2021 TKPOA Laminar Flow Aeration End of Season Report

Prepared Pursuant to
California Water Boards General 401 Water Quality Certification Order (SB14007IN) Requirements and Basin Plan Prohibition Exemption for the Tahoe Keys Property Owners Association Laminar Flow Aeration Trial Project, El Dorado County

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TAHOE KEYS
WATER QUALITY PROGRAM

2021 Laminar Flow Aeration Report

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1.0 INTRODUCTION

The Tahoe Keys Property Owners Association (TKPOA) has been working to create an adaptive, integrated plan to significantly reduce the bio volume of the aquatic invasive species (AIS), or aquatic invasive plants (AIP), infestation in the Tahoe Keys lagoons. There are now 3 macrophyte species causing poor water quality and problems for boaters in the Keys, and threatening Lake Tahoe's prestigious clarity with their fragments migrating out through the channels. These species are Eurasian watermilfoil (*Myriophyllum spicatum*), curlyleaf pondweed (*Potamogeton crispus*), and the native coontail (*Ceratophyllum demursum*). Since the 1980s, the TKPOA has been combating increasing amounts of invasive and nuisance aquatic vegetation, with harvesting and fragment collection being the only methods currently available for management at the scale required. As the AIP density has increased in the waterways, so has the accumulation of organic material at the benthic layer. This accumulation of detritus, referred to herein as the "muck layer", promotes aquatic vegetation growth and creates ideal conditions for harmful algal blooms (HABs) (Hoyer, et al., 2017). In the past few years, the incidence of HAB's has increased to the extent their release of toxins required pet-warning signs (Appendix C) to be posted (California Cyanobacterial and Harmful Algal Bloom (CCHAB) Network, 2022).

In 2019, the TKPOA implemented a Laminar Flow Aeration (LFA) project in an attempt to reduce the muck layer and circulate the water column. LFA is a technology originally used for improving water quality in wastewater treatment plants by assisting in the organic breakdown of sludge. LFA has recently been adapted for water body restoration by accelerating a water body's natural capability to process nutrients, purge harmful gases like ammonia and hydrogen sulfide, precipitate iron and manganese, and keep down algae growth.

2.0 PROJECT DESCRIPTION

Laminar Flow Aeration (LFA) is a technology used for improving water quality where there is consistently low dissolved oxygen and a buildup of fine organic sediment. LFA uses microporous ceramic disks, called diffusers, that are placed throughout the area needing oxygen. These disks are connected by self-sinking hoses connected to an air compressor. Air is pumped through the system, creating an abundance of bubbles that rise and create laminar flow, and provide oxygenation to the bottom of the water column where dissolved oxygen is typically the lowest.

Increasing dissolved oxygen in the sediment layer triggers a reaction that converts (through oxygenation) ammonia into nitrite and then nitrate. This process is believed to be in part responsible for the reduction in the organic matter seen in other LFA studies. Organic matter, a combination of animal and plant detritus, contains carbon and nitrogen as carbohydrates, lipids, amino acids, and proteins. The increase in dissolved oxygen and the disruption of organic matter aid in hydrolysis of carbohydrates and lipids, and proteolysis of proteins to amino acids, which can lead to nitrification and denitrification. Figure 1 below shows water circulation with the LFA system.

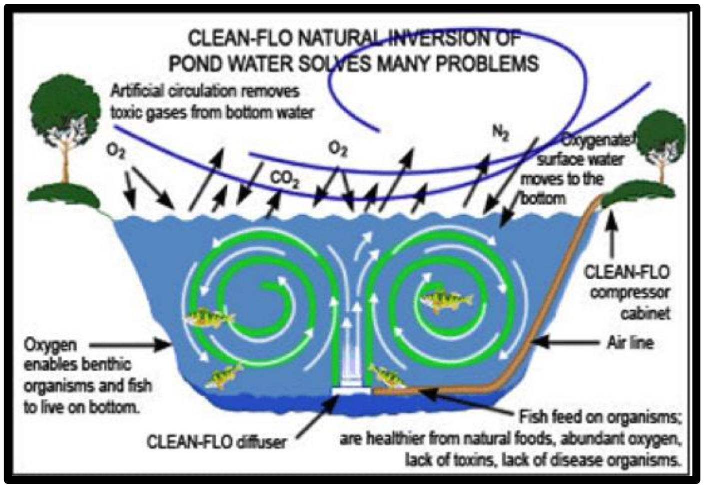

Figure 1. LFA Circulation

SOLitude Lake Management, a natural resources management company, states "Maintaining a constant high dissolved oxygen level throughout the water column from top-to-bottom (including the sediment and water boundary layer) delivers the following benefits to the water body:

1. Control of the nutrients that lead to excessive aquatic weed and algae growth (P, N, and CO_2 /HCO_3
2. Prevention of the formation of undesirable toxic gasses, hydrogen sulfide, methane and ammonia, formed as a result of the persistent anaerobic conditions
3. Prevention of the chemical release of Manganese, Iron, and other metals through redox reactions under anaerobic conditions
4. Increase in biological activity in the benthic layer, accelerating the decomposition of organic "muck" at the bottom
5. Fishery improvement as a result of enabling fish to live and feed all the way to the bottom of the water body"

LFA reduces the organic sediment that accumulates at the bottom of lakes and ponds by increasing dissolved oxygen content. This dissolved oxygen is utilized by organic microbes that break down the organic material. Typical installations include the use of additional microbes to stimulate the system. However, due to local permitting restrictions, the TKPOA was not able to use additional microbes, but rather relied on the native microbial populations.

The test site represents typical conditions within the Tahoe Keys lagoons, including dead-end coves and open water areas, to assess water quality and sediment variation by location (refer to Figure 2). The control site, Site 6, is representative of these same conditions and has been monitored without diffuser installation for comparison. LFA equipment was installed in April 2019 and will be kept in place for a minimum of three years.

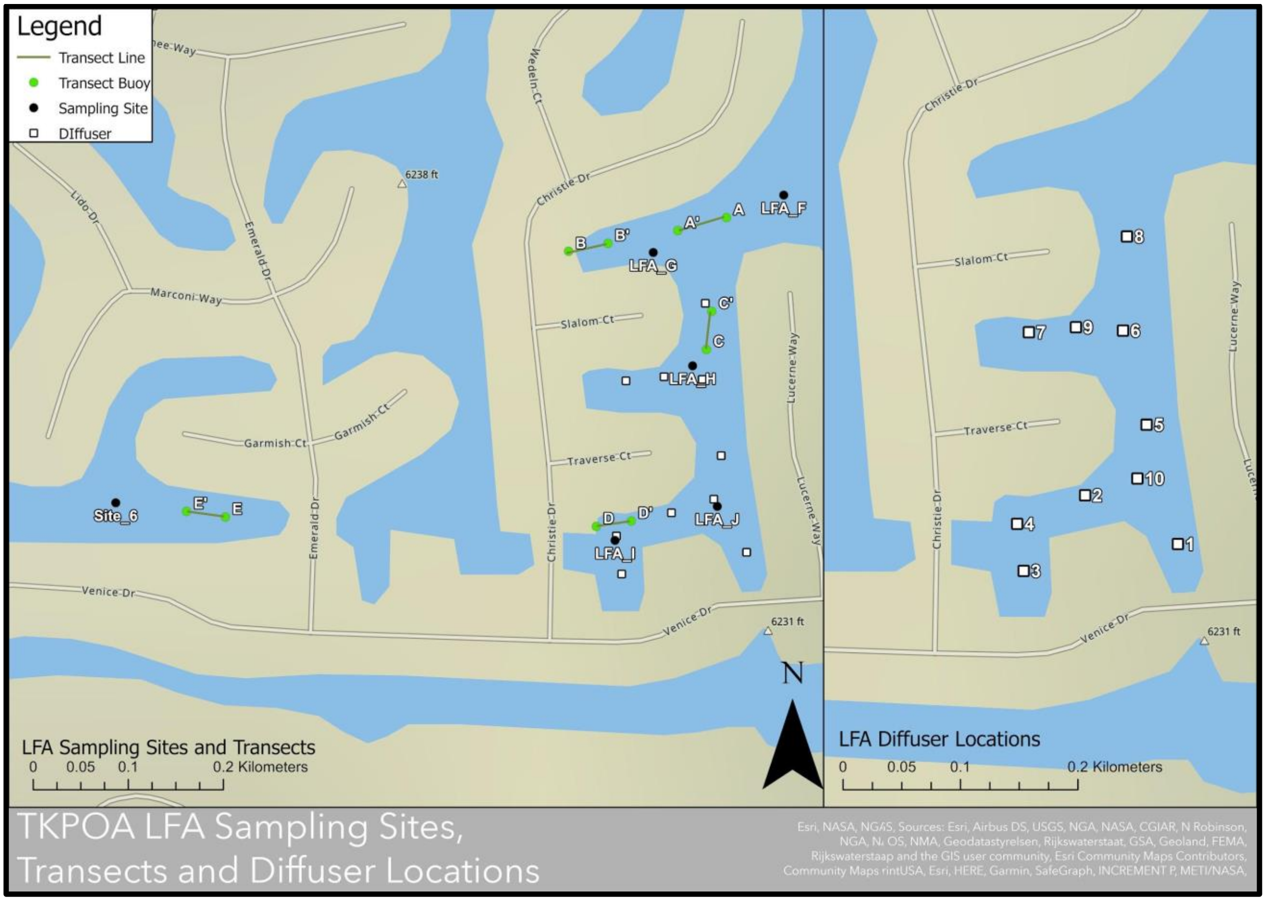

Figure 2. 2021 LFA Project Map

3.0 OBJECTIVES FOR LFA SYSTEM

The system is operating continuously for the minimum three-year test period with sediment, organic matter, dissolved oxygen, and nutrients in the pore (interstitial) water being monitored. The WQ Department has been monitoring the LFA system for 3 years and will follow the sampling/monitoring guidelines in Figure 5 for the fourth year of operation to determine its performance. In accordance with the California Water Boards *General 401 water quality certification order (SB14007IN) Requirements and Basin Plan Prohibition Exemption for the Tahoe Keys Property Owners Association Laminar Flow Aeration Trial Project, El Dorado County* of the objectives for the LFA system are:

1. Increase Dissolved Oxygen (DO) levels at the sediment-water interface and throughout the water column to promote a healthy ecosystem, and encourage chemical reduction of sediments
2. Reduce organic matter in sediments around the LFA diffusers
3. Circulate the water column to decrease the opportunity for Harmful Algal Bloom (HAB) occurrences
4. Reduce the habitable environment for aquatic macrophyte growth

4.0 METHODS

Water samples were collected by TKPOA Water Quality staff according to the monitoring schedule created in accordance with the TKPOA and California State SWAMP Protocols.

Sampling is further discussed in the following sections. Sediment level reduction is monitored and evaluated by TKPOA Water Quality staff. Evaluations are conducted using Lowrance hydroacoustic scanners. The geo-referenced output from the scans were uploaded for analysis and to generate "heat maps" using the BioBase software. The scanners use sonar technology to measure underwater topography. Sediment organic matter reduction is monitored twice a year by sediment sampling at the 6 sampling sites.

