PLANKTOTHRIX AGARDHII RESPONSE TO HYDROGEN PEROXIDE TREATMENT IN SPAHRS QUARRY

by

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ABSTRACT

Cyanobacteria blooms are a major environmental concern and are becoming more frequent occurrences in aquatic environments. Those that produce the hepatotoxin microcystin, like *Planktothrix agardhii*, are of particular concern. This study tested the effectiveness of treating an entire quarry with a single application of H_2O_2 to suppress a bloom of *P. agardhii.* While many laboratory studies have tested the effectiveness of H_2O_2 as a cyanocide, only a few field studies have tested its effectiveness against *Planktothrix* when applied to an entire waterbody. Results show that the bloom persisted in the presence of 1.4 mg H_2O_2/L. In addition to not achieving the minimum recommended concentration of 2.0 mg/L, a number of factors were not measured which can influence the success of H_2O_2 treatment. Further studies of *P. agardhii* blooms in Spahrs Quarry would be needed to determine whether different methods or rates of H_2O_2 application would be successful in that environment.

ACKNOWLEDGEMENT AND SPONSORSHIP

First and foremost, I thank Dick Klein, the owner of Spahrs Quarry, for hosting this work on his property. This study would not have been possible without his hospitality. Thank you to Bob Foote for applying the hydrogen peroxide treatment to Spahrs Quarry, making this study possible. Thank you to Dr. Kevin Sellner for granting me the opportunity to lead this study, for all his guidance and patience throughout the process, and for continuing his support even into his retirement. Thank you to Dr. Drew Ferrier for stepping in as my project advisor when Dr. Sellner retired, for his guidance, and for motivating me to finish this paper. For all their assistance during the 2018 field season, I thank the Center for Watershed and Coastal Studies staff who accompanied me in the field: Alexander Marinelli, Nathan Purser, and Lillian Myers. Thank you to Sandra Demas for all the miles driven and time spent lending her microbiology expertise and assisting with cell counts in the lab. For their unwavering love, patience, and encouragement over all the years it has taken me to get here, I thank my friends and family – most of all, my husband, Ben and my dear friend, Marie Beam.

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INTRODUCTION

Cyanobacteria blooms are a major environmental concern that are becoming more frequent occurrences in aquatic environments that are influenced by anthropogenic changes such as nutrient inputs, hydrologic alterations (i.e., water withdrawal), climate change, and modifications to benthic and planktonic habitats (Paerl 2014). Blooms are generally increasing world-wide due to stable water columns, nutrient enrichment, and increasing temperatures (Mattheiss et al. 2017).

Cyanobacteria blooms are harmful to the environment. When they decay through bacterial decomposition, oxygen depletion occurs which can lead to hypoxia and anoxia (Paerl 2014). They reduce water clarity, in turn impacting photosynthesis and impairing the growth of submerged aquatic vegetation, which also impacts habitats for other aquatic organisms such as invertebrates and fish (Paerl 2014). The presence of surface cyanobacteria blooms can also negatively impact populations of eukaryotic phytoplankton, which are an important part of the food web through shading and production of allelopathic substances (Paerl 2014). Cyanobacteria blooms are not only an environmental threat, but they are a threat to human health as well, as many of them produce harmful hepato- and neurotoxins (Paerl 2014).

The focus of this experiment was to suppress a species of cyanobacteria known as *Planktothrix agardhii*. It is found in shallow, turbid, eutrophic lakes (Tonk et al. 2005) and has been discovered in local lakes including Spahrs Quarry and Lake Anita Louise, both in Frederick County, MD (Mattheiss et al. 2017). It is also widely distributed in temperate areas (Oberhaus et al. 2007).

Generally speaking, *P. agardhii* is more predominant in cooler fall months when temperatures and irradiance are lower. P. *agardhii* tends to concentrate around the summer hypolimnion or thermocline in lakes where it exhibits little to no growth, rapidly increasing in growth when isothermal mixing occurs in the fall (Reynolds 1984, Kurmayer et al. 2016). A study of a shallow eutrophic lake dominated by *P. agardhii* in France observed *P. agardhii* throughout the entire year with highest density in the winter (Briand et al. 2002). Additionally, *P. agardhii* is most likely to form a bloom in water temperatures ranging from 10.3-22.6 °C (Mischke et al. 2003, Toporowska et al. 2010, Toporowska et al. 2016).

P. *agardhii* can produce the hepatotoxin microcystin (Kurmayer 2005, Mattheiss et al. 2016, Paerl 2014). The production of this toxin makes the genus *Planktothrix* a particularly important cyanobacterium (Kurmayer 2005). Microcystins are hepatoxic, monocyclic polypeptides that have been found to cause liver damage through the inhibition of protein phosphatases (MacKintosh et al. 1990, Yoshizawa et al. 1990) and promote liver tumors (Nishiwaki-Matsushima et al. 1992). MacKintosh et al. (1990) suggested that protein phosphatase inhibition caused by microcystin may explain why both floral and faunal species diversity is commonly reduced in waters with toxic cyanobacterial blooms.

Both toxicity and growth rate of *P. agardhii* are influenced by light (Tonk et al. 2005). The specific growth rate of P. *agardhii* has been found to be limited by photon irradiance up to approximately 60 µmol/m^2/s and inhibited at levels greater than approximately 100 µmol/m^2/s (Tonk et al. 2005), suggesting that growth rate is greatest at a photon irradiance between 60-100 µmol $m^{-2}s^{-1}$. Microcystin-DeLR, a more toxic variant of microcystin produced by *P. agardhii*, has been found to increase with increasing photon irradiance, while the less toxic variant, microcystin-DeRR, decreases with increasing photon irradiance (Tonk et al. 2005). These

findings confirm that a more toxic variant of microcystin can be produced by *P. agardhii* during sunny periods of weather when recreational water activities are more popular (Tonk et al. 2005).

Many methods have been tested to suppress cyanobacteria blooms such as effective microorganisms (a blend of approximately 80 species of microorganisms which can include fermenting fungi, photosynthetic bacteria, yeasts, actinomycetes, and lactic acid bacteria), golden algae, ultrasound, conventional compounds (herbicides, copper-based compounds, and potassium chloride), and natural compounds including plant/tree extracts (barley straw, rice straw, *Ephedra equisetina* root extract, anthraquinones, L-Lysine, sanguinarine, and hydrogen peroxide) (Lurling et al. 2016, Matthijs et al. 2016). While a full review of the benefits and drawbacks of each potential treatment will not be provided here, it is important to note the wide variety of methods that have been explored and that many appear to either lack effectiveness, pose risks to aquatic life and water quality, and/or are only effective for small volume applications, with the exception of hydrogen peroxide (Matthijs et al. 2016).

Hydrogen peroxide acts rapidly and quickly degrades into water and oxygen, leaving no trace of chemical application; a lake can be safe for swimming or other activities within just three days of application (Matthijs et al. 2016). Hydrogen peroxide treatment causes very little or no damage to other plankton species at/below a concentration of five mg/L, and hence, is considered safe for most other aquatic biota (Matthijs et al. 2016).

Effectiveness of hydrogen peroxide treatment in suppressing cyanobacteria blooms has been shown to be impacted by a number of factors including genetic variation in H_2O_2 sensitivity in different strains (Lurling et al. 2020; Schuurmans et al 2018), degradation/residency time (impacted by a number of factors including biomass, water reductive power (influenced by dissolved solutes or organic matter and presence of green algae (which degrades hydrogen

peroxide at a faster rate than cyanobacteria)(Arvin and Pedersen 2015, Matthijs et al. 2012, Weenink et al. 2015, Weenink et al. 2021), availability (Sandrini et al. 2020), and light intensity (Piel et al. 2019).

While many laboratory studies have been conducted to test the effectiveness of hydrogen peroxide as a cyanocide as noted by Matthijs et al. (2016) and Matthijs et al. (2011), only a few field studies have occurred to test its effectiveness against cyanobacterial blooms containing *Planktothrix* when applied to an entire waterbody (Barrington et al. 2013, Lusty and Gobler 2023; Piel et al. 2021, Sinha et al. 2018, Mattheiss et al. 2017, Matthijs et al. 2012, Weenink et al. 2021, Yang et al. 2018).

This study tested the effectiveness of hydrogen peroxide in erradicating cyanobacteria (*Planktothrix agardhii*) from Spahrs Quarry in Thurmont, MD where there had been a persistent bloom for 5-10 years. The owner of this quarry reached out to Hood College's Center for Coastal and Watershed Studies (CCWS) for assistance in treating the bloom. The CCWS collected water samples and observed that the quarry had a sawdusty color and high near-surface turbidity in mid-to late April of 2018. Maryland Department of Natural Resources (DNR) confirmed the taxon was *Planktothrix agardhii* and that microcystin levels reached 92 ppb on April 21, 2018, far exceeding the recreational exposure limit of 10 ppb suggested by the World Health Organization and adopted by Maryland in its water contact advisories (Maryland DNR, 2010).

This study tested the null hypothesis that *P. agardhii* will persist in the presence of the treatment and the alternative hypothesis that a treatment of 1.0-2.5 mg/L hydrogen peroxide will suppress the bloom, with the treatment and analyses extending from July 18 – November 12, 2018.

MATERIALS AND METHODS

Hydrogen Peroxide Application

The experiment was conducted at Spahrs Quarry in Thurmont, MD where a bloom of *P. agardhii* dominated quarry phytoplankton in the winter-spring, remaining present throughout the year (K. Sellner, pers. comm.). The entire 5-acre quarry was treated with GreenClean PRO®, a hydrogen peroxide cyanocide, by dispersing the hydrogen peroxide crystals across the surface of the quarry from a small motorized jon boat with a lawn fertilizer spreader modified to deliver the peroxide granules from the boat's bow (Figure 1).

