in both copper products for the duration of the trial. H_2O_2-based products, liquid H_2O_2 and granulated PAK-27, both significantly reduced total phytoplankton and cyanobacteria at concentrations ≥5 mg/L as H_2O_2 (Online Resource Figs. 2 and 6). Although biomass did decrease in the first three days, both total phytoplankton and cyanobacteria again increased over the 14-day trial, but remained lower than the control. Peracetic acid-based products, Peraclean and VigorOx SP-15, significantly reduced phytoplankton and cyanobacteria with concentrations ≥2 mg/L as volume, with the greatest effects observed at concentrations ≥10 mg/L ($p \leq 0.05$; Online Resource Fig. 3 and 7). Cyanobacteria remained at or near-to zero after day 1 in concentrations ≥5 mg/L. Phytoplankton increased over the 14-day experiment in concentrations less than ≤5 mg/L and ≤12 mg/L in the Peraclean and VigorOx SP-15 treatments, respectively. However, phytoplankton and cyanobacteria still remained lower than that of the control throughout the

entire experiment after treatment. For Phoslock, only the ratio of 50:1 (kg phoslock:kg waterbody phosphorus) reduced phytoplankton abundance when compared to the control (Online Resource Fig. 4) while the 200:1 Phoslock treatment was the only treatment to reduce cyanobacteria relative to the control (Online Resource Fig. 8).

From the various concentrations that the seven algaecides were tested, it was determined that the following concentrations were to be tested in the field experiment: 0.4 mg/L of $CuSO_4$ and Captain, 10.2 mg/L of liquid H_2O_2 and PAK-27, 10 mg/L of VigorOx SP-15 and Peraclean, and 200:1 ratio for Phoslock (Table 2). Between these products, all had at least one concentration that significantly reduced both total phytoplankton (Fig. 2a and c) or cyanobacteria (Fig. 2b and d) over the 14-day experiment when compared to the control.

Field experiment

A 35-day field mesocosm experiment evaluated seven algal control products on phytoplankton (as chlorophyll and biovolume), cyanobacteria (as phycocyanin), and zooplankton biomass relative to a control. Although some treatments caused large, rapid declines in chlorophyll (starting values averaged ~56 µg/L), all treatments returned to near initial conditions within 21 days (Figs. 3a and 3c). Significant effects of treatment ($p = 0.00170$) and time ($p < 0.001$) in the field experiment were observed, but treatment x time interaction was not significant ($p = 0.293$) on chlorophyll (RM-ANOVA). Only $CuSO_4$ decreased chlorophyll more than the control across the entire 35 day experiment ($p < 0.05$; Fig. 3a). In the first seven days, Captain and $CuSO_4$ significantly reduced phytoplankton before increasing over time (Figs. 3a and 3c). Liquid H_2O_2 also reduced chlorophyll, but this reduction was short-lived considering that chlorophyll peaked on day 7 in this treatment (Figs.

3a and 3c). Chlorophyll concentrations for liquid H_2O_2 and the controls were statistically similar (Fig. 3a). Several treatments, including Peraclean, VigorOx SP-15, Phoslock, and PAK-27, had similar chlorophyll concentrations relative to the control the entire experiment (Figs. 3a and 3c).

Initial cyanobacterial concentrations (as phycocyanin) averaged ~15 µg/L at the start of the experiment (Fig. 3b and d). Although there were significant effects of treatment ($p < 0.00001$) and a treatment x time interaction ($p < 0.00001$) in the field experiment, time was not significant ($p = 0.425$) on phycocyanin (RM-ANOVA). All products reduced cyanobacterial densities after 1 day except for Phoslock. Interestingly, liquid H_2O_2 increased in cyanobacteria relative to the control by day 7 (Figs. 3b and 3d). In total, all products except for liquid H_2O_2 had a significantly lower cyanobacterial concentration than that of the control during the 35-day experiment ($p \leq 0.05$, Figs. 3b and 3d).

Phytoplankton biovolume

Phytoplankton biovolume was estimated for all mesocosms for days 0, 1, 7, and 35 of the field experiment. Average starting phytoplankton biovolume averaged ~1.17 x 10^7 μm^3/mL across all products (Fig. 4a). Chlorophytes were the dominant phytoplankton, averaging 9.89 x 10^6 μm^3/mL (55.9% of starting biovolume) between all enclosures. Cyanobacteria next dominated the mesocosms, averaging 1.66 x 10^6 μm^3/mL (9.4% of starting biovolume) between all enclosures (Fig. 5a). Additional phytoplankton groups observed included cryptophytes, dinoflagellates, euglenoids, and diatoms, but the presence of these taxa were generally not substantial (Fig. 5a).