4.1 System Layout

Clean-Flo International was granted the contract to design an LFA system for the Tahoe Keys, strategically placing ten diffusers in the locations shown below in Figure 3. Due to the 2019 cyanobacteria bloom, the TKPOA moved two of the ten diffusers on August 12, 2020 after consulting with Clean-Flo International, LLC. This provides better treatment with more diffusers in a smaller area, approximately 2 diffusers per acre. The compressor is located in TKPOA's Well #2, located near the bridge on Venice Drive. The diffuser airlines run from Well #2, under the bridge on Venice Drive, and out to their designated waterway locations.

Figure 3. Original Diffuser Locations (Left) and New Diffuser Locations (Right)

This system was specifically designed for the project area's size and water depth. It consists of a 7.1 horsepower compressor, variable frequency drive, 10 microporous ceramic diffusers (refer to Figure 4), stainless manifold with equal number of ports and control valves, and approximately 9,000 feet of self-sinking airline.

Figure 4. Diffuser

4.2 System Maintenance

In Spring and Fall, both compressors received an oil and filter change. On site visual monitoring occurred daily to assure the system was active and each diffuser was functioning properly. Every time maintenance was performed, it was recorded in the LFA Maintenance Logbook (Appendix D) as well as any issues/power outages affecting the system.

4.3 Monitoring

Water quality monitoring (turbidity, pH, dissolved oxygen (DO), oxidation reduction potential (ORP), specific conductance (SPC), temperature, phycocyanin (PC) and chlorophyll-a (chl-a) took place bimonthly while nutrient samples (total nitrogen (TN), orthophosphate (P)) and cyanobacteria samples were taken monthly. Sediment samples were taken bi-annually, once in the Spring and once in the Fall, along with an annual macrophyte survey. Hydroacoustic scans occurred bimonthly as well to determine plant density/biomass (refer to Figure 5 for LFA monitoring schedule).

TKPOA LFA Monitoring Schedule	
YEAR 3 - April - November 2021	
Water	
Turbidity / pH / DO / ORP / SPC / Temp / PC / Chl-a	Bimonthly-Tuesdays 0900-1300
Total Kjeldahl Nitrogen / Nitrate + / Nitrite Nitrogen / Ammonia / Orthophosphate	Monthly-Tuesdays 0900 - 1300
Cyanobacteria	Monthly-Tuesdays 0900 - 1300
Sediment	
Sediment Surface Total Kjeldahl Nitrogen / Nitrate + / Nitrite Nitrogen / Ammonia / Orthophosphate	Bi-annual - Spring / Fall 2021
Aquatic Plants	
Plant composition	Annual Macrophyte Survey
Plant Density/Biomass Hydroacoustic Scanning	Bimonthly- Tuesdays 0900-1300

Figure 5. LFA monitoring schedule

1. Equipment
   Water Quality data was collected using the YSI ProDSS and the YSI Pro1020, both portable multiparameter water quality instruments. The ProDSS and Pro1020 require regular calibration for accurate readings. The TKPOA also uses an HDS 7 Lowrance system with a 3 in 1 Active Imaging sonar transducer for hydroacoustic scans.

2. Calibration
   The TKPOA Water Quality staff calibrated the ProDSS and Pro1020 bimonthly and no later than one day prior to each scheduled sampling event. Both meters were calibrated according to the manufacturer's instructions. On the day of sampling, DO was calibrated for more accurate readings, a method suggested by the manufacturer. The Pro1020 and ProDSS calibration information was logged on a calibration worksheet and then archived with the sampling data sheets for that event.

3. Parameters
   The monitored parameters for this project consisted of atmospheric and physical attributes such as:
   a. Time Start / End
   b. Air Temperature (°F)
   c. Cloud Cover (%)
   d. Last Precipitation
   e. Wind Speed (mph)
   f. Wind Direction
   g. General Comments

4. Water Quality

Parameters measured at each of the designated sites for water quality are shown in the following table:

Parameter	Method of Measurement	Description
Time of Day	Watch	Time of sampling at each site
Depth	YSI ProDSS or Water Sounder	Depth in meters of water at each site. Used to determine the 5 sampling points in the water column.
Temperature	YSI ProDSS and YSI Pro1020	Measure of thermal energy in a substance, or a measure of how hot or cold a substance is. Temperature influences several other parameters and can alter the physical and chemical properties of water (Fondriest Environmental Inc. 2016)
Specific Conductance	YSI ProDSS	Measure in micro Siemens per centimeter (µS/cm) of dissolved ionic particles in the water. Acts as a good indicator of Total Dissolved Solids.
Dissolved Oxygen	YSI ProDSS and YSI Pro1020	Amount (in parts per million) of oxygen present in water. An important parameter in water quality assessment due to its influence on aquatic organisms. (Fondriest Environmental Inc. 2016).
pH	YSI ProDSS	Measure of acidity or alkalinity of water, with pH 7 being neutral. Surface, mid-point, and bottom were collected during the season to monitor effects of plant biomass on overall pH.
Phycocyanin (PC)	YSI ProDSS	A measure of Phycocyanin in the water column. Phytocyanin is a blue-copper containing pigment found in harmful algae.
Chlorophyll (Chl-a)	YSI ProDSS	Chlorophyll content in the water column.
Oxidation Reduction Potential	YSI ProDSS and YSI Pro1020	Oxidation Reduction Potential (ORP) recorded in millivolts. This is a key component in water quality to determine the health of an ecosystem.
Turbidity (FNU)	YSI ProDSS	Measurement of water clarity using Formazin Nephelometric Units

Table 1. Water Quality Parameters

5. <u>Hydroacoustic Scans</u>

Bathymetry maps were made in April and November with the BioBase analysis software, to compare depths at the LFA sites. Once completed, scans were immediately uploaded to the BioBase system and then reviewed by BioBase analysts. TKPOA Water Quality staff analyzed scan results next to lake level data for more accurate measurements. Lake level data was retrieved from the USGS database.

4.4 Sampling Procedures

During the 2021 season, staff collected samples for sediment, nutrients, and cyanobacteria to comply with the project permits. Samples for nutrients and cyanobacteria were collected according to the procedures instructed by Lahontan Regional Water Quality Control Board (LRWQCB) (Appendix A). Samples for nutrients require field filtration and were conducted by TKPOA Water Quality staff using the method instructed by Babcock Laboratories.

1. Sampling Checklist
    a. Check the weather forecast for sampling day to determine if conditions are appropriate for sampling to occur.
    b. Verify sampling materials delivery.
    c. Verify, if applicable, that the selected analytical lab is scheduled to pick up samples the day after they are to be collected, as hold times on parameters (such as nutrients) require quick processing.
    d. Calibration of the YSI ProDSS and YSI Pro1020 should occur monthly and take place no later than a day prior to the scheduled sampling event. Sampling should not occur if calibration is not completed. Calibrate according to the manufacturer's instructions.
2. Water Quality / Cyanobacteria Sampling Procedure
    a. Review the Sampling Checklist.
    b. Verify that all required sampling equipment is gathered.
    c. Once on the boat with all necessary materials, AIS staff will collect and record the following monitoring parameters onto the data sheet: date, sample collector, boat driver, start time of each sample, air temperature, cloud coverage, last precipitation, wind speed, and wind direction.
    d. YSI: Data will be collected at each site with the YSI ProDSS and the YSI Pro1020. Data collected will include:
        i. Depth (m), water temperature (℃), pH, dissolved oxygen (%), oxidation-reduction potential (ORP), phycocyanin (PC), chlorophyll-a (chl-a).
        ii. Turbidity (FNU) and specific conductivity (uS/cm) are collected at each site midpoint.
        iii. Observations (i.e. the presence of algae, odor, fish, insects, or amphibians in a sample site etc.) are recorded at each site, if applicable.
    e. YSI monitoring protocol includes:
        i. Check that the blue calibration cup is not covering the YSI sensors. If so, remove the calibration cup. Do not pour out the water in the calibration cup.
        ii. Lower the instrument to the desired depth in the water column, according to the data sheet (Bottom, Q1, Mid, Q3, Surface).
        iii. Allow adequate time to ensure the YSI data stabilizes before recording information onto the data sheet.
        iv. For each depth, record YSI reading for each parameter onto the data sheet.
        v. Verify that all required data has been collected before moving on to the next site.
3. Sample Grabs: The following information shall be recorded on each sample bottle at the time of sampling:

a. Sample ID Number (for contract laboratories)
b. Sampling Date and Time
c. Site Name/ Station Code
d. Preservative (optional depending on sampling)
e. Collector's Initials

4. Nutrient Surface Grabs: The Sample Collector should be wearing arm-length, disposable, powder-free gloves when handling the sample containers to prevent contamination of the sample.
   a. Holding the correct sample bottle, lower into the water column until the water reaches two inches below the top of the Collector's elbow-length glove.
   b. Triple rinse the collection bottles before collecting the actual sample, filling roughly three-quarters of the bottle.
   c. Secure sample bottle cap and place in iced cooler for preservation.
5. Cyanobacteria Surface Grabs: **Do not rinse the sample container prior to sample collection.**
   a. The Collector will remove the PETG plastic bottle cap, invert and slowly lower the bottle into the water.
   b. Once the bottle has reached the desired depth, between 1 inch and 11.8 inches, the Collector will again invert the bottle in the water to collect the sample.
   c. Return the container to the surface quickly and, if necessary, pour out a small volume of the sample to allow for homogenization.
   d. Quickly replace the cap and tighten securely.
   e. Place in an iced cooler for preservation.
6. Sediment Samples
   a. Triple rinse sample bucket, Van Veen sediment sampler and sample jar.
   b. Set the Van Veen sampler and lower it to the bottom
   c. Lift the sampler and empty content into sample bucket
   d. Scoop contents into sample jar with gloved hand.
   e. Place the sample jar into an iced cooler for preservation.

4.5 Laboratory Analysis

Laboratory analysis for the water quality and sediment samples were collected to assess ambient water quality conditions in the Tahoe Keys lagoons. The following laboratory analysis results include:

1. Nutrients
   a. Orthophosphorus ("SRP"= soluble reactive phosphate) – Dissolved inorganic phosphorus that is readily available for aquatic plants and algae.
   b. Total Phosphorus – Amount of all forms, dissolved and particulate, of phosphorus present in the sample.
   c. Nitrate-Nitrogen – Amount of nitrogen bound to a nitrate ion present in the sample.
   d. Nitrite-Nitrogen –Amount of nitrogen bound to a nitrite ion present in the sample.
   e. Total Kjeldahl Nitrogen – Measure of ammonia and organic forms of nitrogen.
   f. Total Nitrogen – Sum of all forms of nitrogen, including Nitrate-Nitrogen, Nitrite-Nitrogen, and TKN.
2. Cyanobacteria
   a. Anatoxin-A – A secondary, bicyclic amine alkaloid and cyanotoxin with acute neurotoxicity, produced by seven different genera of cyanobacteria.

b. Cylindrospermopsin – An alkaloid consisting of tricyclic guanidine coupled with hydroxymethyluracil. Zwitterionic, highly water soluble molecule; resistant to high temperatures, sunlight, and pH extremes. Often released from cells into the surrounding water, it bioaccumulates, particularly in organisms low in the food chain such as gastropods, bivalves, and crustaceans.
c. Microcystin – Cyclic non-ribosomal peptides produced by cyanobacteria, known to cause severe hepatic damage principally by inhibiting protein phosphatases. May be released into the surrounding water when cyanobacteria cells disintegrate. Typical environmental half-life of 10 weeks, the breakdown rate is increased under direct sunlight, at high environmental temperatures (>40° C), and extremely low pH (<1) or high pH (>9).
d. Saxitoxin – Produced in freshwater and marine environments. In freshwaters, saxitoxins are produced by cyanobacteria in the genera *Anabaena* sp., *Aphanizomenon* sp., *Planktothrix* sp., *Cylindrospermopsis* sp., *Lyngbya* sp., and *Scytonema* sp. can accumulate in freshwater fish. Also known as paralytic shellfish poisons (PSPs).
e. Total Cyano (16S) – 16S rRNA is a genetic characterization of cyanobacterial strains. Quantitative polymerase chain reaction; process used to enumerate pathogens, algae or specific genes responsible for production of undesirable compounds (i.e., 16S gene[1], microcystin, anatoxin-a).
f. Chlorophyll-a - A pigment found in algal/phytoplankton species and used to measure algal growth in a waterbody.

