2020 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

Tahoe Keys

2020 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) infestation in the Tahoe Keys lagoons. Since the 1980s, the TKPOA has been combating increasing amounts of invasive and nuisance aquatic vegetation, with harvesting and fragment collection being the main methods available for management. As the vegetation 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 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.

LFA has been used successfully with shallow, warmer waters with minimal circulation at low elevation; however, it has not been fully tested in an area such as Lake Tahoe, which has deeper, cold water at a much higher elevation.

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 turns 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. The 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 the organic matter aid in hydrolysis of carbohydrates and lipids, and protolyzation of proteins to amino acids, which can lead to nitrification and denitrification. Figure 1 below shows water circulation with the LFA system.

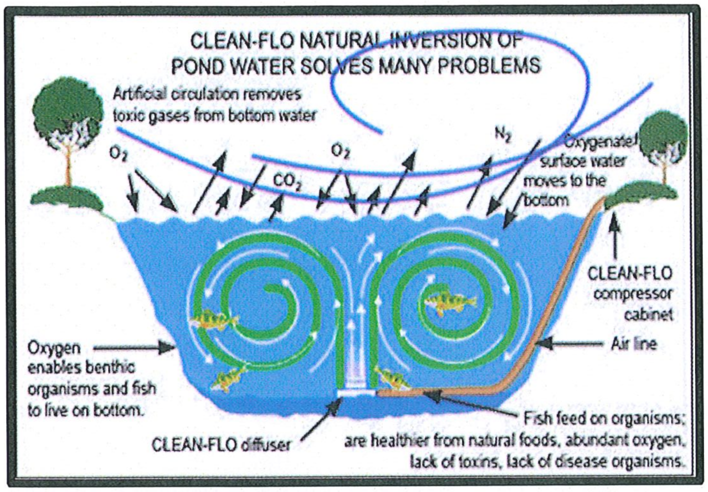

Figure 1. LFA Circulation.

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 CO2)
2. Prevention of the formation of undesirable toxic gases, 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 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. 2020 LFA Project Map

3.0 Objectives for LFA System

The system is operating continuously for the 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 2 years and will follow the sampling guidelines in figure 5 for the third 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 in companion with the BioBase analysis 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 2 of the 10 diffusers on August 12, 2020 after consulting with Clean-Flo International, LLC. This will provide better treatment with more diffusers in a smaller area. 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. Diffuser map with depths (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.

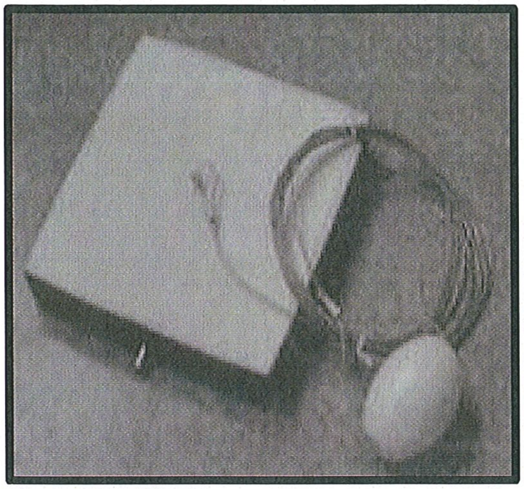

Figure 4. Diffuser.

4.2 Monitoring

Bimonthly monitoring for the project for water quality was conducted from April to November 3rd, 2020 according to the monitoring schedule (refer to Figure 5). Monthly cyanobacteria samples also were taken along with biannual sediment samples as required by the LRWQCB permit.

Water	Pre-Project		Installation		Post Monitoring			
	Week Prior	Day Prior	Day 1	Day 2-7	Week 2-4	Month 2/3	Month 4-12	Year 2-3
Turbidity / pH / DO / ORP / SPC / Temp	LFA-1 0830 / 1430 LFA-2 0915 / 1515	LFA-1 0830 / 1430 LFA-2 0915 / 1515	LFA-1 0830 / 1430 LFA-2 0915 / 1515	LFA-1 0830 / 1430 LFA-2 0915 / 1515	LFA-1 0830 / 1430 LFA-2 0915 / 1515	Bi-Monthly Tues- LFA-1 0830 / 1430 Bi-MonthlyTues- LFA-2 0915 / 1515	Monthly Monthly	Quarterly Quarterly
Total Kjeldahl Nitrogen / Nitrate + / Nitrite Nitrogen /	One week from installation date	N/A	N/A	Day 7	Week 3	Once per month	Monthly	Quarterly

Sediment	Pre-Project	Installation		Post Monitoring		
	Two Weeks Prior	Day 1	Day 2	Week 2-4	Month 2-12	Year 2-3
Core Sample	Two Weeks from Installation	N/A	N/A	N/A	October	Bi-Annual
Sediment Surface Total Kjeldahl Nitrogen / Nitrate + / Nitrite Nitrogen / Ammonia / Ortho-	Two Weeks from Installation	N/A	N/A	N/A	October	Bi-Annual

Aquatic Plants	Pre-Project	Installation	Post Monitoring	
			Year 2-3	
Plant Composition	TKPOA Annual Macrophyte Survey	N/A	Annual Macrophyte Survey	
Plant Densitiy / Bio Mass Hydro-	Weekly Hydro-Acoustic Scanning Until October			Weekly Scanning Map

Figure 5. LFA Project timeline.

1. <u>Equipment</u>

   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 sonar transducer for hydroacoustic scans. Initial muck levels were assessed by using a telescoping pole with a plate mounted to the end. The pole has marks every decimeter to indicate depth. Sediment samples were taken with a Van Veen grab sampler.

2. Calibration
   The TKPOA Water Quality staff calibrated the ProDSS and Pro1020 monthly 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 Temp Start / End
   c. Cloud Cover Start / End
   d. Last Precipitation
   e. Wind Speed Start / End
   f. Wind Direction Start / End
   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 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.
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. Hydroacoustic Scans
   Muck levels were monitored bi-monthly using Lowrance hydroacoustic scanners in companion with the BioBase analysis software. 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.

4.3 Sampling Procedures

During the 2020 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 orthophosphorus require field filtration and were conducted by TKPOA Water Quality staff using the method instructed by Delta Environmental Laboratories (Appendix B).

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, the Sample Collector will begin to complete the data sheet, indicating Date, Sample Collector, Boat Driver, Start time, 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 to be collected:

i. Depth (m), Water Temperature (℃), pH, Dissolved Oxygen, Oxidation-Reduction Potential (ORP), Algae Content, Ammonium.
ii. Turbidity (FNU) and Electric 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.
iv. 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.
v. Lower the instrument to the desired depth in the water column, according to the data sheet (Bottom, Q1, Mid, Q3, Surface).
vi. Allow adequate time to ensure the YSI data balances before recording information onto the data sheet.
vii. Complete for each column of each category on the data sheet.
viii. Verify that all required data has been collected before moving on to the next site

3. <u>Sample Grabs: The following information shall be recorded on each sample bottle at the time of sampling:</u>
   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. <u>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.</u>
   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. <u>Cyanobacteria Surface Grabs:</u> <u>**Do not rinse the sample container prior to sample collection.**</u>
   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. <u>Sediment Samples</u>
   a. Triple rinse sample bucket, VanVeen 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.

4.4 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 – 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.
    g. Blue-Green Algae – Identification of abundant classes of cyanobacteria as well as potential toxicity and quantification of chlorophyll-a.
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).
3. Sediments
    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 NH4
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. Cyanobacteria samples were sent to Bend Genetics, LLC in Sacramento, California. Nutrient samples were sent to Delta Environmental Laboratories in Benicia, California and sediment samples were sent to Western Environmental Testing Laboratory (WetLabs) 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 4 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.