Figure 1. Hydrogen peroxide application in Spahrs Quarry, Thurmont, MD on 7/19/2018.

The amount of GreenClean PRO® needed to achieve the target concentration was pre-determined by the chemical applicator and project advisor based on the amount of GreenClean PRO® that had been applied to a smaller 3-acre Lake Anita Louise to achieve a 3 mg/L

concentration (K. Sellner, pers. comm.).Three hundred fifty pounds of GreenClean PRO® was applied to Lake Anita Louise, while 550 pounds was applied to Spahrs Quarry (K. Sellner, pers. comm.). The target concentration was between 1.0-2.5 mg H_2O_2/L.

Sampling Timing, Distribution and Field Measurements

The quarry was sampled in five locations; surface samples were taken at all sites throughout the lake with one in the center/deepest part of the lake (approximately 12.8 m) (Figure 2). Sampling occurred one day prior to treatment to establish a baseline (7/18/2018), the day of treatment (7/19/2018), one day after treatment (7/20/2018), four days after treatment (7/23/2018), seven days after treatment (7/26/2018), and monthly thereafter for four months (8/22, 9/18, 10/15, and 11/12/2018).

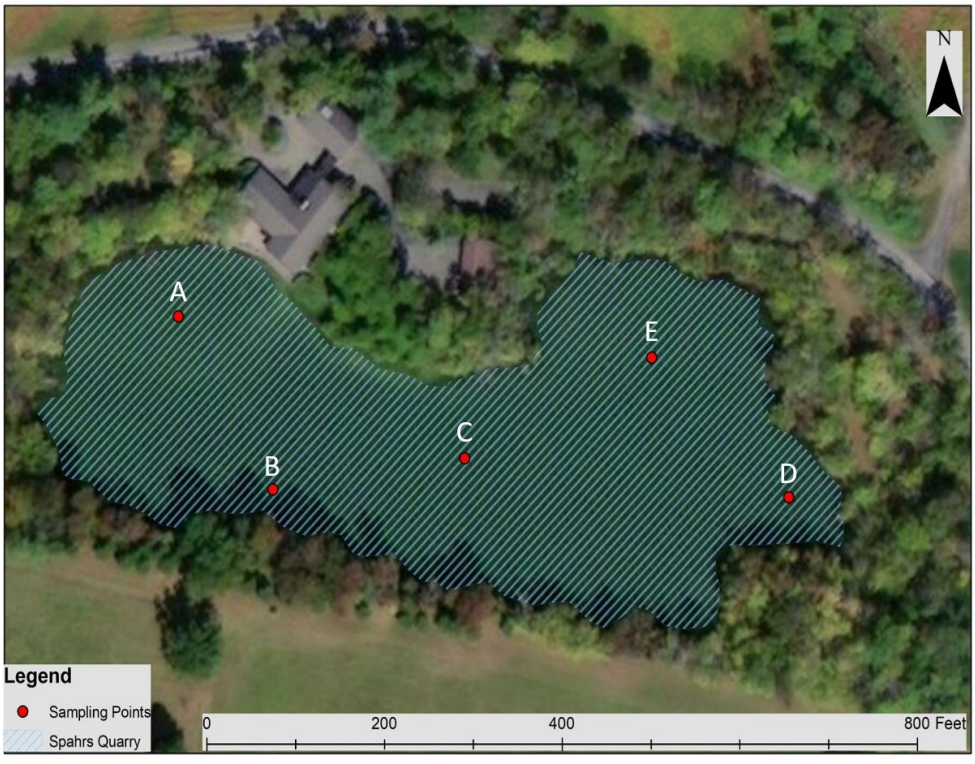

Figure 2. Map of Spahrs Quarry, Thurmont, MD with sampling sites indicated as A through E.

At station C, a vertical profile was taken to measure dissolved oxygen (DO), temperature (^{o}C), and conductivity (µS/cm) using a YSI Pro2030 meter. DO throughout the vertical profile identified the location of the oxycline where *P. agardhii* was expected to concentrate. Light penetration was also measured at each sampling location using a Secchi disc.

A surface sample (10 cm below the surface) was collected at all five sampling locations during each sampling event. Using a Nansen bottle, samples were taken at the oxycline depth/mid-depth in the center of the quarry for each sampling event and two below, the oxycline and near-bottom, to determine *Planktothrix* sp. presence and hydrogen peroxide concentrations through the water column, resulting in eight samples for each sampling event.

Fluorescence

All samples were transported back to a dock where measurements of relative fluorescence of chlorophyll *a* and phycocyanin were taken using a Turner Designs AquaFluor® portable fluorometer. While chlorophyll levels are correlated with levels of phytoplankton and algae in general (Higgins 2014), phycocyanin is strongly correlated with concentrations of cyanobacteria (Brient et al. 2008) and *Planktothrix agardhii* is known to have the blue-green pigment phycocyanin (Kurmayer et al. 2016). Phycocyanin:chlorophyll ratios were also calculated as an indicator of cyanobacteria, and likely dominance by *Planktothrix agardhii*; a ratio exceeding 0.25 indicated increasing abundances of the procaryotes.

Hydrogen Peroxide

Hydrogen peroxide (H_2O_2) was measured for all samples using a Hach 0.2-10 mg/L Model Hyp-1 meter and test kit.

Cell Counts

At least one surface sample and one subsurface sample were selected for each sampling date for cell counts. Samples with the highest fluorescence values were processed. Fifty mL was treated with 5 drops of acid Lugol's iodine solution to preserve all plankton. Samples were concentrated via centrifugation using an Allegra-X14 centrifuge to 2 mL final volume. Following removal of the supernatant, samples were re-suspended by tapping gently on a counter top. Two drops of sample were applied to the slide (25 drops = 1 g or 1 ml, so 2 drops = 0.08 ml). *Planktothrix* filaments and cells, as well as phytoplankton group (diatoms, dinoflagellates, flagellates, colonial species, and miscellaneous) abundances were determined using a Nikon H550L Eclipse CI compound microscope at 100X magnification. Only organisms larger than the smallest unit of measurement (0.10 mm) on the ocular micrometer were counted in five view fields. Cells in colonies were quantified and cells per filament were converted to total cell numbers from the product of filaments and the average number of cells per filament determined from counting cells in 10 filaments. Using sample volumes referenced above and areas of the view field and slide, absolute *Planktothrix* abundance was calculated (filaments and cells/mL).

Statistical Analysis

Two separate 2-way ANOVA and Lavene's tests were used to analyze whether the independent variables sampling date and sampling depth had a significant effect on the dependent variables phycocyanin fluorescence and phycocyanin:chlorophyl ratios, as these variables are the most specific indicators of *P. agardhii*.

RESULTS

Peroxide Concentrations

Ambient peroxide concentrations at the surface and through the water column (obtained from vertical profiles at Station C) were non-detectable on 18 July, the day before treating the quarry with GreenCleanPro® (Figures 3-4). The maximum concentration measured at the surface or below reached 1.4 mg/L at the surface the day of treatment. Following treatment, peroxide levels declined at all stations except for Maximum Depth (12.8 m), which peaked at 0.8 mg/L on 20 July, the day after treatment, and remained at 0.6 mg/L through 18 September, declining to 0.4 mg/L through 12 November. At other stations and depths, ambient concentrations of peroxide rose to 0.2 mgL in October and November.

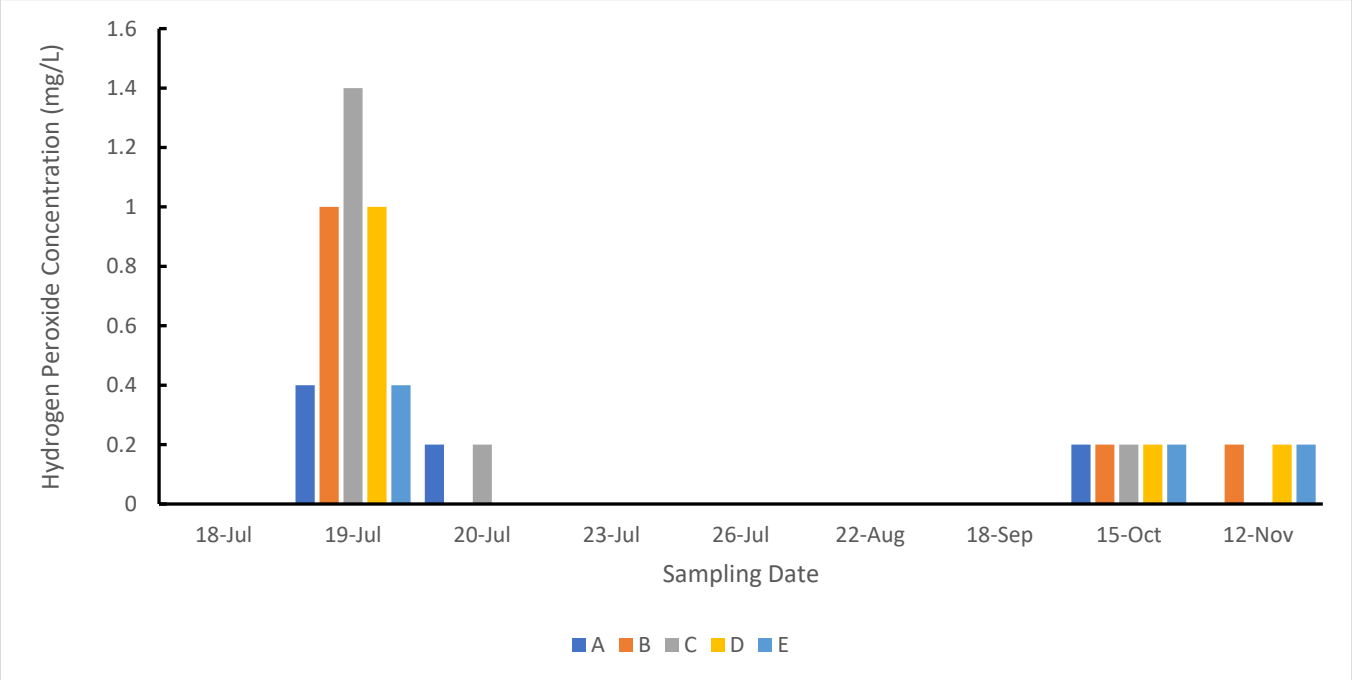

Figure 3. Surface hydrogen peroxide concentrations in Spahrs Quarry.. Bars A through E represent hydrogen perioxide levels at 5 sites within the quarry. See Figure 2 for the locations of each site.

