Deliverable 5: Final Report

DEP Agreement No.:	**INV017**		
Grantee Name:	Florida Gulf Coast University		
Grantee Address:	10501 FGCU Blvd. South, Fort Myers, FL 33965-6565		
Grantee's Grant Manager:	Donna Gilmore	**Telephone No.:**	(239) 590-7022
Reporting Period:	July 1, 2020 – October 15, 2023		
Project Number and Title:	INV017 Harmful Algal Bloom Innovative Technology Project: Chemical-Free Harmful Algal Bloom Control		

This Task 5, Final Report includes:

- Section 1: Project location and background, project description and timeline, grant award amount and anticipated benefits.
- Section 2: 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.
- Section 3: 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.
- Section 4: 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.
- Section 5: Photo documentation of work performed (before, during and after), appropriate figures (site location, site plan[s]. etc.), appropriate tables summarizing data/information relevant to Grant Work Plan tasks, and appropriate attachments relevant to the project.
- Section 6: 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).
- Section 7: Summary of monitoring activities completed and any not completed and why, monitoring results, and an interpretation of data based on planned versus realized results.

Section 1: Project location and background, project description and timeline, grant award amount and anticipated benefits.

This project used nanobubble injection technology to address persistent and hard to treat freshwater harmful algae blooms (HABs) in Pahokee, Florida. The goal of this project was to comprehensively evaluate the use of Moleaer oxygen and ozone nanobubbles as an innovative technology for the mitigation and control of HABs and to evaluate the fate of cyanobacteria, their cyanotoxins, and the non-target eukaryotic algae after nanobubble exposure in the Lake Okeechobee Pahokee Marina.

Nanobubbles are high-pressure nanosized bubbles that have several unique properties that distinguish them from larger bubbles. They are extremely small with an approximate diameter of 100nm which enables the generation of high concentrations of 10^7 or higher bubbles per mL. The high surface area from the bubble concentration contributes to hyper-efficient oxygenation. Further, they create a mild oxidative stress that has been suggested to impact the viability of cyanobacterial cells. Intrinsic to the property of the bubble itself and not the gas that it contains, nanobubbles have shown to generate reactive oxygen species upon bubble collapse resulting in an oxidation effect that degrades easily oxidizable organic materials, which may lead to the degradation of cyanobacteria and their toxins.

The INV17 grant project took place within the South Florida Water Management District at Pahokee Marina and Campground [190 N Lake Ave, Pahokee, FL 33476 (26.825080, -80.667972)] located on the southeast corner of Lake Okeechobee as shown in Figure 1. The marina was chosen as an optimal location to conduct the grant work given its size, history of intense and routine blue-green algal blooms, and need for a novel remediation strategy to address the persistent algae blooms.

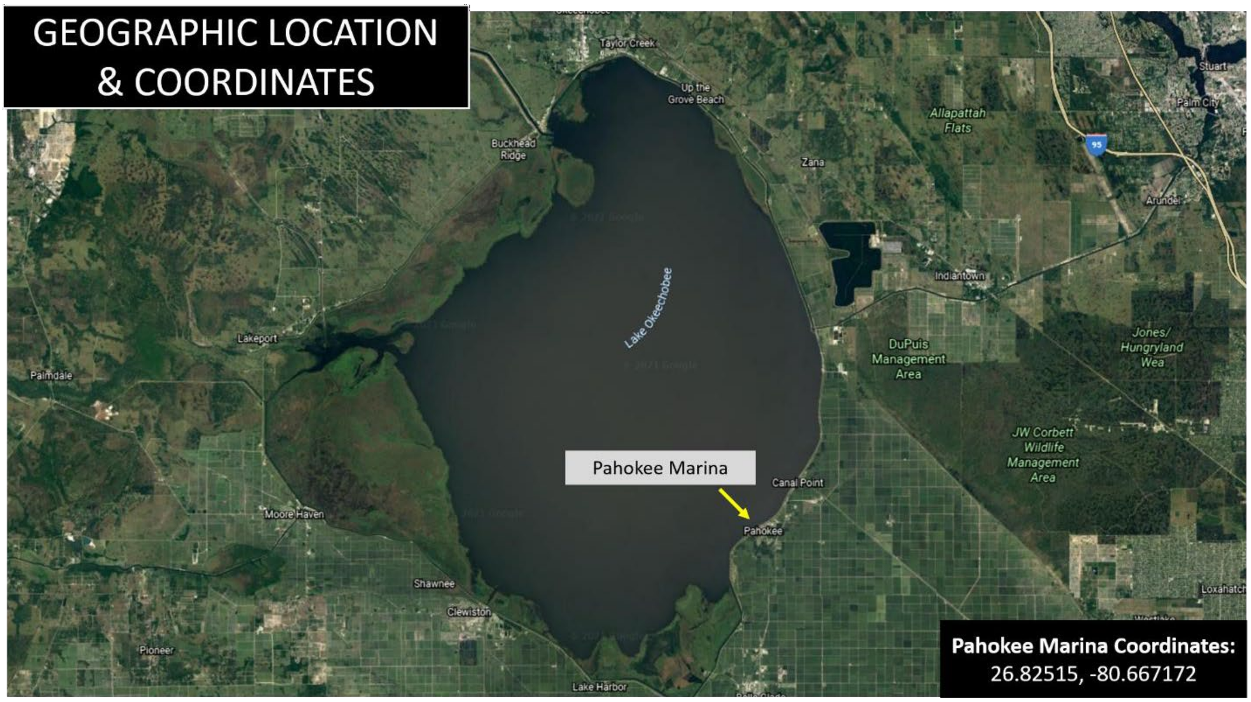

Figure 1: The location of Pahokee Marina within Lake Okeechobee in central Florida.

The nanobubble generators were installed to treat approximately 9 acres of Lake Okeechobee, the Pahokee Marina in Pahokee, Florida. This marina was identified in partnership with the South Florida Water Management District (SFWMD) as a site that routinely experienced HAB's. It was selected as it had the required power availability, accessibility, and space for installation of the Moleaer nanobubble generators. Florida Gulf Coast University (FGCU) subcontracted with Moleaer Inc. to coordinate and manage the installation, operation, and maintenance of the nanobubble generators throughout the approximately 18-month period of the evaluation.

In Pahokee Marina, four dock-mounted Moleaer Clear nanobubble generators injected oxygen and ozone nanobubbles into a recirculating stream of water from the marina. The technology consisted of a pump, oxygen generator, ozone generator, and flow-based core nanobubble generator technology. The marina water was recirculated through the nanobubble generator through 2.5" intake and discharge pipes. To determine the effect of the treatment, the treatment area sampling was conducted in a spatial pattern to understand the distance and area of impact. A monitored control site located on the north-eastern side of the marina but away from the nanobubble treated area was included to compare water quality nearby the nanobubble generators to an area considered outside the area of treatment. In-Situ water quality sondes equipped with six sensors (temperature, conductivity, ORP, pH, dissolved oxygen, and phycocyanin) and cloud-connected telemetry devices were deployed at two locations in the marina at the treatment and control locations. The continuous water monitoring was complimented by discrete water samples collected by a 3rd-party sampling service. These samples were analyzed by a 3rd party National Environmental Laboratories Accreditation Conference (NELAC) certified lab for nutrients (nitrate, nitrite, ortho-phosphate and total phosphorus) and by FGCU for the identification and quantification of algal populations as well as microcystins, cylindrospermopsin, saxitoxin, and anatoxin-a concentrations. The locations of the nanobubble generators and sampling points are shown in Figure 2. These data were used to evaluate the temporal and spatial efficacy of algae treatment from the fall of 2021 through the spring of 2023. This treatment schedule enabled the opportunity to understand the seasonal fluctuations of water quality in the marina and better capture the impact of the nanobubble generators through two warm-weather periods when blooms were more likely to occur.

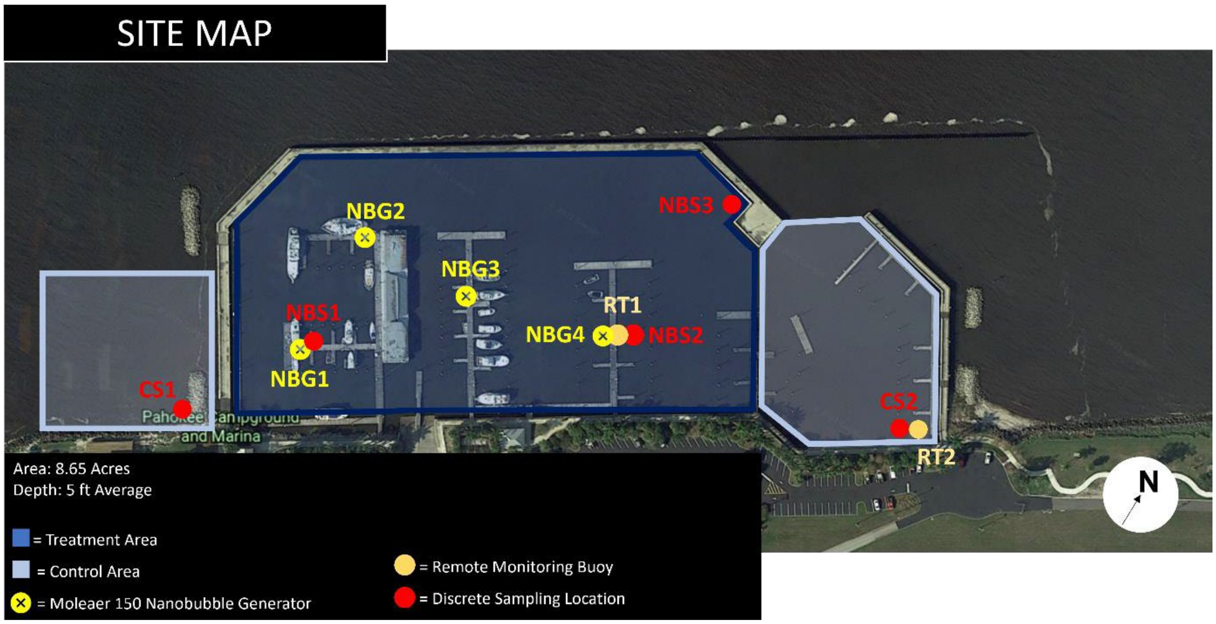

Figure 2: Pahokee Marina treatment and control areas.

The total grant award amount was $685,787 to FGCU. Of this amount, $270,373 was subcontracted to Moleaer to install and operate the nanobubble generators. The budget summary is shown in Table 1.

Task No.	Budget Category	Budget Amount
1	Salary	$2,419.67
	Fringe	$479.90
	Contractual Services	$9,071.00
	Overhead/Indirect (17%)	$2,035.00
	Total for Task	**$14,005.57**
2	Salary	$2,425.16
	Fringe	$524.00
	Contractual Services	$8,487.00
	Overhead/Indirect (17%)	$1,944.15
	Total for Task	**$13,380.31**
3	Salary	$15,000.02
	Fringe	$4,185.00
	Contractual Services	$15,869.00
	Overhead/Indirect (17%)	$4,526.59
	Total for Task	**$39,580.61**
4	Salary	$205,196.15
	Fringe	$60,251.10
	Contractual Services	$219,824.00
	Miscellaneous/Other	$355.36
	Materials and Supplies	$46,977.26
	Overhead/Indirect (17%)	$57,443.75
	Total for Task	**$590,047.62**
5	Salary	$7,494.00
	Fringe	$2,323.00
	Contractual Services	$17,247.00
	Overhead/Indirect (17%)	$1,668.89
	Total for Task	**$28,732.89**
	Total for all Tasks	**$685,747**

Category Totals	Grant Funding, Not to Exceed, $
Salary	$232,535.00
Fringe	$67,763.00
Contractual Services	$270,498.00
Miscellaneous/Other	$355.36
Materials and Supplies	$46,977.26
Overhead/Indirect (17%)	$67,618.38
Total:	**$685,747.00**

Table 1: Budget summary for total award amount.

This budget was allocated over the period of 7/1/2020 through 7/15/2023. The grant work was organized into 5 tasks- Site Confirmation, Installation Design and Permitting, Quality Assurance Project Plan (QAPP), Operation and Monitoring, and Final Report. The breakdown of the amount awarded per task of the grant is shown in Table 2.

Task No.	Task or Deliverable Title	Task Start Date	Task End Date
1	Site Confirmation	7/1/2020	3/28/2022
2	Installation Design and Permitting	7/1/2020	3/28/2022
3	Quality Assurance Project Plan	7/1/2020	3/28/2022
3a	Draft Quality Assurance Project Plan	7/1/2020	3/28/2022
3b	Final Quality Assurance Project Plan	7/1/2020	3/28/2022
4	Nanobubble Generator Installation, Operations and Monitoring	7/1/2020	10/24/2023
5	Final Report		
5a	Draft Final Report	7/1/2020	9/15/2023
5b	Final Report	5/15/2023	10/15/20223

Table 2: A summary of the project timeline and budget detail.

<u>Section 2: Financial summary of actual costs versus the budget, along with any changes required to the budget.</u> 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.

Invoicing still needs to take place for tasks 4 and 5 not to exceed $618,780.51. This will happen once all encumbered items and salary have been fully processed. Task 5 is not complete until this report is approved.

Subcontractor (Moleaer) Financial Summary:

The subcontractor budget, costs, and contributions are shown in Table 3. Tasks 1, 2, 3, and 5 were billed through single invoices for the entire task whereas Task 4 was billed monthly due to the length of time for task execution. A list of the monthly costs for Task 4 is shown in Table 4. Task 4 also included an estimated in-kind contribution of $14,000 to cover unexpected costs of service to the equipment and the sensors due to environmental impacts on the equipment. The $14,000 is estimated from 8x Moleaer service trips ($1,750/site visit) in addition to the number of service trips initially expected. In summary, the overall billed costs for the grant matched the initial expected budgeted amount.

Task No.	Task or Deliverable Title	Budgeted Amount (USD)	Billed Costs (USD)	Contributions (USD)
1	Site Confirmation	$9,071	$9,071	--
2	Installation Design and Permitting	$8,487	$8,487	--
3	Quality Assurance Project Plan (QAPP)	$15,869	$15,869	--
4	Operations and Monitoring	$219,699	$219,698	$14,000
5	Final Report	$17,247	$17,247	--
	Total:	**$270,373**	**$270,372**	**$14,000**

Table 3: Summary of subcontractor budget vs the actual costs.

Task or Deliverable Title	Billed Costs (USD)
Operations and Monitoring - 4.1	$25,134
Operations and Monitoring - 4.2	$13,882
Operations and Monitoring - 4.3	$13,882
Operations and Monitoring - 4.4	$16,297
Operations and Monitoring - 4.5	$17,907
Operations and Monitoring - 4.6	$18,346
Operations and Monitoring Extension - 4.1	$13,882
Operations and Monitoring Extension - 4.2	$13,882
Operations and Monitoring Extension - 4.3	$13,882
Operations and Monitoring Extension - 4.4	$13,882
Operations and Monitoring Extension - 4.5	$13,882
Operations and Monitoring Extension - 4.6	$13,882
Operations and Monitoring Extension - 4.7	$13,882
Operations and Monitoring Extension - 4.8	$17,076

Table 4: Breakdown of individual subcontractor costs for Task 4.

Section 3: Project Schedule. 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.

The grant project was ultimately executed on time without major disruptions or unexpected site conditions. The project schedule was adjusted from the original (Table 5) and extended (Table 6) from the original finish date. The project was extended to allow for minor delays due to unexpected site conditions, for more severe weather conditions than expected, and most significantly, to allow for the operation of the nanobubble generators over a period of time where algae blooms are more likely to occur in the marina. Amendment #2 updated task end dates as shown in Table 5.

Task No.	Task or Deliverable Title	Task Start Date	Task End Date
1	Site Confirmation	7/1/2020	3/28/2022
2	Installation Design and Permitting	7/1/2020	3/28/2022
3	Quality Assurance Project Plan	7/1/2020	
3a	Draft Quality Assurance Project Plan	7/1/2020	3/28/2022
3b	Final Quality Assurance Project Plan	7/1/2020	3/28/2022
4	Nanobubble Generator Installation, Operations, and Monitoring	7/1/2020	10/24/2023
5	Final Report		
5a	Draft Final Report	7/1/2020	9/15/2023
5b	Final Report	5/15/2023	10/15/2023

Table 5: Original project timeline

Task No.	Task or Deliverable Title	Task Start Date	Task End Date
1	Site Confirmation	7/1/2020	7/11/2021
2	Installation Design and Permitting	7/1/2020	7/11/2021
3	Quality Assurance Project Plan	7/1/2020	12/11/2021
3a	Draft Quality Assurance Project Plan	7/1/2020	6/11/2021
3b	Final Quality Assurance Project Plan	7/1/2020	7/11/2021
4	Nanobubble Generator Installation, Operations, and Monitoring	7/1/2020	12/17/2021
5	Final Report	7/1/2020	3/28/2022

Task No.	Task or Deliverable Title	Task Start Date	Task End Date
1	Site Confirmation	7/1/2020	3/28/2022
2	Installation Design and Permitting	7/1/2020	3/28/2022
3	Quality Assurance Project Plan	7/1/2020	3/28/2022
3a	Draft Quality Assurance Project Plan	7/1/2020	3/28/2022
3b	Final Quality Assurance Project Plan	7/1/2020	3/28/2022
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Table 6: Final project timeline after extension.

The disruptions to the timeline included unexpected site conditions, harsh weather events, and a lack of alignment with the seasonal behavior of algae blooms. The unexpected site conditions included challenges with the electrical supply for the nanobubble generators at the marina. While the availability of electricity was confirmed during Task 2, *Installation Design and Permitting*, after equipment installation the electrical supply was found to be unreliable with routine voltage drops or power outages that would result in the shutoff of the Moleaer equipment. The root cause of this behavior was identified by installing voltage monitors in the nanobubble generators and monitoring for irregularities in electrical supply. This resulted in a high frequency of unit restarting events and a lower than anticipated equipment uptime (Figure 18) during the first two quarters of operation. The problem was solved by adding voltage regulators to the equipment which made up for lower voltage during low voltage events and prevented voltage spikes.

Disruptions to the project also occurred due to environmental conditions including severe weather events and algae bloom seasonality. The initial timeline of the project planned for the operation of the equipment from approximately August through December. However, due to the timeline of equipment installation, equipment operation, and the algae bloom seasonality, the units were not treating the marina during a period of high algae growth inhibiting a clear analysis of the impact of the nanobubble generators. For this reason, Task 4 was extended from December 2021 through April 2023. This enabled the nanobubble treatment to occur over a period of higher algae growth – traditionally the warmer, drier months in the region- and for monitoring to occur over more than an entire year allowing for the consideration of seasonal variability in data analysis. During this period of treatment, severe weather events also occurred, including a hurricane in the near vicinity which caused damage to the equipment during Q4 2022. These unexpected events resulted in changes to the timeline, however, all work for the grant has been completed on schedule according to the updated timeline.

Section 4: Summary of activities. Activities completed as well as those not completed and why, as well as a brief summary of any additional phases yet to be completed.

Task No.	Task or Deliverable Title	Status
1	Site Confirmation	Complete
2	Installation Design and Permitting	Complete
3	Quality Assurance Project Plan (QAPP)	Complete
4	Operations and Monitoring	Complete
5	Final Report	Complete

Table 7: Summary of tasks and completion status.

Section 5: Photo documentation.

Photos of work performed (before, during and after), appropriate figures (site location, site plan[s]. etc.), appropriate tables summarizing data/information relevant to Grant Work Plan tasks, and appropriate attachments relevant to the project.

The Moleaer nanobubble generators were installed at four locations within the marina as shown earlier in Figure 2. A pre-installation site visit was conducted with Moleaer, FGCU, and Pahokee staff to select nanobubble generator locations. The sites were selected to maximize the distribution of nanobubble treatment within the target treatment region of the marina. A photo of B Dock during the pre-installation site visit on May 20, 2021, is shown in Figure 3. Additionally, locations were selected based on the availability of space and electricity on the docks, the frequency of boat traffic near the generators, and the integrity of the docks. For example, generators could only be mounted on Docks B, C, and D as other docks within the marina lacked electrical access or were in insufficient condition to support a nanobubble generator and technician staff.

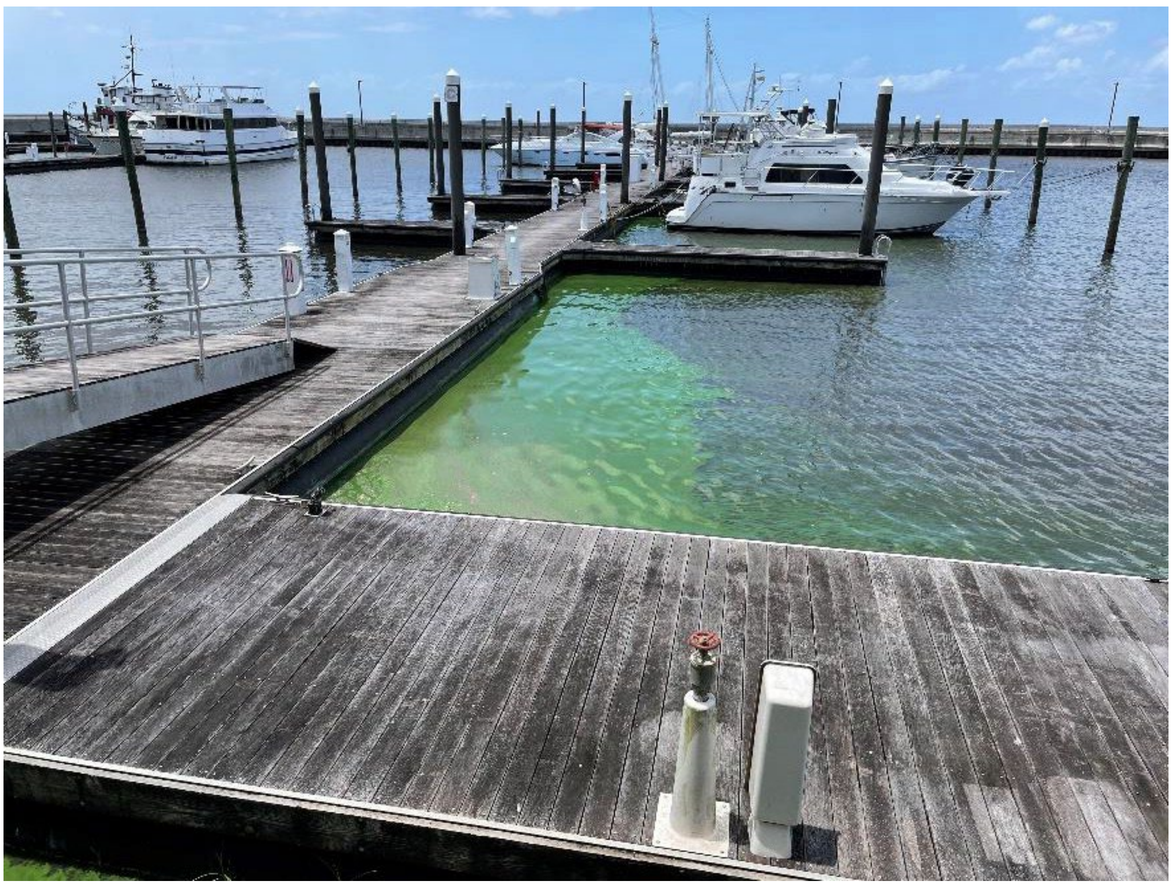

Figure 3: Photo of Dock B prior to nanobubble generator installation.

After the pre-installation site visit, locations on docks B, C, and D were chosen to install the nanobubble generators. Dock slips B14, B25, C14, and D4 were selected, and photos of each installation site are shown in Figures 4-7 respectively. The photos were taken on September, 21 2021 after each generator had been installed, tested, and commissioned.

Figure 4: Photo of nanobubble generator located at dock space B14.

Figure 5: Photo of nanobubble generator site located at dock space B25.

Figure 6: Photo of nanobubble generator site located at dock space C14.

Figure 7: Photo of nanobubble generator site located at dock space D4.

The continuous water quality monitoring sensors were deployed to Pahokee Marina on September 22, 2021 on D Dock, identified as the *Treatment Sensor or D Dock Sensor* and on the F Dock, identified as the *Control Sensor.* The Treatment and Control sensors are shown in Figures 8 and 9 respectively. Each sensor consisted of a telemetry device, a cable, a sonde, and a package of 4 sensor housings. The telemetry devices were mounted to a dock cleat with the sensor suspended by a communications cable no more than 2 ft below the water's surface. As the sensors were mounted on a floating dock, the sensor depth from the water's surface was consistent even with fluctuating water depth.

Figure 8: Treatment area In-Situ water quality monitoring sensor mounted on D Dock.

Figure 9: Control area In-Situ water quality monitoring sensor mounted in the F Dock.

After the completion of the monitoring and operating period of the nanobubble generators (April 14, 2023), all equipment was removed from the marina. Photos of dock space B14, B25, C14, and D4 after the nanobubble generators were removed are shown in Figures 10-13 respectively.

Figure 10: Photo of dock space B14 after nanobubble generator removal.

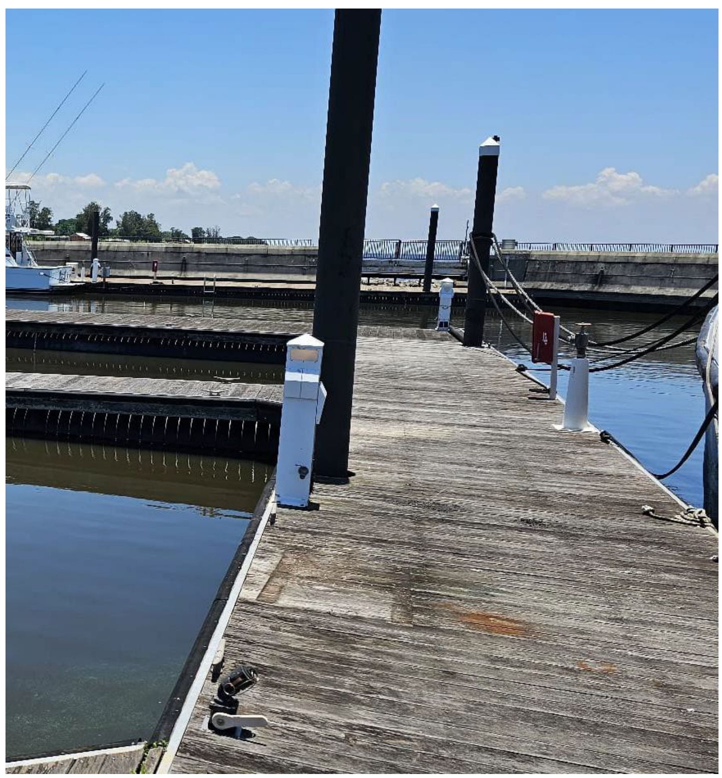

Figure 11: Photo of dock space B25 after nanobubble generator removal.

Figure 12: Photo of dock space C14 after nanobubble generator removal.

Figure 13: Photo of dock space D4 after nanobubble generator removal.

The nanobubble generator uptime reports for nanobubble generators B14, B25, C14, and D4 as well as ozone usage are provided in Figures 14-17 respectively. These reports include the operating status (ON/OFF) for each nanobubble generator on the left and for the ozone equipment on the right. Included in each report is the overall uptime percentage which represents the percent of time that the equipment is considered operating which includes the operation of the liquid pump, the gas supply compressor, and the nanobubble generator core technology. Additionally, the usage of ozone, shown as percent usage, on each unit is included. This represents the amount of time ozone was injected into the water through the nanobubble generator. The ozone uptime value is impacted both by the operating condition of the equipment as well as by the estimated requirement for ozone based on the likelihood of algae growth. This data was captured by the remote equipment monitoring incorporated into each nanobubble generator system. The summary of all equipment uptime and ozone usage is shown in Figure 18. The total uptime for all equipment was 69% and the average of ozone usage across all units was 48%.

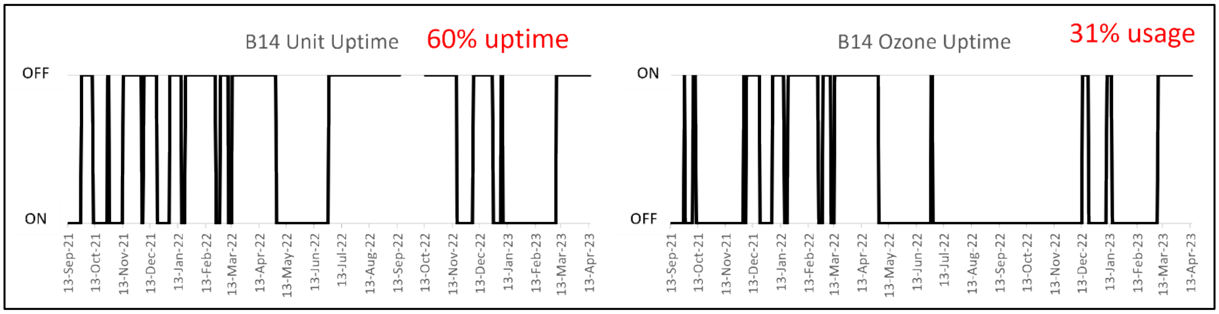

Figure 14: Total Operating Data (left) and Ozone Utilization (right) for unit B14.

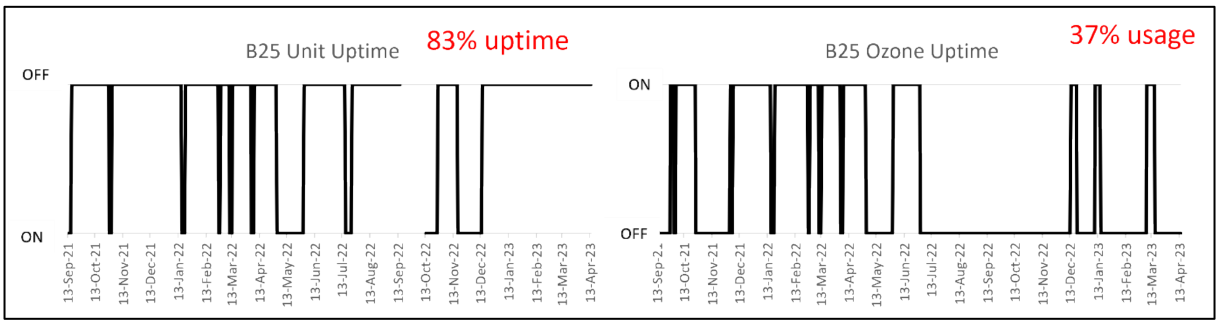

Figure 15: Total Operating Data (left) and Ozone Utilization (right) for unit B25.

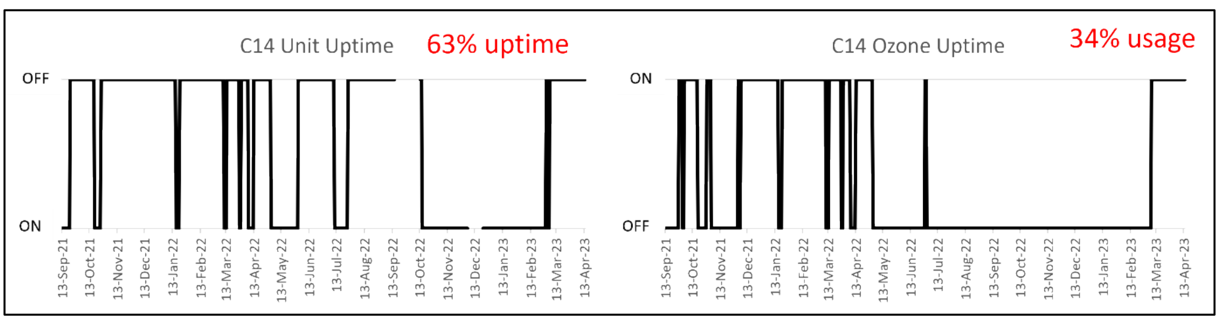

Figure 16: Total Operating Data (left) and Ozone Utilization (right) for unit C14.

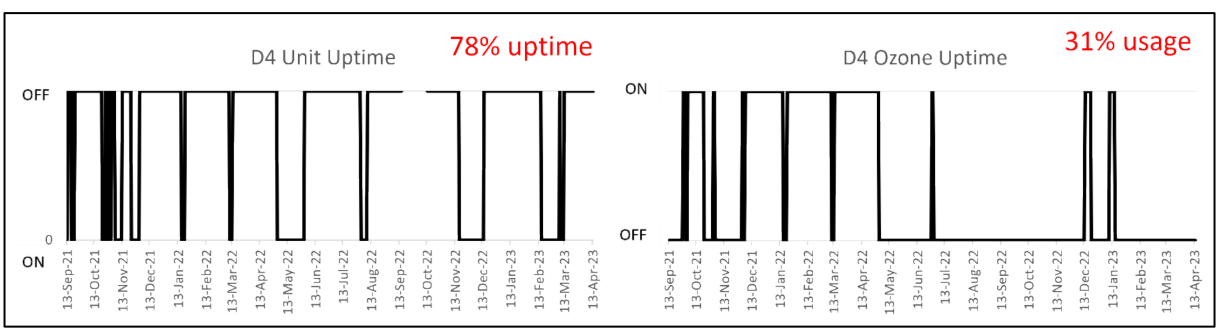

Figure 17: Total Operating Data (left) and Ozone Utilization (right) for unit D4.

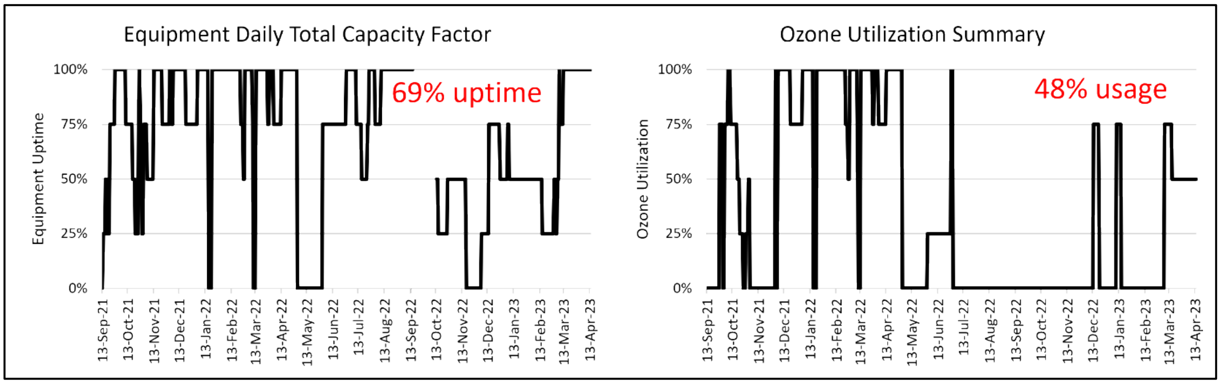

Figure 18: Summary of Total Operating Data (left) and Ozone Utilization (right) for all units.

<u>Section 6: Discussion of whether the anticipated benefits have been/will be realized</u>.

Nanobubble technology offers the potential to mitigate and control persistent HABs in difficult to treat areas. Ozone nanobubble generator sizing was conducted considering factors such as marina water volume and quality. The equipment size and treatment range were determined by the size and estimated depth of the marina resulting in an approximate volume. Further, as the marina is a partially isolated region within the much larger Lake Okeechobee, water exchange between the marina and the lake was considered to estimate a hydraulic residence time, or a rate of turnover of the marina water. Water exchange between the marina and broader water body was the hardest factor to estimate considering the variability of currents depending on water depth and wind intensity. Moreover, the removal of barriers in the sea wall during the project likely increased water exchange. The systems were sized also considering water quality parameters such as algae density. Units were placed throughout the marina to best distribute treatment based on estimated water currents and accumulation zones for algae.

This project investigated the real-world relationships occurring between oxygen and ozone nanobubbles and known HAB-related environmental conditions in Pahokee Marina. Primary interest was given to water quality parameters: phycocyanin (PC), oxidation-reduction potential (ORP), dissolved oxygen), nutrient levels (ammonia, nitrite, nitrate, ortho-phosphate, total phosphorus), and algae community characteristics (cell concentrations, cyanotoxin levels, colony disruption). General conclusions are discussed below and elaborated upon in Section 7.