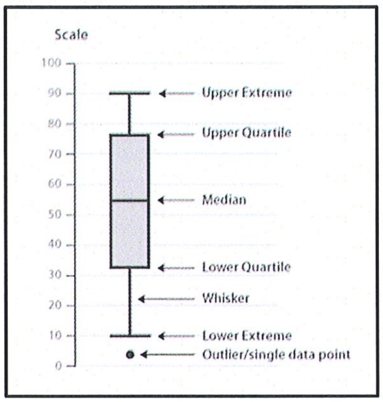

Figure 6: Box and whisker plot description

5.1 Dissolved Oxygen Levels

The figures below show dissolved oxygen (DO) data from the 2020 season. Figure 7 shows the spread of recorded DO levels from April through November. 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). The lowest DO levels are from before the LFA system was turned on. There was an error with monitoring equipment for a few weeks in August 2020 therefore there is a gap in the data. It can also be noted that LFA G has a wide range of DO throughout the year. This is likely because the diffuser near that site was moved and the bottom became anaerobic due to lack of introduced oxygen. Figure 8 shows the drop in DO at LFA G after the diffusers were moved on August 12, 2020.

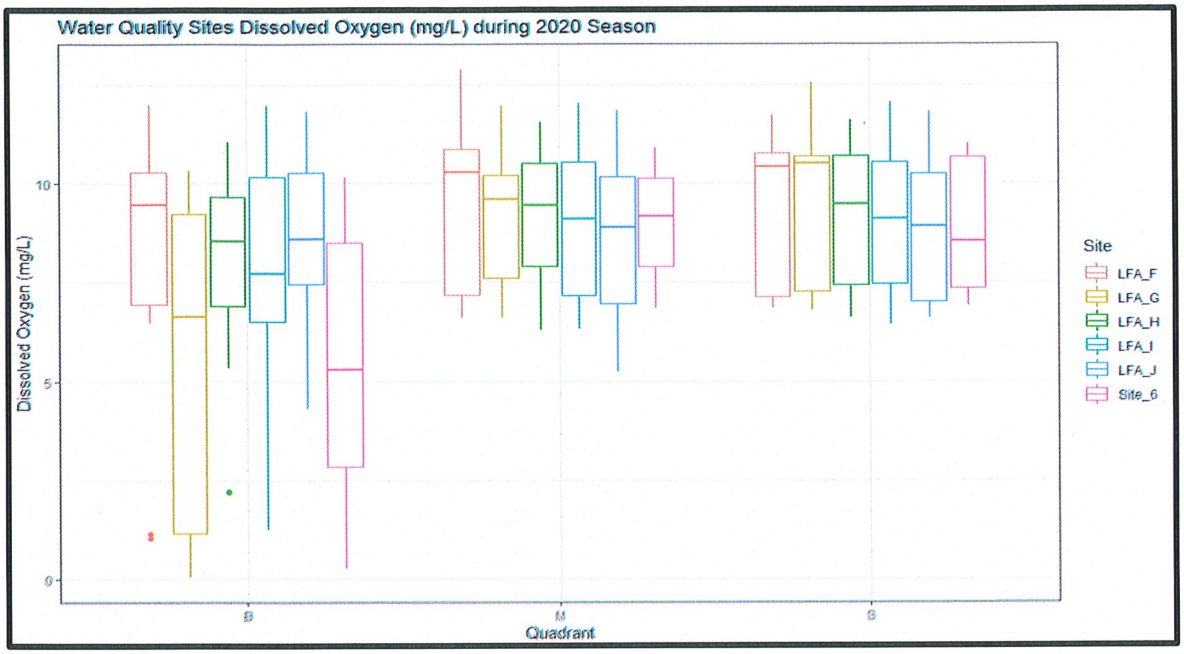

Figure 7. DO for the 2020 season.

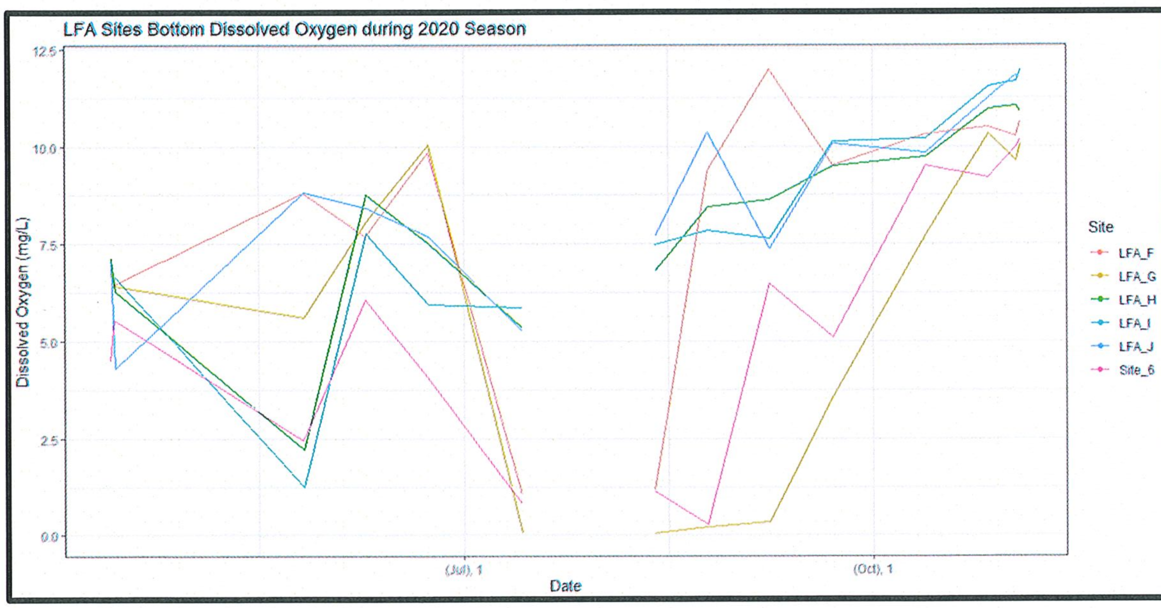

Figure 8. DO for all sites at the bottom quadrant throughout 2020.

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. Figures 9 and 10 display the results of turbidity monitoring from the 2020 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.