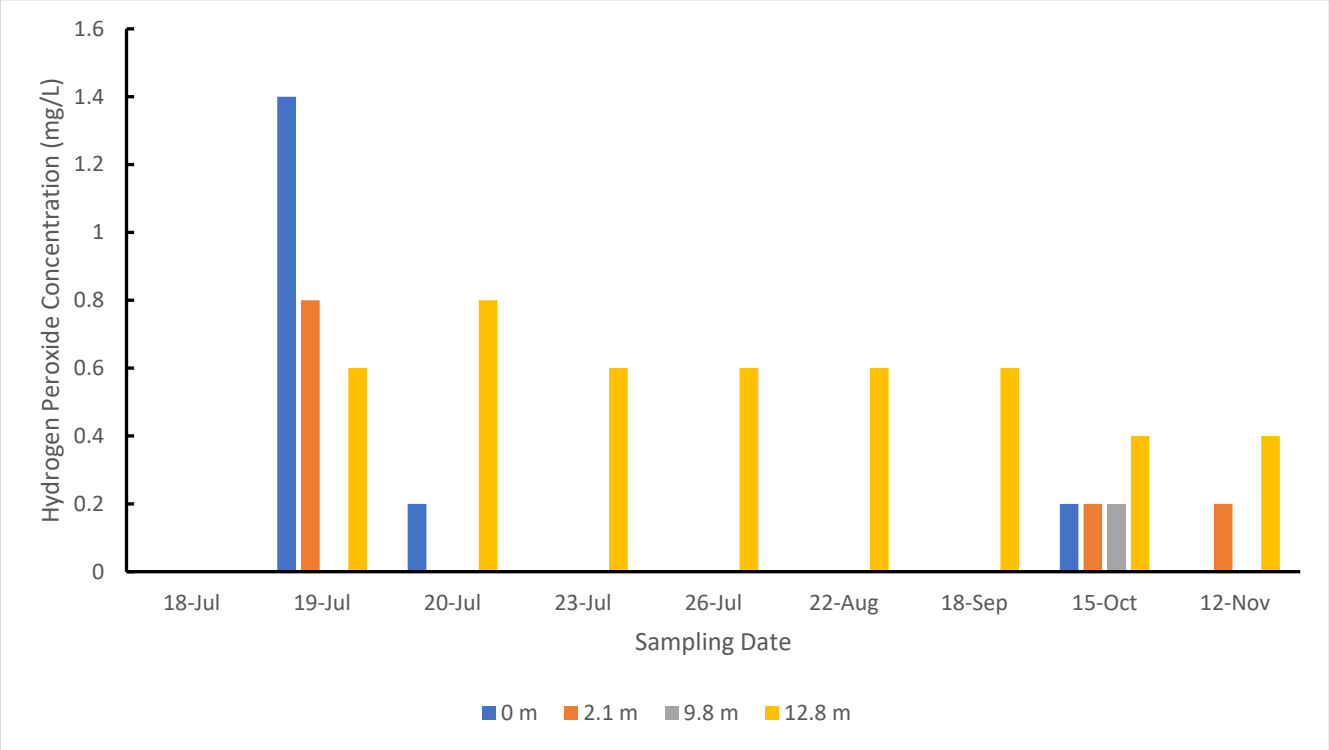

Figure 4. Hydrogen peroxide concentrations at all sampling depths at vertical station (sampling site C) in Spahrs Quarry. See Figure 2 for the location of the vertical station.

Water Transparency

Secchi depth increased initally from 1.9 m the day before treatment to 2 m the day of and day aftet treatment, decreasing again to 0.8 m within 4 days after peroxide treatment, the lowest secchi depth measured during the study (Figure 5). A increase in secchi depth would be expected to occur in coincidence with bloom suppression following treatment. Secchi depth remained relatively constant and did not increase from 1.1 m from 1 week following treatment through November, indicating a potential increase in suspended particles such as algae, cyanobacteria, and/or sediment.

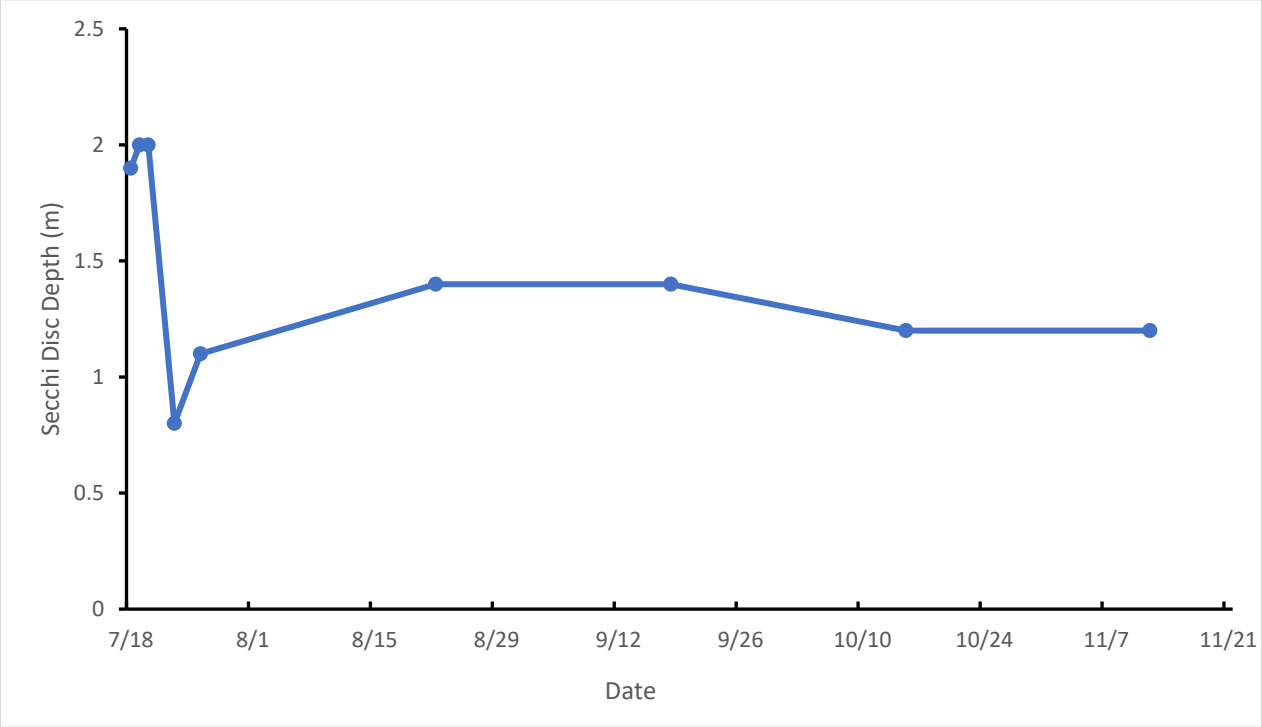

Figure 5. Secchi disc depth (m) at vertical station (sampling site C) in Spahrs Quarry. See Figure 2 for location the vertical station.

Water Temperature

Water temperature remained similar at surface and 2.1 m throughout the study, and the same was true for 9.8 m and 12.8 sampling depths. Temperature fluctuated very little at 9.8 and 12.8 m, with the lowest temperatures reading 5.7 °C on in July, and the highest temperature reading 6.8 °C n November. At shallower depths, the highest temperature was observed at the surface in July at 29.6 °C, with surface and shallower (2.1 m) temperatures steadily trending down through November, when they reached a low of 10.5 °C. A 22.9 °C temperature difference was observed between shallow (surface and 2.1 m) and deeper (9.8 and 12.8 m) sampling depths in July, vs. only a 3.7 °C difference in the fall (Figure 6).

