

2017 Cyanobacteria Sampling Report for the Tahoe Keys Lagoons



April 19, 2018

2017 Cyanobacteria Sampling Report for the Tahoe Keys Lagoons

Prepared for



*Tahoe Keys Property Owners Association
South Lake Tahoe, California*

Prepared by



Sierra Ecosystem
Associates



TAHOE KEYS INTEGRATED
MANAGEMENT PLAN

April 19, 2018

-Final-

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1.0 INTRODUCTION

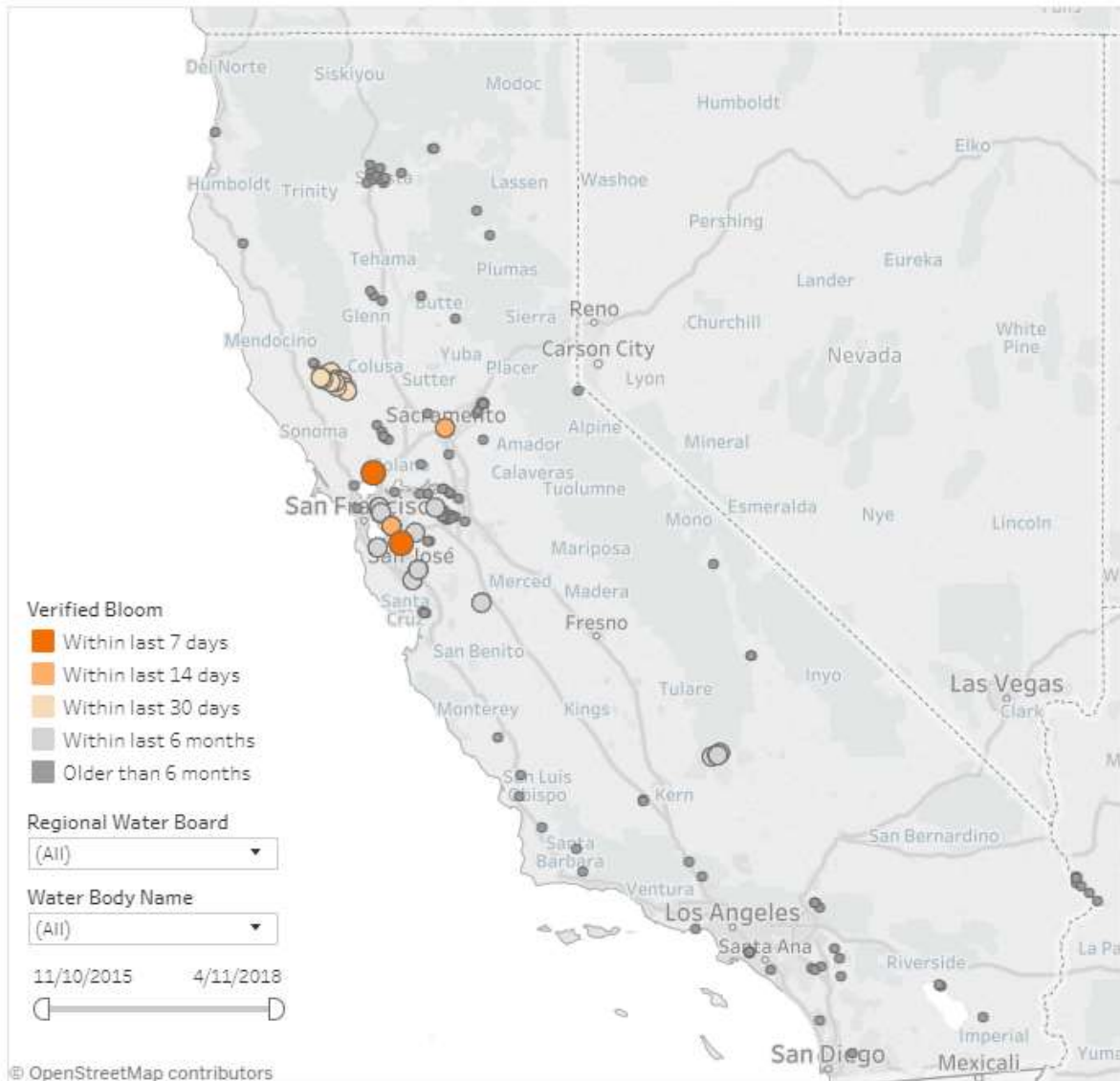
Cyanobacteria, or blue-green algae, are naturally occurring photosynthetic prokaryotes that can be found in almost all bodies of water, both fresh and saltwater. These organisms are considered to be the dominant primary producers on Earth, contributing more than 25% of photosynthesis worldwide. Cyanobacteria are true bacteria, often unicellular. However, they have the ability to grow into large colonies that may be visible to the naked eye, often referred to as a cyanobacteria harmful algal bloom (HAB) (UC Berkeley 2006, Frontier 2015).

Some cyanobacteria have the ability to produce secondary metabolites, such as cyanotoxins, which can be harmful to eukaryotes. Currently, there is still a lack of information on why or when these cyanotoxins are produced. For example, it has been noted in previous studies that these toxins may be present in the water column prior to the visible bloom (i.e., discolored water or mat-like scum accumulation on surface or shoreline) (USGS 2012, EPA 2018).

Cyanotoxin producing cyanobacteria blooms have become a growing global hazard, especially in surface waters that have been affected by anthropogenic nutrient loads. According to the US Environmental Protection Agency (EPA), these blooms can be caused by a number of factors, including: still water, large amounts of sunlight, warmer water temperatures, and elevated concentrations of nutrients (i.e., phosphorus and nitrogen) (USGS 2012, EPA 2018).

Cyanobacteria blooms have been a frequent occurrence in California since 2015. Figure 1 indicates California blooms from November 2015 to February 2018.

Figure 1. California Cyanobacteria Blooms 2015-2018



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2.0 CYANOBACTERIA EVALUATION

The analytical laboratory Bend Genetics, LLC was selected for cyanobacteria analysis of samples collected from the Tahoe Keys lagoons. Bend Genetics, LLC is located in Sacramento, CA and was selected due to its ability to conduct a variety of cyanobacteria analyses. Parameters and methods for testing are listed below in Table 1.