3. <u>Sediments</u>
   a. Aluminum - Amount of aluminum in the sediment sample.
   b. Phosphorus - Amount of all forms, dissolved and particulate, of phosphorus present in the sample.
   c. Orthophosphorus - Dissolved inorganic phosphorus that is readily available for aquatic plants and algae.
   d. Organic Matter - Total organic material present in the sample.
   e. Ammonia - Measure of nitrogen in the form of NH_4
   f. Total Solids - Measures percent of total solids in the sample
   g. Nitrate Nitrogen – Amount of nitrogen bound to a nitrate ion present in the sample.
   h. Nitrite Nitrogen –Amount of nitrogen bound to a nitrite ion present in the sample.

Three separate laboratory shipments were prepared once sampling was completed (Appendix B). Cyanobacteria samples were sent to Bend Genetics, LLC in Sacramento, California. Nutrient samples were sent to Babcock Laboratories in Riverside, California and sediment samples were sent to WetLab Environmental Testing Laboratory in Sparks, Nevada. Pigment samples were analyzed at the Lahontan Regional Water Quality Control Board's South Lake Tahoe, California, location.

5.0 RESULTS

Box-and-Whisker Plots are used to show results from collected data. Figure 6 represents the upper quartile, median, and lower quartile for the dataset. Extending arms from the box represent the maximum and minimum dataset values while dots outside the arms are outliers.

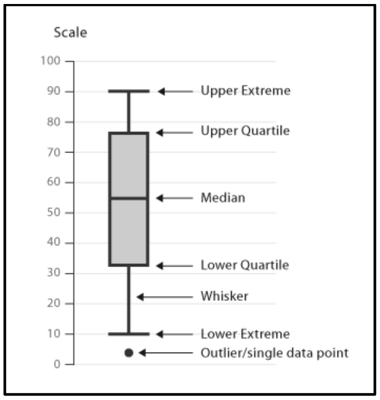

Figure 6. Box and whisker plot description

5.1 Dissolved Oxygen Levels

The figures below show dissolved oxygen (DO) data from the 2021 season. Results are presented for data measured in the bottom quarter of the water column (labeled "B" on the x-axis of Figure 7), the middle of the water column (labeled "M on the x-axis of Figure 7), and the upper, or surface, quarter of the water column (labeled "S" on the x-axis of Figure 7). Due to a battery malfunction with the YSI ProDSS, which held the DO sensor, staff was unable to collect DO data from mid-June to mid-September. TKPOA sent the ProDSS to the manufacturer in mid-June and did not receive it until mid-September. Therefore, 2021 DO data presented may not be considered an accurate representation for the season.

Figure 7 shows the spread of recorded DO levels at each quadrant from April through November, 2021, minus data from mid-June through mid-September. Data from 2021 was then compared to data from 2020 and 2019 shown in Figure 8. The standard deviations show high variability within the data which is inherent in small volume systems with high biomass and seasonal changes.

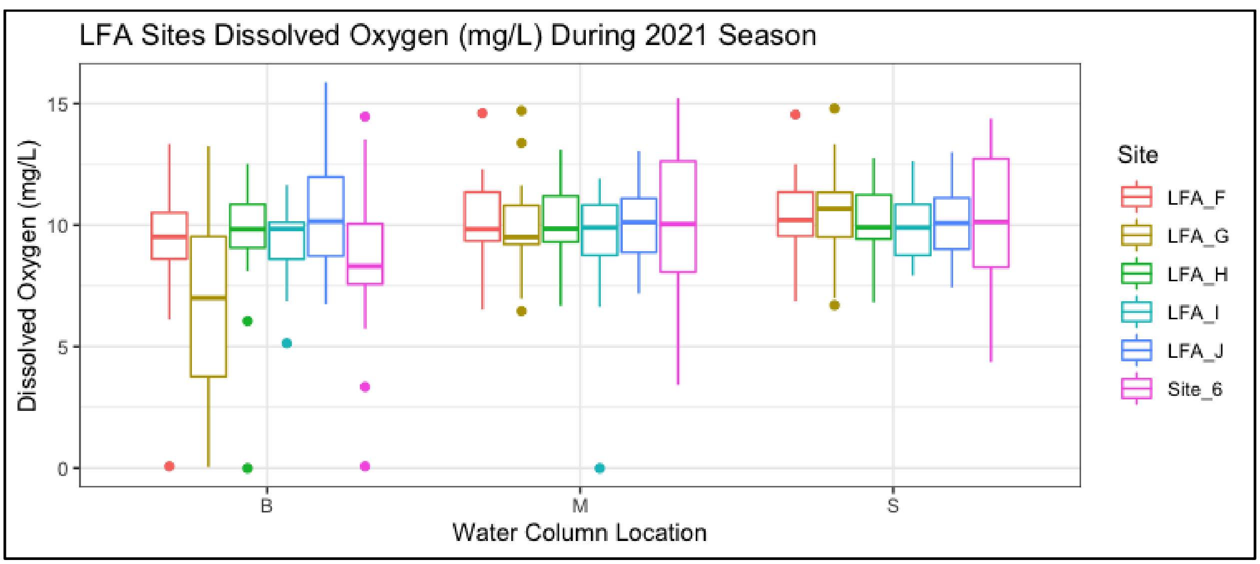

Figure 7. DO for the 2021 season

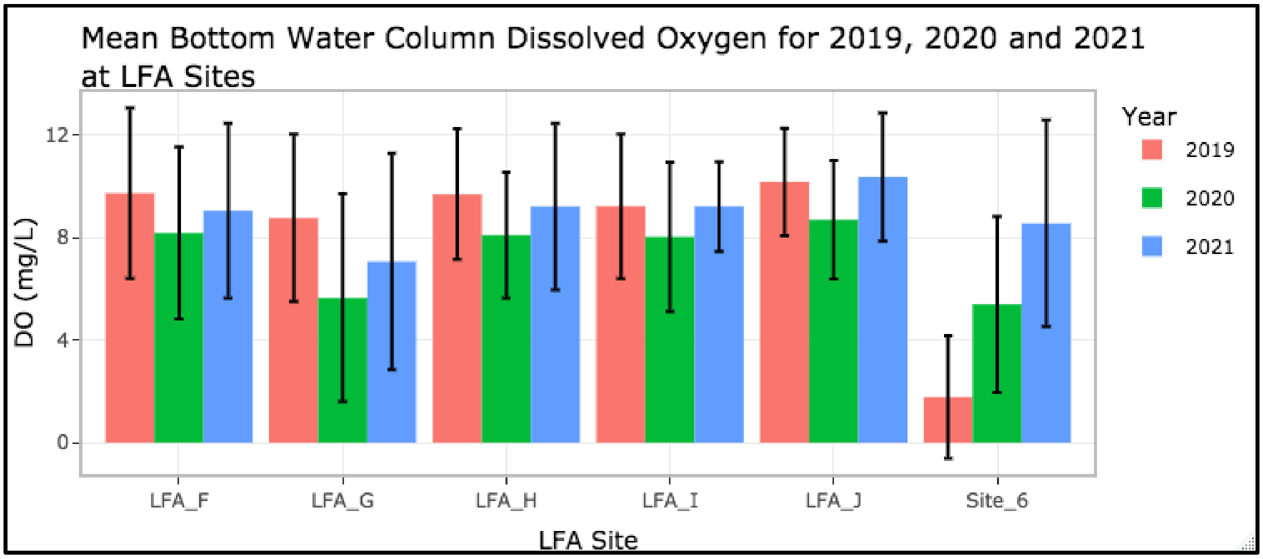

Figure 8. DO means and standard deviation at LFA sites for 2021, 2020 and 2019

5.2 Turbidity

The TKPOA is required to monitor turbidity as a part of the permit NO. WDID6A091810005 issued by the LRWQCB. According to this permit, the TKPOA must keep turbidity in the project site below 3 NTU. If the turbidity exceeds 3 NTU, then the TKPOA must notify LRWQCB. Figure 11 displays the results of turbidity monitoring from the 2021 season. It can be seen that the turbidity did rise above 3 NTU. TKPOA WQ Staff submitted all data sheets to LRWQCB which stated the turbidity levels. However, it is uncertain if the turbidity increase was related solely to operation of the LFA system since the turbidity at the control site, Site 6, also increased above 3 NTU for a short period. During the 2021 season, turbidity levels exceeded the past two years' highest levels, (refer to Figure 12), and all sites were over 3.0 (FNU). This could be due to the effects from the Caldor Fire (additional nutrients from ash) along with shallower waters (~3ft lower in 2021 than 2020). For all three years, a pattern shows high turbidity levels that coincide with the algal blooms which have occurred mid-late July and peak in September. The highest turbidity levels were recorded at the sites located in dead end areas (LFA H, LFA I and LFA J). The mid-summer decrease in between the peaks could be due to the sequestration of water column nutrients by the macrophytes that in turn reduces phytoplankton blooms. It is unknown if high turbidity is primarily due to algae or particulates. With fall senescence (e.g. *P. crispus*) nutrients are released which can add to turbidity (via algal growth) as well as plant tissue particulates (decomposing plants within the water column).