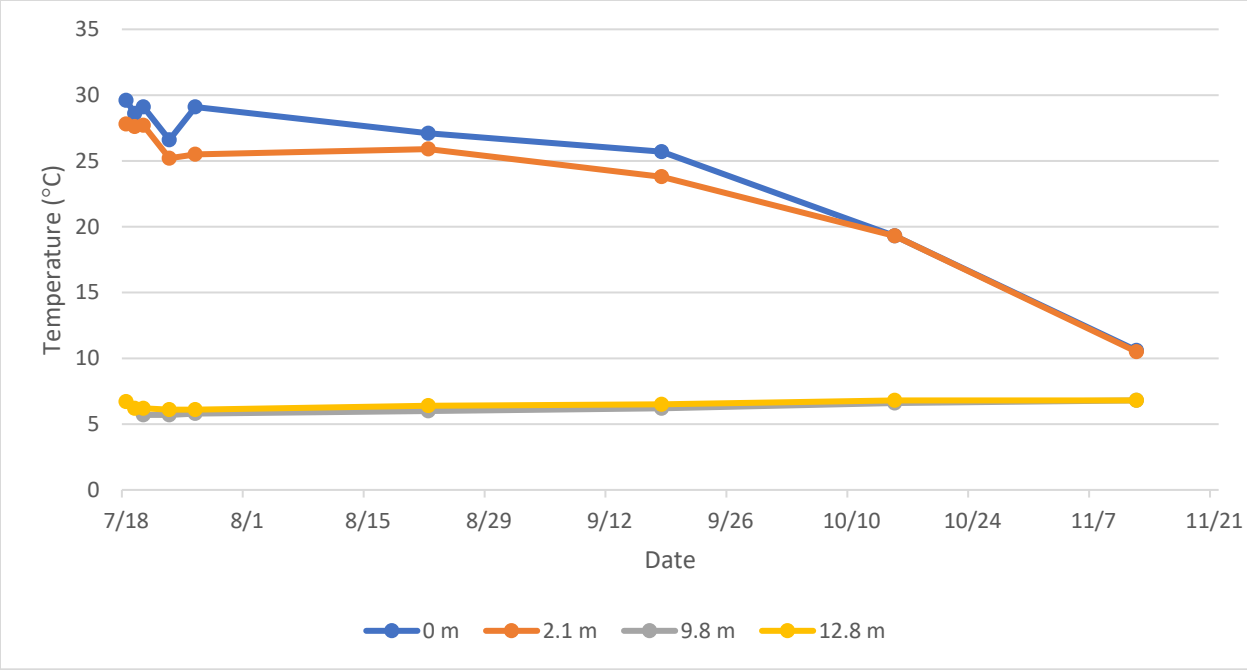

Figure 6. Temperature (℃) at all sampling depths at vertical station (sampling site C) in Spahrs Quarry. See Figure 2 for location of the vertical station.

Total Phytoplankton and Planktonic Cyanobacteria (*P. agardhii* and non- *P. agardhii)*

Abundance of all phytoplankton (cyanobacteria and non-cyanobacteria) rose the day after treatment from 1,361 to 3,289 cells/mL at surface and 16,065 to 72,513 cells/mL at depth. Thereafter, abundance at depth decreased 4 days after treatment and continued to decrease until late August when abundances began to consistently increase thorugh October when it peaked at 115,354 cells/mL, decreasing again in November to 81,312 cells/mL. Surface abundance remained low through August and gradually rose from August through October when it peaked at 186,917 cells/mL, decreasing again in November to 85,306 cells/mL (Figure 7).

Total phytoplankton abundances did not appear to respond to the treatment. However, *P. agardhii* always represented 87% or more of the cell count at depth and over 61% at the surface on all sampling dates except 7/23 and 7/26 (4 and 7 days after treatment), where there were no

cyanobacteria cells observed in surface samples. Non-cyanobacteria cells counts were also low on those days (11 and 5 cells/mL respectively).

While Figure 7 represents total phytoplankton abundances, the trend depicted is most representative of cyanobacteria cell counts and corresponds closely to changes seen in *P. agardhii* filament counts (Figure 8), phycocanin fluorescense (Figures 11-12) and phycocyanin: chlorophyl rations (Figures 13-14).

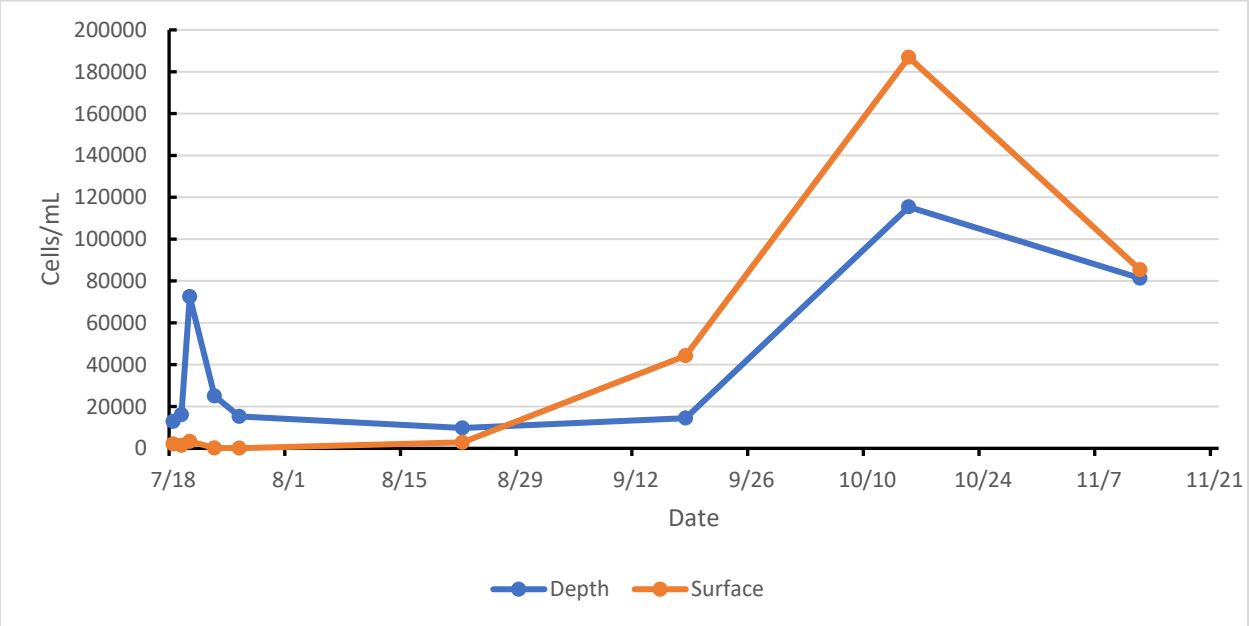

Figure 7. Abundance of cyanobacteria and non-cyanobacteria combined (total phytoplankton abundances) expressed as cells/mL at surface and subsurface depths in Spahrs Quarry.

The day before treatment, absolute *Planktothrix* abundances began at 170 filaments/mL (at depth) and 105 filaments/mL (at the surface). Abundances rose slightly deeper in the water column the day of treatment to 216 filaments/mL, rising further the day after treatment to 563 filaments/mL, while surface abundance dropped to 13 filaments/mL the day of treatment and rose slightly to 92 filaments/mL the day after. By four days after treatment, there was no *Planktothrix* observed at the surface and numbers remained low until September. Abundances at depth fluctuated from July through September and were never reduced to zero. Depth values

actually increased on the day of and day after treatment from pretreatment values (170 filaments/mL), reaching 563 filaments/mL the day after treatment. Abundances at depth did not drop below pre-treatment levels until August (92 filaments/mL). A sharp rise in filaments was seen at the surface in September (2 months after treatment), and levels in both surface and depth samples continued to increase substantially from October-November, reaching highs of 1400 filaments/mL (at the surface) and 1361 filaments/mL (at depth) (Figure 8).

P. agardhii distribution over time also follows the same patterns as phycocyanin fluorescence (Figures 11-12) and phycocyanin: chlorophyll ratios (Figures 13-14), and inversely corresponds to water temperature at the surface. As indicators of *P. agardhii* increased at the surface from September - November, the water temperature steadily decreased (Figure 6). Additionally, as secchi depth dropped and remained low from August-November (Figure 5), total phytoplankton and cyanobacteria abundances were steadily increasing. Generally, secchi depth was observed to be higher when abundances were low and vice versa.

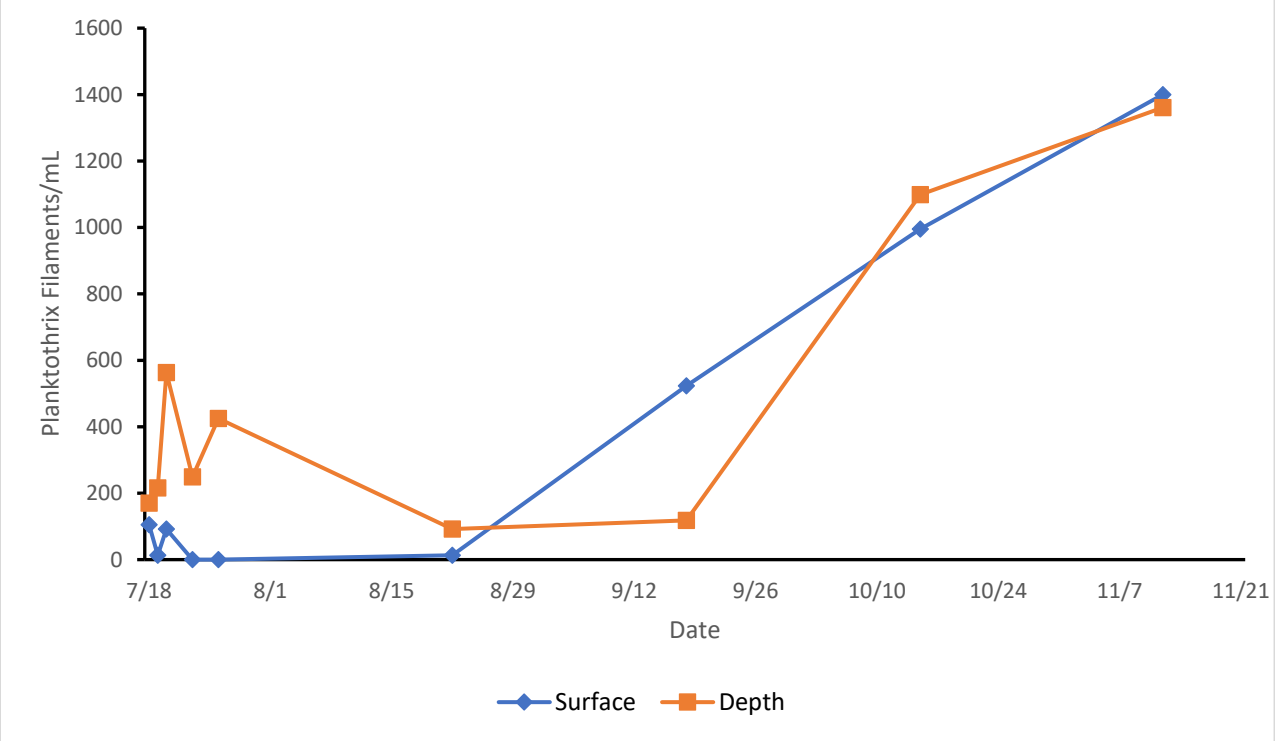

Figure 8. Absoulute abundances of Planktothrix agardhii *expressed as filaments/mL at surface and subsurface (depth) sampling locations.*

Cyanobacteria (*P. agardhii*) at depth remained higher than abundances of all other organisms throughout the study, while more fluctuation was seen at the surface (Figure 9). Generally speaking, it appears that the *P. argardhii* bloom was more stable and less impacted by the treatment at subsurface depths as compared to *P. agardhii* at the surface.