Continuous water quality parameter monitoring was performed for the duration of this project. Despite sonde technology issues, the following insights were made. PC concentrations appear to trend lower in the treatment area as compared to the control area, however a quantitative reduction in algae concentration could not be provided with PC data alone given the uncertainties with probe calibration. ORP remained positive and consistent across both treatment and control areas. The consistent ORP conditions across the control and treatment areas suggest that the ozone nanobubble treatment was not introducing any harmful impacts to the waterbody because of the over-application of ozone. Dissolved oxygen levels remained slightly higher in the treatment area, and fluctuated with decreasing intensity throughout the project. Increased dissolved oxygen levels along with improved dissolved oxygen stability suggests possible benefit to aquatic health.

Nutrient sampling was performed successfully for the duration of the project. Throughout the monitoring period ammonia, nitrate, nitrite, ortho-phosphate, and total phosphorus concentrations in the control and treatment regions followed similar trends. As such, it is difficult to distinguish the impact of nanobubble treatment from natural water quality variations. As an interesting note, total phosphorus showed little to no correlation with nitrogen-compound concentrations, suggesting that the sources of nutrient loading may be different for the two categories of nutrients.

Discrete algae sampling was performed in tandem with nutrient sampling for the duration of this project. A lack of statistical differences in algae concentration averages indicates that nanobubble generators had no significant impact on major algae genera concentrations or total community algae concentrations. *Microcystis* natural unit composition and microcystin concentration similarly lacked statistical differences, indicating that nanobubble generator operation did not significantly disrupt *Microcystis* colonies or reduce the production rate of microcystins across treatment sites.

A multivariate correlation table was created utilizing all measured responses collected during this project. While causation cannot be claimed, the following noteworthy relationships were uncovered, pointing to promising avenues of future study. Firstly, *Dolichospermum* concentration is negatively correlated with nitrate presence across all sampling sites, possibly indicating that *Dolichospermum*, as a nitrogen-fixing cyanobacteria, is outcompeting other dominant algae under nitrogen-limited conditions. A relationship between the diatom *Cyclotella* and ortho-phosphate also emerged, as cell concentrations appear to be positively correlated in nanobubble treatment areas, while no correlation exists in control areas. Finally, correlations suggest there is a negative relationship between nanobubble generator operation and ammonia and nitrate concentrations, indicating nanobubble treatment may hinder the accumulation of these nutrients. Additional study of specific algae genera, nanobubbles, and nutrient interactions are required to fully understand these correlations.

While these findings provide insight into the use of nanobubble technology as a method for HAB control, definitive conclusions are hindered by a lack of understanding of nanobubble treatment range. If nanobubble dosing were significantly different than designed, either through technology efficiency or marina hydrodynamics, analysis of the technology would be impossible due to the misclassification of sampling sites. Hence the efficacy of the nanobubble technology for HAB control in Pahokee Marina was inconclusive. Without first significantly improving the understanding of nanobubble treatment distribution in Pahokee Marina, nanobubble technology, as examined by this project, cannot be recommended as a viable mitigation technique for HABs. Additional research into HAB mitigation and control through nanobubble technology should include further differentiation of the treatment and control regions to isolate the impact of the technology.

Section 7: Summary of monitoring activities completed and any not completed and why, monitoring results, and an interpretation of data based on planned versus realized results.

The following section includes a discussion on the activities completed and if they were completed according to expectations as well as datasets and an interpretation of data. The data and interpretation sections, Section 7.2 and 7.3, are separated into three sections which capture the results from the real-time monitoring, the discrete nutrient sampling, and the algal concentration and algal toxin analysis.

7.1 Activities Completed

Over the installation and monitoring period, the marina and surrounding area water quality was monitored through a combination of continuous and discrete monitoring. The continuous sensors consisted of two In-Situ AquaTroll 500 Sondes equipped with Temperature, Conductivity, Dissolved Oxygen, ORP, pH, and Phycocyanin probes. Each AquaTroll 500 was connected via a cable to a VuLink In-Situ Telemetry unit that provided power and relayed data daily to the cloud. One troll was mounted on D Dock to monitor water quality conditions in the Treatment Area. The second troll was mounted on the F Dock to monitor conditions near the F Dock in the Control or Untreated Area. The data reported from the sondes is shown in detail in the previously submitted quarterly report as well as included in entirety for Phycocyanin, Dissolved Oxygen, ORP, and Temperature in section 7.3.1. The probes were installed and operated according to the project schedule. Challenges were encountered with the operation of the individual sensors and the sondes leading to gaps in the data recorded. The Phycocyanin Probe encountered the most significant challenges not only with data recording but also with maintaining calibration. The manufacturer, In-Situ, worked closely with the project throughout the monitoring period, including several site visits, to increase sensor reliability. Further discussion on the data presented in Section 7.3.1 is provided in Section 8.

The discrete water quality samples were pulled every two weeks throughout the monitoring period to complement the continuous monitoring data. The sampling trips were completed as planned by FGCU for the original monitoring period and were subsequently conducted by Short Labs for the extension of the monitoring period. The discrete water quality samples were analyzed for Nitrate, Nitrite, Nitrate + Nitrite, Orthophosphate, and Total Phosphorus by AEL Laboratory. The nutrient results from the entire monitoring period are presented in section 7.3.2. Discrete water samples were also analyzed for algal community composition, algal cell concentrations, and cyanotoxin concentration. This monitoring data is provided in section 7.2.3, with further analysis presented in 7.3.3. Additionally, discrete water quality monitoring trips presented the opportunity to capture observational data from the marina. Photo documentation of the site visits is presented in section 7.3.4. All discrete monitoring activities were completed as planned.

7.2 Monitoring Results

7.2.1 Real Time, In-Situ Monitoring and Weather Data:

The following section includes data captured from In-Situ, real-time water quality probes located within the treatment and control areas of the marina. The probe carried Phycocyanin (Figures 19-23), ORP (Figures 24-26), Dissolved Oxygen (Figures 27-29), and Temperature Probes (Figures 30-32). Weather data showing daily average, minimum, and maximum temperatures and daily precipitation is shown in Figures 33 and 34 respectively.

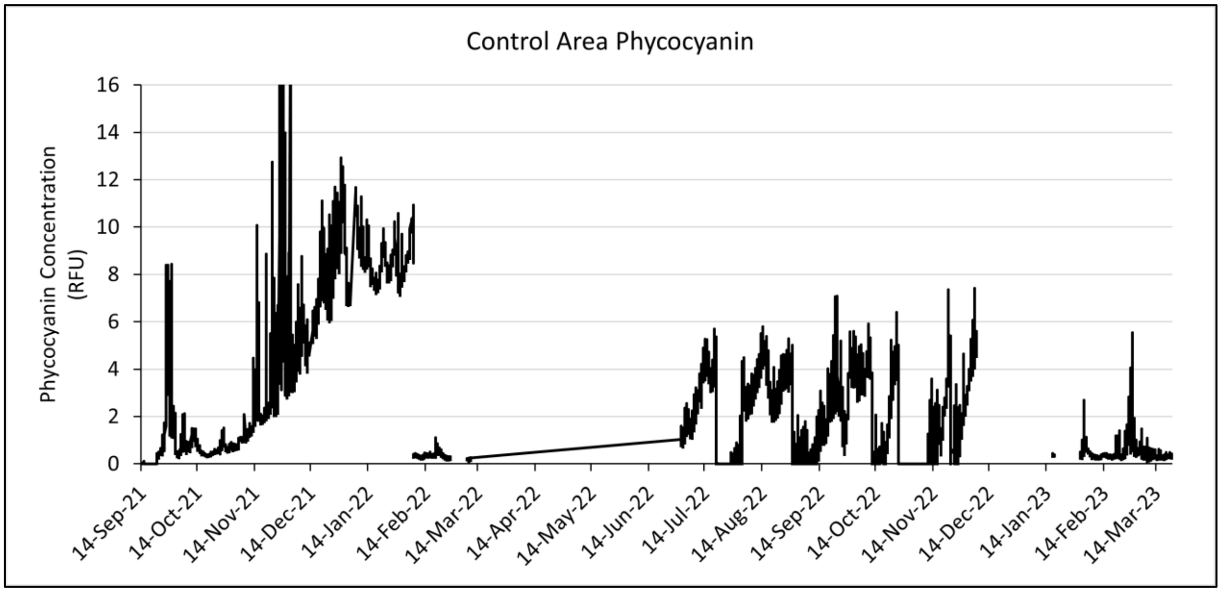

Figure 19: Control area phycocyanin reported as relative fluorescent units.

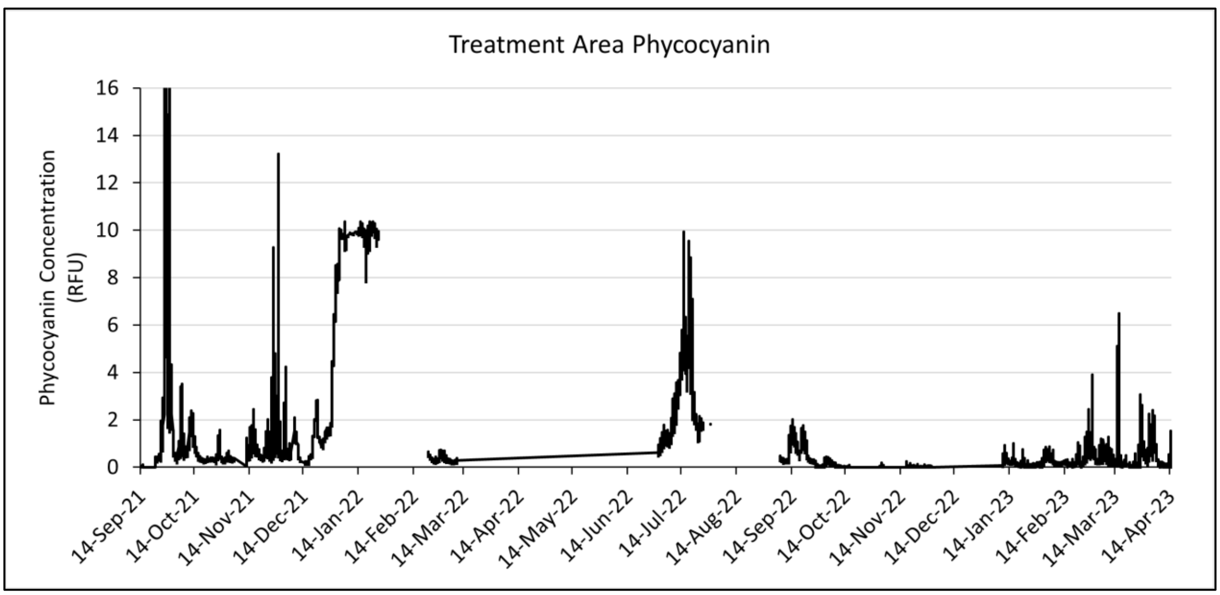

Figure 20: Treatment area phycocyanin reported as relative fluorescent units.

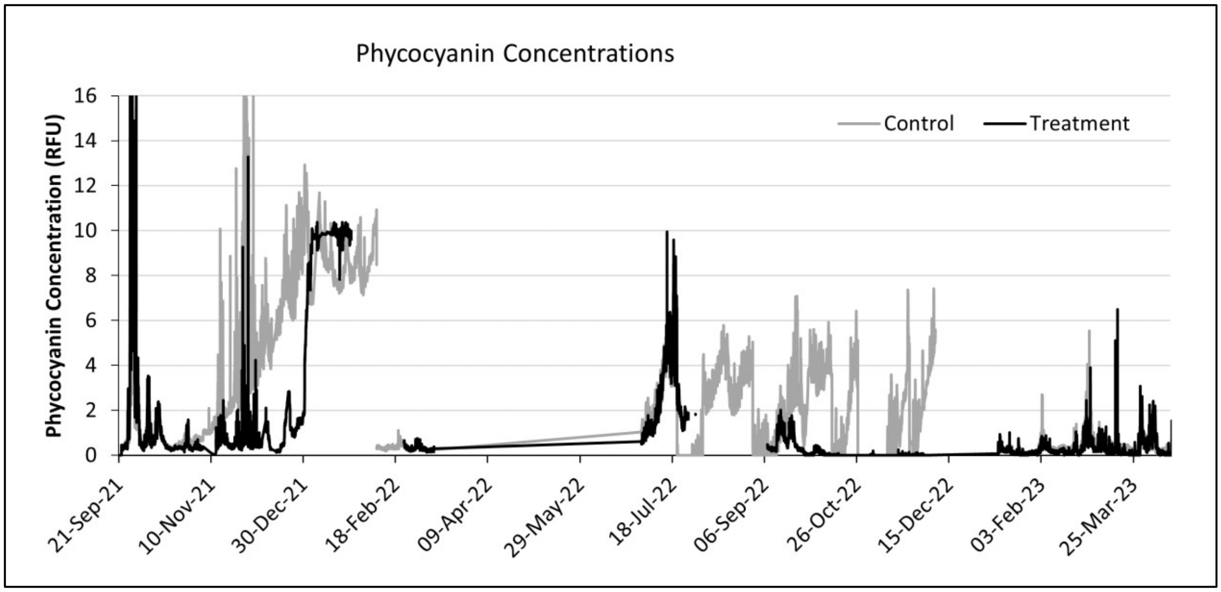

Figure 21: Control and Treatment area phycocyanin reported as relative fluorescent units.

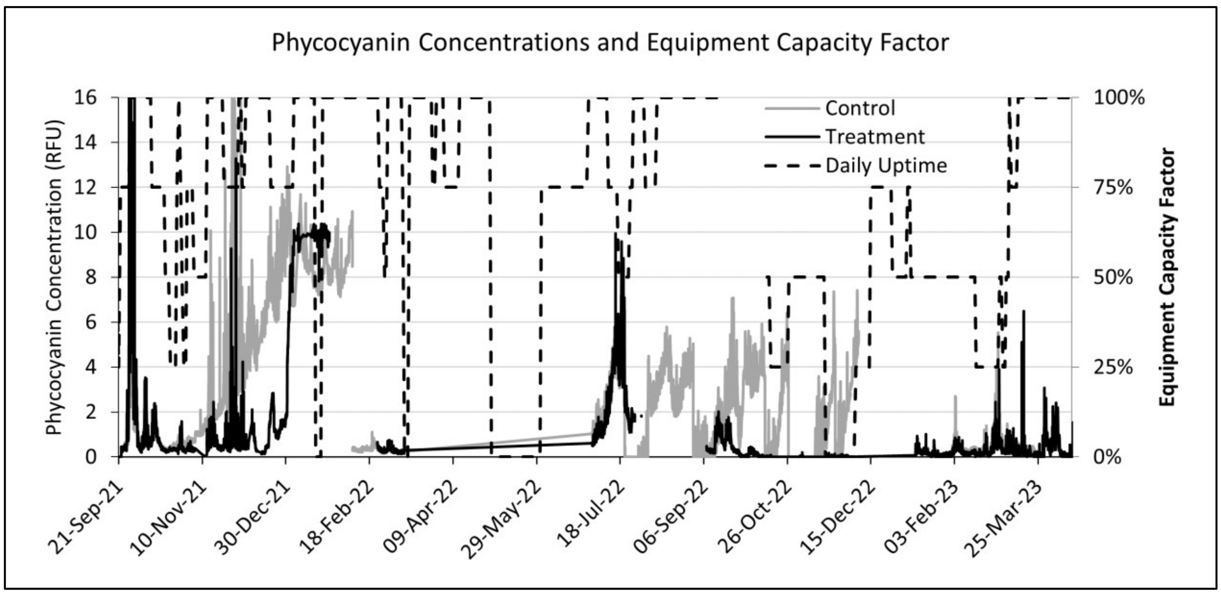

Figure 22: Combined relative phycocyanin concentrations along with equipment capacity factor.

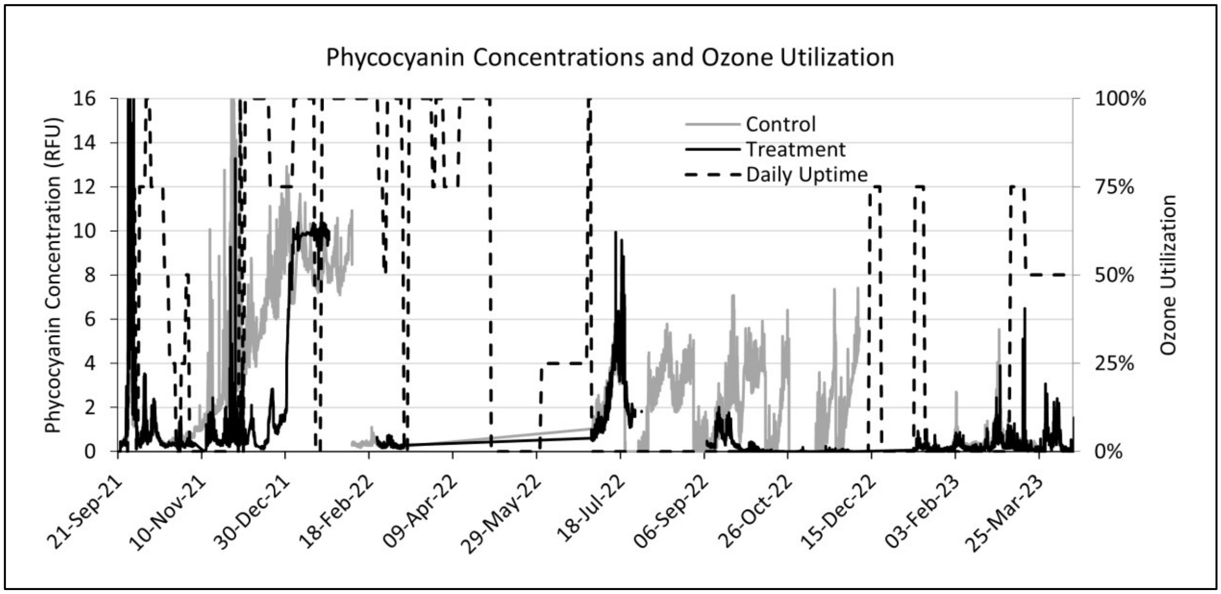

Figure 23: Combined relative phycocyanin concentrations along with total ozone utilization.

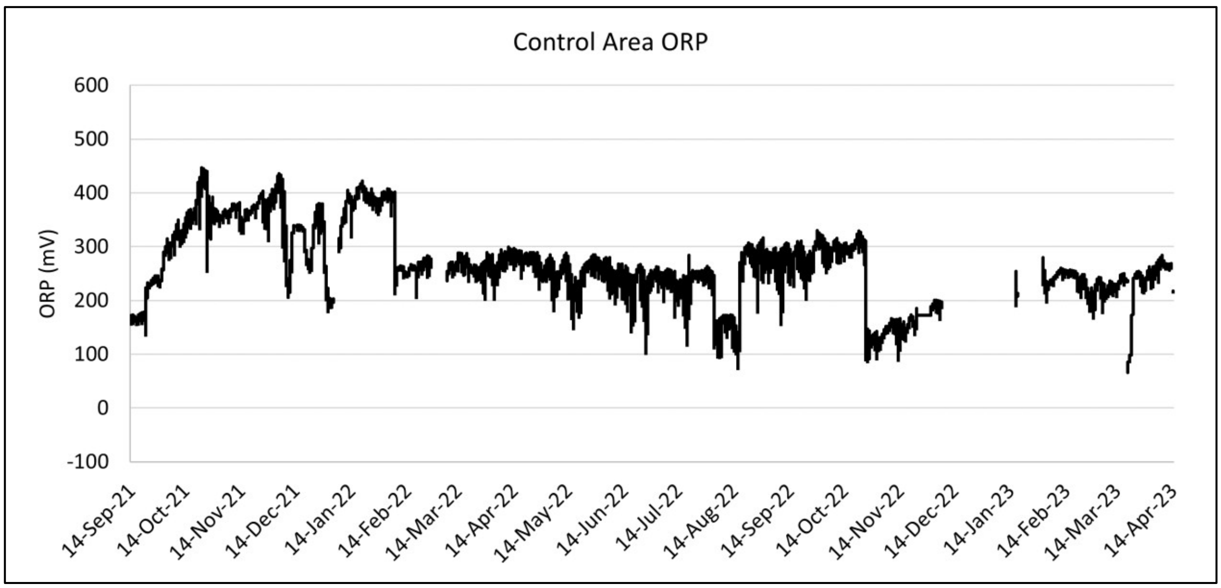

Figure 24: Control area ORP values.

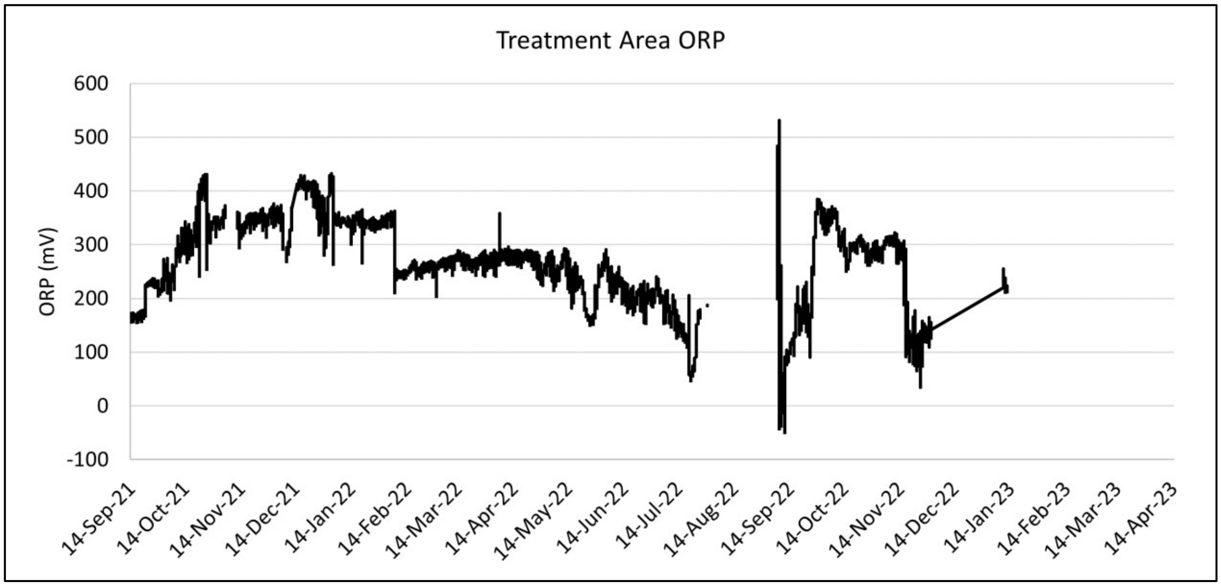

Figure 25: Treatment area ORP values.

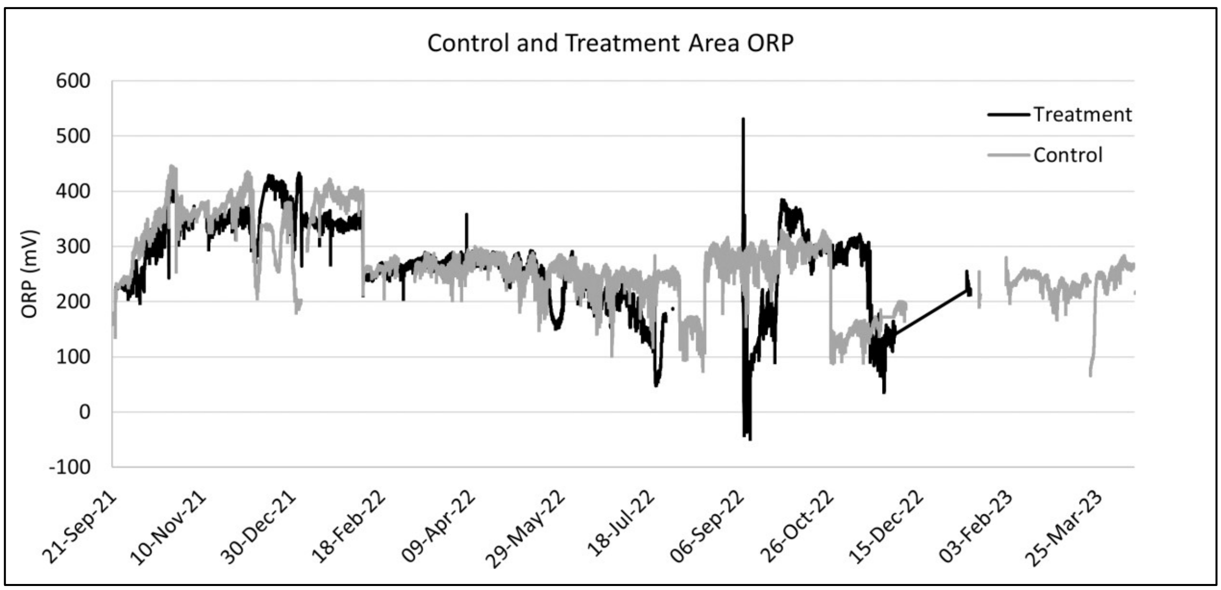

Figure 26: Comparison of ORP values from the control and treatment regions.

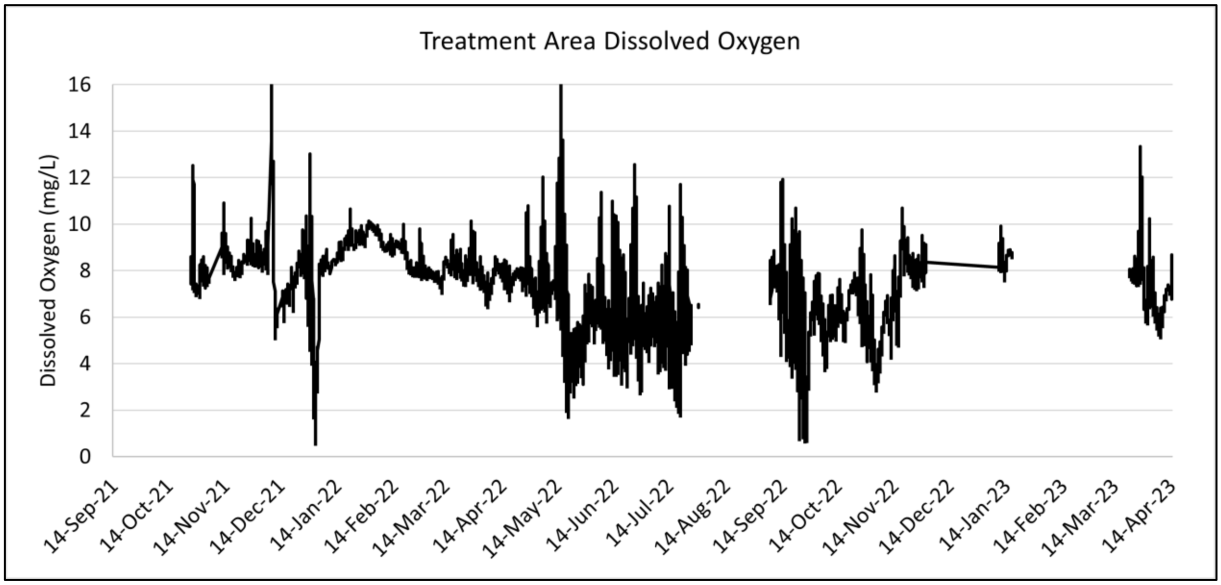

Figure 27: Control area dissolved oxygen concentrations.

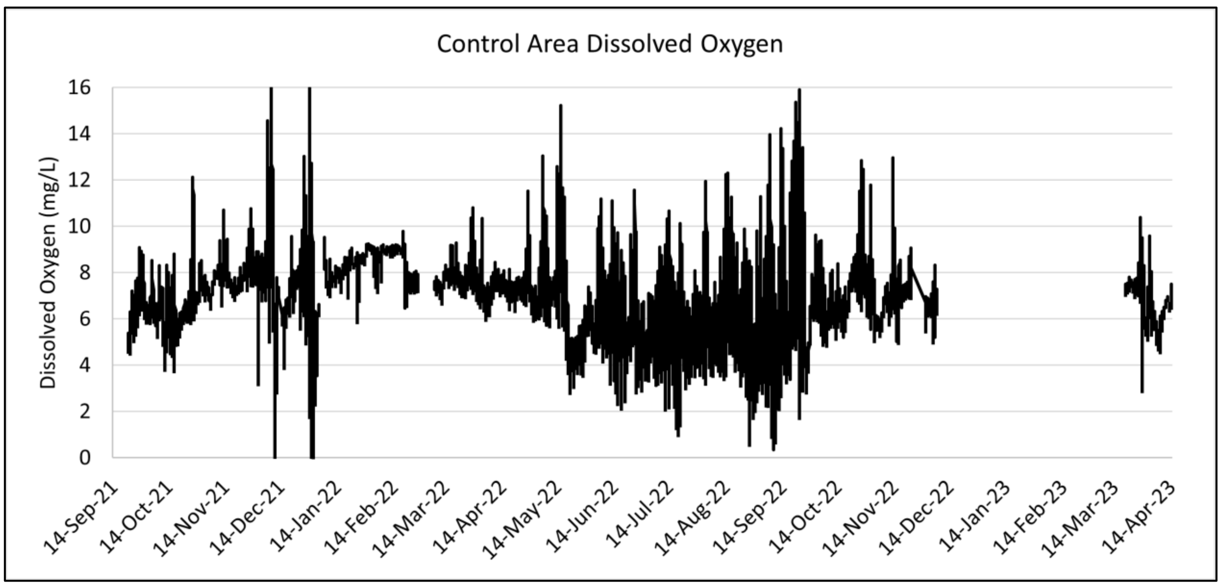

Figure 28: Treatment area dissolved oxygen concentrations.

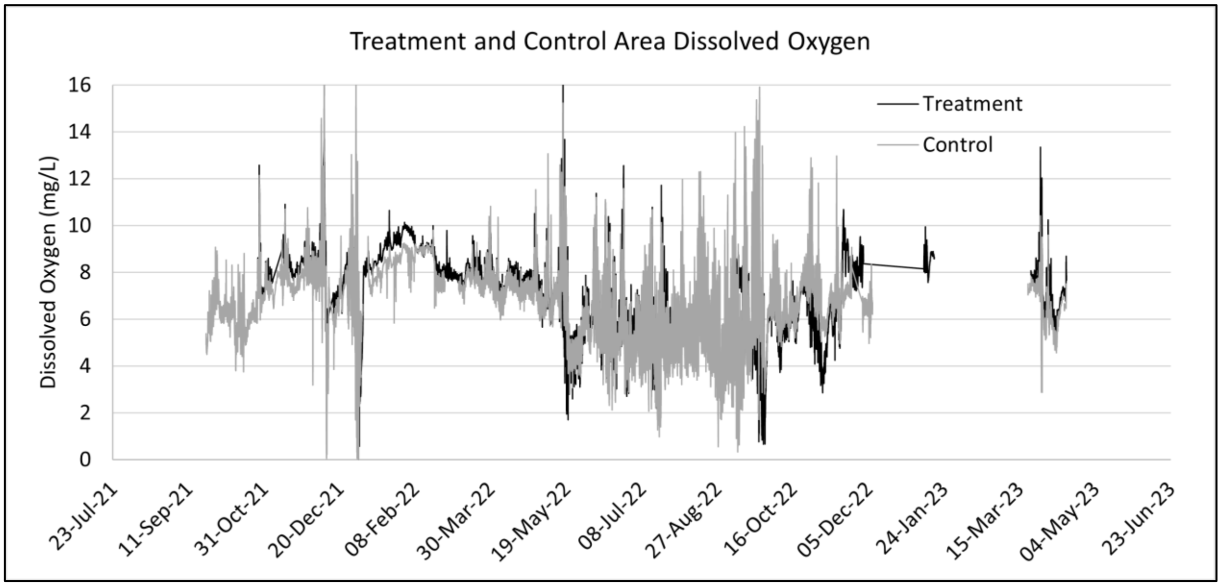

Figure 29: Comparison of control and treatment dissolved oxygen concentrations.

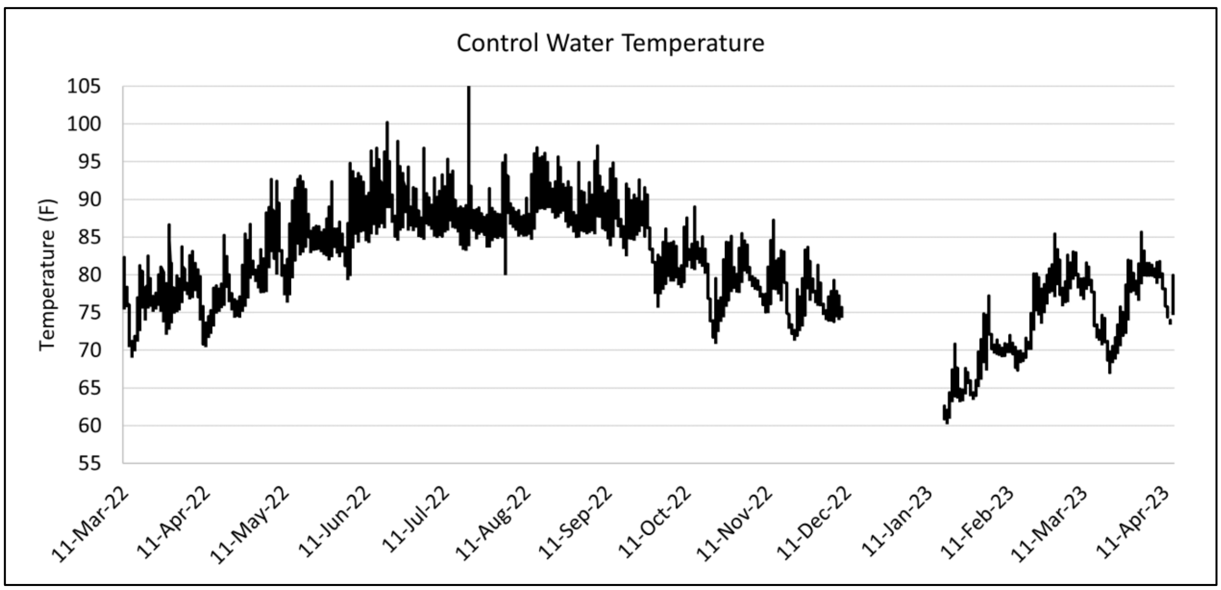

Figure 30: Control area water temperatures.

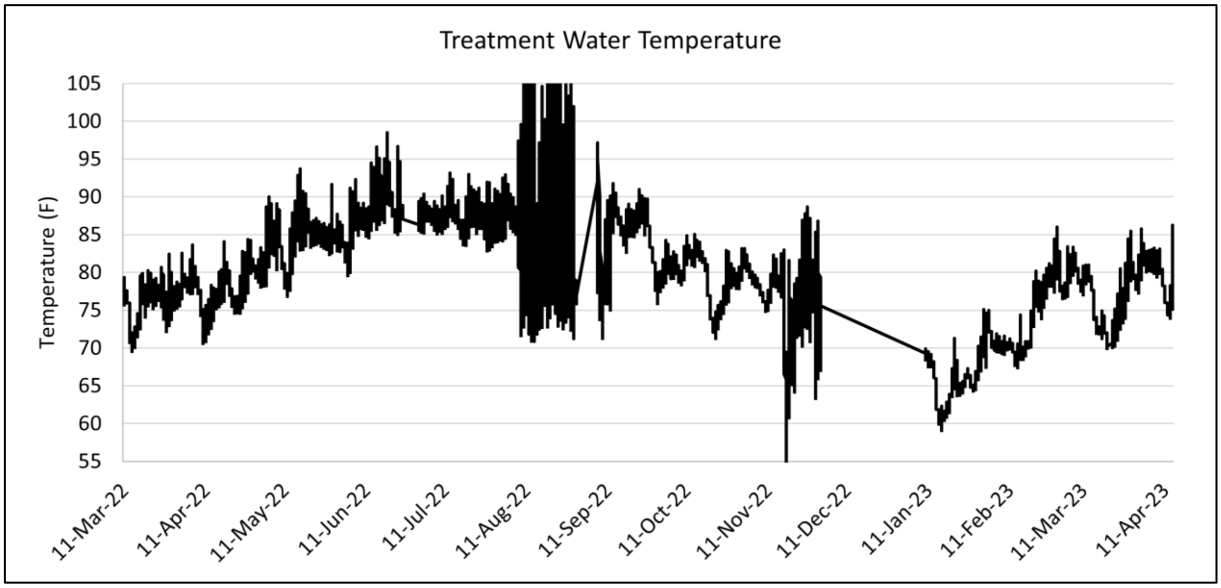

Figure 31: Treatment area water temperatures.

