

**DEPARTMENT OF ENVIRONMENTAL PROTECTION  
Progress Report Form**

**Exhibit A**

<b>DEP Agreement No.:</b>	<b>INV006</b>		
<b>Grantee Name:</b>	Florida Gulf Coast University		
<b>Grantee Address:</b>	10501 FGCU Blvd. South, Fort Myers, FL 33965-6565		
<b>Grantee's Grant Manager:</b>	Donna Gilmore	<b>Telephone No.:</b>	(239) 590-7582
<b>Reporting Period:</b>	JULY 2020–FEBRUARY 2022		
<b>Project Number and Title:</b>	Monitoring, predicting, and controlling harmful algal blooms by buoy ultrasonic technology in a range of lakes in southwest Florida		
<p><b>Provide the following information for all tasks and deliverables identified in the Grant Work Plan: a summary of project accomplishments for the reporting period; a comparison of actual accomplishments to goals for the period; if goals were not met, provide reasons why; provide an update on the estimated time for completion of the task and an explanation for any anticipated delays and identify by task.</b></p> <p><b>NOTE: Use as many pages as necessary to cover all tasks in the Grant Work Plan.</b></p> <p><b><u>See Attached Final Report Draft</u></b></p>			

This report is submitted in accordance with the reporting requirements of DEP Agreement No. INV006 and accurately reflects the activities associated with the project.



\_\_\_\_\_  
Director of Research  
Signature of Grantee's Grant Manager

\_\_\_\_\_  
April 4, 2022  
Date

**STATE OF FLORIDA  
DEPARTMENT OF ENVIRONMENTAL PROTECTION**

**FINAL REPORT (DRAFT)  
JULY 2020–FEBRUARY 2022  
DEP AGREEMENT NO. INV006**

Monitoring, predicting, and controlling harmful algal blooms by buoy ultrasonic  
technology in a range of lakes in southwest Florida

Submitted by

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## **Task 5: Final Report**

**Task Description:** The Grantee will prepare a Final Report summarizing the results of the project, including all tasks in the Grant Work Plan. The Final Report must include at a minimum:

- Project location and background, project description and timeline, grant award amount, and anticipated benefits.
- Financial summary of actual costs versus the budget, along with any changes required to the budget. Include any match or locally pledged contributions provided, along with other related project work performed outside of this Agreement to identify the overall project cost.
- Discussion of project schedule versus actual completion, including changes required to the schedule, unexpected site conditions and adjustments, significant unexpected delays and corrections, and/or other significant deviations from the original project plan.
- Summary of activities completed as well as those not completed and why, as well as a brief summary of any additional phases yet to be completed.

- Photo documentation of work performed (before, during and after), appropriate figures (site location, site plans, etc.), appropriate tables summarizing data/information relevant to Grant Work Plan tasks, and appropriate attachments relevant to the project.
- Discussion of whether the anticipated benefits have been/will be realized (e.g., why a Best Management Practice [BMP] approach did or did not exceed the expected removal efficiency).
- Summary of monitoring activities completed and any not completed and why, monitoring results, and an interpretation of data based on planned versus realized results.

**Deliverable 5a:** An electronic copy of the draft Final Report in Word format submitted to the Department's Grant Manager for review prior to submission of the Final Report. Upon request, the Grantee will provide a hardcopy of the draft Final Report.

**Performance Standard:** The Department's Grant Manager will review the submitted draft Final Report to verify that it meets the specifications in the Grant Work Plan and this task description and provide any comments to the Grantee for incorporation into the Final Report.

**Deliverable 5b:** An electronic copy of the Final Report, with all Department comments/concerns, addressed and incorporated, in PDF format submitted to the Department's Grant Manager for review and approval. Upon request, the Grantee will provide a hardcopy of the Final Report.

**Performance Standard:** Upon review and written approval by the Department's Grant Manager of the Final Report, the Grantee may proceed with payment request submittal for this task.

**Payment Request Schedule:** Grantee may submit a payment request for cost reimbursement upon completion of the task and Department approval of all associated task deliverables.

## 1. Project location and description

Description of project: The project was located at one multi-lake location in southwest Florida: Treviso Bay Naples, a golf community in Collier County (26°4'42.3"N, 81°44'18.0"W) with 42 available lakes covering 182 acres (Figure 1). LG-Sonic MPC-Buoy ultrasonic technology can potentially deactivate cells from the photic zone at initial cyanobacterial bloom locations within impacted south Florida bodies of water, such as Lake Okeechobee and the lakes emphasized in this study. This project united a technical firm (LG-Sonic) with significant experience in restoring lakes and aquatic ecosystems with MPC-Buoy ultrasonic technology with one of the most active water quality/wetland biochemistry laboratories in the United States – FGCU's Everglades Wetland Research Park (EWRP) in Naples, Florida.

This two-year project was a test of the MPC-Buoy ultrasonic technology to eliminate the symptoms of eutrophication by cyanobacteria for a scale of a few to 50 or more acres. If the results were significant and reliable at this scale, it could be tested at a much larger scale, such as in the outflow areas of Lake Okeechobee. Reliable gas vesicle operation is essential to harmful algae (such as *M. aeruginosa*) behavior for their proliferation. Existing LG-Sonic ultrasonic technology (MPC-Buoy) can exclude cells from the surface at initial cyanobacterial bloom locations within a lake's water column, preventing vertical movement and suppressing the proliferation of the cells. Expected benefits included safe cell decomposition, a restored competitive environment for primary producers, and enhanced downstream water quality.

Each MPC-Buoy can treat up to 49 acres (20 hectares), an area equivalent to 37 football fields. Buoy dimensions are 8.3 ft x 7.2 ft x 2.8 ft (2.5 m x 2.2 m x 0.8 m) (L x W x H) and each weigh 441 pounds (200 kg).

Seven of the nine MPC-buoys had automatic water quality monitoring capabilities to monitor chlorophyll-a, phycocyanin (a light-harvesting pigment, more specifically found in a cyanobacterial bloom), pH, turbidity, dissolved oxygen, and temperature. This data was logged by the buoys and downloaded to a server every 30 minutes. Monthly water samples were measured to verify these results. FGCU's Everglades Wetland Research Park (EWRP) performed and oversaw monthly water quality measurements of Treviso Bay Naples. Surface water was analyzed *in-situ* by EWRP staff at nine locations (Figure 2) throughout the lake system. At each of the nine locations, in-situ water quality parameters were measured using a YSI data sonde to collect data for water temperature, pH, dissolved oxygen, and phycocyanin. Water samples were collected by EWRP staff for analysis of chlorophyll-a and turbidity by staff at Lee County Environmental Laboratory. Table 1 and Figure 2 list and show the locations of all 9 sampling points that were sampled for the project throughout Treviso Bay Naples.

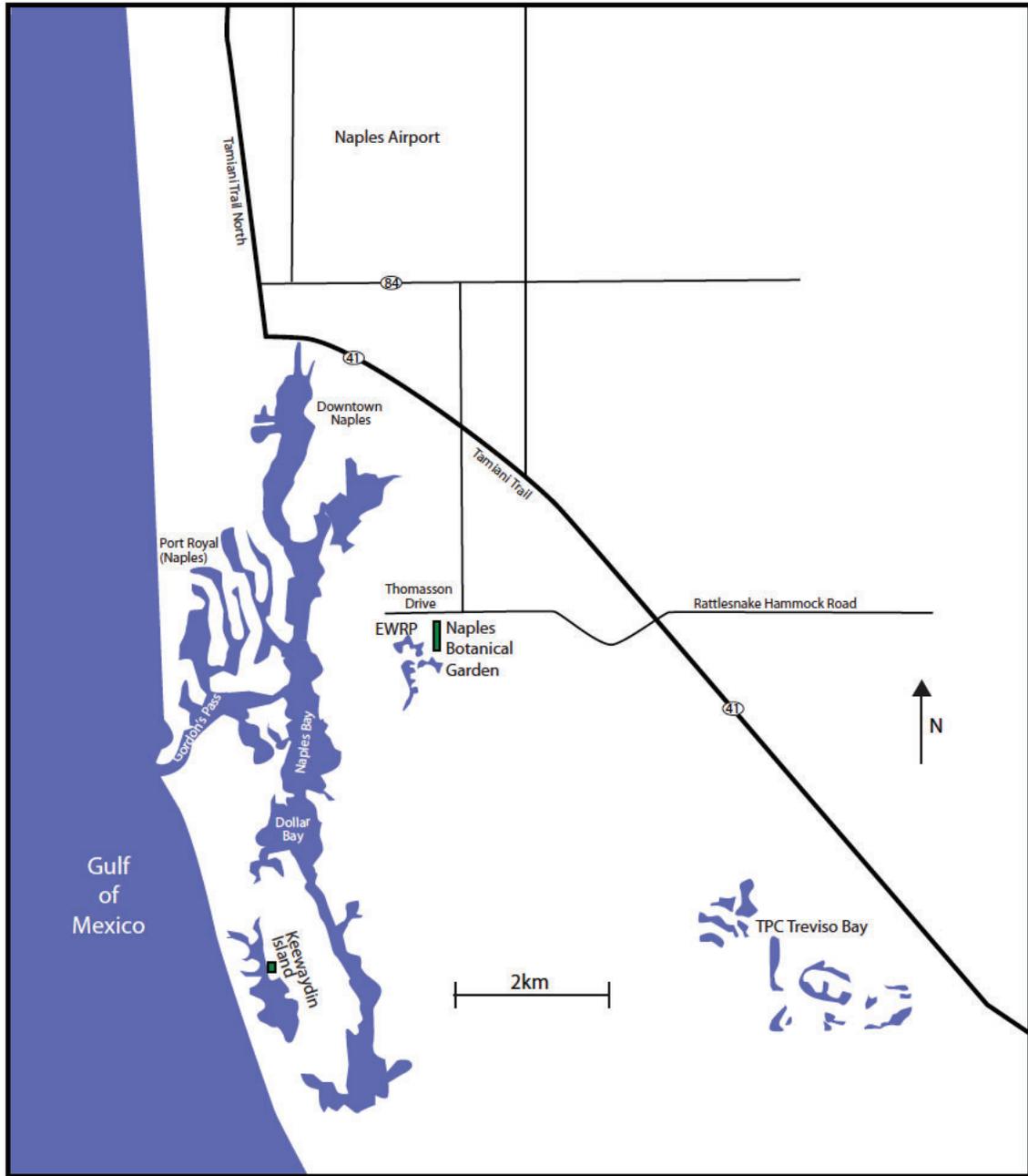


Figure 1. A map depicting the location of Treviso Bay and the Everglades Wetland Research Park (EWRP) within the larger City of Naples landscape.