Table 1. Parameters and Analysis Methods

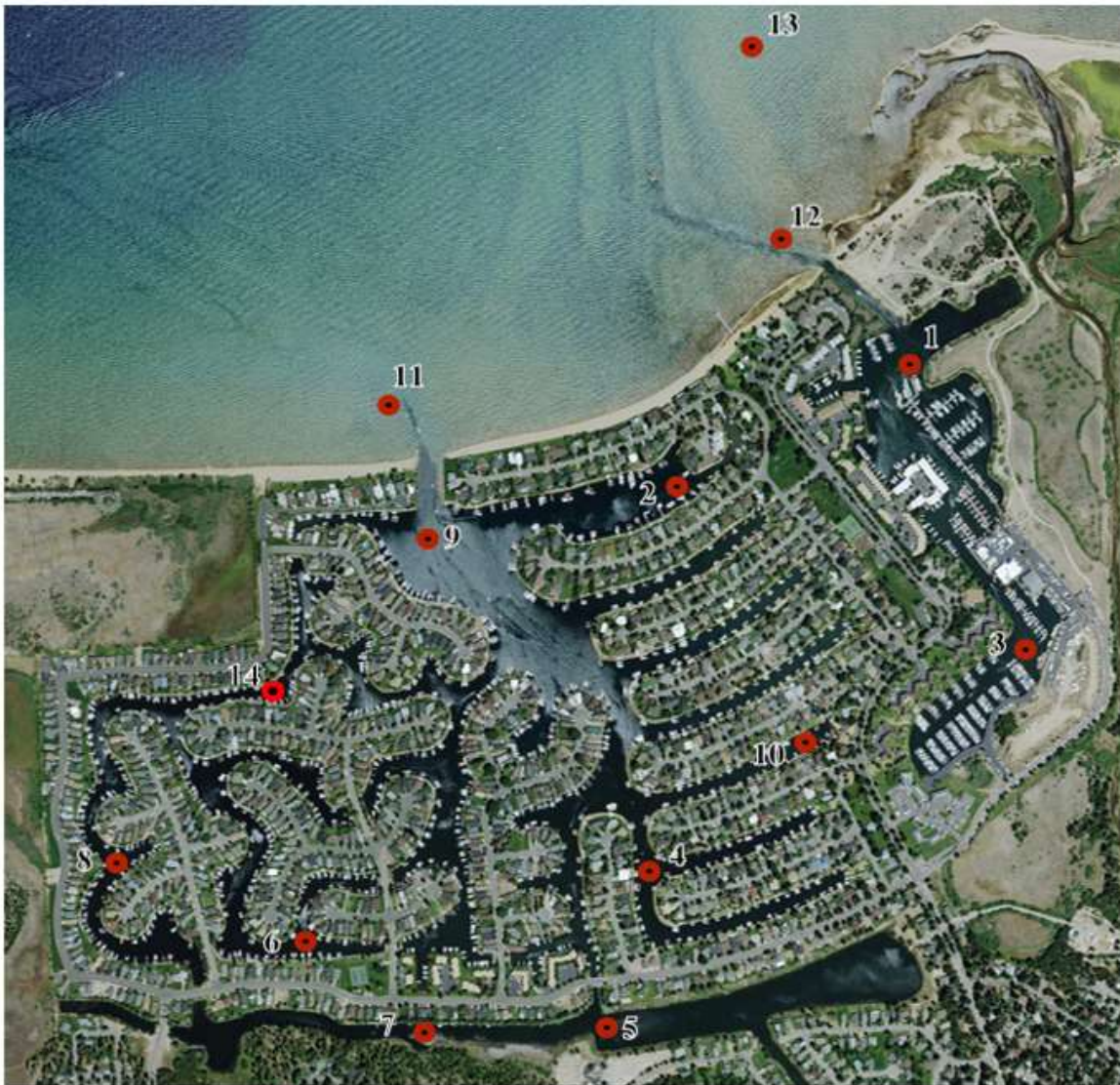
Parameter	Method	Units	Description
PTOX Identification	Microscope Analysis	-	Collected samples are plated and observed under a microscope to identify potentially toxic cyanobacteria species
Total 16S rRNA	QPCR	Copies/mL	Quantitative polymerase chain reaction; process used to enumerate pathogens, algae or specific genes responsible for production of undesirable compounds (i.e., 16S gene ¹ , microcystin or anatoxin-a)
Quantification of Total Cyanobacterial Toxins	ELISA	µg/L	Enzyme-linked immunosorbent assay; rapid test used to detect substances with antigenic properties (i.e., hormones, bacterial antigens, antibodies)
Quantification of Total Anatoxin-a (<i>anaC</i> gene)	QPCR	Copies/mL	(See above definition)
Quantification of Total Microcystin (<i>mcyE</i> gene)	QPCR	Copies/mL	(See above definition)

Sampling sites for water quality, sediment and cyanobacteria sampling are shown in the map below (Figure 2). A total of 13 sites were sampled, a combination of lagoon and lake sites, for water and sediment parameters. Of these 13 sites, six were selected initially for cyanobacteria sampling. The originally selected sites include the following: 1, 2, 5, 6, 10, and 11.

Sampling methods can be found in Appendix A of this document.

¹ rRNA sequence typically used to identify bacterial pathogens; responsible for the production of some cyanobacterial secondary metabolites

Figure 2. Sampling Map for the Tahoe Keys Lagoons



In mid-August, following reports from homeowners, it was determined that a cyanobacteria bloom was underway in the lagoons and an additional sampling site (Site 14) was included for the Sierra Ecosystem Associates (SEA)/Tahoe Keys Property Owners Association (TKPOA) August 28, 2017 and October 20, 2017 sampling events. Figure 3 shows the section of a narrow channel off the Main lagoon near Site 14 at the start of the bloom.

TKPOA notified both the US EPA as well as the Lahontan Regional Water Quality Control Board (LRWQCB) of the bloom. Overall, TKPOA does not have the legal, regulatory, or technical obligations to be responsible for monitoring or for public protection. However, local veterinarians were alerted and signage was posted throughout the Tahoe Keys lagoons. E-Blasts were distributed to residents and rental agencies and various public media articles (news and radio) were released.

LRWQCB and the US EPA requested TKPOA undertake further testing at a total of 16 sites. These sites were to be sampled weekly until the results showed non-detectable (ND) rates of anatoxin-a. This report does not include the additional data collected from the weekly samples. This data was delivered directly to LRWQCB.

Figure 3. Tahoe Keys Cyanobacteria Bloom (August 23, 2017)



3.0 RESULTS

Results of each of the three sampling events undertaken by TKPOA during the course of the 2017 season, not including the additional sampling required following the HAB, showed that the most prevalent cyanobacteria species found in the lagoon was *Dolichospermum* sp., followed by the *Worochinia* sp. Figures 4 and 5 are images of *Dolichospermum* sp. detected during the microscope analysis of the samples collected July 3 (Site 5) and August 28 (Site 14).

Figure 4. *Dolichospermum* sp. (July 3, 2017)



Dolichospermum is a diverse freshwater genera of cyanobacteria that grow in filamentous, multicellular clumps that resemble a chain-like pattern. Various species of this cyanobacteria 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, UCSC 2018).

The larger, darker cells featured in the Figure 4 *Dolichospermum* sp. are called akinetes, thick-walled and dormant cells found in filamentous cyanobacteria that is resistant to cold and desiccation and has the ability to store various essential materials to permit survival. Akinetes are often found near heterocysts. Heterocysts, a differentiated cell for nitrogen fixation that arises from vegetative cells were also detected during the July and August sampling events and are shown in the smaller of the darker cells shown in Figure 4 (Li et al. 2016).

Figure 5. Dolichospermum sp. (August 28, 2017)

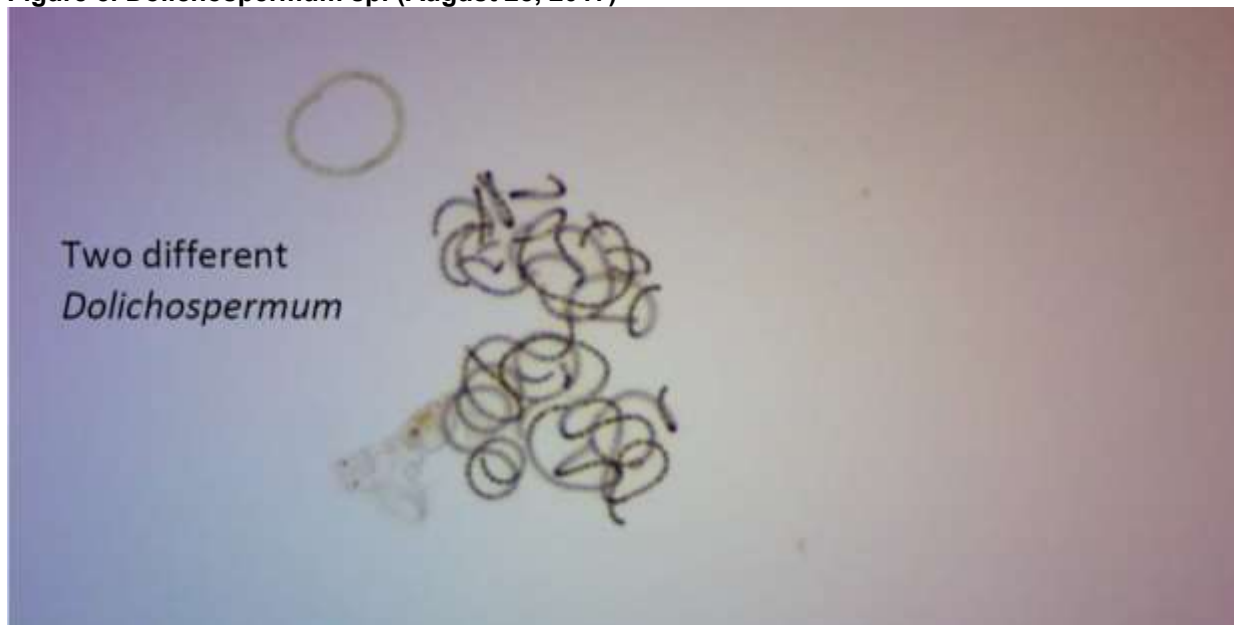


Figure 5, an image from microscope analysis of a sample collected during the August sampling event, shows two different species of Dolichospermum present in the Tahoe Keys lagoons. *Dolichospermum* sp. are common worldwide, with a total of 110 described species (UCSC 2018).

