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HAB Cyanobacteria in Northeastern SC Retention Ponds: Time trends in abundance and relationship to water quality

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**HAB Cyanobacteria in Northeastern SC Retention Ponds:
Time trends in abundance and relationship to water quality**

By

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Marine Science Major, Biology Minor, Honors

Submitted in Partial Fulfillment of the
Requirements for the Degree of Bachelor of Science
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Coastal Carolina University

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Abstract

The accumulation of polluted runoff in retention ponds can create water quality conditions that favor development of harmful algal blooms, including toxin-producing cyanobacteria. This can present a health risk to pedestrians and nearby residents, as many such ponds are surrounded by walkways or housing complexes. Blooms can also cause harm to natural ecosystems, periodically causing hypoxic conditions and toxin buildups. This study aims to form a more complete understanding of the population dynamics of certain potentially harmful phytoplankton genera, in relation to water quality parameters in retention ponds. Sampling was performed in five ponds chosen to reflect a variety of adjacent land uses. Eleven sets of samples were collected every other week for a period of six months. Cell abundance was quantified for five common and potentially harmful phytoplankton genera, which were chosen due to their prevalence in harmful algal blooms and potential for toxin production. Time trends in genera diversity and abundance were compared to water quality data to investigate relationships. A bloom event was captured at one location, and additional samples and data were collected over the course of the bloom to generate a more comprehensive data set.

Introduction

Harmful algal blooms (HABs) are an emergent threat to the health of humans, animals, and local ecosystems. Freshwater harmful algal blooms in particular are often made up of cyanobacteria. Some cyanobacteria are able to produce toxins, such as microcystins, which are harmful to pets, wild animals, and even humans (Song et al. 1998). These toxins can be produced by certain genera, including *Microcystis*, *Aphanizomenon*, *Raphidiopsis*, *Dolichospermum*, and *Planktothrix* (Litvinchuk et al., 2023). When algae reproduce quickly due to certain favorable environmental conditions, they can accumulate in large numbers, forming a bloom. Eventually these blooms die off, which can release their toxins into the water. Not all species of cyanobacteria or phytoplankton have the capability to produce toxins however, and not all algal blooms of toxin-producing species release toxins. More research is needed to determine the link between environmental conditions and toxin production. Additionally, these toxins can be present in multiple forms in the water column. Toxins may be dissolved following cell lysis, particulate within the cell, or even aerosolized through mechanisms such as fountain spray (Murby and Haney, 2015).

In South Carolina, retention ponds may be a hotspot for HABs due to their widespread use for stormwater management and their favorable conditions for supporting blooms. Due to rapid population growth and the resulting increased demand for impervious surfaces (such as roads, houses, and buildings), artificial retention ponds are ubiquitous along the SC coast in order to manage flooding and capture stormwater runoff (Greenfield et al., 2019). Retention ponds are associated with high nutrient levels, which can lead to cyanobacterial blooms (Lewitus et al., 2003). These high nutrient levels are often caused by nutrient-dense runoff from urban surroundings, such as parking lots (Silva et al., 2019). Additionally, the ponds often experience

warm temperatures which promote cyanobacterial blooms, as their growth rates are optimized at higher temperatures (Paerl and Paul, 2012). The widespread use of retention ponds can create issues for nearby residents and their pets who may be exposed to any toxins present in the ponds via skin contact or inhalation of aerosolized cells. As such, it is in the best interest of both residents and local ecosystems that algal blooms are monitored and better understood.

Oxygen saturation, chlorophyll, and pH are all parameters that may be used to track ongoing algal blooms in freshwater ponds. High oxygen saturations are characteristic of ongoing algal blooms as the cells photosynthesize rapidly, releasing large amounts of oxygen into the water (Zang et al., 2010). Chlorophyll *a* is an algal photosynthetic pigment that has been shown to serve as a good indicator for algal abundance in freshwater habitats not dominated by aquatic plants, and as such, high levels of chlorophyll can also serve as an indicator for blooms (Zang et al., 2010; Du et al. 2019). Finally, a high pH can also result from high algal cell densities due to uptake of CO₂ during photosynthesis (Zang et al., 2010). In South Carolina, the Class FW water quality criterion for dissolved oxygen states that concentrations should not drop below 4 mg/L, and pH should remain between 6.0 and 8.5 (SC R.61-68). These water quality criteria can be used to evaluate whether an algal bloom is occurring in a pond.

The presence of microcystins, toxins produced by many HAB cyanobacteria, in recreational and even drinking water presents a serious health concern for freshwater ponds and lakes, marine ecosystems, and public health. Exposure to levels over the 1 µg/L drinking water criterion can lead to weakness, stomach pains, high temperature, anorexia, pallor, nausea, labored breathing, vomiting and diarrhea (WHO, 1998). High levels of exposure can lead to liver damage, or even death (Massey et al., 2018). Pets and children are especially at risk from the toxin due to their lower body mass and increased likelihood for ingesting contaminated water

(Farrer et al., 2015). A water quality criterion of 8 µg/L for microcystins was recently added to SC R. 61-68 to protect recreational water usage (2020). While stormwater ponds are not considered recreational waters, they can be utilized in similar ways by residents, regardless of local guidelines and regulations (SC DHEC, 2020).

This research addresses three main questions: Are the target genera of HAB cyanobacteria present and/or blooming in retention ponds on Coastal Carolina University's campus? How does this change over time? And do water quality parameters indicate the presence of these blooms if they are present? These questions were addressed by monitoring several local stormwater ponds for: (1) water quality parameters, (2) the abundance of certain species of cyanobacteria which can produce toxins, and (3) where a HAB was suspected, qualitative screening for the presence of the microcystin toxins. This required identification and counts of cyanobacteria genera, specifically *Aphanizomenon*, *Dolichospermum*, *Raphidiopsis*, *Microcystis*, and *Planktothrix*. These genera are also monitored by NOAA's Freshwater Phytoplankton Monitoring Network (NCCOS PMN) due to their prevalence in harmful algal blooms and potential for toxin production.

Materials and Methods

Study Area

In order to represent the various types of stormwater ponds in the region, sampling locations were selected to cover a variety of land uses, such as residential, undeveloped, under construction, or hypothesized heavy fertilizer use (Figure 1). Three sampling ponds were located on the main campus of Coastal Carolina University: Wall, Brittain, and HTC (Figure 2c). Wall Pond was chosen due to hypothesized heavy foot traffic and possible fertilizer use, Brittain Pond for foot and motor traffic, and the HTC Pond for parking lot runoff. The remaining two locations (Destiny Lane and University Place) are in the area surrounding CCU's main campus. The former is a stormwater pond adjacent to duplex and apartment housing mainly utilized by students, and a gas station, but is surrounded by trees on all sides (Figure 2a). The pond at UP is surrounded by student housing complexes, and therefore was hypothesized to have heavy residential and motor runoff due to the vast number of parking lots in the surrounding area (Figure 2b).

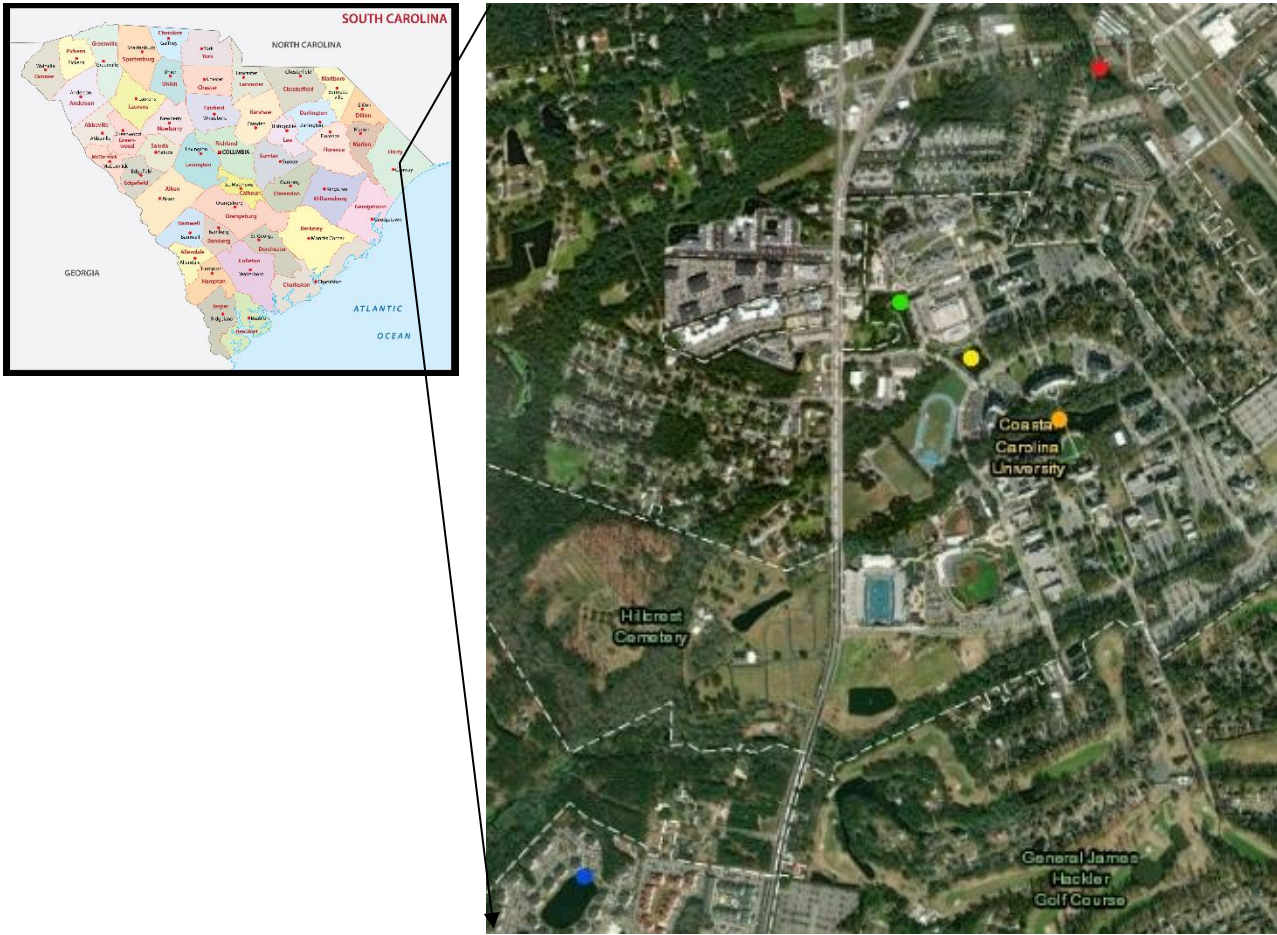


Figure 1. Map of sample locations. Red circle is the Pond at Destiny Lane, Wall Pond is in orange, Brittain Pond is in yellow, HTC Pond is in green, and UP pond in blue.