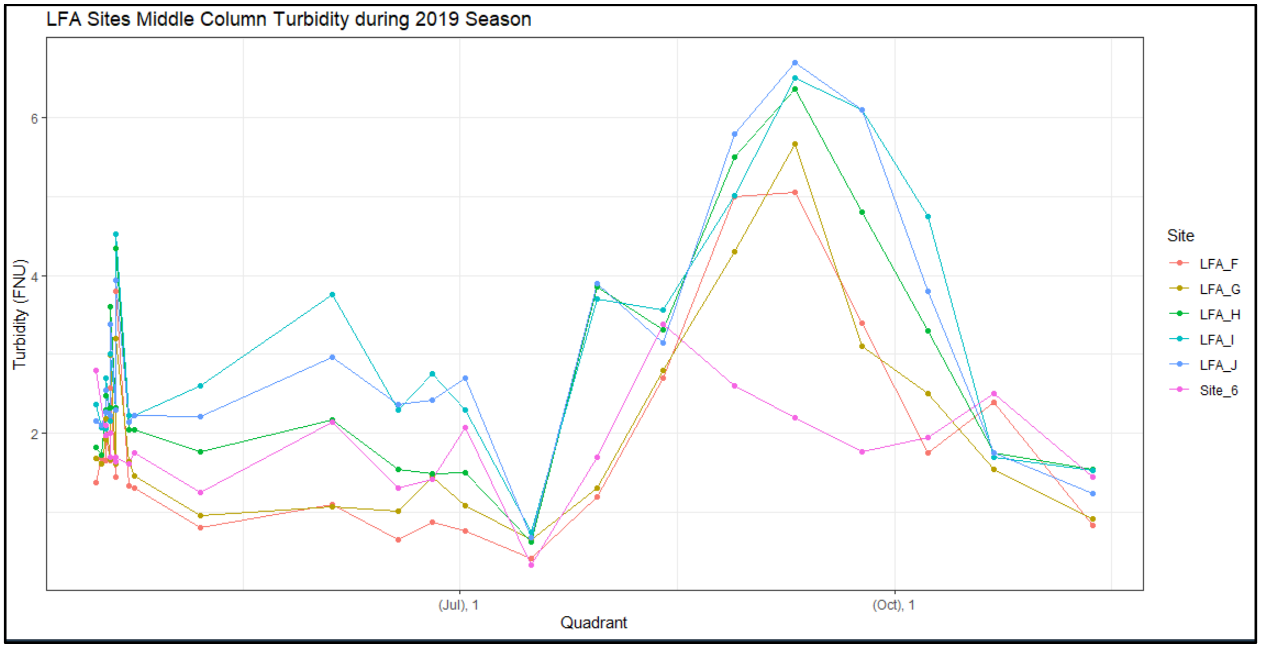

Figure 9. Turbidity During 2019 Season

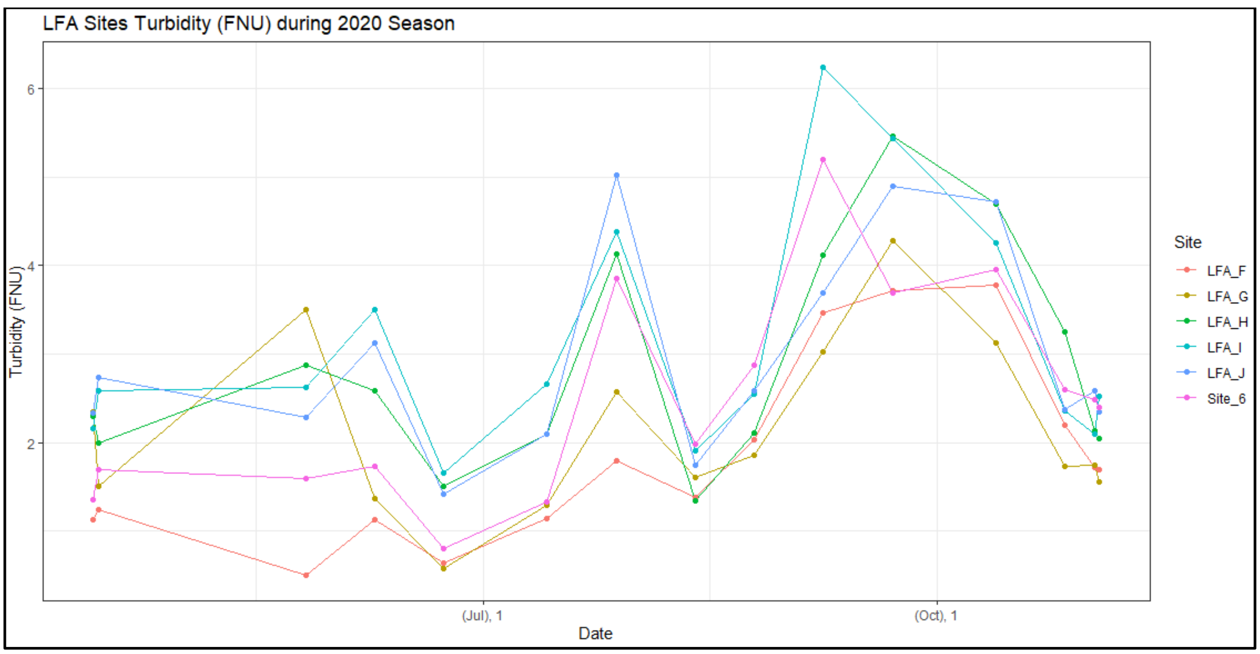

Figure 10. Turbidity throughout the 2020 Season

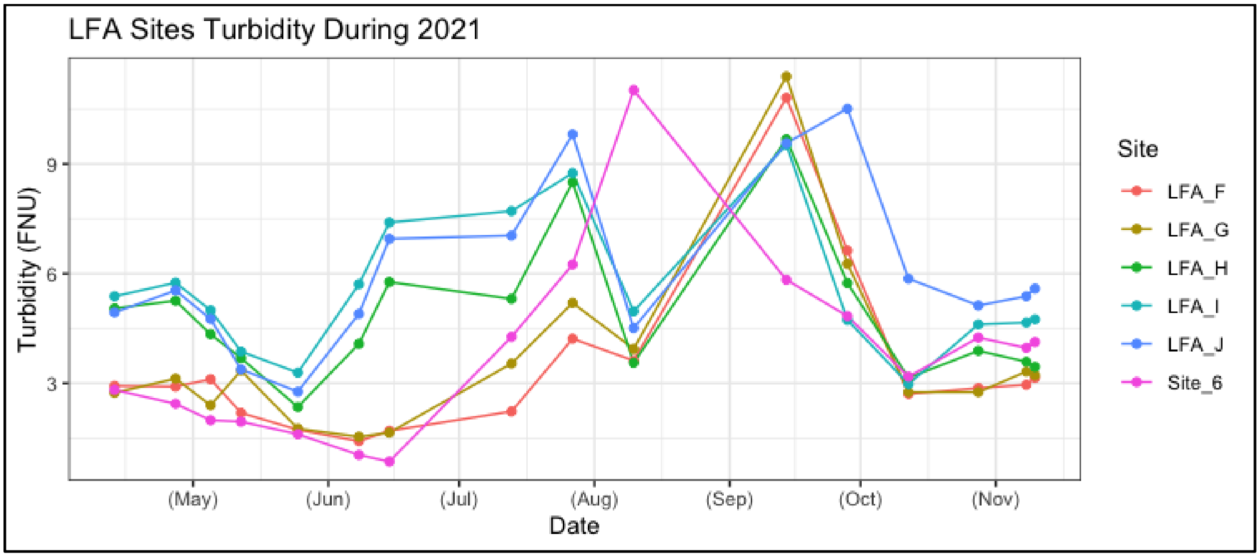

Figure 11. Turbidity throughout the 2021 Season

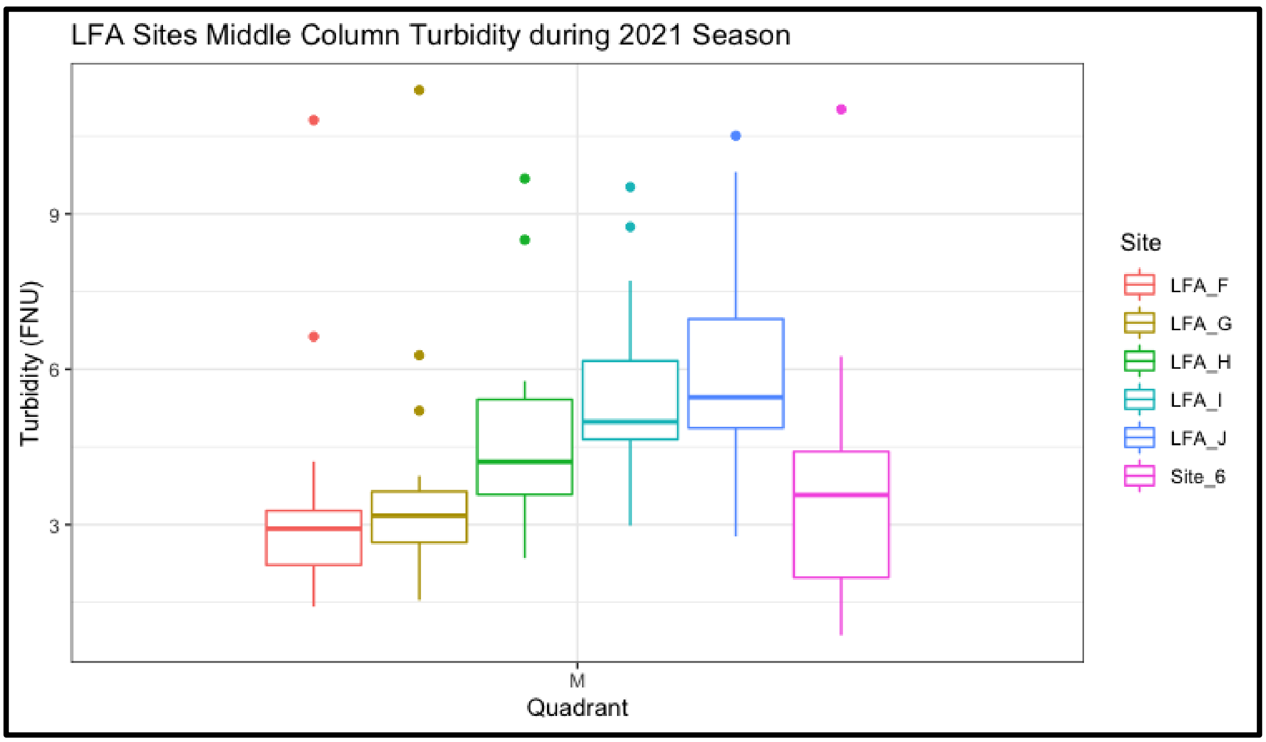

Figure 12. Turbidity spread for the 2021 season

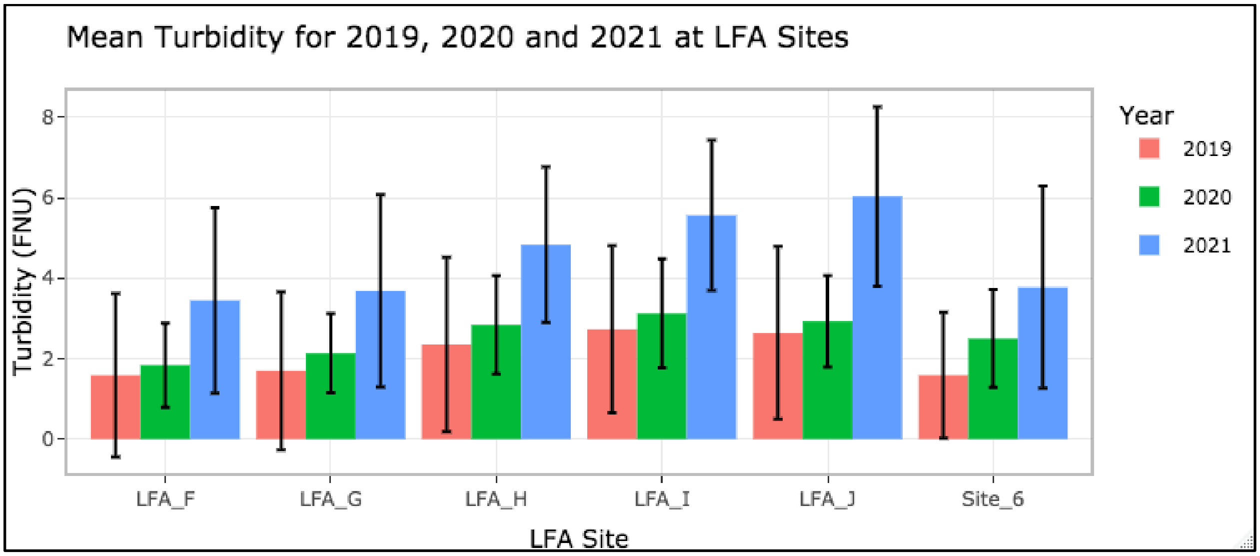

Figure 13. Turbidity Means and SD for 2019, 2020 and 2021

5.3 Hydroacoustic Scans

An objective of the LFA project is to reduce the muck layer/organic matter around the diffusers, thus reducing the availability of nutrients for aquatic plants and algae. In the past, hydroacoustic scans were used in an attempt to monitor the change in muck depth, but due to dense vegetation, the readings were not very accurate. TKPOA will thus refer to organic matter percentages, which are shown in section 5.5, for its muck layer analysis.