Other species present are listed in Table 2 below.

Table 2. Species Present at Each Sampling Event

Species	SAMPLING EVENT		
	07/03/2017	08/28/2017	10/20/2017
<i>Dolichospermum</i> sp.	Present	Present	Present
<i>Worochinia</i> sp.	Not Present	Present	Present
<i>Microcystis</i> sp.	Not Present	Present	Not Present
<i>Snowella</i> sp.	Not Present	Not Present	Present
<i>Asterionella</i> sp.	Not Present	Not Present	Present
<i>Wilmottia</i> sp.	Not Present	Present	Not Present

Following the first detection of the cyanobacteria bloom in August 2017, samples were screened for possible toxicity. Low levels of anatoxin-a² (Sites 5, 6, 10, and 14) and microcystin³/Nod (Site 14), as well as multiple 16S gene copies/mL for each toxin, were detected. Low levels were also detected from the October 2017 samples. Please see Bend Genetics, LLC results memorandums for sampling events in Appendix B.

² A bicyclic secondary amine cyanotoxin that is rapidly degraded, with a half-life between 1 to 2 hours; known to be produced by *Dolichospermum*, *Aphanizomenon*, and *Oscillatoria* (Li et al. 2016).

³ A cyclic heptapeptide cyanotoxin that is noted as the most ubiquitous; known to be produced by multiple species of *Dolichospermum* cyanobacteria.

4.0 DISCUSSION

Results indicate the presence of a variety of cyanobacteria within the Tahoe Keys lagoons. Furthermore, the occurrence of a cyanobacteria bloom in August 2017 and detected levels of both anatoxin-a as well as microcystin further indicate the presence of potentially toxic cyanobacteria in the Tahoe Keys lagoons. Samples taken in and near the Main and Marina Channels in Lake Tahoe had no detections of cyanobacteria, rather various forms of eukaryotic algae, protozoan grazers, diatoms, and flagellates were identified as well as low levels of 16S gene copies.

Exposure to cyanotoxins can cause allergic, respiratory, liver, kidney and nervous system reactions in mammals, and large blooms of cyanobacteria (not necessarily toxin producing) can outcompete beneficial phytoplankton and cause a depletion of available oxygen in the water column (USGS 2006, Paerl and Otten 2013). Therefore, monitoring of cyanobacteria, especially for the presence of potentially toxic secondary metabolites, is important to protect both the health of the lagoon ecosystem as well as the health of residents and recreationists. Monitoring of a body of water provides entities, such as public health and resource agencies, the ability to implement a response plan, including timely notifications to the public.

Currently, the recommended method to reduce the occurrence of cyanobacteria blooms in surface water is to address nutrient pollution, especially that of nitrogen and phosphorus (Schwartz et al. 2013, EPA 2018). The reduction of available nutrients for the cyanobacteria will decrease the probability of a bloom from occurring. As part of TKPOA's Integrated Management Plan (IMP), Nonpoint Source (NPS) Plan and other ongoing programs (e.g., water conservation), continued actions to improve and enhance overall water quality of the Tahoe Keys lagoons will help address the nutrients available for both aquatic macrophyte and cyanobacteria growth.

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6.0 GLOSSARY

Akinetes	Thick-walled and dormant cells found in filamentous cyanobacteria that is resistant to cold and desiccation and has the ability to store various essential materials to permit survival.
Anatoxin-a	A bicyclic secondary amine cyanotoxin that is rapidly degraded, with a half-life between 1 to 2 hours; known to be produced by <i>Dolichospermum</i> , <i>Aphanizomenon</i> , and <i>Oscillatoria</i> (Li et al. 2016).
Anthropogenic	Arising from human activity; primarily environmental pollutants or pollution.
DNA	Deoxyribonucleic acid; hereditary material in nearly all organisms on Earth coded by four nucleobases: adenine, guanine, thymine, cytosine.
Eukaryote	Organisms, either uni- or multi-cellular, with DNA as the genetic material; eukaryotic cells contain membrane-bound specialized organelles, including a nucleus containing chromosomes.
Heterocysts	A differentiated cell for nitrogen fixation that arises from vegetative cells.
Microcystin	A cyclic heptapeptide cyanotoxin that is noted as the most ubiquitous; known to be produced by multiple species of <i>Dolichospermum</i> cyanobacteria.
Prokaryote	Organism, often microscopic and single-celled, that has no distinct nucleus or other specialized organelles; includes bacteria and cyanobacteria.
RNA	Ribonucleic acid; responsible for various roles including regulation, coding, expression of genes.
rRNA	Component of RNA that is essential for the synthesis of proteins; links amino acids together.
Secondary Metabolites	Organic compounds (produced by plants, cyanobacteria, etc) which are not involved in development, reproduction or growth of an organism.
16S	rRNA sequence typically used to identify bacterial pathogens; responsible for the production of some cyanobacterial secondary metabolites.

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- APPENDICES -

**2017 Cyanobacteria Sampling Report for the
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April 19, 2018

Appendix A
Sampling Protocols

-FINAL-

**2017 Baseline Water Quality Sampling Protocols
for the
Tahoe Keys Lagoons**



June 30, 2017

**2017 Baseline Water Quality Sampling Protocols
for the
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Prepared for



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Prepared by



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1.0 BACKGROUND

The Tahoe Keys, a residential and commercial development located along the south shore of Lake Tahoe, is comprised of three water features: Lake Tallac Lagoon (a storm water collection basin for South Lake Tahoe), the Main Lagoon (western water access for most residences of the Tahoe Keys), and the independent, separately owned Marina Lagoon (eastern water access for the Keys Marina, other commercial, and many townhome residences of the Tahoe Keys). The Tahoe Keys encompass 172 acres of waterways with 1,529 homes as well as townhouses, marinas, and a commercial center. Property in and around the Tahoe Keys lagoons is controlled by multiple landowners and waterway land ownership includes individual property owners, association ownership (e.g., TKPOA common property and Tahoe Keys Beach and Harbor Association), and commercial and governmental ownership. Through various agreements, TKPOA maintains the waterways for boating and other recreation. Both the Main and Marina lagoons have direct connections to Lake Tahoe via the West and East channels, respectively.

The Waste Discharge Requirements (WDRs) permit that was issued to the TKPOA by the Lahontan Regional Water Quality Control Board's (LRWQCB) Executive Order No. R6T-2014-0059 specifies that the TKPOA improve the control of aquatic invasive plants in the Tahoe Keys lagoons and that an IMP for Aquatic Plants and a Nonpoint Source Plan for Water Quality (NPS Plan) be implemented by the TKPOA (Lahontan 2014). The Monitoring and Reporting Program for the WDRs specifies that water quality parameters including dissolved oxygen, temperature, nitrate and nitrite nitrogen, ammonia, total ammonia, total Kjeldahl nitrogen, total phosphorus, and orthophosphorus be collected and analyzed for the Tahoe Keys lagoons during use of the circulation system. The TKPOA voluntarily added the 2016 Baseline Water Quality Program.