Table 1. Geographic coordinates of buoys deployed in Treviso Bay lakes

Sampling location	Latitude, Longitude
TB-1	26°04'02.4"N 81°43'21.8"W
TB-2	26°04'09.8"N 81°43'44.6"W
TB-3	26°04'11.5"N 81°43'52.7"W
TB-4	26°04'20.1"N 81°43'50.8"W
TB-5	26°04'44.5"N 81°44'18.3"W
TB-6	26°05'01.1"N 81°44'54.2"W
TB-7	26°05'15.2"N 81°44'50.0"W
TB-8	26°05'24.9"N 81°44'42.7"W
TB-9	26°05'24.7"N 81°44'04.7"W

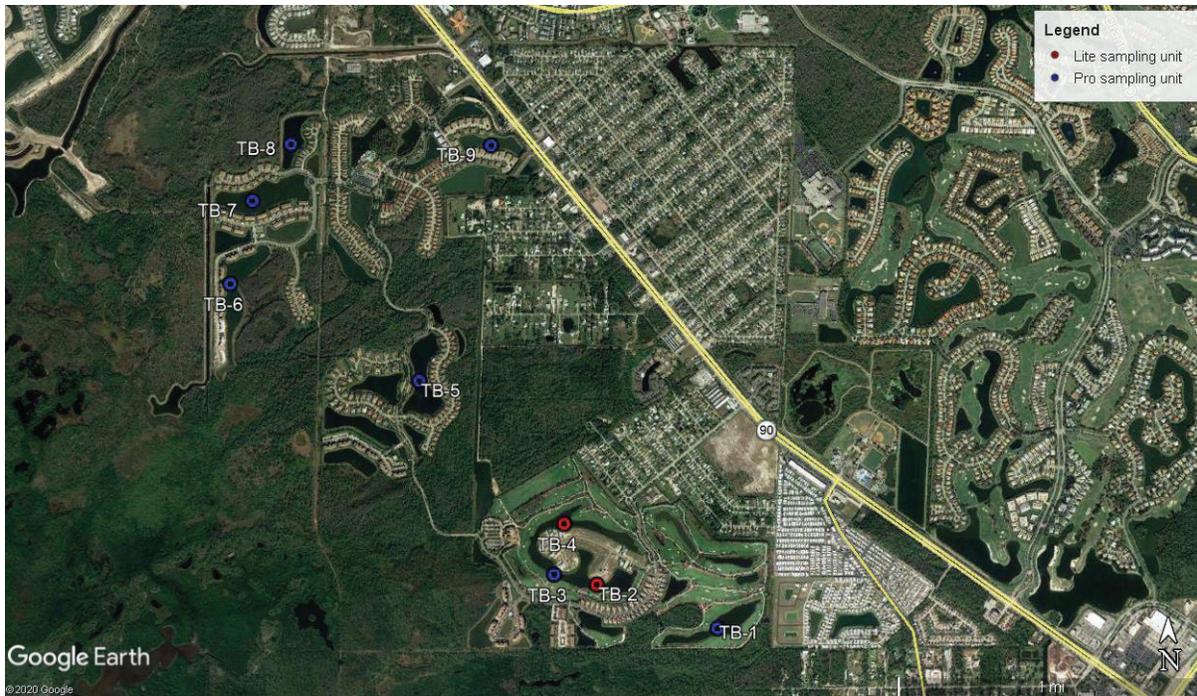


Figure 2. Nine sampling locations in seven of the lakes of Treviso Bay Naples. Blue dots indicate Pro sampling units which contain full monitoring, prediction, and control capability; red dots indicate Lite units which contain only control capability.

## 2. Financial summary of actual costs versus the budget

Table 2. Current balance for DEP AGREEMENT NO. INV006 as of March 31, 2022.

Original Award \$983,685 – Expenditures to date \$803,157.13 – Balance remaining \$180,527.87. There are several outstanding expenses still in process and subaward invoices to be received.

## 3. Discussion of project schedule versus actual completion

The schedule laid out in the QAPP involved a 21-month project beginning July 1, 2020 and ending March 31, 2022. The data collection period (Task 4) of the project was to begin January 2021 and end December 2021. Task 1 (Design and Permitting) was completed on schedule on September 1, 2020. Task 2 (QAPP) was completed on schedule in October 2020. Task 3 (Construction and Implementation) occurred from December 28, 2020 to January 13, 2021 about two weeks behind schedule due to several delays in buoy component shipping from the Netherlands to Miami, and transport from Miami to the Treviso Bay community. Task 4 (Monitoring and Verification) occurred from January 2021 to December 2021, and included all sampling collection events and Quarterly Report submissions, all of which were completed on schedule. Task 5 is the submission of this Final Report, which will be submitted on schedule.

## 4. Summary of activities completed and problems encountered

All study lakes were sampled as described above for 12 months (January 2021 through December 2021). Seven of the nine buoys were Pro sampling units that automatically collected data, which was verified and analyzed by EWRP staff. Water quality results collected by EWRP staff once per month during the sampling period, and automated buoy sampling results, are described below.

### Overall findings of our study

- The LG-Sonic buoys did not function as efficiently as anticipated in the Treviso Bay study lakes which were mostly dominated by filamentous algae as often found in Florida lakes. In other deployments in different locations, these buoys have been shown to be effective where unicellular algal blooms are more common. Ultrasonic treatment is better equipped to treat microalgae compared to the "more plant-like" filamentous algae which has more complex structure and tends to be benthic or accumulate around the shoreline away from the buoys.
- Overall, conclusions were clouded by the continued application of chemical algal treatment, with significant phycocyanin (PC) spikes seen in only one lake (TB-3) which was surrounded on all sides by construction projects and golf courses. Although significant PC spikes appearing in only one lake would normally suggest successful treatment, it is difficult to differentiate the impact made by the buoys versus the impact made by unanticipated and "not-agreed-upon" chemical algae treatments throughout the study.
- The water data that we gathered with monthly manual field sampling generally agreed with the much more frequent (48 times per day) water quality monitoring data collected by the LG Sonic buoys. Monthly data collection did miss spikes in poor water quality on several occasions especially for chlorophyll-a, phycocyanin, and turbidity in one lake (TB3).
- Water quality data collected as part of this study rarely indicated trophic conditions of "eutrophic" with one exception—TB2 sampling site in The Peninsula Lake. This is not surprising because of continual home construction and golf-course fertilization nearby.
- We recommend the use of LG-Sonic buoys as an excellent monitoring device for water quality itself. They do not appear to work well for managing water quality at small lakes in Florida because they do not work well with certain types of algae found frequently in small Florida lakes (filamentous algae) and because of the universal use of chemical spraying to control algal blooms that make conclusions on effectiveness essentially impossible.
- Surrounding artificial lakes with littoral zone and buffer wetlands is probably one of the most effective approaches for controlling lakes such as these study lakes from ultimately becoming eutrophic.

#### 4 a) Activities completed

### Monthly sampling data

Water bodies can be classified by trophic status as oligotrophic, mesotrophic, eutrophic or hypertrophic, which is a response to nutrient add-ins to the water (Bougarne & Abbou, 2019). The classification of a trophic state of an aquatic system is often evaluated by measuring several criteria, including chlorophyll concentration that indicates the extent of algal biomass and excessive eutrophication (Smith, 2003). Figure 3 illustrates chlorophyll concentrations observed at each of the study sites. Among the sites, TB-2 site had the highest average chlorophyll concentrations, while TB-8 had the lowest. Sites TB-5, TB-7, TB-8 and TB-9 showed concentrations that fell between 2~4  $\mu\text{g/L}$ , with TB-5 and TB-7 being very close to 3  $\mu\text{g/L}$  and TB-8 ~2.00  $\mu\text{g/L}$ . Sites TB-3, TB-6, and the Control lake had chlorophyll concentrations between 5~6  $\mu\text{g/L}$  while sites TB-1, TB-2, and TB-4 had concentrations above 6  $\mu\text{g/L}$ .

Using a system developed by the Organization for Economic Cooperation and Development (OECD), which is used internationally, the trophic status of the sites can be determined based on the chlorophyll concentrations (Bougarne & Abbou, 2019): Oligotrophic <2.5; mesotrophic 2.5 - 8; and eutrophic lakes 8-25  $\mu\text{g/L}$ . According to these guidelines, most of the lakes from this study would be considered 'mesotrophic'. TB-8 conditions were considered oligotrophic while TB-5 and TB-7 were slightly above that threshold. Amongst the lakes categorized as mesotrophic, TB-1 was the one lake nearing eutrophic conditions. TB-2 was the only buoy to be considered in eutrophic waters with a chlorophyll value of 9.77  $\mu\text{g/L}$ . It should be noted that buoys TB-2, TB-3, and TB-4 were all placed in different locations within the same narrow U-shaped lake. Additionally, all data depicted here are annual averages so the trophic status of any given lake is subject to change over time or within the year.

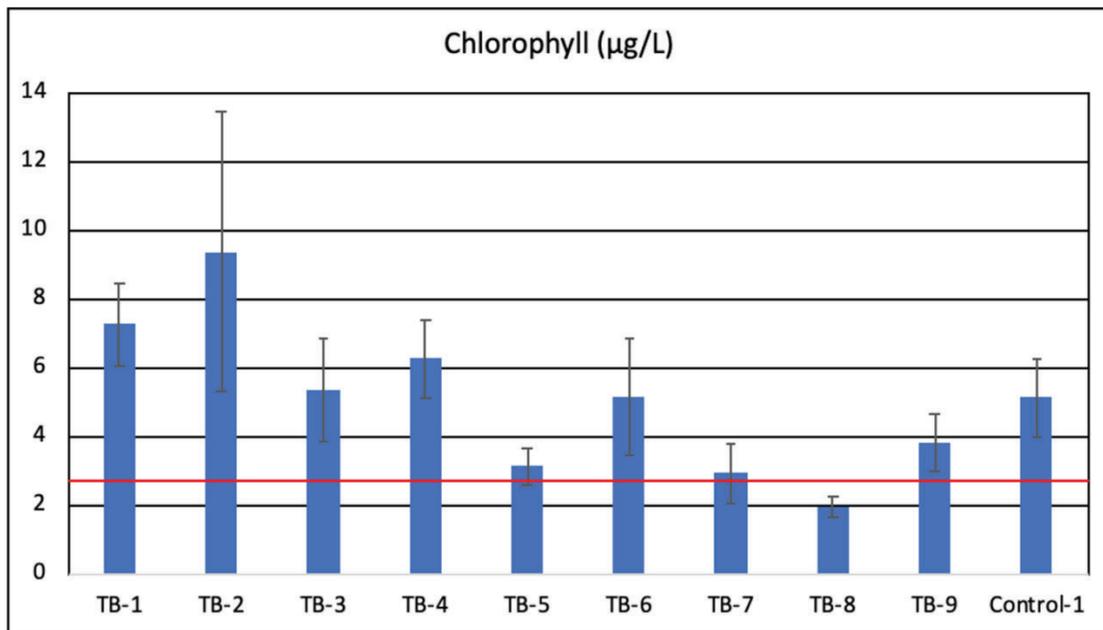


Figure 3. Average  $\pm$  standard error chlorophyll-a at each buoy location based on monthly water samples collected January–December 2021. The red line represents the threshold between oligotrophic and mesotrophic lakes.

Figure 4 depicts the same chlorophyll-a data as Figure 3, except it is divided into wet and dry season categories. The wet season is considered from May to October, while the dry season is considered from November to April. Chlorophyll-a concentrations were greater in the wet season than the dry season for all buoy locations. This is the expected outcome, as the wet season includes the warmest summer months with the longest sunlight hours, increasing the potential for phytoplankton and algal growth.

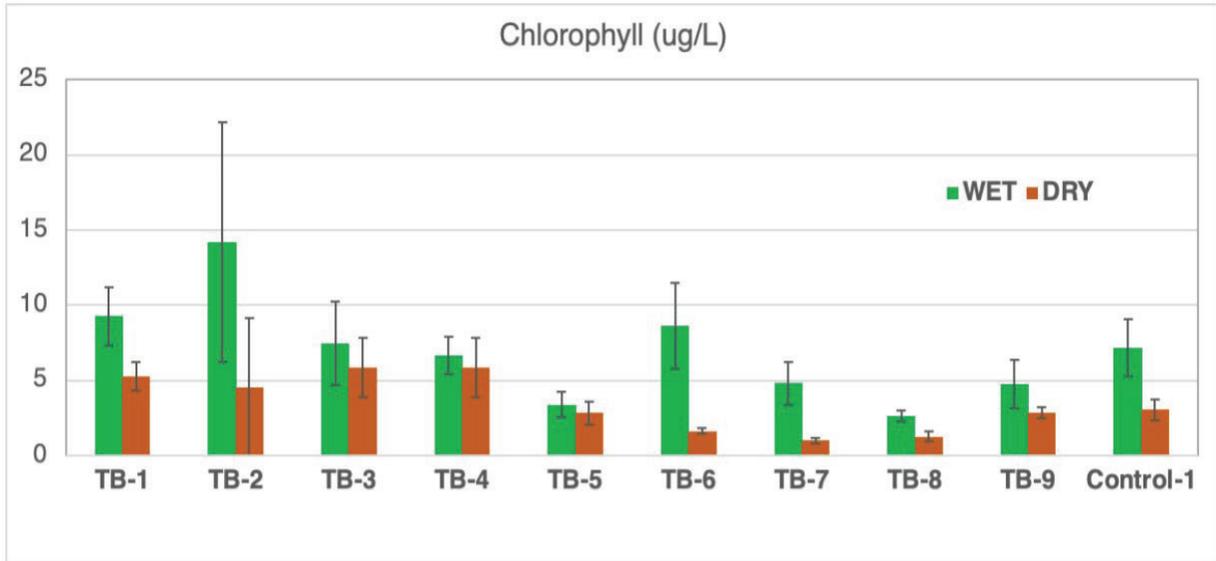


Figure 4. Average  $\pm$  standard error chlorophyll-a at each buoy location grouped into wet and dry season categories, based on monthly water samples collected January–December 2021.