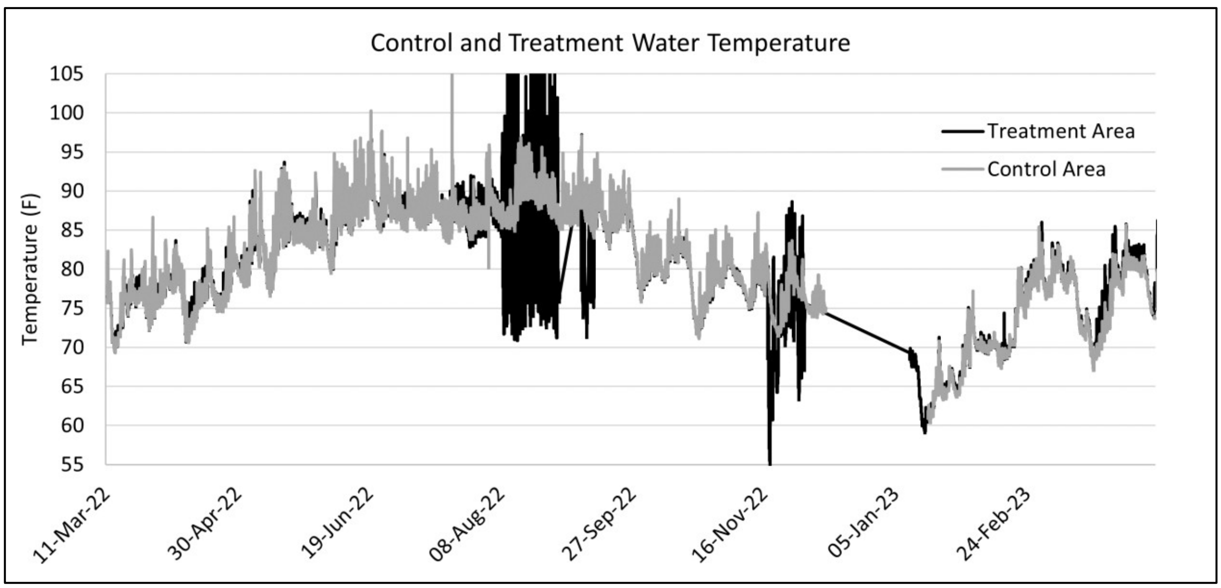

Figure 32: Control and Treatment area water temperatures.

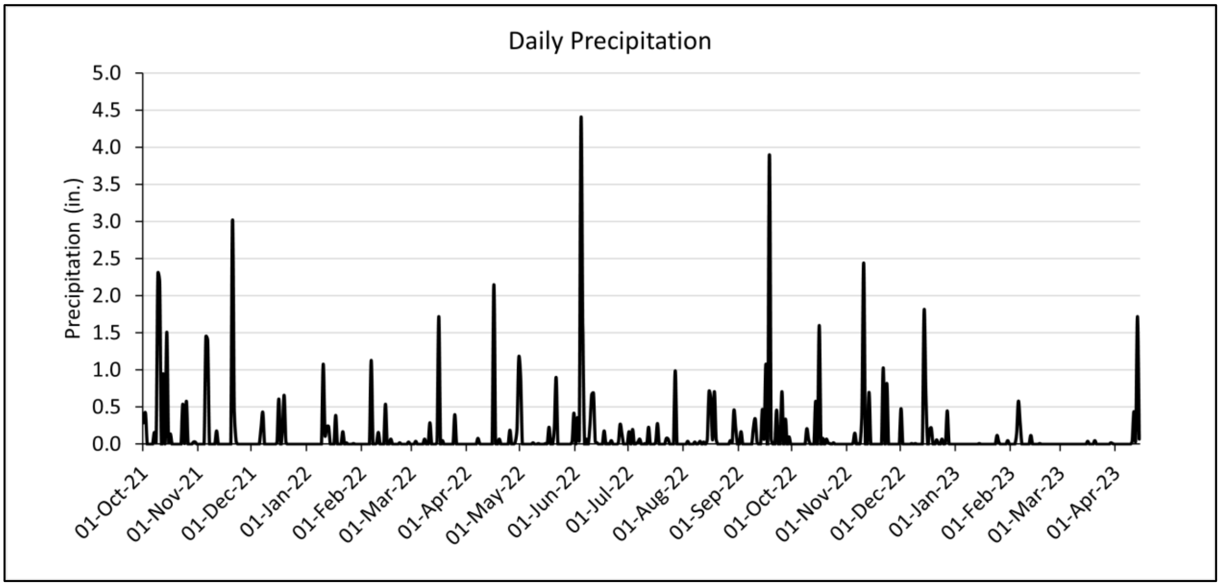

Figure 33: The daily average, minimum, and maximum air temperature.

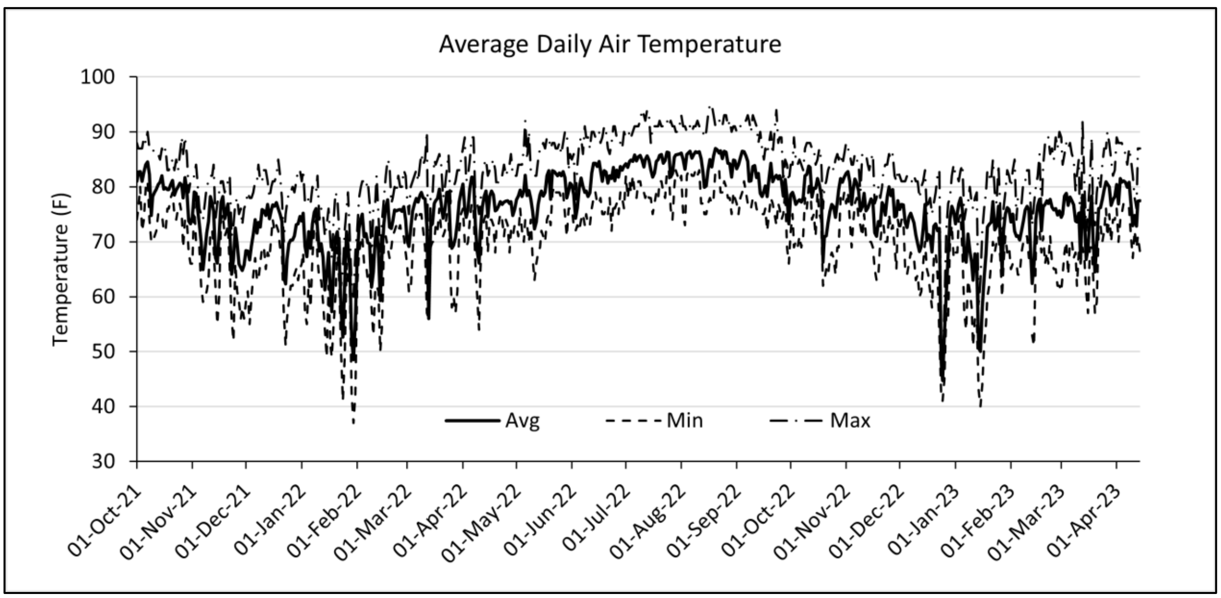

Figure 34: The daily precipitation for Pahokee Marina area.

7.2.2 Nutrient Grab Sample Data

The water quality nutrient data is captured in detail in the eight Quarterly Reports provided each quarter between Q3 2021 and Q2 2023. A summary of all nutrient data for Ammonia, Nitrate, Nitrite + Nitrite, Nitrite, Ortho-phosphorus, and Total Phosphorus are shown in Figures 35 through 40 respectively.

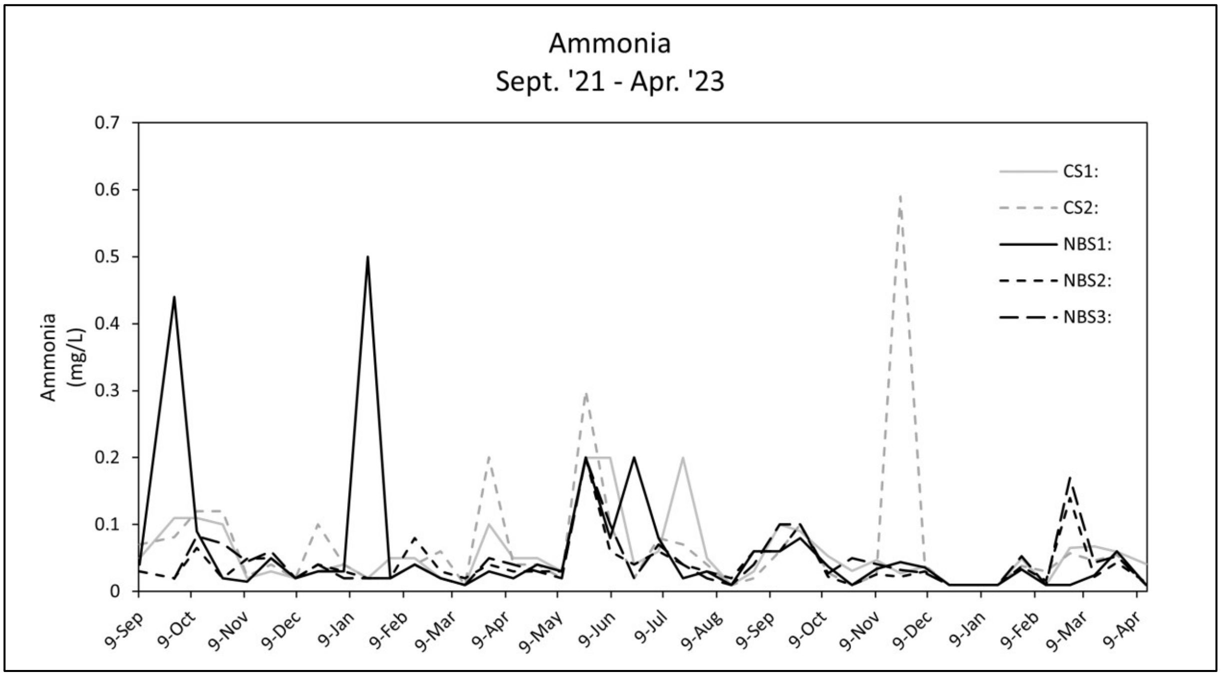

Figure 35: Ammonia discrete sample results for the total monitoring period.

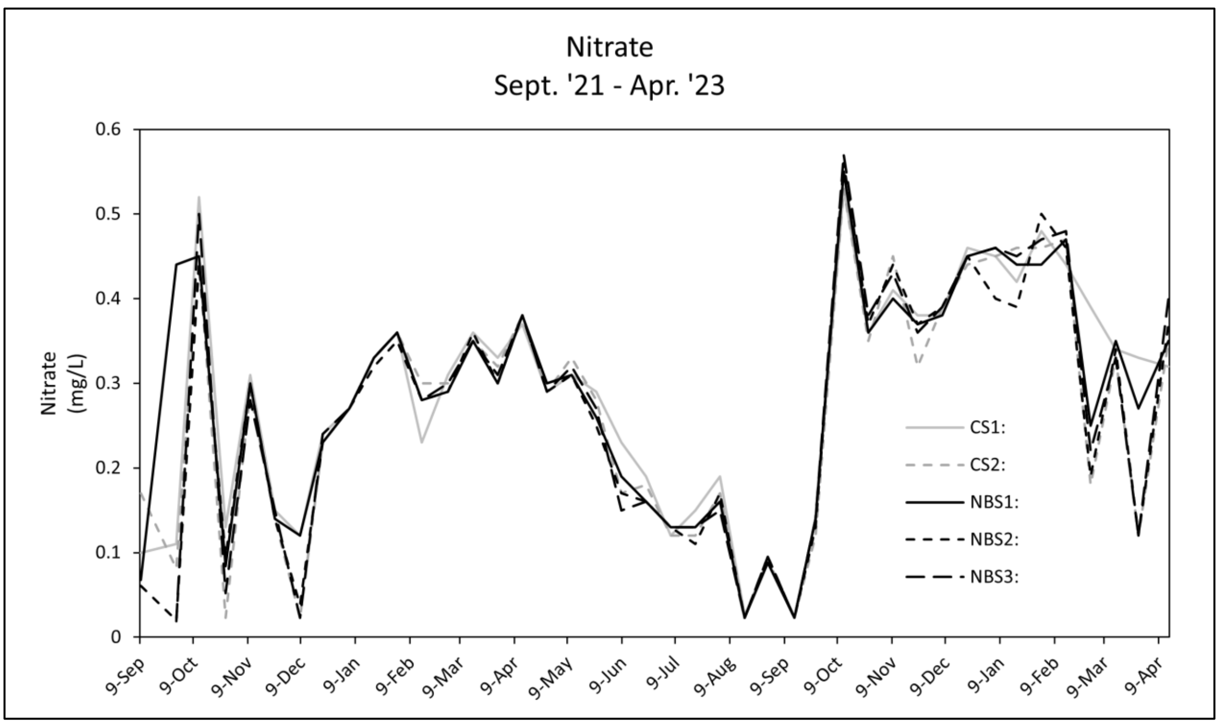

Figure 36: Nitrate discrete sample results for the total monitoring period.

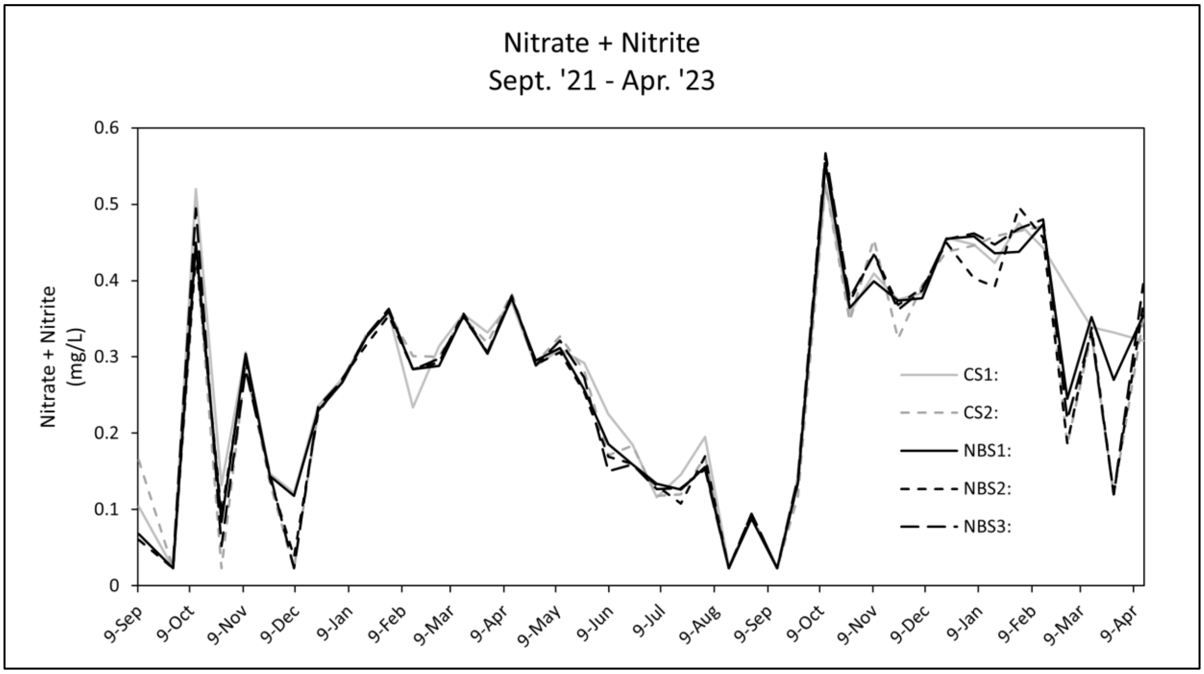

Figure 37: Nitrate + Nitrite discrete sample results for the total monitoring period.

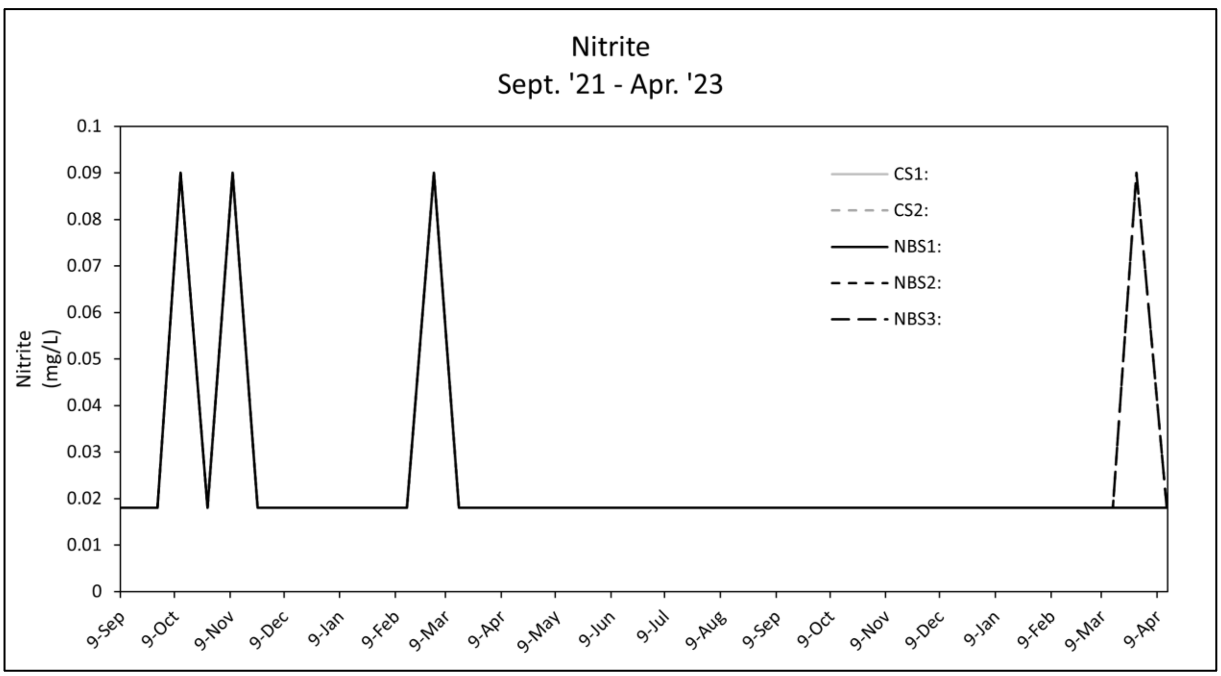

Figure 38: Nitrite discrete sample results for the total monitoring period.

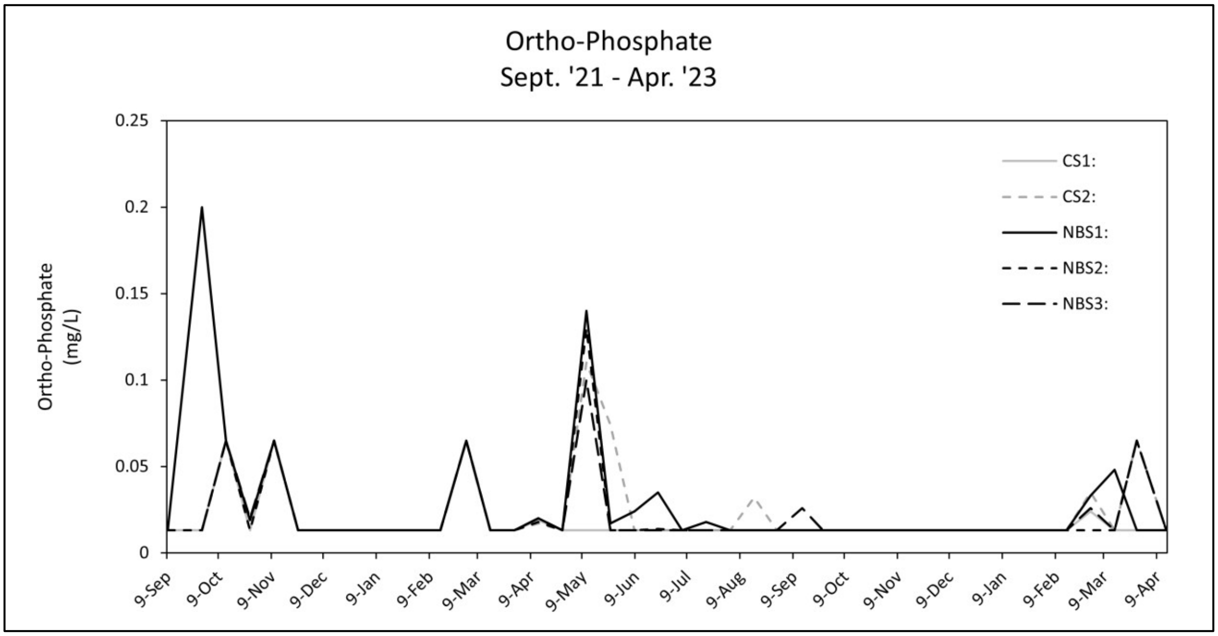

Figure 39: Ortho-phosphate discrete sample results for the total monitoring period.

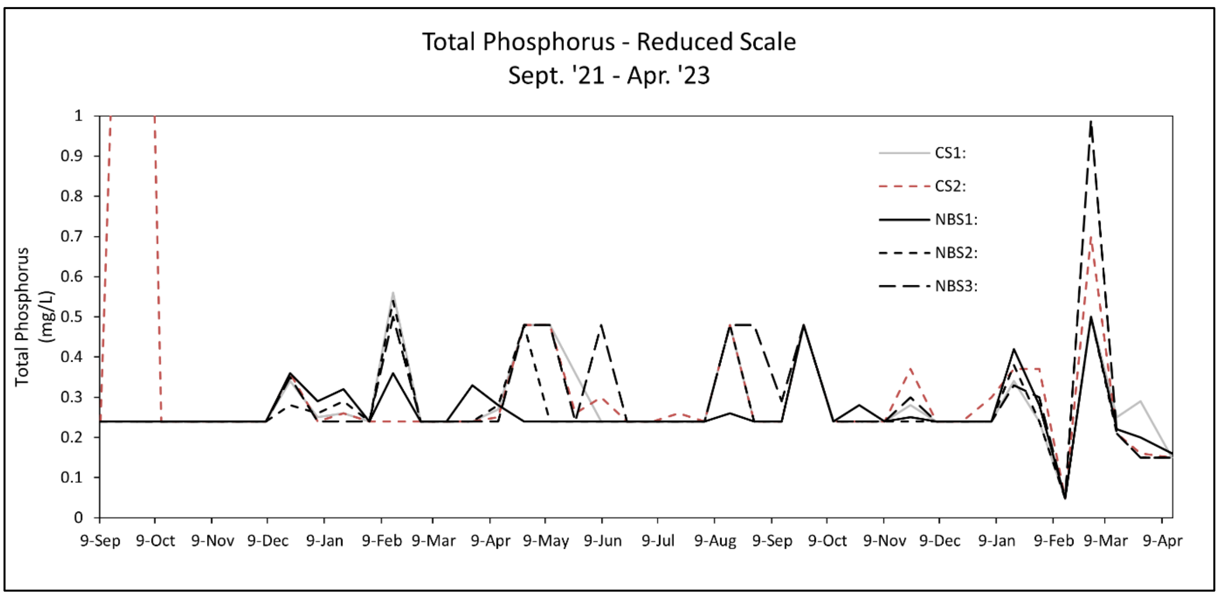

Figure 40: Total phosphorus discrete sample results for the total monitoring period.

7.2.3 Algal Cell Density and Cyanotoxin Data

Bi-weekly discrete algae sample grabs were conducted at each sampling site in Pahokee Marina from September 9, 2021, through April 14, 2023, as shown in Table 8. Note, collection site NBS3 was inaccessible on 9/9/21 due to a locked access gate in the marina. All other sample collections during this project were performed successfully.

Sampling Date	CS1	CS2	NBS1	NBS2	NBS3	IFCB Processing
9/9/2021	✓	✓	✓	✓	X	9/14/2021
9/29/2021	✓	✓	✓	✓	✓	9/30/2021
10/12/2021	✓	✓	✓	✓	✓	11/5/2021
10/27/2021	✓	✓	✓	✓	✓	11/5/2021
11/10/2021	✓	✓	✓	✓	✓	11/19/2021
11/23/2021	✓	✓	✓	✓	✓	2/8/2022
12/8/2021	✓	✓	✓	✓	✓	2/8/2022
12/21/2021	✓	✓	✓	✓	✓	2/9/2022
1/5/2022	✓	✓	✓	✓	✓	2/9/2022
1/19/2022	✓	✓	✓	✓	✓	2/9/2022
2/1/2022	✓	✓	✓	✓	✓	2/10/2022
2/15/2022	✓	✓	✓	✓	✓	2/16/2022
3/2/2022	✓	✓	✓	✓	✓	3/7/2022
3/16/2022	✓	✓	✓	✓	✓	3/29/2022
3/30/2022	✓	✓	✓	✓	✓	4/1/2022
4/13/2022	✓	✓	✓	✓	✓	4/18/2022
4/27/2022	✓	✓	✓	✓	✓	4/27/2022
5/11/2022	✓	✓	✓	✓	✓	6/14/2022
5/25/2022	✓	✓	✓	✓	✓	6/14/2022
6/8/2022	✓	✓	✓	✓	✓	6/14/2022
6/22/2022	✓	✓	✓	✓	✓	6/23/2022
7/6/2022	✓	✓	✓	✓	✓	7/21/2022
7/20/2022	✓	✓	✓	✓	✓	7/21/2022
8/3/2022	✓	✓	✓	✓	✓	8/19/2022
8/17/2022	✓	✓	✓	✓	✓	8/19/2022
8/31/2022	✓	✓	✓	✓	✓	10/11/2022
9/14/2022	✓	✓	✓	✓	✓	10/11/2022
9/28/2022	✓	✓	✓	✓	✓	10/12/2022
10/12/2022	✓	✓	✓	✓	✓	12/8/2022
10/26/2022	✓	✓	✓	✓	✓	12/8/2022
11/9/2022	✓	✓	✓	✓	✓	12/9/2022
11/23/2022	✓	✓	✓	✓	✓	12/9/2022
12/7/2022	✓	✓	✓	✓	✓	12/9/2022
12/21/2022	✓	✓	✓	✓	✓	12/22/2023
1/6/2023	✓	✓	✓	✓	✓	1/10/2023
1/18/2023	✓	✓	✓	✓	✓	2/1/2023
2/1/2023	✓	✓	✓	✓	✓	2/16/2023
2/15/2023	✓	✓	✓	✓	✓	2/17/2023
3/1/2023	✓	✓	✓	✓	✓	3/15/2023
3/15/2023	✓	✓	✓	✓	✓	4/7/2023
3/28/2023	✓	✓	✓	✓	✓	4/7/2023
4/14/2023	✓	✓	✓	✓	✓	4/17/2023

Table 8. Bi-weekly discrete algae sample grabs were conducted at each sampling site in Pahokee Marina from September 9, 2021, through April 14, 2023.

Algae samples were placed on ice within 15 minutes of collection to preserve algal community features. Samples were transported back to The Water School at Florida Gulf Coast University within 8 hours. A 50 mL aliquant from each sample site was preserved using 0.5% glutaraldehyde by volume and refrigerated until further analysis occurred.

Algal enumeration was conducted using an Imaging Flow Cytobot (IFCB) with post-processing done with Matlab (as per McLane Research Laboratories, Inc.). Sample IFCB processing dates are noted in Table 8. Five dominant algae genera were identified and successfully classified to enable robust and accurate cell counts via Matlab's machine learning through random tree classification. *Microcystis*, the genus primarily responsible for harmful algal blooms in Pahokee Marina, was further classified into "colony" and "single cell" to enable analysis of potential natural unit disruption caused by nanobubble presence. An "unclassified" class was created and monitored throughout to track possible changes to the dominant genera of the algal community. Algae classes were added and/or modified as needed throughout the project. Algal concentrations for all classes spanning the duration of this project can be seen in Table 9 through Table 11.

	Aphanocapsa'						'Cyclotella'				
	CS1	CS2	NBS1	NBS2	NBS3		CS1	CS2	NBS1	NBS2	NBS3
9/9/2021	13.673	7.283	17.316	24.905	X	9/9/2021	17.354	6.763	27.811	63.646	X
9/29/2021	11.157	314.538	20.008	8.636	8.625	9/29/2021	7.634	21.692	51.270	11.334	9.703
10/12/2021	3.097	5.220	6.704	1.545	7.963	10/12/2021	2.065	5.220	24.238	2.060	4.550
10/27/2021	4.183	5.690	4.757	2.056	2.028	10/27/2021	2.615	5.173	15.856	4.626	0.507
11/10/2021	82.969	21.050	9.200	10.483	5.129	11/10/2021	5.319	30.765	31.389	9.435	6.667
11/23/2021	18.469	25.187	18.476	15.640	19.849	11/23/2021	0.637	0.630	0.210	0.617	0.207
12/8/2021	6.263	3.641	4.630	4.250	12.940	12/8/2021	1.414	1.011	0.403	0.809	3.906
12/21/2021	1.006	0.401	0.602	0.401	0.803	12/21/2021	0.000	0.000	0.000	0.201	0.402
1/5/2022	1.809	0.201	2.415	3.831	1.208	1/5/2022	2.010	1.004	1.207	1.613	4.833
1/19/2022	1.205	6.852	14.633	8.907	7.663	1/19/2022	0.201	1.411	5.487	2.227	0.807
2/1/2022	3.040	2.816	5.944	3.641	4.246	2/1/2022	7.700	0.804	17.832	4.247	4.853
2/15/2022	20.228	15.443	25.017	22.062	18.378	2/15/2022	14.507	12.192	33.286	27.266	28.402
3/2/2022	5.057	25.608	21.922	8.197	6.065	3/2/2022	2.427	94.535	197.744	29.305	6.267
3/16/2022	1.207	5.876	1.004	1.406	2.623	3/16/2022	1.006	7.902	1.004	1.406	7.063
3/30/2022	6.477	19.709	447.620	154.948	266.085	3/30/2022	4.858	5.748	59.021	32.514	55.709
4/13/2022	1.406	0.401	5.853	1.005	2.823	4/13/2022	0.402	0.401	2.422	0.804	2.218
4/27/2022	8.488	12.361	4.226	6.454	4.845	4/27/2022	1.819	4.053	2.012	2.219	3.230
5/11/2022	0.804	1.005	0.604	1.406	0.603	5/11/2022	0.201	0.804	1.207	0.201	1.408
5/25/2022	0.603	0.401	0.201	0.402	0.402	5/25/2022	0.804	0.401	1.406	2.614	1.608
6/8/2022	22.772	206.676	12.501	40.635	168.273	6/8/2022	4.103	366.459	9.222	40.184	154.392
6/22/2022	5.668	3.063	8.281	3.687	7.778	6/22/2022	4.049	9.800	20.910	12.291	11.351
7/6/2022	0.604	2.818	2.822	3.222	3.545	7/6/2022	1.208	3.824	6.048	3.021	2.085
7/20/2022	125.033	57.968	56.540	46.794	47.922	7/20/2022	19.340	95.250	69.948	19.658	17.757
8/3/2022	23.375	79.611	69.233	60.098	56.756	8/3/2022	3.724	6.599	12.178	9.489	6.950
8/17/2022	84.614	173.738	183.660	166.874	164.015	8/17/2022	18.509	31.124	20.844	27.034	43.178
8/30/2022	41.367	41.045	31.497	30.474	30.161	8/30/2022	189.238	22.596	84.205	25.256	9.643
9/14/2022	22.408	118.487	86.107	103.724	81.172	9/14/2022	23.287	20.922	17.859	7.197	6.536
9/26/2022	38.933	28.690	30.506	28.415	41.774	9/26/2022	2.610	3.279	2.662	2.453	4.562
10/12/2022	6.646	0.806	4.265	2.623	2.627	10/12/2022	51.924	6.247	10.156	4.642	8.284
10/26/2022	14.667	13.399	14.431	2.252	8.165	10/26/2022	108.065	51.294	17.523	32.752	65.738
11/9/2022	27.774	4.448	7.680	6.666	10.325	11/9/2022	5.310	8.694	2.829	2.626	3.442
11/23/2022	19.227	71.821	16.175	33.317	28.399	11/23/2022	14.318	284.019	14.128	32.689	33.410
12/7/2022	35.945	62.438	18.986	18.126	10.525	12/7/2022	45.091	137.864	14.033	4.277	2.429
12/21/2022	39.604	59.718	33.719	22.376	28.850	12/21/2022	13.063	11.606	50.157	8.006	86.551
1/6/2023	100.059	222.221	79.908	67.561	57.767	1/6/2023	15.655	75.757	49.155	30.644	15.993
1/18/2023	30.420	9.632	22.516	13.944	13.724	1/18/2023	134.559	10.862	48.677	23.309	13.929
2/1/2023	138.753	50.252	29.607	12.158	18.277	2/1/2023	56.712	170.858	72.548	34.128	108.969
2/15/2023	19.015	11.241	26.102	18.654	25.929	2/15/2023	50.634	13.322	49.729	16.322	64.479
3/1/2023	4.855	213.217	59.689	22.782	134.844	3/1/2023	4.248	239.206	55.300	19.829	117.792
3/15/2023	18.364	12.026	11.378	9.930	10.922	3/15/2023	2.040	4.484	2.235	2.837	1.416
3/28/2023	724.429	281.384	262.073	316.896	65.334	3/28/2023	239.767	84.489	63.980	54.189	20.576
4/14/2023	1364.440	2014.165	375.009	134.746	115.922	4/14/2023	295.056	406.962	81.040	32.430	44.690

Table 9: *Aphanocapsa* (left) and *Cyclotella* (right) cell counts by treatment site for all sampling dates.