P. agardhii densities dropped to their all-time low of zero at the surface on the sampling dates of 7/23 and 7/26 (4 and 7 days after treatment), at which time diatoms and flagellates increased from 0 to 14 cells/mL on 7/23 before decreasing again to non-detectable on 7/26. There does appear to be some corresponding pattern between the rise of *P. agardhii* and fall of colonial forms from August through November at surface and depth, with zero colonial forms observed in October and November, when *P. agardhii* reached maximum levels. There is less of

an observable pattern displayed for other organisms, which do not appear to correspond directly to fluctuations in *P. agardhii* cell counts. This may be due to the fact that *P. agardhii* levels generally remained high throughout the study and did not reach levels low enough for a long enough period to allow other phytoplankton groups to grow. Flagellates, colonial forms, diatoms, and "other" comprised the majority of the phytoplankton community outside of cyanobacteria. No dinoflagellates were observed in any samples (Figures 9-10).

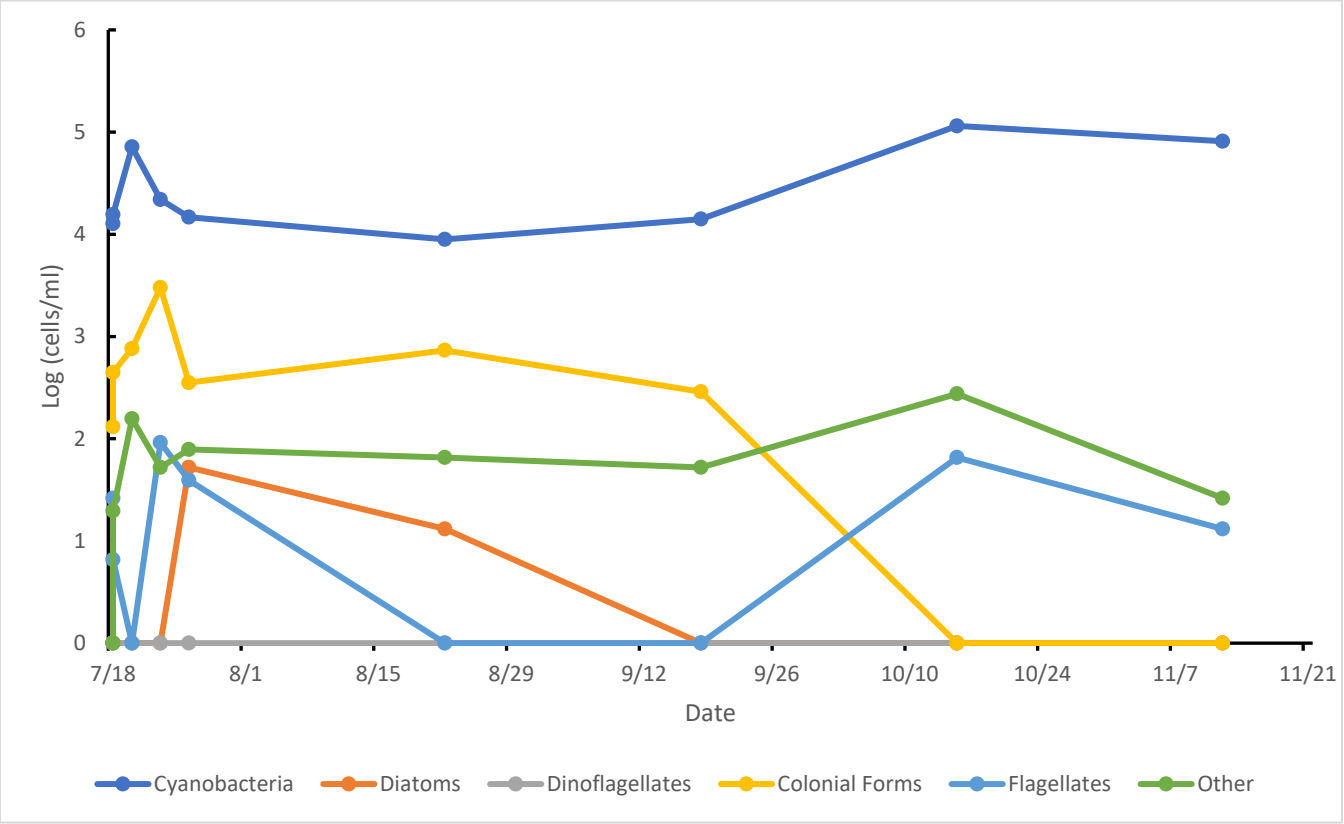

Figure 9. Abundances of 5 major groups of phytoplankton at select ***subsurface depths*** *of the vertical station (sampling site C) in Spahrs Quarry. Cyanobacteria represents* P. agardhii *cells. See Figure 2 for location of vertical station.*

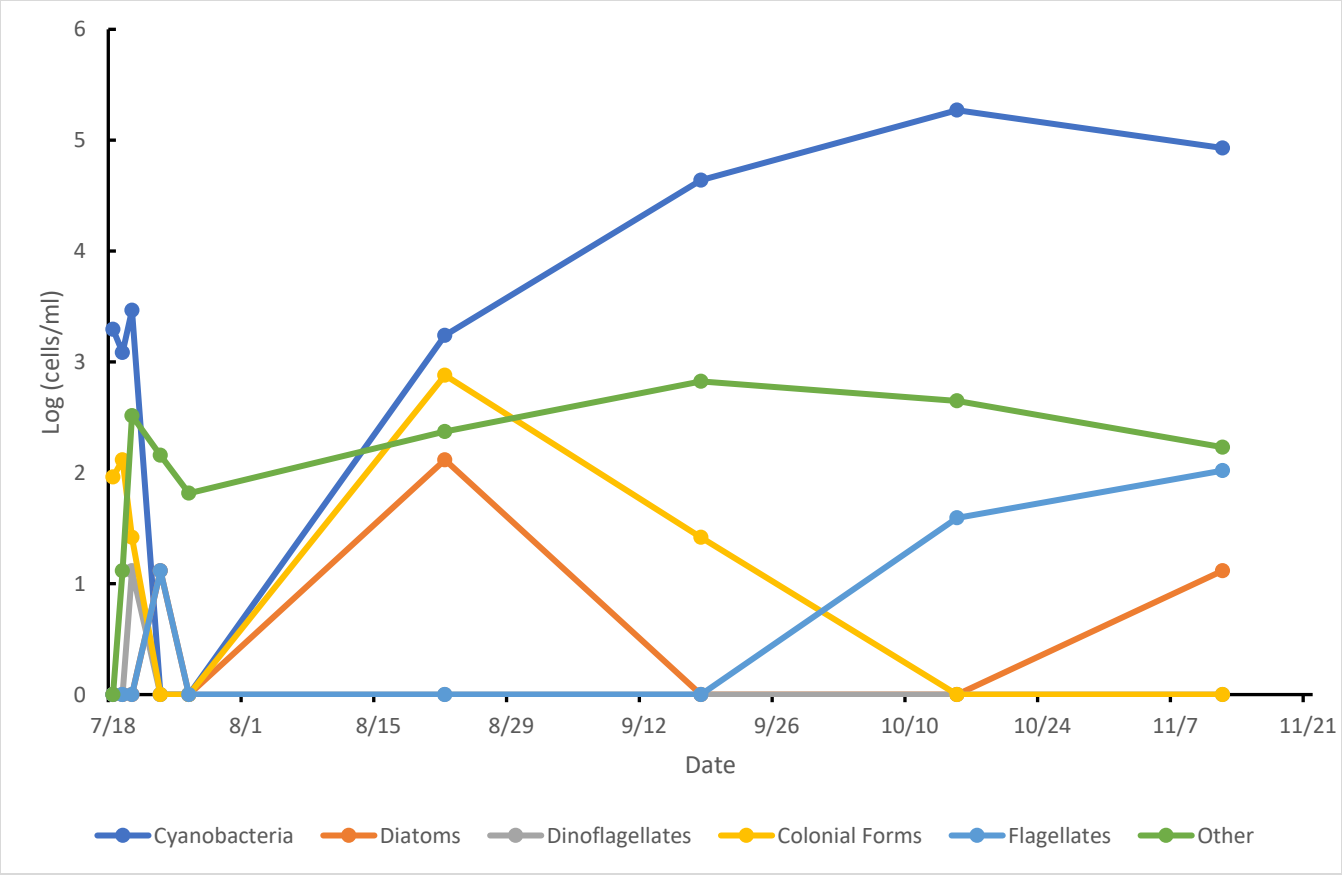

*Figure 10. Abundances of 5 major groups of phytoplankton at select sampling sites on the **surface** of Spahrs Quarry. Cyanobacteria represents of* P. agardhii *cells.*

Phycocyanin Fluorescence

Phycocyanin (PC) fluorescence generally increased throughout the quarry from the beginning to the end of the study (7/18-11/12). Subsurface phycocyanin fluorescence initially decreased on 7/19, the day of treatment from 20.91 to 9.75 RFU at 2.1 m and 37.87 to 7.43 RFU at 12.8 m, while surface levels rose slightly from 0.00 to 2.71 RFU. The day after application (7/20), levels decreased again at all surface sampling locations (from 2.71 to -1.08 RFU at vertical station), as well as at the 2.1 m and 9.8 m sampling depths (from 9.75 to 6.38 RFU at 2.1 m and 78.97 to 54.20 RFU at 9.8 m), rising at the 12.8 m sampling depth from 7.43 to 28.45 RFU.