In 2021, TKPOA purchased and implemented a new Lowrance HDS7 Live with a 3 in 1 Active Imaging Transducer which is an upgraded system that provides more accurate readings. Figure 14 shows a storyboard for 2019, 2020 and 2021 of biovolume percent within the LFA area. These scans clearly show that the final objective for the LFA system, to reduce the habitable environment for aquatic macrophyte growth, is not being met.

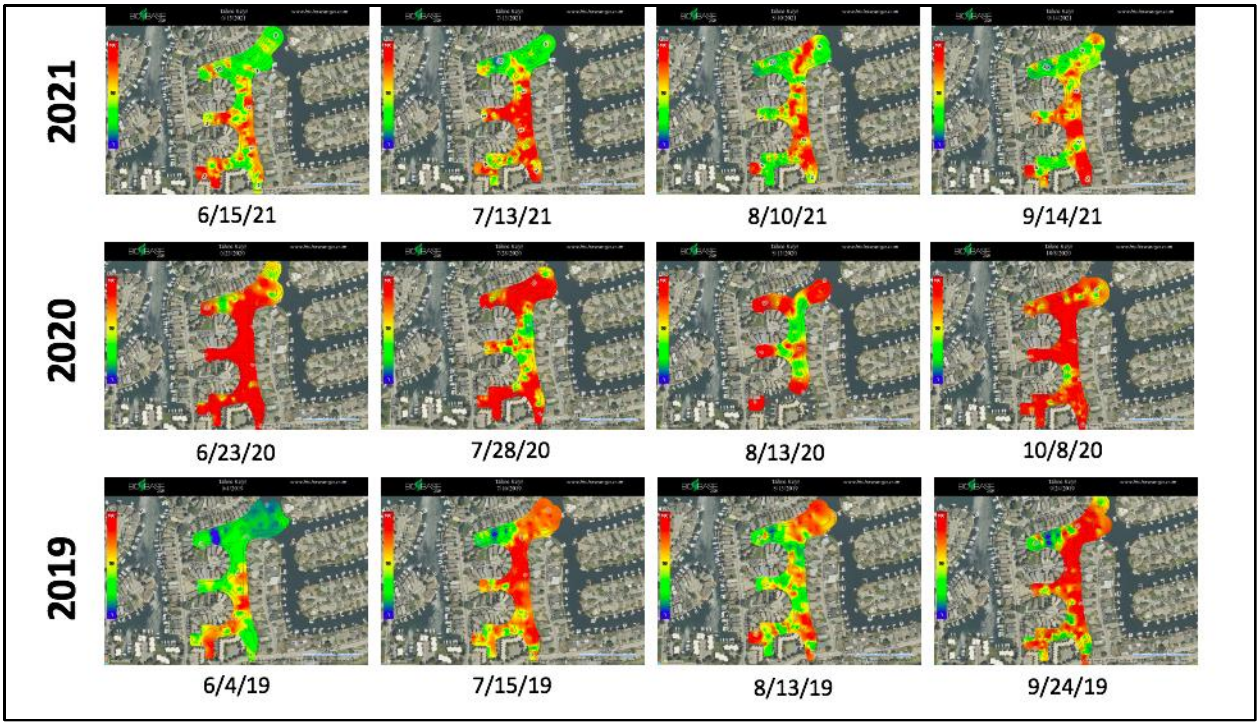

Figure 14. LFA area biovolume percent storyboard for 2019, 2020, 2021

5.4 Cyanobacteria Results

An objective of the LFA system is to reduce cyanobacteria in the project area. To maintain continuity with how the project has progressed the results from 2019 and 2020 are compared to the 2021 results in the section below.

In 2019, anatoxin-a was first detected on 7/16/2019 at Control Site 6. By the next sampling on 7/30/2019, every site had anatoxin-a present. After anatoxin-a was detected, the TKPOA sampled certain water quality sites based on appearance and levels of scum present. The TKPOA posted caution signs (Appendix C) at all of the entrances to the Tahoe Keys and along the waterways to warn boaters and homeowners of the potential danger. The last sampling event to detect anatoxin-a in the 2019 season was on 9/24/21. The next sampling event on 10/8/2019 had non-detects at all sites. Therefore, anatoxin-a was present at the LFA sampling sites for approximately 70 days.

During 2020, TKPOA sampled for cyanobacteria once a month, this is a change from the twice a month sampling that occurred during 2019. In 2020, anatoxin-a was first detected on 7/14/2020 at all sites though the highest levels were at Site 6. The TKPOA followed state procedures and posted caution signs. Anatoxin-a levels peaked on 9/8/20 and was last detected in very low amounts at Site 6 on 10/13/20. Therefore, anatoxin-a was present in the LFA sampling sites for approximately 91 days. The caution signs were posted until cyanobacteria was not detected during the 11/3/2020 sampling event.

During 2021, TKPOA continued to sample for cyanobacteria once a month. Blooms of anatoxin-a were first detected on 7/20/2021 at all sites sampled (LFA F, LFA H, LFA I, Site 6). While no anatoxin-a was detected above 0.20 ug/L on the 8/10/21 sampling event, several cyanobacteria species were present in moderately low to low levels, including *Aphanizomenon* sp., *Woronichinia* sp. and *Dolichospermum* sp. Anatoxin-a was detected again and peaked on 9/14/2021 with larger blooms in LFA I and Site 6. Anatoxin-a was not detected again above 0.20 ug/L throughout the season, though cyanobacteria species *Woronichinia* sp. was still present in low levels in the last sampling event on 11/10/21. Therefore, anatoxin-a was present in the LFA sampling sites for approximately 56 days.

Historically, sampling results show that the cyanobacteria blooms (of anatoxin-a) begin in July and peak in September with a sharp decline into October. This is likely due to the longer days with hotter temperatures in July-September compared to cooling temperatures with shorter days in October. During 2019, the highest amounts of anatoxin-a were found at Sites LFA I, LFA J, and LFA F. In 2020, the highest amounts of anatoxin-a were found at LFA I and Site 6. In 2021, the highest levels of anatoxin-a were also found in LFA I and Site 6.

The following box plots are used to show the differences between the sites while Table 2 is used to show the differences between the years. Figures 15 and 16 show the spread of anatoxin-a at each site in µg/L for 2020 and 2021.These figures show that concentrations were highest at LFA I and Site 6 for both 2020 and 2021. LFA I is a dead end area with no natural shorelines inside the test site while Site 6 is in a more open area that is untreated.

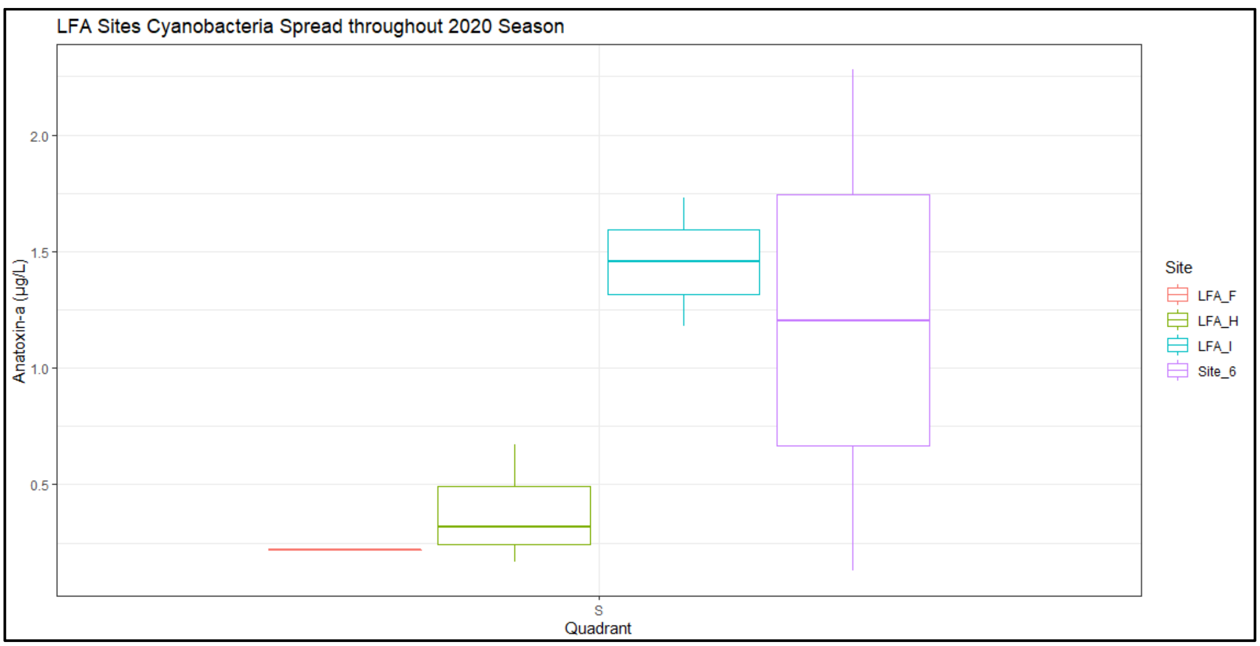

Figure 15. Cyanobacteria spread during the 2020 season

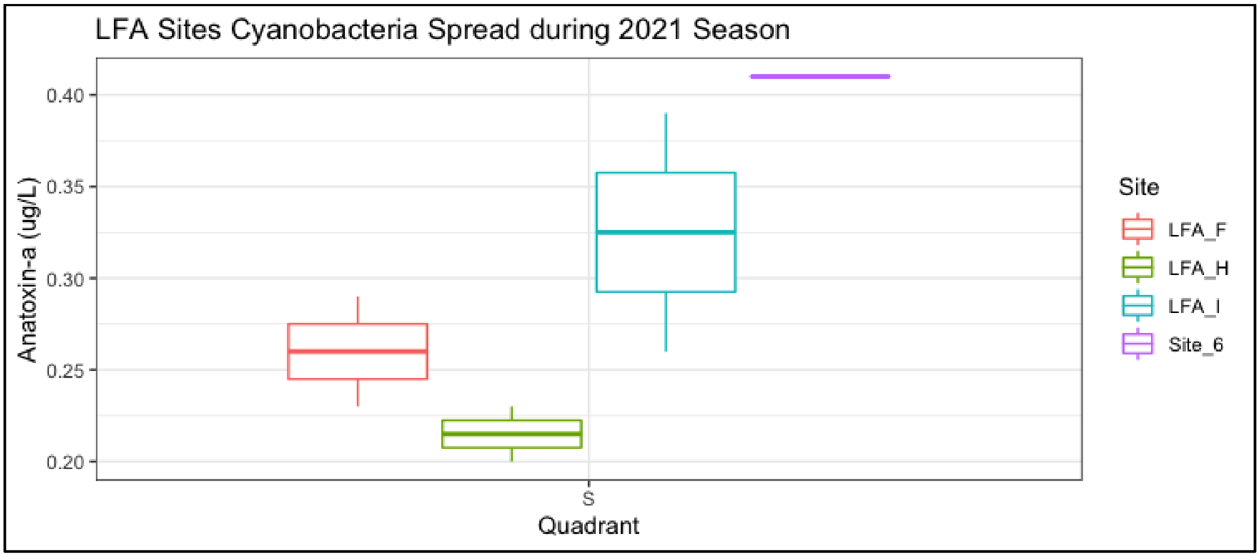

Figure 16. Cyanobacteria spread during 2021 season

Anatoxin-a (µg/L) Means			
Site	2019	2020	2021
LFAF	0.49	0.22	0.26
LFAH	1.79	0.39	0.22
LFAI	0.3	1.45	0.33
Site 6	0.49	1.21	0.41