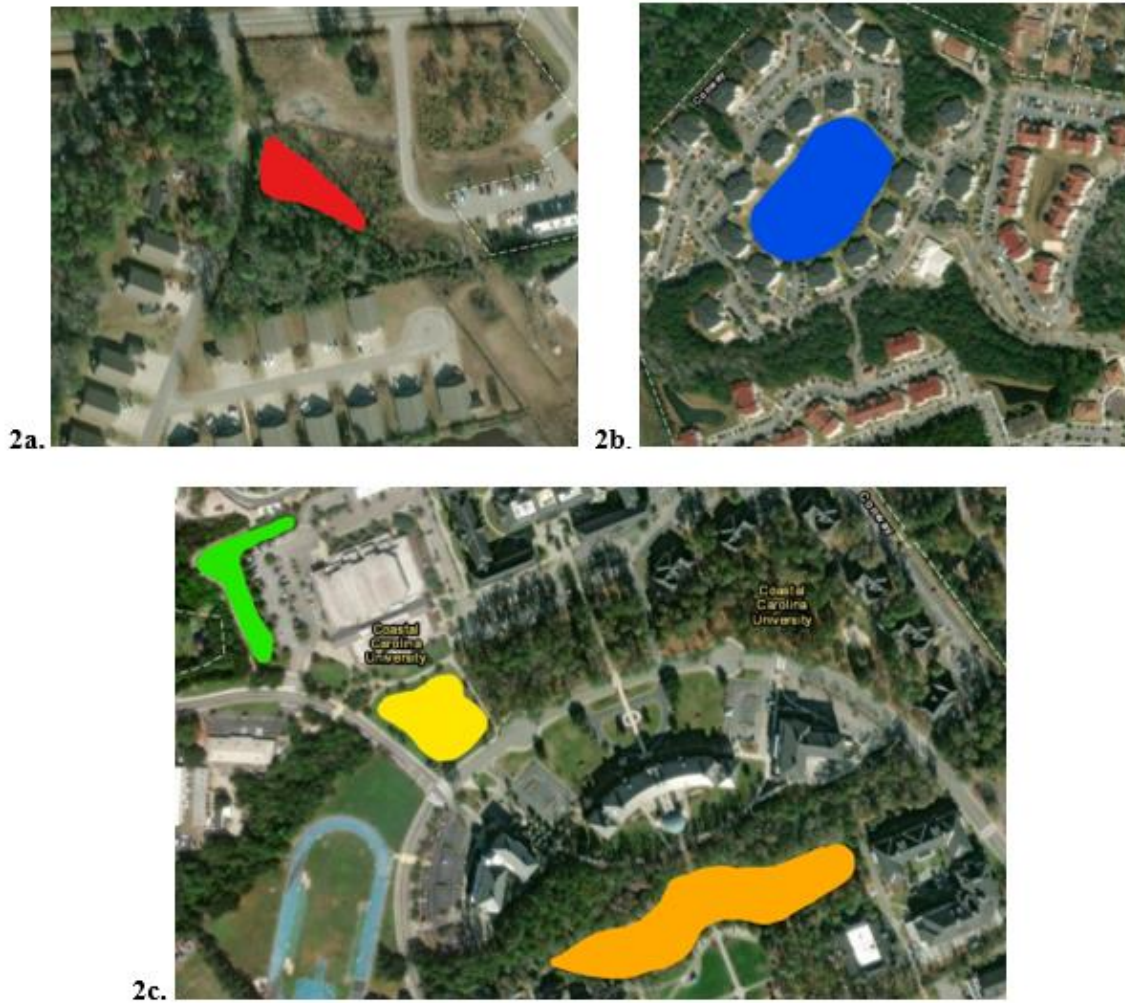


Figure 2. Figure 2a. shows the boundaries of the ponds at Destiny Lane in red, 2b shows University Place (UP) in blue, and 2c. shows Wall Pond in orange, Brittain Pond in yellow, and HTC Pond in green.

Sampling techniques

Samples for microscopic counts were collected using a pole sampler from the surface layer of the water column and stored in amber glass bottles. Samples were preserved using

Lugol's preservative and stored on ice for transportation (Vollenweider 1974). Lugol's preservative can be used to store samples for up to 12 months in dark bottles and a cool environment, provided that the preservation is checked every three months and additional Lugol's is added if necessary (Anderson and Karlson, 2017). Water samples for total microcystins analysis, if collected, were stored on ice in clear glass vials. All 5 sites were tested for the toxin on 8/24/23 and 9/28/23 to investigate relative amounts of the toxin in the ponds when no algal bloom was visible. Ten additional tests per site were run for two sites within Wall pond over the course of a *Microcystis* bloom from 10/27/23 to 11/30/23 (Table 1).

Table 1. Microcystin toxin sampling dates. All ponds were sampled on the first two dates, and Wall pond only was sampled for the remaining nine dates where the test was run.

Date	Sites tested		
	Wall pond only		All ponds
	WP1	WP3	
8/24/2023			X
9/28/2023			X
10/27/2023	X	X	
10/31/2023	X	X	
11/2/2023	X	X	
11/9/2023	X	X	
11/14/2023	X	X	
11/16/2023	X	X	
11/20/2023	X	X	
11/28/2023	X	X	
11/30/2023	X	X	

Water quality parameters

YSI EXO multiparameter sondes were for in-situ measurement of temperature, pH, dissolved oxygen saturation and concentration, conductivity and specific conductivity, turbidity, and chlorophyll. Meter calibrations were performed according to (EQL SOP 358¹). At each site, the meter was deployed near the water's surface and allowed to equilibrate prior to data collection. At sampling sites where the bottom of the pond was shallow enough to contact the meter when it was deployed, a buoy was used to keep the meter from contacting the pond floor. This was done to prevent variation in meter readings due to positioning in the water column, especially in silty bottomed ponds where turbidity may increase drastically closer to the bottom.

Phytoplankton enumeration

A 1-mL Sedgewick Rafter chamber was used to obtain phytoplankton density estimates in each sample, and results were recorded in phytoplankton individuals (cells or filaments/mL), or in the case of *Microcystis*, colonies/mL. Disposable 1-mL pipettes were used to fill the chambers, and plankton were allowed to settle 7 minutes before counting using a light microscope. Of the chamber's 1000 fields, 100 fields were counted as 5 random columns of 20 fields. A table of identification features for each genus was constructed to aid in enumeration (Table 2).

¹ This SOP is used by CCU's Environmental Quality Lab and complies with SC DHEC certification requirements. The SOP follows Standard Methods protocols (American Public Health Association, American Water Works Association, Water Environment Federation).

Microcystis counts were enumerated as colonies as opposed to individual cells or filaments, so an estimate of 300 cells per colony was used to make a conversion. This estimate was based on an average colony size of 109.3 μm (n=83) in the retention ponds, which according to Liu et al. (2017) is a large colony, and as such, likely contains 100+ cells.

Thirteen additional HAB count samples were collected at Wall pond from 10/26/2023 to 11/30/2023 over the course of a visible *Microcystis* bloom on the surface of the pond in addition to samples for microcystin toxin testing. These samples were collected with microcystin toxin samples, and sampling dates are the same as those seen in table 1 for WP1 and WP3.

Table 2. Identification rubric for HAB genera quantified in this study. This key was used to determine if individuals (or colonies in the case of *Microcystis*) should be counted in circumstances where degradation or unusual angle of viewing was an issue. If individuals were not distinguishable according to the key, they were not counted.

HAB genera	Counting criteria
<i>Microcystis</i> (colony)	Clumps of 10+ well-defined round cells, usually in contact with each other, no specific arrangement around a central point. Clumps were counted as a single colony.
<i>Dolichospermum</i>	Curly strands with round cells, may have akinetes or heterocysts, tend to appear darker with Lugol's. Filaments were counted as one individual, despite containing multiple cells.
<i>Planktothrix</i>	Long and thick, tapered at the ends, tend to appear very dark with Lugol's
<i>Raphidiopsis</i>	Visible terminal arrowhead shape, or short "squiggles"
<i>Aphanizomenon</i>	Barrel-to-cylinder shaped cells, may have akinetes or heterocysts, end cells may appear empty or tapered, cells tend to be more spotted than <i>Dolichospermum</i> . Filaments were counted as one individual.