Values continued to gradually rise and fall at the deepest sampling depths (9.8 and 12.8 m) throughout the remainder of the study (7/23-11/12). However, beginning in August, values at the surface and shallower sampling depth (2.1 m) began to rise each month, sharply increasing from the pre-treatment values. Fluorescence at the 2.1 m increased from 20.91 RFU pre-treatment to a maximum value of 263.27 RFU in October. The value at the surface increased from 0.00 RFU pre-treatment to a max value of 320.5 RFU in November (Figure 12).

Phycocyanin fluorescence averages at the surface of the quarry fluctuated throughout July, dropping from 9.72 on the day of treatment to 0.04 RFU the day after, rising again 4 days after treatment to 40.6 RFU, and falling to 6.83 RFU eight days after treatment. Levels rose steadily from 33.18 in August to a high of 390.99 RFU in November. (Figure 11).

A 2-Way ANOVA concluded that date of sampling and depth alone both had statistically significant effects on phycocyanin fluorescence, as well as the interaction of date and depth, meaning PC levels also varied significantly as a result of the combined effects of date and depth at which measurements were taken ($p<.001$, $p = 0.026$, and $p<0.001$ respectively).

Surface levels, when compared to one another, were not significantly different from pre-treatment levels until October and November, when phycocyanin fluorescences significantly increased ($p < 0.001$). Fluorescences at depth were not significantly different throughout the study ($p > 0.05$ for all samples). Fluorescences at the surface and depth were not significantly different from one another on a given sampling date until October and November ($p = 0.003$ and $p <0.001$ respectively), when levels at the surface and 2.1 m increased significantly and levels at deeper depths (9.8 and 12.8 m) remained relatively the same, with some fluctuation (Figure 12).

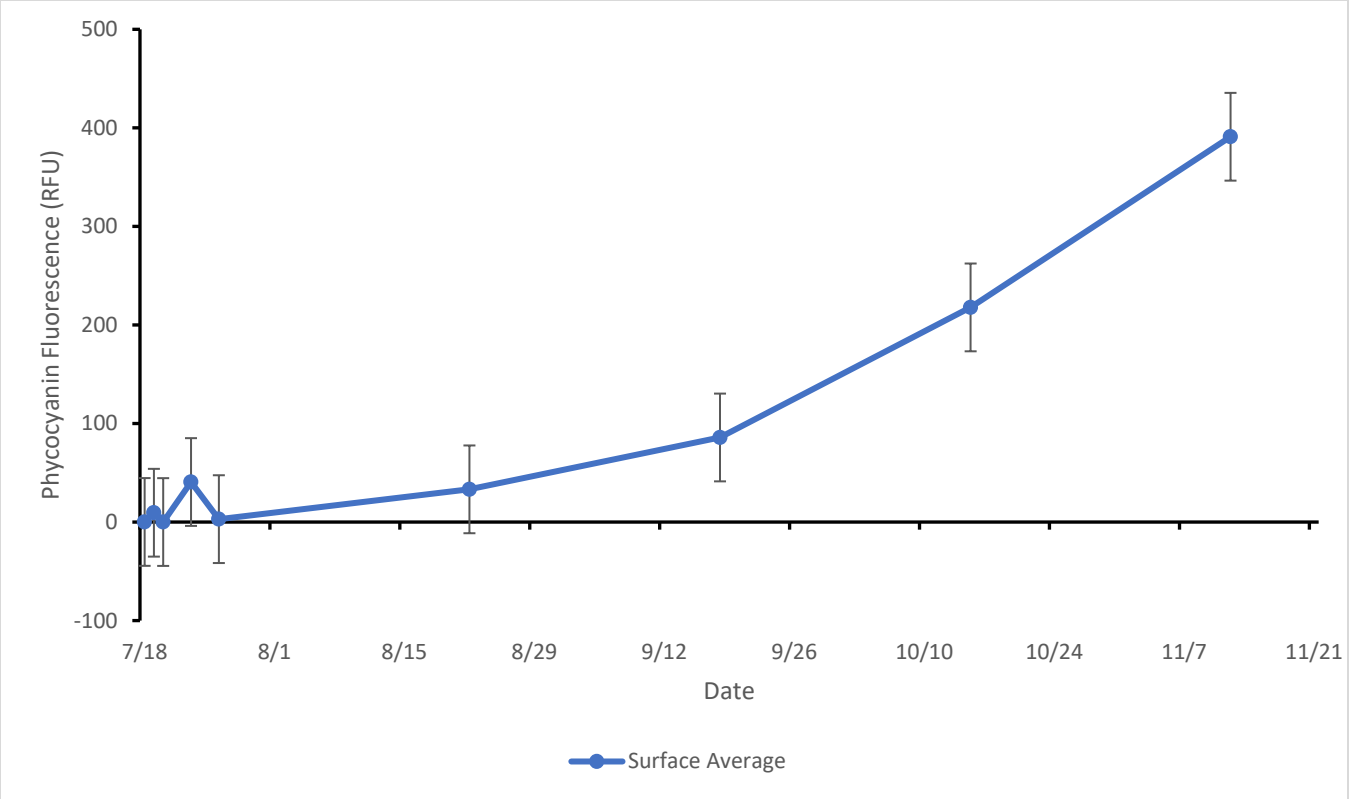

Figure 11. Surface phycocyanin fluorescence averages in Spahrs Quarry. Markers represent averages of 5 surface sampling sites within the quarry. See figure 2 for locations of each site.

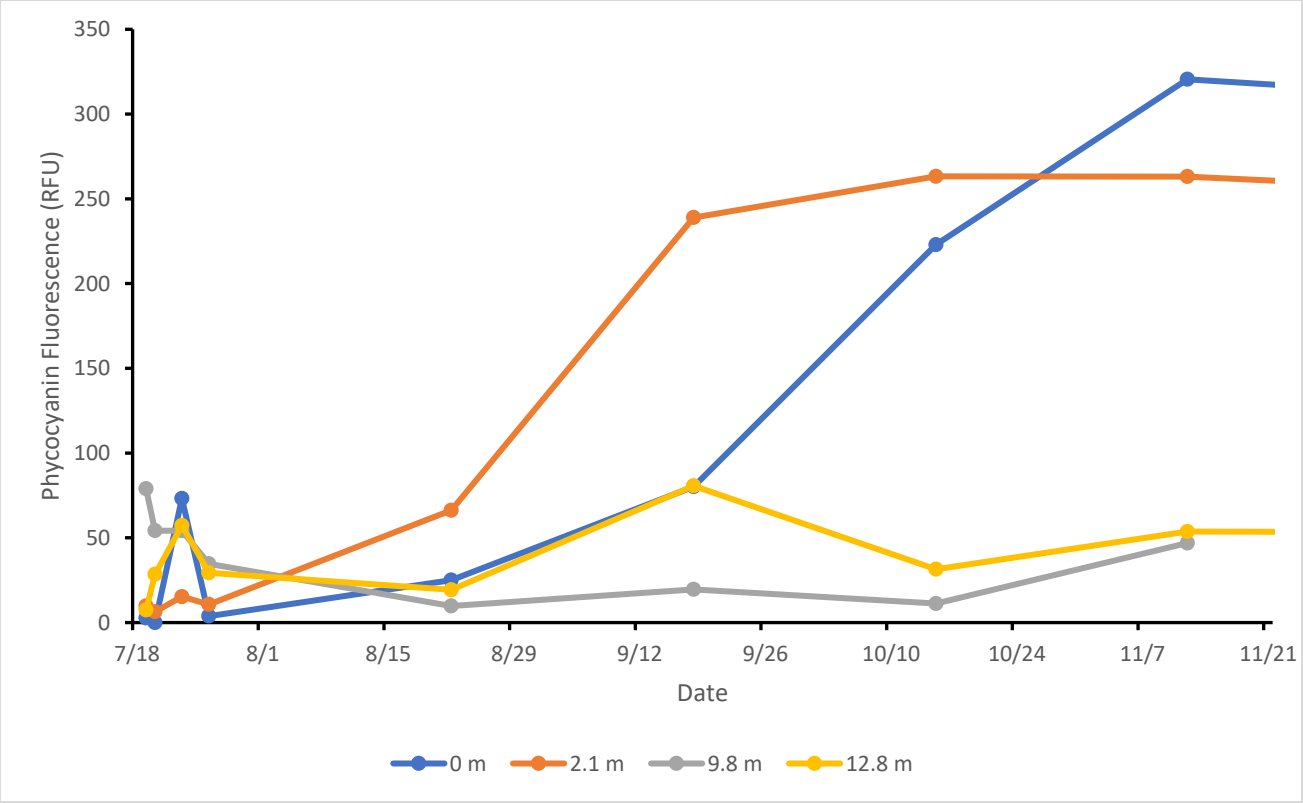

Figure 12. Phycocyanin fluorescences at 4 sampling depths at vertical station (sampling site C) in Spahrs Quarry. Lines represent depths from 0 m (surface) down to 12.8 m. See Figure 2 for location of vertical station.