Table 2. Anatoxin-a (µg/L) means for 2019, 2020 and 2021 season

Overall during the 2021 season, mean cyanobacteria levels were higher in LFA F than during 2020 (by 0.04 ug/L) but were lower in LFA H, LFA I and Site 6 than during 2020 (by 0.17, 1.12 and 0.8 ug/L respectively).

The most prevalent cyanobacteria species found in the lagoons is *Dolichospermum* sp. Sampling results show that *Dolichospermum* sp. colonies formed in the lagoons by June. *Dolichospermum* sp. is a diverse freshwater genera of cyanobacteria that are able to produce different forms of cyanotoxins, including nonribosomal peptide toxin (microcystin), alkaloid toxins (cylindrospermopsin, saxitoxin, and anatoxin-a), and lipopolysaccharides (LPS) (Li et al. 2016). Other species of cyanobacteria that were found that also produce anatoxin-a include *Woronichinia* sp., *Aphanizomenon* sp., *Aphanocapsa* sp., *Microcystis* sp., *Trichodesmium* sp., *Anagnostidinema* sp., *Planktothrix* sp. and *Cuspidothrix* sp. (Appendix D).

5.5 Sediment Sampling Results

The TKPOA Water Quality Staff sampled sediment twice during the 2021 season. The results from these sampling events are shown below. Blank columns represent a non-detect.

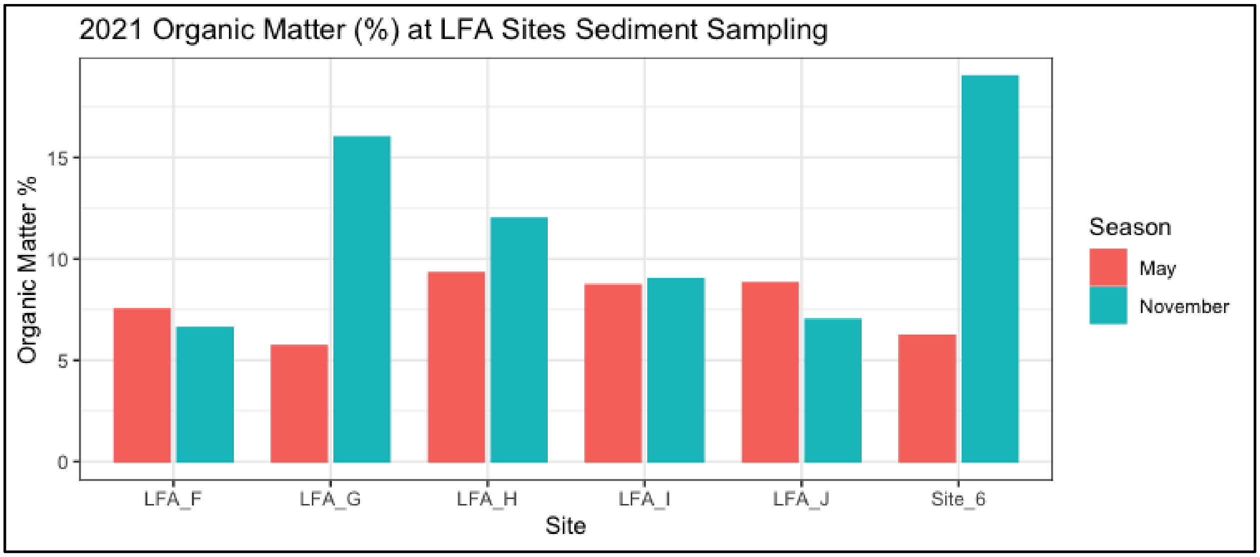

Figure 17. Organic Matter % Results from Spring and Fall Sediment Sampling 2021

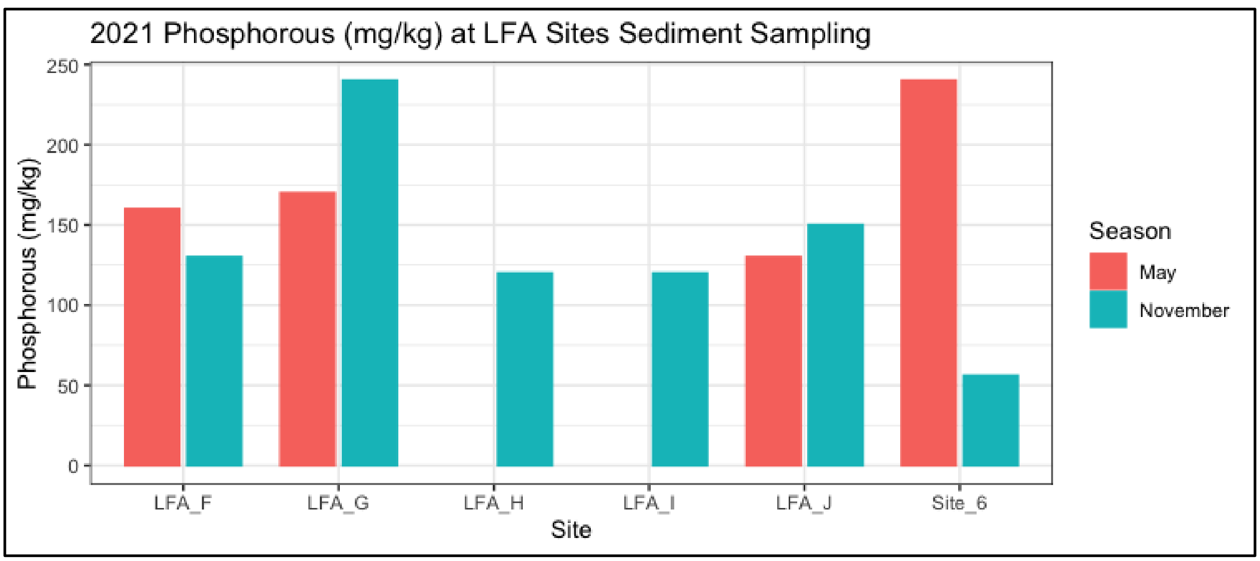

Figure 18. Phosphorous Results from Spring and Fall Sediment Sampling 2021

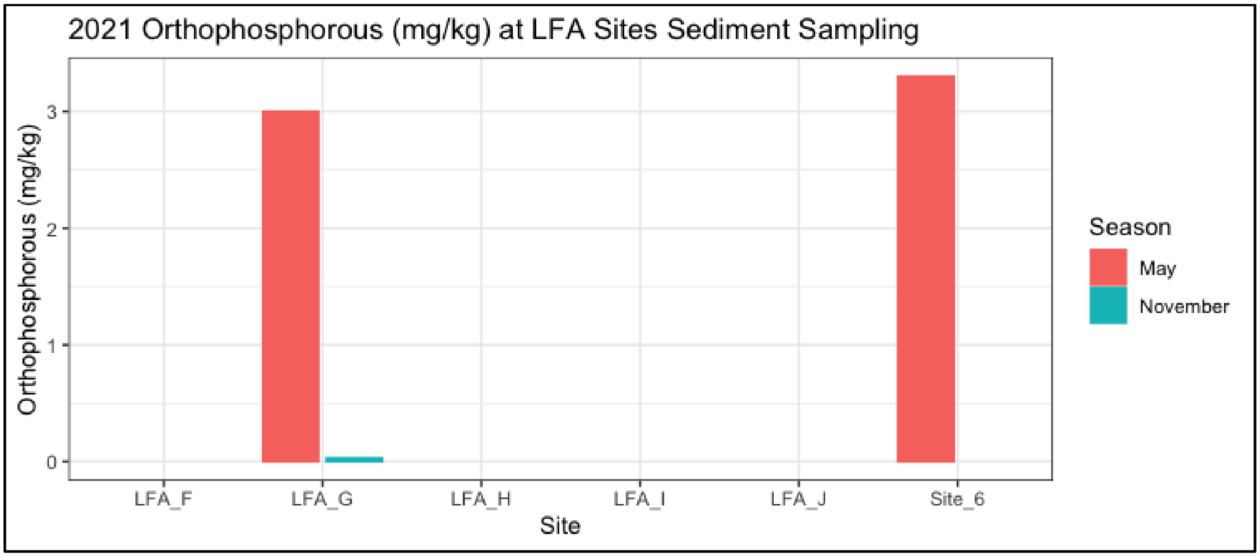

Figure 19. Orthophosphorus Results from Spring and Fall Sediment Sampling During 2021