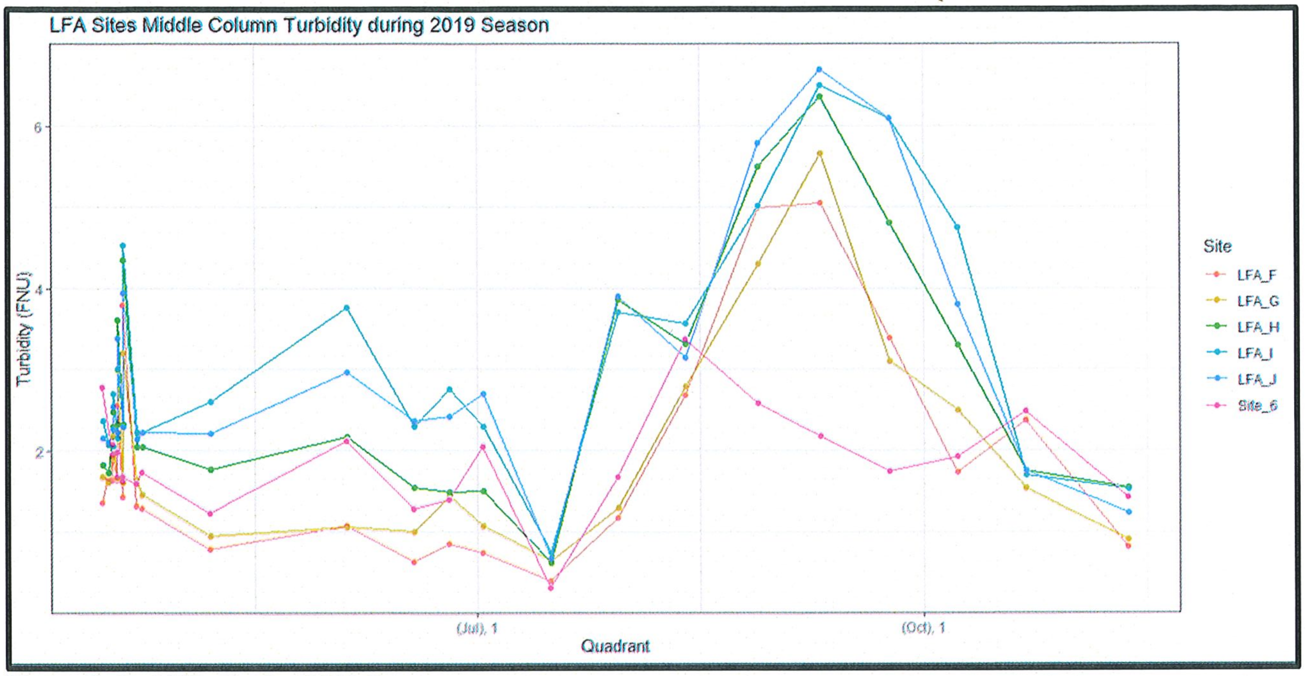

Figure 9. Turbidity During 2019 Season.

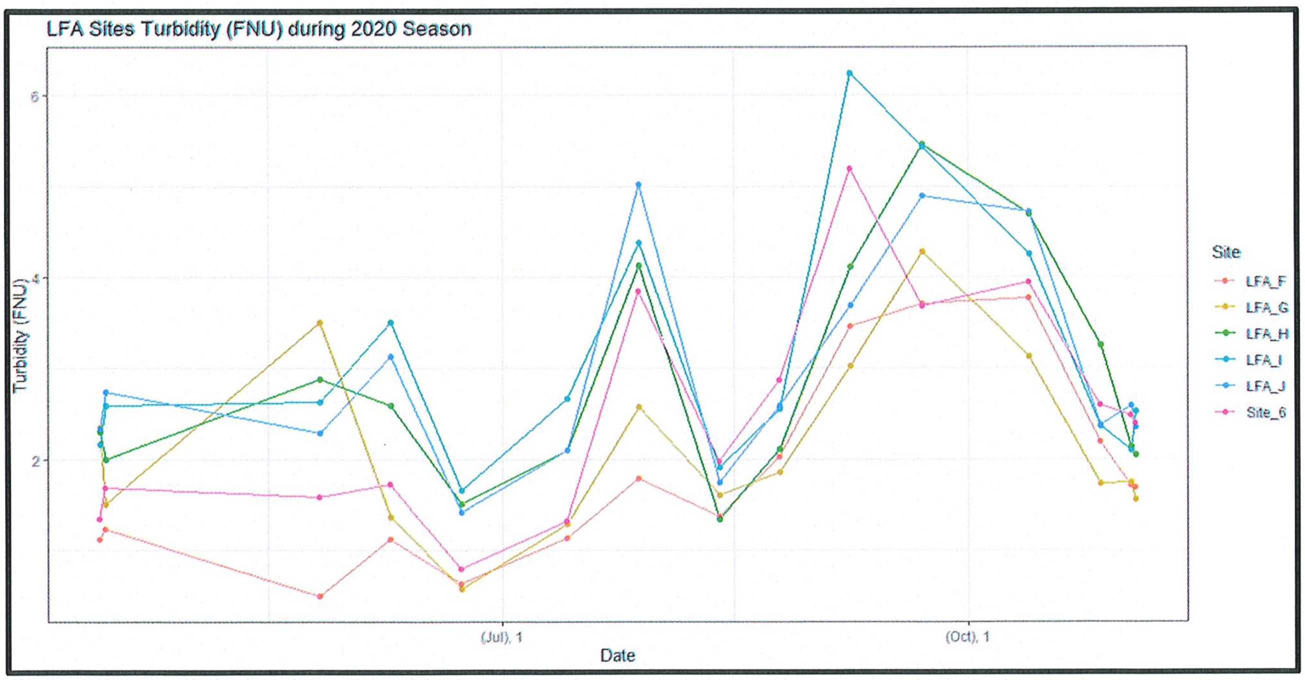

Figure 10. Turbidity throughout the2020 Season.

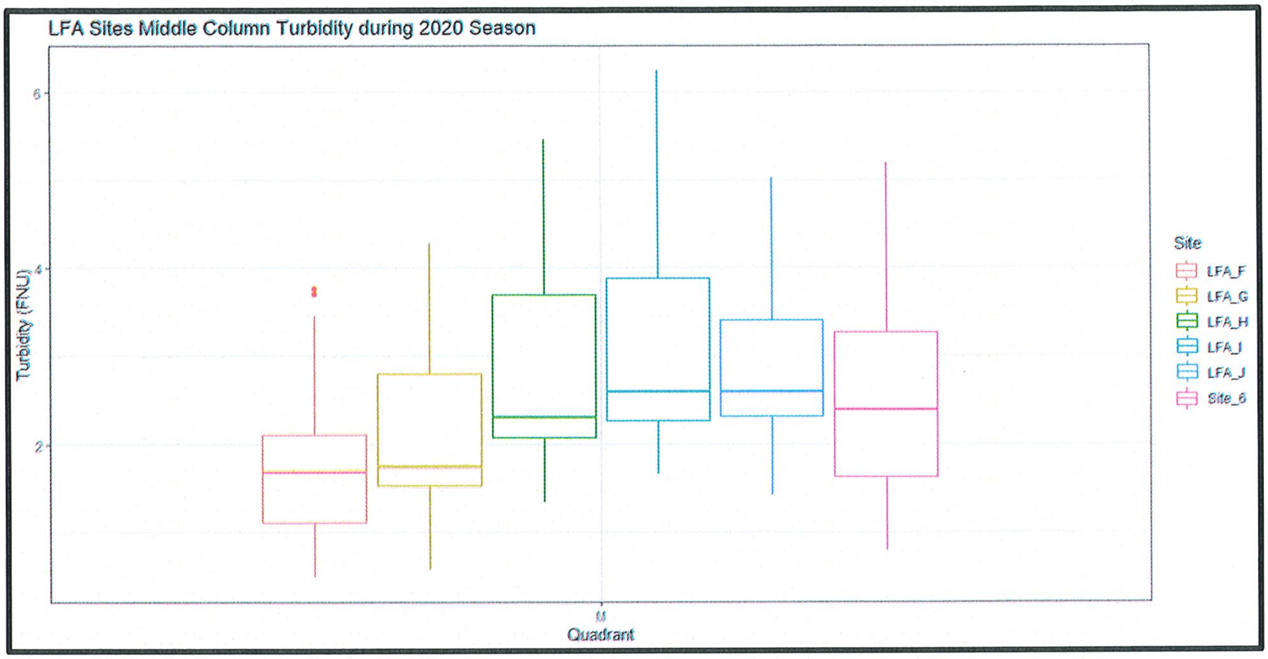

Figure 11. Turbidity range for the 2019 season.

5.3 Muck Levels

An objective of the LFA project is to reduce organic matter around the diffusers, thus reducing the availability of nutrients for aquatic plants and algae. Most of these nutrients are in the organic layer at the bottom of the water column. At the beginning of the project, the organic layer was measured to be 3-5 feet thick. Table 2 below shows the results from measured depths from April installation compared to an October measurement. All diffusers saw an increase in depth to the top of the muck layer, which is equivalent to a reduction in the muck layer thickness. This reduction of muck could be due to the decomposition of organic material but also could be due to the physical movement of the muck away from the diffusers. The TKPOA attempted to monitor depth throughout the area using hydroacoustic scans (Figure 12), but due to dense vegetation, the readings were not very accurate. All diffuser locations combined have increased their depth by ¾ of a foot on average (See Table 2).