Phycocyanin: Chlorophyll Ratio

Phycocyanin:Chlorophyll ratios were very low prior to addition of hydrogen peroxide and remained very low through September, increasing significantly in October and November. Ratios generally increased from the beginning to the end of the study and followed similar trends for both shallow depths (surface and 2.1 m) (Figure 13) and deeper depths (9 .8 and 12.8 m) (Figure 14).

Ratios below 0.3 are indicative of low levels of cyanobacteria in the phytoplankton community (K. Sellner, pers. Comm.). Surface ratios on the day of treatment was 0.33, indicating that cyanobacteria comprised a significant portion of the phytoplankton population (Figure 13). Ratios dipped below 0.33 following treatment and did not reach levels above 0.30

again until August and from there, continued to increase monthly through November, reaching a high of 7.79 in November.

The day before treatment (7/18), values were highest at 12.8 m at 1.14, indicating cyanobacteria dominated the phytoplankton population at that depth (Figure 14). Values were below 0.3 at all other depths, indicating that the *P. agardhii* bloom was mostly found at depth prior to treatment. A sample was not taken at 9.8 m the day before treatment. The ratio at 12.8 m declined below 0.30 the day of treatment (7/19) to 0.16, and values were below 0.30 for all depths except 9.8 m, which was 1.53, indicating a high level of cyanobacteria at that depth. From there, ratios continue to fluctuate at all depths, remaining above 0.30 below the surface at 9.8 and 12.8 m, and below 0.30 at the surface and at 2.1 m. Ratios rose above 0.30 for the first time at the surface in August and continue to rise sharply through November at the surface and 2.1 m to highs of 7.66 and 6.95, respectively. As surface and shallow depth ratios rose in the fall, subsurface ratios at 9.8 and 12.8 m decreased to levels that fluctuated between 0.20 - 0.94.

A 2-Way ANOVA concluded that there was a statistically significant difference in phycocyanin: chlorophyll ratios by date ($p<0.001$); however, there was not a statistically significant difference by depth ($p=0.092$). Ratios were significantly impacted by the interaction of sampling date and depth combined ($p<0.001$), meaning the combined effects of date and depth at which samples were taken had a significant impact on ratios. Pairwise comparisons indicated that there was no significant difference from pre-treatment values at the surface until September, October, and November ($p=0.017$ and $p<0.001$), when values were highest. Ratios at depth did not differ statistically from one another across the study ($p>0.05$ for all dates). Surface and depth averages were significantly different from one another in October and November ($p=0.008$ and $p<0.001$).

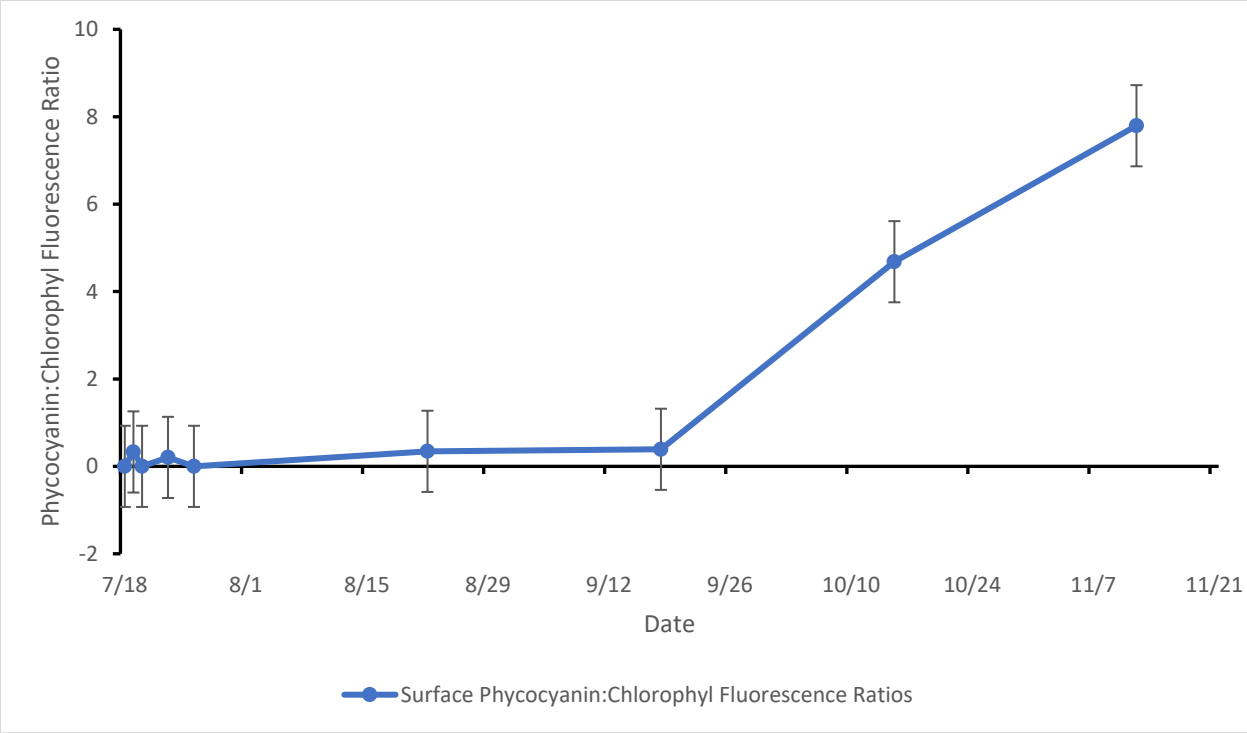

Figure 13. Surface phycocyanin:chlorophyl fluorescence ratios in Spahrs Quarry. Markers represent averages of 5 surface sampling sites within the quarry. See figure 2 for locations of each site.

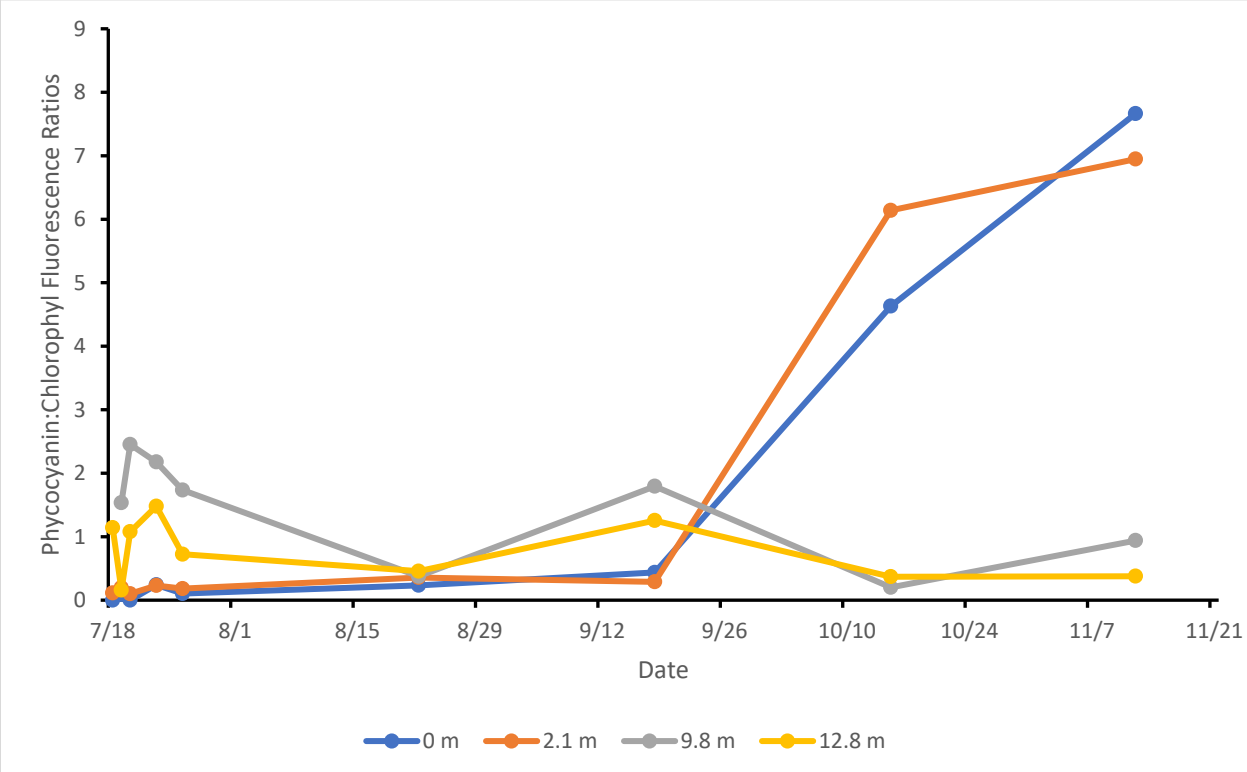

Figure 14. Phycocyanin:chlorophyl fluorescence ratios at 4 sampling depths at vertical station (sampling site C) in Spahrs Quarry. Lines represent depths from 0 m (surface) down to 12.8 m. See Figure 2 for location of vertical station.

DISCUSSION

The use of hydrogen peroxide in controlling concentrations of cyanobacteria is a generally accepted mitigation strategy (Matthijs et al. 2016). In this project, the experiment closely replicated the approach that Mattheiss et al. (2017) used to test the effectiveness of hydrogen peroxide as a cyanocide for *P. agardhii* in Spahrs Quarry, Frederick County, MD. Those researchers found suppression of the species in another local spring-fed pond (Lake Anita Louise), by monitoring *Planktothrix* levels over eight days post-treatment and then monthly thereafter. This experiment replicated some of that work in the short- and long-term for presence of the peroxide as well as *P. agardhii* abundances for months after treatment.