	'Dolichospermum'						'Euglena'				
	CS1	**CS2**	**NBS1**	**NBS2**	**NBS3**		**CS1**	**CS2**	**NBS1**	**NBS2**	**NBS3**
9/9/2021	13.147	7.804	14.168	30.439	X	9/9/2021	7.362	2.601	15.742	13.283	X
9/29/2021	7.047	197.942	13.130	7.016	7.008	9/29/2021	10.570	67.788	136.930	19.430	5.930
10/12/2021	13.420	18.791	4.641	11.329	134.232	10/12/2021	0.000	0.522	2.063	0.000	0.569
10/27/2021	30.328	5.173	23.784	11.307	1.014	10/27/2021	0.523	3.621	0.529	1.028	3.549
11/10/2021	2.659	9.715	20.566	13.628	6.667	11/10/2021	0.532	0.000	2.165	0.524	0.513
11/23/2021	53.496	72.413	83.351	43.010	49.209	11/23/2021	1.061	0.840	0.840	0.617	2.274
12/8/2021	5.051	3.641	2.013	7.487	196.049	12/8/2021	0.202	0.202	0.000	0.000	0.488
12/21/2021	2.213	0.803	0.201	0.201	0.402	12/21/2021	0.000	0.000	0.000	0.000	0.000
1/5/2022	0.603	1.004	0.805	1.210	2.215	1/5/2022	0.000	0.000	0.000	0.202	0.000
1/19/2022	1.004	0.403	1.829	0.810	1.008	1/19/2022	0.000	0.000	0.610	0.202	0.000
2/1/2022	0.608	1.006	1.845	0.809	1.415	2/1/2022	0.811	0.000	0.820	0.000	0.000
2/15/2022	2.861	1.219	2.968	2.498	1.044	2/15/2022	1.839	1.219	9.541	4.579	10.442
3/2/2022	5.259	8.536	15.944	6.763	4.448	3/2/2022	0.607	11.737	8.858	1.025	0.404
3/16/2022	1.811	0.203	0.000	0.201	1.211	3/16/2022	0.201	0.000	0.000	0.000	0.000
3/30/2022	0.607	3.901	19.587	12.333	15.882	3/30/2022	3.036	11.497	64.767	26.012	68.659
4/13/2022	0.602	0.602	2.220	1.206	1.815	4/13/2022	0.000	0.000	0.202	0.000	0.000
4/27/2022	1.617	0.811	0.604	0.000	0.000	4/27/2022	2.021	2.229	0.604	4.034	3.432
5/11/2022	0.402	1.005	1.006	1.004	2.012	5/11/2022	0.201	0.000	0.000	0.000	0.201
5/25/2022	0.603	0.000	0.402	0.603	0.000	5/25/2022	0.000	0.000	0.000	0.201	0.000
6/8/2022	3.077	9.726	2.664	6.547	18.697	6/8/2022	2.667	690.540	8.607	78.787	227.197
6/22/2022	1.012	9.800	20.082	9.832	11.351	6/22/2022	1.012	5.513	8.488	5.121	3.574
7/6/2022	0.403	0.201	0.403	1.007	7.925	7/6/2022	0.000	0.000	0.000	0.604	0.000
7/20/2022	50.373	39.100	52.294	28.419	30.379	7/20/2022	3.148	8.866	2.235	1.709	1.284
8/3/2022	9.516	12.772	27.513	9.489	17.143	8/3/2022	0.414	0.639	1.353	0.633	0.463
8/17/2022	34.595	34.608	30.571	156.306	383.802	8/17/2022	1.763	3.949	2.779	2.458	2.998
8/30/2022	18.741	6.841	16.284	11.480	11.900	8/30/2022	6.171	4.768	7.928	2.713	0.205
9/14/2022	131.593	17.079	36.356	20.533	30.993	9/14/2022	0.439	1.067	0.850	0.635	0.633
9/26/2022	66.991	7.377	3.071	3.884	199.989	9/26/2022	0.218	0.410	0.614	0.613	0.240
10/12/2022	1.246	0.403	3.047	1.614	0.808	10/12/2022	1.038	0.403	1.016	0.605	0.606
10/26/2022	2.588	1.884	1.443	1.228	2.303	10/26/2022	1.294	1.675	2.268	0.819	0.419
11/9/2022	2.859	0.809	1.415	1.414	1.620	11/9/2022	0.408	0.202	0.202	0.000	0.000
11/23/2022	1.227	12.305	2.252	3.353	2.506	11/23/2022	0.409	55.247	1.638	1.467	0.626
12/7/2022	3.828	5.925	1.857	2.037	1.417	12/7/2022	16.590	32.130	2.064	2.037	0.405
12/21/2022	4.354	5.909	4.847	3.900	3.633	12/21/2022	0.829	2.321	5.479	3.285	3.633
1/6/2023	9.529	8.417	6.302	6.032	3.819	1/6/2023	120.252	1648.136	347.866	282.790	288.120
1/18/2023	4.885	3.074	5.361	4.370	2.253	1/18/2023	9.992	14.141	12.008	10.198	1.844
2/1/2023	1.569	1.546	1.356	0.640	1.388	2/1/2023	5.380	4.639	0.678	1.066	0.231
2/15/2023	2.211	1.873	2.700	1.484	2.065	2/15/2023	5.307	1.665	3.825	0.636	2.754
3/1/2023	2.225	188.554	29.844	40.080	252.112	3/1/2023	0.607	20.950	4.389	0.211	16.003
3/15/2023	4.897	4.892	2.641	0.811	1.416	3/15/2023	2.040	4.484	1.625	2.027	1.214
3/28/2023	8.120	2.694	3.384	0.499	0.636	3/28/2023	1826.672	41.632	999.997	117.868	10.182
4/14/2023	18.538	17.694	5.297	3.197	3.403	4/14/2023	388.516	359.286	219.815	79.934	83.709

Table 10: *Dolichospermum* (left) and *Euglena* (right) cell counts by treatment site for all sampling dates.

	'Micro Colony'				
	CS1	CS2	NBS1	NBS2	NBS3
9/9/2021	9.466	13.526	16.267	23.798	X
9/29/2021	155.613	11217.607	269.483	125.216	129.373
10/12/2021	13.936	17.747	16.502	10.299	27.301
10/27/2021	9.412	11.898	11.099	5.654	2.535
11/10/2021	35.634	28.067	28.683	14.152	8.206
11/23/2021	5.519	8.396	7.348	4.322	3.308
12/8/2021	1.414	8.900	0.604	4.047	320.319
12/21/2021	5.230	1.004	0.000	0.201	1.004
1/5/2022	0.804	0.602	2.817	4.840	1.208
1/19/2022	1.004	1.814	8.129	5.871	3.025
2/1/2022	12.159	1.810	26.440	10.518	9.705
2/15/2022	2.861	1.626	60.423	34.342	39.471
3/2/2022	6.270	21.980	21.701	6.148	3.639
3/16/2022	1.207	3.242	0.000	0.201	0.605
3/30/2022	4.655	6.775	111.775	42.157	95.781
4/13/2022	0.602	0.401	1.615	0.804	4.033
4/27/2022	1.819	3.647	0.604	1.412	1.009
5/11/2022	1.407	1.206	1.610	0.602	0.805
5/25/2022	0.603	0.000	0.402	0.804	0.402
6/8/2022	6.565	69.471	4.509	60.953	204.534
6/22/2022	4.251	4.900	5.383	9.423	58.439
7/6/2022	3.623	0.201	1.411	0.201	63.815
7/20/2022	44.526	45.693	43.355	32.051	34.230
8/3/2022	22.755	18.519	134.633	12.441	137.835
8/17/2022	56.189	72.933	76.892	108.137	423.081
8/30/2022	68.793	7.463	23.783	22.125	5.540
9/14/2022	68.762	11.315	14.032	12.701	15.391
9/26/2022	60.031	2.869	4.709	3.680	166.138
10/12/2022	4.777	0.806	4.469	1.614	1.414
10/26/2022	16.177	10.049	9.483	2.456	6.281
11/9/2022	3.880	0.607	3.031	2.626	2.227
11/23/2022	1.023	48.718	4.505	10.687	11.067
12/7/2022	11.911	17.091	14.033	2.037	1.417
12/21/2022	0.622	3.587	2.107	2.053	2.564
1/6/2023	6.353	25.252	11.848	7.480	3.819
1/18/2023	16.875	1.640	38.384	13.319	6.965
2/1/2023	14.794	12.885	6.328	3.413	5.784
2/15/2023	4.864	1.873	13.726	5.299	17.210
3/1/2023	6.877	165.217	45.864	43.666	210.661
3/15/2023	11.223	8.153	5.079	5.269	3.439
3/28/2023	85.906	57.550	23.377	29.467	16.546
4/14/2023	167.224	187.261	39.461	15.530	10.662

	'Micro Single'				
	CS1	CS2	NBS1	NBS2	NBS3
9/9/2021	13.147	6.243	12.594	15.496	X
9/29/2021	24.076	3964.260	52.521	14.573	17.789
10/12/2021	7.742	10.439	9.798	3.090	36.971
10/27/2021	7.844	3.104	4.757	2.570	0.507
11/10/2021	19.147	17.811	12.989	6.814	8.206
11/23/2021	11.251	14.483	10.708	8.026	14.887
12/8/2021	4.243	0.405	1.409	0.405	87.893
12/21/2021	0.604	0.201	0.201	0.201	0.402
1/5/2022	0.603	0.000	0.805	1.613	0.604
1/19/2022	0.603	0.605	2.642	1.620	1.613
2/1/2022	4.458	1.207	10.043	2.225	3.033
2/15/2022	9.194	7.721	29.682	20.605	12.322
3/2/2022	6.068	14.938	26.573	5.328	1.415
3/16/2022	0.604	1.013	0.201	0.402	1.211
3/30/2022	0.607	3.285	83.831	27.805	54.488
4/13/2022	0.602	0.000	1.615	1.407	1.412
4/27/2022	4.648	3.445	2.817	1.210	3.028
5/11/2022	1.608	0.201	1.409	0.803	0.805
5/25/2022	0.603	1.004	0.201	0.201	0.201
6/8/2022	6.975	282.746	6.148	45.827	418.133
6/22/2022	3.037	6.533	12.629	7.169	21.862
7/6/2022	0.201	0.201	0.605	0.000	10.427
7/20/2022	18.890	13.412	13.409	7.692	7.916
8/3/2022	2.482	6.599	12.854	4.217	7.876
8/17/2022	4.627	15.098	21.539	22.610	60.569
8/30/2022	27.426	3.731	14.356	5.636	3.488
9/14/2022	5.492	3.629	4.252	4.869	3.584
9/26/2022	8.918	1.025	2.047	1.227	30.731
10/12/2022	8.308	1.411	2.437	1.816	1.818
10/26/2022	15.962	7.118	5.154	3.480	7.746
11/9/2022	19.401	4.650	6.265	5.858	7.895
11/23/2022	6.955	76.341	7.576	14.039	12.738
12/7/2022	11.060	33.497	9.287	5.091	3.036
12/21/2022	12.856	28.276	21.917	8.827	26.072
1/6/2023	29.269	89.646	26.972	16.649	16.710
1/18/2023	25.979	9.017	21.658	9.781	6.760
2/1/2023	26.002	23.451	11.526	7.679	14.344
2/15/2023	17.689	6.661	20.477	9.539	22.258
3/1/2023	4.855	141.880	30.941	22.361	184.689
3/15/2023	27.342	21.606	22.553	12.970	8.697
3/28/2023	445.769	93.060	160.566	89.150	17.818
4/14/2023	446.060	736.266	171.349	61.207	60.116

Table 11: *Microcystis* colony (left) and *Microcystis* single cell (right) counts by treatment site for all sampling dates.

All Pahokee Marina samples were analyzed for total cyanotoxin concentration. Following algal community examination and identification, the following four cyanotoxins were determined most likely to be significant and monitored throughout the project; microcystins, cylindrospermopsin, anatoxin-a, and saxitoxin. All cyanotoxin samples went through three cycles of complete freeze/thaw to lyse algal cells and release intracellular toxins into solution. Samples were then filtered using glass fiber filters. Approximately 2-mL of filtered sample were transferred into a 4-mL amber glass vial and analyzed using Enzyme-Linked Immunosorbent Assay (ELISA) via a CAAS Cube Auto-Analyzer. If any sample's cyanotoxin concentrations were above the maximum limit for detection, a dilution was performed with the appropriate diluent and the analysis was run again. Resulting values were then multiplied by the dilution factor to achieve accurate cyanotoxin concentrations. A summary of cyanotoxin concentration results is shown below.

	Microcystins (ppb)					Cylindrospermopsin (ppb)					Anatoxin-a (ppb)					Saxitoxin (ppb)				
	CS1	CS2	NBS1	NBS2	NBS3	CS1	CS2	NBS1	NBS2	NBS3	CS1	CS2	NBS1	NBS2	NBS3	CS1	CS2	NBS1	NBS2	NBS3
9/9/2021	0.266	0.299	<0.15	0.26	N/A	<0.05	<0.05	<0.05	<0.05	N/A	<0.15	<0.15	<0.15	<0.15	<0.15	<0.02	<0.02	<0.02	<0.02	N/A
9/29/2021	104.04	3528	81.12	34.04	13.08	<0.05	<0.05	<0.05	<0.05	<0.05	<0.15	<0.15	<0.15	<0.15	<0.15	<0.02	<0.02	<0.02	<0.02	<0.02
10/12/2021	<0.15	<0.15	0.206	0.262	<0.15	<0.05	<0.05	<0.05	<0.05	0.062	<0.15	<0.15	<0.15	<0.15	<0.15	<0.02	<0.02	<0.02	<0.02	<0.02
10/27/2021	<0.15	<0.15	<0.15	<0.15	0.577	<0.05	<0.05	<0.05	<0.05	<0.05	<0.15	<0.15	<0.15	<0.15	<0.15	<0.02	<0.02	<0.02	<0.02	<0.02
11/10/2021	<0.15	<0.15	0.255	<0.15	<0.15	<0.05	<0.05	<0.05	<0.05	<0.05	<0.15	<0.15	<0.15	<0.15	<0.15	<0.02	<0.02	<0.02	<0.02	<0.02
11/23/2021	1.105	<0.15	0.646	0.292	0.492	<0.05	<0.05	<0.05	<0.05	<0.05	<0.15	<0.15	<0.15	<0.15	<0.15	<0.02	<0.02	<0.02	<0.02	<0.02
12/8/2021	<0.15	0.18	<0.15	0.329	9.36	<0.05	<0.05	0.068	<0.05	<0.05	<0.15	<0.15	<0.15	<0.15	<0.15	<0.02	<0.02	<0.02	<0.02	<0.02
12/21/2021	<0.15	<0.15	<0.15	<0.15	<0.15	<0.05	<0.05	0.052	<0.05	<0.05	<0.15	<0.15	<0.15	<0.15	<0.15	<0.02	<0.02	<0.02	<0.02	<0.02
1/5/2022	<0.15	<0.15	0.181	<0.15	<0.15	<0.05	<0.05	<0.05	0.062	<0.05	<0.15	<0.15	<0.15	<0.15	<0.15	<0.02	<0.02	<0.02	<0.02	<0.02
1/19/2022	<0.15	<0.15	<0.15	<0.15	<0.15	<0.05	<0.05	<0.05	<0.05	<0.05	<0.15	<0.15	<0.15	<0.15	<0.15	<0.02	<0.02	<0.02	<0.02	<0.02
2/1/2021	<0.15	<0.15	<0.15	<0.15	<0.15	<0.05	<0.05	<0.05	<0.05	<0.05	<0.15	<0.15	<0.15	<0.15	<0.15	<0.02	<0.02	<0.02	<0.02	<0.02
2/15/2021	<0.15	<0.15	<0.15	<0.15	<0.15	<0.05	<0.05	<0.05	<0.05	<0.05	<0.15	<0.15	<0.15	<0.15	<0.15	<0.02	<0.02	<0.02	<0.02	<0.02
3/2/2022	<0.15	<0.15	<0.15	<0.15	<0.15	<0.05	<0.05	<0.05	<0.05	<0.05	<0.15	<0.15	<0.15	<0.15	<0.15	<0.02	<0.02	<0.02	<0.02	<0.02
3/16/2022	<0.15	<0.15	<0.15	<0.15	<0.15	<0.05	<0.05	<0.05	<0.05	<0.05	<0.15	<0.15	<0.15	<0.15	<0.15	<0.02	<0.02	<0.02	<0.02	<0.02
3/30/2022	<0.15	<0.15	<0.15	<0.15	<0.15	<0.05	<0.05	<0.05	<0.05	<0.05	<0.15	<0.15	<0.15	<0.15	<0.15	<0.02	<0.02	<0.02	<0.02	<0.02
4/13/2022	<0.15	<0.15	<0.15	<0.15	0.162	<0.05	<0.05	<0.05	<0.05	<0.05	<0.15	<0.15	<0.15	<0.15	<0.15	<0.02	<0.02	<0.02	<0.02	<0.02
4/27/2022	<0.15	<0.15	<0.15	<0.15	<0.15	<0.05	<0.05	<0.05	<0.05	<0.05	<0.15	<0.15	<0.15	<0.15	<0.15	<0.02	<0.02	<0.02	<0.02	<0.02
5/11/2022	<0.15	<0.15	<0.15	<0.15	<0.15	<0.05	<0.05	<0.05	<0.05	<0.05	<0.15	<0.15	<0.15	<0.15	<0.15	<0.02	<0.02	<0.02	<0.02	<0.02
5/25/2022	<0.15	<0.15	<0.15	<0.15	<0.15	<0.05	<0.05	<0.05	<0.05	<0.05	<0.15	<0.15	0.16	<0.15	<0.15	<0.02	<0.02	<0.02	<0.02	<0.02
6/8/2022	<0.15	0.9	<0.15	2.443	14.28	<0.05	<0.05	<0.05	<0.05	<0.05	0.17	0.19	<0.15	<0.15	0.19	<0.02	<0.02	<0.02	<0.02	<0.02
6/22/2022	0.161	<0.15	<0.15	2.916	1.69	<0.05	<0.05	<0.05	<0.05	<0.05	0.21	<0.15	0.18	0.2	0.17	<0.02	<0.02	<0.02	<0.02	<0.02
7/6/2022	<0.15	<0.15	<0.15	<0.15	1.093	<0.05	<0.05	<0.05	<0.05	<0.05	<0.15	<0.15	0.19	0.17	<0.15	<0.02	<0.02	<0.02	<0.02	<0.02
7/20/2022	<0.15	<0.15	<0.15	<0.15	<0.15	<0.05	<0.05	<0.05	<0.05	<0.05	<0.15	0.16	0.16	<0.15	<0.15	<0.02	<0.02	<0.02	<0.02	<0.02
8/3/2022	0.358	0.18	0.338	<0.15	1.858	<0.05	<0.05	<0.05	<0.05	<0.05	<0.15	<0.15	<0.15	<0.15	<0.15	<0.02	<0.02	<0.02	<0.02	<0.02
8/17/2022	<0.15	<0.15	<0.15	0.366	0.621	<0.05	<0.05	<0.05	<0.05	<0.05	<0.15	<0.15	<0.15	0.15	<0.15	<0.02	<0.02	<0.02	<0.02	<0.02
8/31/2022	0.18	<0.15	0.161	<0.15	0.44	<0.05	<0.05	<0.05	<0.05	<0.05	<0.15	<0.15	<0.15	<0.15	<0.15	<0.02	<0.02	<0.02	<0.02	<0.02
9/14/2022	0.25	0.155	<0.15	<0.15	<0.15	<0.05	0.051	<0.05	<0.05	<0.05	0.18	0.23	<0.15	<0.15	<0.15	<0.02	<0.02	<0.02	<0.02	<0.02
9/26/2022	<0.15	<0.15	<0.15	<0.15	<0.15	<0.05	<0.05	<0.05	<0.05	<0.05	<0.15	<0.15	<0.15	<0.15	<0.15	<0.02	<0.02	<0.02	<0.02	<0.02
10/12/2022	<0.15	<0.15	<0.15	<0.15	<0.15	<0.05	<0.05	<0.05	<0.05	<0.05	<0.15	<0.15	<0.15	<0.15	<0.15	<0.02	<0.02	<0.02	<0.02	<0.02
10/26/2022	<0.15	<0.15	<0.15	<0.15	<0.15	<0.05	<0.05	<0.05	<0.05	<0.05	<0.15	<0.15	<0.15	<0.15	<0.15	<0.02	<0.02	<0.02	<0.02	<0.02
11/9/2022	<0.15	<0.15	<0.15	<0.15	<0.15	<0.05	<0.05	<0.05	<0.05	<0.05	<0.15	<0.15	<0.15	<0.15	<0.15	<0.02	<0.02	<0.02	<0.02	<0.02
11/23/2022	<0.15	<0.15	<0.15	<0.15	<0.15	<0.05	<0.05	<0.05	<0.05	<0.05	<0.15	<0.15	<0.15	<0.15	<0.15	<0.02	<0.02	<0.02	<0.02	<0.02
12/7/2022	<0.15	<0.15	0.217	<0.15	<0.15	<0.05	<0.05	<0.05	<0.05	<0.05	<0.15	<0.15	<0.15	<0.15	<0.15	<0.02	<0.02	<0.02	<0.02	<0.02
12/21/2022	<0.15	<0.15	<0.15	<0.15	<0.15	<0.05	<0.05	<0.05	<0.05	<0.05	<0.15	<0.15	<0.15	<0.15	<0.15	<0.02	<0.02	<0.02	<0.02	<0.02
1/6/2023	<0.15	<0.15	<0.15	<0.15	<0.15	<0.05	<0.05	<0.05	<0.05	<0.05	<0.15	<0.15	<0.15	<0.15	<0.15	<0.02	<0.02	<0.02	<0.02	<0.02
1/18/2023	0.33	<0.15	<0.15	<0.15	0.41	<0.05	<0.05	<0.05	<0.05	<0.05	<0.15	<0.15	<0.15	<0.15	<0.15	<0.02	<0.02	<0.02	<0.02	<0.02
2/1/2023	<0.15	<0.15	<0.15	<0.15	<0.15	<0.05	<0.05	<0.05	<0.05	<0.05	<0.15	<0.15	<0.15	<0.15	<0.15	<0.02	<0.02	<0.02	<0.02	<0.02
2/14/2023	<0.15	<0.15	0.182	<0.15	<0.15	<0.05	<0.05	<0.05	<0.05	<0.05	<0.15	<0.15	<0.15	<0.15	<0.15	<0.02	<0.02	<0.02	<0.02	<0.02
3/1/2023	0.328	>5.0	1.867	>5.0	>5.0	<0.05	<0.05	<0.05	<0.05	<0.05	<0.15	<0.15	<0.15	<0.15	<0.15	<0.02	<0.02	<0.02	<0.02	<0.02
3/15/2023	<0.15	<0.15	<0.15	<0.15	<0.15	<0.05	<0.05	<0.05	<0.05	<0.05	<0.15	<0.15	<0.15	<0.15	<0.15	<0.02	<0.02	0.03	0.03	<0.02
3/28/2023	<0.15	<0.15	0.183	<0.15	<0.15	<0.05	<0.05	<0.05	<0.05	<0.05	<0.15	<0.15	<0.15	<0.15	<0.15	<0.02	<0.02	<0.02	<0.02	<0.02
4/14/2023	<0.15	0.166	<0.15	0.185	<0.15	<0.05	<0.05	<0.05	<0.05	<0.05	<0.15	<0.15	<0.15	<0.15	<0.15	<0.02	<0.02	<0.02	<0.02	<0.02

Table 12: Summary of cyanotoxin presence in Pahokee Marina

Additional cyanotoxin analysis was conducted as a QA/QC reference for ELISA data provided in Table 12. Sample volume allowed for QA/QC quantification of 3 cyanotoxins for select sampling events performed via LC-MS/MS by Lumigen Instrument Center at Wayne State University. Results (Table 13) are primarily consistent with ELISA results, with slightly more clarity on low toxin levels due to LC-MS/MS's lower minimum detection limit.

Sampling Date	Sampling Location	LC-MS/MS Microcystins (ppb)	ELISA Microcystins (ppb)	LC-MS/MS Anatoxins (ppb)	ELISA Anatoxin (ppb)	LC-MS/MS Cylindrospermopsins (ppb)	ELISA Cylindrospermopsin (ppb)
11/23/2022	CS1	0.01	<0.15	ND	<0.15	ND	<0.15
11/23/2022	CS2	0.01	<0.15	ND	<0.15	ND	<0.15
11/23/2022	NBS1	ND	<0.15	ND	<0.15	ND	<0.15
11/23/2022	NBS2	0.01	<0.15	ND	<0.15	ND	<0.15
12/21/2022	CS1	0.01	<0.15	ND	<0.15	ND	<0.15
12/21/2022	CS2	ND	<0.15	ND	<0.15	ND	<0.15
12/21/2022	NBS1	0.01	<0.15	ND	<0.15	ND	<0.15
12/21/2022	NBS2	0.01	<0.15	ND	<0.15	ND	<0.15
2/28/2023	CS1	0.14	0.33	ND	<0.15	ND	<0.15
2/28/2023	CS2	8.05	>5.0	ND	<0.15	ND	<0.15
2/28/2023	NBS1	0.27	1.87	ND	<0.15	ND	<0.15
2/28/2023	NBS2	2.97	>5.0	ND	<0.15	ND	<0.15
3/28/2023	CS1	0.13	<0.15	ND	<0.15	ND	<0.15
3/28/2023	CS2	1.00	<0.15	ND	<0.15	ND	<0.15
3/28/2023	NBS1	ND	0.18	ND	<0.15	ND	<0.15
3/28/2023	NBS2	0.01	<0.15	ND	<0.15	ND	<0.15
4/14/2023	CS1	0.59	<0.15	ND	<0.15	ND	<0.15
4/14/2023	CS2	0.01	0.17	ND	<0.15	ND	<0.15
4/14/2023	NBS1	0.03	<0.15	ND	<0.15	ND	<0.15
4/14/2023	NBS2	0.01	0.19	ND	<0.15	ND	<0.15

Table 13: ELISA and LC-MS/MS cyanotoxin result comparison

7.2.4 Observational Data

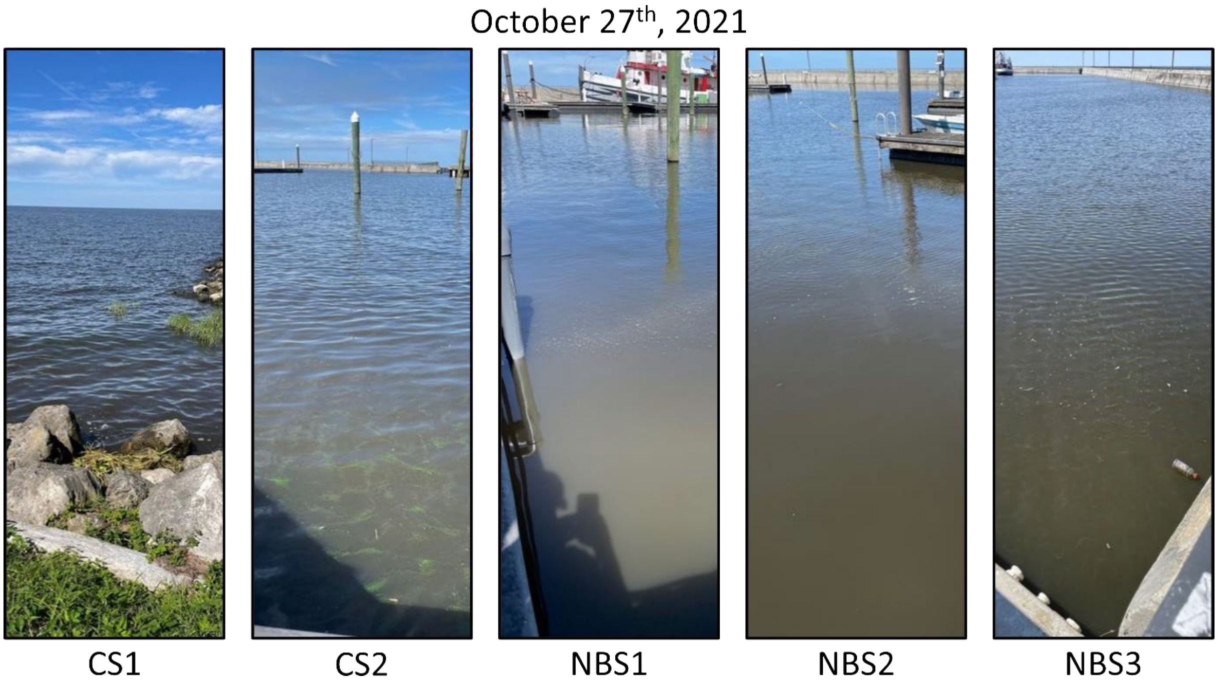

Figure 41: October 27th, 2021 side-by-side photos of control and nanobubble treatment locations.

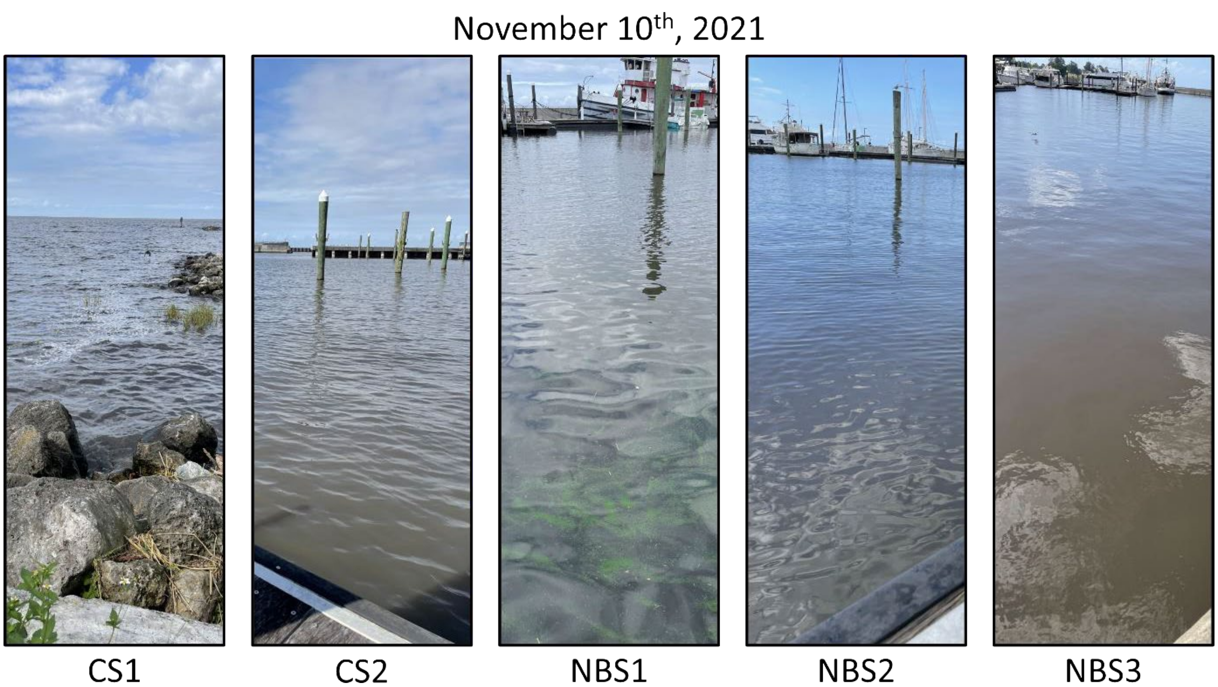

Figure 42: November 10th, 2021 side-by-side photos of control and nanobubble treatment locations.

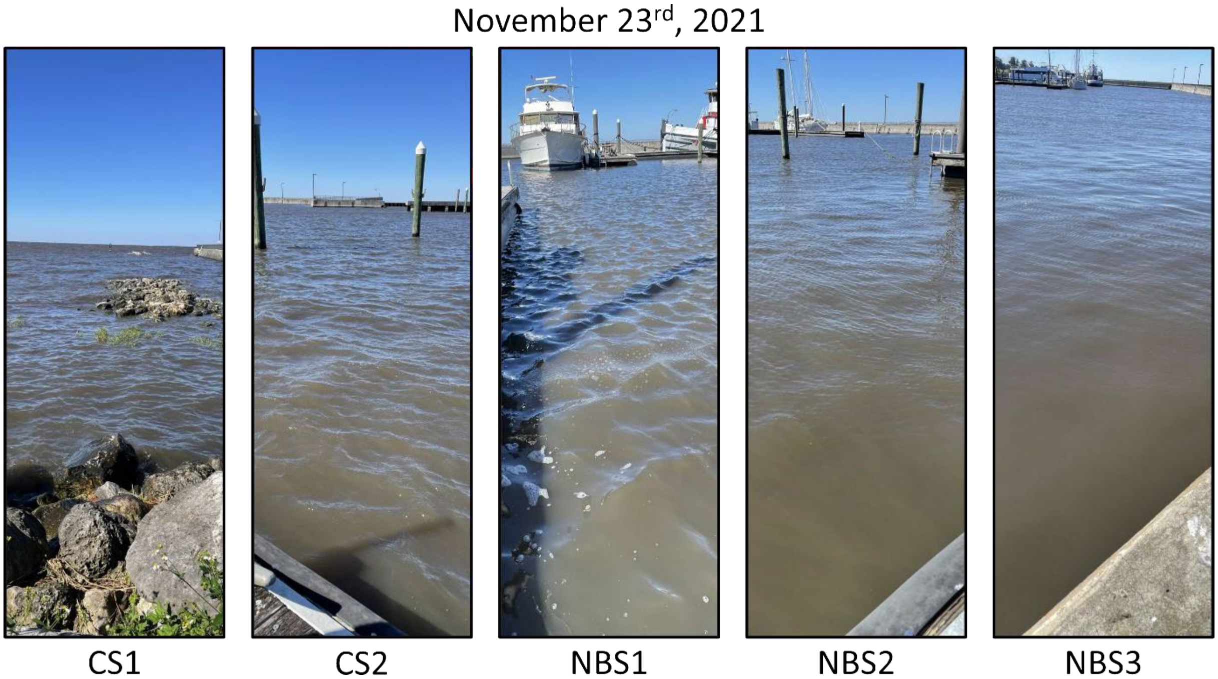

Figure 43: November 23rd, 2021 side-by-side photos of control and nanobubble treatment locations.

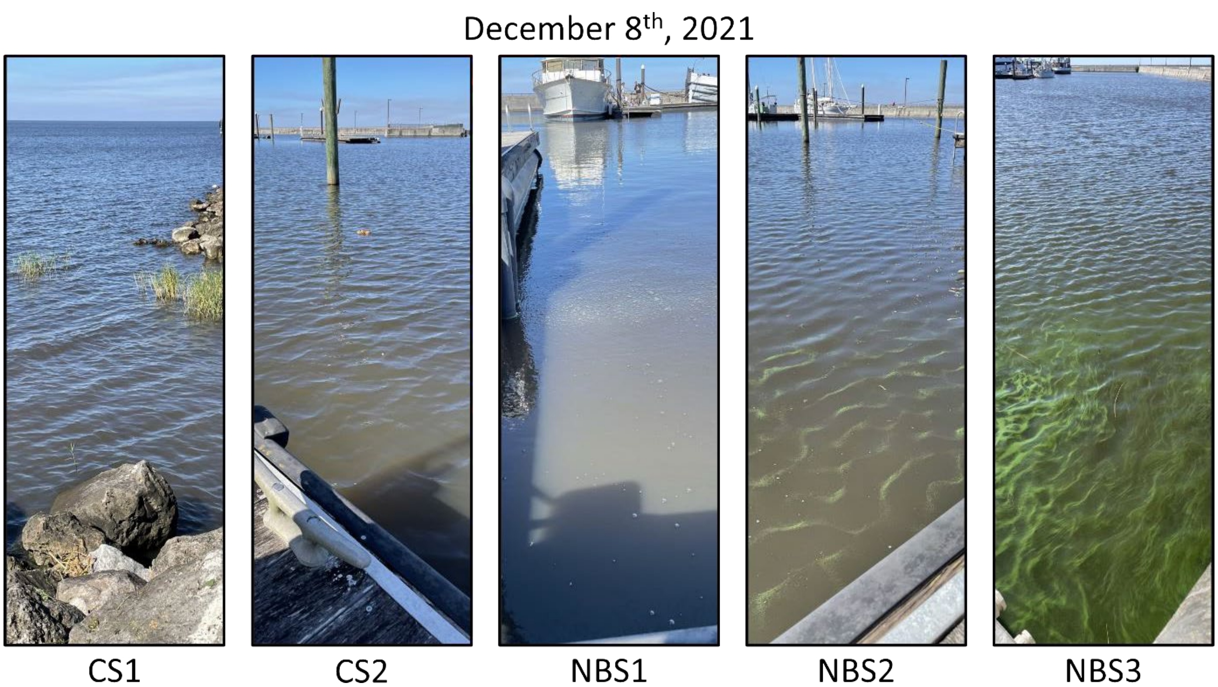

Figure 44: December 8th, 2021 side-by-side photos of control and nanobubble treatment locations.