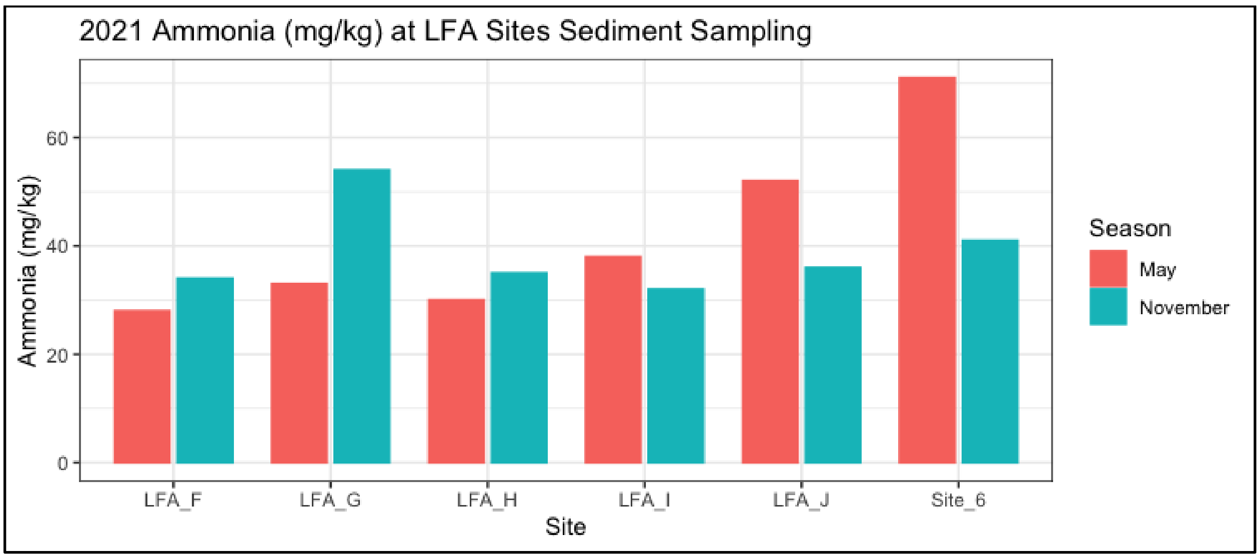

Figure 20. Ammonia Results from Spring and Fall Sediment Sampling During 2021

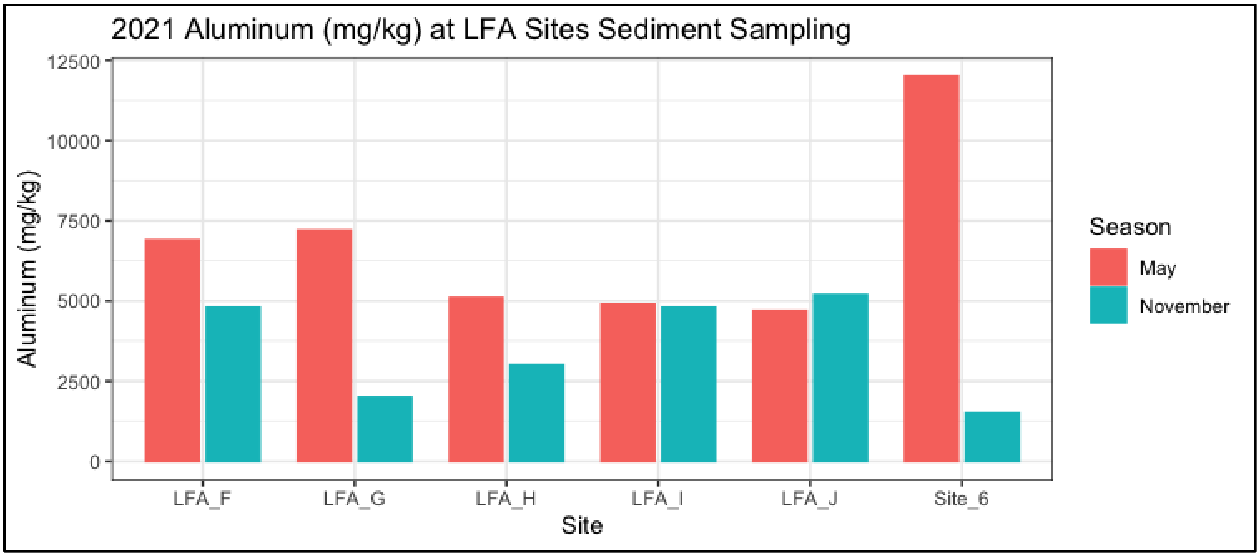

Figure 21. Aluminum (mg/kg) results from Spring and Fall Sediment Sampling During 2021

Sediment sampling occurred on 5/5/2021 (Spring) and 11/8/2021 (Fall). Figures 17- 21 show the sediment sampling results from 2021. Figure 17 shows organic matter percent detected in the samples collected from the 6 different sampling sites. LFA G, LFA H and Site 6 saw an increase in organic matter percent while LFA I stayed the same and LFA J and LFA F had a slight decrease. Figure 19 shows that orthophosphorus (mg/kg) was only detected at LFA G and Control Site 6 in the Spring and detected in a very low amount in the Fall only at LFA G. Figure 21 shows that aluminum levels decreased at LFA F, LFA G, LFA H and Control Site 6 and stayed the same at LFA I and LFA J. Figure 20 shows that ammonium (mg/kg) stayed relatively the same at LFA F, LFA H and LFA I, increased at LFA G and then decreased at LFA J and Control Site 6.

5.6 Nutrient Sampling

The TKPOA staff conducted nutrient sampling twice during the 2021 season at 4 out of the 6 sites: LFA F, LFA H, LFA I and Control Site 6. Samples were analyzed for orthophosphate, ammonia, total phosphorus as P, total nitrogen, nitrate + nitrite nitrogen and total Kjeldahl nitrogen. Figures 22 and 23 below display the total phosphorus and total nitrogen results from these samplings, blank columns are non-detects. Orthophosphate was only detected at LFA I in November at 0.022 (mg/L). The rest of the analyzed elements returned as non-detects. It is likely that if the results were analyzed in µg/mL there could have been more detects for orthophosphate.

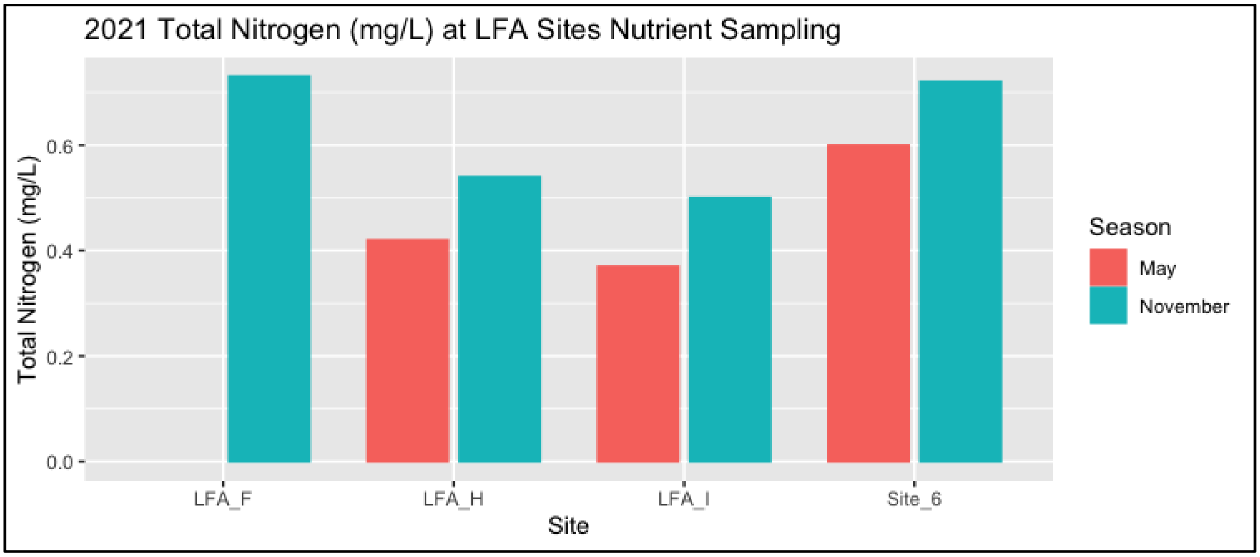

Figure 22. Total Nitrogen Results from Spring and Fall Nutrient Sampling During 2021

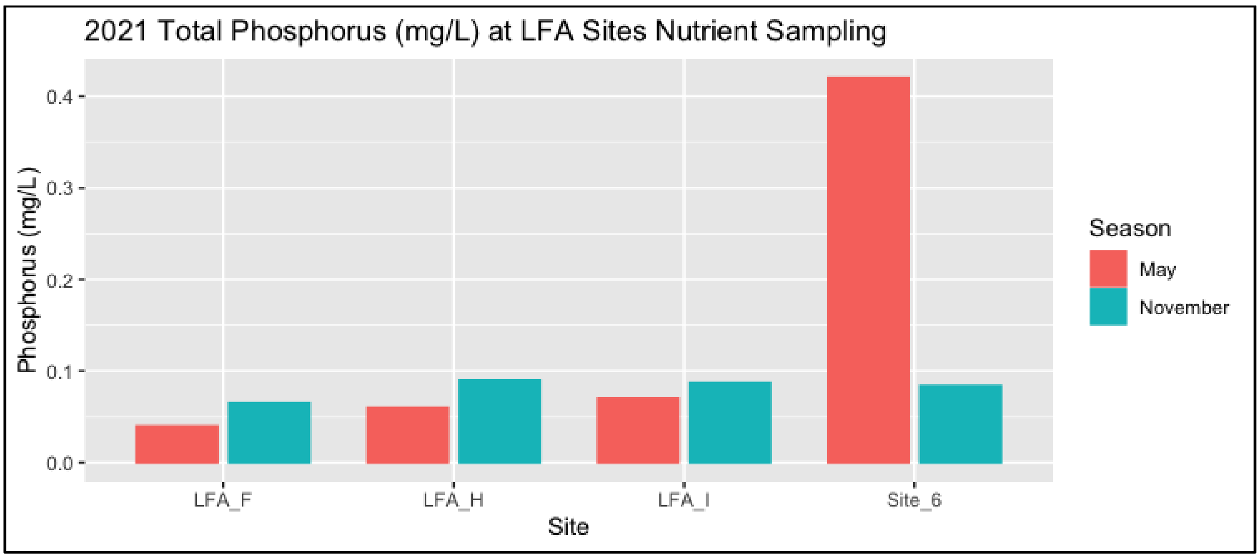

Figure 23. Total Phosphorus Results from Spring and Fall Nutrient Sampling During 2021

Results show that total nitrogen (mg/L) increased at all four sites. There was a very small increase in total phosphorus (mg/L) at LFA F, LFA H and LFA I and a decrease in Control Site 6.

6.0 DISCUSSION

The LFA project has four main objectives with the common goal of improving water quality in the Tahoe Keys lagoons. The objectives are to:

1) Increase DO levels at the sediment-water interface and throughout the water column to promote a healthy ecosystem, and encourage chemical reduction of sediments,
2) Reduce organic matter in sediments around the LFA diffusers,
3) Circulate the water column to decrease the opportunity for HAB occurrences, and
4) Reduce the habitable environment for aquatic macrophyte growth.

Data collected during the 2019, 2020 and 2021 seasons indicates that bottom dissolved oxygen increases when the system is activated. At the Control Site 6, there was significantly lower DO at the bottom of the water column than at the rest of the LFA sites during 2019 but not significantly lower during 2020 and 2021. This is likely due to lower water levels allowing for better mixing in 2020 and 2021. Both Site 6 and LFA G appear to have lower bottom DO levels in 2019, 2020 and 2021 since Site 6 is untreated and LFA G is not near any diffusers and in a dead-end area. As previously stated, 2021 data may be skewed due to the 3-month period where DO monitoring was not conducted, especially since it was during the warmest period of the season where significant changes can occur.

Sediment sampling data does appear to support the second objective of the LFA project. Figure 17 shows that organic material from the LFA G and Control Site 6 have a dramatic increase in organic matter % in the Fall while LFA F, LFA I, LFA H, LFA J stay relatively the same indicating that the diffusers have an effect on organic matter % as the plants are decomposing. Clean Flo International, LLC recommends that the project be operated with microorganisms to increase the rate of organic material decomposition. The entire area would likely have seen a larger decrease in organic content in the sediment if this project was permitted to use microorganisms with the aeration.