The Tahoe Keys Property Owners Association (TKPOA) began collecting baseline data on water quality in 2016 to help inform the Tahoe Keys Integrated Management Plan (IMP) and create an inventory for several water quality and sediment parameters. The program is continuing from 2016 to 2017, with the 2017 Baseline Water Quality Program beginning in the last week of April (April 27, 2017) and will continue through the summer into October. Water quality samples are to be taken every month at 13 different locations throughout the Tahoe Keys Lagoons as shown in Figure 1. Sediment sampling will occur twice during the 2017 season, once in the Spring and once in the Fall. Protocols for sediment sampling are discussed separately.

2.0 BASELINE WATER QUALITY

The Baseline Water Quality Program was initiated in 2016 to produce baseline data for nutrient concentrations, turbidity levels, and other water quality parameters (refer to Table 1) during the course of the growing season.

2.1 Overview of Program

Fifteen water quality parameters will be measured during at least 7 sampling events over the course of the aquatic plant growing season from April to October (Table 2). The following section describes the selection of sampling sites, sampling schedule, monitored parameters, and lab analysis details.

2.1.1 Sampling Sites

Similar to the 2016 Baseline Water Quality Monitoring program, water will be sampled at 13 sites in the Tahoe Keys lagoons. The sites for data collection include dead-end coves and open water areas to assess water quality and sediment variation by location. Using geo-referenced locations will allow future monitoring to occur at the same sites. Figure 1 shows all sampling sites for both water quality and sediment sampling. Refer to Table 1 for more information on sampling sites.

Cyanobacteria samples will be collected at 6 out of the 13 TKPOA Water Quality Monitoring program. These sites include: 1, 2, 5, 6, 10, and 11.

Table 1. Summary of Sample Sites

Site Number	Location	Comments
1	Marina Lagoon	Near entrance of the East Channel
2	Main Lagoon	---
3	Marina Lagoon	---
4	Main Lagoon	---
5	Lake Tallac	---
6	Main Lagoon	---
7	Lake Tallac	---
8	Main Lagoon	Dead-end cove
9	Main Lagoon	Open water area
10	Main Lagoon	Dead-end cove
11	Lake Tahoe	Near West Channel
12	Lake Tahoe	Near East Channel and Upper Truckee River delta
13	Lake Tahoe	Between East and West Channels

Figure 1. Water Quality and Cyanobacteria Sampling Sites



2.1.2 Sampling Schedule

Water quality sampling will begin in late April and will occur monthly into October. Sampling will occur on Monday or Wednesday and WETLab courier service will be used to pick up the samples Tuesday or Thursday.

One cyanobacteria sampling event will occur in the following months: June, August, October.

2.1.2 Monitored Parameters

Parameters that will be measured at each of the thirteen sites for water quality include: depth of water column, pH (of surface, mid-point, and bottom), specific conductivity, dissolved oxygen (DO), temperature, turbidity, orthophosphorus, total phosphorus (TP), nitrate-nitrogen, nitrite-nitrogen, total Kjeldahl nitrogen (TKN), total nitrogen (TN), and blue-green algae (cyanobacteria).

Table 2 below summarizes all parameters to be monitored.

Table 2. List of Parameters to be Monitored

Constituent	Method of Measurement	Brief Description
Time of Day (TOC)	Watch/YSI ProDSS	Each site is given a specific time frame in which samples will be collected. Time of sample collection must be the same during each sampling event. For example, Site X collected each month between 10:00am and 10:30am.
Depth	YSI ProDSS and water level sounder	Depth, in feet, of water level. Used to determine mid-depth, for sample collection and YSI data collection, as well as monitoring of snowmelt and potential storm runoff.
pH	YSI ProDSS.	Measure of acidity or alkalinity of water, with pH 7 being neutral. Surface, mid-point, and bottom will be collected during the season to monitor effects of plant biomass on overall pH.
Specific Conductance	YSI ProDSS	Measure in micro Siemens per centimeter ($\mu\text{S}/\text{cm}$) of dissolved ionic particles in the water. Acts as an indicator of Total Dissolved Solids.
Dissolved Oxygen	YSI ProDSS	Amount (in parts per million) of oxygen present in water. An important parameter in water quality assessment due to its influence on aquatic organisms. Concentrations of DO that are either too high or too low can be harmful to aquatic life and can affect water quality (Fondriest Environmental Inc. 2016).
Temperature	YSI ProDSS	Temperature, in degrees Celsius ($^{\circ}\text{C}$), of the water when sample is collected. Aquatic macrophytes begin growing in water around 50°C . Numerous biological and chemical processes are influenced by temperature changes.
Turbidity	YSI ProDSS	According to the USGS, turbidity is the measure, in a liquid, of clarity. In this case measured in Formazin Nephelometric Unit (FNU). Turbidity is caused by phytoplankton, algae, clay, silt, and fine suspended particles in the water column that scatter light (Perlman 2016). Higher levels of turbidity scatter more light and can cause a reduction in photosynthetic activity and lower the concentration of oxygen in the water body. Wildlife in the ecosystem can also be negatively impacted by higher levels, sometimes leading to low survival rates (Lenntech 2016).
Ortho-phosphorus	Lab Analysis	Dissolved inorganic phosphorus that is readily available for aquatic plants and algae.
Total Phosphorus	Lab Analysis	Amount of all forms, dissolved and particulate, of phosphorus present in the sample.
Nitrate-Nitrogen	Lab Analysis	Amount of nitrogen bound to a nitrate ion present in the sample.
Nitrite-Nitrogen	Lab Analysis	Amount of nitrogen bound to a nitrite ion present in the sample.
Total Kjeldahl Nitrogen	Lab Analysis	Measure of ammonia and organic forms of nitrogen.
Total Nitrogen	Lab Analysis	Sum of all forms of nitrogen, including Nitrate-Nitrogen, Nitrite-Nitrogen, and TKN.
Blue-Green Algae	Lab Analysis	Identification of abundant classes of cyanobacteria as well as potential toxicity and quantification of chlorophyll a.

2.1.3 Analytical Laboratory Testing

Western Environmental Testing (WET) Lab was selected to conduct the analysis of collected water quality samples for constituents that could not be completed in the field. The analytical lab located in Sparks, NV was used because it serves the South Lake Tahoe area.

TKPOA will utilize Wet Lab for test supply delivery, including: coolers, sample containers, and any necessary preservatives. The samples will be collected on Mondays or Wednesdays and the WET Lab courier service will collect all samples on Tuesday or Thursday, respectively.

Bend Genetics, LLC was selected for cyanobacteria analysis. The analytical lab is located in Sacramento, CA and was selected due to its ability to conduct a variety of cyanobacteria analyses. Samples will be collected with water quality samples and will either be hand delivered or shipped overnight to the lab for analysis.

2.2 Materials and Methods

Specific equipment and supplies are required to perform both water quality and sediment sampling. The necessary items are to be obtained by the TKPOA prior to the end of April 2017 and the initiation of field sampling.

The following section provides information on the required equipment utilized for water quality and the methods to be used by the TKPOA Water Quality Department throughout the season.

2.2.1 Required Materials

The following materials are required for water quality sampling:

- Pre-Sampling Checklist
- YSI ProDSS
- YSI Calibration Log
- Calibration Solutions
- Sample pump
- Pen/Pencil/Sharpie
- Sample location map
- Wet ice
- Disposable, powder-free gloves
- Water Quality Data Collection Sheet
- Water level sounder
- Portable battery

The following will be provided by WET Lab:

- Cooler(s)
- 1 L bottles
- 500mL bottles
- Sample bottle labels
- Sulfuric acid preservative

The following materials are required for cyanobacteria sampling:

- 250mL PETG Plastic Sample Bottles
- YSI ProDSS
- Water Quality Data Collection Sheet
- Water level sounder
- Pen/Pencil/Sharpie
- Sample location map
- Disposable, powder-free gloves
- Wet ice / ice packs
- Cooler(s)
- Preservative (optional)

2.2.2 Water Quality Sample Methods

a. Pre-Sampling Checklist:

- Check weather forecast for sampling day to determine if conditions are appropriate for sampling to occur.
- Verify sampling materials delivery
- Verify that WETLab (or 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.
- Calibration of the YSI ProDSS should occur monthly and take place no later than a day prior to scheduled sampling event. Sampling should not occur if calibration is not completed. Calibrate according to manufacturer's instructions.

b. Field Sampling:

On the day of sampling, once on the boat with all necessary materials, the sample collector will complete the title section of the data sheet, indicating sample event number, boat driver, sample collector, and start time. Lake elevation, Truckee River discharge, and recent weather conditions were recorded and later input into the water quality database.