Phycocyanin (PC) is a bluish green photosynthetic pigment found extensively in cyanobacteria, and is used as an indicator for harmful algal blooms (HAB) in freshwater lakes (Marion et al., 2012; Mchau et al., 2019). Figure 5 depicts annual PC concentration averages in the study lakes. TB-1, TB-2, TB-5, and TB-8 all had PC values lower than 0.10 RFU (relative fluorescence unit) with TB-8 having the lowest value. TB-3, TB-4, TB-6, TB-7, and the Control lake all had RFU values between 0.10 and 0.4 RFU. TB-9 had the highest RFU value out of the nine test sites with a value of 1.47 RFU, though it should be noted that this average was skewed by a single large outlier measurement taken in October. It is possible an algae bloom was occurring in the lake the day of that measurement, though no bloom was visibly evident. Using RFU values found in McQuaid et al.'s study (2011) these values can be compared to values found in other water bodies. Any value below 1.7 RFU is considered below the WHO alert level 1 standard. RFU values can be calibrated with PC standard solution to convert the RFU to actual PC concentrations ( $\mu\text{g/L}$ ). A PC concentration of  $4 \mu\text{g/L}$  is used to identify water bodies affected by cyanobacterial blooms that could have potential adverse health effects (Zhang et al., 2015). Based on RFU numbers, all locations in this study averaged PC estimates lower than the WHO alert level 1 and lower than the  $4 \mu\text{g/L}$  benchmark for adverse health during the monitoring period from January to December 2021.

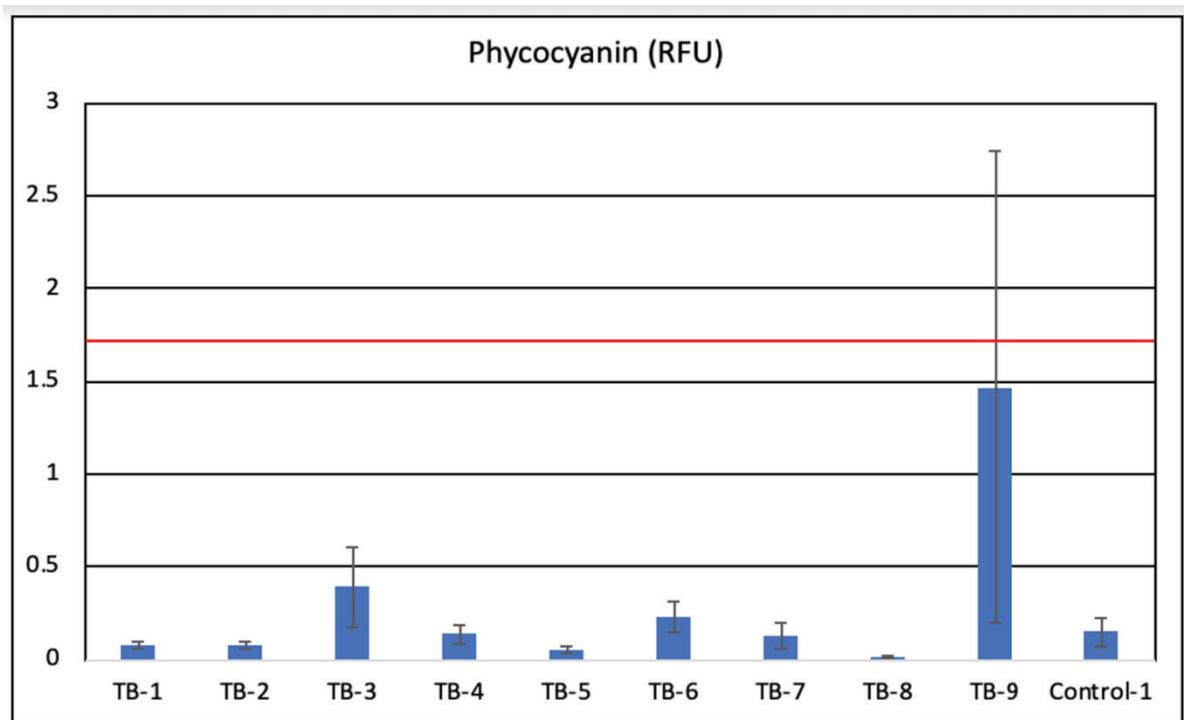


Figure 5. Average  $\pm$  standard error phycocyanin at each buoy location based on monthly water samples taken January– December 2021. The red line represents the threshold for the WHO alert level 1.

Turbidity is a crucial parameter to examine when assessing water quality because it is one of the factors effecting the health of primary producers in a body of water as well as other particles. A harmful algae bloom can potentially increase the turbidity of a water source, blocking light availability for beneficial algae and other aquatic plant life and fish (Davies-Colley & Smith, 2007). Figure 6 shows that TB-1 and TB-2 had NTU values just below 2.00, while TB-3, TB-4, and TB-5 had NTU values between 2.00 and 3.00. TB-6 had the highest NTU value (~ 4.00) out of the nine test sites, with the Control lake just below that value. TB-7, TB-8, and TB-9 all had values below or near 1.00 NTU. When comparing these values to others in the literature, all values are relatively low and should not cause any negative impacts on organisms within the water body such as fish foraging success or primary production. A value below 5 NTU is allowed for recreational purposes.

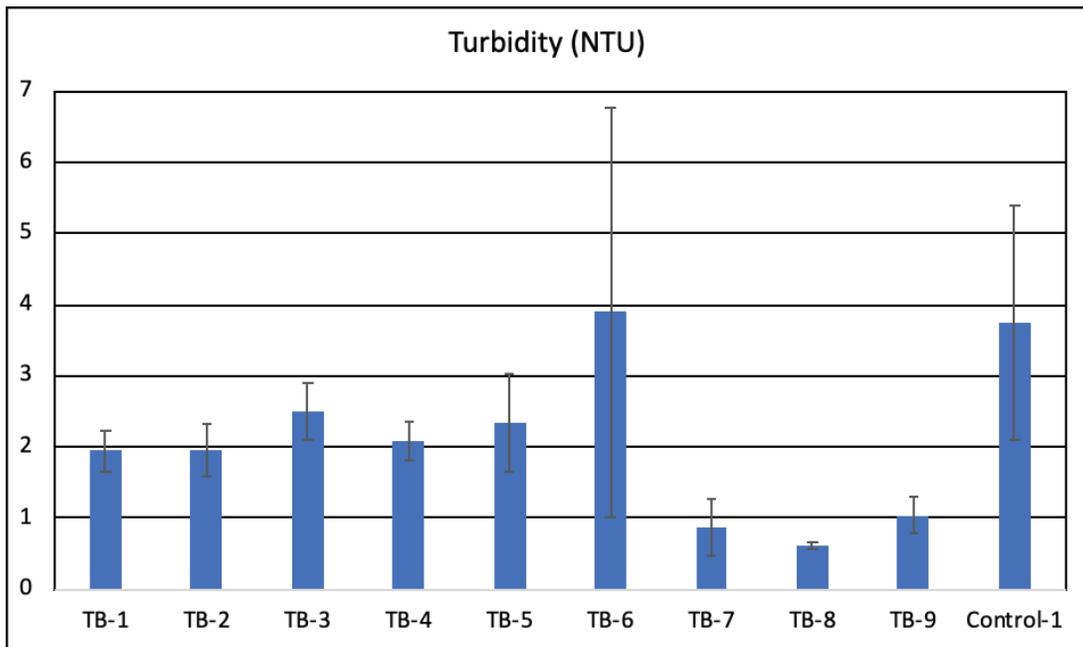


Figure 6. Average  $\pm$  standard error turbidity at each buoy location based on monthly water samples taken January– December 2021.

Dissolved oxygen (DO) is one of the most important indicators of water quality in lakes because it is essential for the survival of fish and other aquatic organisms. Figure 7 shows that dissolved oxygen concentrations observed at study sites TB-1, TB-3, TB-4, TB-9, and the Control lake had values below 8.00 mg/L, while TB-2, TB-5, TB-6, TB-7 and TB-8 had concentrations between 8.00 mg/L and 10.00 mg/L. Ideal DO concentrations for fish survival are at least 5 mg/L, though it depends on species (Boyd et al., 2017). These data indicate that all lakes' average DO concentrations are sufficient for fish.

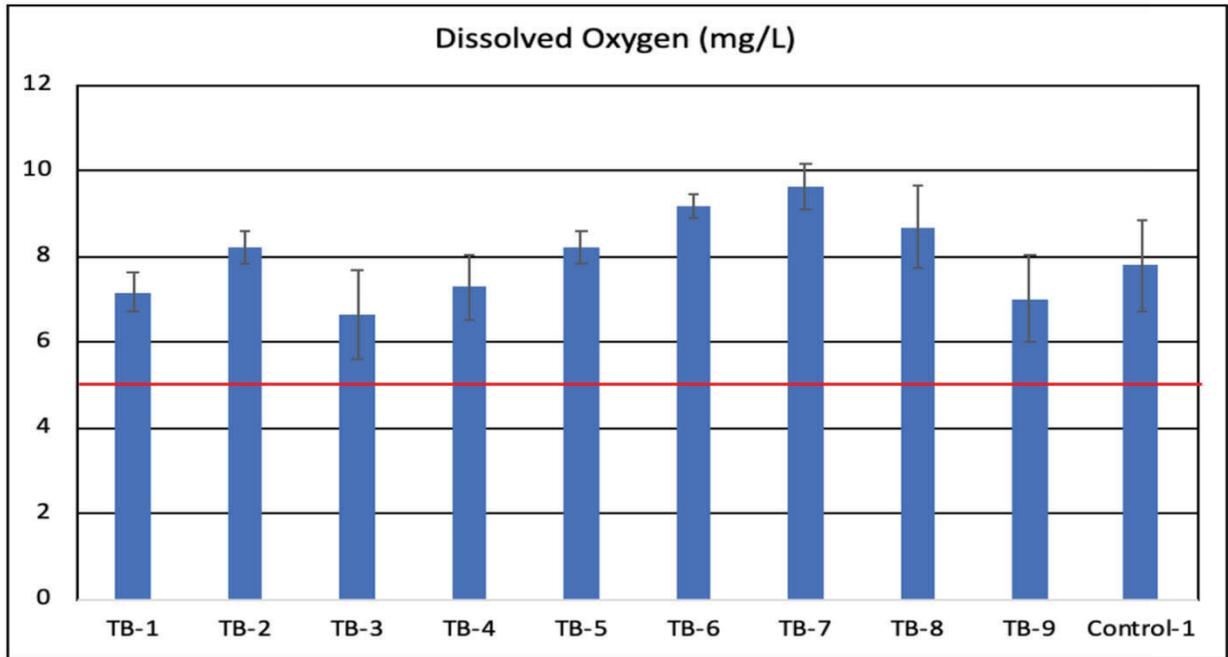


Figure 7. Average  $\pm$  standard error dissolved oxygen at each buoy location based on monthly water samples taken January– December 2021. The red line represents the threshold for the minimum DO for fish survival.

pH values in water affect the solubility and availability of chemical constituents, such as nutrients. Thus pH is used as a parameter for water quality testing, especially when the testing is being used to gauge algal bloom probabilities (Zerpernick et al., 2021). Figure 8 shows that the pH values for all sites fall between 8.00 and 10.00. Alkaliphiles thrive in high pH environments. The optimal growth pH for alkaliphiles is at or above a pH of 9. This information indicates that most lakes had near-optimal pH values for alkaliphiles, which includes many photosynthetic organisms, including cyanobacteria (López-Archilla et al., 2004). In addition, algae and cyanobacteria (photosynthetic organisms) utilize carbon dioxide from the water column as part of their photosynthesis, potentially resulting in increased pH.