282 Across all products during the 35-day experiment, there were significant effects of
283 treatment ($p < 0.000001$), time ($p = 0.0448$), and treatment x time interaction ($p = 0.0022$) on
284 phytoplankton biovolume (RM-ANOVA). All products, except Phoslock, reduced
285 phytoplankton biovolume during the experiment first day, however phytoplankton rebounded
286 to initial concentrations over the duration of the experiment (Fig. 4a). Phytoplankton
287 biovolume in the two copper-based treatments (Captain and $CuSO_4$) were the only products to
288 remain significantly lower to that of the control across the 35-day experiment ($p \leq 0.05$; Fig.
289 4a). The final ratio of cyanobacteria to total phytoplankton varied greatly between product
290 treatments with Captain, $CuSO_4$, and H_2O_2 having ≥50% of their total biovolume comprised of
291 cyanobacteria (Fig. 5a). Although some variation in findings did occur, phytoplankton
292 biovolume generally mirrored the trends observed in the algal pigment data (Fig. 3).
293
294 *Zooplankton dry biomass*
295 Zooplankton biomass was estimated for all mesocosms on days 0, 1, 7, and 35 of the field
296 experiment. The average starting zooplankton dry biomass was ~602 µg/L across all treatments
297 (Fig. 4b). Mesocosms contained a mixture of cladoceran and copepod taxa, comprising of 38%
298 and 62% of the total biomass, respectively, at the start of the experiment. Starting densities of
299 these genera varied. On average, mesocosms contained *Ceriodaphnia* (1% of total starting
300 biomass), *Diaphanosoma* (10%), Bosmina (25%), copepod nauplii (15%), calanoid copepods
301 (46%), and cyclopoid copepods (4%).
302 There were large effects of treatment ($p < 0.000001$), time ($p < 0.000001$), and treatment
303 x time interaction ($p < 0.000001$) on zooplankton dry biomass (RM-ANOVA) during the 35-day
304 experiment. Only $CuSO_4$, Captain, and PAK-27 treatments were significantly lower than the

control for zooplankton biomass ($p \leq 0.05$; Fig. 4b) while the four other products, although oscillating in value over time, were not significantly different to that of the control. $CuSO_4$ zooplankton biomass remained the lowest over the 35 days. Interestingly, liquid H_2O_2 contained the lowest zooplankton biomass of any product after day 1, but steadily rebounded in number over the next 35 days. Final (day 35) relative biomass between zooplankton groups were *Ceriodaphnia* (2% of final biomass), *Diaphanosoma* (47%), *Bosmina* (3%), copepod nauplii (3%), calanoid copepods (44%), and cyclopoid copepods (0.4%), although diversity and abundance in biomass varied between products (Fig. 5b).

Discussion

This study utilized both a short, microcosm laboratory and five-week, field mesocosm experiment to evaluate seven algal control products in an aquaculture pond. In doing so, both the short- and long-term effectiveness of each product was assessed. The effects of each product on algal pigments representing phytoplankton and cyanobacteria, phytoplankton biovolume, and zooplankton biomass will be described in the following sections, with the focus of this discussion on to the findings of the field experiment. As the chlorophyll pigment and total phytoplankton biovolume data are both assessments of total phytoplankton densities in the field experiment, the results of these two assessments will be described within a single section.

Effects on phytoplankton (using chlorophyll and phytoplankton biovolume data)

Phytoplankton communities in the mesocosms at the start of the experiment were dominated by green algae (Fig. 5). Cyanobacteria were the next largest taxa present. Of the products tested, Captain and $CuSO_4$ best reduced phytoplankton abundance in the field experiment (Figs. 3 and 4). When assessing the chlorophyll data, both Captain and $CuSO_4$ significantly reduced chlorophyll within the first 7 days of the experiment, and $CuSO_4$ was the only product to significantly lower chlorophyll levels to that of the control for the duration of the 35-days (Fig. 3). Similarly, phytoplankton biovolume data in Captain and $CuSO_4$ treatments were significantly lower than the control (Fig. 4).

The broad-spectrum toxicity and extended duration of select copper products have been observed in prior studies (Murray-Gulde 2002; Viriyatum and Boyd 2016). The efficiency of copper does vary and can often be attributed to the form it is applied. For instance, Viriyatum and Boyd (2016) observed that a single treatment of $CuSO_4$ encapsulated in a slow-release

337 coating had an equally comparable reduction in phytoplankton over four months when compared
338 to ponds treated with basic $CuSO_4$ applied weekly. Although differences between Captain and
339 $CuSO_4$ were observed in this study, both products were found to be the most efficient at reducing
340 phytoplankton over time (when assessing chlorophyll and algal biovolume data).

341 VigorOx SP-15 and Peraclean reduced phytoplankton similar to that of the copper-based
342 products in the laboratory experiment (Fig. 2) but caused negligible effects on phytoplankton in
343 the field (Figs. 3 and 4). Indeed, it was observed in the field experiment that phytoplankton of
344 both products increased from day 0 to 1 (Figs. 3 and 4). On one hand, this significant difference
345 between the laboratory and field studies is likely due to contact time, species assemblages, and
346 more ideal conditions in the laboratory. Such discrepancies between lab and field-based studies
347 may indicate how short-term, laboratory studies poorly reflect what happens in nature. On the
348 other hand, VigorOx SP-15 and Peraclean reduced cyanobacteria while having small effects on
349 other algae, including beneficial green algae. Such findings would benefit farmers as they seek to
350 balance the presence of algae to support dissolved nutrient removal and promote oxygenation
351 within ponds while selecting against cyanobacteria.

352 Granulated PAK-27 and liquid H_2O_2 produced similar reductions of phytoplankton in the
353 laboratory and field study. However, unlike PAK-27, liquid H_2O_2 produced an immediate
354 decline in phytoplankton that quickly rebounded to values greater than that of the control in the
355 following days and weeks. Interestingly, only the granulated H_2O_2-based product selectively
356 reduced cyanobacteria. The effectiveness of H_2O_2 as an algaecide has been noted to vary between
357 cyanobacterial species and phytoplankton taxa for both PAK-27 (Sinha et al. 2018) and liquid
358 H_2O_2 (Yang et al. 2018) and may be of use to keep some amount of algae present within farm
359 ponds.