Figure 12. Left: Bathymetry map of the LFA site on April 16th, 2019 (day of installation), and Right: November 5th 2020 (most recent scan).

Diffuser	Depth on 4/17/2019 for Diffuser 1-8 and 8/12/2020 for 9 and 10 (feet)	Depth on 11/16/2020 (feet)	Lake Difference (feet)	Change in Depth (feet)
1	7.9	5.9	-2.16	-0.2
2	10.6	10.2	-2.16	-1.7
3	9.3	7.6	-2.16	-0.4

4	10.7	9.0	-2.16	-0.5
5	7.3	7.6	-2.16	-2.4
6	9.4	8.2	-2.16	-1.0
7	9.5	7.9	-2.16	-0.5
8	9.4	8.3	-2.16	-1.0
9	8.5	7.9	-1.41	-0.8
10	10.1	9.2	-1.41	-0.4

Table 2: LFA Muck Depths

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 are compared to the 2020 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 D) 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 cyanobacteria in the 2019 season was on September 24th. The next sampling event on 10/8/2019 had non-detects at all sites. In the 2019 season, cyanobacteria were present for approximately 72 days.

During 2020 the 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 again and posted caution signs (Appendix D). Cyanobacteria was detected in high concentrations from all sites on 10/13/2020 sampling event, indicating that the bacteria were around for longer during this season. However, this could be due to multiple factors such as lower water level and warmer water temperatures later into the year. The caution signs were posted until cyanobacteria was not detected during the 11/3/2020 sampling event. During the 2020 season cyanobacteria was present for approximately 111 days.

The 2019 and 2020 sampling results show that the cyanobacteria bloom peaked from August to September and cyanobacteria lingered until mid-October. During 2019 the highest amounts of Anatoxin-a were found at Sites LFA I, LFA J, and LFA F. In 2019 results of the 9/10/2019 sampling found both Anatoxin-a and Microcystin at every site.

Figures 13 and 14 shows the spread of anatoxin-a at each site in µg/L. The figure shows that concentrations were highest at LFA I, LFA J and LFA G. These are dead-end areas inside of the test site, as shown on Figure 2. Lower concentrations were detected at Site 6 and LFA F, which are open water areas.

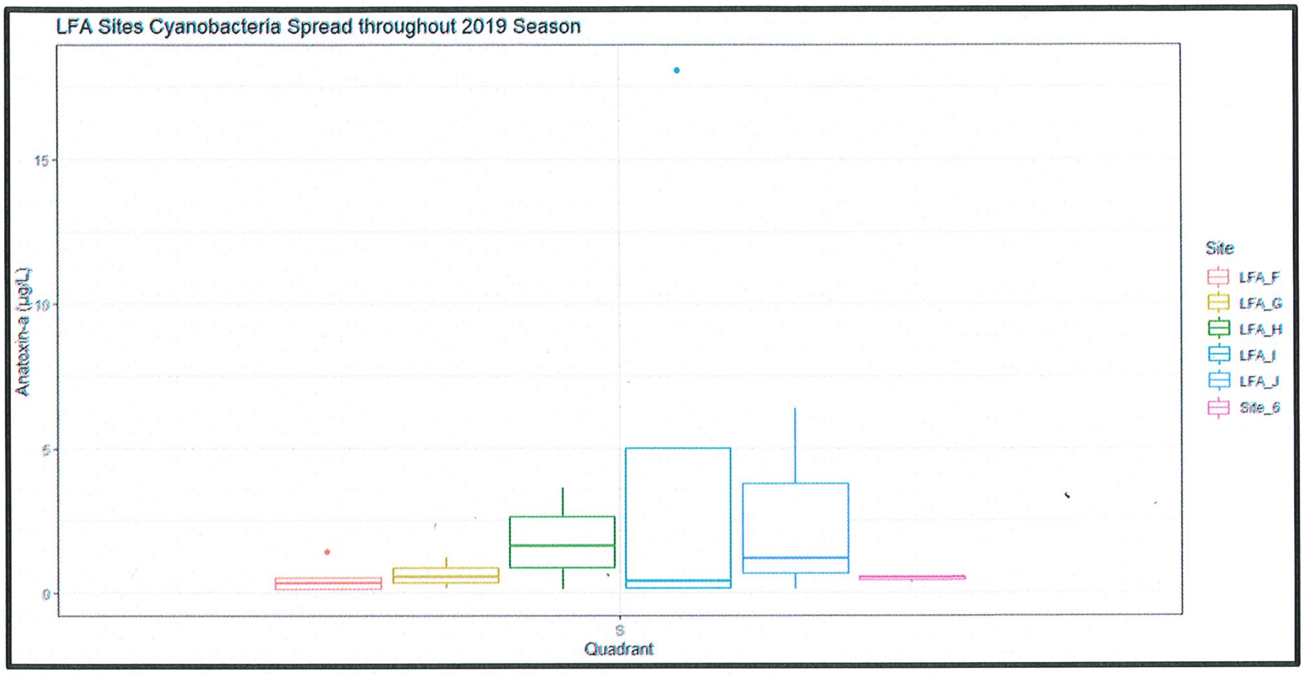

Figure 13. Cyanobacteria spread during the 2019 season.

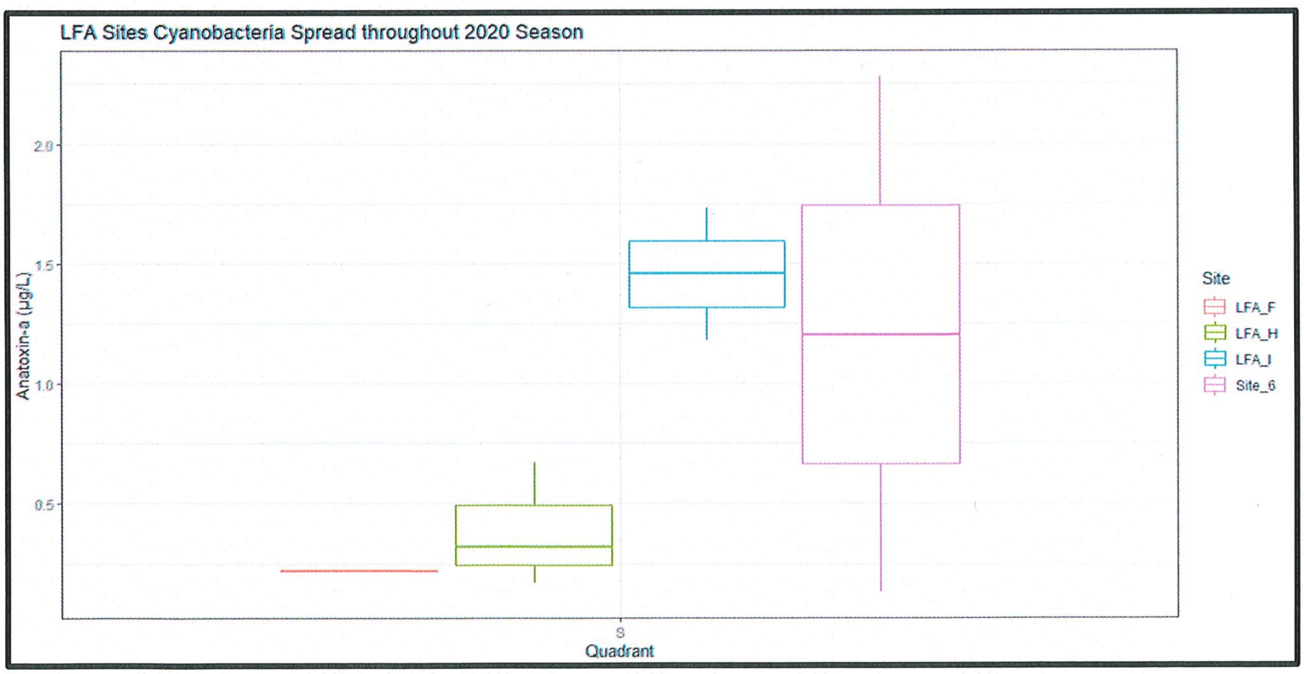

Figure 14. 2020 Cyanobacteria spread during 2020 Season

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, in smaller quantities, include *Aphanizomenon* sp., *Aphanocapsa* sp., *Microcystis* sp., *Snowella* sp., and *Woronichinia* sp.