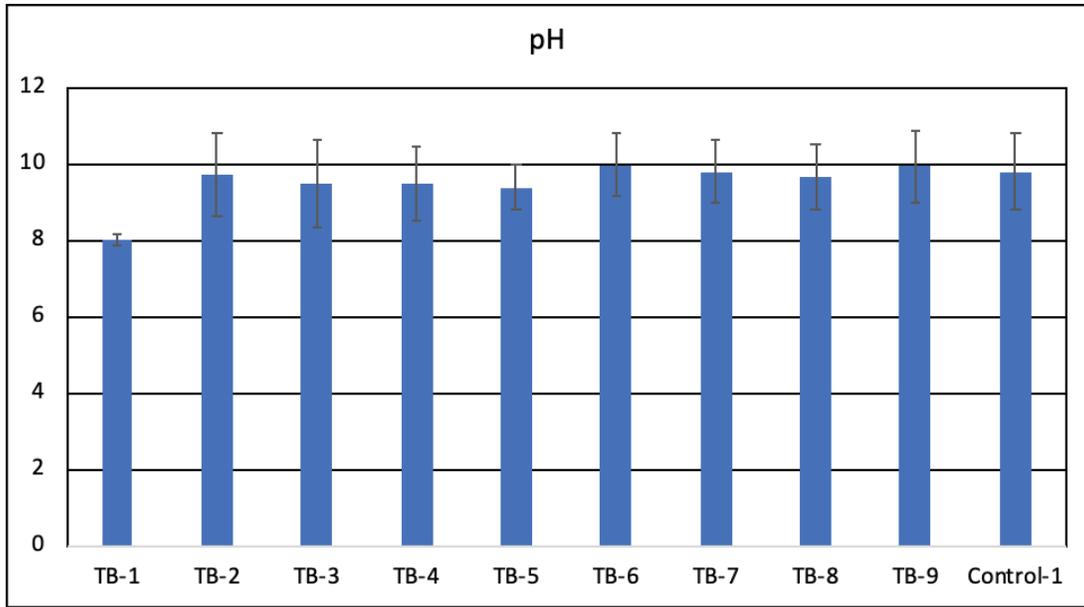


Figure 8. Average  $\pm$  standard error pH at each buoy location based on monthly water samples taken January– December 2021.

Water temperature is one of the parameters that controls the dynamics of microalgae in freshwater lakes. High water temperatures can largely influence the potential for harmful algae blooms (Larras, et al., 2013). The data in Figure 9 shows that each monthly average water temperature from all sites were higher than 26°C. Among the sites, TB-8 had highest value (27.48°C) with TB-4 representing the lower end of the temperature range (~26.0°C). Previous studies have shown that higher average air and surface water temperatures, including longer summers, contribute to the incidence and abundance of harmful algal blooms in lakes (Ho & Michalak, 2019). The lakes in this study are therefore all at an increased risk for harmful algal blooms, once the nutrient concentrations (N and P) and other physical factors trigger HABs, due to their average surface water temperatures, and the prolonged warmth in South Florida. Among the physical factors that predict HABs, water temperature is the most significant (Rouso et al., 2020).

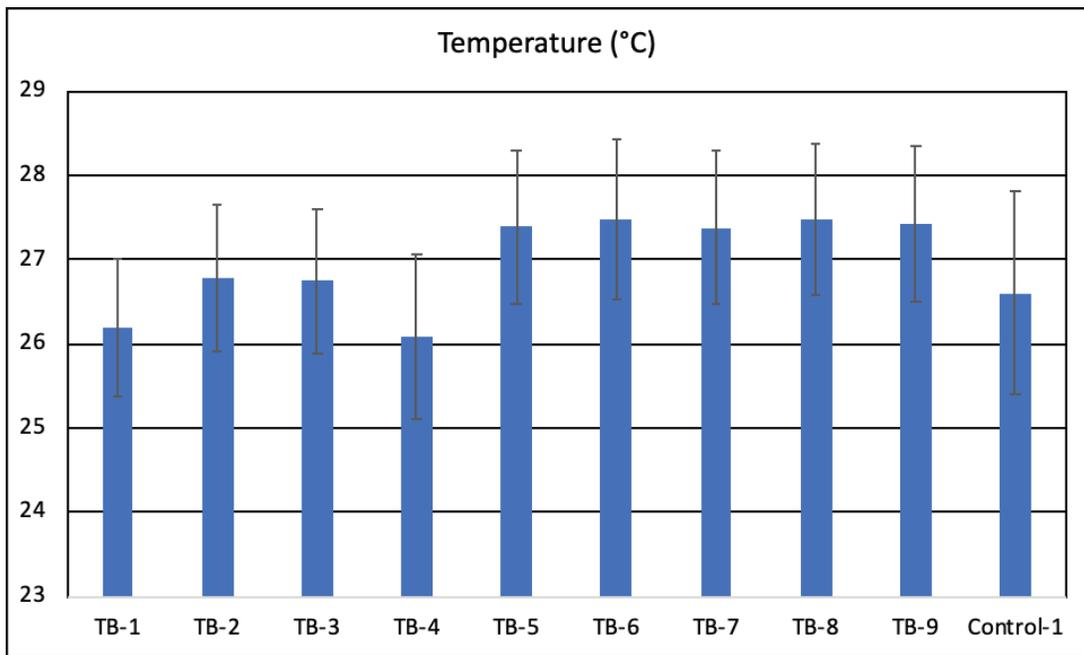


Figure 9. Average  $\pm$  standard error temperature at each buoy location based on monthly water samples taken January–December 2021.

### Daily averages from MPC-buoys

Most of the 30-min chlorophyll-a readings at the buoys seen in Figure 10 remained low until mid-summer TB-6 had a small spike that almost reached 50  $\mu\text{g/L}$  between May and June. TB-3 then peaked multiple times to 200  $\mu\text{g/L}$  from July – October 2021. These were the highest concentrations of chlorophyll viewed throughout the study. TB-5 had a small spike at the end of May that was slightly above 10  $\mu\text{g/L}$ , but it quickly dropped back down. TB-7 and TB-8 seemed to have very little change throughout the year and did not have any extreme spikes. TB-3 and TB-6 concentrations fluctuated at slightly elevated levels from October to December.

Chlorophyll data for buoys TB-8 and TB-9 were not available for the period of June through August. Conditions promoted the encrusting of algae on the sensor preventing the collection of data. Mechanisms, such as an automatic cleaning brush, were implemented prior to deployment that would prevent this event from occurring. However, the accumulated algae inhibited the cleaning brush motor's function, ultimately shorting out the electronic motor.

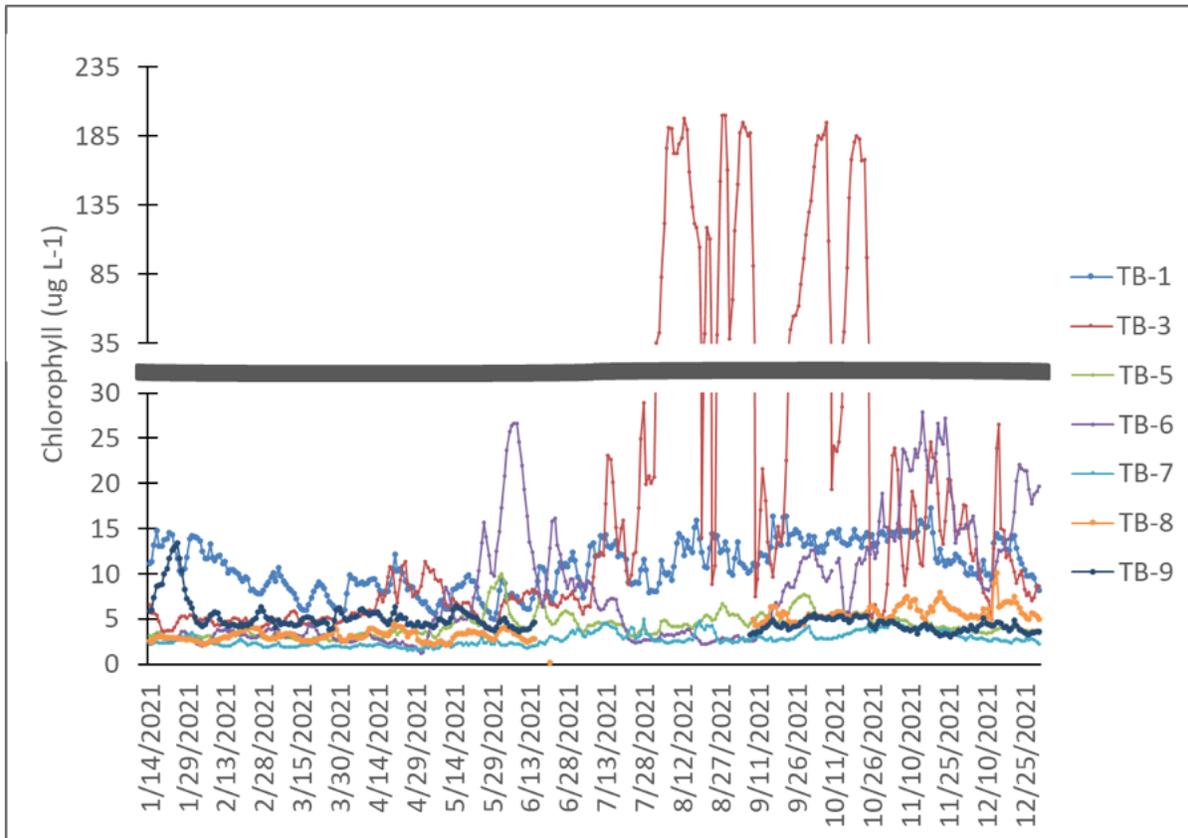


Figure 10. Daily average chlorophyll at each buoy based on readings by the MPC-buoy collected every 30 minutes. A break in the Y-axis is displayed to differentiate between smaller values and larger outlier data.

As seen in Figure 11, phycocyanin (PC) levels remained low from January to April, then increased for almost all sites in May, with the months following containing the highest levels of PC. TB-1 and TB-3 showed the highest PC levels during the months of January and February compared to other sites. By mid-June all sites were experiencing an increase in PC levels with notable spikes between July and November at TB-3. On November 9, TB-3 peaked at 186  $\mu\text{g/L}$ , only 8  $\mu\text{g/L}$  less than its highest peak in August. Phycocyanin levels continued to increase from August-November for TB-6. Since PC is an indicator for cyanobacteria blooms, it can be stated that harmful algae blooms in Naples, FL lakes are most likely to occur beginning in May with potential to recur through December.

During most of the year, phycocyanin levels remained below 30  $\mu\text{g/L}$ , equivalent to WHO ‘alert level 1’ or 20,000 cyanobacteria cells/ml. At ‘alert level 1’, weekly water monitoring is necessary. Beginning in July, TB-6 and TB-3 exceeded the 30  $\mu\text{g/L}$  limit, now classified as a WHO ‘alert level 1’ requiring weekly water monitoring. By the end of July, TB-3 approached 90  $\mu\text{g/L}$ . At this level, measurements are equivalent to 100,000 cells/ml, transitioning into ‘alert level 2’. ‘Alert level 2’ restricts water access due to the high potential risk of cyanotoxin (Mchau et al., 2019). Throughout July and November, TB-3 periodically exceeds 90  $\mu\text{g/L}$  reaching levels of  $\sim 180 \mu\text{g/L}$  in early August and early November. These daily averages reveal that utilizing only monthly phycocyanin averages may be misleading when used to influence health-based decisions. Phycocyanin data for buoys TB-8 and TB-9 were not available for the period of June through August for the same technical difficulties explained in Figure 10.