Phoslock did not significantly reduce phytoplankton relative to the control in the laboratory or field experiment. Phoslock targets phosphorus by binding and removing it to the sediments (Bishop et al. 2018). The efficiency of this product is meant for the long-term control of phosphorus in systems leading to the eventual change in nutrient ratios and thereby a reduction in phytoplankton density. This is likely the reason for its undetectable effect in the short-term in the laboratory experiment as well as small effects in the field experiment. The constant addition of nutrients to the water column by way of feed and fish waste-products may further reduce the success of Phoslock in intensive aquaculture. However, the long-term effect of Phoslock on removing cyanobacteria showed promise in this study (to be discussed).

Effects on cyanobacterial biomass

Captain and $CuSO_4$ both effectively reduced cyanobacteria in the laboratory and field experiments (Fig. 2 and 3) reflecting the results documented in prior studies (Murray-Gulde 2002; Viriyatum and Boyd 2016). Although a concentration of 0.4 mg/L as copper was used in this study, others have used smaller, repeated doses to remove cyanobacterial genera capable of producing off-flavors in farm ponds (Schrader et al. 2005). Moreover, treatments comparable to that used in this study have been shown to reduce cyanobacterial genera capable of producing microcystin (Greenfield 2014). Off-flavors and microcystin were too low to be detectable in the collected water samples of this study, and therefore not reported. Kansole and Lin (2017) found that hydrogen peroxide (20 mg/L) could degrade microcystin compounds while $CuSO_4$ (2 mg/L) could not and that both treatments had a deleterious effect on bacterial populations that could degrade microcystin naturally. Such reports reflect ability of copper to reduce phytoplankton, but not cyanotoxins, at environmentally relevant concentrations. In addition, It was observed that

383 Captain and $CuSO_4$ enclosures both were both dominated by cyanobacteria by the end of the 35-
384 day field experiment (Fig. 5). Although phytoplankton in Captain and $CuSO_4$ treatments were
385 the lowest observed across the tested products, such a shift in the dominant phytoplankton taxa
386 could promote cyanobacterial blooms in the future.
387
388 Similar to that of the copper-based algaecides, VigorOx SP-15 and Peraclean (peracetic
389 acid-based) significantly reduced cyanobacteria in both the laboratory and field experiments.
390 Yet, both products did not significantly reduce phytoplankton in the field experiment, which
391 were dominated by green algae (Fig. 2 and 3). This selective effectiveness has been observed for
392 other algaecides, such as H_2O_2 (Yang et al. 2018), which is a chemical also present in VigorOx
393 SP-15 and Peraclean. Reasons for this selectiveness may be attributed to the lack of a cell wall in
394 prokaryotes (e.g., cyanobacteria; Yang et al. 2018), the proximity of the photosynthetic
395 apparatuses to the plasma membrane (Yang et al. 2018), or the overall ability to degrade
396 bacterial cell membranes (Mikula et al. 2012). Once hydrogen peroxide enters into the cell of
397 cyanobacteria, it induces oxidative stress, damaging proteins, genes, and photosystems (Liu et al.
398 2005; Latifi et al. 2008), and can be compounded by UV light exposure (Drábková et al. 2012)
399 and/or the presence of iron (Zepp et al. 1992). The selective effect of H_2O_2 against cyanobacteria
400 was observed in the field experiment for most treatments, except liquid H_2O_2. However the
401 selectivity of peracetic acid among phytoplankton taxa is understudied and should be further
402 researched.
403
404 Liquid H_2O_2 and PAK-27 had similar reductions in cyanobacterial densities in the
405 laboratory experiment (Figs. 2 and 5). However, substantial differences were observed between
406 both the findings of laboratory and field experiments as well as between the two products in the

field (Figs. 2, 3, and 5b). It was observed in the field experiment that liquid H_2O_2 first reduced cyanobacteria, but phycocyanin then increased greater than the control. In contrast, granulated H_2O_2 kept densities well below that of the control for the duration of the experiment (Fig. 3b and d). Such differences again reflect the dissonance between laboratory and field studies. It should be noted in the field experiment that both liquid H_2O_2 and PAK-27 reduced cyanobacteria for the first three days of the experiment. This finding may support that H_2O_2-based products are effective at quickly removing toxic and problematic cyanobacterial species, as has been suggested in prior studies (Barrington et al. 2013; Sinha et al. 2018; Yang et al. 2018), but repeated treatments may be required for the continual suppression of a bloom (as suggested by Barrington et al. 2013). Prior research has also observed that hydrogen peroxide may degrade cyanotoxins, negating their negative effects once released from the cells of cyanobacteria (Barrington et al. 2013; Kansole and Lin 2017); however, concentrations needed to achieve this are relatively high (e.g., 20 mg/L Kansole and Lin 2017) and may not be economically feasible for fish farmers to utilize (to be discussed) or may directly harm farmed fish.