Overall during the 2020 season, cyanobacteria levels were lower than during 2019 at all sites except for the control site 6. During 2020 the TKPOA activated the circulation system flowing through control site 6. The system was operational from July until October but only at full capacity for a couple weeks in August. This may have influenced the Cyanobacteria levels detected at site 6.

5.5 Sediment Sampling Results

The TKPOA Water Quality Staff sampled sediment twice during the 2020 season. The results from these sampling events are shown below.

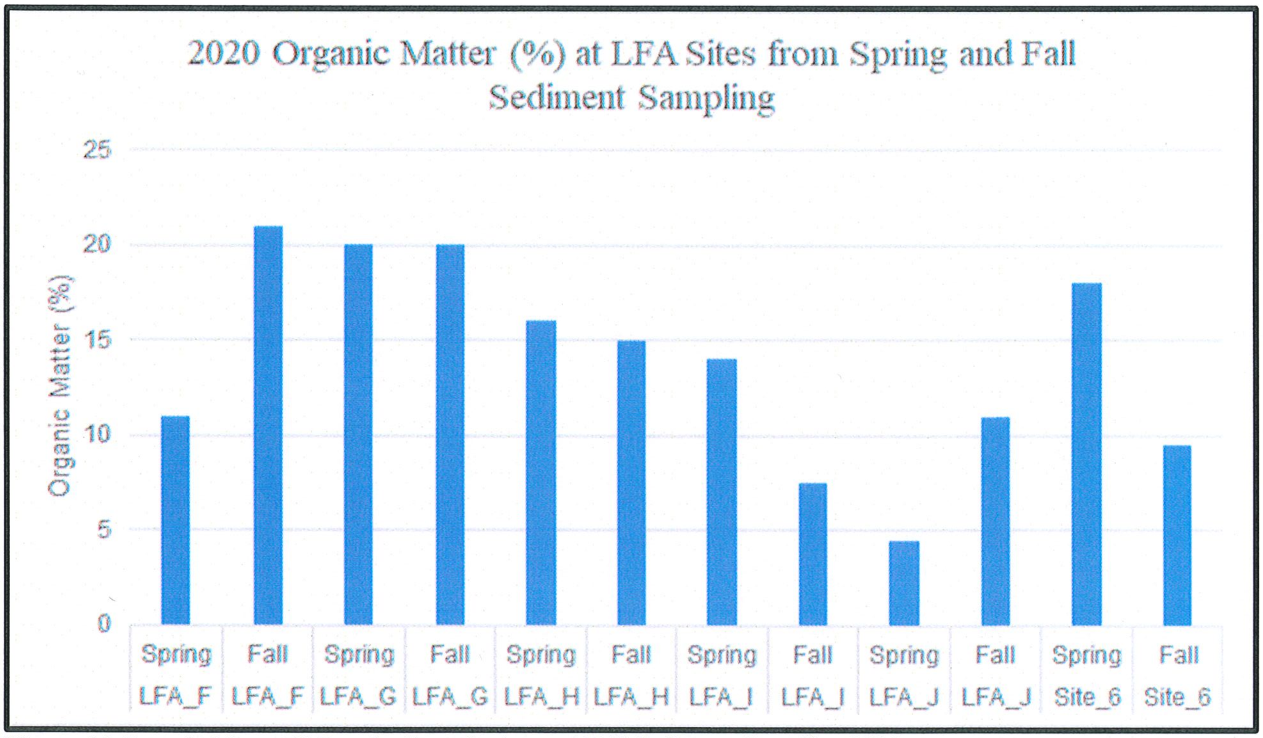

Figure 15 Organic Matter % Results from Spring and Fall Sediment Sampling 2020

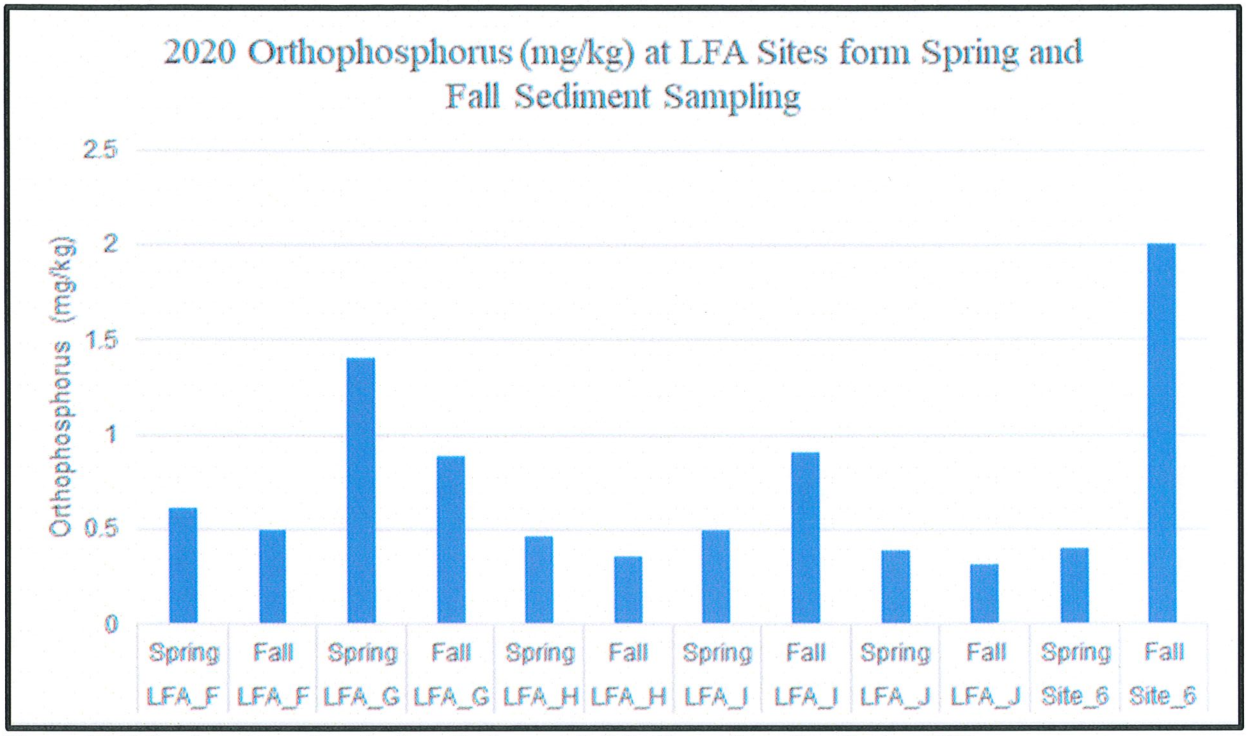

Figure 16 Orthophosphorus Results from Spring and Fall Sediment Sampling During 2020