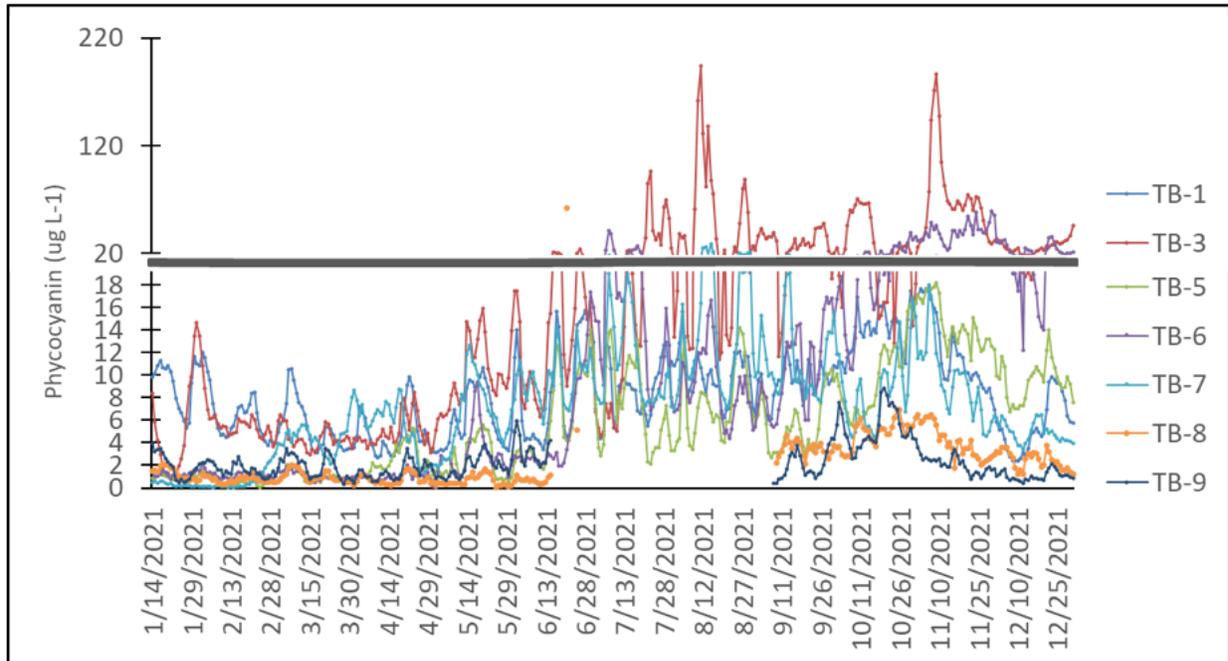


Figure 11. Daily average phycocyanin (PC) at each buoy based on readings by the MPC-buoy collected every 30 minutes. A break in the Y-axis is displayed to differentiate between smaller values and larger outlier data.

The TB-3 peak from mid-March through April seen in Figure 12 may be a result of community development along the peninsula residential area of Treviso Bay. The cause of increased turbidity at buoys TB-7 and TB-9 from October-December 2021 is unclear, though variability in those months was high. One possible explanation is that shallower lakes experienced increased turbidity due to resuspension of sediments in windy conditions. Turbidity data for buoys TB-8 and TB-9 were not available for the period of June through August for the same technical difficulties explained in Figure 10.

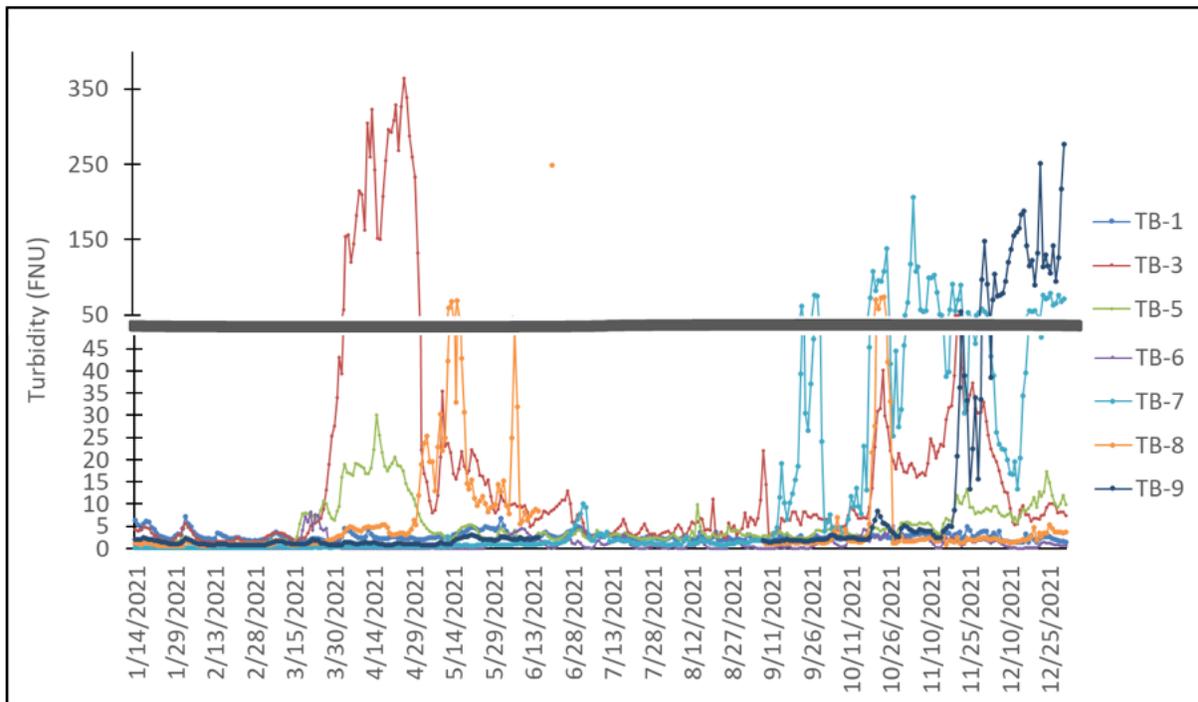


Figure 12. Daily average turbidity at each buoy based on readings by the MPC-buoy collected every 30 minutes. A break in the Y-axis is displayed to differentiate between smaller values and larger outlier data.

The pattern of dissolved oxygen in the nine buoys over the twelve months of data collection seen in Figure 13 showed overall averages in the lakes decreased from about 10 ppm to 5 ppm in the summer months, caused primarily by increasing water temperatures and thus lower DO saturation levels. TB-3 exhibited DO levels lower than the other buoys. The higher PC levels seen at TB-3 suggest the presence of cyanobacteria, which would decompose when eliminated by the buoys, stripping dissolved oxygen from the water column. DO was highly variable at TB-7, possibly due to large overall productivity in the lake. Although Chlorophyll-a was not elevated in TB-7, it is possible that a larger quantity of submerged aquatic vegetation contributed to DO, and also that more organisms were respiring in the lake, contributing to variability. A die-off of such vegetation potentially explains drop to 2ppm in August. TB-7 was also located in the lake with the largest surface area of any study lakes, potentially increasing the impact of wind action on oxygen diffusion. Dissolved oxygen data for buoys TB-8 and TB-9 were not available for the period of June through August for the same technical difficulties explained in Figure 10.

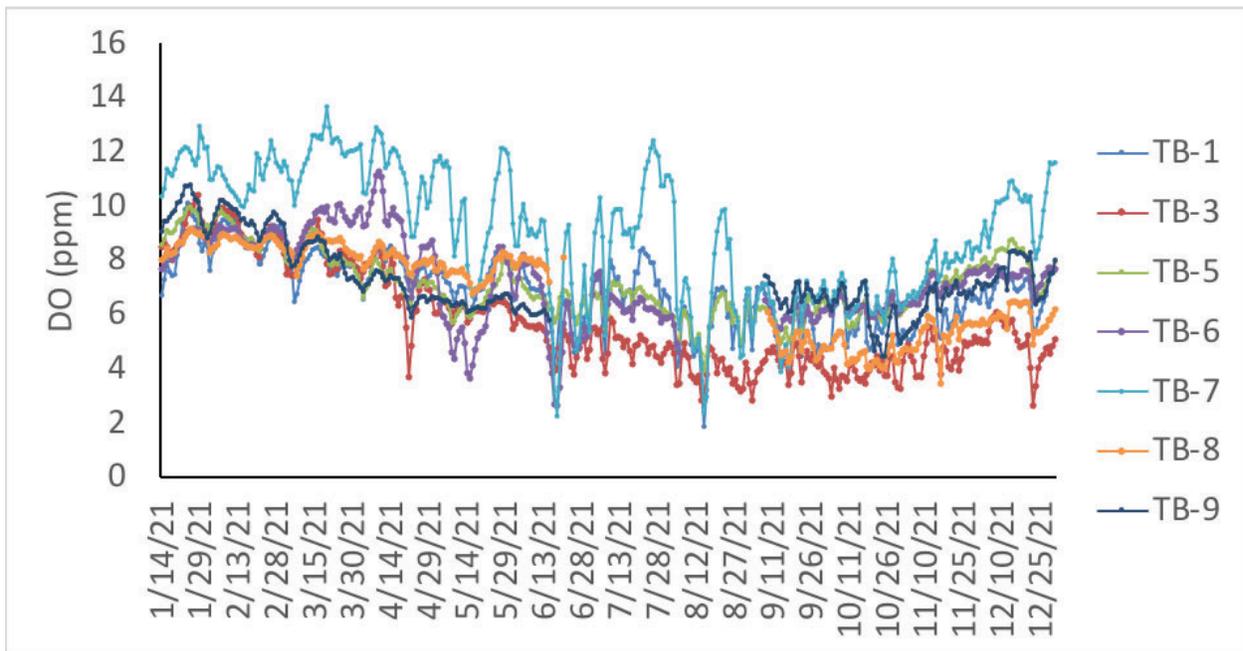


Figure 13. Daily average dissolved oxygen at each buoy based on readings by the MPC-buoy collected every 30 minutes.

Figure 14 shows that pH generally fluctuated between 8.0 and 9.0 for most buoys throughout the study, indicating slightly basic conditions. These conditions suggest a high amount of primary productivity, though the reason for large variability seen at TB-7 and TB-5 is unclear. pH data for buoys TB-8 and TB-9 were not available for the period of June through August for the same technical difficulties explained in Figure 10.

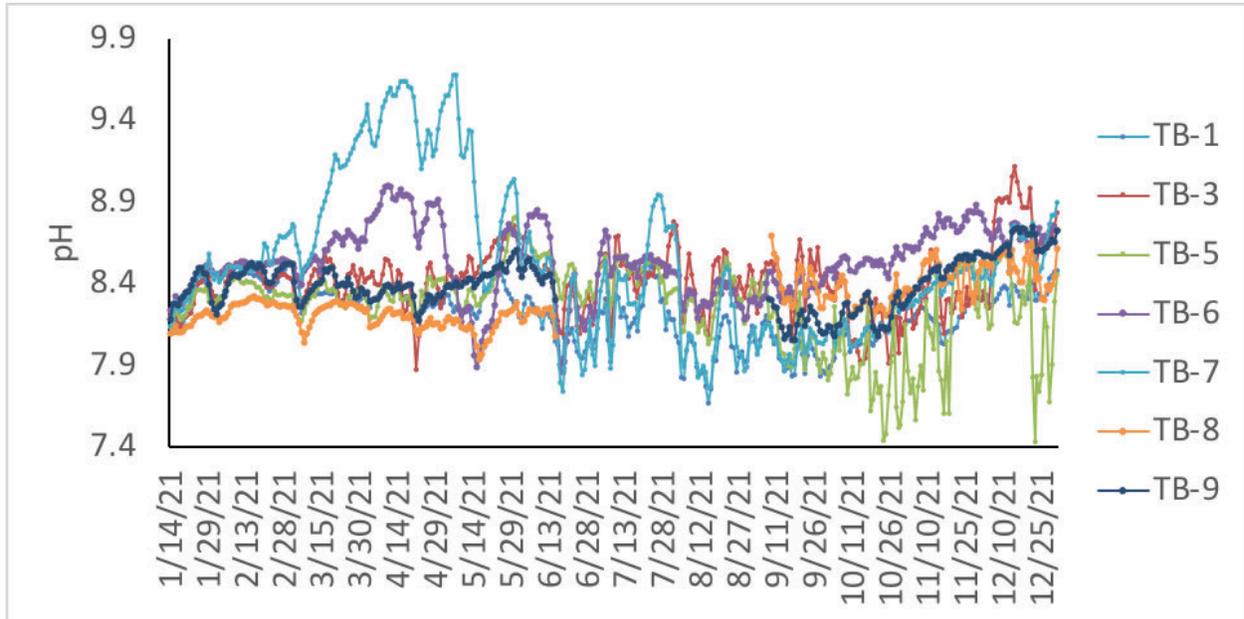


Figure 14. Daily average pH at each buoy based on readings by the MPC-buoy collected every 30 minutes.