Microcystins analysis

Abraxis ELISA test strips (Gold Standard Diagnostics, ABRAXIS® Microcystins Strip Test) were used at some sites and dates to screen for microcystins. These strips are a rapid immunochromatographic testing technique designed for qualitative screening of total

microcystins and nodularins in recreational waters. The lower limit of detection is 1 µg/L and the upper limit is 10 µg/L.

Data analysis

Box plots were constructed using R (boxplot ()) where the boxes represent the interquartile range (Q_1 to Q_3). The hinges represent the data range exclusive of any outliers, where outliers have values greater than $Q_3 + (1.5 * \text{interquartile range})$ or less than $Q_1 - (1.5 * \text{interquartile range})$. Outliers, if present, are shown as circles.

Site-to-site differences were detected using two tests: a Friedman test and a pairwise Wilcoxon rank sum test. The Friedman test is a nonparametric ANOVA on ranks for repeated measures (R, `friedman.test()`). Results with p values lower than 0.05 were considered significant. The pairwise Wilcoxon rank sum test was performed to determine which sites were significantly different using a Bonferroni correction for water quality parameters and a Hochberg correction for HAB counts (R, `pairwise.wilcox.test()`). Results with p values lower than 0.05 were considered significant. These tests were performed for both water quality parameters, HAB counts and a pond ranking index for bloom probability.

Spearman's correlation coefficient and p values were calculated using Sigma Plot 15.1 to compare association between water quality parameters. Correlation coefficients greater than 0.7 were considered to represent strong associations. Results with p values lower than 0.05 were considered significant.

Results

Water Quality parameters

Results from the measurements performed with the YSI EXO sondes are presented in Figures 3–9. For temperature, a difference was detected between sites with a Friedman test, however the pairwise Wilcoxon test did not detect differences between any of the five sampling locations over the course of eleven sampling dates (Figure 3). For pH, Destiny was significantly lower than all other locations, while Wall was significantly higher pH than all locations but UP (Figure 4). Most of the samples in Wall Pond had pH's that exceeded the Class FW water quality criterion (8.5, SC R.61-68). This was due in part to the high conductivity of the water in this pond and to photosynthesis, as evidenced by supersaturations.

Dissolved oxygen saturation and concentration both showed similar results, with Destiny's dissolved oxygen levels being significantly lower than all other locations (Pairwise Wilcoxon Rank Sum test; Figure 5, 6). UP and Wall Ponds had a significant fraction of supersaturated samples whereas all of the samples from Destiny pond exceeded the Class FW criterion, and most were hypoxic (<2.0 mg/L, SC R.61-68).

For specific conductivity, the Friedman test did not detect any significant site-to-site differences. All sites had significantly different specific conductivities according to the Wilcoxon test; however, Wall had a noticeably higher specific conductivity even with all sites being different (Figure 7). The higher conductivity may reflect the source of the water in this pond from groundwater pumping. The pond with the lowest conductivity was UP.

For turbidity, the Wilcoxon test detected significantly higher turbidities at Wall and Brittain than the other three sites but were not significantly different from each other, and UP had

a lower turbidity than all other sites (Figure 8). A Friedman test did not detect any differences for this parameter.

Brittain and Destiny ponds both had significantly higher chlorophyll concentrations than UP and Wall, while UP's chlorophyll was significantly lower than all other locations (Pairwise Wilcoxon test; Figure 9). HTC and Wall ponds both had a significantly higher chlorophyll level than UP, but only Wall pond's chlorophyll was significantly lower than both Brittain and Destiny (Pairwise Wilcoxon Rank Sum test; Figure 9). Almost all of the samples from the ponds with the highest median chlorophyll concentrations (Brittain, Destiny, and HTC) exceeded the EPA (2017) threshold for eutrophication for freshwaters in coastal plains. The Friedman test for this parameter did not detect any differences.

Spearman's Correlation Coefficient was calculated to investigate relationships between water quality parameters. Very few parameters had significant correlations ($p < 0.05$) except for oxygen saturation versus pH. As shown in Table 3, the correlations of oxygen saturation with pH were significant and strong at all five sites.

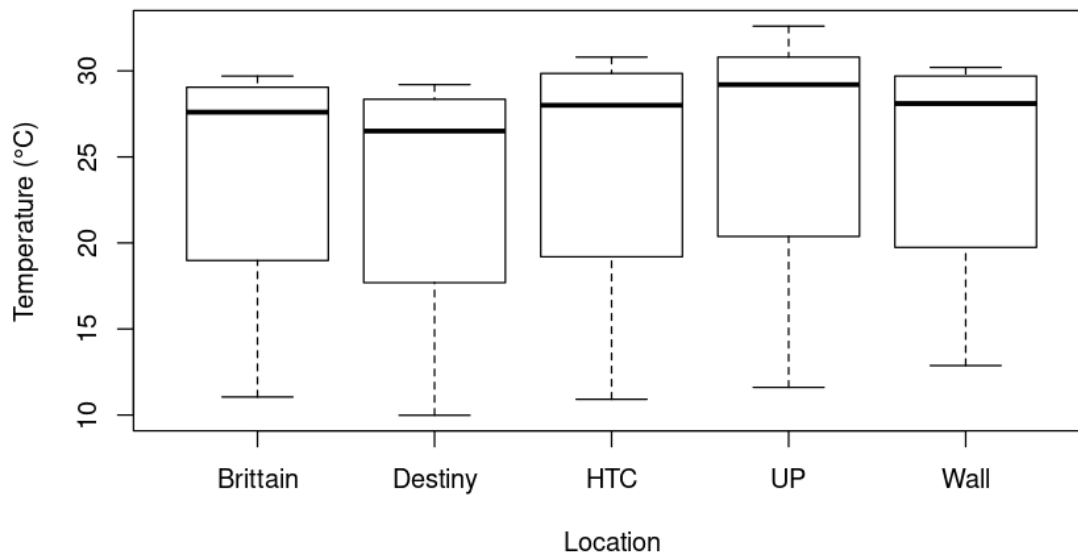


Figure 3. Water quality parameter box plot for temperature over 11 sampling dates. No significant differences were found between any sites (Pairwise Wilcoxon rank sum test, $p < 0.05$).

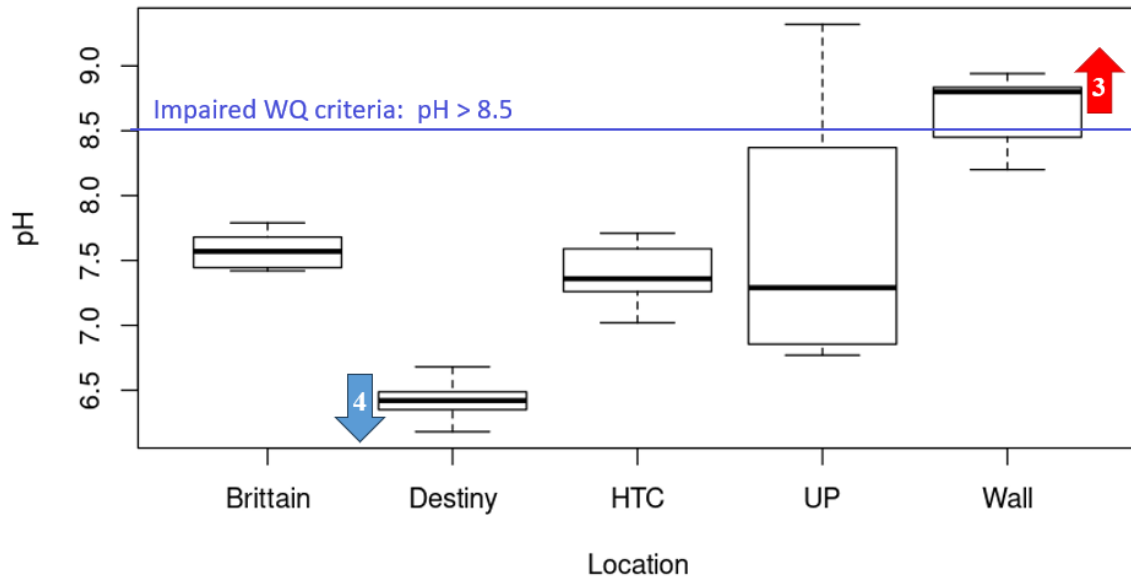


Figure 4. Water quality parameter box plot for pH over 11 sampling dates. A blue arrow indicates the number of other sites the indicated site is significantly lower than, and a red arrow indicates the number of other sites it is significantly higher than (Pairwise Wilcoxon rank sum test, $p < 0.05$). The blue line marks the Class FW water quality criterion for pH at 8.5 (SC R.61-68).