Similar to the H_2O_2- and peracetic acid-based products, Phoslock was also found to have a significant effect on cyanobacteria in the field experiment, but not on phytoplankton in general (Fig. 3). Such a reduction was likely due to the removal of phosphorus out of the water column as the decrease of cyanobacteria was gradual in the field experiment (Van Oosterhout and Lürling 2013). However, in the laboratory experiment, the removal of cyanobacteria was much more rapid and did not have a similar effect on other phytoplankton taxa (Fig. 2). This finding may suggest that Phoslock bound and removed cyanobacteria upon its application into the jars and that its removal is taxon-specific. Phoslock and other clay compounds have been shown to bind directly with phytoplankton (including cyanobacteria) and remove them from the water

column (Pan et al. 2011; Van Oosterhout and Lürling 2013). The selectivity of such clays on their possible selectivity against cyanobacteria is understudied and should be further studied.

Effects on zooplankton biomass

The seven algal control products revealed varying effects on zooplankton biomass during the field experiment (Fig. 4b). Although zooplankton biomass was reduced by most treatments relative to the controls, zooplankton returned to values similar to that of the control in the Phoslock, liquid H_2O_2, Peraclean, and VigorOx SP-15 treatments (Fig. 4b). In contrast, $CuSO_4$, Captain, and PAK-27 each significantly reduced zooplankton densities below that of the control over the 35 days (Fig. 4b). Significant reductions of zooplankton after a treatment of copper-based algaecides have been observed in prior studies. McIntosh and Kevern (1974) reported that treatments of 3 mg/L of $CuSO_4$-$5H_2O$ significantly reduced copepods and cladocerans in field treatments. However, it has also been observed that water quality factors such as dissolved organic matter will "buffer" the toxicity of copper to zooplankton (De Schamphelaere et al. 2004). These factors may influence the effect when copper is applied to cyanobacterial blooms in more productive systems than that used in this study, although such variables were not measured in our field experiment.

VigorOx SP-15 and Peraclean had minimal effects on zooplankton biomass in this study. As with copper-based products, the toxicity of peracetic acid to zooplankton has been found to be dependent on water quality variables (e.g., dissolved organic matter, salt; Liu et al 2015). Interestingly, Liu et al. (2015) found that the toxicity of peracetic acid products to zooplankton will increase with the amount of H_2O_2 that a product also contains. Of the H_2O_2-based products used in this study, PAK-27 also significantly reduced zooplankton biomass, and liquid H_2O_2

453 greatly reduced biomass after the initial treatment by day 1, but the densities in the liquid H_2O_2
454 treatment rebounded by the end of the 35-day experiment. The toxicity of H_2O_2 to zooplankton
455 has been assessed on numerous occasions (Barrington et al. 2013; Reichwaldt et al. 2012; Yang
456 et al. 2018), and findings of these past studies are aligned with the results from our field
457 experiment.
458 Lastly, the effect of Phoslock on zooplankton biomass was minimal. Lürling and Tolman
459 (2010) observed that the active ingredient (lanthanum) of Phoslock was not toxic to *Daphnia* at
460 concentrations up to 1000 µg/L. It is likely that the rapid removal of Phoslock out of the water
461 column or limited toxicity (relative to that of the other products tested) reduced its effectiveness
462 on the zooplankton biomass in this study.
463
464 *Costs per product treatment*
465 The average cost to treat a 20 acre-foot pond were calculated based on an example
466 dosage of each product used in this study as well as prices for these products as of April 2020
467 (Table 3). Copper sulfate had a remarkably lower cost and application volume than any other
468 product. This relatively low price likely reflects the wide availability and popularity of
469 $CuSO_4$, and the relatively lower application volume contributes to the use of copper for fish
470 farmers. Conversely, PAK-27 had the highest cost. It should be noted that all costs are subject
471 to change and may be lower if a product is purchased at a larger quantity. Further, prices may
472 be influenced if an algaecide gains USEPA approval for use in food-fish aquaculture. At this
473 time, $CuSO_4$ is the only product fully allowed by the EPA. However, PAK-27, Captain, and
474 Phoslock are approved to control nuisance algae and cyanobacterial blooms in some states.
475

Disclaimers

An algaecide must first receive USEPA approval before its use in food fish aquaculture in the U.S., requiring significant effort and costs. It should be noted that some algaecides are approved for use to combat nuisance plants and algae in non-aquaculture ponds. Such approvals vary from state to state. In general, "any product or device that is used or implied to control algae (including cyanobacteria) must be registered by the USEPA under FIFRA" **(**Laughinghouse et al. 2020). Moreover, guidelines and directions provided by the vendor on the labeled instructions should be explicitly followed. The objectives of these experiments were to compare efficacy in a demonstration/research environment and not to endorse the use of any specific product. Local, state and federal authorities should be consulted before any chemical is applied to surface waters.

The assessment of oxygen during the night hours or amounts levels of ammonia were not checked during this study to minimize contamination between enclosures and treatments. Such factors can be major issues to fish after major a phytoplankton or plant die-off as oxygen concentrations will be depleted through microbial disposition (Chislock et al 2013a) and ammonia concentrations may increase through the breakdown of organic material (Farnsworth-Lee and Bake 2000) or through the lack of uptake by phytoplankton (Boyd et al. 1975). Moreover, both off-flavors and microcystin can be released from cyanobacteria as their cells rupture, an issue that can be promoted by algaecide applications (Jones and Orr 1994; Jüttner and Watson 2007). Applicators should monitor their ponds for these parameters after an application of algaecide to avoid serious issues.