Figure 15 shows that temperatures remained consistent among all study lakes with highs and lows correlating to Florida’s summer and winter climate. Temperature data for buoys TB-8 and TB-9 were not available for the period of June through August for the same technical difficulties explained in Figure 10.

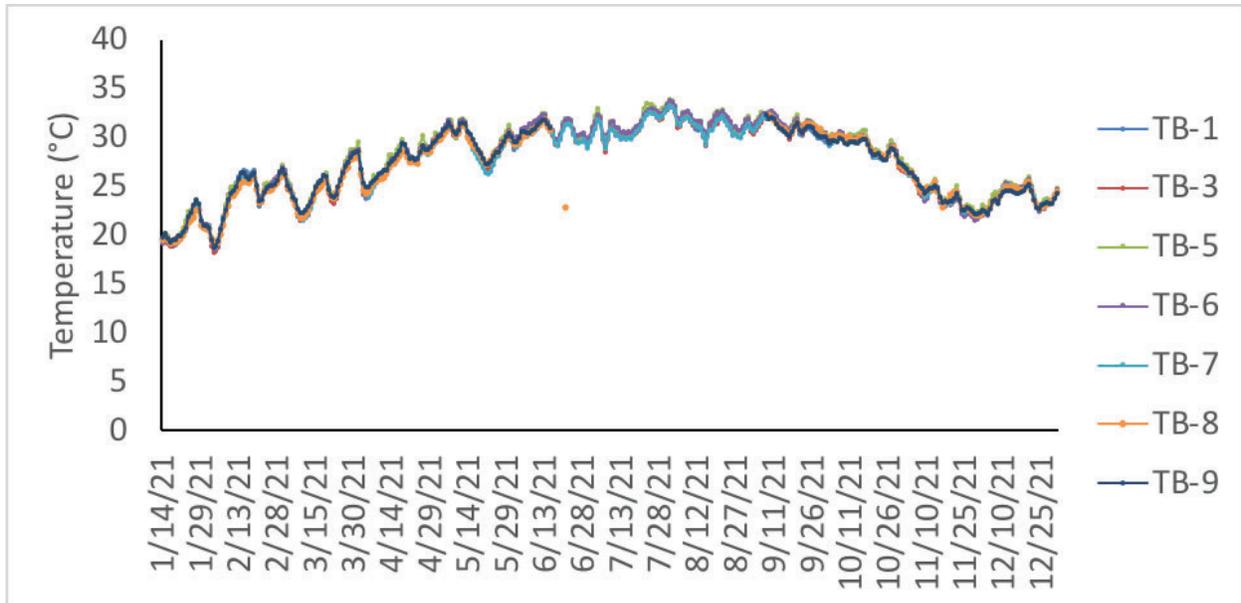


Figure 15. Daily average temperature at each buoy based on readings by the MPC-buoy collected every 30 minutes.

### Monthly averages from MPC-buoys

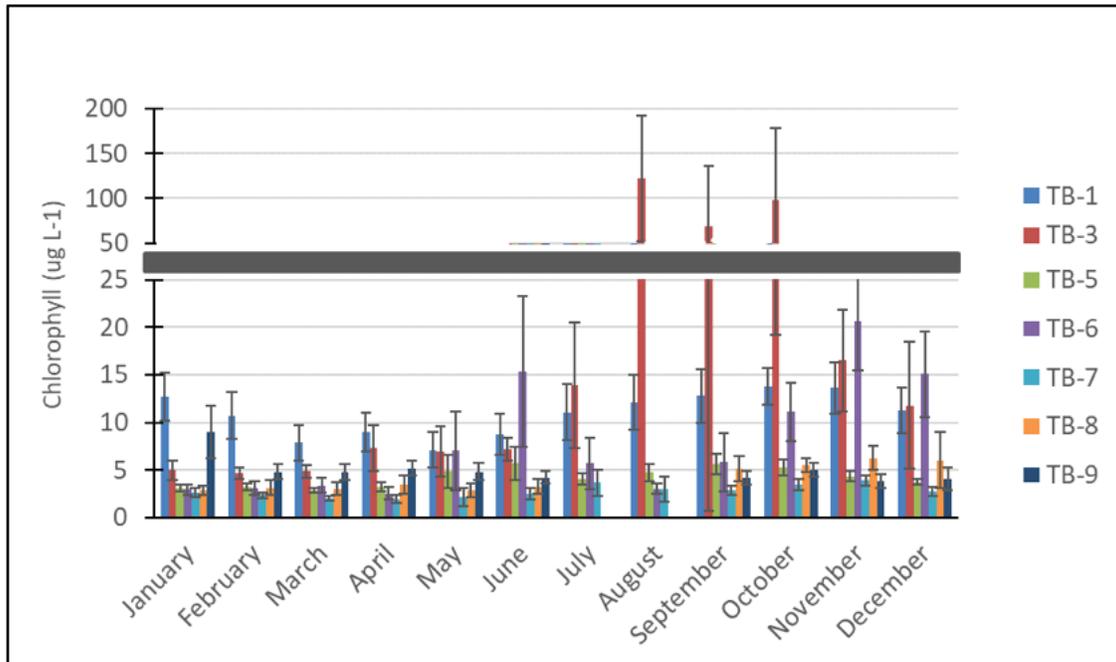


Figure 16 Monthly average  $\pm$  standard deviation chlorophyll-a at each buoy based on readings by the MPC-buoy collected every 30 minutes. Chlorophyll-a data for buoys TB-8 and TB-9 were not available for the period of June through August for the same technical difficulties explained in Figure 10. A break in the Y-axis is displayed to differentiate between smaller values and larger outlier data.

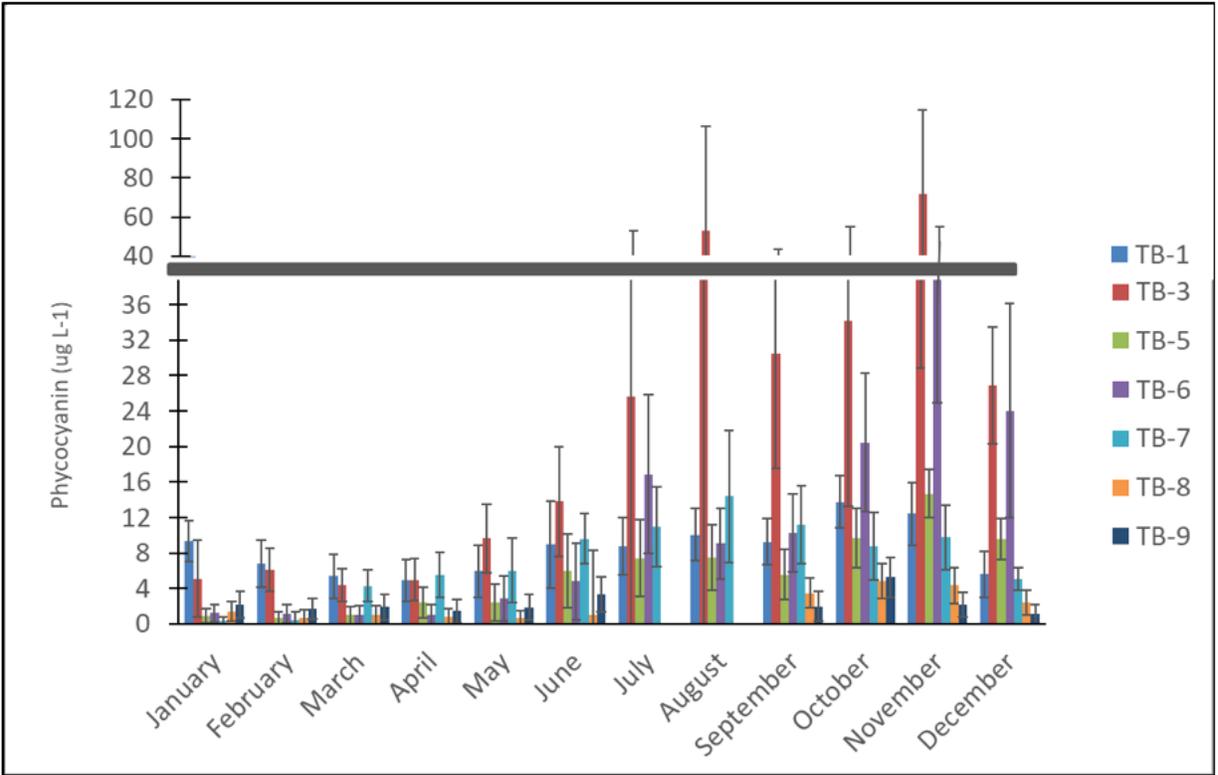


Figure 17. Monthly average  $\pm$  standard deviation phycocyanin at each buoy based on readings by the MPC-buoy collected every 30 minutes. Phycocyanin data for buoys TB-8 and TB-9 were not available for the period of June through August for the same technical difficulties explained in Figure 10. A break in the Y-axis is displayed to differentiate between smaller values and larger outlier data.

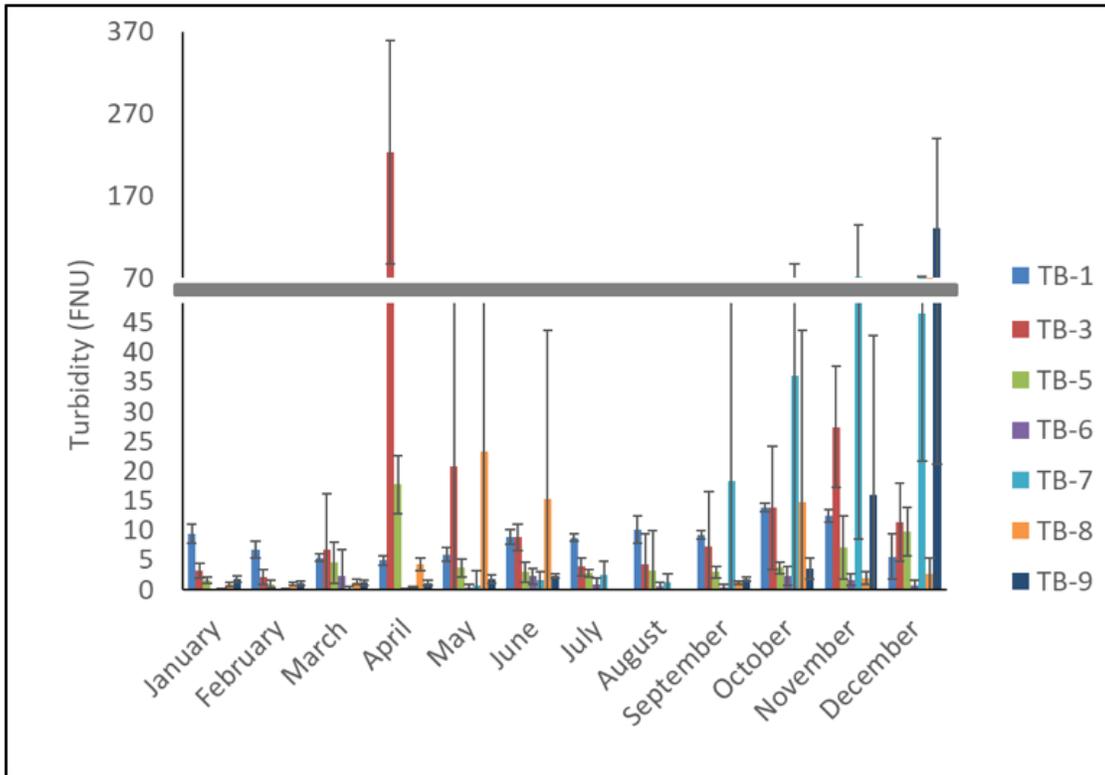


Figure 18. Monthly average  $\pm$  standard deviation turbidity at each buoy based on readings by the MPC-buoy collected every 30 minutes. Turbidity data for buoys TB-8 and TB-9 were not available for the period of June through August for the same technical difficulties explained in Figure 10. A break in the Y-axis is displayed to differentiate between smaller values and larger outlier data.