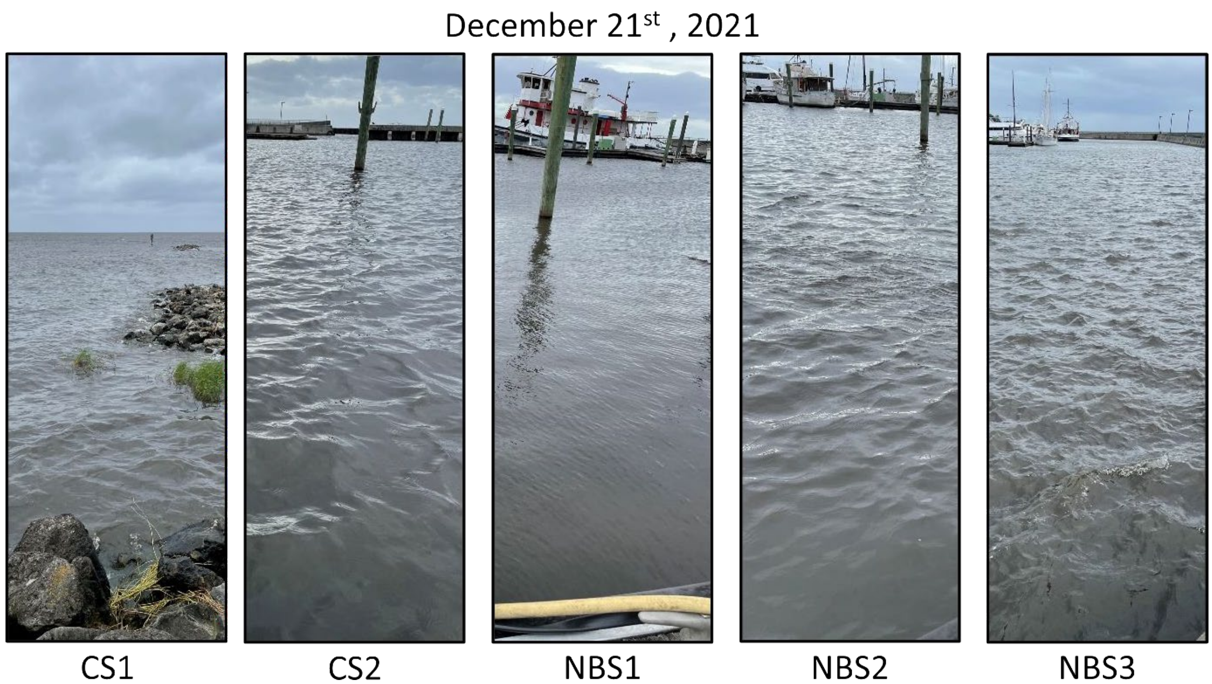

Figure 45: December 21st, 2021 photos of control and nanobubble treatment locations.

Figure 46: January 5th, 2022 photos of control and nanobubble treatment locations.

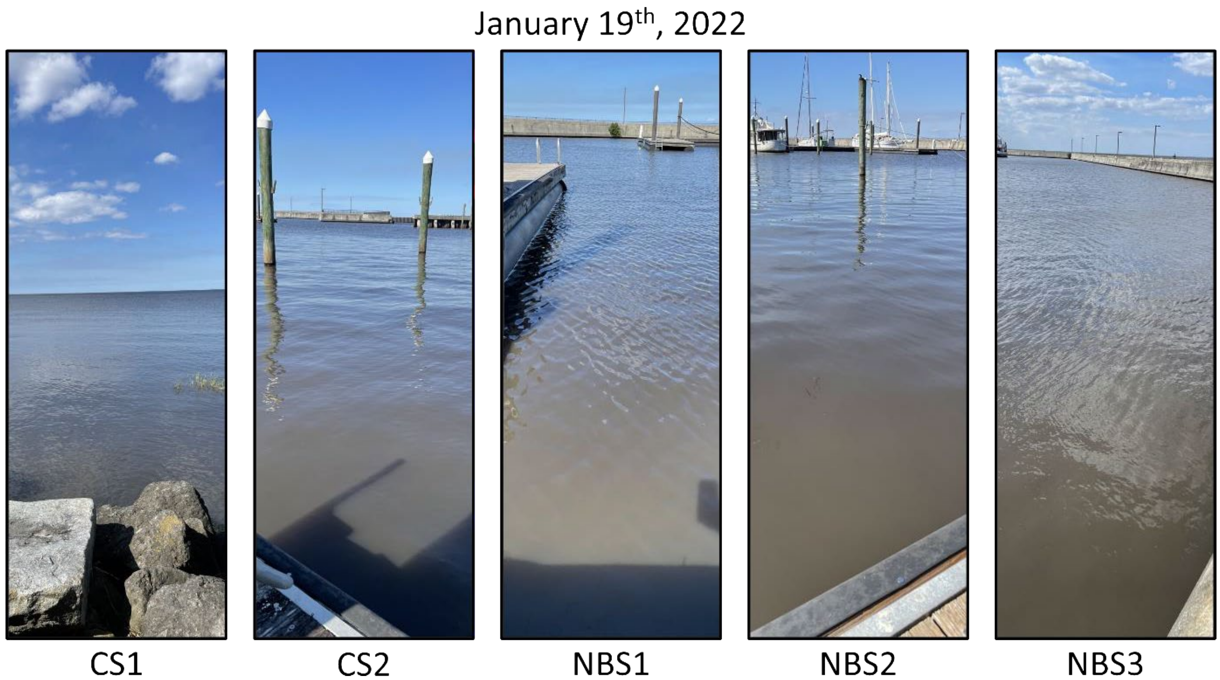

Figure 47: January 19th, 2022 photos of control and nanobubble treatment locations.

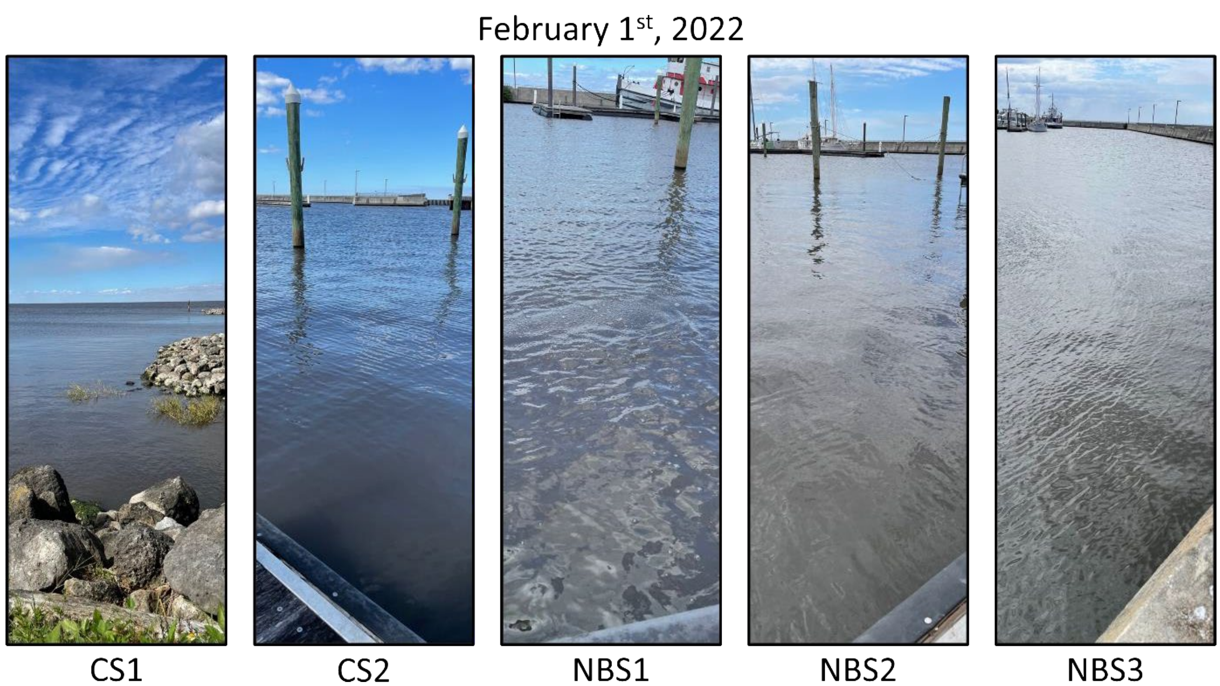

Figure 48: February 1st, 2022 photos of control and nanobubble treatment locations.

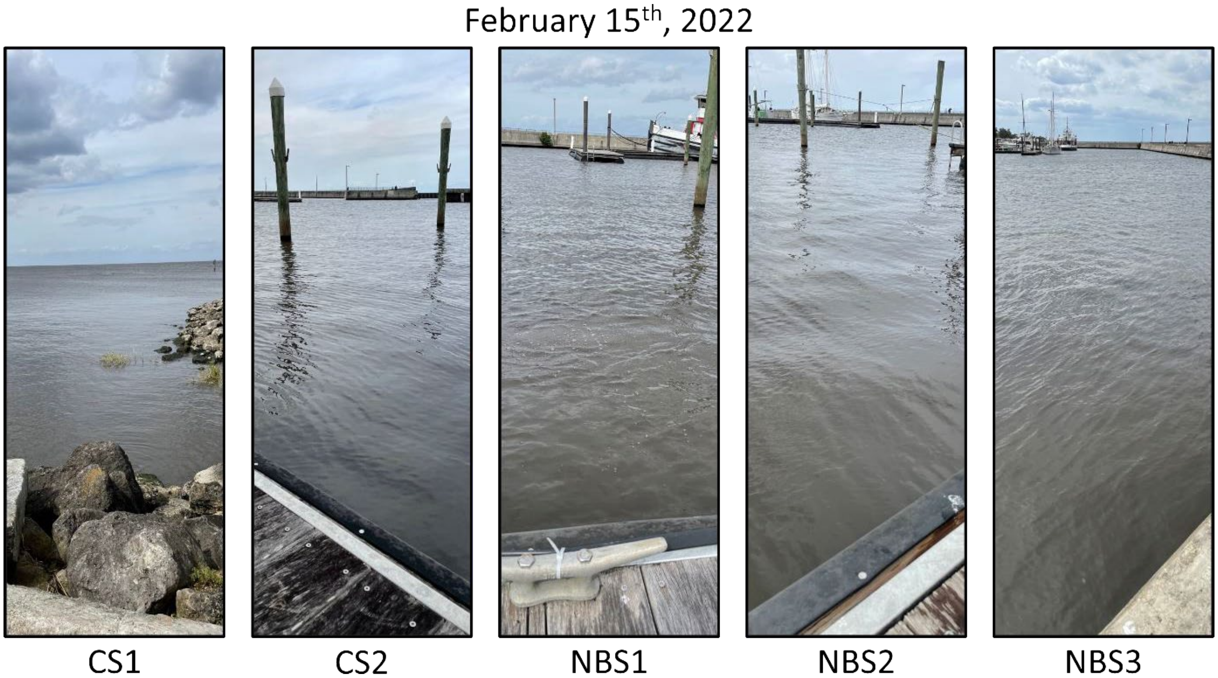

Figure 49: February 15th, 2022 photos of control and nanobubble treatment locations.

Figure 50: March 2nd, 2022 photos of control and nanobubble treatment locations.

Figure 51: March 16th, 2021 photos of control and nanobubble treatment locations.

Figure 52: March 30th, 2022 photos of control and nanobubble treatment locations.

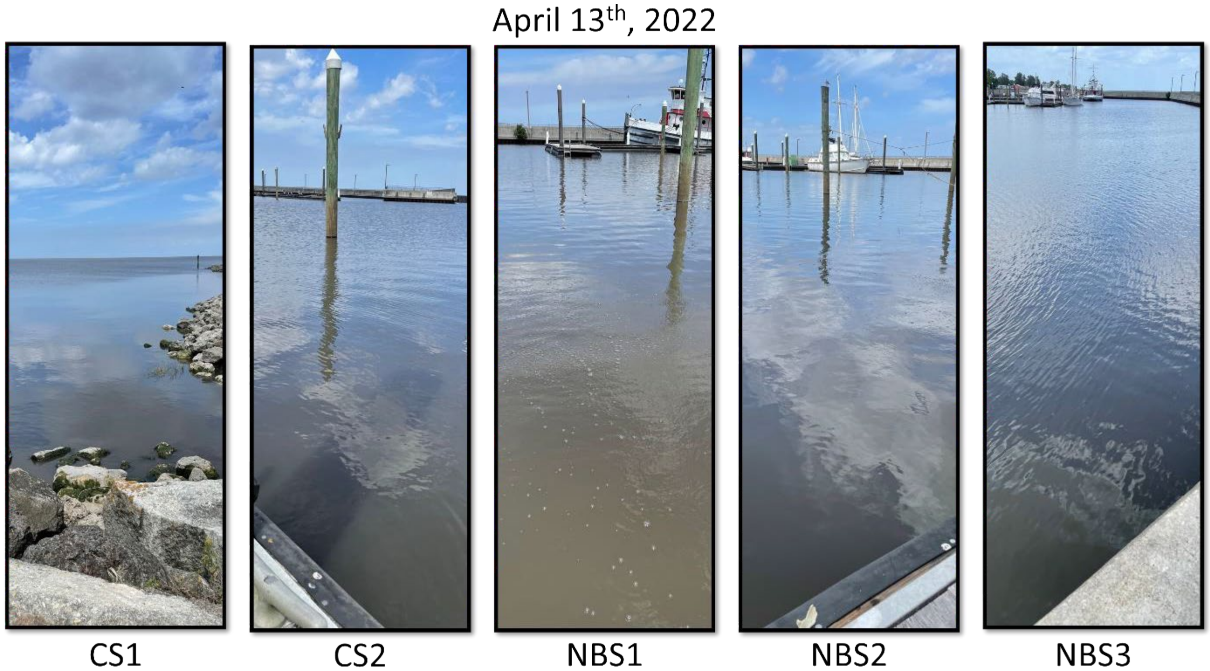

Figure 53: April 13th, 2022 photos of control and nanobubble treatment locations.

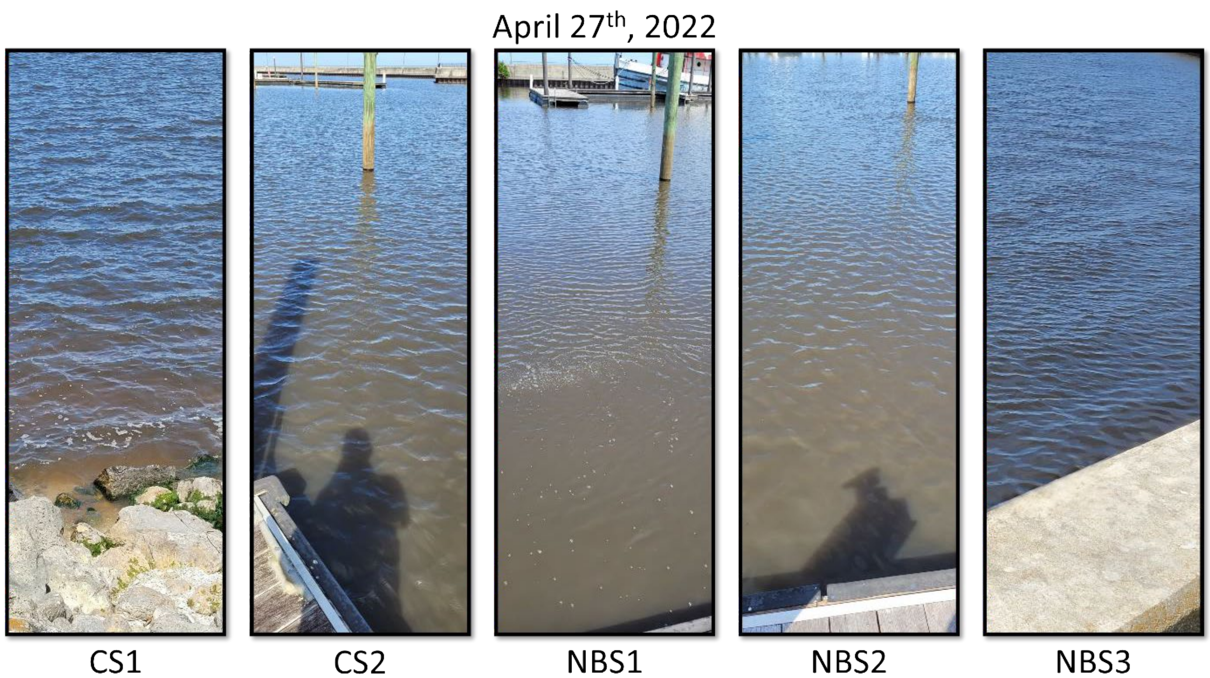

Figure 54: April 27th, 2022 photos of control and nanobubble treatment locations.

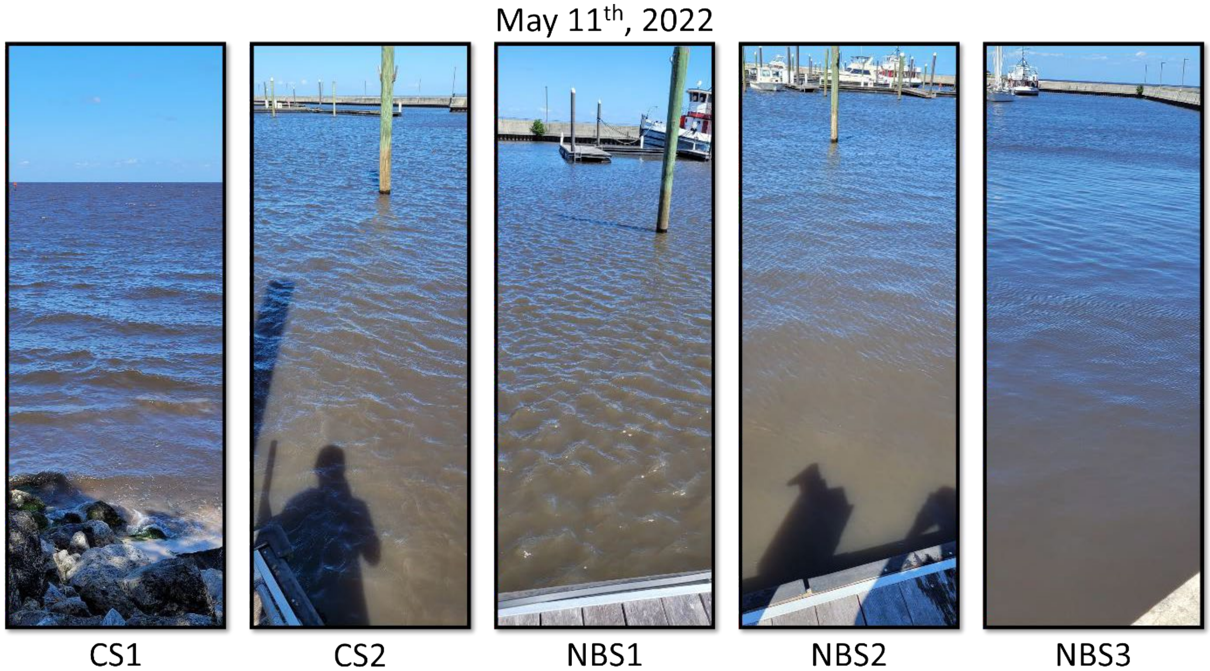

Figure 55: May 11th, 2022 photos of control and nanobubble treatment locations.

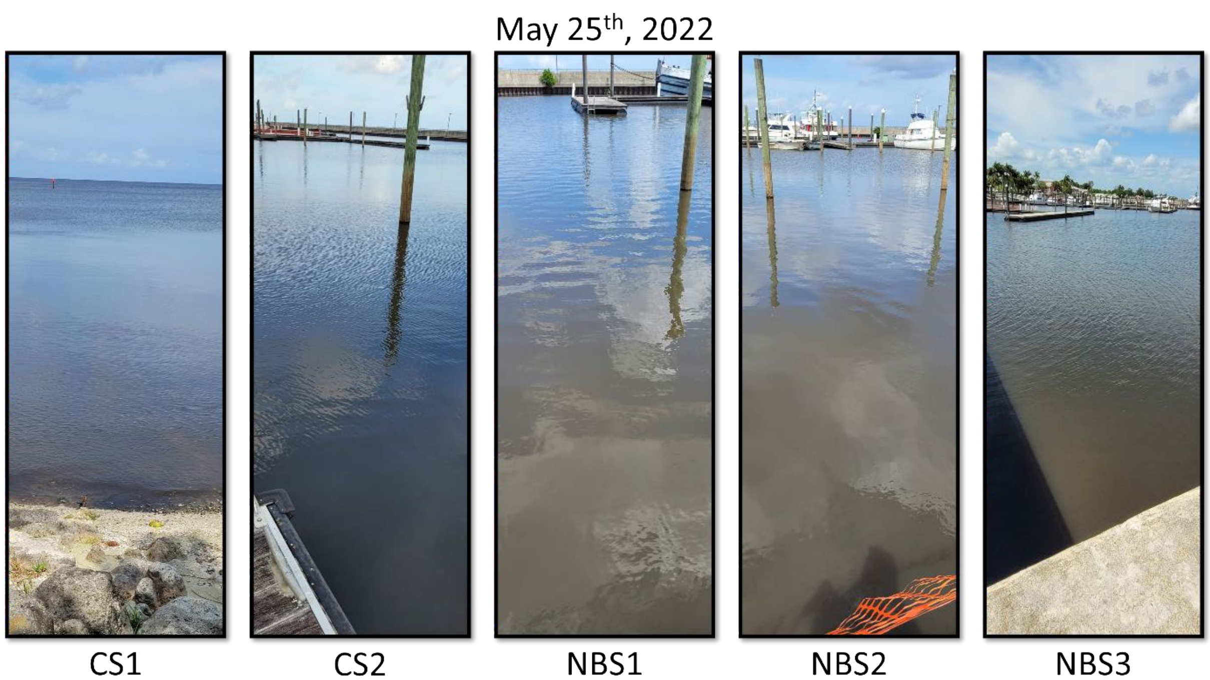

Figure 56: May 25th, 2022 photos of control and nanobubble treatment locations.

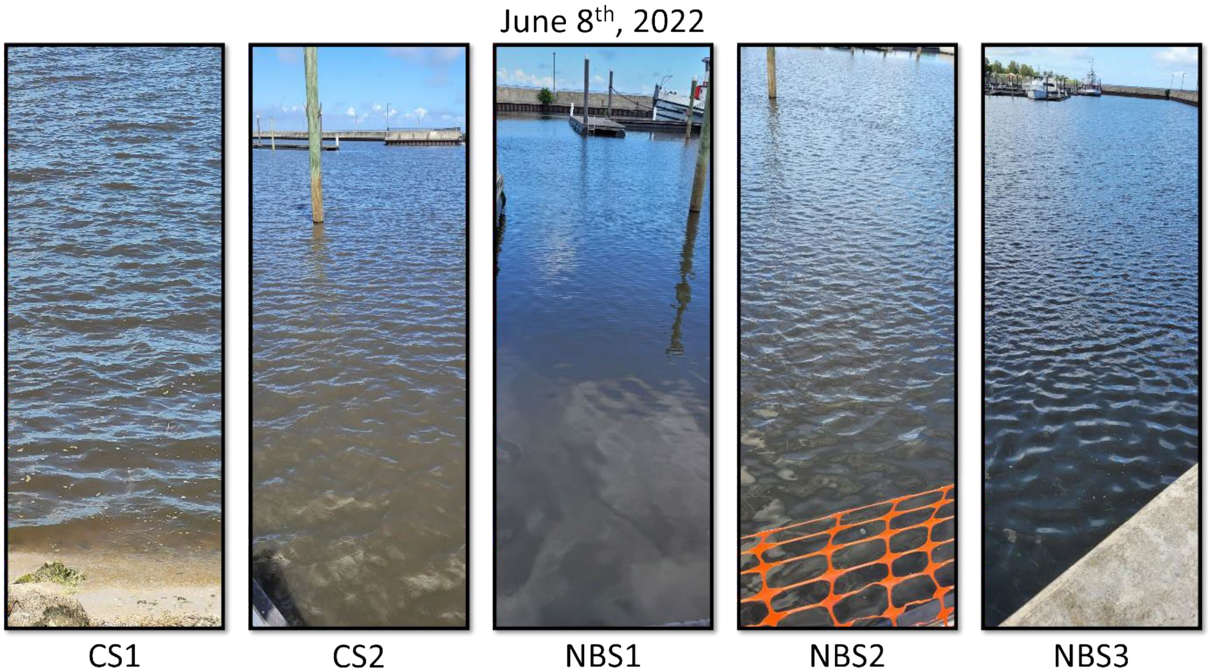

Figure 57: June 8th, 2022 photos of control and nanobubble treatment locations.

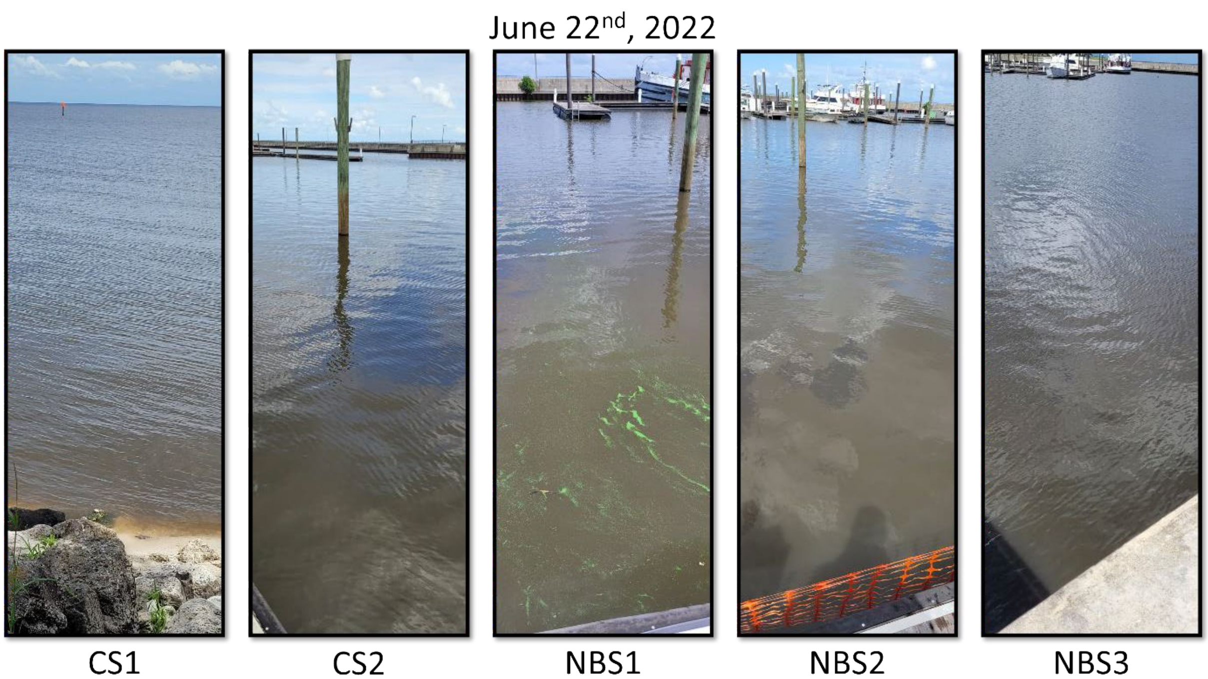

Figure 58: June 22nd, 2022 photos of control and nanobubble treatment locations.

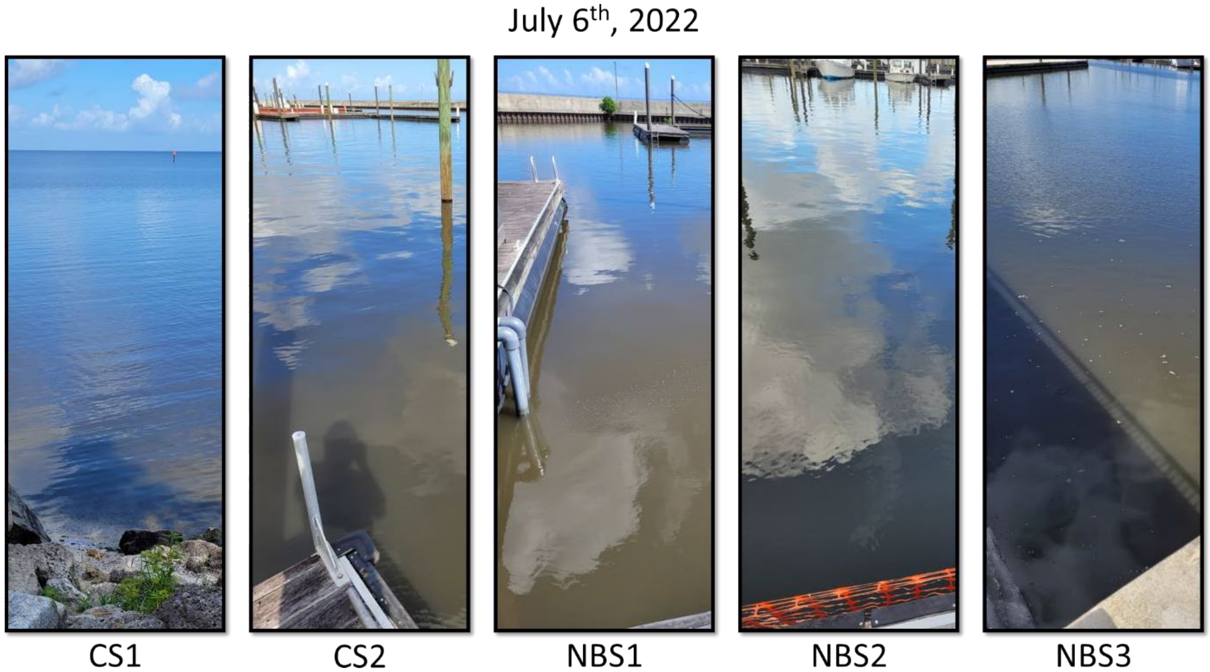

Figure 59: July 6th, 2022 photos of control and nanobubble treatment locations.

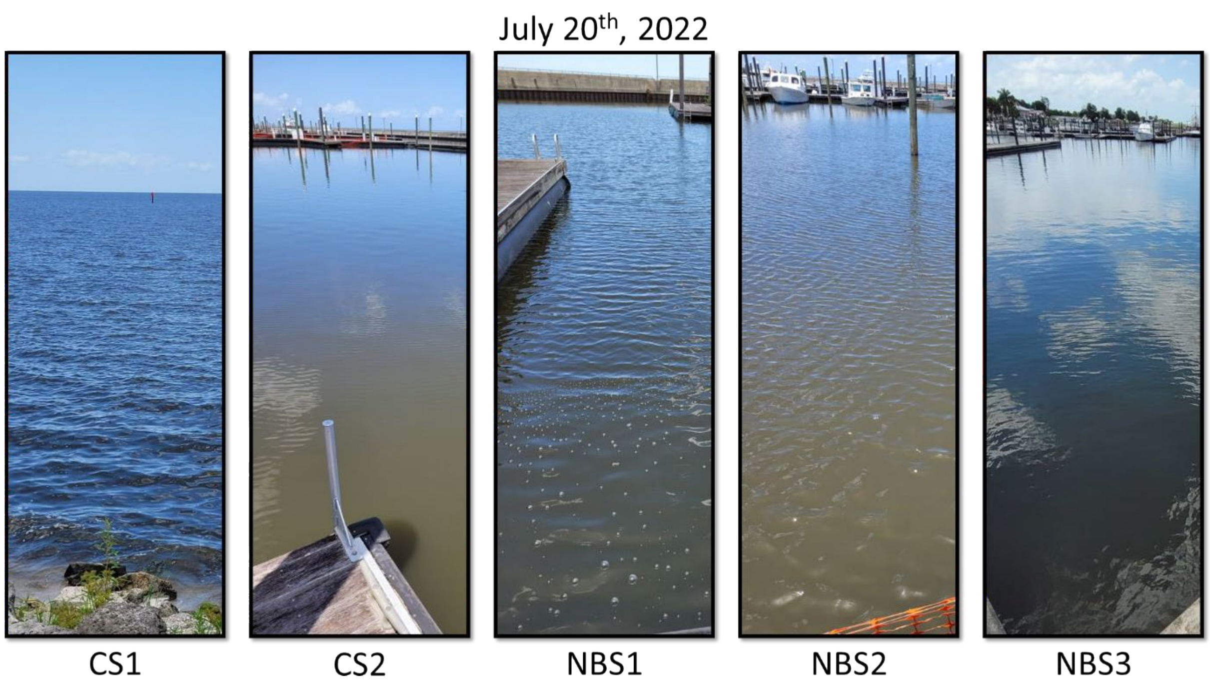

Figure 60: July 20th, 2022 photos of control and nanobubble treatment locations.

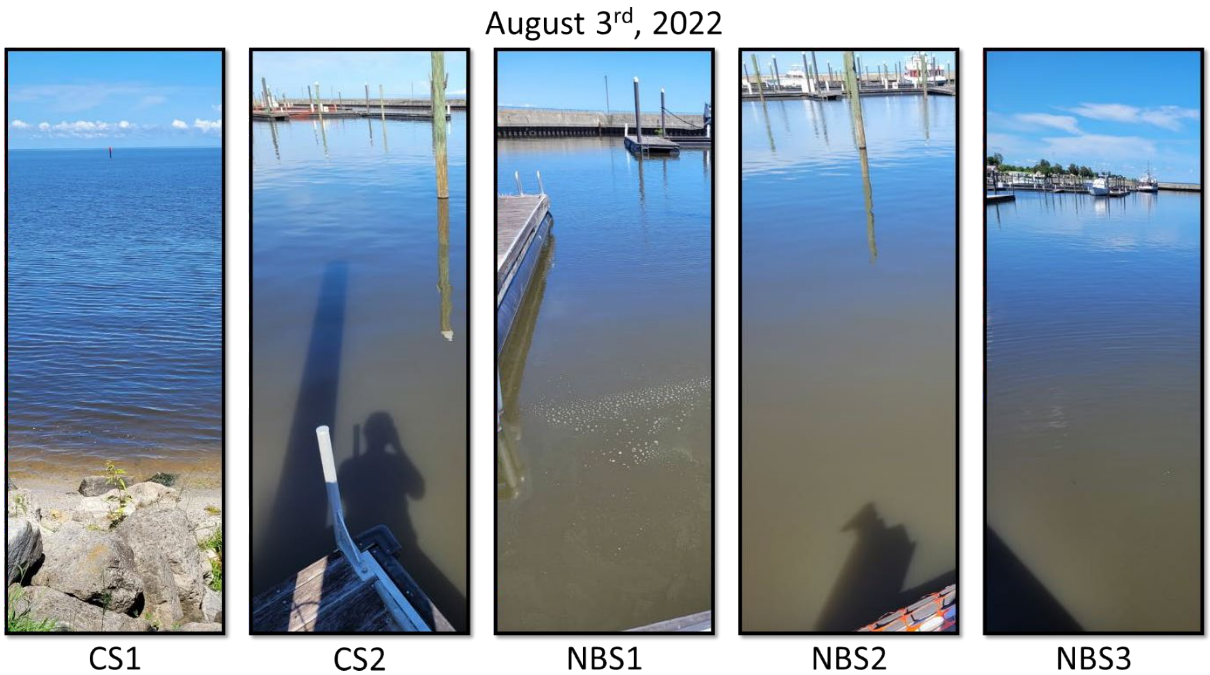

Figure 61: August 3rd, 2022 photos of control and nanobubble treatment locations.

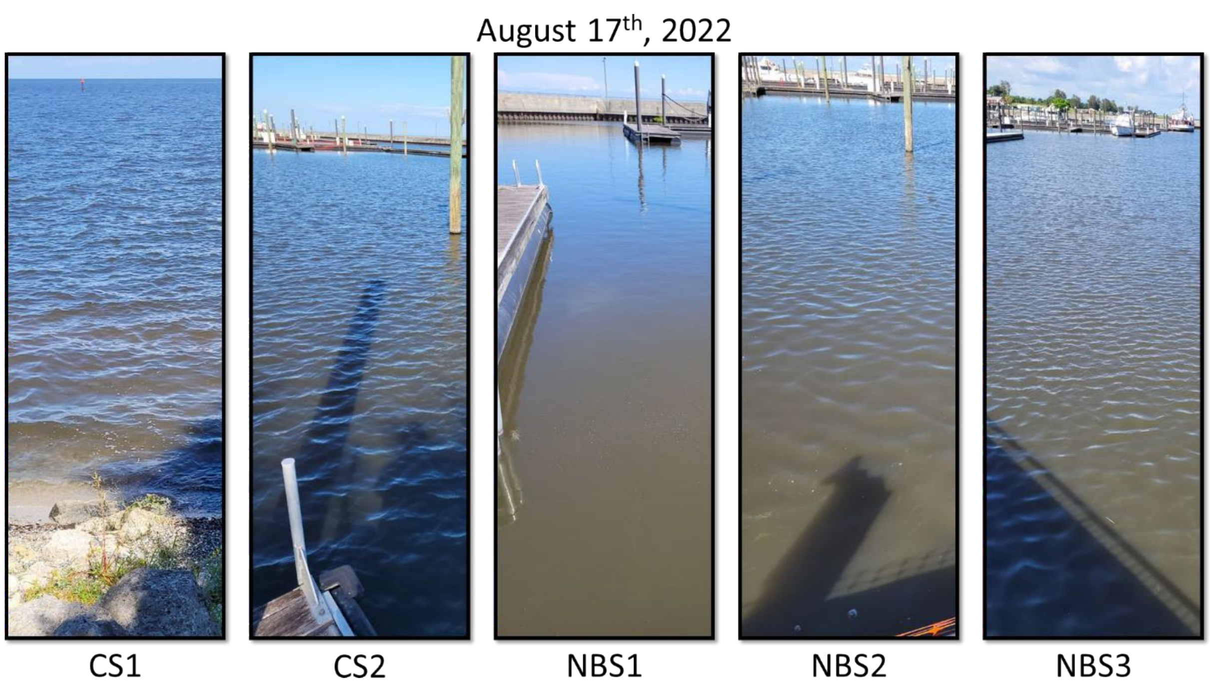

Figure 62: August 17th, 2022 photos of control and nanobubble treatment locations.