Conclusions

This study utilized both a laboratory and field study to compare algal control products to one routinely used ($CuSO_4$) in farm-pond aquaculture as well as a treatment-less control. Our findings indicate that copper-based products, Captain and $CuSO_4$, had the greatest reduction of phytoplankton and cyanobacteria in both the laboratory and field studies. Copper sulfate also had the lowest treatment costs relative to the other algaecides tested and is the only algaecide approved for use in food-fish aquaculture to date. However, it was observed that copper-based products had significant adverse effects on zooplankton densities and its broad-spectrum toxicity may not be useful in all situations.

Peracetic-acid based products, VigorOx SP-15 and Peraclean, as well as a granulated H_2O_2-based product (PAK-27), significantly removed cyanobacteria while having small effects on other phytoplankton, specifically beneficial green algae, during the field experiment. Moreover, peracetic acid-based products had small effects on zooplankton when compared to the control treatment. Surprisingly, liquid H_2O_2 showed to have short-lasting effects on phytoplankton abundance while also promoting cyanobacteria by the end of the field experiment. In addition, large negative effects of both H_2O_2-based products on zooplankton was observed. The cost of the peracetic acid- and H_2O_2-based products ranged from moderate-to-high relative to the others tested.

The clay product, Phoslock, showed little significant effect on phytoplankton in the field experiment, but significantly reduced cyanobacterial abundance. Given that the mechanism that Phoslock exploits to control phytoplankton is by binding phosphorus and making it unavailable for phytoplankton, it may take some time for this treatment to show effects relative to true algaecides tested in this study. In the laboratory experiment, cyanobacterial densities were

immediately reduced upon the application of Phoslock and may indicate its ability to bind and selectively remove cyanobacteria from the water column. The cost of Phoslock was the second highest treatment used in this study, but perhaps may be circumvented if fewer applications are needed.

In this study, it was made clear that extended results from the tightly controlled lab studies to the field should be done with caution. Also, the effects of most algaecides on phytoplankton are short-lived. As this study was performed in floating mesocosms, we encourage the use of full-scale pond trials to rigorously test multiple algaecides under uniform conditions to evaluate their efficacy. Aspects such as mixing, sedimentation, and application methods may influence treatment effectiveness and longevity.

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672

Figure legends

Fig. 1. Floating dock that held the mesocosms for the field experiment. Additional mesocosms pictured here were not used as part of this study.

Fig. 2. Dynamics of phytoplankton (as chlorophyll (µg/L)) or cyanobacteria (as phycocyanin (µg/L)) across a 14-day laboratory, microcosm (0.4 L) experiment where seven algaecides were tested relative to an algaecide-less control (0.0 mg/L). Only data for the targeted concentration used in the field experiment for each algaecide are shown. Data for other algaecides concentrations are available in the Supplementary Materials. The Phoslock application rate was calculated as 200 units (µg) of Phoslock for every unit (µg) of total phosphorus in a waterbody given an estimated volume. Panels A and B show absolute data, while panels C and D show relative concentrations (calculated as (product treatment mean – control mean)/control mean) for each sampling day. Error bars in panels A and B represent one standard error. Letters in brackets after each product are results from Tukey's multiple comparison tests. Products sharing the same letter are not statistically different ($p \geq 0.05$) using repeated measures ANOVA.

Fig. 3. Dynamics of phytoplankton (as chlorophyll (µg/L)) or cyanobacteria (as phycocyanin (µg/L)) across a 35-day field mesocosm (1,310 L) experiment where seven algaecides were tested relative to an algaecide-less control (0.0 mg/L or no Phoslock added). The Phoslock application rate was calculated as 200 units (µg) of Phoslock for every unit (µg) of total phosphorus in a waterbody given an estimated volume. Panels A and B show absolute data, while panels C and D show relative concentrations (calculated as (product treatment mean – control mean)/control mean) for each sampling day. Error bars in panels A and B represent one

standard error. Letters in brackets after each product are results from Tukey's multiple comparison tests. Products sharing the same letter are not statistically ($p \geq 0.05$) different using repeated measures ANOVA.

Fig. 4. Dynamics of (A) phytoplankton biovolume (μm^3/ml) and (B) zooplankton dry biomass (µg/L) across a 35-day field mesocosm (1,310 L) experiment where seven algaecides were tested relative to an algaecide-less control (0.0 mg/L or no Phoslock added). The Phoslock application rate was calculated as 200 units (µg) of Phoslock for every unit (µg) of total phosphorus in a waterbody given an estimated volume. Error bars in panels A and B represent one standard error. Letters in brackets after each product are results from Tukey's multiple comparison tests using $\log_{10}$-transformed data. Products sharing the same letter are not statistically ($p \geq 0.05$) different using repeated measures ANOVA.

Fig. 5. Trends in (A) phytoplankton and (B) zooplankton community structure across four sampling days (0 (pre-treatment), 1, 7, and 35) of a 35-day field mesocosm (1,310 L) experiment where seven algacides were tested relative to an algacide-less control (0.0 mg/L or no Phoslock added). The Phoslock application rate was calculated as 200 units (µg) of Phoslock for every unit (µg) of total phosphorus in a waterbody given an estimated volume.