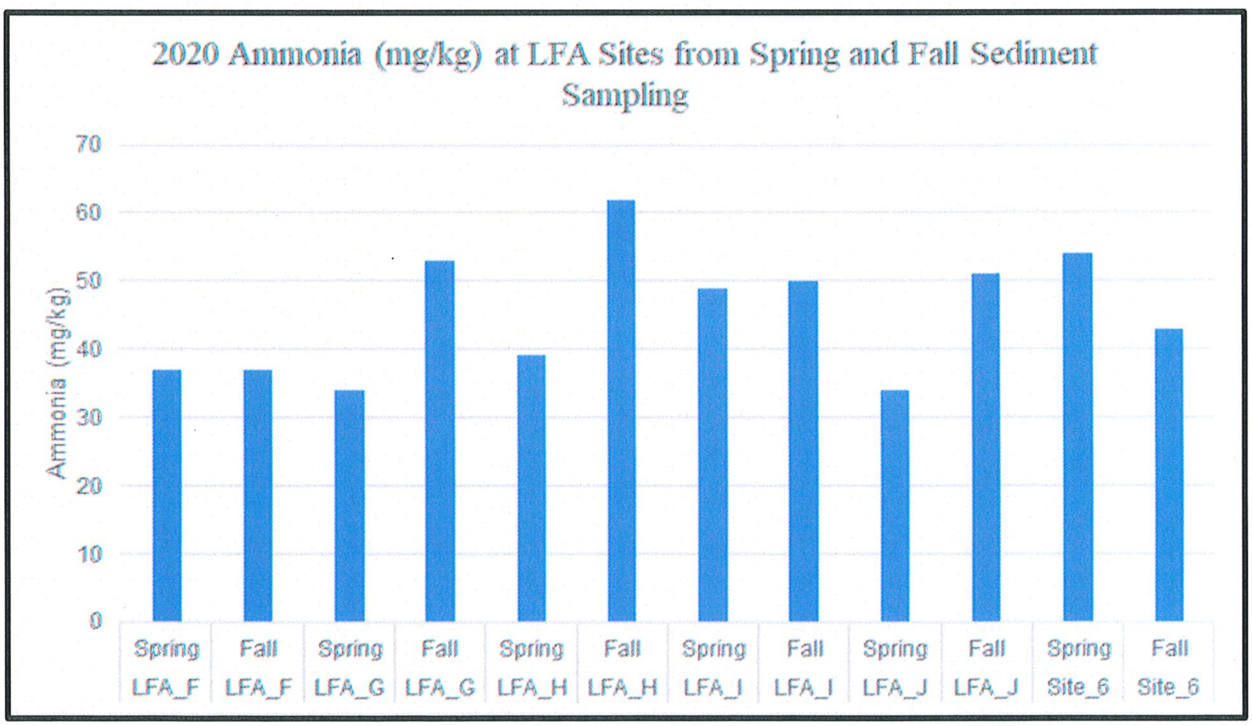

Figure 17 Ammonia Results from Spring and Fall Sediment Sampling During 2020

Sediment sampling occurred on 5/27/2020 (spring) and 11/2/2020 (fall). Figures 15, 16 and 17 show the sediment sampling results from 2020. Figure 15 shows organic matter percent detected in the samples collected from the 6 different sampling sites. LFA F and LFA J saw an increase in organic matter percent while LFA G and LFA I stayed the same and LFA H had a slight decrease. Control Site 6 also recorded an increase. Figure 16 shows that LFA F, LFA G, LFA H, and LFA J recorded a decrease in orthophosphorus while LFA I and site 6 saw an increase. Figure 17 shows that ammonia levels increased at LFA G, LFA H and LFA J, decreased at site 6 and stayed the same at LFA F.

5.6 Nutrient Sampling

The TKPOA staff conducted nutrient sampling twice during the 2020 season at the middle of the water column at all 6 sites as required by the State permit. Samples were analyzed for Orthophosphate, Ammonia and Nitrogen, Total Phosphorus as P, Total Nitrogen, Nitrate + Nitrite Nitrogen and Total Kjeldahl Nitrogen. Figures 18 and 19 below display the Total Phosphorus and Total Nitrogen results from these samplings, blank columns are non-detects. 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 detects for orthophosphate and phosphorus.

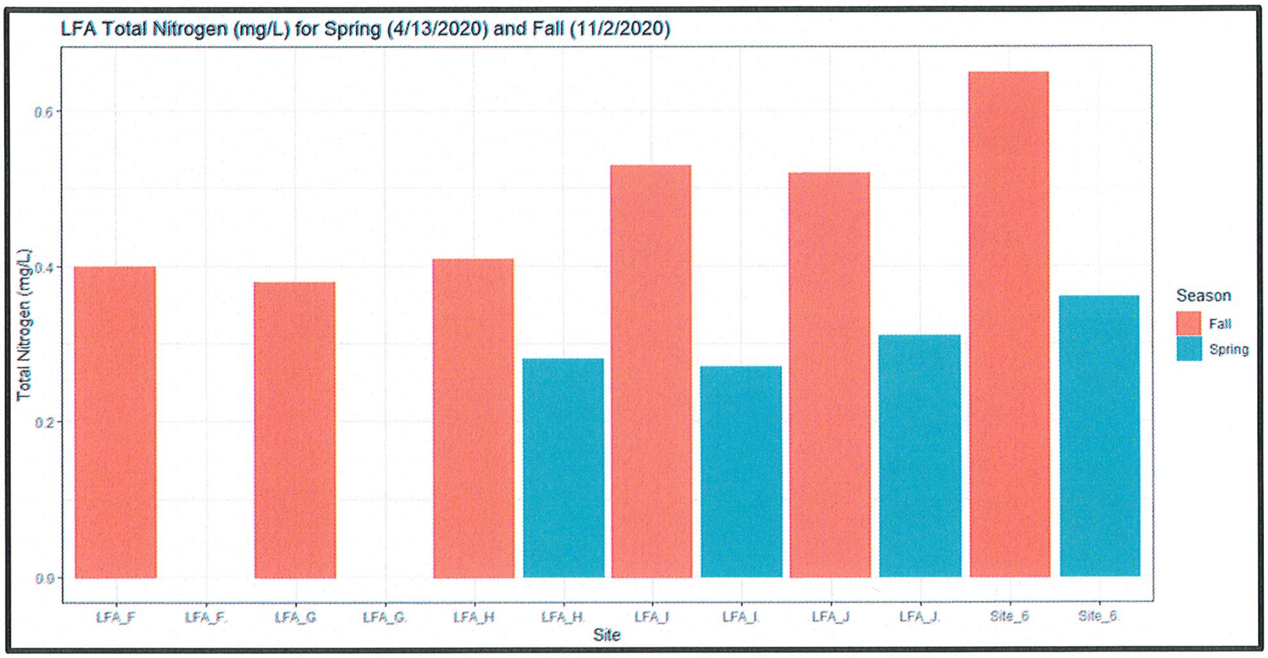

Figure 18 Total Nitrogen Results from Spring and Fall Nutrient Sampling During 2020