The objective of using the LFA system to reduce cyanobacteria is unclear. Cyanobacteria blooms (measured in anatoxin-a levels) occurred in higher concentrations at LFA I and Site 6 in 2020 than 2019, but lower concentrations in LFA F and LFA H in 2020 than 2019. In 2021 anatoxin-a levels were lower than 2020 in LFA H, LFA I and Site 6 but slightly higher in LFA F. Cause for these variances in levels is unknown therefore TKPOA cannot determine a relationship between the LFA system and cyanobacteria levels. Figure 24 shows paint like algae near LFA I in 2021.

Figure 24. Cyanobacteria 'paint' in site LFA I taken September 14 2021

The cause of these blooms is not fully understood and further analysis is needed to reach a conclusion. The TKPOA WQ Department has hypotheses that may explain the cyanobacteria blooms in the lagoons such as:

1. One explanation, supported by peer reviewed papers, is that *P.crispus* die off in mid-July releasing phosphorus into the water column that ends up fueling cyanobacteria growth (Wang, L, et. al., 2018). While cyanobacteria were detected in early June, before the *P. crispus* died off, they were in relatively low concentrations. There was a spike in PC in mid-late July in both 2020 and 2021 that may indicate that the cyanobacteria present were allowed to multiply faster due to increased nutrients in the water.
2. The high cyanobacteria concentrations could also have been a symptom of the LFA system being activated and stirring up nutrients from the sediment into the water column. Without any natural shorelines in a dead-end area, a buildup of nutrients along the edges creates the ideal habitat for an algal bloom.

The TKPOA WQ staff has continued to monitor during the 2021 season to further evaluate possible causes of cyanobacteria blooms and to propose a solution that will reduce cyanobacteria concentrations, if not occurrences.

The final objective of the LFA system is to reduce the habitable environment for aquatic macrophyte growth. Based on biovolume percent taken from hydroacoustic scans, it does not appear this objective is being met. To get a better understanding of temporal changes in macrophyte composition and abundance, during the 2022 season, staff will compare differences between the 2019, 2020, and 2021 macrophyte survey conducted by SEA.

8.0 2021 RECOMMENDATIONS FOR 2022 SEASON

8.1 Transect-Specific Macrophyte Survey

During the 2020 season, the TKPOA WQD installed transect lines to monitor the species of AIS growing within that area. In 2021, these transects were not used and taken out completely in August. In 2022, WDQ recommends reinstalling these transects and utilizing the underwater drone (FiFish ROAV) to conduct a more detailed survey of these plants. This process could be repeated biannually to determine changes in vegetation over the years.

8.2 RWT Dye Study

Due to the relatively recent adaptation of the LFA technology for use in water body restoration, not much is known about how rapidly the water column cycles. During the 2022 season, the TKPOA WQ Department will conduct a RWT dye study. This study will help determine flow directions within the LFA test area and how rapidly the water column is inverted.

8.3 Additional Vegetation Control Methods

The TKPOA WQD would like to implement combined methods to go along with the LFA project. These additional methods could include; Diver assisted hand pulling, Floating Treatment Wetlands, Ultrasound and UVC light. These additional methods would assist with vegetation reduction along with other project objectives. Along with these additional control methods, TKPOA will increase the number of sample sites to monitor the influence area of diffusers.

8.4 Use of Microorganisms along with LFA

As stated earlier, organic material reduction would likely have increased with the use of microorganisms recommended by Clean-Flo. Other LFA projects elsewhere have proved the efficacy of these microorganisms to reduce organic content. If the TKPOA would be allowed to implement this method it may help the project meet its objectives and reduce available nutrients for aquatic macrophytes. It is recommended that the Water Quality Department seek out permission to implement said microorganisms. This will be difficult due to Lake Tahoe's classification as a tier 3 waterbody.

8.5 Readjustment of Diffusers

Historically, cyanobacteria was observed to be concentrated in the dead-end areas of the lagoons. In 2022, TKPOA will adjust diffuser locations in LFA I closer to the shoreline to disrupt accumulation of nutrients.

9.0 ACKNOWLEDGEMENTS

The League to Save Lake Tahoe authorized a $100,000 grant to the TKPOA over a four-year period (2017, 2019-2021) to support this project and the AIS Program. This grant disperses $25,000 per year with installments given each December.

10.0 List of Preparers

The following individuals prepared the text presented in this report.

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Gregory J Hoover TKPOA	Graduate Certificate in Fish and Wildlife Management B.S. Biology and Environmental Sciences	Principle in Charge Contributing Author Data Collection
Kathryn Terwilliger TKPOA	MSc Coastal & Marine Environments; Physical Processes, Policy, Practice B.A. Biology	Primary Author Data Collection Data Analysis
Meghan Hoffmann TKPOA	B.A. Biology and Secondary Education	Contributing Author Data Collection Data Analysis
Erin Harkins TKPOA	B.A. Biology with Zoology concentration	Contributing Author Data Collection
Moire Breslin TKPOA	B.S. Food Marketing with Environmental and Sustainability Studies	Contributing Author Data Collection
Colleen Hoskins TKPOA	B.S. Environmental Science	Data Collection

11.0 References

California Cyanobacterial and Harmful Algal Bloom (CCHAB) Network. (2022). *California Voluntary Guidance for Response to HABs in Recreational Inland Waters*. https://mywaterquality.ca.gov/habs/resources/habs_response.html

Hoyer, Mark V., et al. "A Beginner's Guide to Water Management—Muck: Causes and Corrective Actions." *Florida Lakewatch*, 2017, p. 13.

Lahontan Regional Water Quality Control Board (Lahontan) 2014. Water Quality Certification and Waste Discharge Requirements for TKPOA. https://www.waterboards.ca.gov/lahontan/water_issues/programs/tahoe_keys_weed_control/docs/TKPOA_WDR.pdf

Six Benefits of Lake and Pond Aeration and Fountains. (2021). Retrieved 23 December 2021, from https://www.solitudelakemanagement.com/six-benefits-of-pond-and-lake-aeration/

Wang, L., Liu, Q., Hu, C., Liang, R., Qiu, J., & Wang, Y. (2018). Phosphorus release during decomposition of the submerged macrophyte Potamogeton crispus. *Limnology*, *19*(3), 355–366. doi: 10.1007/s10201-018-0538-2

Appendix A

LRWQCB Sample Bottle Collection Protocol

Appendix B

These are the shipping and handling procedures for sampling as of May 2021. As distributors and shipping methods are subject to change, information in the following sections should be updated as changes arise.

For all Sampling: Proper Chain of Custody (COC) forms are to be filled out to ensure proper handling of samples. Samples are to be packed in a cooler with fresh ice packs. The COC forms and a copy of the datasheet are to be placed within a ziplock bag inside the cooler.

Harmful Algal Blooms Sampling (Bend Genetics): The HAB samples require two shipping labels to be made; one for sending (from AIS Staff to Tim Otten) and for returning (from Tim Otten to AIS Staff). The cooler is taken to Mountain Postal before 2:30 PM (located next to Raleys).

Sediment and Water Sampling (WetLab): The sediment samples are to be left in the TKPOA Pavilion located at 365 Ala Wai Blvd. Arrange to have WetLab pick up the samples on the following day.

LFA Specific Sampling (Babcock): A shipping label is affixed to the outside of the package. The label is sent with the sampling kit. The package is taken to Mountain Postal before 2:30 PM (located next to Raleys).

Appendix C

HAB signs

Appendix D

Cyanobacteria species present 2021

Site	Date	Dominant	Levels	Sub-dominant	Levels	Present	Levels
LFA_F	4/13/21	Woronichinia	Low				
LFA_H	4/13/21						
LFA_I	4/13/21						
Site_6	4/13/21	Aphanocapsa	Low				
LFA_F	5/12/21						
LFA_H	5/12/21						
LFA_I	5/12/21	Aphanocapsa	Low				
Site_6	5/12/21	Trichodesmium	Low				
LFA_F	6/15/21	Dolichospermum	Moderately Low				
LFA_H	6/15/21	Dolichospermum	Moderate				
LFA_I	6/15/21	Dolichospermum	Moderate				
Site_6	6/15/21	Dolichospermum	Low				
LFA_F	7/13/21	Dolichospermum	Low				
LFA_H	7/13/21	Microcystis	Moderately Low	Aphanizomenon	Low	Dolichospermum, Woronichinia	Low
LFA_I	7/13/21	Microcystis	Moderately Low	Woronichinia	Low	Dolichospermum	Very Low
Site_6	7/13/21	Dolichospermum	Moderate	Aphanizomenon	Low	Microcystis	Low
Cove_2	7/13/21	Dolichospermum	Moderate	Woronichinia	Moderately Low		
Lighthouse Shores	7/13/21	Dolichospermum	Moderately Low	Planktothrix	Low		
Alpine_Venice	7/13/21	Dolichospermum	Low	Planktothrix	Low	Anagnostidinema	Very Low
Site_16	7/13/21	Dolichospermum	Low	Aphanizomenon	Low	Anagnostidinema	Low
Tallac_15th	7/13/21	Microcystis	Moderately Low	Trichodesmium	Moderately Low	Woronichinia	Low
Site_8	7/13/21	Dolichospermum	Moderately Low	Anagnostidinema	Low	Aphanizomenon	Low
LFA_F	7/20/21	Aphanizomenon	Moderate	Dolichospermum	Moderately Low	Woronichinia	Low
LFA_H	7/20/21	Aphanizomenon	Moderate	Dolichospermum	Moderately Low	Microcystis	Moderately Low
LFA_I	7/20/21	Aphanizomenon	Moderately Low	Woronichinia	Moderately Low	Dolichospermum, Microcystis	Low
Site_6	7/20/21	Dolichospermum	Moderate	Aphanizomenon	Moderate		
LFA_F	8/10/21	Aphanizomenon	Moderately Low	Woronichinia	Moderately Low	Dolichospermum, Microcystis	Low
LFA_H	8/10/21	Aphanizomenon	Moderately Low	Dolichospermum	Moderately Low	Woronichinia, Microcystis	Low
LFA_I	8/10/21	Woronichinia	Moderately Low	Dolichospermum	Low	Microcystis	Low
Site_6	8/10/21	Aphanizomenon	Moderately Low	Dolichospermum	Low	Woronichinia	Low
LFA_F	9/14/21	Dolichospermum	High	Aphanizomenon	High	Woronichinia, Microcystis	Moderately Low, Low
LFA_H	9/14/21	Dolichospermum	Moderately High	Aphanizomenon	Moderately High	Woronichinia, Aphanocapsa	Moderate, Very Low
LFA_I	9/14/21						
Site_6	9/14/21	Aphanizomenon	Moderately High	Dolichospermum	Moderate	Woronichinia	Moderately Low
LFA_F	10/12/21	Woronichinia	Moderately Low	Dolichospermum	Low	Cuspidothrix	Very Low
LFA_H	10/12/21	Dolichospermum	Moderately Low	Woronichinia	Low	Cuspidothrix	Very Low
LFA_I	10/12/21	Woronichinia	Moderately Low	Dolichospermum	Low		
Site_6	10/12/21	Woronichinia	Moderately Low				
LFA_F	11/10/21	Woronichinia	Low				
LFA_H	11/10/21	Woronichinia	Very Low				
LFA_I	11/10/21	Woronichinia	Low				
Site_6	11/10/21	Woronichinia	Low				