At each sampling location, two sets of duplicate samples will be taken for a total of four bottles at each site. One set will be unpreserved and the other will be preserved with H₂SO₄. Bottle labels should be filled out before water collection and will include the following information:

- Company Name (TKPOA)
- Sample ID (WQ-instance number-site number A (B for duplicate))
 - ex: WQ-01-01A
- Sampled By (collector's initials)
- Date of Sample

Samples will be taken by placing a submersible pump at mid-depth in the water column. Attach the sample pump to the portable battery and allow water to run through the attached hose for at least one minute to flush the system prior to rinsing the collection bottles.

Depth of the sample site is determined with the YSI ProDSS or a water level sounder.

The sample collector should be wearing disposable, powder free gloves when handling the sample containers to prevent contamination of sample. Triple rinse the collection bottles before collecting the actual sample, filling roughly three quarters of the bottle. For samples that require preservative, once sample is collected carefully add in the H₂SO₄.

Additional data will be collected at each site with the YSI. Lower the instrument to mid-depth in the water column. Data to be collected:

- Water Temperature (°C)
- pH
- Dissolved Oxygen (%)
- Turbidity (FNU)
- Electric Conductivity (uS/cm)
- Observations (i.e. the presence of algae, odor, fish, insects, or amphibians in sample site etc.)

This data will be recorded on a data sheet along with the site number, time, and depth.

Refer to sample parameter section for more information on monitored constituents.

c. Chain of Custody:

The Chain of Custody (COC) Form supplied by WETLab will be filled out completely as shown in Figure 2. This form will be signed by the collector when dropped off at the TKPOA Pavilion. Samples are to be picked up by a WETLab courier or dropped off at the lab located in Sparks, NV within 24hrs of collection. Samples should have enough ice to keep them cool until pickup/drop off occurs.

Current analysis to be carried out by the lab includes:

- Total Phosphorous
- Orthophosphorous
- Total Nitrogen
- Nitrate Nitrogen
- Nitrite Nitrogen
- Total Kjeldahl Nitrogen

d. Data Handling:

Enter the data into the database or scan the data sheet and email to SEA staff. Data should be entered into database or transmitted to SEA within one day of sample collection. SEA will enter the collected data into the 2017 Water Quality workbook (refer to Figure 3).

2.2.3 Cyanobacteria Sample Methods

a. Field Sampling:

Prior to each cyanobacteria sampling event, field staff will verify that all equipment is present before heading out onto the water as well as that the analytical lab is aware that sampling is occurring and when materials will be shipped or hand delivered.

On the day of sampling, once on the boat with all necessary materials, the sample collector will complete the title section of the data sheet, indicating sample event number, boat driver, sample collector, and start time. Lake elevation, Truckee River discharge, and recent weather conditions were recorded and later input into the water quality database. If sampling is occurring alongside water quality monitoring, the data for both water quality sampling and cyanobacteria will be documented on one Water Quality Data Collection Sheet.

Bottles should be labeled following sample collection. For each site, the collector will use a sharpie to label the bottles with field identification numbers. Bottles should include the following information:

- Company Name (TKPOA)
- Sample ID (CY – sample event number – site number)
 - Ex: CY-01-02
- Sampled by (collector's initials)
- Date of Sample
- Time of Sample

Depth of the sample site is determined with the YSI ProDSS or a water level sounder. The following data will be collected by lowering the instrument to mid-depth in the water column:

- Water Temperature (°C)
- pH
- Dissolved Oxygen (%)
- Turbidity (FNU)
- Electric Conductivity (uS/cm)
- Observations (i.e. the presence of algae, odor, fish, insects, or amphibians in sample site etc.)

This data will be recorded on a data sheet along with the site number, time, and depth.

The sample collector should be wearing disposable, powder free gloves when handling the sample containers to prevent contamination of sample.

DO NOT RINSE THE SAMPLE CONTAINER PRIOR TO SAMPLE COLLECTION.

The collector will remove the PETG plastic bottle cap, invert and slowly lower the bottle into the water. 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. Return the container to the surface quickly and, if necessary, pour out a small volume of the sample to allow for homogenization. Quickly replace the cap, tighten securely, wash the bottle with water, dry, and then label with sharpie following the above instructions. Place the container into the cooler with ice immediately following completion of the above instructions.

b. COC Forms:

The Chain of Custody (COC) Form supplied by Bend Genetics, LLC will be filled out completely as shown in Figure 4. This form will be signed by the collector when dropped off at the TKPOA Pavilion. Samples are to be shipped (next day/overnight) or hand delivered to the lab located in Sacramento, CA within 24hrs of collection. Samples should have enough ice to keep them cool until drop off occurs.


Current analysis to be carried out by the lab includes:

- Microscope Identification
- QPCR Total Cyanobacteria (16S rRNA)

c. Data Handling:

Once the data has been sent via email from the lab, SEA will enter the data into the 2017 Water Quality workbook.

Figure 2. Example of COC Form



WETLAB
WESTERN ENVIRONMENTAL TESTING LABORATORY
Specializing in Soil, Hazardous Waste and Water Analysis

475 E. Grog Street #119 | Sparks, Nevada 89431 | www.WETLaboratory.com
tel (775) 355-0808 | fax (775) 355-0817

1084 Lamotte Highway | Elko, Nevada 89801
tel (775) 777-9833 | fax (775) 777-9833

3230 Palaris Ave., Suite 4 | Las Vegas, Nevada 89102
tel (702) 475-8898 | fax (702) 776-8152

WETLAB Order ID. _____

Sparks Control # _____

Elko Control # _____

LV Control # _____

Report Due Date _____

Page 1 of 2

Client: Tahoe Keys POA

Address: 356 Ala Wai Blvd

City, State & Zip: South Lake Tahoe, CA, 96150

Contact: Chad Johnson / Greg Hoover

Phone: 530-626-1401 Collector's Name: Kristen Hunter

Fax: _____ PWS/Project Name: Bi-weekly Sampling

P.O. Number: _____ PWS/Project Number: _____

Email: chad@sierra.ecos.com

Turnaround Time Requirements

Standard: 5 Day* (20%) 72 Hour** (80%)

48 Hour* (100%) 24 Hour* (200%)

*Surcharge WA Appx

Sample Collected From: Well State? Other

Report Results Via: PDF EGG

Compliance Monitoring? Yes No

Report to Regulatory Agency? Yes No

Standard OC Required? Yes No

Billing Address (if different than Client Address)

Company: _____

Address: _____

City, State & Zip: _____

Contact: _____

Phone: _____ Fax: _____

Email: _____

S
A
M
P
L
E
T
Y
P
E

Analyses Requested

SAMPLE ID/LOCATION	DATE	TIME	PRES TYPE	NO. OF CONTAINERS	Total Phos	Ortho Phos	Total Nitrogen	Spl. No.
WQ-01-01A	5/24/16	10:10A	1 SW	1	X	X	X	
WQ-01-01B	5/24/16	10:10A	1 SW	1	X	X	X	
WQ-01-01A	5/26/16	10:15A	2 SW	1	X	X	X	
WQ-01-01B	5/26/16	10:15A	2 SW	1	X	X	X	

Instructions/Comments/Special Requirements: _____

Sample Matrix Key: DW = Drinking Water WW = Wastewater SW = Surface Water MW = Monitoring Well SD = Solid/Dudge SO = Soil HW = Hazardous Waste OTHER: _____

*SAMPLE PRESERVATIVES: 1=Unpreserved 2=H2SO4 3=NaOH 4=HCl 5=HNO3 6=Na2S2O3 7=ZnOAc+NaOH 8=HCl/VOA Vial

Temp	Custody Seal	# of Containers	DATE	TIME	Samples Relinquished By	Samples Received By
°C	Y N None	4	5/24/16	5:15p	Sample Signature	
°C	Y N None					
°C	Y N None					
°C	Y N None					

WETLAB'S Standard Terms and Conditions apply unless written agreements specify otherwise. Payment terms are Net 30.