Figures

Fig. 1

Fig. 2

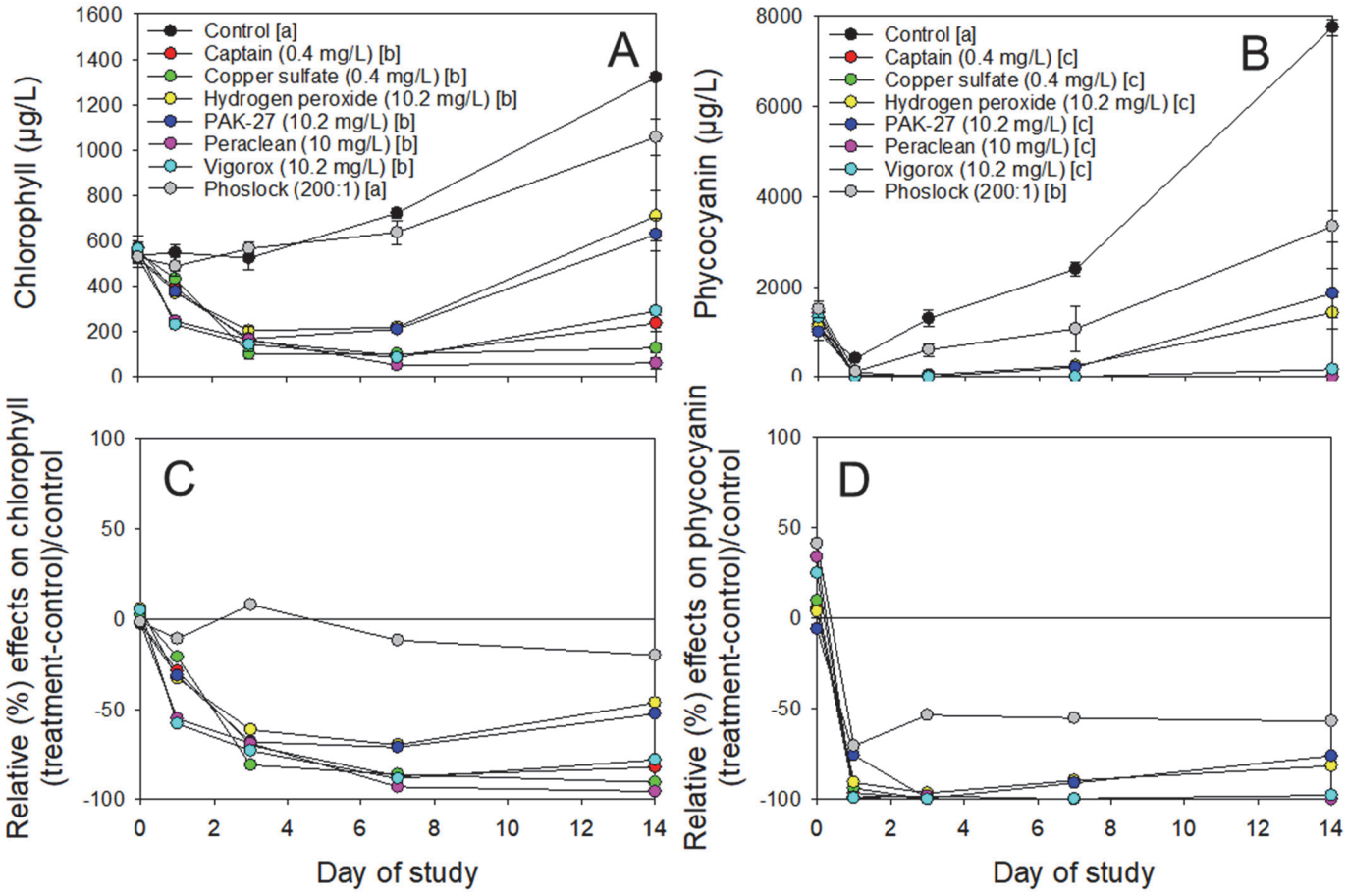