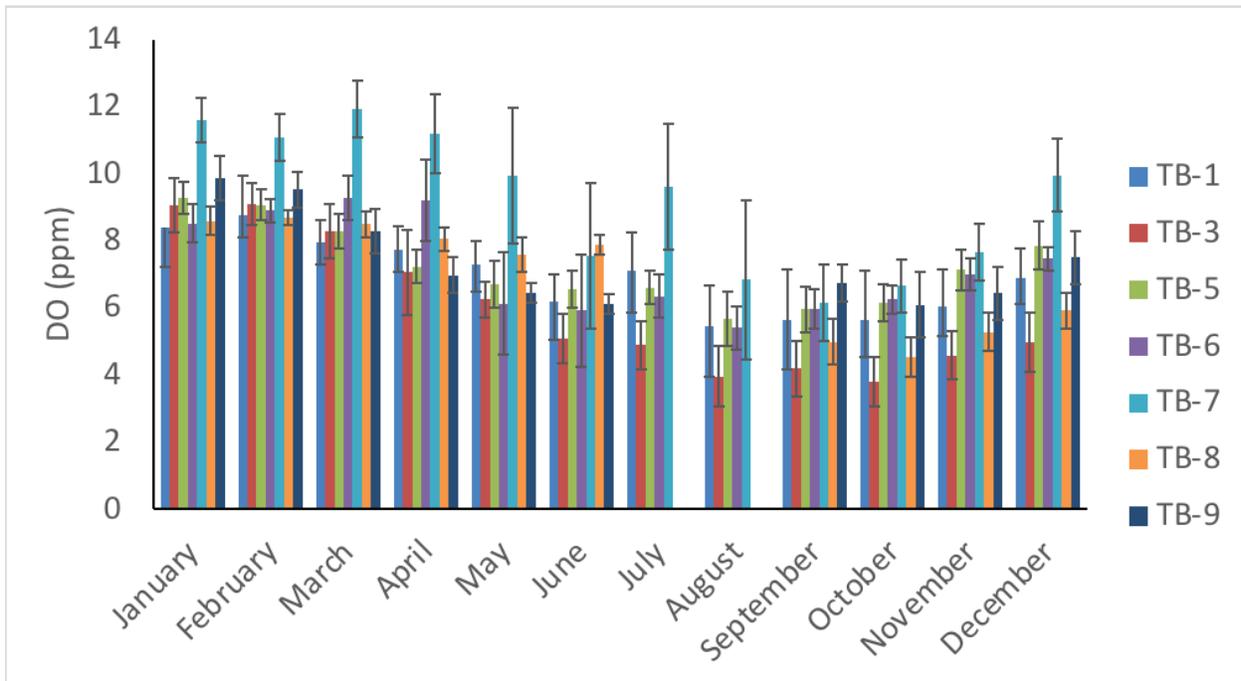


Figure 19. Monthly average  $\pm$  standard deviation dissolved oxygen at each buoy based on readings by the MPC-buoy collected every 30 minutes. Dissolved oxygen data for buoys TB-8 and TB-9 were not available for the period of June through August for the same technical difficulties explained in Figure 10.

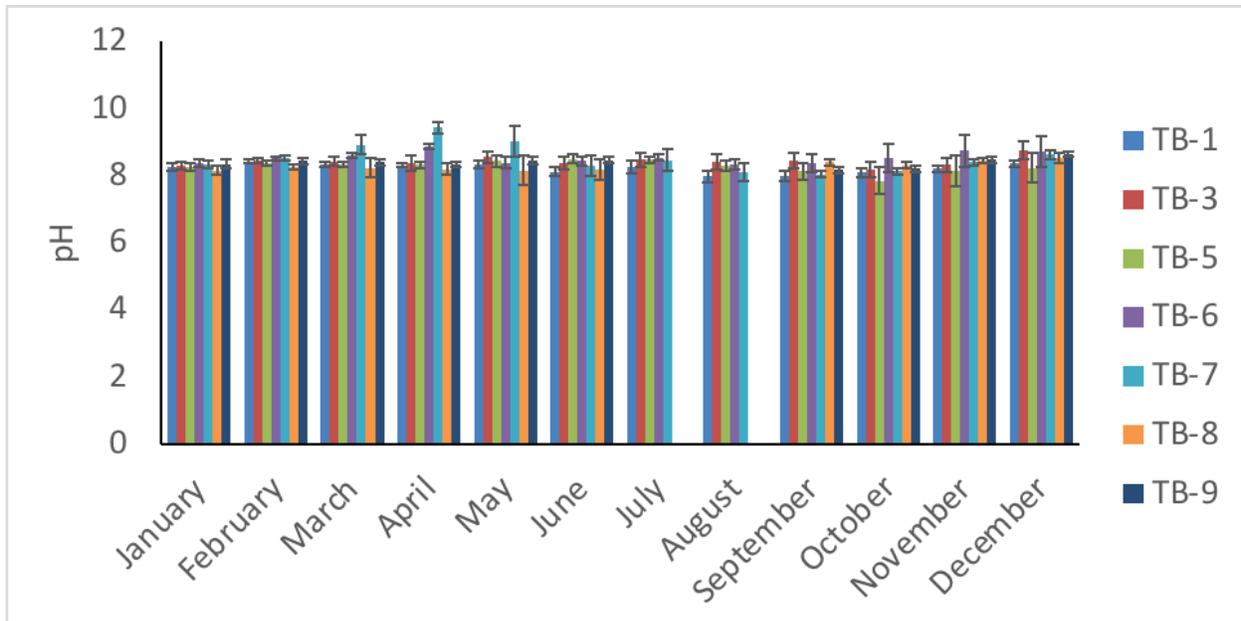


Figure 20. Monthly average  $\pm$  standard deviation pH at each buoy based on readings by the MPC-buoy collected every 30 minutes. Despite large variability of TB-7 and TB-5 pH as seen in Figure 13, monthly averages are consistent with other lakes. pH data for buoys TB-8 and TB-9 were not available for the period of June through August for the same technical difficulties explained in Figure 10.

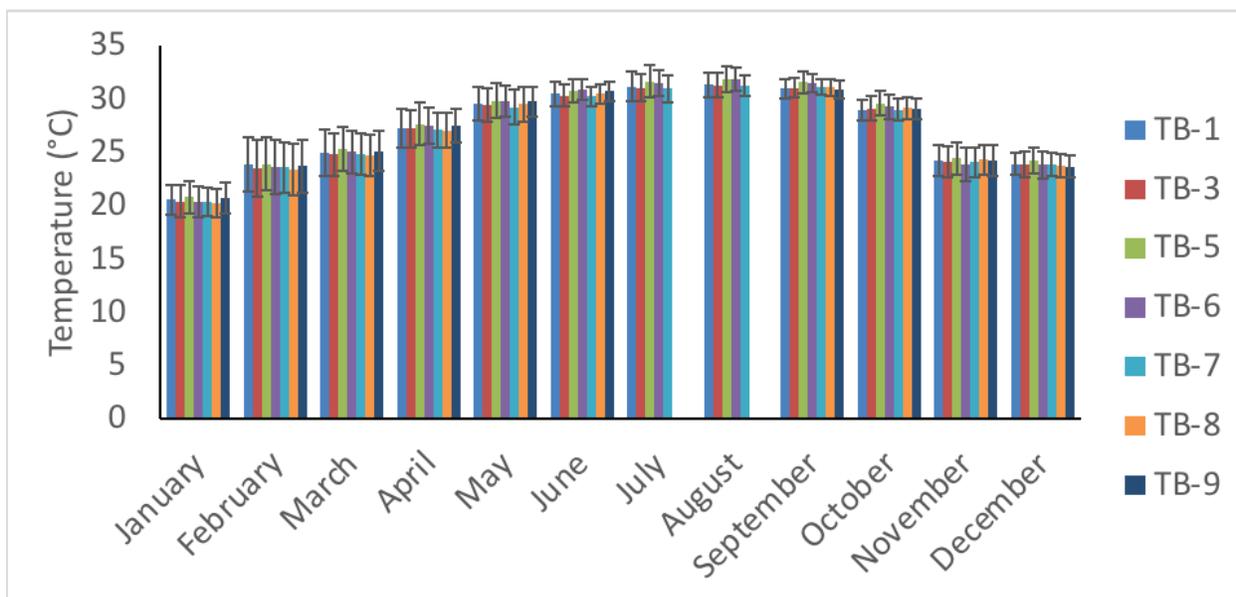


Figure 21. Monthly average  $\pm$  standard deviation temperature at each buoy based on readings by the MPC-buoy collected every 30 minutes. Temperature data for buoys TB-8 and TB-9 were not available for the period of June through August for the same technical difficulties explained in Figure 10.

### Statistical Analysis: T-Test

There are no significant differences ( $p < 0.01$ ) between monthly buoy data and monthly sampling data for all parameters in Table 3, except DO (ppm) in TB-6. The DO sensor on the YSI had a technical issue for a time, so some data is missing which could lead to less accurate statistical analyses.

There is no significant difference ( $p < 0.01$ ) for monthly sampling parameters Chlorophyll ( $\mu\text{g L}$ ), Turbidity (NTU), Phycocyanin (RFU), DO (mg/L), pH and Temperature ( $^{\circ}\text{C}$ ) between a designated test lake (TB-1) and a designated control lake (Table 4). Table 4 also shows lower values in Turbidity, Phycocyanin and pH in the buoy test lake (TB-1) than in those same parameters in the control lake, which is the expected outcome assuming proper buoy function. However, there was a higher value of Chlorophyll ( $7.26 \pm 1.19 \mu\text{g L}$ ) for the buoy test lake than in the control lake, which is an unexpected outcome that may have been caused by the impact of chemical algae treatment administered from the CDD (see Table 6).

Table 3: T-test results for parameters: Chlorophyll ( $\mu\text{g L}$ ), Turbidity (FNU), DO (ppm), pH and Temperature ( $^{\circ}\text{C}$ ) between monthly buoy data and monthly sampling data in the study lakes: TB-1, TB-3, TB-5, TB-6, TB-7, TB-8 and TB-9 at the significance level  $p < 0.01$ .

Parameters	TB-1	TB-3	TB-5	TB-6	TB-7	TB-8	TB-9
Chlorophyll ( $\mu\text{g L}$ )	0.01	0.07	0.09	0.22	0.85	0.01	0.32
Turbidity (FNU)	0.01	0.19	0.01	0.31	0.07	0.04	0.27
DO (ppm)	0.26	0.56	0.03	0.00*	0.90	0.05	0.94
pH	0.20	0.37	0.08	0.12	0.13	0.12	0.14
Temperature ( $^{\circ}\text{C}$ )	0.03	0.29	0.59	0.93	0.59	0.40	0.63

\* indicates statistically significant difference at  $p < 0.01$

Table 4: Mean, Standard Error and paired t-test for monthly sampling parameters: Chlorophyll ( $\mu\text{g L}$ ), Turbidity (NTU), Phycocyanin (RFU), DO (mg/L), pH and Temperature ( $^{\circ}\text{C}$ ) between the study lakes: TB-1 and Control-1 at the significance level  $p < 0.01$ .