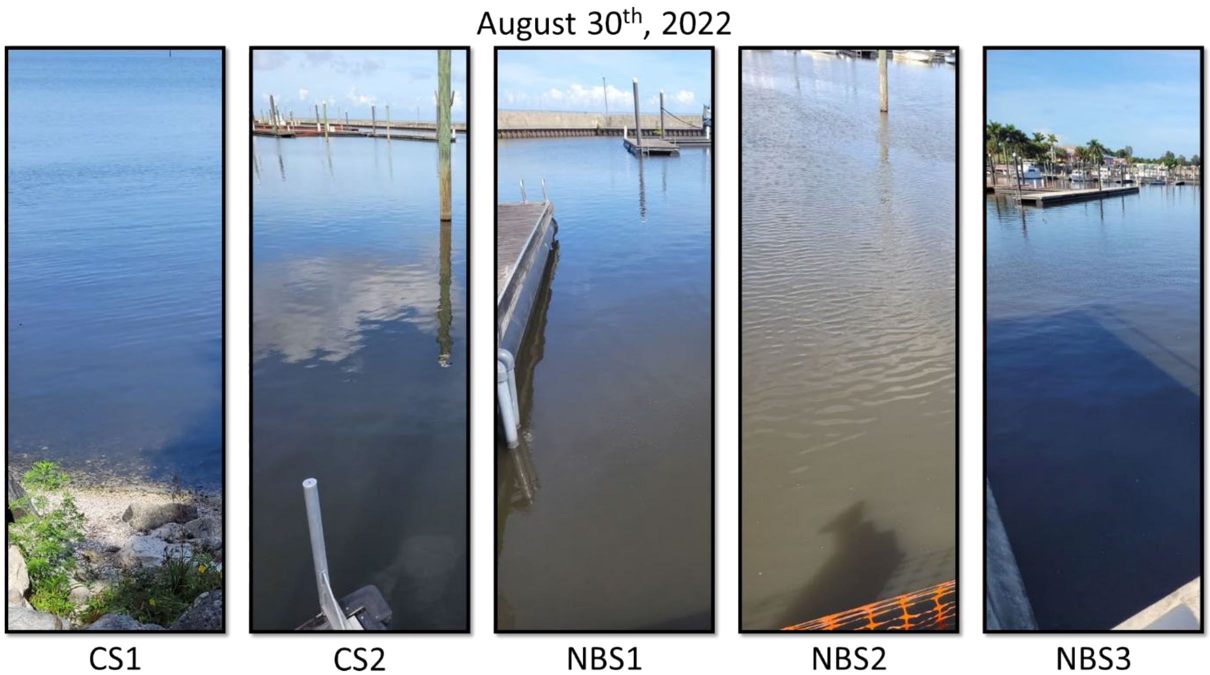

Figure 63: August 30th, 2022 photos of control and nanobubble treatment locations.

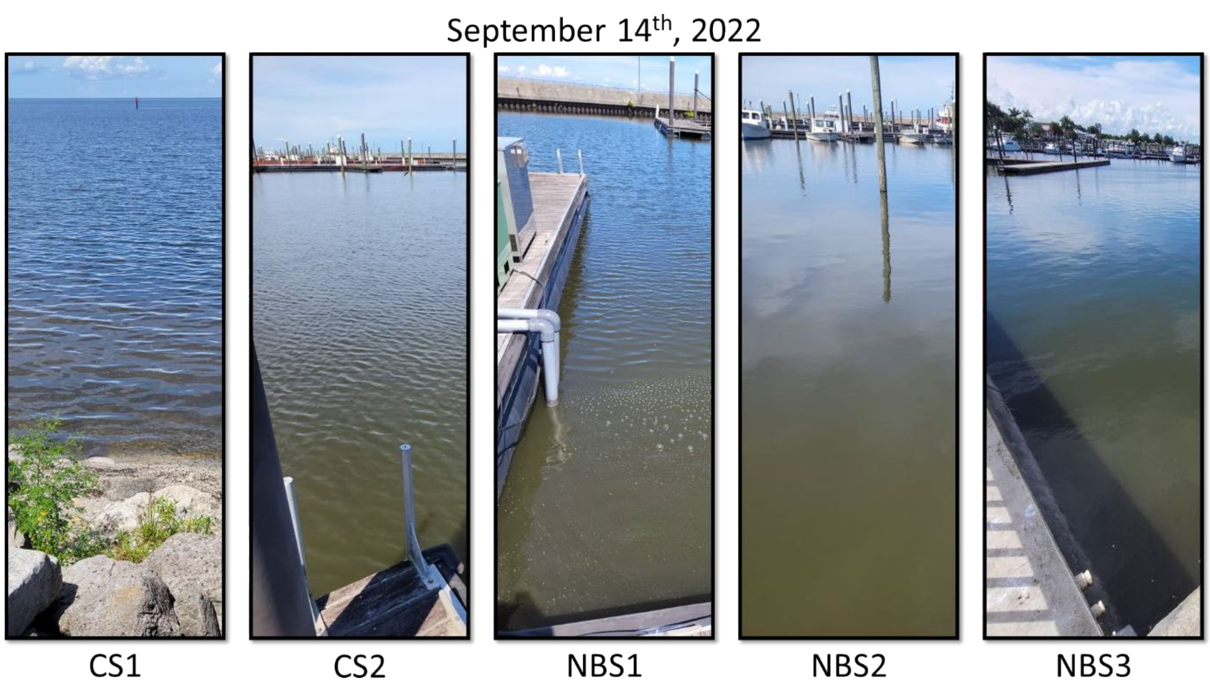

Figure 64: September 14th, 2022 photos of control and nanobubble treatment locations.

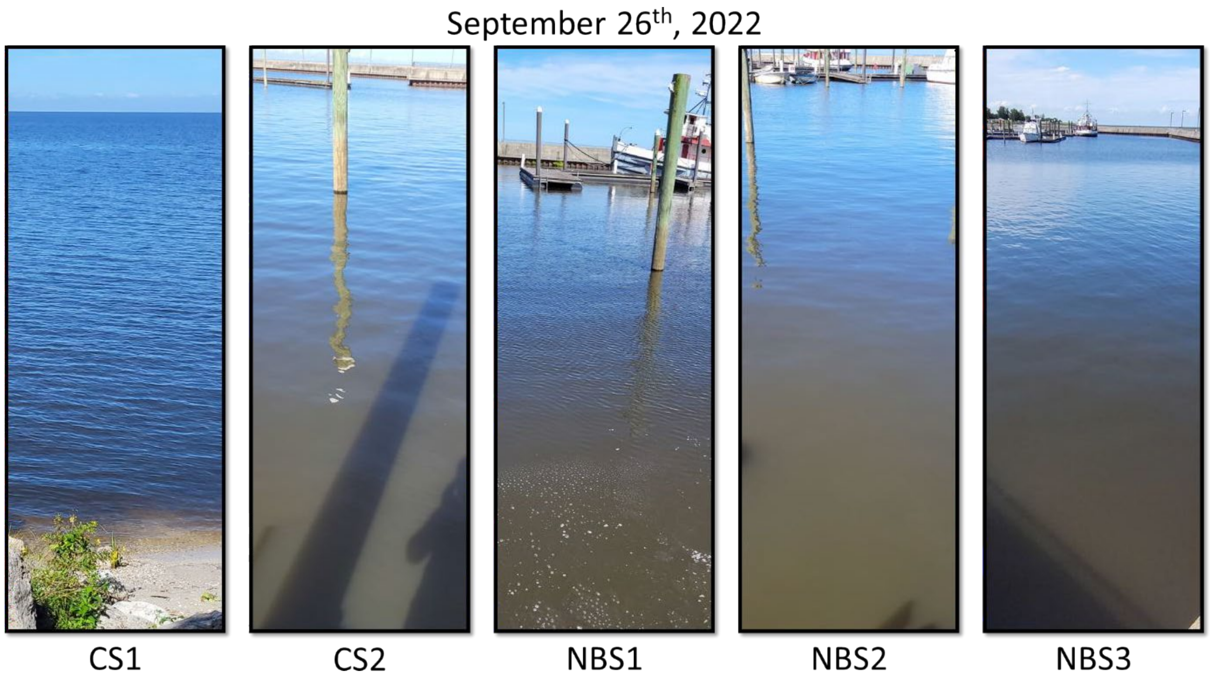

Figure 65: September 26th, 2022 photos of control and nanobubble treatment locations.

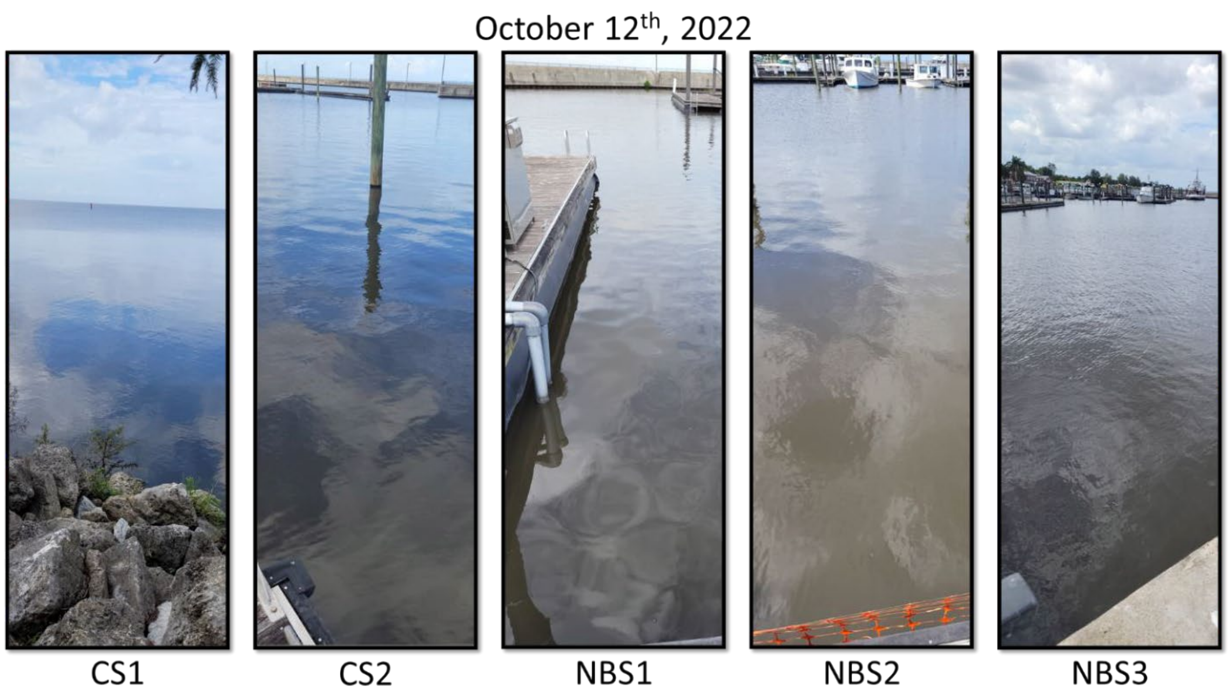

Figure 66: October 12th, 2022 photos of control and nanobubble treatment locations.

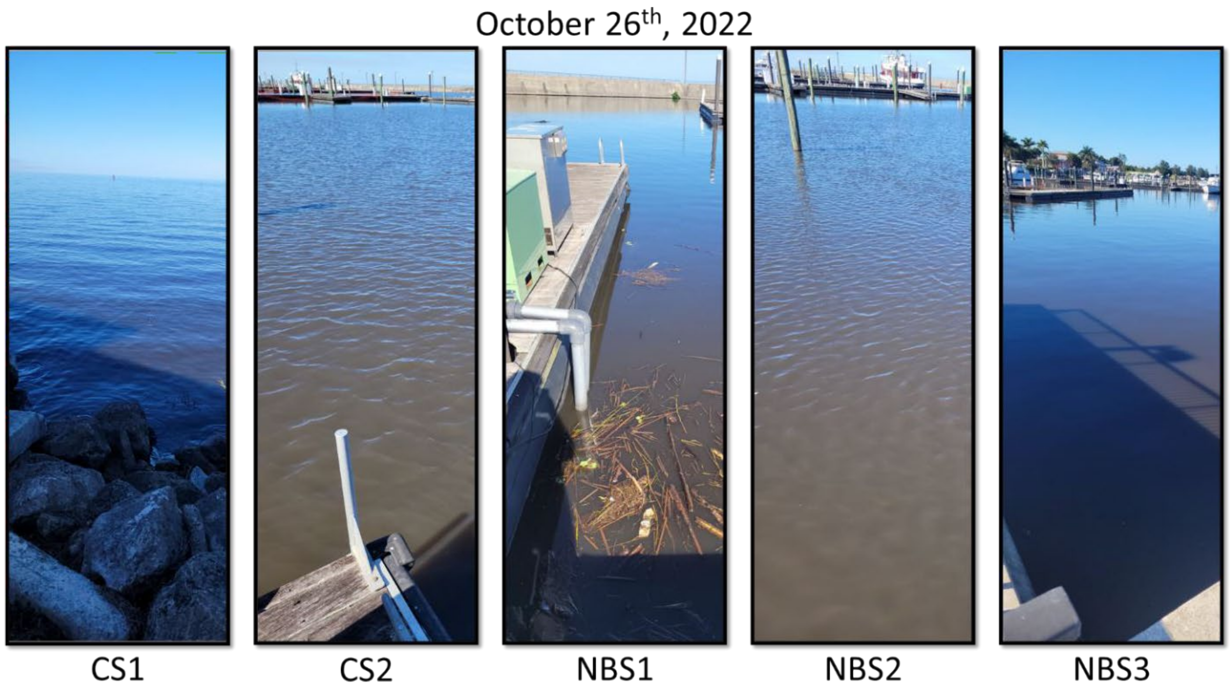

Figure 67: October 26th, 2022 photos of control and nanobubble treatment locations.

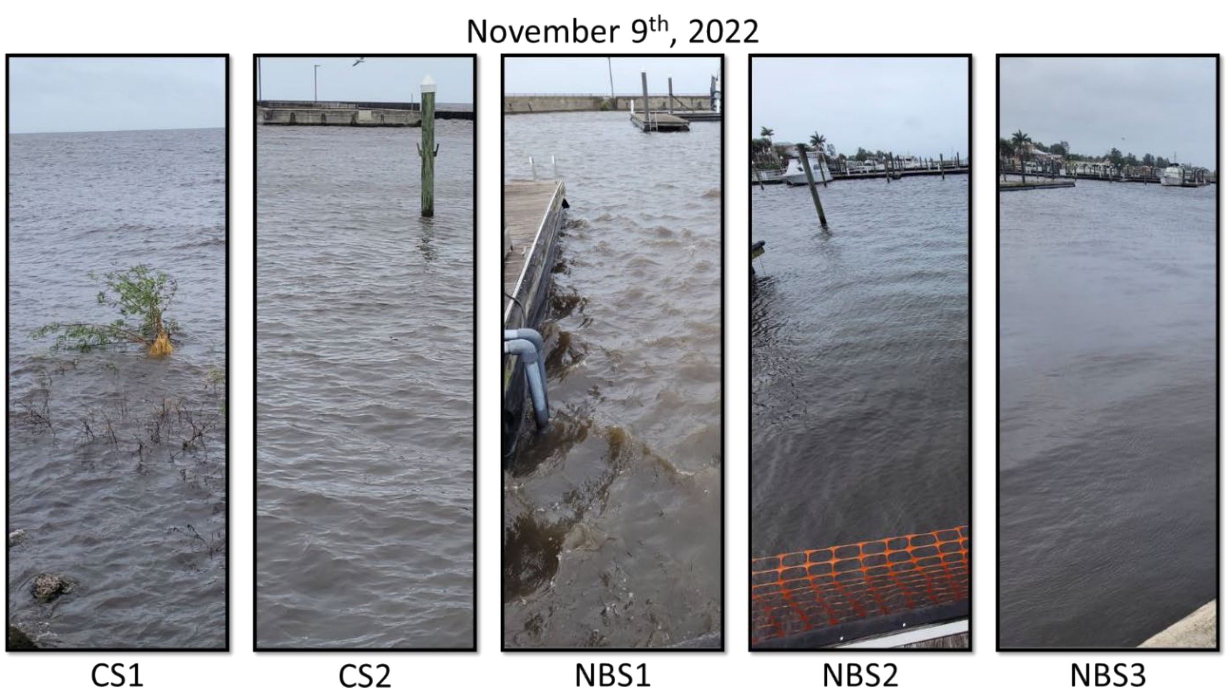

Figure 68: November 9th, 2022 photos of control and nanobubble treatment locations.

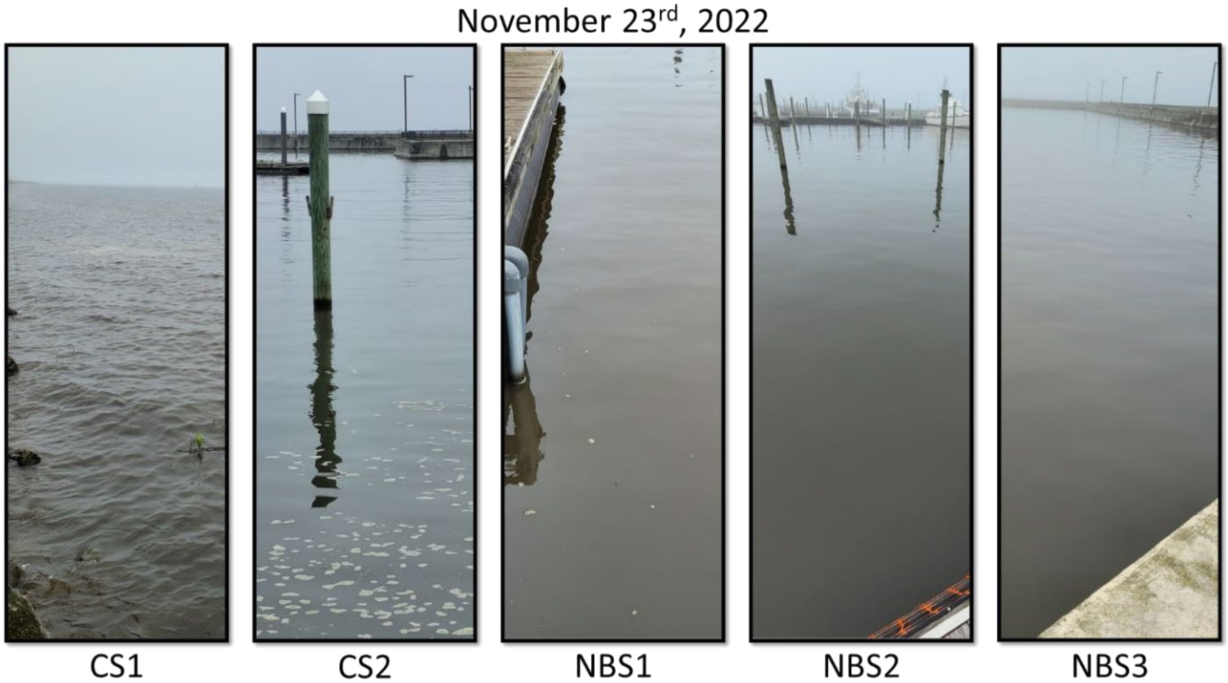

Figure 69: November 23rd, 2022 photos and nanobubble treatment locations

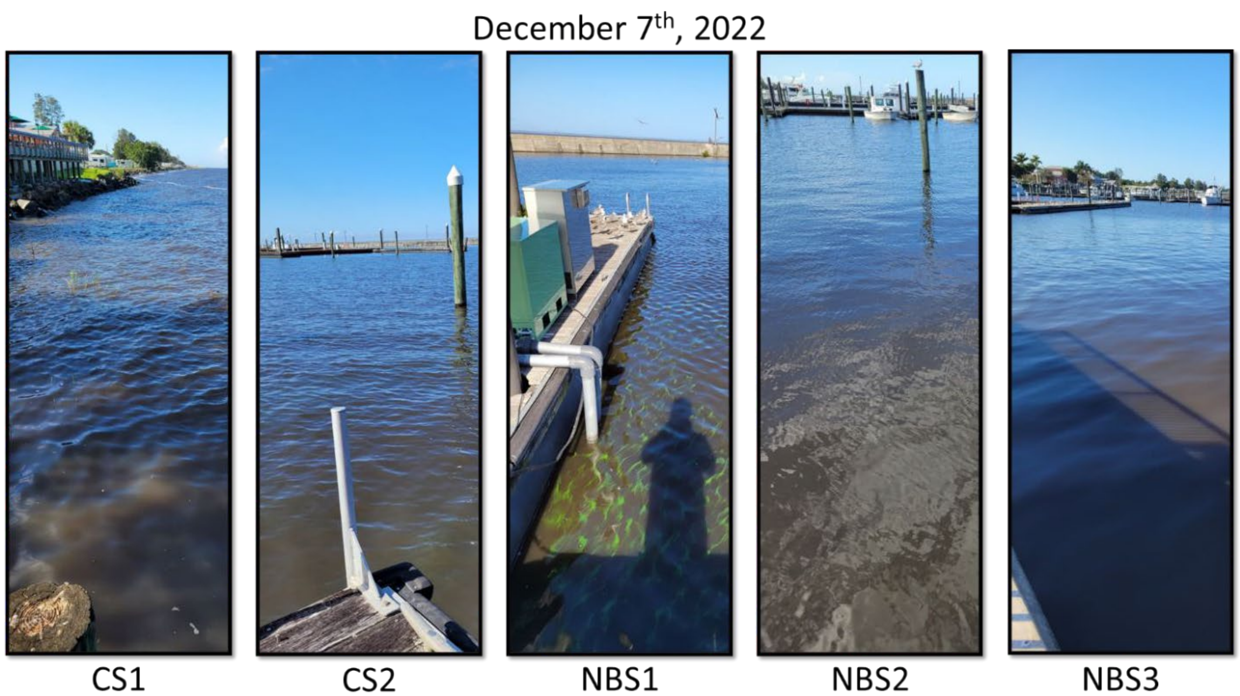

Figure 70: December 7th, 2022 photos and nanobubble treatment locations

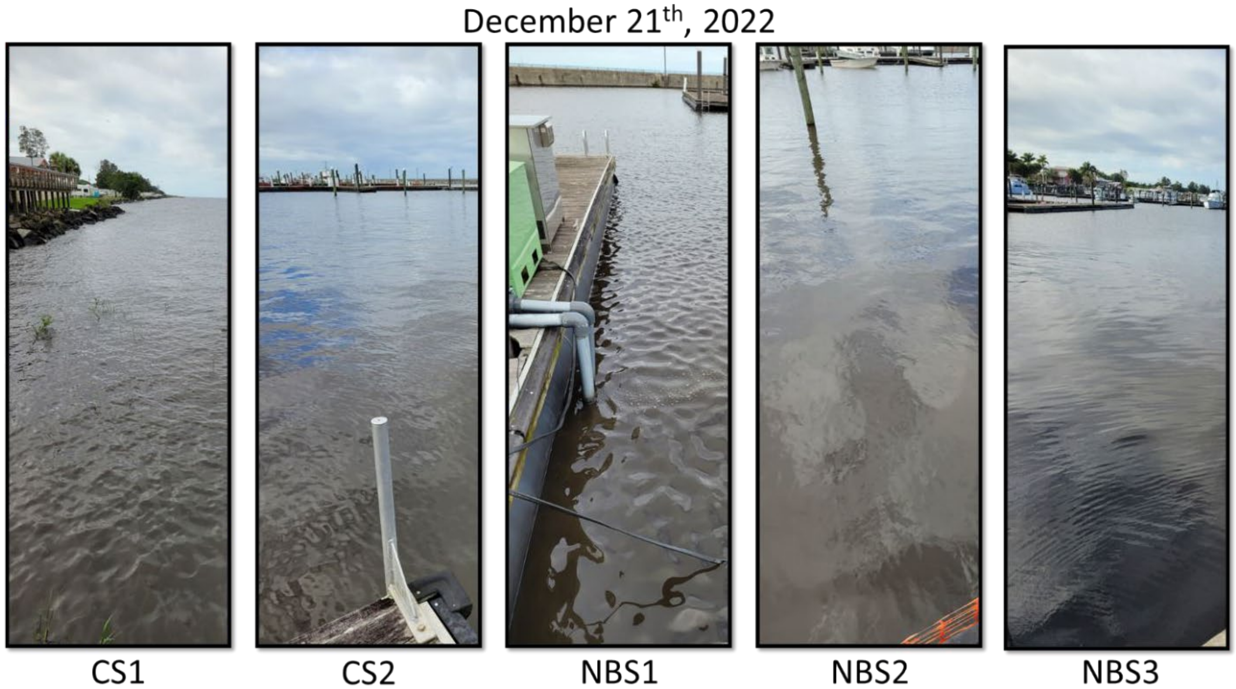

Figure 71: December 21st, 2022 photos of control and nanobubble treatment locations.

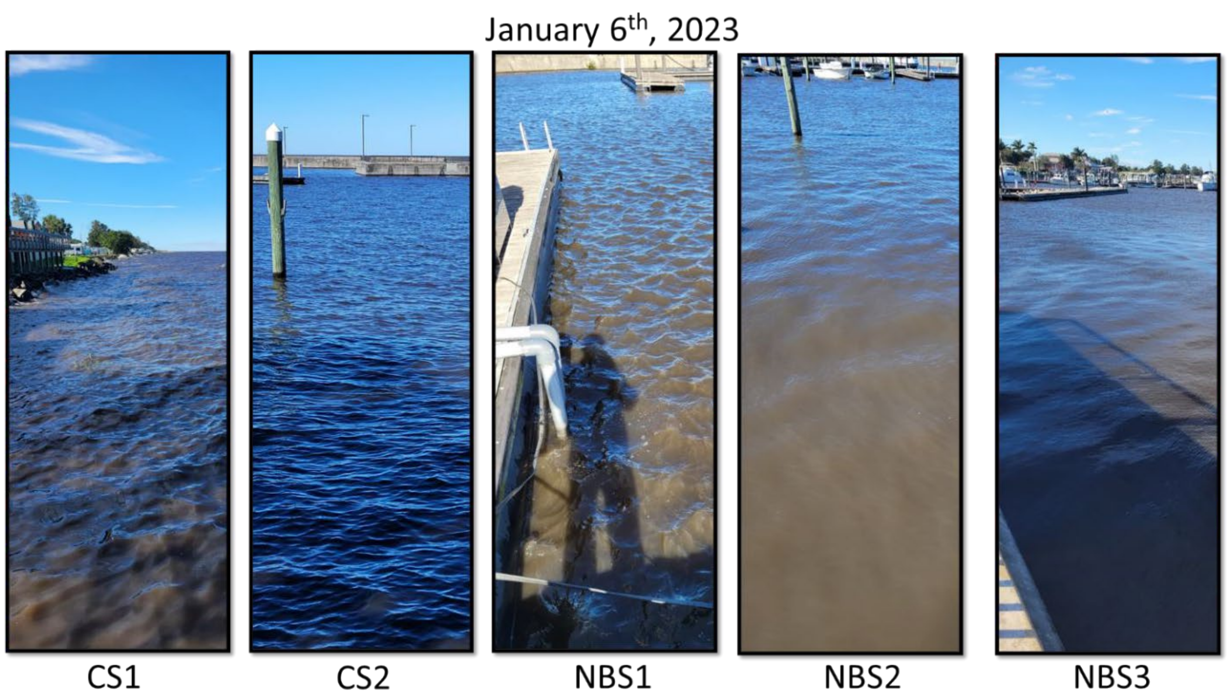

Figure 72: January 6th, 2023 photos of control and nanobubble treatment locations.

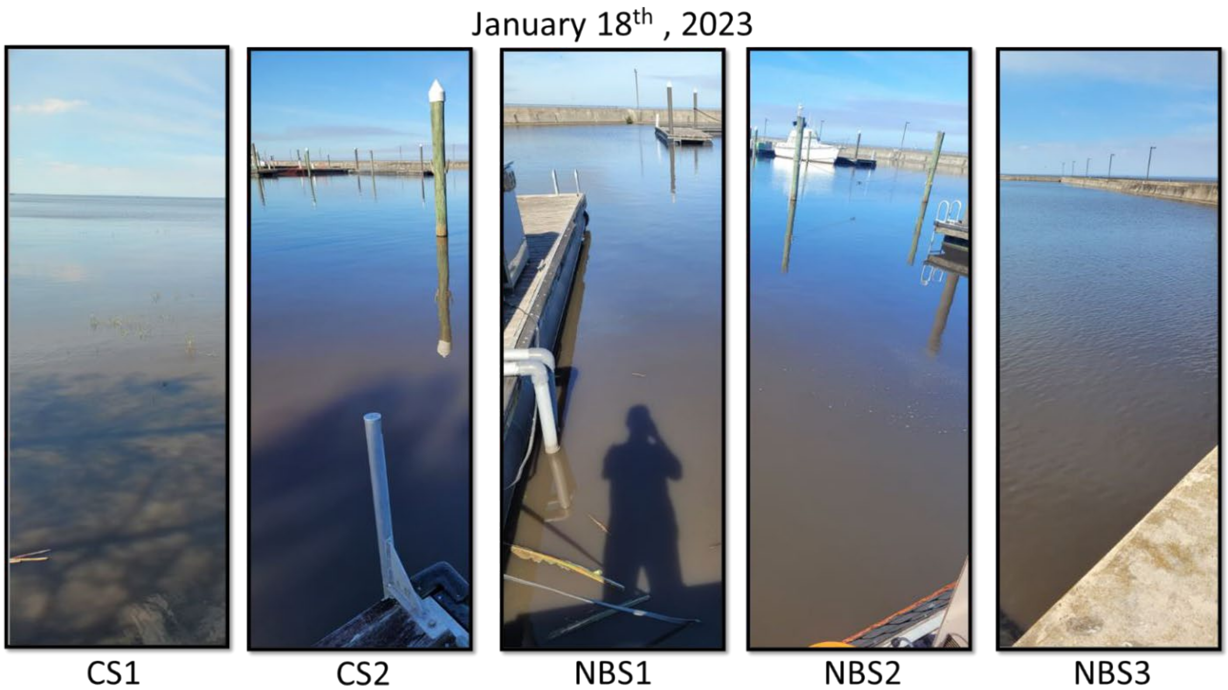

Figure 73: January 18th, 2023 photos of control and nanobubble treatment locations.

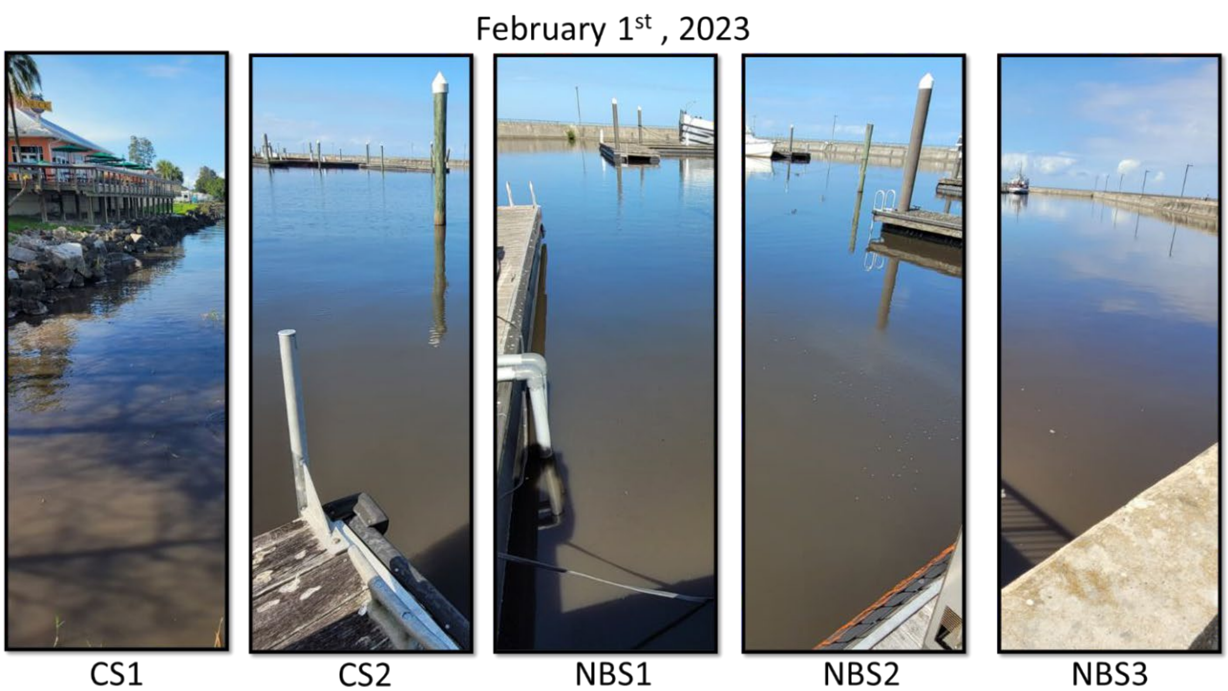

Figure 74: February 1st, 2023 photos of control and nanobubble treatment locations.

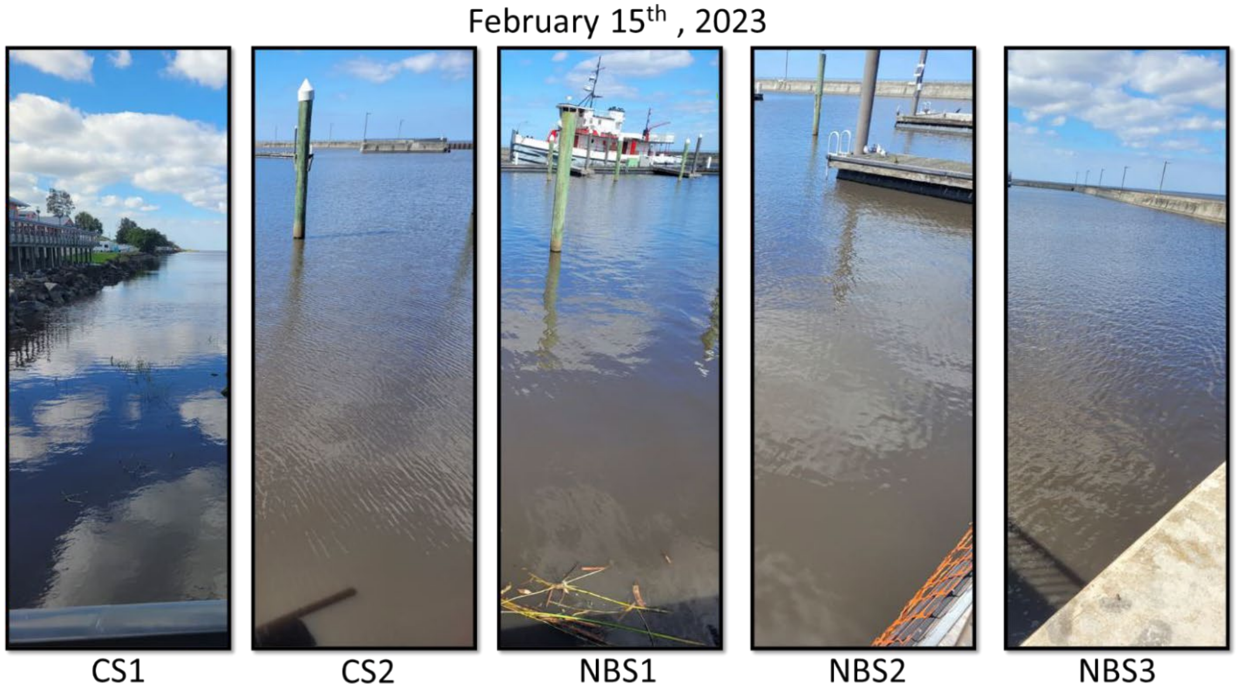

Figure 75: February 15th, 2023 photos of control and nanobubble treatment locations.