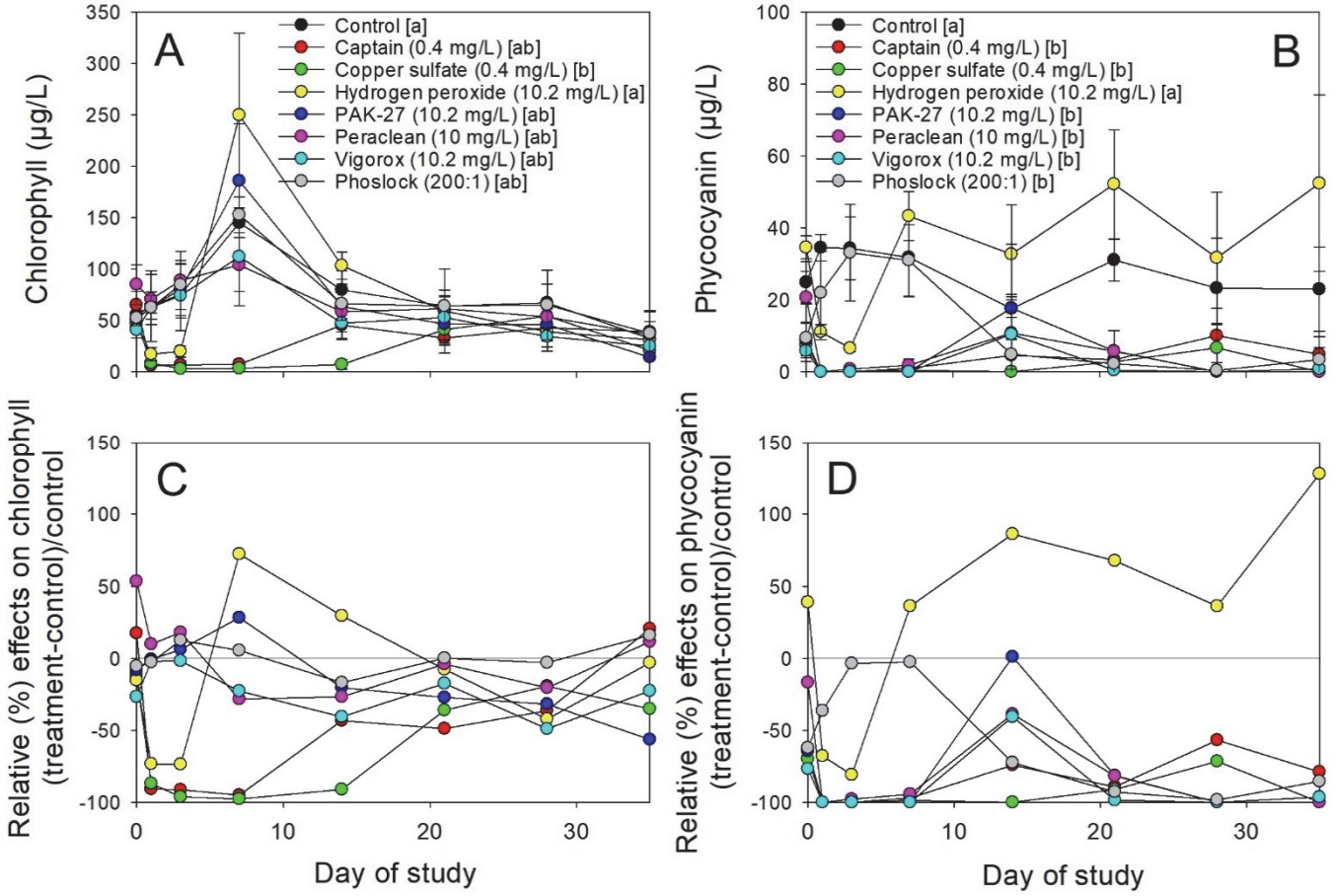
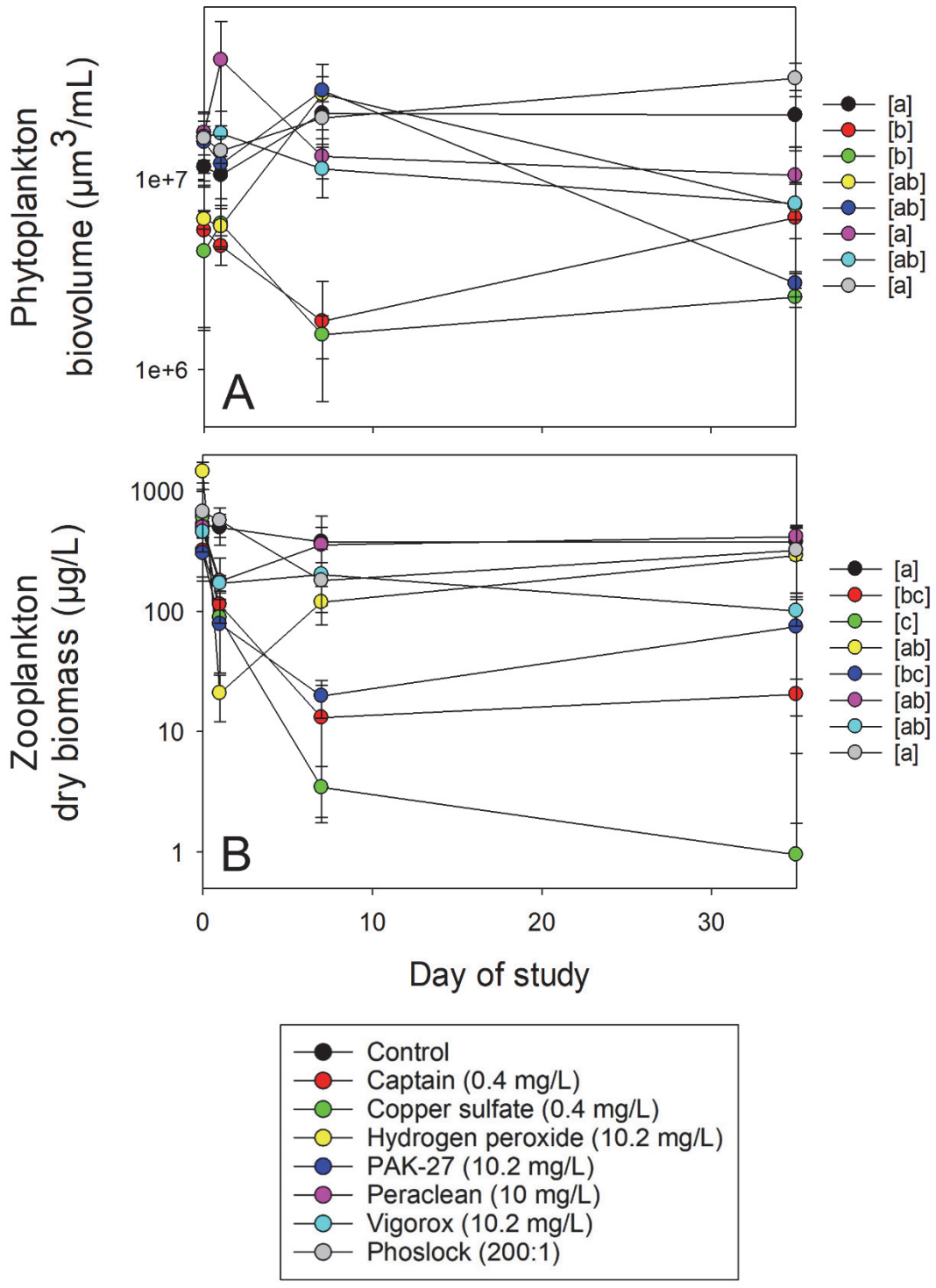