Many studies testing the effectiveness of a single H_2O_2 application in the field have documented short-term success, i.e., looking at impacts over the course of several days (Barrington et al. 2013, Dziga et al. 2019, Piel et al. 2021, Santos et al. 2021, Mattheiss et al. 2017, Weenink et al. 2021, Yang et al. 2018). Few studies have examined impacts beyond that, some measuring impacts of a single treatment up to approximately 1.5-2 months after treatment (Matthijs et al. 2012, Sinha et al. 2018) and one measuring impacts of repeated treatments over the course of 2 years (Lusty and Gobler 2023). This study sought to determine long term impacts and if a single treatment of H_2O_2 resulted in long-term bloom suppression for 4 months after treatment.

While Mattheiss et al. (2017) were able to successfully treat a lake using a dosage of 2 mg/L, Matthijs et al. (2012) found that while a concentration of at least 1 mg/L is required to suppress cyanobacteria, a slightly higher concentration is required in field applications due to the rapid degradation rate of hydrogen peroxide. Additionally, a dosage concentration greater than

2.5 mg/L is not recommended by Matthijs et al. (2011) due to the sensitivity of zooplankton, although phytoplankton and macrofauna were found to thrive at concentrations ranging from 0-8 mg/L in their 2011 study.

Field studies have found success in treating cyanobateria blooms containing *Planktothrix* sp. (in some cases, co-occurring with other genera of cyanobacteria such as *Microcystis*, *Dolichospermum*, and *Cylindrospermopsis*) with hydrogen peroxide concentrations as low as 1.2 mg/L (Barrington et al. 2013) and as high as 10 mg/L (Santos et al. 2021). Successful bloom suppression was observed for as little as two weeks (Lusty and Gobler 2023) and up to 7 weeks (Matthijs et al 2012) before blooms returned.

The patterns observed in the various *P. agardhii* indicators measured (cell counts, filaments, phycocyanin fluorescence, and phycocyanin: chlorophyll ratios) (Figures 7-14) all indicate that the *P. agardhii* bloom was smaller in size and found at deeper depths in the lake throughout the summer, and in the fall the bloom increased in size and migrated to the surface of the lake. This seasonal behavior is expected of *P. agardhii* when isothermal mixing occurs in the fall (Reynolds 1984, Kurmayer et al. 2016). Temperature data indicate a 22.9° C difference in temperature between surface and subsurface depths measured, which gradually decreased every month until there was only a 3.8° C difference in temperature between the surface and bottom of the quarry in November, indicating that mixing was occurring (Figure 6).

Additionally, *P. agardhii* indicators all rose at surface and shallow sampling depths from September – November as water temperature steadily declined, supporting that *P. agardhii* does better in colder temperatures (Briand et al. 2002) and that blooms are most likely to form when water temperatures range from 10.3-22.6 °C (Mischke et al. 2003, Toporowska et al. 2010, Toporowska et al. 2016), with the most dramatic increases seen in October and November when

surface/shallow water temperatures declined below 22.6 °C for the first time during the study to to 19.3 °C and 10.5 °C, respectively.

P. agardhii did appear to demonstrate some initial response to the application of hydrogen peroxide at the surface, in that within 1 day after treatment, absolute abundances of *P. agardhii*, phycocyanin fluorescence, and phycocyanin:chlorophyll ratios all increased at depth (Figures 8, 12, and 14) and decreased at the surface (Figures 8, 11, and 13) Increases at depth and decreases at surface directly after treatment could represent peroxide impact on upper water column *Planktothrix* population, inducing settling of dying portions of the shallower *P. agardhii* population.

The decline seen at the surface was minimal and the reduction/low levels at the surface through September may have been more closely associated with seasonality of *P. agardhii* growth (Kurmayer et al. 2016, Mischke and Nixdorf 2003, Reynolds 1984, Toporowska et al. 2010, Toporowska et al. 2016) than the treatment itself, especially given that absolute abundances of *P. agardhii*, phycocyanin fluorescence, and phycocyanin:chlorophyll ratios all indicated such little response at depth (1.2-12.8 m, where levels fluctuated, but *P. agardhii* persisted throughout the summer (Figures 8, 11, and 13)).

Overall, the single treatment of hydrogen peroxide yielding 1.4 mg/L in Spahrs Quarry was ineffective for limiting the *Planktothrix* population as abundances rapidly increased to expected late fall-early winter concentrations as noted for non-treated local ponds like Lake Anita Louise (Mattheiss et al. 2017). The temporal limitations of this study and the peroxide concentrations employed present an opportunity for a wide variety of future research, particularly in Spahrs Quarry.

A number of factors could have resulted in this concentration being unsuccessful in suppressing the bloom days-to-months post-treatment: 1.) The recommended minimum concentration of 2.0 mg/L was not achieved during treatment (Matthijs et al. 2016); 2.) Greater mitigation success may have been achieved with repeated treatments (Piel et al. 2021, Lusty and Gobler 2023); 3.) the *P. agardhii* strain present in Spahrs quarry may have been more resistant to H_2O_2, as some strain sensitivity to H_2O_2 levels has been documented (Lurling et al. 2020; Schuurmans et al 2018); 4.) Light intensity has been shown to impact sensitivity to H_2O_2 in another cyanobacterium, *Microcystis* (Piel et al. 2019, Sandrini et al. 2020) where concentrations of H_2O_2 as low as 1/mg/L was effective when the cyanobacterium was exposed to high light intensities (Piel et al. 2019); 5.) In conjunction with low light intensity, Sandrini et al. (2020) found that *Microcystis* was less sensitive to H_2O_2 when populations were nitrogen or phosphorous limited, as compared to nutrient-replete assemblages. Nutrient availability was not found to be as influential on sensitivity to H_2O_2 when light intensity was high. Future treatments in Spahrs Quarry should be paired with measurements of light intensity and nutrients. 6.) The presence of green algae can decrease the effectiveness of H_2O_2 treatment. Green algae degrade hydrogen peroxide at a faster rate than cyanobacteria because they contain higher levels of antioxidant and H_2O_2-degrading enzymes than cyanobacteria (Weenink et al. 2021). High biomass, in general, also decreases effectiveness as well (Weenink et al. 2021), likely attributable to increased demand of the peroxide's oxidation potential from the elevated carbon content (see point 7 below). Weenink et al. (2015) recommend a minimum concentration of 2.3 mg/L and Schuurmans et al. (2018) suggest when treating waterbodies with hydrogen peroxide, the dose depends on initial cell density in order to reach the necessary concentration per cell. Matthijs et al. (2016) recommend a minimum concentration of 2.0 mg/L; however, they also

agree that strain type and cell density impact the required dose. And 7.) Waters with elevated levels of dissolved solutes and organic matter (DOM) may also reduce peroxide effectiveness, due to the influence of DOM on the reductive power of water and oxidation of DOM by H_2O_2 (Arvin and Pedersen 2015, Weenink et al. 2015, Borikar et al. 2015) . Although DOM concentrations were not available for the quarry, the system was surrounded by trees and leaf litter and submersed grasses were visible in the littoral zone.

For future treatments, it would be beneficial to estimate the minimal effective concentration the day before treatment (Matthijs et al. 2016). To avoid impacts on other system biota, treatment is not recommended if the concentration required to mitigate the bloom would exceed 5 mg/L (Matthijs et al. 2016, Weenink et al. 2015). Prior to treatment, a minimal effective dosage could be determined using laboratory incubations of water from the quarry. Generally, a minimum of 2 mg/L sustained for at least 5 hours after treatment, and no more than 5 mg/L as a maximum concentration, is suggested to successfully treat a bloom without harming the biotic community (Matthijs et al. 2016).

Once these factors are taken into consideration, it can be determined whether treatment is a viable option, at what concentration, and whether multiple treatments should be considered. Lusty and Gobler (2023) applied multiple treatments over a 2-year period and found that the cyanobacteria would rebound in two weeks or less after each treatment, so it may be beneficial to re-treat within that time frame after the initial treatment has degraded and concentrations of hydrogen peroxide have returned to ambient levels.

Alternative application methods could also be considered for future treatments at Spahrs Quarry. It would be interesting to see if other methods, such as applying a liquid solution of H_2O_2 and injecting it at depth (e.g., Matthijs et al. 2012) would differ in effectiveness at similar

concentrations. Other application methods suggested by distributors of GreenClean Pro® include subsurface application by placing the granules in burlap bags and dragging through the water using a boat until all granules have dissolved or creating a foam by making a solution and spraying it across the surface using a foamer (DoMyOwn 2022). Similar peroxide-based products could also be explored, such as PAK 27 (Kannan and Lenca 2013, SePRO 2022). Further research may be needed to compare effectiveness of treatment based on product and application method.

Interestingly, based on visual observations, the *Planktothrix* bloom no longer appears to be present in Spahrs Quarry as of summer 2022 (4 years after treatment) (K. Sellner, pers. comm). Natural succession of the bloom could have occurred due to environmental changes within the quarry. Since the treatment applied in 2018 was in the form of dissolving granules, one may speculate that granules may have sunk to the bottom and slowly dissolved over time. This could account for low levels of hydrogen peroxide consistently detected at 12.8 m after treatment through the end of the study, whereas hydrogen peroxide was not detectable at any depth pre-treatment (Figures 3-4). Additional monitoring of Spahrs Quarry is needed to determine whether *P. agardhii* is still present and further research should address dissolution rates of GreenClean PRO® under different environmental conditions.

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