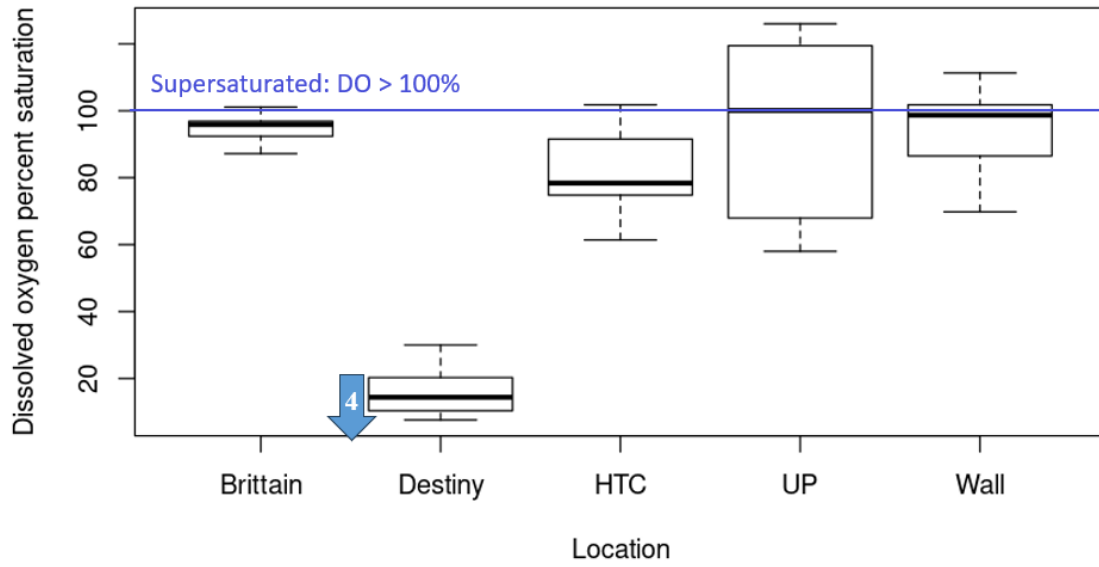


Figure 5. Water quality parameter box plot for dissolved oxygen saturation over 11 sampling dates. A blue arrow indicates the number of other sites the indicated site is significantly lower than (Pairwise Wilcoxon rank sum test, $p < 0.05$). The blue line indicates the minimum level for a supersaturated sample at 100% saturation.

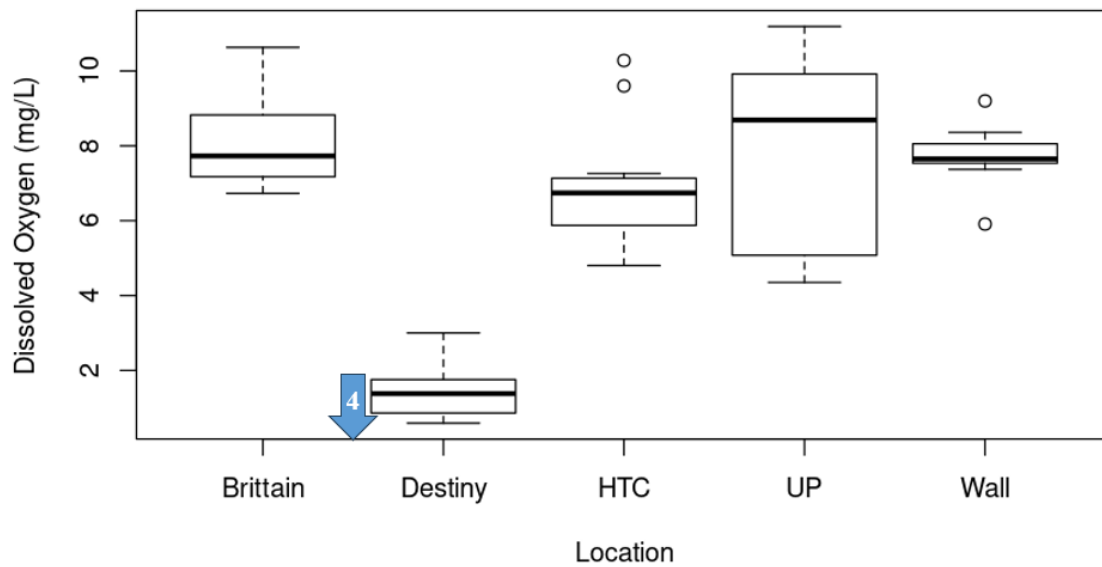


Figure 6. Water quality parameter box plot for dissolved oxygen concentration over 11 sampling dates. A blue arrow indicates the number of other sites the indicated site is significantly lower than (Pairwise Wilcoxon rank sum test, $p < 0.05$). The blue line marks the Class FW water quality criterion (4.0 mg/L; SC R.61-68).

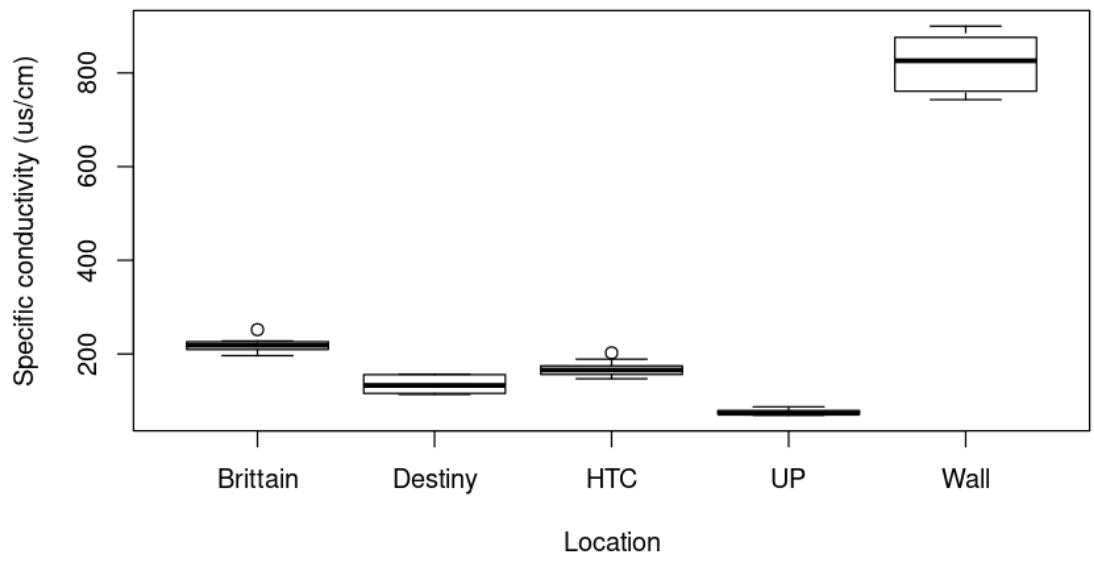


Figure 7. Water quality parameter box plot for specific conductivity over 10 sampling dates. All sites are significantly different (Pairwise Wilcoxon rank sum test, $p < 0.05$).

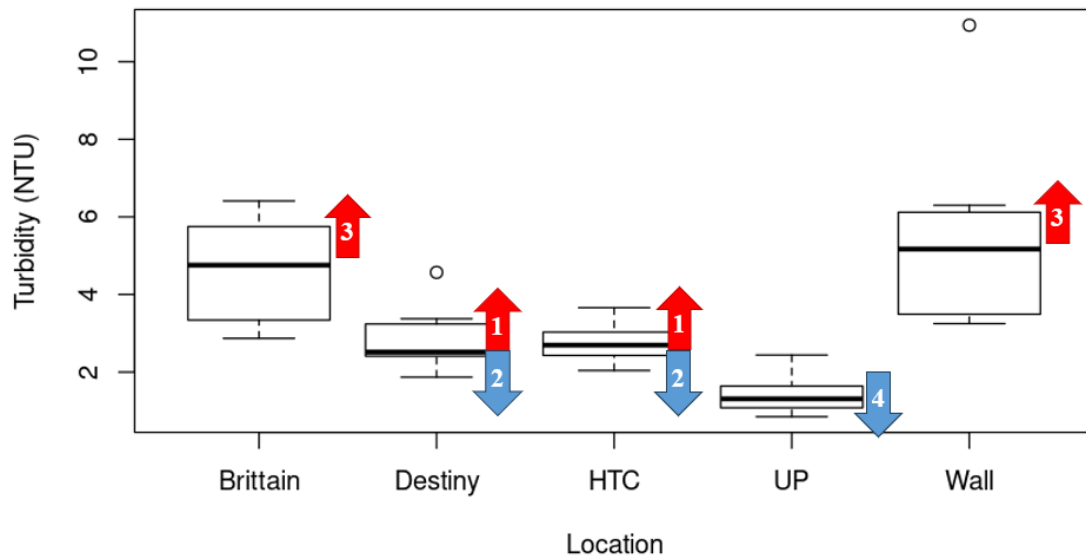


Figure 8. Water quality parameter box plot for turbidity over 10 sampling dates. A blue arrow indicates the number of other sites the indicated site is significantly lower than, and a red arrow indicates the number of other sites it is significantly higher than (Pairwise Wilcoxon rank sum test, $p < 0.05$).

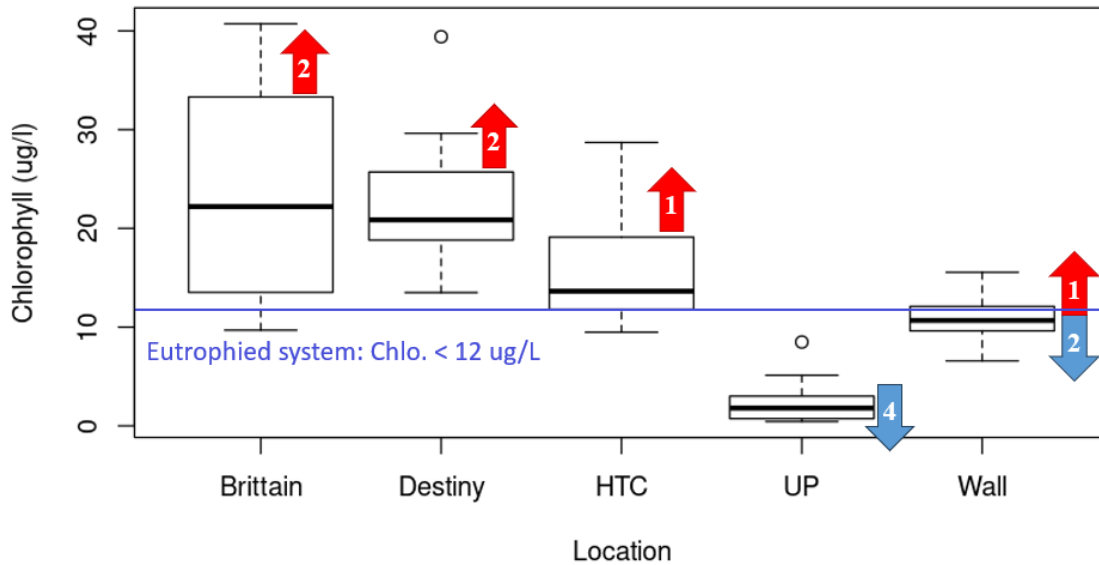


Figure 9. Water quality parameter box plot for chlorophyll over 10 sampling dates. A blue arrow indicates the number of other sites the indicated site is significantly lower than, and a red arrow indicates the number of other sites it is significantly higher than (Pairwise Wilcoxon rank sum test, $p < 0.05$). The blue line indicates the chlorophyll threshold for a eutrophied system (Coastal Plain National Lake Assessment, EPA 2017).