Client/Collector attests to the validity and authenticity of this (these) sample(s) and, is (are) aware that tampering with or intentionally mislabeling the sample(s) location, date or time of collection may be considered fraud and subject to legal action (NAC445.0536). SS Initial

To the maximum extent permitted by law, the Client agrees to limit the liability of WETLAB for the Client's damages to the total compensation received, unless other agreements are made in writing. This limitation shall apply regardless of the cause of action or legal theory filed or asserted. SS Initial

WETLAB will dispose of samples 90 days from sample receipt. Client may request a longer sample storage time for an additional fee. 301 2E

Please contact your Project Manager for details. SS Initial

Figure 3. Example 2017 Water Quality Workbook Layout

Sampling event no:

Date: _____

Collector: _____

Boat Driver: _____

Truckee Discharge: _____

Start time: _____

Lake Tahoe dam elevation: _____

End time: _____

Wind speed and direction: _____

Storm since last sample (duration, accumulation): _____

Site #	Time	Depth to bottom	Depth at measurement	pH	SPC (us/cm)	DO (ppm)	temperature °C	turbidity (FNU)
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
13								

WET Lab Results

Site #	Orthophos	Ammonia	Total Phosphor	Nitrate Nitrogen	Nitrite Nitrogen
1A					
1B					
2A					
2B					
3A					
3B					
4A					
4B					
5A					
5B					
6A					
6B					
7A					
7B					
8A					
8B					
9A					
9B					
10A					
10B					
11A					
11B					
12A					
12B					
13A					
13B					

WET Lab Sediment Results

Site #	Aluminum (mg/kg)	Phosphorus (mg/kg)	Organic Matter (%)	Orthophosphorus (mg/kg)	Ammonia (mg/kg)	Total Solids (%)	Nitrate Nitrogen (mg/kg)	Nitrite Nitrogen (mg/kg)
1A								
1B								
2A								
2B								
3A								
3B								
4A								
4B								
5A								
5B								
6A								
6B								
7A								
7B								
8A								
8B								
9A								
9B								
10A								
10B								
11A								
11B								
12A								
12B								
13A								
13B								

3.0 2017 BASELINE WATER QUALITY DOCUMENTS

Contents:

- Baseline Water Quality Pre-Sampling Checklist
- YSI ProDSS Calibration Log
- TKPOA Water Quality Data Collection Sheet

Baseline Water Quality Pre-Sampling Checklist

(Check each box to mark complete; To be completed prior to every sampling event)

Month	YSI ProDSS		WETLab			Weather Check (safe conditions)	Materials	TKPOA Water Quality Data Collection Sheet	Initials
	Calibration Complete	Calibration Log Complete	Materials Delivery	Courier Service Set-up	COC Forms				
April									
May									
June									
July									
August									
September									
October									

YSI Multiprobe Calibration / Maintenance Log

Date: _____	Time: _____	Employee Name: _____
-------------	-------------	----------------------

Calibration

Function	Temp of Standard	Value of Standard	Initial Reading	Calibrated to	Comments
Specific Conductivity (high) 1000 mS/cm					
Specific Conductivity (med) 100 mS/cm					
Specific Conductivity (low) 1 mS/cm					
pH calibrated (at pH 10)					
pH calibrated (at pH 7)					
pH calibrated (at pH 4)					
Turbidity (high) 1000 NTU					
Turbidity (med) 100 NTU					
Turbidity (low) 1 NTU					
Dissolved Oxygen (ppm)					
Dissolved Oxygen (% sat)					

Data Needed for Dissolved Oxygen (% sat) Calibration

Altitude (A) = _____ ft above mst	Barometric Pressure (BP) _____ inches
	Barometric Pressure Options
	Barometric Pressure Formulas
Barometer	BP (in) ____ x 25.4 = BP ____ mm
Local Source After Correction _(CBP)	BP ____ mm = CBP ____ mm - 2.5 (altitude __/100)
	Estimated from Altitude Only

Employee Signature: _____

TKPOA Water Quality Data Collection Sheet

Date: _____

Entered by: _____

Collector: _____

QC'd by: _____

Boat Driver: _____

Start time (boat out)

End Time (boat in)

Site #	Time	Depth to bottom (feet)	Depth at measurement	pH			SPC (µs/cm)	DO (ppm)	Temp °C	Turbidity (FNU)
				Bottom	Mid	Surface				
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										
11										
12										
13										

Observations

Appendix B

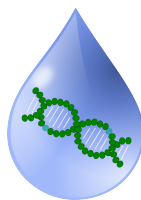
Bend Genetics, LLC Sampling Results

Index:

July 3, 2017

August 28, 2017

October 20, 2017



Bend Genetics, LLC
87 Scripps Drive, Ste. 108
Sacramento, CA 95825
Tel: (916) 550-1048

Date: 7/6/2017

Subject: Cyanobacteria analysis results

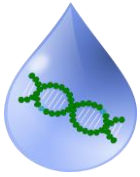
From: Tim Otten, Laboratory Director

To: Kristen Hunter
Sierra Ecosystem Associates

Attached are the results of microscopy and DNA analyses conducted on samples collected on 7/3/2017. These data have been reviewed and are considered final.

Analyses included in this report:

- Quantification of total cyanobacteria (16S rDNA) by real-time quantitative polymerase chain reaction (QPCR) method.
- Identification of potentially toxigenic cyanobacteria (PTOX) via microscopy.



Bend Genetics, LLC
87 Scripps Drive, Ste. 108
Sacramento, CA 95825
Tel: (916) 550-1048

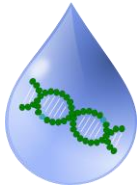
Project: Sierra Ecosystem Associates
Analysis for Toxigenic Cyanobacteria

Agreement #:

Reported: 7/6/2017 16:00

ANALYTICAL REPORT FOR SAMPLES

Sample ID	BG_ID	Date Collected	Date Received	Matrix	Preserved
CY-01-01	SE01	7/3/2017 12:35	7/3/2017 15:50	Water	N
CY-01-02	SE02	7/3/2017 12:10	7/3/2017 15:50	Water	N
CY-01-05	SE03	7/3/2017 10:26	7/3/2017 15:50	Water	N
CY-01-06	SE04	7/3/2017 11:25	7/3/2017 15:50	Water	N
CY-01-10	SE05	7/3/2017 11:50	7/3/2017 15:50	Water	N
CY-01-11	SE06	7/3/2017 12:20	7/3/2017 15:50	Water	N

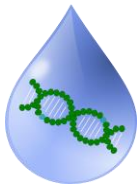


Bend Genetics, LLC
87 Scripps Drive, Ste. 108
Sacramento, CA 95825
Tel: (916) 550-1048

Project: Sierra Ecosystem Associates
Analysis for Toxigenic Cyanobacteria
Agreement #:
Reported: 7/6/2017 16:00