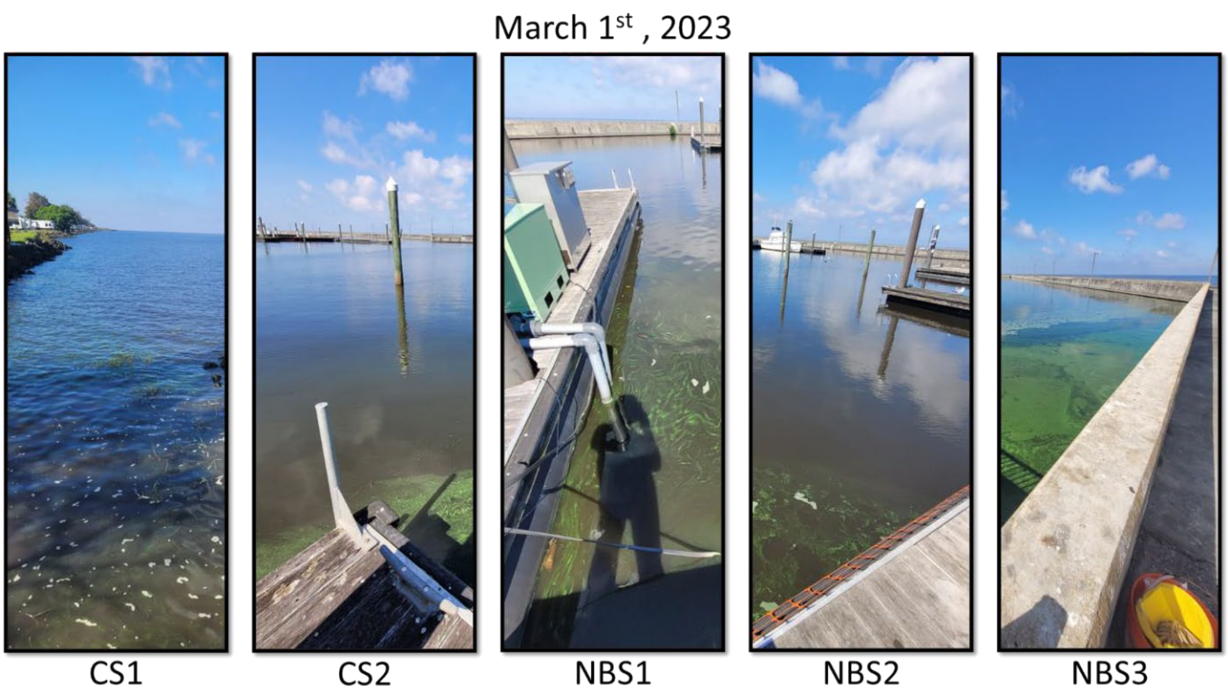

Figure 76: March 1st, 2023 photos of control and nanobubble treatment locations.

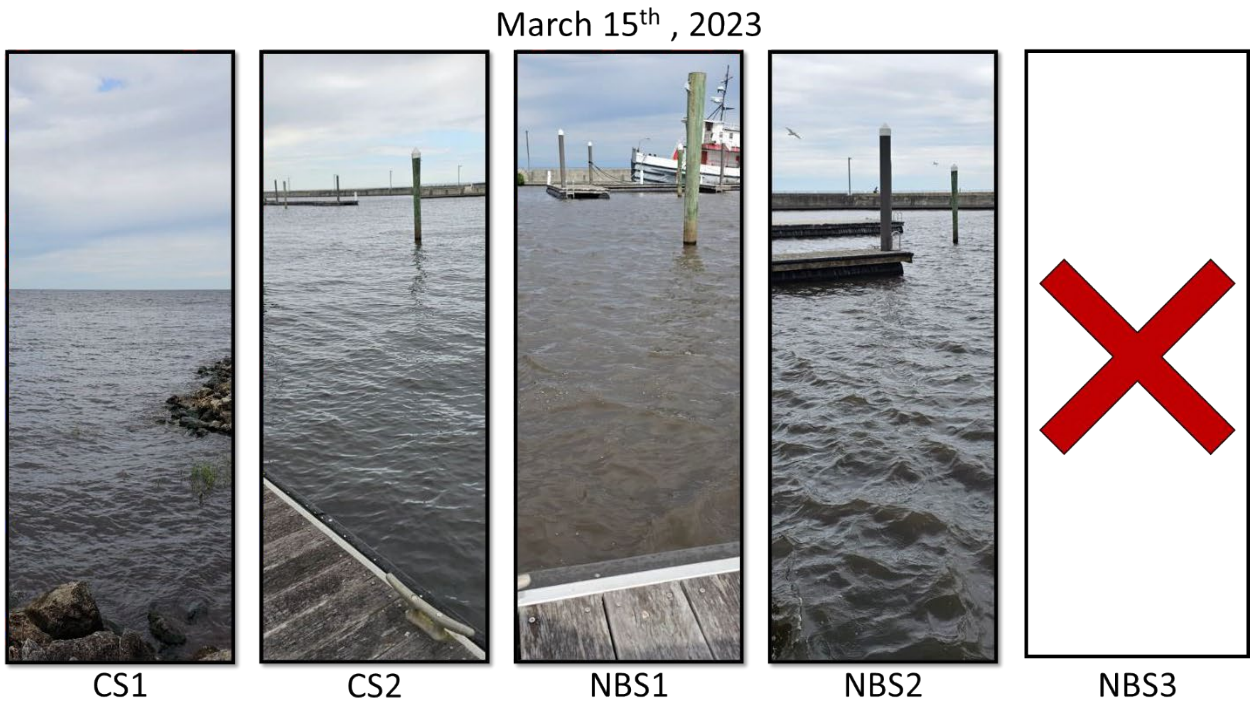

Figure 77: March 15th, 2023 photos of control and nanobubble treatment locations. NBS3 photo unavailable due to inclement weather.

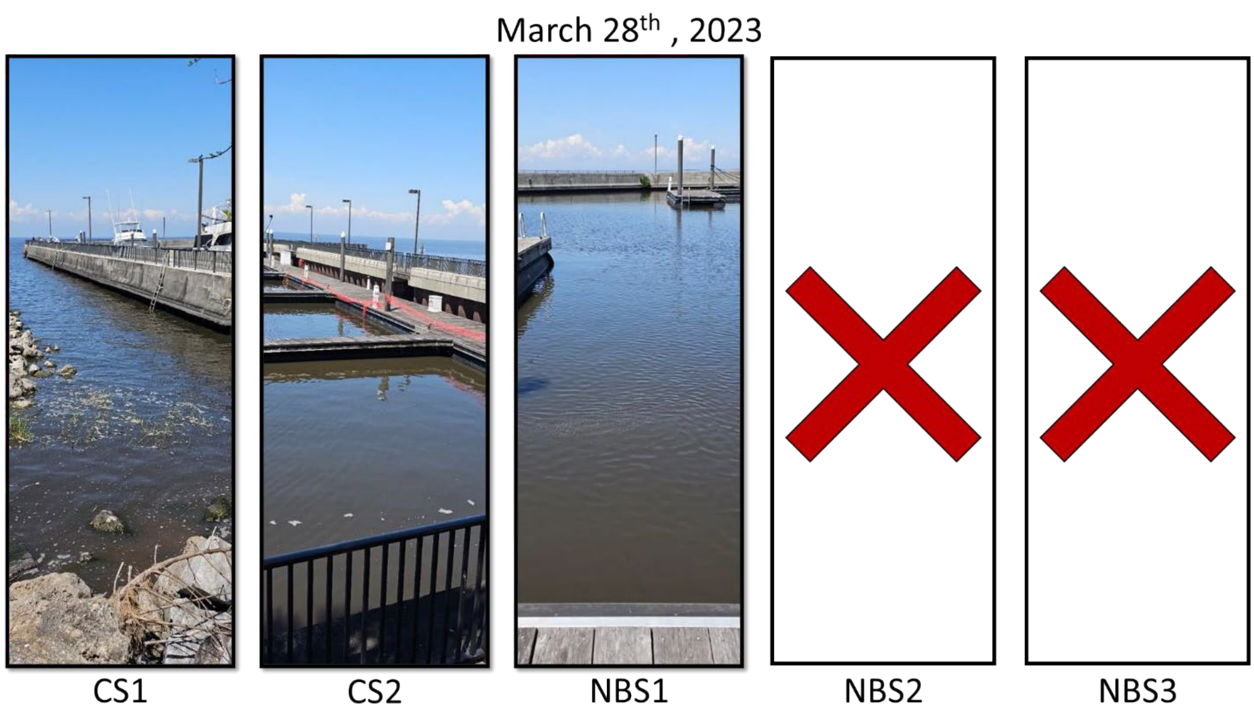

Figure 78: March 28th, 2023 photos of control and nanobubble treatment locations. NBS2 and NBS3 unavailable due to technical issues.

7.3 Interpretation of Data

7.3.1 Real Time, In-Situ Monitoring Data Interpretation

In-Situ probes captured various water quality parameters. This discussion will focus on three main parameters – phycocyanin (PC), oxidation-reduction potential (ORP), and dissolved oxygen as they provide the most context and support for a difference in water quality conditions between the treatment and control regions.

The phycocyanin probe was provided by In-Situ and is a fluorescence-based probe. This probe presented significant operational challenges throughout the monitoring period, limiting the usability of the data that was collected. Challenges were encountered with probe calibration, with zero-point drift and, to a lesser extent, with probe connectivity to the sonde. Probe calibration challenges were first observed by a significant drift upwards on control side probe between install in September 2021 and January 2022. During this period, the treatment area PC probe appeared to be providing correct data until January 2022. In January 2022, the treatment area PC probe began to drift high. The drift in the probe zero-point is evident when probe data is compared to the visual observation of the marina as captured in the photos from the sampling events. Extensive efforts were undertaken to restore probe functionality including close collaboration with probe manufacturer, In-Situ, who provided several replacement probes. PC probes were also returned to In-Situ's engineering team for special investigation. Several water quality parameters were explored as potential interferences with the probe including natural organic matter, however these interferences were ruled out. The root cause of the probe drift was not ultimately identified however the issue appeared to have reduced in severity through several modifications to the probe and probe operation including software updates by the manufacturer, replacement of the probe, and more frequent calibrations.

The PC data collected suggests that PC concentrations trended lower in the treatment area compared to the control area however a quantitative reduction in algae concentration cannot be provided with PC data alone given the concerns with probe calibration. Further, the measurement of PC concentration as a method to indicate a differentiated level of treatment between the treatment and control areas proved challenging given the lack of information on marina circulation/mixing and possible interference by wind on the accumulation of PC. For example, wind was observed to accumulate pockets of high-density algae within catchment basins of the marina such as in the corner of docks or along seawalls. In this way, a high-density pocket of algae may be encountered by the sensor resulting in a significant increase in PC fluorescence but may not be representative of the broader algae conditions in the marina.

While there were blooms observed during nanobubble treatment, the PC probes did not capture any significant blooms or occurrences of algae. However, no severe blooms occurred in the control region inhibiting a quantitative comparison on the reduction in bloom intensity. Given the well-established knowledge of ozone as a method for algae control and the PC data combined with observational and photo data, nanobubbles may be an effective method for applying ozone as a treatment method to diminish algae bloom intensity in Pahokee Marina.

ORP is an important water quality parameter in surface waters and was monitored to capture the general health of the waterbody as well as to ensure that ozone was injected at appropriate levels to reduce algae but not result in potential harm to non-target organisms. As a measure of waterbody health, ORP is used as a quantitative metric for classifying microbial and chemical activity. Positive ORP values are associated with an oxic environment and with microbial and chemical conditions which are generally considered to be healthy whereas negative ORP values are typically measured in waterbodies with poor health and are often accompanied by low/no dissolved oxygen. ORP was also measured to ensure that no significant oxidative impact was occurring in the waterbody due to ozone residual from the ozone nanobubble injection. Residual ozone can be measured by ORP where an increased level of ORP represents a higher ozone concentration. Measuring ozone via ORP is always relative to the background ORP levels which are generated by a combination of all oxidizers and reducers in the water.

For the entirety of the monitoring period, ORP values remained positive in both treatment and control regions. The positive ORP often indicates conditions in which aerobic microbial activity such as nitrification can occur however ORP was measured near the surface and full water column monitoring would be required to fully understand the microbial activity in the waterbody. Large and abrupt fluctuations in ORP such as that observed in early February 2022 on both the control and treatment probes or that observed in September 2022 on the treatment probe are likely artifacts of the behavior of the ORP probes rather than reflections of water quality. The ORP data indicated no clear differences between control and the treatment areas. Considering the positive ORP conditions across both control and treatment areas, increased ozone levels in the treatment region as algae control would likely not result in further increasing in ORP. Additionally, the consistent ORP conditions across the control and treatment areas suggest that the ozone nanobubble treatment was not introducing any harmful impact to the waterbody because of over-applying ozone.

Oxygen is critical to monitor as it reflects several different key behaviors of waterbody such as the growth of algae. During algae growth periods, diurnal swings in dissolved oxygen occur as algae photosynthesize during the day causing dissolved oxygen to increase. Further, when blooms continue over an extended period, the increased organic loading from algae sinking to the bottom of the water column causes a spike in aerobic microbial activity and a crash in dissolved oxygen. The dissolved oxygen data measured in the treatment and control regions was analyzed along with phycocyanin data to understand the growth of algae in the marina. Additionally, the nanobubble generators injected oxygen during both standard non-ozone operation and during the injection of ozone. An increase in the dissolved oxygen is a by-product of the nanobubble treatment process as the technology is an effective method of efficiently transferring oxygen into a waterbody.

Dissolved oxygen level remained elevated across both treatment and control areas throughout the entire monitoring period. However, some differences in concentrations were observed between the areas of comparison. Dissolved oxygen concentrations ranged between 4 mg/L and 10 mg/L with brief fluctuations as high as approximately 14 mg/L and as low as 1 mg/L. The large swings in dissolved oxygen often accompanied periods when algae were detected in the marina either through phycocyanin monitoring or visually. Concentrations of dissolved oxygen varied significantly for the period of June through October 2022 in the control while reduced fluctuations were observed in the treatment area. This was likely a consequence of reduced algae activity in the treatment area reducing photosynthesis. While dissolved oxygen concentrations were expected to rise slightly in the nanobubble treatment region, the high turnover and subsequent low residence time of water in the marina meant that no significant increases of dissolved oxygen were expected. However, the dissolved oxygen was consistently higher in the treatment region by approximately 0.25mg/L to 0.5 mg/L. The reduction in dissolved oxygen fluctuations along with the increase in dissolved oxygen may suggest reduced algae activity along with an increase in aquatic health in the treatment region.

7.3.2 Nutrient Sample Data Interpretation

Samples for nutrient and algae analysis were collected manually every two weeks throughout the entire monitoring period. Nutrient samples were analyzed for Ammonia, Nitrate, Nitrite, Orthophosphate, and Total Phosphorus. These nutrients were selected as they are known to be contributors to algae growth.

Ammonia is a nitrogen pollutant and key to understanding the nitrogen cycles within an aquatic environment. Ammonia is commonly introduced to an environment from non-source pollution such as water run-off from agricultural areas, from animal waste, or it can be sourced from oxygen-deprived sediments which are rich in organic material. Ammonia samples revealed that concentrations fluctuated seasonally with spikes in concentrations during warmer months. Ammonia concentrations had a baseline value in both control and treatment regions of approximately 0.03 – 0.05 mg/L as shown in Figure 35. Fluctuations in concentrations were observed on what may be a seasonal basis. In both treatment and control regions, concentrations rose to 0.2 mg/L, a more than six-fold increase between May and July in 2022. In several instances throughout the observation period, intermittent spikes in concentrations lasting one sampling period were observed. As nanobubble generator operational frequency was increased after January 2022, the severity in concentration spikes reduced. Spikes in control regions continued throughout the monitoring period and were higher and more frequent than across the three nanobubble treatment samples. However, the high rate of mixing in the marina environment may have muted benefits that occurred because of the nanobubble treatment. Throughout the monitoring period ammonia concentrations in the control and treatment regions followed similar trends.

Nitrite is an intermediate oxidized form of ammonia and can be introduced to the environment from the nitrification of ammonia or from environmental pollution. Nitrate sampling revealed minimal variation in concentrations were captured above the minimum detection limit (MDL) of the analysis method. The value of 0.018 mg/L represents the MDL of the analysis method by Advanced Environmental Laboratories (AEL). The value shown on the charts reported as 0.018 mg/L indicates that levels were at or below 0.018 mg/L and variability in concentrations. Baseline levels were consistent for all but four sampling points over the 19-month monitoring period. During the four sampling points where increased nitrite concentrations were observed, the levels rose to 0.09 mg/L in all cases. The consistency of the baseline nutrient concentrations suggests that either nitrite loading on the system was low or that nitrification was rapidly oxidizing the available nitrite to nitrate through the nitrification cycle. The low fluctuations in nitrite concentrations supports that loading on the system was low while the rapid reduction in concentrations during the elevated concentration samples supports that higher levels of nitrite were rapidly removed from the system. No period of elevated concentrations persisted for longer than one sampling point. Further, there were no seasonal fluctuations observed as might be expected if nitrite loading on system contributed significantly to the waterbody's nitrogen balance.

The consistency of the nitrite concentrations extended to both the control and treatment regions. The results across all five sampling points revealed identical concentrations except for sampling on 3/28/23 where the increase in nitrite concentration was only observed on the sample site NBS2. While nitrite concentrations remained consistent throughout the monitoring period there was variation in algae concentrations. Therefore, algae growth is unlikely to be strongly contributed to by the nitrite concentrations as they are consistently low and did not correlate with even mild periods of algae growth in the marina.

Nitrate is the final oxidized form of ammonia in the nitrification process and is important to monitor to characterize the nitrate and nitrification balance in the marina. Nitrate concentrations showed a varying concentration throughout the monitoring period that may be seasonally and environmentally driven. Initial samples from 9/9/21 to 10/12/21 suggested there may be varying concentrations between control and treatment regions. However, samples subsequently aligned between control and treatment locations with little variation in nitrate between the control and treatment regions. For the monitoring period of 12/8/21 through 9/14/22 a seasonal trend in nitrate concentrations appeared to emerge. For this period, concentrations were lowest during the cool period of 12/8/21 ranging from 0.023 mg/L to 0.12 mg/L.

Concentrations then rose to 0.23 mg/L to 0.36 mg/L until 5/11/22 when concentrations began to decrease back to baseline conditions of about 0.023 mg/L. During this period, nitrate concentrations were variable sample to sample although consistent across sampling points.

The weather event of Hurricane Ian in early October 2022 introduced a strong increase in nitrate concentration that resulted in elevated concentrations for the remainder of the monitoring period. Concentrations rose sharply after the hurricane in late September 2022 from 0.023 mg/L to 0.57 mg/L. Due to the high volume of precipitation, the source of the nitrates is likely to be from non-point source runoff rather than from natural environmental sources such as nitrification. Concentrations remained high between 0.19 mg/L and 0.5 mg/L for the remainder of the sampling period. For both periods before and after Hurricane Ian, the control and treatment sampling points remained similar in nitrate concentration. Therefore, ozone nanobubble treatment had no significant effect on nitrate concentrations between treatment and control sites. The selection of a control region location that is influenced by the treatment region water quality may also contribute to the observed nitrate trends. Additionally, a high rate of mixing within the waterbody may result in diminished observable differences between treatment and control site. The significant increase in nitrate concentration after the hurricane in October 2022 suggested that the nitrate concentration alone is not sufficient to drive algae blooms. However, the elevated concentrations leading into the summer 2023 months may have contributed to the wide-spread blooms that were observed after the removal of the nanobubble generators.

Ortho-phosphate, a subset of total phosphorus representing the biologically available phosphorus which is understood to be a major contributor to algae growth. Bi-weekly sampling revealed a baseline concentration of 0.013 mg/L. Given that baseline total phosphorus concentrations were 0.24 mg/L, ortho-phosphate consisted of about 5% of the total phosphorus concentration. Spikes in concentrations occurred in both treatment and control regions, however the increases in concentration often quickly subsided by the subsequent sampling period. Large increases in concentration comprising increases more than 0.1 mg/L did not persist for more than one sampling across any sampling points. However, there were periods of small increases in concentrations comprising increases of less than 0.1 mg/L that persisted up to three sampling periods, or six weeks of time. Overall, concentrations as high as 0.2 mg/L were seen in the beginning of the monitoring period. May 2022 saw a spike of 0.14 mg/L with the final season of monitoring seeing a spike of only 0.065 mg/L. The reducing spikes in concentration may be a consequence of nanobubble injection or a consequence of natural water quality fluctuations. Since similar trends were observed across the treatment and control regions, it is difficult to distinguish the impact of nanobubble treatment from the natural water quality variations. The overarching trends suggests an improvement in water quality due to a reduction in the fluctuations of ortho-phosphate however limitations of the test location inhibit a decisive conclusion on the impact of nanobubble treatment on ortho-phosphate concentrations.

Total phosphorus is an important water quality parameter to characterize water body health and to understand conditions for algae blooms. While ortho-phosphate conditions varied significantly over the monitoring period, total phosphorus concentrations were more consistent. Baseline concentrations of total phosphorus were 0.24 mg/L with brief periods of deviation from baseline. The variations from baseline were observed year-round with concentrations rising as high as 0.56 mg/L during 2022 and, for one sampling period, rising as high as 0.99 mg/L in February 2023. One significant increase in concentration was observed in the location CS2 on 9/29/21 however this point is an outlier from all other testing and from the trends in concentrations at other sites. The high concentration may be a consequence of sampling error. Different from other nutrients, total phosphorus concentrations varied from each sampling location. Although increases in concentrations were observed in all sampling sites, sites NBS2, NBS3, and CS2 tended to either experience the highest fluctuations in concentrations while NBS1 tended to experience more muted fluctuations in comparison to other sampling sites.

Total phosphorus showed little to no correlation with nitrogen-compound concentrations suggesting that sources of nutrient loading were different for the two categories of nutrients. For example, the hurricane in September 2022 resulted in a significant increase in nitrate, beyond the average amplitude of concentration fluctuations observed during other sampling periods. However, the hurricane did not significantly increase total phosphorus concentrations above levels observed throughout the year. Overall, no significant difference between control and treatment locations were observed. As with the other monitored nutrients, the consistency in total phosphorus concentrations may have resulted from the impact of the nanobubble treatment impacting conditions within the control zone or from a lack of impact on the nutrient parameter. Further studies should be considered where the impacts of mixing can be ruled out and the benefits of nanobubble treatment can be isolated.

7.3.3 Algal Concentration and Cyanotoxin Data Analysis and Interpretation

To explore the broad influence of nanobubbles on Pahokee Marina's algae community, all major algae genera cell count values were summed together to represent the total algal community concentration. Total community concentration was then graphed based on sampling date and treatment site (Figure 79). Major trends are not immediately discernable based on this macro view of the algae concentration.

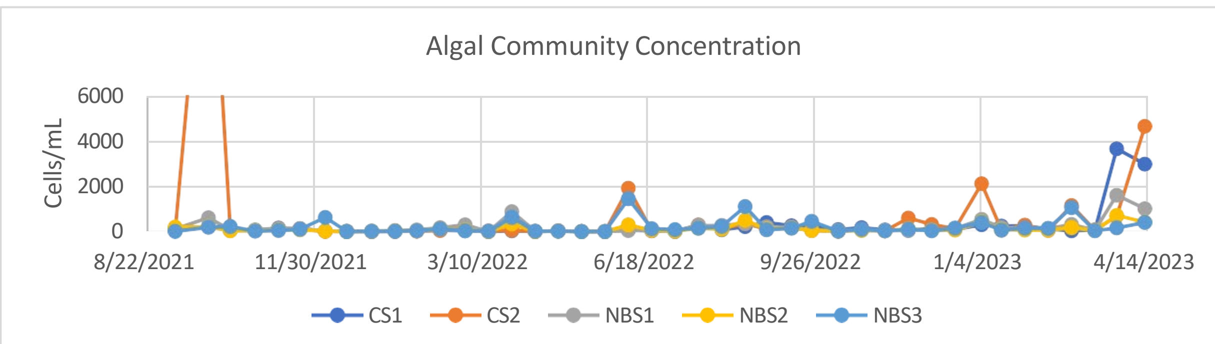

Figure 79: Algal community concentration (cells/mL) of Pahokee Marina spanning the duration of the project.

To further investigate the impact of ozone nanobubbles on algal cell concentrations, nanobubble generator uptime was taken into consideration. The operational status of the four nanobubble generators was monitored for the duration of the project and recorded daily as either operational (denoted by 1) or inoperative (denoted by 0). Special attention was given to operational status data on the day prior to and the day of discrete algae sampling. The operational status of each unit over this critical 48-hour period was averaged to create a nanobubble generator uptime percentage for each sampling date (Table 14).

	B14	B25	C14	D4	Daily Ave	Sampling Ave
9/8/2021	Data Unavailable					
9/9/2021						
9/28/2021	1	1	1	1	100%	100%
9/29/2021	1	1	1	1	100%	
10/11/2021	0	1	1	1	75%	75%
10/12/2021	0	1	1	1	75%	
10/26/2021	0	1	1	1	75%	88%
10/27/2021	1	1	1	1	100%	
11/9/2021	0	1	1	0	50%	50%
11/10/2021	0	1	1	0	50%	
11/22/2021	1	1	1	1	100%	88%
11/23/2021	1	1	1	0	75%	
12/7/2021	1	1	1	1	100%	100%
12/8/2021	1	1	1	1	100%	
12/20/2021	1	1	1	1	100%	88%
12/21/2021	0	1	1	1	75%	
1/4/2022	1	1	1	1	100%	100%
1/5/2022	1	1	1	1	100%	
1/18/2022	0	0	0	0	0%	0%
1/19/2022	0	0	0	0	0%	
1/31/2022	1	1	1	1	100%	100%
2/1/2022	1	1	1	1	100%	
2/14/2022	1	1	1	1	100%	100%
2/15/2022	1	1	1	1	100%	
3/1/2022	1	1	1	1	100%	100%
3/2/2022	1	1	1	1	100%	
3/15/2022	1	1	1	1	100%	100%
3/16/2022	1	1	1	1	100%	
3/29/2022	1	1	0	1	75%	88%
3/30/2022	1	1	1	1	100%	
4/12/2022	1	1	0	1	75%	88%
4/13/2022	1	1	1	1	100%	
4/26/2022	1	1	1	1	100%	100%
4/27/2022	1	1	1	1	100%	
5/10/2022	0	0	0	0	0%	0%
5/11/2022	0	0	0	0	0%	
5/24/2022	0	0	0	0	0%	0%
5/25/2022	0	0	0	0	0%	
6/7/2022	0	1	1	1	75%	75%
6/8/2022	0	1	1	1	75%	
6/21/2022	0	1	1	1	75%	75%
6/22/2022	0	1	1	1	75%	

	B14	B25	C14	D4	Daily Ave	Sampling Ave
7/5/2022	1	1	1	1	100%	100%
7/6/2022	1	1	1	1	100%	
7/19/2022	1	0	0	1	50%	50%
7/20/2022	1	0	0	1	50%	
8/2/2022	1	1	1	0	75%	75%
8/3/2022	1	1	1	0	75%	
8/16/2022	1	1	1	1	100%	100%
8/17/2022	1	1	1	1	100%	
8/29/2022	1	1	1	1	100%	100%
8/30/2022	1	1	1	1	100%	
9/13/2022	1	1	1	1	100%	100%
9/14/2022	1	1	1	1	100%	
9/25/2022	Data Unavailable					
9/26/2022						
10/11/2022	Data Unavailable					
10/12/2022						
10/25/2022	1	0	0	1	25%	25%
10/26/2022	1	0	0	1	25%	
11/8/2022	1	1	0	1	50%	50%
11/9/2022	1	1	0	1	50%	
11/22/2022	0	0	0	0	0%	0%
11/23/2022	0	0	0	0	0%	
12/6/2022	1	0		0	25%	25%
12/7/2022	1	0		0	25%	
12/20/2022	1	1		1	75%	75%
12/21/2022	1	1		1	75%	
1/5/2023	0	1	0	1	50%	63%
1/6/2023	1	1	0	1	75%	
1/17/2023	0	1	0	1	50%	50%
1/18/2023	0	1	0	1	50%	
1/31/2023	0	1	0	1	50%	50%
2/1/2023	0	1	0	1	50%	
2/14/2023	0	1	0	1	50%	50%
2/15/2023	0	1	0	1	50%	
2/28/2023	0	1	0	0	25%	25%
3/1/2023	0	1	0	0	25%	
3/14/2023	1	1	1	1	100%	100%
3/15/2023	1	1	1	1	100%	
3/27/2023	1	1	1	1	100%	100%
3/28/2023	1	1	1	1	100%	
4/13/2023	1	1	1	1	100%	100%
4/14/2023	1	1	1	1	100%	

Table 14: Operational status of nanobubble generators during project sampling events.

For data analysis, sampling sites were considered dosed with nanobubbles when nanobubble generator operational status (NBG) was at or above 75% (highlighted green). Sampling events that occurred when generator operational percentage was at or below 25% were not considered to be treated with nanobubbles (highlighted in red). Samples collected when generator operational status was greater than 25% but under 75% was considered nebulous and omitted from statistical analysis. If generator operation status was unavailable during a sampling event, the data was omitted from statistical analysis. Table 15 illustrates how sampling dates were categorized based on nanobubble generator operational status.

Nanobubble Treatment Dates	Non-Treatment Dates	Intermediate Treatment Dates	Lack of Data
9/29/2021	1/19/2022	11/10/2021	9/9/2021
10/12/2021	5/11/2022	7/20/2022	9/26/2022
10/27/2021	5/25/2022	11/9/2022	10/12/2022
11/23/2021	10/26/2022	1/6/2023	
12/8/2021	11/23/2022	1/18/2023	
12/21/2021	12/7/2022	2/1/2023	
1/5/2022	3/1/2023	2/15/2023	
2/1/2022			
2/15/2022			
3/2/2022			
3/16/2022			
3/30/2022			
4/13/2022			
4/27/2022			
6/8/2022			
6/22/2022			
7/6/2022			
8/3/2022			
8/17/2022			
8/30/2022			
9/14/2022			
12/21/2022			
3/15/2023			
3/28/2023			
4/14/2023			

Table 15: Sample collection dates categorized based on nanobubble generator operational status.

Total algal community concentration was graphed by sample date, utilizing only sampling data taken when nanobubble generator operation was at or above 75% (Figure 80).

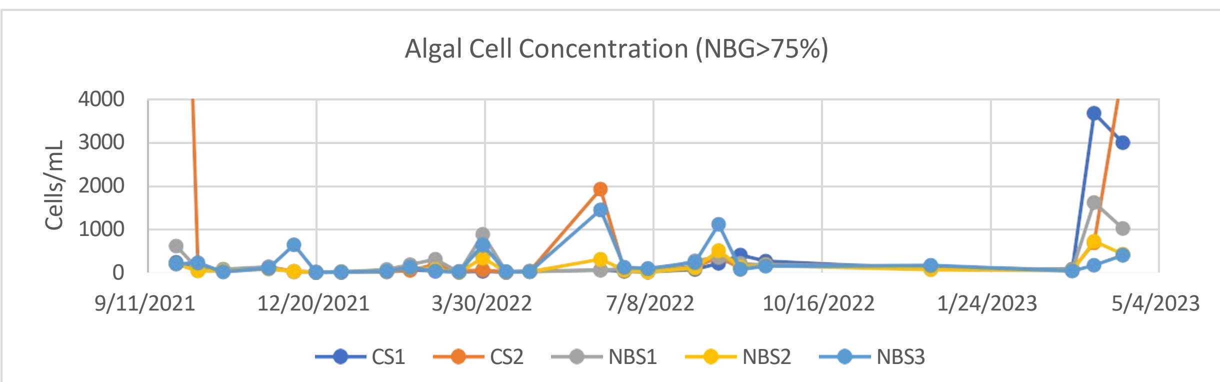

Figure 80: Algal community concentration (cells/mL) of Pahokee Marina when NBG>75% spanning the duration of the project.

To clarify patterns or trends in the data, each dominant genera was graphed in isolation using the same 75% generator operation status threshold as above.