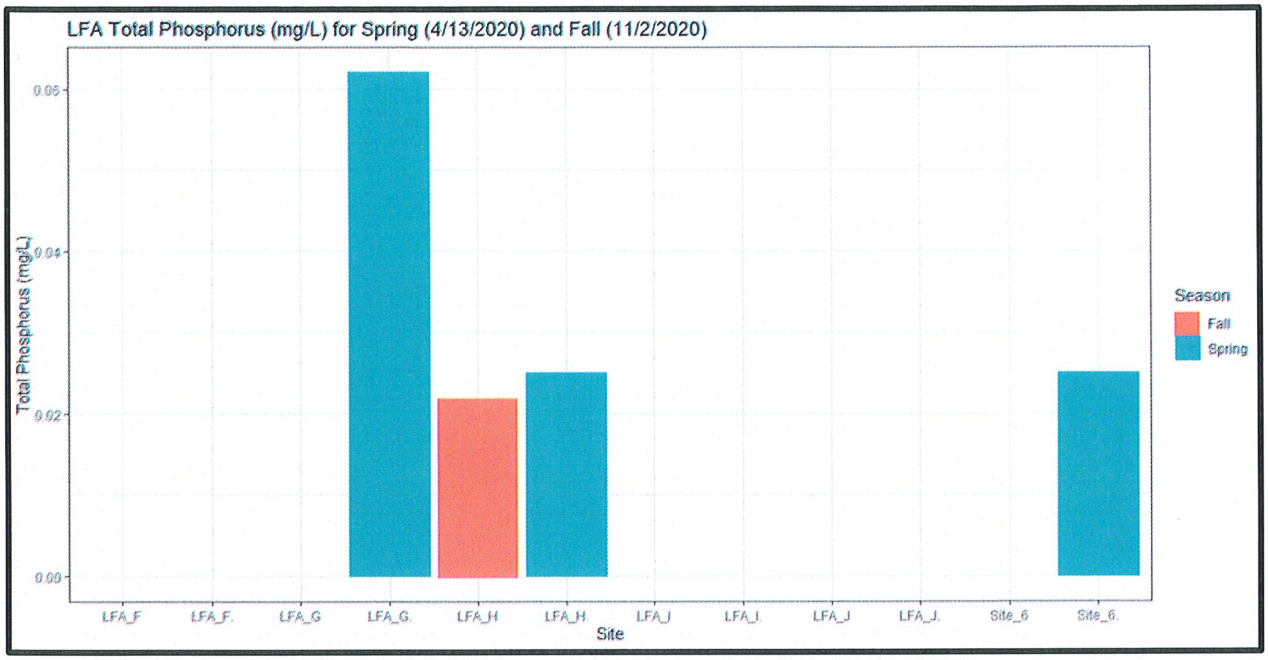

Figure 19 Total Phosphorus Results from Spring and Fall Nutrient Sampling During 2020

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 and 2020 seasons indicates that dissolved oxygen increased when the system was activated. Figure 6 shows that at the Control site, Site 6, there was lower DO at the bottom of the water column than at the rest of the LFA sites during 2019 but not during 2020. This is likely due to lower water levels allowing for better mixing. Figure 8 shows a drop in DO when the diffusers were moved away from the site.

Sediment sampling data does appear to support the 2nd objective of the LFA project. Figure 15 shows that organic material from the sample sites either increased or stayed relatively similar while content decreased at the control site. This is possibly due to plant material being deposited in the sediment after the plants growing season. Another possibility is that organic content near the diffusers may have decreased but not at the sample sites where the sediment samples were taken. When compared to 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 was not met during 2019 but was during the 2020 season. Cyanobacteria blooms occurred in higher concentrations in 2019 than the 2018 season but were in lower concentrations during 2020. Figure 20 shows paint like algae near LFA I on 10/8/2020.

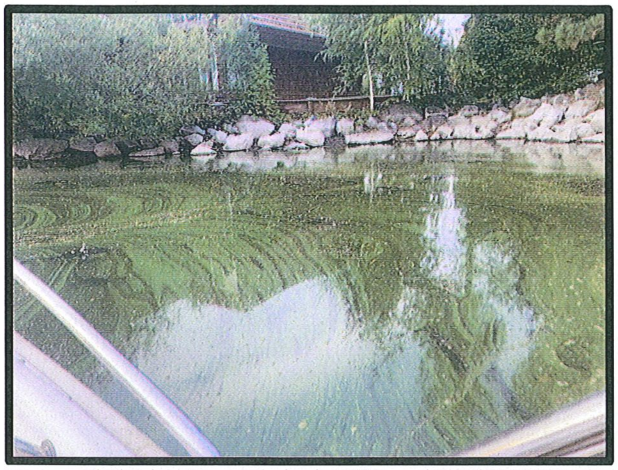

Figure 20 Cyanobacteria near Diffuser 4, 10/8/2020

The cause of these blooms is not fully understood, and further analysis of the available data 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 curly leaf pondweed dieoff in mid-July releases phosphorus into the water column that ends up fueling cyanobacteria growth (Wang, L, et. al., 2018). While cyanobacteria was detected in early June, before the curly leaf pondweed die off, they were in relatively low concentrations. There was a spike in PC in mid-July 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. This was supported by lower concentrations detected during the 2020 season.

The TKPOA WQ staff has continued to monitor during the 2020 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. A decrease in the muck levels due to oxidizing organic material in the sediment is intended to achieve this objective. Table 2 shows the change of depth observed at each diffuser from the project's implementation in April 2019 to the last depth measurement in November 2020. This change of depth, after accounting for change in depth of the lake, could be attributed to the organic breakdown of material in the sediment or the physical movement of sediment away from the diffusers. However, sediment sampling does not indicate that there was a reduction of organic content in the sediment. Diffusers averaged a ¾ of a foot increase in depth. Continued monitoring will provide additional data for WQ staff to evaluate changes in the thickness of the muck layer. Of particular interest is the potential for the level to decrease over the winter season due to lack of vegetation. During the 2021 season staff will compare differences between the 2019 and 2020 macrophyte survey conducted by SEA to determine changes in vegetation.

TKPOA Staff also noted that there was an increase of vegetation growth away from the diffusers mostly next to the bulkheads. This was different from the growth patterns observed during 2019 where staff observed coontail blooms around the diffusers. It could be speculated that this was caused by the diffusers pushing nutrients away from the diffusers.

7.0 2019 Recommendations Implemented during 2020

7.1 Sediment Monitoring

Per the 2019 recommendation, the TKPOA WQ Department monitored the change in sediment levels at the diffusers during the 2020 season. It was noted that there was reduction at each diffusers and the sediment sampling helps determine if this is due to breakdown of organic material or if the material is simply being moved away from the diffusers. Staff also installed muck monitoring sticks next to visibly quantify the muck reduction.

7.2 Transect-Specific Macrophyte Survey

The permit issued by the LRWQCB to the TKPOA states that the project must not increase the abundance of AIS in the project area. The WQ staff assisted in the monitoring and observations of AIS during SEA's 2019 Macrophyte Survey. For the 2020 season, the TKPOA WQ Department installed underwater transects that follow a set line. This transect determined what species are growing along the line and was monitored through the use of hydroacoustic scanning. During 2020 staff were unable to conduct transects along the predetermined lines due to equipment failure of the remote-controlled underwater drone.

7.3 Readjustment of Diffusers

During the 2019 season, cyanobacteria was observed to be concentrated in the dead-end areas of the lagoons. The TKPOA WQ Department consulted with Clean-Flo International, LLC in 2020 to adjust the position of the diffusers. This has been perceived to disrupt the cyanobacteria growth and possibly has reduced the severity of blooms.

8.0 2020 Recommendations for 2021 Season

8.1 Sediment Monitoring

For the 2021 season, the WQ Department plans to conduct sediment depth monitoring from different locations throughout the LFA area. This sampling would help determine the reduction of organic matter that has occurred since the start of the project. The department would also like to bring in a diver to help determine sediment hardness. This would collect data to determine the bottom hardness and sediment composition as a part of one of the main objectives for the LFA project.

8.2 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, WQD recommends 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.

8.3 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 2021 season, the TKPOA WQ Department has planned and budgeted to 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.

8.4 New Sampling Sites to Determine Influence Area of Diffusers

The influence area of the diffusers and how their circulation is influenced is relatively unknown. In order to determine how the circulation is affected the TKPOA staff will monitor DO content at a few new sites throughout the LFA area during 2021. The DO concentration could also be monitored at measured increments from a diffuser to determine how far from the diffuser the DO increase is observed.