SAMPLE RESULTS

Sample ID	Method	Target	Result	Units	Quantitation	
					Limit	Notes
CY-01-01	QPCR	Total Cyano (16S)	65,245	copies/mL	100	
CY-01-02	QPCR	Total Cyano (16S)	206,307	copies/mL	100	
CY-01-05	QPCR	Total Cyano (16S)	471,018	copies/mL	100	
CY-01-06	QPCR	Total Cyano (16S)	1,347,398	copies/mL	100	
CY-01-10	QPCR	Total Cyano (16S)	669,113	copies/mL	100	
CY-01-11	QPCR	Total Cyano (16S)	57,854	copies/mL	100	



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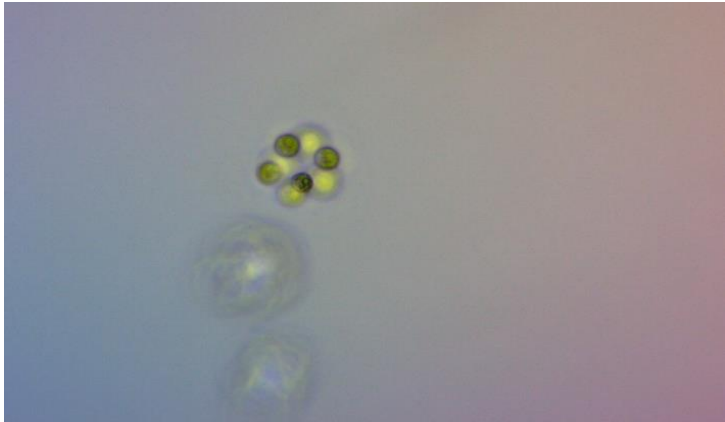
Project: Sierra Ecosystem Associates
Analysis for Toxigenic Cyanobacteria

Agreement #:


Reported: 7/6/2017 16:00

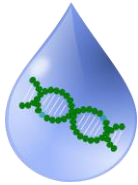
Microscope evaluation of potentially toxigenic cyanobacteria (PTOX)

Sample ID	Dominant	Sub-dominant	Also present	Notes
CY-01-01	NA			No PTOX cyanobacteria were observed in this sample, although various coccoidal cyanobacteria were observed. The photomicrograph was taken under 400X magnification.



Sample ID	Dominant	Sub-dominant	Also present	Notes
CY-01-02	NA		<i>Dolichospermum</i>	Although not highly abundant, there was a low amount of <i>Dolichospermum</i> sp. filaments present as well as some coccoidal cyanobacteria in this sample.





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Tel: (916) 550-1048

Project: Sierra Ecosystem Associates
Analysis for Toxigenic Cyanobacteria

Agreement #:

Reported: 7/6/2017 16:00

Microscope evaluation of potentially toxigenic cyanobacteria (PTOX)

Sample ID	Dominant	Sub-dominant	Also present	Notes
-----------	----------	--------------	--------------	-------

CY-01-05 *Dolichospermum*



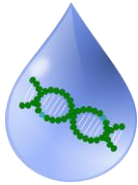
This sample contained a considerable amount of *Dolichospermum* sp. containing both heterocysts and akinetes. The photomicrograph was taken under 400X magnification.

Sample ID	Dominant	Sub-dominant	Also present	Notes
-----------	----------	--------------	--------------	-------

CY-01-06 *Dolichospermum*



Dolichospermum sp. was highly abundant in this sample, along with a moderate amount of coccoidal cyanobacteria. Notably, the morphology of the *Dolichospermum* filaments were different than those at site CY-01-05, instead they were more similar to the filaments observed at sites CY-01-02 and CY-01-10. The photomicrograph was taken under 400X magnification.



Bend Genetics, LLC
87 Scripps Drive, Ste. 108
Sacramento, CA 95825
Tel: (916) 550-1048

Project: Sierra Ecosystem Associates
Analysis for Toxigenic Cyanobacteria

Agreement #:

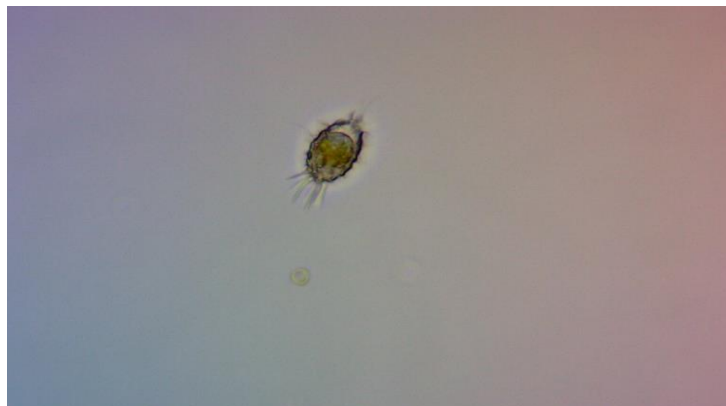
Reported: 7/6/2017 16:00

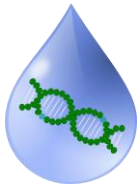
Microscope evaluation of potentially toxigenic cyanobacteria (PTOX)

Sample ID	Dominant	Sub-dominant	Also present	Notes
CY-01-10			<i>Dolichospermum</i>	This sample contained a moderate amount of <i>Dolichospermum</i> sp. filaments, but considerably less than observed in the CY-01-05 and CY-01-06 samples. The photomicrograph was taken under 40X magnification.



Sample ID	Dominant	Sub-dominant	Also present	Notes
CY-01-11	NA			No PTOX cyanobacteria were observed in this sample. Instead there were various flagellates and protozoan grazers. The photomicrograph was taken under 400X magnification.





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Tel: (916) 550-1048

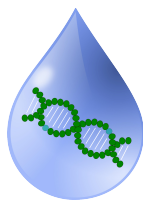
Project: Sierra Ecosystem Associates
Analysis for Toxigenic Cyanobacteria
Agreement #:
Reported: 7/6/2017 16:00

QUALITY CONTROL

Method	Analyte	Result	Qualifiers / Comments	Units	Expected Value	%REC	%REC Limits
QPCR	Cy16S - Blank	ND	U	copies/mL	0		
QPCR	Cy16S - Matrix Sp	62,812		copies/mL	65,000	96.6	70-130

QUALIFIERS/COMMENTS/NOTES

- C1 The reported concentration for this analyte is below the quantification limit.
- C2 The reported concentration for this analyte is above the calibration range of the instrument.
- J The reported result for this analyte should be considered an estimated value.
- U Undetected



Bend Genetics, LLC
87 Scripps Drive, Ste. 108
Sacramento, CA 95825
Tel: (916) 550-1048

Date: 10/20/2017

Subject: Cyanobacteria testing results

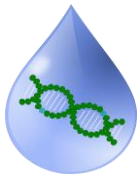
From: Tim Otten, Laboratory Director

To: Gregory Hoover
Tahoe Keys Property Owners Association

Attached are the results of microscopy, ELISA and QPCR analyses conducted on 21 samples collected from the Tahoe Keys on 10/16/2017. These data have been reviewed and are considered final.

Analyses included in this report:

- Quantification of total anatoxin-a and microcystin/nodularin by enzyme linked immunosorbent assay (ELISA).
- Quantification of total anatoxin-a producing cyanobacteria (*anaC* gene) and total microcystin producing cyanobacteria (*mcyE* gene) inferred by real-time quantitative polymerase chain reaction (QPCR) method.
- Microscope identification and photographs of all potentially toxic (PTOX) cyanobacteria.