Table 3. Spearman’s Correlation Coefficient for oxygen saturation and pH values at all five sites. Three asterisks (***) indicate a highly significant corresponding p-value ($p < 0.001$) and two asterisks (**) indicate a very significant corresponding p-value ($p < 0.01$).

Site	Oxygen percent saturation and pH Correlation Coefficient
Wall	0.785 (**)
HTC	0.979 (***)
Destiny	0.720 (**)
Brittain	0.932 (***)
UP	0.937 (***)

HAB cyanobacteria counts

Results from the microscopic counts of phytoplankton are shown in Figures 10 – 15 as boxplots, and in Table 4. As with the water quality results, repeated measures tests were used to detect site-to-site differences.

According to the Pairwise Wilcoxon Rank Sum test, Wall pond had a significantly higher *Aphanizomenon* count than Destiny and UP, which both had zero counts for this genus (Figure 10). Wall pond had a significantly higher *Raphidiopsis* count than all other locations, including two sites with zero counts (Destiny and UP), and two sites with low numerical counts (HTC and Brittain) (Pairwise Wilcoxon Rank Sum test; Figure 11). No locations were significantly different for *Planktothrix*, *Dolichospermum*, and *Microcystis* counts (Pairwise Wilcoxon Rank Sum test; Figures 12, 13, and 14). Total genera counts were computed by summing the counts of the five genera after converting *Microcystis* from colonies to cells (individuals).

Wall pond had a significantly higher total genera count (consisting of 5 genera) than Brittain, Destiny, and UP, but no significant difference was detected between Wall and HTC (Pairwise Wilcoxon Rank Sum test; Figure 15). Table 4 shows total HAB cyanobacteria counts by location and date. HTC and Wall ponds both had algal counts exceeding the HAB bloom criteria on two sampling dates (Graham et al. 2008).

Friedman tests detected a difference between *Microcystis* and *Planktothrix* counts, but not other genera or total counts. Friedman tests for *Aphanizomenon*, *Raphidiopsis*, and total counts detected no significant differences, while the Wilcoxon test did detect differences. Friedman tests for *Planktothrix* and *Microcystis* data sets showed significant differences which were not reflected in the Wilcoxon tests. Only Friedman and Wilcoxon tests run on *Dolichospermum* count

data showed the same results, as both failed to find significant differences between sites. All count data sets cover the same number of sampling dates, which is ten.

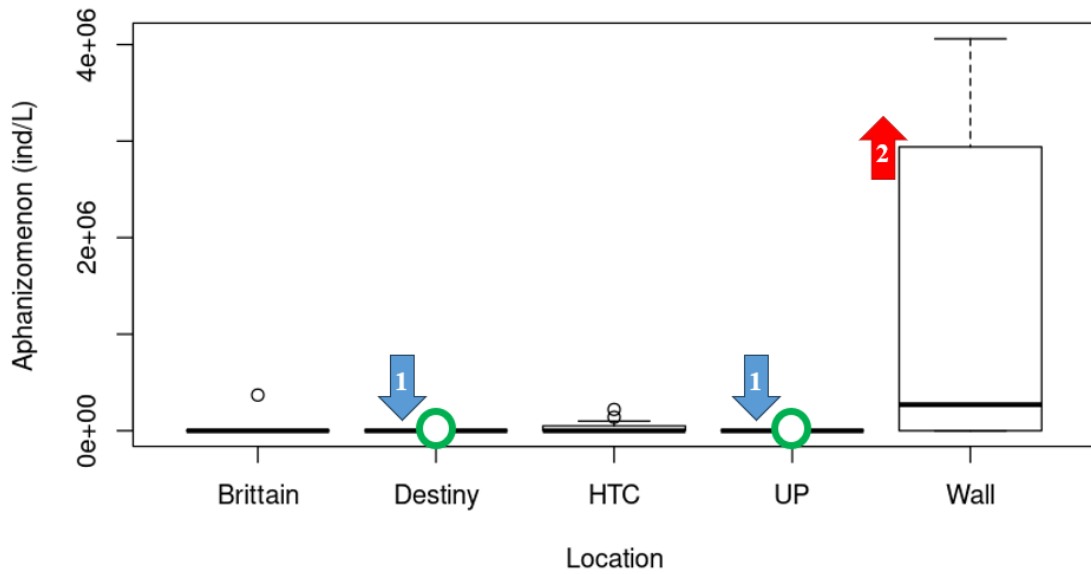


Figure 10. *Aphanizomenon* counts for each location over 11 sampling dates. A blue arrow next to a site's box indicates the number of other sites the parameter for the indicated site is significantly lower than, and a red arrow indicates number of sites an indicated site is significantly higher than (Pairwise Wilcoxon rank sum test, $p < 0.05$). Green circles indicate a total count of zero for the indicated genus and location.

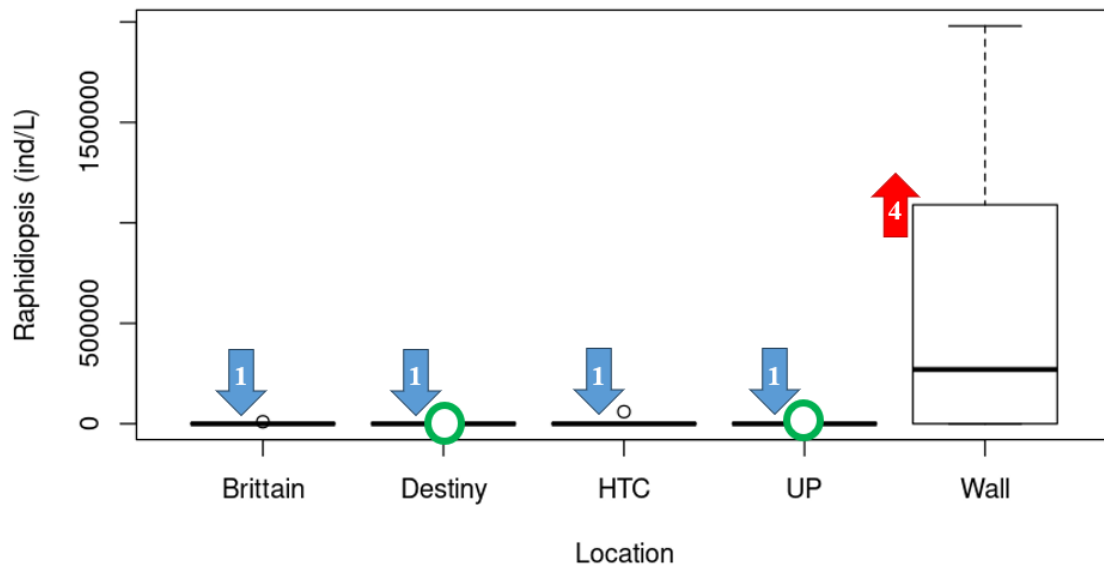


Figure 11. *Raphidiopsis* counts for each location over 11 sampling dates. A blue arrow next to a site's box indicates the number of other sites the parameter for the indicated site is significantly lower than, and a red arrow indicates number of sites an indicated site is significantly higher than (Pairwise Wilcoxon rank sum test, $p < 0.05$). Green circles indicate a total count of zero for the indicated genus and location.

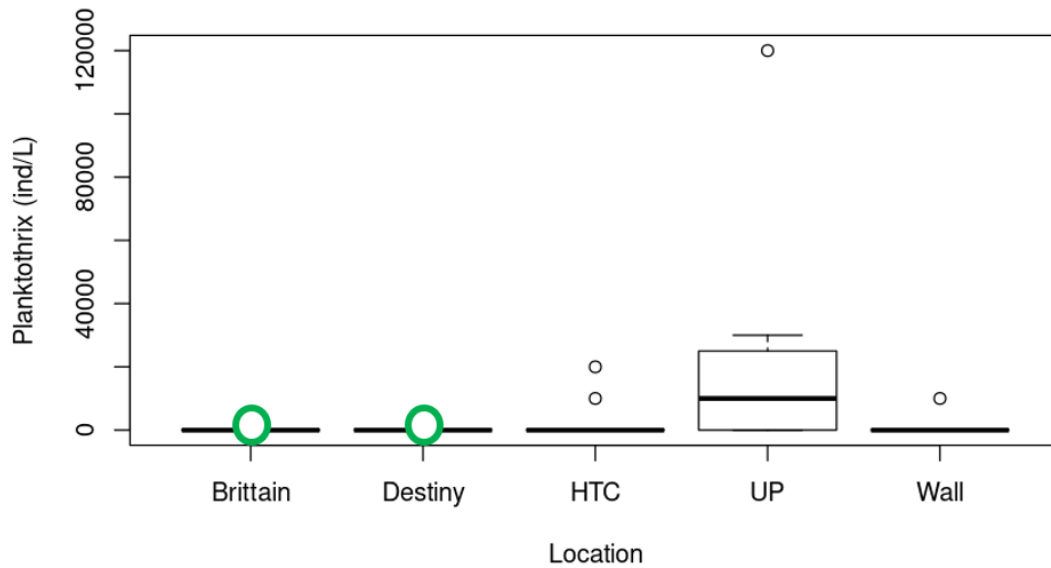


Figure 12. *Planktothrix* counts for each location over 11 sampling dates. Green circles indicate a total count of zero for the indicated genus and location.