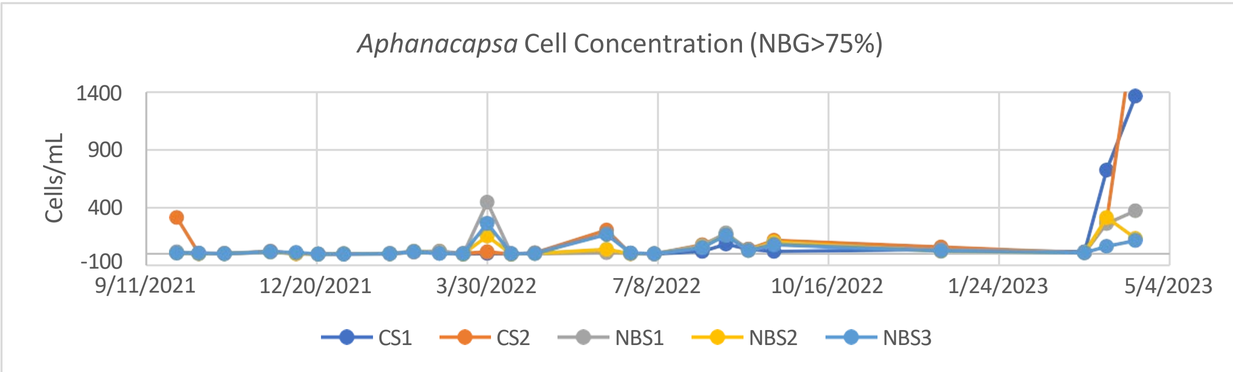

Figure 81: *Aphanocapsa* concentration (cells/mL) of Pahokee Marina when NBG>75%

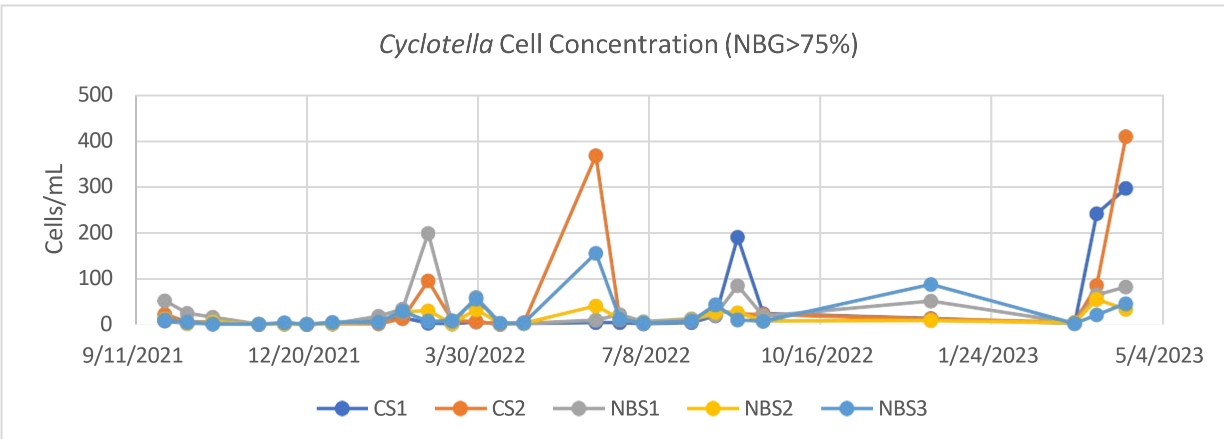

Figure 82: *Cyclotella* concentration (cells/mL) of Pahokee Marina when NBG>75%

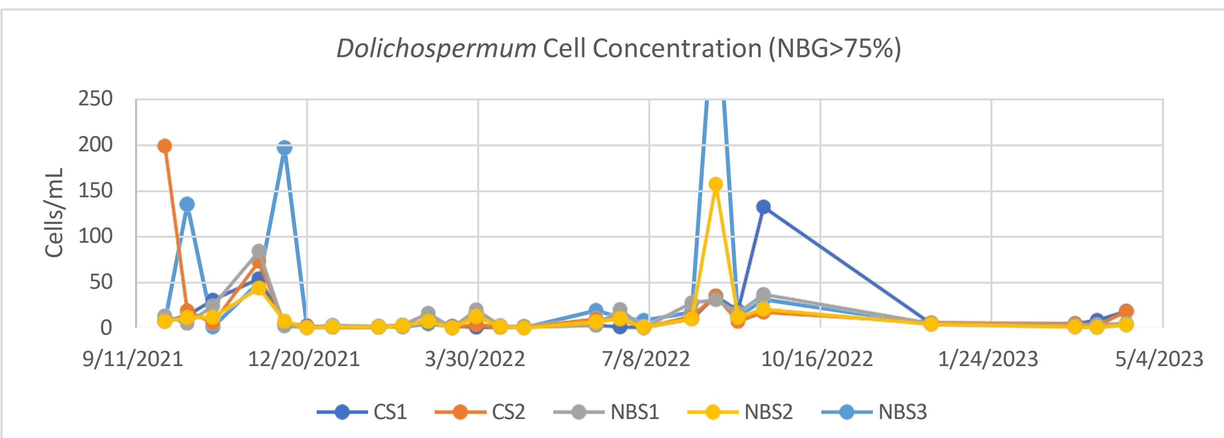

Figure 83: *Dolichospermum* concentration (cells/mL) of Pahokee Marina when NBG>75%

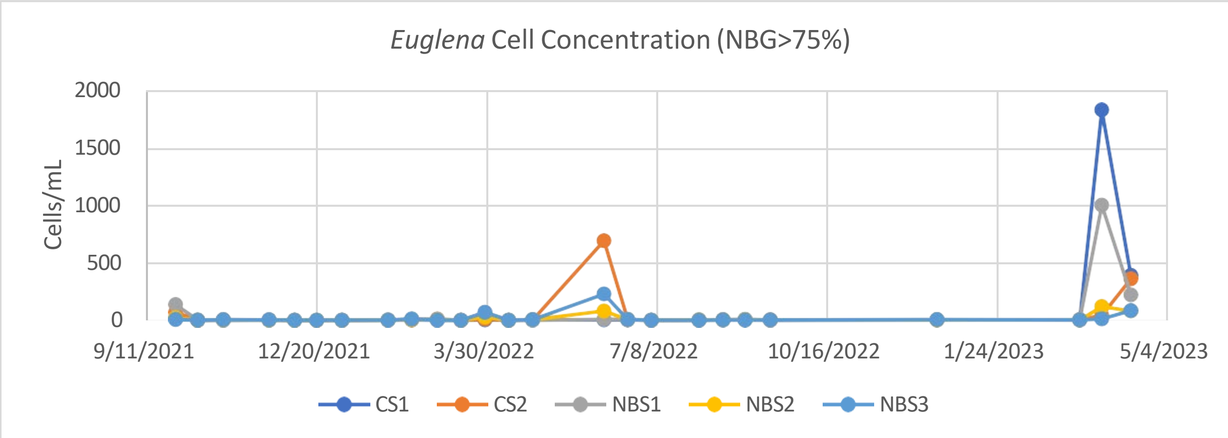

Figure 84: *Euglena* concentration (cells/mL) of Pahokee Marina when NBG>75%

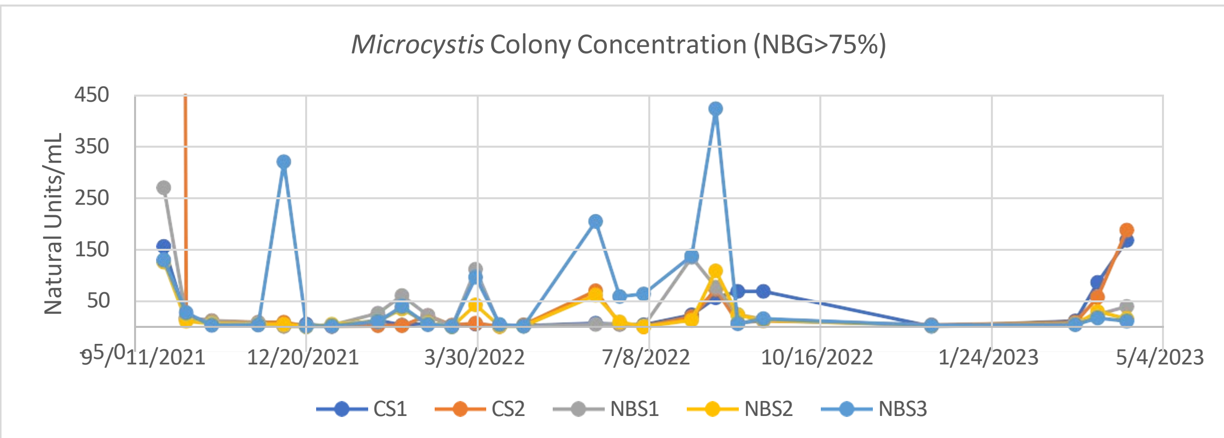

Figure 85: *Microcystis* colony concentration (cells/mL) of Pahokee Marina when NBG>75%

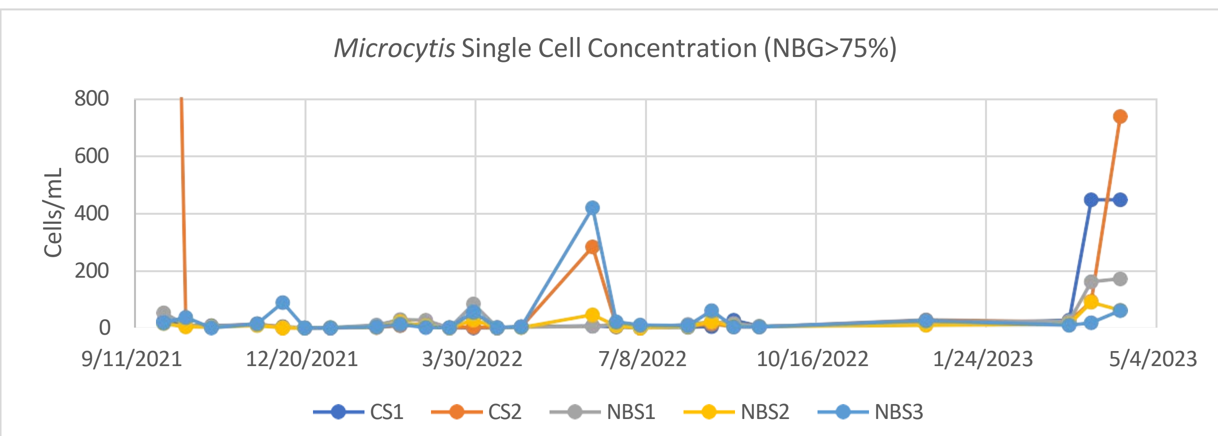

Figure 86: Single-cell *Microcystis* concentration (cells/mL) of Pahokee Marina when NBG>75%

The volume and scale of data from Pahokee Marina's algae community makes definitive conclusions from visual analysis of graphs difficult. Formal statistical analysis was used to gain greater understanding of algae populations at each sampling site during this project. One-way ANOVAs (analysis of variance) with post hoc Tukey HSD and connecting letters reports were used to determine if any algae population averages were significantly different between treatment sites. If required, data was uniformly transformed, and outliers omitted to meet unequal variance and normal residuals assumptions before formal statistical analysis was performed. Averages not connected by the same letter are significantly different. Results are shown in Figure 87 to Figure 92.

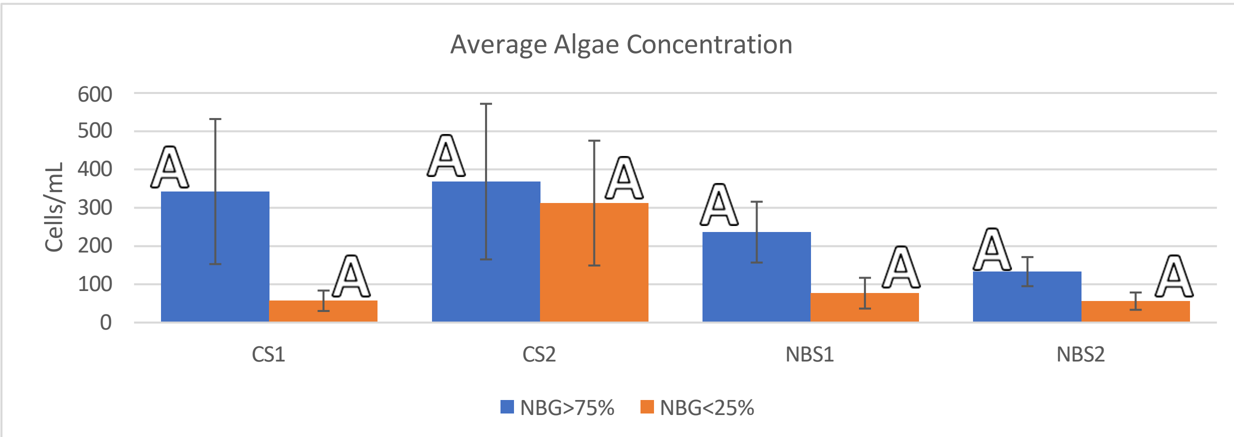

Figure 87: Algal community concentration averages by site and nanobubble generator status. No significantly different averages present.

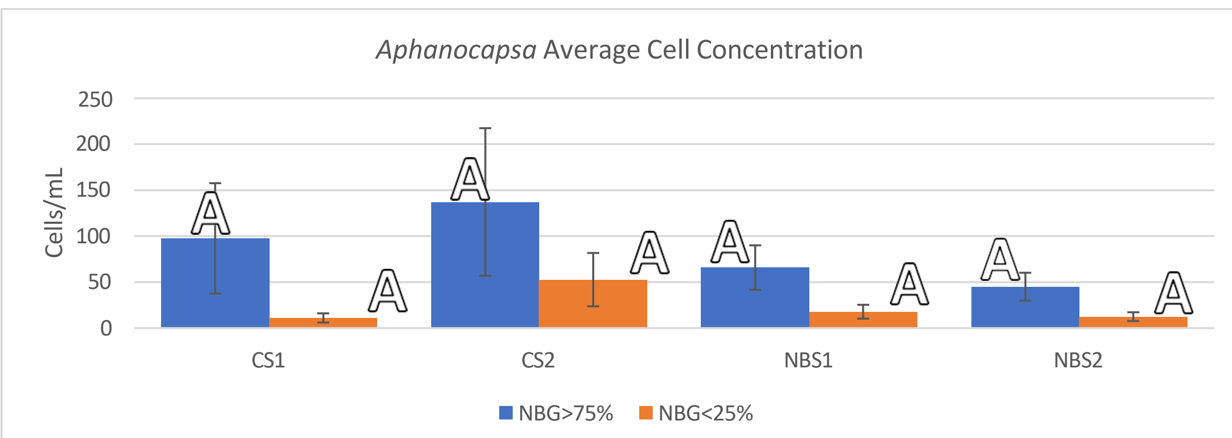

Figure 88: *Aphanocapsa* cell concentration averages by site and nanobubble generator status. No significantly different averages present.

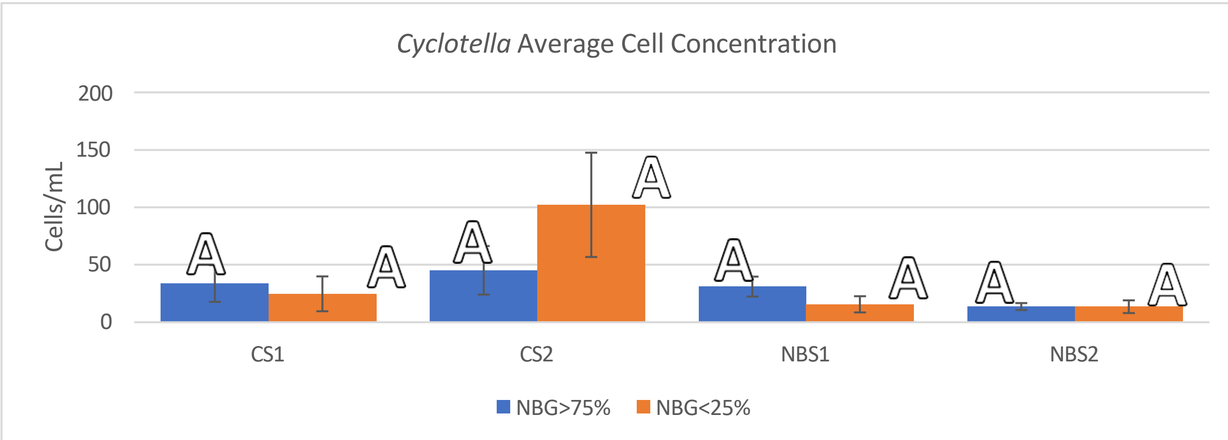

Figure 89: *Cyclotella* cell concentration averages by site and nanobubble generator status. No significantly different averages present.

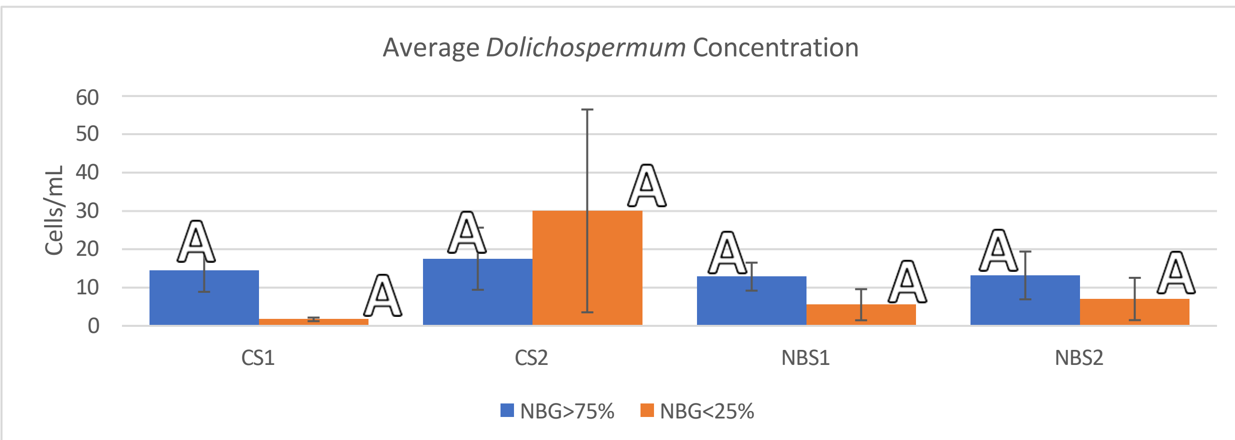

Figure 90: *Dolichospermum* cell concentration averages by site and nanobubble generator status. No significantly different averages present.

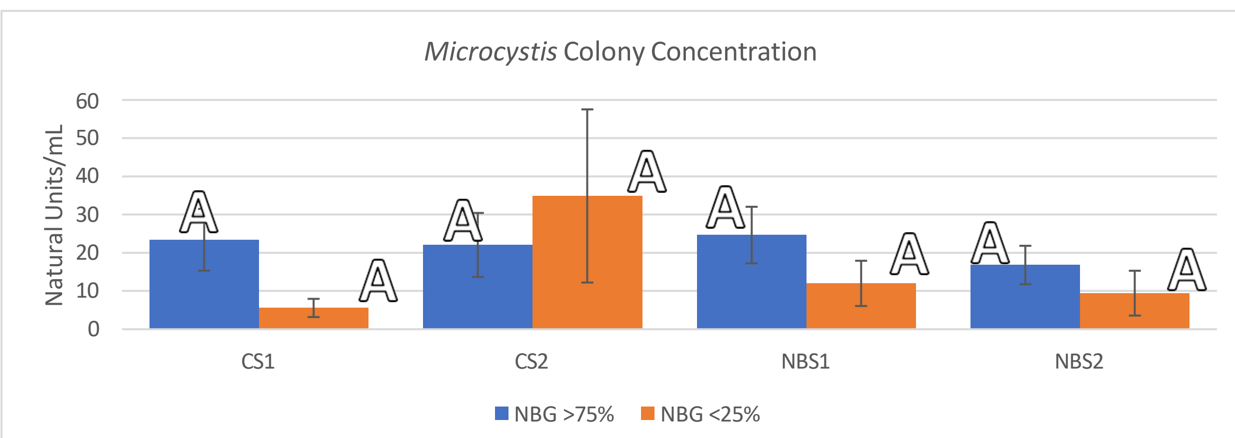

Figure 91: *Microcystis* colony concentration averages by site and nanobubble generator status. No significantly different averages present.

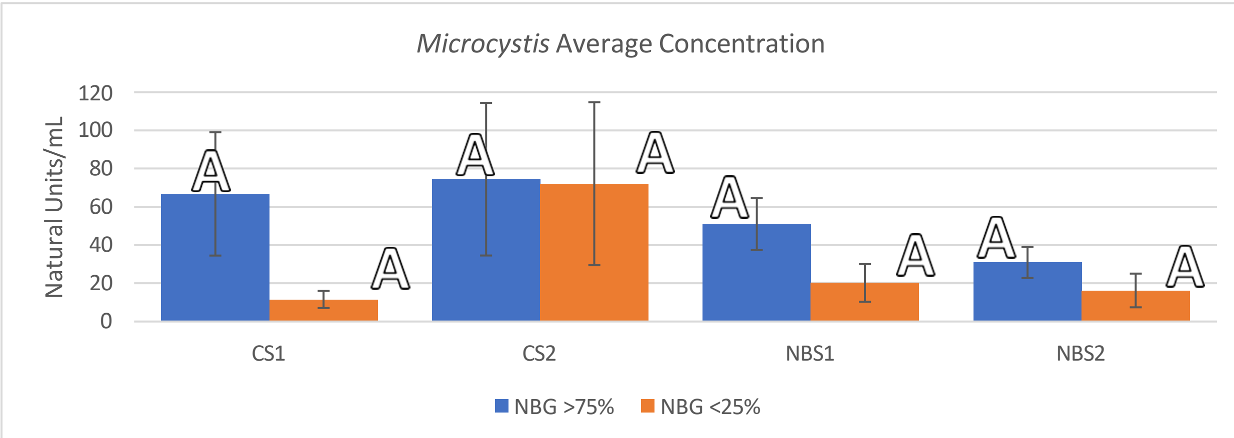

Figure 92: Total *Microcystis* cell concentration averages by site and nanobubble generator status. No significantly different averages present.

All connecting letters reports indicate that there are no significant statistical differences between average algal cell concentrations in control areas as compared to nanobubble treatment areas for *Aphanocapsa*, *Cyclotella*, *Dolichospermum*, *Microcystis* colonies, total *Microcystis*, and total algae community (*Euglena* data was unable to be transformed to meet required assumptions and was therefore excluded from individual statistical analysis. *Euglena* cell counts were successfully included in total algal concentration statistical analysis). The lack of statistical differences in algae concentration averages on sampling dates where nanobubble generators were above 75% (NBG>75%) indicates that nanobubble generators had no impact on major algae genera concentrations or total community algae concentrations during this project. The lack of statistical differences in cell concentration averages when nanobubble generators were not operational (NBG<25%) may suggest mixing within the marina. This could be a factor in interpreting the viability of nanobubble generators as a harmful algal bloom deterrent through direct control of algae concentration. If mixing in the marina homogenized nanobubbles levels across all sampling areas, results will be compromised. Similarly, if marina circulation influenced algal cell distribution, similar unclear conclusions will be generated. Additional investigation into factors promoting marina mixing needs to be conducted before definitive conclusions can be made about the efficacy of nanobubble generators as a treatment for harmful algae blooms.

Microcystis cells commonly form multicellular groups, or colonies, to improve survival rates in their environment. The ability to disrupt these natural units may hinder *Microcystis* survival and therefore contribute to HAB prevention. The ratio of *Microcystis* colonies to *Microcystis* solitary cells was monitored during this project. Potential *Microcystis* colony disruption by nanobubbles was analyzed using one-way ANOVA with connecting letters report (Figure 93).

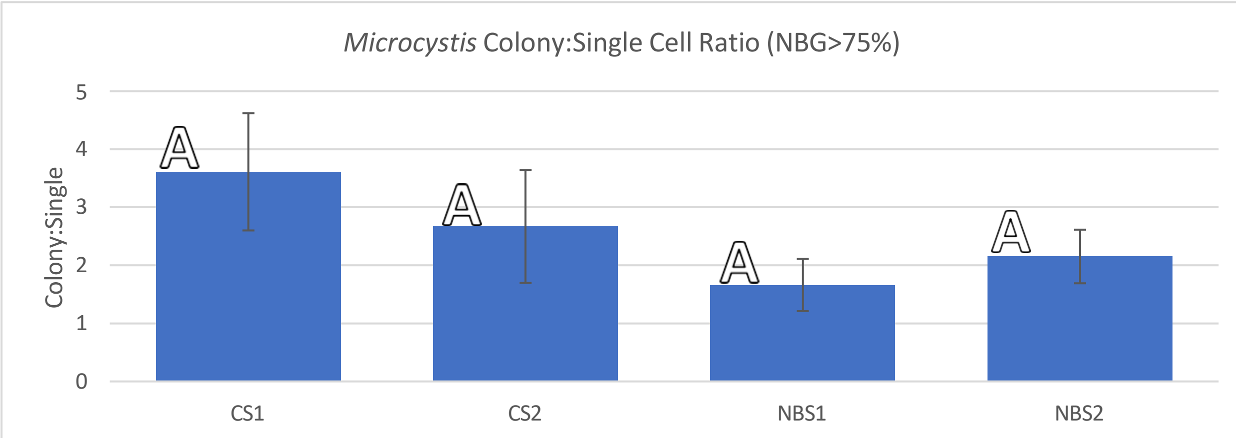

Figure 93: Average *Microcystis* colony to solitary cell ratio at each treatment site. Data from dates with NBG>75%. No significantly different averages present.

Consistent connecting letters report indicates that the average *Microcystis* colony to single cell ratio across sampling sites is not significantly different when nanobubble generators are operational. This indicates that nanobubble generator operation did not cause a significant difference in the disruption of *Microcystis* colonies between the control and treatment zones in Pahokee Marina during this study period.

While all four cyanotoxins (microcystins, cylindrospermopsin, anatoxin-a, saxitoxin) were present in Pahokee Marina during this project (Table 12), only microcystins were present at the requisite frequency as to allow formal statistical analysis. To examine if nanobubble presence influenced the production of microcystins, toxin production per colony of *Microcystis* was calculated for each site. The average toxin per colony for each site was then analyzed using one-way ANOVA and connecting letters report (Figure 94).

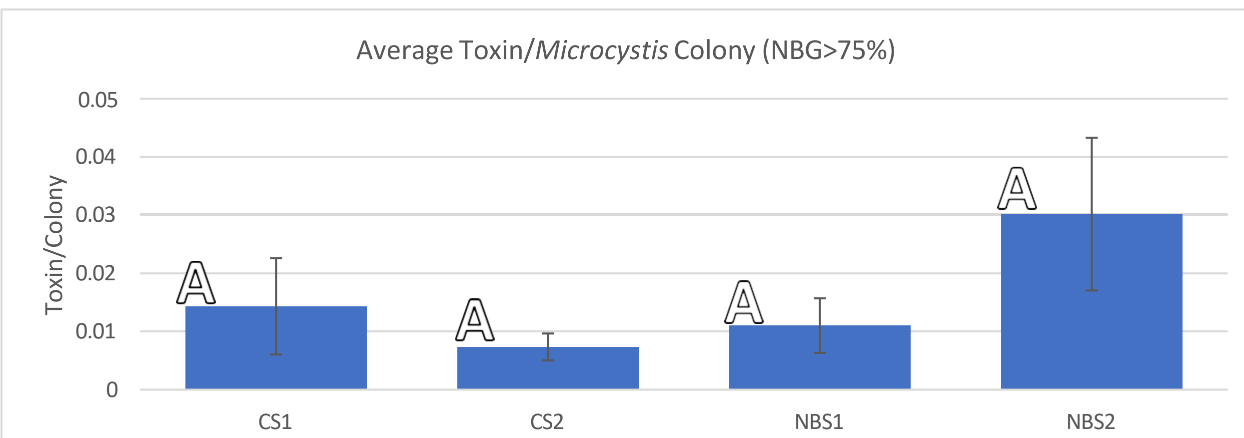

Figure 94: Average microcystins toxin level per *Microcystis* colony at each treatment site. Data from dates with NBG>75%. No significantly different averages present.

Consistent connecting letters report indicates that the average cyanotoxin to colony ratio between sites is not significantly different when nanobubble generators are operational. This suggests that nanobubble generator operation did not influence the production of microcystins between the control and treatment regions of Pahokee Marina during this study.

While primary data analysis focused on the relationship between nanobubble presence and the algal community features (concentration, colony formation, and cyanotoxin generation), additional relationships were also investigated. To examine other possible interactions between nanobubbles, nutrient levels, and the algal community, all measured responses gathered during this project (when the NBG>75% threshold was met) were used to generate a multivariate correlation table. Correlation coefficients (ranging from -1 to 1) were generated between all measured responses and are shown in Table 16 to Table 18. Note that values from 0.3 to 0.5 indicate weak positive correlations, 0.5 to 0.7 indicate moderate positive correlation, and 0.7 and above indicate strong positive correlations. Inversely, values from -0.3 to -0.5 indicate weak negative correlations, -0.5 to -0.7 indicate moderate negative correlation, and -0.7 and below indicate strong negative correlations. Negative correlations have been indicated with shades of red; positive correlations with shades of green (the darker the color, the stronger the correlation).

		Ammonia			Nitrate			Nitrate, Nitrite		
	CS1	-0.06586	0.14855		0.143232	0.124394		0.147154	0.125346	
Aphanacapsa	CS2	-0.20651		-0.11093	0.11953		0.072985	0.118339		0.074386
	NBS1	-0.1695	0.11956		0.047509	0.07422		0.054987	0.06893	
	NBS2	-0.00412			-0.27889			-0.27796		
	CS1	-0.0989	0.05475		0.015516	0.030976		0.019589	0.033039	
Cyclotella	CS2	-0.02327		-0.02183	0.051795		0.035511	0.052381		0.037194
	NBS1	-0.09817	0.02899		0.133424	0.054875		0.134632	0.057186	
	NBS2	0.204963			-0.16882			-0.16495		
	CS1	0.148	0.049427		-0.52035	0.38726		-0.51966	0.38855	
Dolichospermum	CS2	-0.11095		0.026568	-0.24843		-0.41637	-0.25332		-0.41596
	NBS1	0.101508	0.008248		-0.5082	0.44513		-0.50214	0.44313	
	NBS2	-0.10289			-0.4292			-0.42982		
	CS1	0.003877	0.032354		0.154333	0.104672		0.158724	0.107671	
Euglena	CS2	0.10396		0.053619	0.001036		0.088837	0.002174		0.090819
	NBS1	0.049156	0.061153		0.09727	0.066067		0.098059	0.066817	
	NBS2	0.051436			-0.05036			-0.05191		
	CS1	-0.04414	0.10252		-0.14943	0.08099		-0.14497	0.07908	
Microcystis Colony	CS2	-0.15512		-0.09138	-0.02196		-0.13551	-0.02238		-0.13366
	NBS1	-0.18194	0.09141		-0.13274	0.21131		-0.13109	0.20931	
	NBS2	0.131236			-0.35056			-0.3487		
	CS1	-0.03866	0.08964		0.1781	0.153782		0.18257	0.155339	
Microcystis Single	CS2	-0.12673		-0.05106	0.14284		0.12974	0.142194		0.131309
	NBS1	-0.10098	0.05563		0.224092	0.125018		0.230354	0.129532	
	NBS2	0.048387			-0.06405			-0.06426		
	CS1	-0.04144	0.0946		0.102491	0.104078		0.107123	0.10575	
Microcystis Total	CS2	-0.13419		-0.06502	0.110021		0.065272	0.109414		0.06701
	NBS1	-0.16906	0.08785		0.083837	0.03562		0.08909	0.03152	
	NBS2	0.108267			-0.25293			-0.2519		
	CS1	-0.03514	0.08411		0.12764	0.111085		0.132311	0.112968	
Algae Community	CS2	-0.126		-0.04719	0.099598		0.072399	0.099019		0.074187
	NBS1	-0.07218	0.03747		0.086658	0.02314		0.091336	0.01949	
	NBS2	0.019482			-0.27123			-0.27076		

Table 16: Correlations between major algae genera and nutrient levels per treatment site. All data from

sampling dates with NBG>75%.

		Nitrite			Ortho-P			Total P		
Aphanacapsa	**CS1**	-0.09773	-0.04961	-0.02446	-0.1037	-0.04958	-0.03851	-0.22507	-0.25174	-0.19704
	CS2	-0.02434			-0.02233			-0.28452		
	NBS1	-0.13343	0.04681		-0.22581	-0.00706		-0.1075	-0.11654	
	NBS2	0.311831			0.30429			-0.14141		
Cyclotella	**CS1**	-0.1231	-0.00994	0.088479	-0.13084	-0.01798	0.066321	-0.20738	-0.19294	-0.15378
	CS2	0.054666			0.043742			-0.18005		
	NBS1	0.564315	0.403926		0.413297	0.347248		-0.18184	-0.11193	
	NBS2	0.368012			0.35882			-0.05346		
Dolichospermum	**CS1**	-0.05885	-0.03998	-0.05618	-0.06787	-0.02206	-0.05717	-0.07783	-0.01306	0.142805
	CS2	-0.00016			0.06777			0.090417		
	NBS1	-0.04196	-0.07063		-0.08473	-0.08837		-0.08223	0.307472	
	NBS2	-0.08757			-0.09295			0.417417		
Euglena	**CS1**	-0.07592	-0.07177	-0.05175	-0.08046	-0.08062	-0.06695	-0.03305	-0.02793	-0.07215
	CS2	-0.07415			-0.09013			-0.05518		
	NBS1	-0.07558	-0.02096		-0.12457	-0.04366		-0.31764	-0.19431	
	NBS2	0.317403			0.312453			-0.35277		
Microcystis Colony	**CS1**	-0.10344	0.006599	-0.01515	-0.11388	0.030811	-0.02747	-0.19259	-0.159	0.007546
	CS2	0.09766			0.150203			-0.12799		
	NBS1	-0.04717	-0.04351		-0.16116	-0.09872		0.200255	0.254422	
	NBS2	-0.02345			-0.03234			0.395225		
Microcystis Single	**CS1**	-0.09042	-0.05273	-0.02473	-0.0967	-0.06291	-0.03978	-0.19215	-0.23166	-0.20008
	CS2	-0.03181			-0.04611			-0.27576		
	NBS1	-0.05385	0.06241		-0.10529	0.040448		-0.35184	-0.24022	
	NBS2	0.322338			0.315681			-0.23495		
Microcystis Total	**CS1**	-0.09697	-0.04049	-0.02358	-0.10453	-0.0431	-0.03878	-0.19925	-0.22046	-0.15541
	CS2	-0.00507			-0.00554			-0.24809		
	NBS1	-0.06309	0.016879		-0.16077	-0.0285		-0.13606	-0.01635	
	NBS2	0.164764			0.155547			0.114304		
Algae Community	**CS1**	-0.1019	-0.05642	-0.02527	-0.10857	-0.06129	-0.04294	-0.15968	-0.19978	-0.16619
	CS2	-0.02306			-0.02752			-0.24807		
	NBS1	-0.03797	0.057818		-0.13562	0.008623		-0.27147	-0.14974	
	NBS2	0.279742			0.271236			-0.07703		

Table 17: Correlations between major algae genera and nutrient levels per treatment site. All data from sampling dates with NBG>75%.

Due to 508 compliance requirements, Table 18 has been removed from this document. To access the full document, which does not meet 508 compliance standards, please reach out to InnTech_HAB@FloridaDEP.gov

Table 18: Correlations between major algae genera (left) and nutrient levels (right) as compared to microcystins presence. All data from sampling dates with NBG>75%.

While direct causation cannot be determined from the above tables, several interesting relationships can be noted. For example, *Dolichospermum* concentration is negatively correlated with nitrate presence across all sampling sites (Table 16), possibly indicating that *Dolichospermum*, as a nitrogen fixing organism, is outcompeting other dominant algae during nitrogen limited conditions. Additionally, *Cyclotella* concentration appears to have a positive correlation with ortho-phosphate and nitrate in nanobubble treatment areas, and no correlation with these nutrients in control areas (Table 17). This indicates additional research into the interactions of diatoms, nutrients, and nanobubbles may be required.

Additional relationships present themselves when nanobubble generator operational status is included as a measured response (Table 19)

		NBG
Aphanacapsa	CS1	0.187400569
	CS2	0.149900583
	NBS1	0.239581723
	NBS2	0.262445665
Cyclotella	CS1	0.125004085
	CS2	-0.17782201
	NBS1	0.141995714
	NBS2	0.011138103
Dolichospermum	CS1	0.229808515
	CS2	-0.15072796
	NBS1	0.123709768
	NBS2	0.136693106
Euglena	CS1	0.173053941
	CS2	-0.00334975
	NBS1	0.166040565
	NBS2	0.078063539
Microcystis Colony	CS1	0.272332488
	CS2	-0.02742973
	NBS1	0.14036802
	NBS2	0.16230404
Microcystis Single	CS1	0.205270761
	CS2	0.085710691
	NBS1	0.243575246
	NBS2	0.192995771
Microcystis Total	CS1	0.230825657
	CS2	0.06002818
	NBS1	0.2421066
	NBS2	0.205686101
Algae Community	CS1	0.210344879
	CS2	0.058707088
	NBS1	0.238838624
	NBS2	0.221874308

		NBG
Ammonia	CS1	-0.09375383
	CS2	-0.36469749
	NBS1	-0.3657768
	NBS2	-0.24207276
Nitrate	CS1	-0.39425374
	CS2	-0.34934668
	NBS1	-0.3455363
	NBS2	-0.34899405
Nitrate, Nitrite	CS1	-0.390713
	CS2	-0.35298138
	NBS1	-0.34077231
	NBS2	-0.344672
Nitrite	CS1	0.045401647
	CS2	0.117316499
	NBS1	0.045401647
	NBS2	0.117316499
Ortho-P	CS1	0.051575774
	CS2	-0.30579736
	NBS1	-0.26623494
	NBS2	-0.18779456
Total P	CS1	-0.10583962
	CS2	-0.29062979
	NBS1	-0.16148091
	NBS2	0.043524274

		NBG
Microcystins	CS1	0.057683624
	CS2	-0.19820849
	NBS1	-0.17775704
	NBS2	-0.13144102

Table 19: Correlations between major algae genera concentration (left), nutrient levels (top right), and cyanotoxin levels (bottom right) as compared to nanobubble generator operational status. Data collected on 9/9/21, 9/26/22, and 10/12/22 are omitted from the above correlation as NBG operational status is unknown.

These correlations suggest there is a negative relationship between nanobubble generator operation and some nutrient concentrations (primarily ammonia and nitrate). Additional study of nanobubble/nutrient interactions may be required to fully understand this relationship.

All correlations require more study before any causation can be attributed or conclusions drawn.

7.3.4 Observational Data Interpretation

Visual observations were noted during all sample collection trips to Pahokee Marina. Visible algae were common, but not guaranteed. Mild to moderate bloom conditions were observed during this project, with some irregular algae accumulation occurring due to marina wall and dock catchment. Wildlife presence

was consistent in and around the marina and typically included shorebirds, lizards, snakes, alligators, turtles, and fish. Fish and bird carcasses were observed frequently in the marina, but never in excess. A community presence was often seen at and around the marina, primarily patrons of the adjacent campground and local anglers utilizing the marina/lake.

This report is submitted in accordance with the reporting requirements of DEP Agreement No. INV017 and accurately reflects the activities associated with the project.

10/5/2023

Signature of Grantee's Grant Manager

Date