A
Chlorophyll (µg/L)
Control [a]
Captain (0.4 mg/L) [b]
Copper sulfate (0.4 mg/L) [b]
Hydrogen peroxide (10.2 mg/L) [b]
PAK-27 (10.2 mg/L) [b]
Peraclean (10 mg/L) [b]
Vigorox (10.2 mg/L) [b]
Phoslock (200:1) [a]
B
Phycocyanin (µg/L)
Control [a]
Captain (0.4 mg/L) [c]
Copper sulfate (0.4 mg/L) [c]
Hydrogen peroxide (10.2 mg/L) [c]
PAK-27 (10.2 mg/L) [c]
Peraclean (10 mg/L) [c]
Vigorox (10.2 mg/L) [c]
Phoslock (200:1) [b]
C
Relative (%) effects on chlorophyll (treatment-control)/control
D
Relative (%) effects on phycocyanin (treatment-control)/control
Day of study
Day of study

Fig. 3


A
B
C
D
Control [a]
Captain (0.4 mg/L) [ab]
Copper sulfate (0.4 mg/L) [b]
Hydrogen peroxide (10.2 mg/L) [a]
PAK-27 (10.2 mg/L) [ab]
Peraclean (10 mg/L) [ab]
Vigorox (10.2 mg/L) [ab]
Phoslock (200:1) [ab]
Control [a]
Captain (0.4 mg/L) [b]
Copper sulfate (0.4 mg/L) [b]
Hydrogen peroxide (10.2 mg/L) [a]
PAK-27 (10.2 mg/L) [b]
Peraclean (10 mg/L) [b]
Vigorox (10.2 mg/L) [b]
Phoslock (200:1) [b]
Chlorophyll (µg/L)
Phycocyanin (µg/L)
Relative (%) effects on chlorophyll (treatment-control)/control
Relative (%) effects on phycocyanin (treatment-control)/control
Day of study
Day of study

58 **Fig. 4**

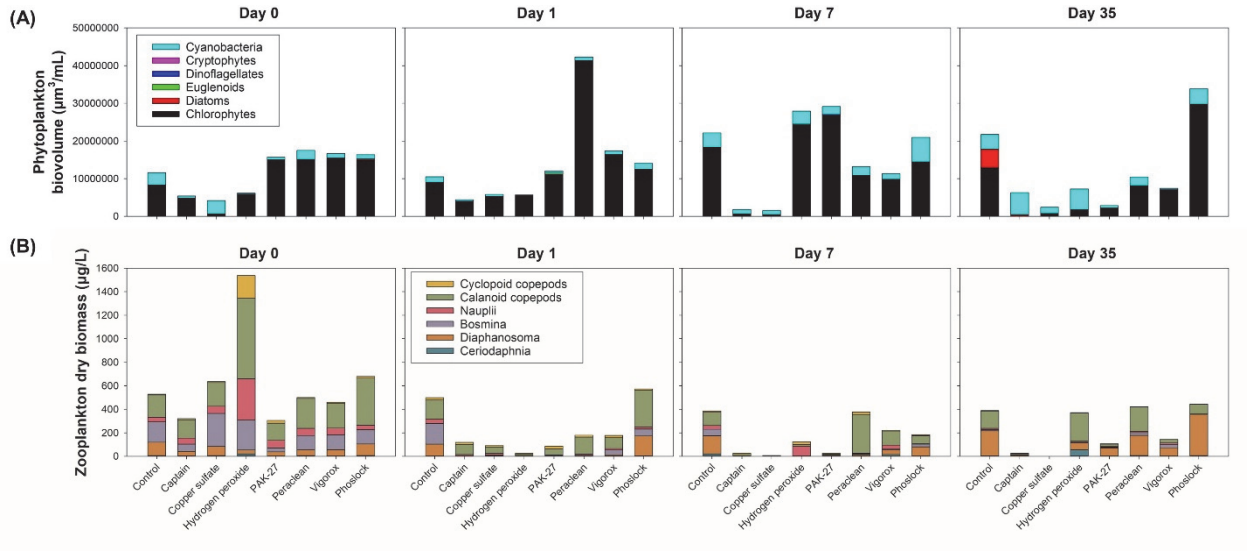
59



60

61

62 **Fig. 5**


(A)
Day 0
Day 1
Day 7
Day 35
Phytoplankton biovolume (µm³/mL)
50000000
40000000
30000000
20000000
10000000
0
Cyanobacteria
Cryptophytes
Dinoflagellates
Euglenoids
Diatoms
Chlorophytes
(B)
Zooplankton dry biomass (µg/L)
1600
1400
1200
1000
800
600
400
200
0
Cyclopoid copepods
Calanoid copepods
Nauplii
Bosmina
Diaphanosoma
Ceriodaphnia
Control
Captain
Copper sulfate
Hydrogen peroxide
PAK-27
Peraclean
Vigorox
Phoslock

63