Parameters	TB-1 (Mean $\pm$ St. err.)	Control-1 (Mean $\pm$ St. err.)	t-test $p < 0.01$
Chlorophyll ( $\mu\text{g L}$ )	7.26 $\pm$ 1.19	5.11 $\pm$ 1.15	0.13
Turbidity (NTU)	1.94 $\pm$ 0.30	3.74 $\pm$ 1.64	0.27
Phycocyanin (RFU)	0.07 $\pm$ 0.02	0.15 $\pm$ 0.07	0.36
DO (mg/L)	7.16 $\pm$ 0.46	7.89 $\pm$ 0.92	0.89
pH	8.00 $\pm$ 0.15	9.16 $\pm$ 1.00	0.11
Temperature ( $^{\circ}\text{C}$ )	26.19 $\pm$ 0.81	26.60 $\pm$ 1.21	0.62

### Statistical Analysis: ANOVA with post-hoc Tukey's HSD test

There are no significant differences ( $p < 0.01$ ) in monthly Turbidity (FNU) and Temperature ( $^{\circ}\text{C}$ ) between the study lakes: TB-1, TB-3, TB-5, TB-6, TB-7, TB-8 and TB-9 (Table 5b and Table 5f). There are no significant differences ( $p < 0.01$ ) in monthly Chlorophyll ( $\mu\text{g L}$ ) between most study lakes, except monthly Chlorophyll ( $\mu\text{g L}$ ) in Lake TB-3 has a significant difference to lakes: TB-5, TB-6, TB-7, TB-8, TB-9 (Table 5a). A similar pattern occurs in monthly Phycocyanin ( $\mu\text{g/L}$ ), with TB-3 having a significant difference from all lakes (Table 5c). This lake was surrounded by golf courses and housing construction which could have led to a higher nutrient input than other lakes. There are no significant differences ( $p < 0.01$ ) in monthly dissolved oxygen (ppm) between most study lakes, except dissolved oxygen in lake TB-7 has a significant difference to study lakes: TB-1, TB-3, TB-5, TB-6 and TB-8 (Table 5d). There are no significant differences ( $p < 0.01$ ) in monthly pH between most study lakes (Table 5e), except pH in lake TB-1 has a significant difference ( $p < 0.01$ ) to lakes TB-6 and TB-7.

Table 5 (a-f): ANOVA analysis for all parameters, with a post-hoc Tukey's HSD test reported here for parameters: Chlorophyll ( $\mu\text{g L}$ ), Turbidity (FNU), Phycocyanin ( $\mu\text{g/L}$ ), DO (ppm), pH and Temperature ( $^{\circ}\text{C}$ ). Monthly average data used from the study lakes: TB-1, TB-3, TB-5, TB-6, TB-7, TB-8 and TB-9. (\* = significance level at  $p < 0.05$  and \*\* = significance level at  $p < 0.01$ )

a) Chlorophyll ( $\mu\text{g L}$ )							
Tukey HSD p-value	TB-1	TB-3	TB-5	TB-6	TB-7	TB-8	TB-9
TB-1	-	0.060	0.900	0.900	0.876	0.900	0.900
TB-3		-	0.003**	0.018*	0.001**	0.003**	0.008**
TB-5			-	0.900	0.900	0.900	0.900
TB-6				-	0.900	0.900	0.900
TB-7					-	0.900	0.900
TB-8						-	0.900
TB-9							-

b) Turbidity (FNU)							
Tukey HSD p-value	TB-1	TB-3	TB-5	TB-6	TB-7	TB-8	TB-9
TB-1	-	0.387	0.900	0.900	0.900	0.900	0.900
TB-3		-	0.504	0.300	0.900	0.625	0.900
TB-5			-	0.900	0.900	0.900	0.900
TB-6				-	0.900	0.900	0.900
TB-7					-	0.900	0.900
TB-8						-	0.900
TB-9							-

c) Phycocyanin ( $\mu\text{g/L}$ )							
Tukey HSD p-value	TB-1	TB-3	TB-5	TB-6	TB-7	TB-8	TB-9
TB-1	-	0.005**	0.900	0.900	0.900	0.719	0.749
TB-3		-	0.005**	0.037*	0.002**	0.001**	0.001**
TB-5			-	0.811	0.900	0.900	0.900
TB-6				-	0.900	0.353	0.384
TB-7					-	0.887	0.900
TB-8						-	0.900
TB-9							-

d) DO (ppm)							
Tukey HSD p-value	TB-1	TB-3	TB-5	TB-6	TB-7	TB-8	TB-9
TB-1	-	0.706	0.900	0.900	0.014**	0.900	0.900
TB-3		-	0.450	0.459	0.001**	0.682	0.334
TB-5			-	0.900	0.049*	0.900	0.900
TB-6				-	0.047*	0.137	0.900
TB-7					-	0.032*	0.167
TB-8						-	0.900
TB-9							-

e) pH							
Tukey HSD p-value	TB-1	TB-3	TB-5	TB-6	TB-7	TB-8	TB-9
TB-1	-	0.283	0.900	0.010**	0.012**	0.900	0.485
TB-3		-	0.645	0.810	0.843	0.750	0.900
TB-5			-	0.059	0.069	0.900	0.827
TB-6				-	0.900	0.107	0.707
TB-7					-	0.122	0.739
TB-8						-	0.900
TB-9							-

f) Temperature (°C)							
Tukey HSD p-value	TB-1	TB-3	TB-5	TB-6	TB-7	TB-8	TB-9
TB-1	-	0.900	0.900	0.900	0.900	0.900	0.900
TB-3		-	0.900	0.900	0.900	0.900	0.900
TB-5			-	0.900	0.900	0.900	0.900
TB-6				-	0.900	0.900	0.900
TB-7					-	0.900	0.900
TB-8						-	0.900
TB-9							-

#### 4b) Problems encountered

##### Problem #1: Glare from buoy solar panels into houses

- January 19 – February 12, 2021
- Homeowner complaint that buoy, maybe the solar panels, was causing “eye damage” from the glare being shined into her house.
- Attempted resolution: covering the buoy with a tarp and anchoring the buoy from two points to limit the spin of the buoy
- Final resolution: on Friday, February 12, 2021, the TB-5 buoy was moved approximately 100 meters (~110 yards) to the south of the original location. This relocation was done by a professional dive team (Adams Commercial Diving, Inc.) and removed the buoy from the homeowner’s view. The buoy was then more than 100 meters from any house.
- See photos of this resolution in the Section 5 Photo Documentation section

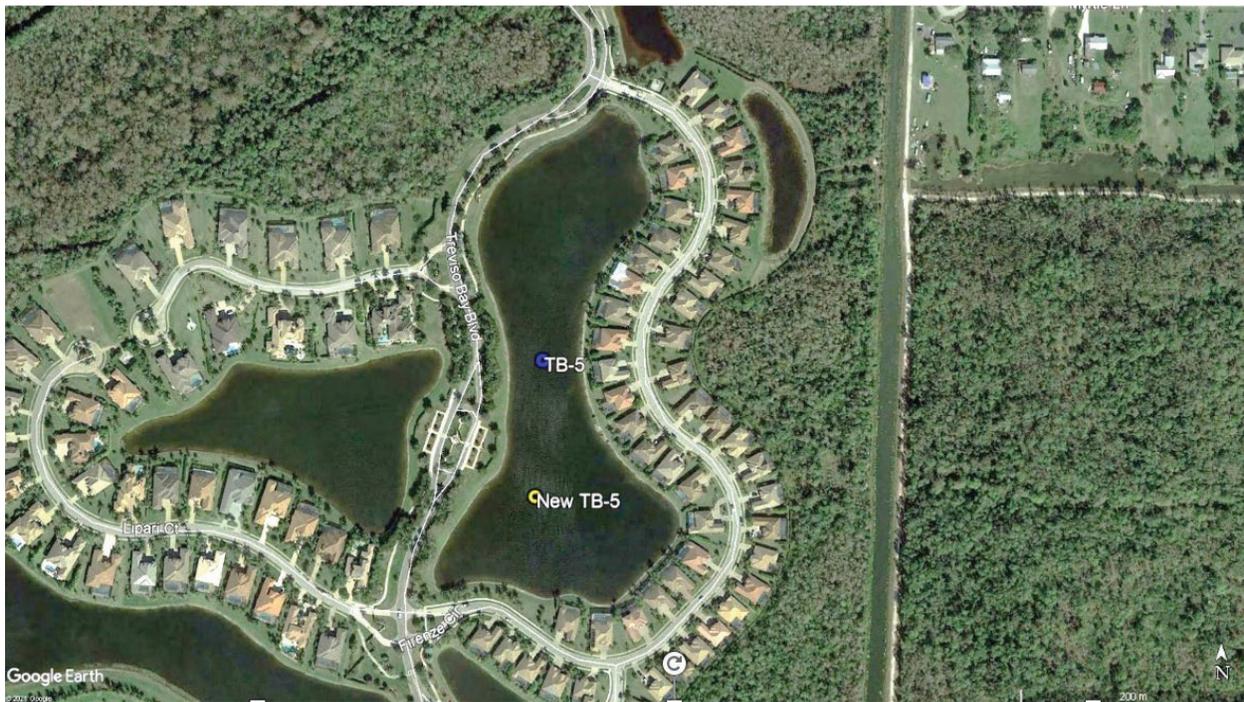


Figure 22. Aerial image indicating the original and modified location of TB-5 to accommodate resident complaints.

Problem # 2. Chemical algae treatment continued throughout our project period at all study and control lakes despite an agreement by Treviso Bay management to not chemically treat lakes throughout the study.

In total, there were 65 chemical treatments administered to all experimental and one control lake throughout the study (Table 6). This means that, on average, each lake was chemically treated 8 times.

Table 6. Each “X” indicates which lakes were chemically treated to eliminate algae and on which date.

<b>Algae Treatment 2021</b>	<b>Lake ID</b>	<b>33</b>	<b>42</b>	<b>20</b>	<b>15</b>	<b>12</b>	<b>10</b>	<b>22</b>	<b>32</b>
<b>DATE</b>	<b>Buoy ID</b>	<b>TB-1</b>	<b>TB-3</b>	<b>TB-5</b>	<b>TB-6</b>	<b>TB-7</b>	<b>TB-8</b>	<b>TB-9</b>	<b>Control-1</b>
1/17/21		X							
1/28/21									X
2/19/21			X					X	
2/26/21									X
3/5/21			X						
3/12/21									
3/19/21			X						
4/2/21			X						X
4/9/21								X	X
4/16/21			X						X
4/23/21								X	X
4/30/21			X		X	X			
5/6/21				X	X				X
5/14/21				X	X				
5/21/21		X							X
5/28/21		X							
7/8/21					X				
7/23/21			X						
8/5/21						X			
8/6/21			X						X
8/13/21									X
8/23/21				X					
8/26/21		X			X	X			
8/27/21									X
9/2/21									
9/3/21		X	X	X					X
9/9/21					X				
9/10/21			X	X					X
9/16/21									
9/17/21									X
9/23/21									
9/25/21			X						X
9/28/21						X			
10/7/21					X	X			
10/8/21			X					X	
10/14/21									
10/15/21			X						
10/21/21			X						
10/29/21					X	X	X		
12/1/21									
12/2/21									X
12/8/21			X						
12/9/21									
12/15/21			X	X					X
12/21/21			X						X
12/23/21									
12/29/21									
12/30/21									

A verbal agreement was established prior to buoy implementation that confirmed chemical algal control would not be implemented throughout the duration of the project. Contrary to the verbal agreement, chemical algal control was applied periodically throughout the duration of the project as displayed in Table 6. A notice of application was not provided by the community prior to the use of chemical algal control. The primary chemical used was copper sulfate, though the amount applied was not specified. Treviso Bay Management breached our verbal agreement, thereby affecting the validity of our results and potentially wasting taxpayer money, time, and resources. Chemical algal control has been taken into consideration when interpreting the data and formulating conclusions. Chemical treatment during the project period might be the reason that we did not see statistical significance between control and study lakes, especially in bloom-related parameters such as chlorophyll-a, phycocyanin and turbidity.

Problem # 3. TB-8 and TB-9 monitoring sensors were down during the months of July and August preventing the daily collection of data such as chlorophyll and phycocyanin concentrations via the buoys. Sensors were replaced by an LG technician on September 9. Immediately after replacement daily data collection resumed.

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