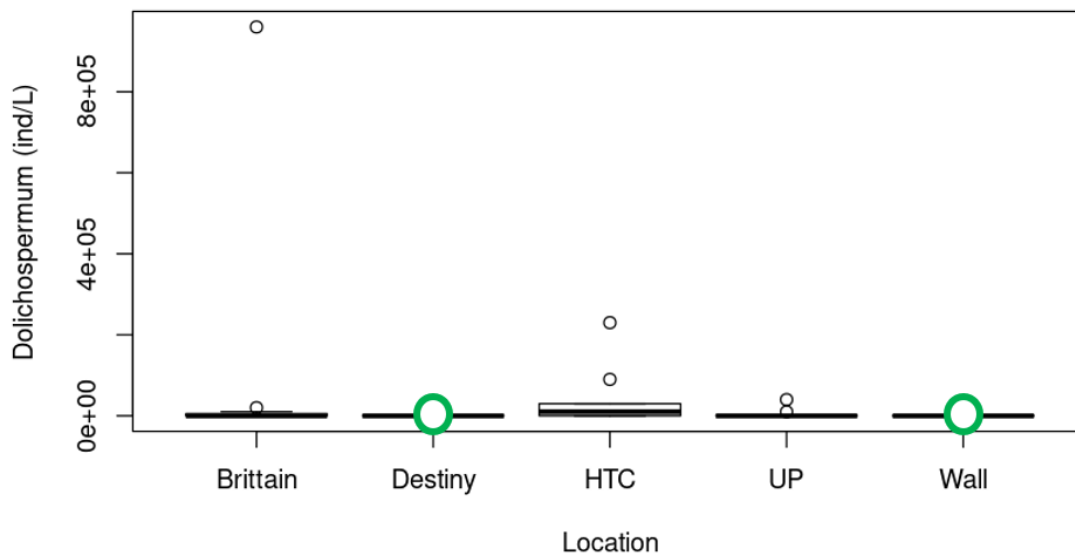


Figure 13. *Dolichospermum* counts for each location over 11 sampling dates. Green circles indicate a total count of zero for the indicated genus and location.

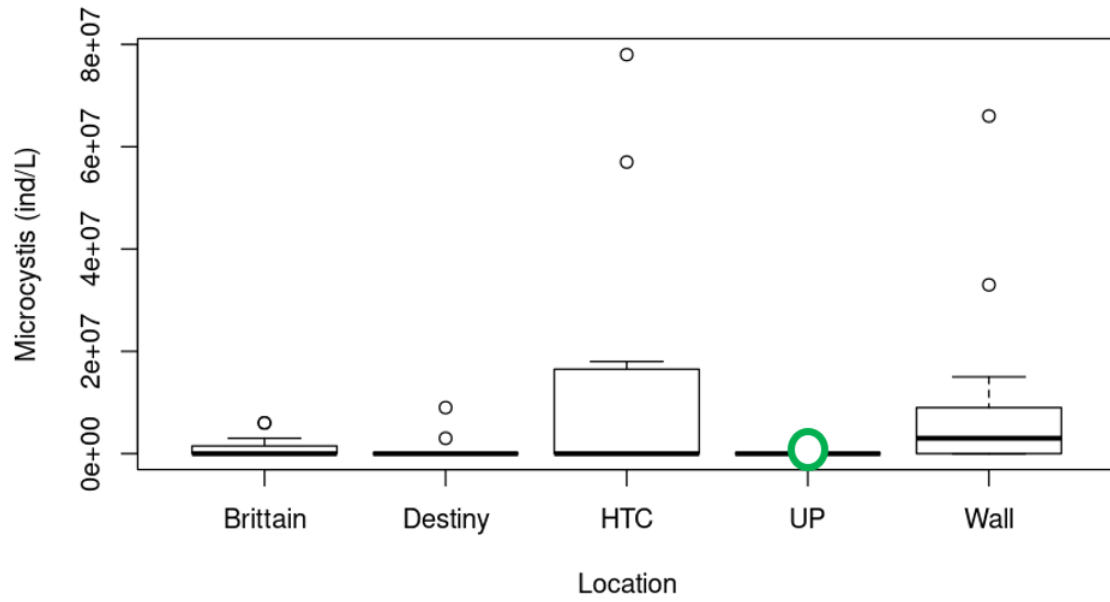


Figure 14. *Microcystis* counts (estimated individual cells/L) for each location over 11 sampling dates. Green circles indicate a total count of zero for the indicated genus and location.

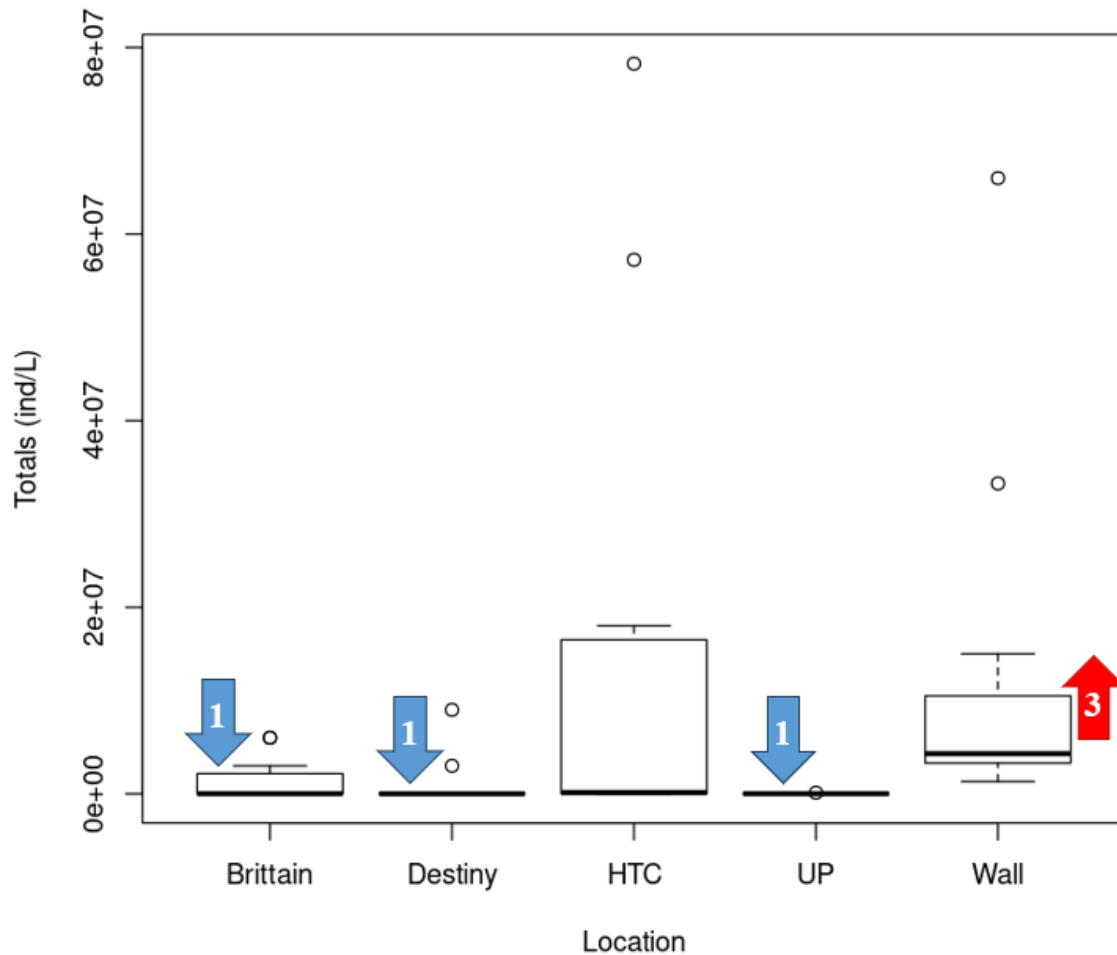


Figure 15. Combined counts from all 5 genera of cyanobacteria (*Aphanizomenon*, *Raphidiopsis*, *Planktothrix*, *Dolichospermum*, and *Microcystis*) for each location over 11 sampling dates. A blue arrow next to a site's box indicates the number of other sites the parameter for the indicated site is significantly lower than, and a red arrow indicates number of sites an indicated site is significantly higher than (Pairwise Wilcoxon rank sum test, $p < 0.05$).

Table 4. Total HAB cell counts (including *Aphanizomenon*, *Raphidiopsis*, *Planktothrix*, *Dolichospermum*, and *Microcystis*) by location and date. Counts highlighted in red indicate HAB cell densities that exceeded the HAB bloom criteria of 2×10^7 cells/L (Graham et al. 2008). Orange indicates that cell densities are below 2×10^7 cells/L, but greater than zero. Green cells indicate a total count of zero for all five genera.