8.5 Suspend Harvesting in the LFA site

In order to properly observe the reduction in vegetation growth caused by the LFA system it is recommended that the TKPOA Water Quality Department cease harvesting operations within the LFA area. This would provide data for vegetation reduction based solely on the effects of laminar flow aeration. The Water Quality department would still conduct regular fragment control operations and pile pickups with skimmer boats, Tiger Cat and Aquaharvester.

8.6 Compare Vegetation Changes Detected During Macrophyte Survey

During 2021 staff will review 2020 and 2019 the macrophyte surveys submitted by SEA to determine changes in vegetation within the LFA project area. At the time that this report was written the 2020 macrophyte survey was yet to be submitted.

8.7 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.

8.8 Use of Microorganisms along with LFA

As stated earlier, organic material reduction would likely have increased with the use of microorganisms recommended by CleanFlow. 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.

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.

Name	Education	Role
Gregory J Hoover TKPOA	Graduate Certificate in Fish and Wildlife Management B.S. Biology and Environmental Sciences	Principle in Charge Contributing Author Data Analysis
Michael Bangs TKPOA	B.S. Environmental Sciences with Applied Geology Emphasis	Primary Author Data Collection Data Analysis
Meghan Hoffmann TKPOA	B.A. Biology and Secondary Education	Contributing Author Data Collection
Moire Breslin TKPOA	B.S. Food Marketing with Environmental and Sustainability Studies	Contributing Author Data Collection
April Hilman	B.S. Environmental Science with Conservation Biology Emphasis	Data Collection

11.0 References

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

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

Appendix A

LRWQCB Sample Bottle Collection Protocol

Bottle Count Spreadsheet

Analysis	Container Size/Type	Collection Method	# bottles
		Pigments	
Chl-A	500 ml or 1 L HDPE	Gloved hands, collect surface grab at least 3 cm below the water surface without touching or disturbing bottom sediments. Triple-rinse sample bottle with sample water and then fill to top	
Phycocyanin	1L HDPE or glass amber		
		Nutrients	
NO3- as N, NO2- as N, TN	1L HDPE	Gloved hands, Triple-rinse bottle by filling it 1/3 full, shake & rinse all internal surfaces. Pour out rinse water without disturbing lake bottom, shake water droplets out of bottle. For rinsing & sampling, fill the bottle by submerging top of bottle with cap on 3 cm below water surface, unscrewing cap with bottle opening tilted slightly up, and screwing cap back on while still underwater.	
TKN, TP, Ammonia as N	500 mL HDPE - H2SO4 pre-preserved	Gloved hands, Triple-rinse bottle by filling it 1/3 full, shake & rinse all internal surfaces. Pour out rinse water without disturbing lake bottom, shake water droplets out of bottle. For rinsing & sampling, fill the bottle by submerging top of bottle with cap on 3 cm below water surface, unscrewing cap with bottle opening tilted slightly up, and screwing cap back on while still underwater. Use this sample to fill 500 ml pre-preserved bottle.	
Ortho-P	60 mL HDPE (Sample must be field filtered within 15 minutes of collection. Syringes and filters will be provided.)	Sample collection and field filtration method coming soon.	
		Cyanobacteria	
Toxin - microcystin/nodularin (MC/NOD); cylindrospermopsin (CYL); anatoxin-a (ANTX); Total MC producing bacteria; Total CYL producing bacteria; Total ANTX - producing cyanobacteria; Identify & photograph potentially toxic algae (PTOX)	250L or 500 mL glass amber	Put on new elbow-length (recommended) gloves and obtain a clean bottle. New sampling bottles should not be rinsed with surface water prior to sample collection. Remove cap from bottle and hold in opposite hand from bottle. Grasp bottle from the bottom and submerge bottle (mouth first) into surface of water, sink bottle downwards 2-4 inches below the surface in a U-shaped motion, then pull the bottle out of water with the mouth facing up. Try to avoid overfilling the bottle; pouring out the sample is discouraged because it is not homogenous. If the sample container is overfilled, shake gently at the elbow 5 times, and then pour out a small volume of water. Immediately cap the bottle. Wipe off exterior of sample bottle and attach label. Place bottle into a cool ice chest. The sample containers should remain in the dark and be cooled to 4-6° C (do not freeze) during the remainder of the field sampling day. To maintain cool ice chests, store in the shade.)	

Note: A total of six (6) sample bottles will be used at each sample location/sample event.

One field dup/sampling event will be collected.

Appendix B
Delta Labs Field Filtration Protocol

Delta Environmental Laboratories

DIRECTIONS FOR USING SYRINGE FILTERS

1. PURPOSE

Syringe filters are generally used to remove particles from a liquid sample prior to analysis which may damage equipment (ion chromatography, ICP) or interfere with results (spectrophotometer). They are typically used for small volumes. For most analysis, a disposable polypropylene syringe is used with a 0.45 um pore size filter.

2. DIRECTIONS

1. Open syringe package and remove the syringe. Most filters provided range from 30-60 ml with a luer-lok tip.
2. Filling the syringe and attacking the syringe filter.

A) Draw a small amount of air into the syringe by pulling the plunger back and release to loosen it. Load the sample into the syringe. Assure that no air bubbles are locked in the syringe.
   i. If air bubbles appear, hold the filter upside down to allow the air to float to the tip of the syringe. Slowly push air out without losing any sample.

B) Remove the syringe filter from its packaging. To reduce contamination, hold the filter with its original packaging when attaching the filter to the syringe. For a luer lock syringe, fix it firmly by rotating the filter in a clockwise motion, but do not overtighten.

C) Hold the assembled syringe and filter vertically. Filter the solution into the receiving vessel by gently pressing down on the syringe plunger to push the sample through the filter. If the back pressure ever increases significantly, change the filter as it may have plugged. Avoid pressing excessively as this could cause the filter housing to burst.

D) If additional sample solution needs to be filtered, remove the syringe filter from the syringe tip. Collect the sample and reattach the syringe filter as described previously.

E) Use a new syringe and syringe filter for each new sample.

Revision #	Reason/Changes	Written by:	Approved by:	Date
1.0	Revision			

Appendix C
Cyanobacteria Caution Level Signage

CAUTION

Harmful algae may be present in this water.
For your family's safety:

You can swim in this water, but **stay away from algae and scum** in the water.

Do not let pets and other animals go into or drink the water, or eat scum on the shore.

Keep children away from algae in the water or on the shore.

Do not drink this water or use it for cooking.

For fish caught here, **throw away guts and clean fillets** with tap water or bottled water before cooking.

Do not eat shellfish from this water.

Call your doctor or veterinarian if you or your pet get sick after going in the water.

For information on harmful algae, go to **mywaterquality.ca.gov/monitoring_council/cyanohab_network**

For local information, contact: **Gregory J Hoover**
TKPOA Water Quality Manager / AIS Management Coordinator
Ghoover@tahoekeyspoa.org
(530) 542-6444

PRECAUCIÓN

Puede haber algas dañinas en estas aguas. Para protección de su familia:

Puede nadar en estas aguas pero **aléjese de las algas o espuma lamosa** en el agua.

Mantenga a los niños alejados de algas en el agua u orilla del agua.

Al pescado que pesque aquí, **quítele los intestinos y tírelos a la basura.** Limpie el filete con agua de la llave o embotellada antes de cocinarlo.

No deje que sus mascotas o animales se metan o beban el agua, o coman la espuma lamosa en la orilla del agua.

No beba de esta agua o use para cocinar.

No coma mariscos de estas aguas.

Llame a su médico o veterinario si usted o su mascota se enferman después de meterse al agua.

Para información sobre algas dañinas, vaya a: **mywaterquality.ca.gov/monitoring_council/cyanohab_network**

Para información local comuníquese con:

Gregory J Hoover
TKPOA Gerente de Qualidad/ El Cordinador de AIS
Ghoover@tahoekeyspoa.org
(530) 542-6444