Total (individuals /L)					
Date	Brittain	Destiny	UP	HTC	Wall
6/30/2023	3.70.E+05	0	0	0	1.31.E+06
7/14/2023	0	0	1.00.E+04	0	5.21.E+06
7/28/2023	0	0	1.00.E+04	5.74.E+07	3.79.E+06
8/11/2023	0	0	1.20.E+05	7.84.E+07	6.01.E+06
8/24/2023	0	0	2.00.E+04	1.40.E+05	4.30.E+06
9/14/2023	0	3.01.E+06	3.00.E+04	1.00.E+04	3.52.E+06
9/28/2023	0	0	3.00.E+04	0	3.11.E+06
10/19/2023	6.03.E+06	9.03.E+06	0	1.81.E+07	3.34.E+07
10/26/2023	3.01.E+06	0	0	1.51.E+07	6.62.E+07
11/9/2023	6.02.E+06	0	0	0	1.51.E+07
11/30/2023	0	0	0	0	3.01.E+06

Wall pond *Microcystis* bloom event

A bloom event was captured at Wall pond from 10/26/2023 to 11/30/2023. Cell densities for two locations within Wall pond, one in the center of the pond off a bridge (WP1) and one on the water’s edge (WP3) can be seen in Figures 16 and 17. The threshold for HAB conditions at

2×10^7 cells/L is indicated as a blue line on both figures (Graham et al., 2008). Both locations exceeded this threshold for multiple dates during the sampling period. Microcystins concentrations and cell counts for each date and site within Wall pond can be seen in Table 5. Both sites had microcystin toxin concentrations exceeding SC DHEC's recreational water criterion at 8 ppb (Table 5; SC DHEC, 2020).

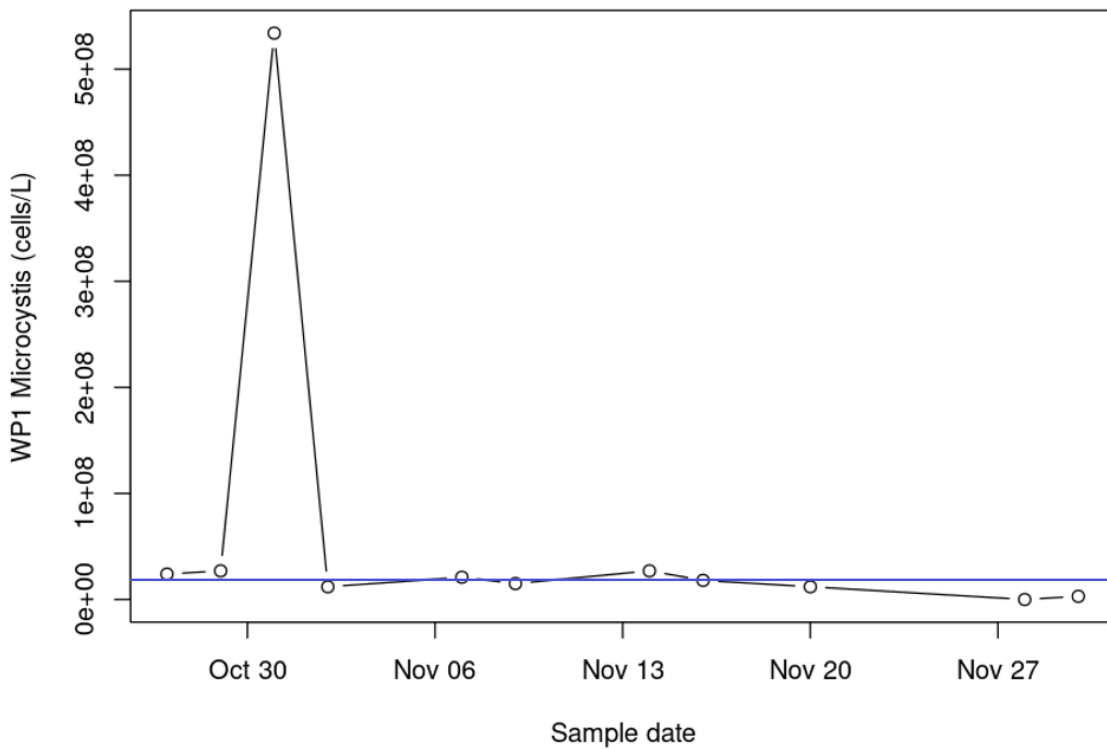


Figure 16. *Microcystis* counts in cells/L (estimated from colony counts) at WP1, a site in the middle of Wall pond, off of a bridge. The blue line indicates the threshold for HAB conditions at 2×10^7 cells/L (Graham et al., 2008). Counts cover 11 sampling dates from 10/27/23 to 11/30/23.

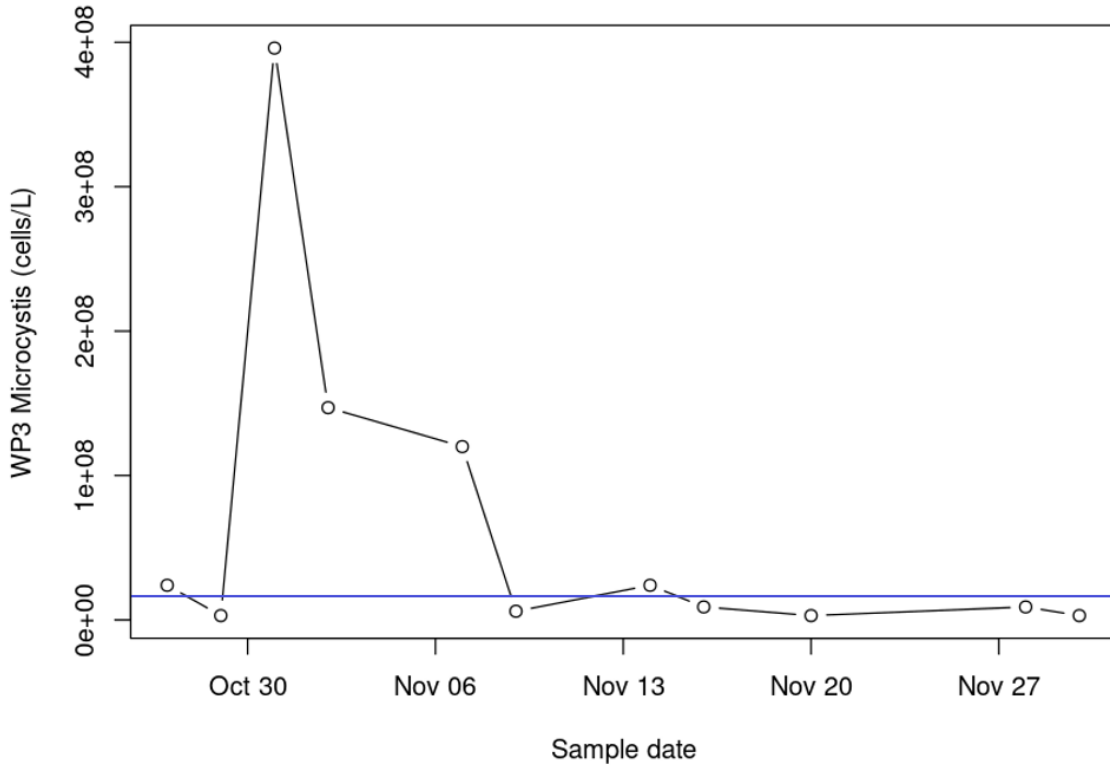


Figure 17. *Microcystis* counts in cells/L (estimated from colony counts) at WP3, a site in the middle of Wall pond, off of a bridge. The blue line indicates the threshold for HAB conditions at 2×10^7 cells/L (Graham et al., 2008). Counts cover 11 sampling dates from 10/27/23 to 11/30/23.

Table 5. *Microcystis* cell counts (estimated from colony counts) for two sites within Wall pond, and microcystins concentration estimates. Cell counts in red exceed the threshold for HAB conditions (Graham et al. 2008), counts in orange are below the threshold, and counts in green are zero. Microcystins concentrations in yellow are greater than the SC DHEC recreational water criterion of 8 ppb (SC DHEC, 2020).

Date	WP1 <i>Microcystis</i> (cells/L)	WP3 <i>Microcystis</i> (cells/L)	Microcystins (ppb)
10/27/2023	2.40.E+07	2.40.E+07	>10
10/29/2023	2.70.E+07	3.00.E+06	No test
10/31/2023	5.34.E+08	3.96.E+08	>10
11/2/2023	1.20.E+07	1.47.E+08	>10
11/7/2023	2.10.E+07	1.20.E+08	>10
11/9/2023	1.50.E+07	6.00.E+06	>10
11/14/2023	2.70.E+07	2.40.E+07	>10
11/16/2023	1.80.E+07	9.00.E+06	2.5-10
11/20/2023	1.20.E+07	3.00.E+06	0-1
11/28/2023	0	9.00.E+06	0-1
11/30/2023	3.00.E+06	3.00.E+06	0

Discussion

Research questions

The three questions addressed in this research are as follows: Are the target genera of HAB cyanobacteria present and/or blooming in retention ponds on Coastal Carolina University's campus? How does this change over time? And do water quality parameters confirm the presence of blooms?

Counts for five HAB genera were analyzed to determine if any one genus reached bloom status on any sampling date. Water quality data were used to compute an index that indicated the likelihood that an algal bloom was underway.

Notable water quality results

The water pH exceeded the Class FW criterion (8.5) in Wall Pond on 8 sampling dates and was above 8.0 on all 11 sampling dates. In UP, the pH was above 8.0 on 4 sampling dates. All ponds except for Destiny had supersaturated oxygen levels during at least one sampling. UP had the highest frequency with 6 dates being supersaturated followed by Wall (5 dates), Brittan (1 date), and HTC (1 date). In Destiny Pond, oxygen concentrations were hypoxic (< 2 mg/L) during 9 samplings. All ponds except for UP had chlorophyll concentrations that exceeded the EPA (2017) threshold for eutrophication (12 µg/L) during at least one sampling. Destiny had the highest frequency with all 10 dates exceeding the threshold, followed by Brittan (9 dates), HTC (7 dates) and Wall (3 dates).

Many parameters measured at UP differed from other sites. This site showed lower chlorophyll *a* concentrations than all other sites, as well as low turbidity, and conductivity. Highly variable oxygen levels and pH were also noted at this site.

Destiny pond had notable results in the form of high chlorophyll *a* concentrations coupled with low pH and oxygen levels. This site also had the lowest frequency of HAB genera detections. The low oxygen and pH in the pond could be due to decomposition by microbial aerobic respiration of detrital organic matter from leaf litter and non-HAB algal blooms, although none were noted over the course of the study.

Water quality parameters as an indicator for ongoing algal activity and HAB risk

Water quality parameters were used to predict which sites had a higher risk for harboring a HAB, based on which sites may have had algal blooms, harmful or not. A system was developed using pH, dissolved oxygen saturation (%), and chlorophyll concentrations to rank sites by their potential for having ongoing blooms, and therefore HABs, during the study period.

The significant positive correlation between oxygen saturation and pH values at all five sites suggests that there is a strong biological control on pH in the ponds (Table 3). This supports the use of oxygen saturation and pH as parameters for detecting algal blooms as pH should increase with increasing net photosynthesis due to the drawdown of dissolved inorganic carbon. Destiny is a likely exception to this. Although chlorophyll concentrations were high, pH and oxygen saturation were low, likely due to organic matter loading.

To provide a ranking for each sampling date, parameter scores were summed, with the highest ranking indicating the greatest likelihood that an algal bloom was occurring. The parameter scores were assigned by placing measurements for each parameter on each date into one of four bins assigned scores of 1, 2, 3, or 4 (Table 6). For pH, the highest score was assigned to values that exceeded the 8.5 SU regulatory water quality criterion with the other three bins established by decreasing steps of 0.5 SU. For chlorophyll, the bins are based on the < 25th percentile, 25th to 50th percentile, 50th to 75th percentile and >75th percentile with the highest

chlorophyll having the highest score. For oxygen saturation, the highest score was applied to data that were supersaturated. The bin with the lowest score was set at <60% as this level is typically equivalent to a warm weather dissolved oxygen concentration of 4 mg/L (Class FW water quality criterion). The rest of the oxygen saturation data were split into values bins of 90% to 100% (moderately elevated) and 60 to 90% (typical values). The site-to-site differences in rankings are summarized in the boxplot in Figure 18. The Wilcoxon test indicted that Brittain pond had a significantly greater likelihood of having ongoing algal blooms as compared to Destiny pond, which had a significantly lower ranking than all sites but UP.

Table 6. Scoring system for chlorophyll, oxygen saturation and pH measurements with highest scores representing greatest likelihood for ongoing algal blooms.

Algal Bloom Likelihood Score	Parameter		
	Chlorophyll (µg/L)	Dissolved Oxygen saturation (%)	pH
1	$9.55 \leq$	≤ 60	≤ 7.0
2	$9.55 < x \leq 12.82$	$60 < x \leq 90.1$	$7.0 < x \leq 7.5$
3	$12.82 < x \leq 20.44$	$90.1 < x \leq 100$	$7.5 < x \leq 8.5$
4	> 20.44	> 100	> 8.5

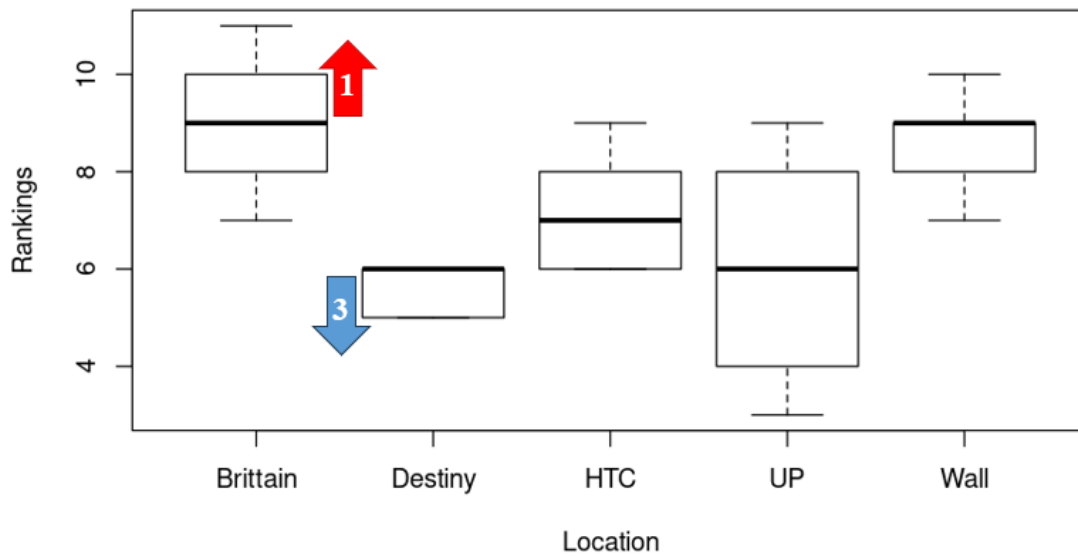


Figure 18. Water quality ranks showing likelihood of ongoing algal blooms. The sites with the highest median rankings were Wall and Brittain. Destiny has a significantly lower ranking than all locations except for UP ($p < 0.05$, Pairwise Wilcoxon rank sum test).

Evidence for HABs from genera identification and counts

The three ponds with the highest median water quality rankings (Figure 18), Brittain, Wall, and HTC, all had some evidence for algal blooms based on the genera counts (Table 4). The strongest evidence was seen in Wall pond, where HAB species were detected on every sampling with concentrations ranging from 1.31 to 66.2×10^6 cells/L with two sampling dates exceeding the bloom threshold of 20×10^6 cells/L. Wall pond's *Microcystis* counts exceeded the threshold for HAB conditions on two regular sampling dates, 10/19/2023 and 10/26/2023. In HTC, HAB genera were detected on six sampling dates with bloom conditions on two dates (7/28/23 and 8/11/23) caused by *Microcystis*. In Brittan Pond, HAB genera were detected on four

sampling dates. Although not a HAB species, a high density of pennate diatoms was observed (Table 7).

As predicted from their low water quality rankings, the least evidence for HABs was found in Destiny and HTC. Destiny had the lowest incidence of HAB genera (two sampling dates). UP had HAB genera detected on six dates, but all at low cell densities.

Table 7. Counts for a potential pennate diatom bloom noted at Brittain pond from 7/28/23 to 11/30/23. This count was not performed for a HAB genus, but rather because a high number of cells were noted. Counting procedures used for HAB genera were utilized to obtain these results.

Sampling date	Pennate diatoms (cells/L)
7/28/2023	8.00E+04
8/11/2023	7.90E+05
8/24/2023	1.74E+06
9/14/2023	2.42E+06
9/28/2023	6.45E+06
10/19/2023	5.66E+06
10/26/2023	3.87E+06
11/9/2023	2.95E+06
11/30/2023	8.00E+04

Wall pond *Microcystis* bloom

A visible *Microcystis* bloom was present at Wall pond from 10/26/2023 to 11/30/2023. Nine additional samples were taken during the course of the bloom in addition to the two scheduled sampling dates for all ponds for a total of 11 HAB count samples from the bloom. The results are shown in Table 5 and plotted as time trend graphs in Figures 16 and 17. Table 5 also includes semi-quantitative measurements of Microcystin from Abraxis screening strips.

At both sites within the pond, cell concentrations exceeded the threshold for HAB conditions. The microcystin toxin levels reached at least 10 ppb, exceeding the SC DHEC recreational water criterion at 8 ppb by at least 2 ppb (SC DHEC, 2020). This represented a human health concern as Wall pond in particular is central to CCU's campus, with foot traffic on nearly all sides and a bridge crossing the pond with fountains on either side. Potential routes to toxin exposure may include aerosolized toxins from fountain spray, or toxins present on the lawn adjacent to the pond, which is watered from the pond itself. Fountains were turned off for a period over the course of the bloom to reduce the possibility of microcystins inhalation.

The bloom was monitored for a little over a month, although the exact start date and end date of the bloom are unknown. The exact cause of the bloom is also unknown, although the end of the bloom may have been hastened by additional algaecide applications once the bloom was confirmed. This indicates that this site may have a high risk for HABs in the future, as no prevention strategies are in place.

Conclusions and further research

Three of five monitored ponds on and around CCU's campus showed evidence of algal blooms over the course of this study: Wall, HTC, and Brittain ponds. HAB genera were detected in all three ponds with bloom conditions occurring in Wall and HTC ponds. For Wall, toxin testing documents that microcystins were present at concentrations exceeding the Class FW water quality criteria.

It is unclear whether toxin levels in Wall pond present a significant health risk to students and staff on CCU's campus. Further studies could evaluate this and should include toxin testing in other ponds on and off CCU's campus, along with nutrient tests to determine if increased nutrient levels may be a cause for HABs at any location.

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