Bend Genetics, LLC
87 Scripps Drive, Ste. 108
Sacramento, CA 95825
Tel: (916) 550-1048

Project: SWAMP_FHAB_2017

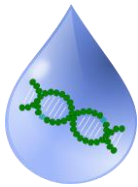
Analysis for Toxigenic Cyanobacteria

Project #: Tahoe Keys POA

Reported: 10/20/2017 12:10

ANALYTICAL REPORT FOR SAMPLES

Sample ID	BG_ID	Date Collected	Date Received	Matrix	Preserved
Cy-05-01	TK04	10/16/2017 9:30	10/17/2017 13:00	Water	Y
Cy-05-11	TK05	10/16/2017 10:30	10/17/2017 13:00	Water	Y
Cy-05-02	TK06	10/16/2017 11:25	10/17/2017 13:00	Water	Y
Cy-05-10	TK07	10/16/2017 12:18	10/17/2017 13:00	Water	Y
Cy-05-14	TK08	10/16/2017 12:50	10/17/2017 13:00	Water	Y
Cy-05-06	TK09	10/16/2017 13:38	10/17/2017 13:00	Water	Y
Cy-05-05	TK10	10/16/2017 14:25	10/17/2017 13:00	Water	Y
Cy-06-03	TK11	10/16/2017 8:00	10/17/2017 13:00	Water	N
Cy-06-01	TK12	10/16/2017 8:30	10/17/2017 13:00	Water	N
Cy-06-12	TK13	10/16/2017 9:49	10/17/2017 13:00	Water	N
Cy-06-13	TK14	10/16/2017 10:10	10/17/2017 13:00	Water	N
Cy-06-11	TK15	10/16/2017 10:30	10/17/2017 13:00	Water	N
Cy-06-09	TK16	10/16/2017 10:50	10/17/2017 13:00	Water	N
Cy-06-02	TK17	10/16/2017 11:25	10/17/2017 13:00	Water	N
Cy-06-04	TK18	10/16/2017 11:58	10/17/2017 13:00	Water	N
Cy-06-10	TK19	10/16/2017 12:18	10/17/2017 13:00	Water	N
Cy-06-14	TK20	10/16/2017 12:50	10/17/2017 13:00	Water	N
Cy-06-08	TK21	10/16/2017 13:00	10/17/2017 13:00	Water	N
Cy-06-06	TK22	10/16/2017 13:38	10/17/2017 13:00	Water	N
Cy-06-05	TK23	10/16/2017 14:25	10/17/2017 13:00	Water	N
Cy-06-07	TK24	10/16/2017 14:45	10/17/2017 13:00	Water	N



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 87 Scripps Drive, Ste. 108
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Project: SWAMP_FHAB_2017

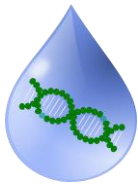
Analysis for Toxicogenic Cyanobacteria

Project #: Tahoe Keys POA

Reported: 10/20/2017 12:10

SAMPLE RESULTS

Sample ID	Method	Target	Result	Quantitation		Notes
				Limit	Units	
Cy-05-01	ELISA	Anatoxin-a	ND	0.165	µg/L	U
Cy-05-01	ELISA	Microcystin	ND	0.165	µg/L	U
Cy-05-01	QPCR	Anatoxin-a	ND	100	copies/mL	U
Cy-05-01	QPCR	Microcystin	ND	100	copies/mL	U
Cy-05-11	ELISA	Anatoxin-a	ND	0.165	µg/L	U
Cy-05-11	ELISA	Microcystin	ND	0.165	µg/L	U
Cy-05-11	QPCR	Anatoxin-a	ND	100	copies/mL	U
Cy-05-11	QPCR	Microcystin	ND	100	copies/mL	U
Cy-05-02	ELISA	Anatoxin-a	ND	0.165	µg/L	U
Cy-05-02	ELISA	Microcystin	ND	0.165	µg/L	U
Cy-05-02	QPCR	Anatoxin-a	ND	100	copies/mL	U
Cy-05-02	QPCR	Microcystin	ND	100	copies/mL	U
Cy-05-10	ELISA	Anatoxin-a	ND	0.165	µg/L	U
Cy-05-10	ELISA	Microcystin	ND	0.165	µg/L	U
Cy-05-10	QPCR	Anatoxin-a	ND	100	copies/mL	U
Cy-05-10	QPCR	Microcystin	ND	100	copies/mL	U
Cy-05-14	ELISA	Anatoxin-a	ND	0.165	µg/L	U
Cy-05-14	ELISA	Microcystin	0.11	0.165	µg/L	C1,J
Cy-05-14	QPCR	Anatoxin-a	74	100	copies/mL	C1,J
Cy-05-14	QPCR	Microcystin	462	100	copies/mL	
Cy-05-06	ELISA	Anatoxin-a	ND	0.165	µg/L	U
Cy-05-06	ELISA	Microcystin	0.12	0.165	µg/L	C1,J
Cy-05-06	QPCR	Anatoxin-a	ND	100	copies/mL	U
Cy-05-06	QPCR	Microcystin	79	100	copies/mL	C1,J
Cy-05-05	ELISA	Anatoxin-a	ND	0.165	µg/L	U
Cy-05-05	ELISA	Microcystin	ND	0.165	µg/L	U
Cy-05-05	QPCR	Anatoxin-a	ND	100	copies/mL	U
Cy-05-05	QPCR	Microcystin	ND	100	copies/mL	U



Bend Genetics, LLC
87 Scripps Drive, Ste. 108
Sacramento, CA 95825
Tel: (916) 550-1048

Project: SWAMP_FHAB_2017

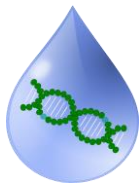
Analysis for Toxicogenic Cyanobacteria

Project #: Tahoe Keys POA

Reported: 10/20/2017 12:10

SAMPLE RESULTS

Sample ID	Method	Target	Result	Quantitation		Notes
				Limit	Units	
Cy-06-03	ELISA	Anatoxin-a	ND	0.15	µg/L	U
Cy-06-03	ELISA	Microcystin	ND	0.15	µg/L	U
Cy-06-03	QPCR	Anatoxin-a	ND	100	copies/mL	U
Cy-06-03	QPCR	Microcystin	ND	100	copies/mL	U
Cy-06-01	ELISA	Anatoxin-a	ND	0.15	µg/L	U
Cy-06-01	ELISA	Microcystin	ND	0.15	µg/L	U
Cy-06-01	QPCR	Anatoxin-a	ND	100	copies/mL	U
Cy-06-01	QPCR	Microcystin	ND	100	copies/mL	U
Cy-06-12	ELISA	Anatoxin-a	ND	0.15	µg/L	U
Cy-06-12	ELISA	Microcystin	ND	0.15	µg/L	U
Cy-06-12	QPCR	Anatoxin-a	ND	100	copies/mL	U
Cy-06-12	QPCR	Microcystin	ND	100	copies/mL	U
Cy-06-13	ELISA	Anatoxin-a	ND	0.15	µg/L	U
Cy-06-13	ELISA	Microcystin	ND	0.15	µg/L	U
Cy-06-13	QPCR	Anatoxin-a	ND	100	copies/mL	U
Cy-06-13	QPCR	Microcystin	ND	100	copies/mL	U
Cy-06-11	ELISA	Anatoxin-a	ND	0.15	µg/L	U
Cy-06-11	ELISA	Microcystin	ND	0.15	µg/L	U
Cy-06-11	QPCR	Anatoxin-a	ND	100	copies/mL	U
Cy-06-11	QPCR	Microcystin	ND	100	copies/mL	U
Cy-06-09	ELISA	Anatoxin-a	ND	0.15	µg/L	U
Cy-06-09	ELISA	Microcystin	ND	0.15	µg/L	U
Cy-06-09	QPCR	Anatoxin-a	ND	100	copies/mL	U
Cy-06-09	QPCR	Microcystin	ND	100	copies/mL	U
Cy-06-02	ELISA	Anatoxin-a	ND	0.15	µg/L	U
Cy-06-02	ELISA	Microcystin	ND	0.15	µg/L	U
Cy-06-02	QPCR	Anatoxin-a	ND	100	copies/mL	U
Cy-06-02	QPCR	Microcystin	ND	100	copies/mL	U

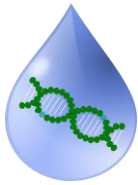


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 Sacramento, CA 95825
 Tel: (916) 550-1048

Project: SWAMP_FHAB_2017
 Analysis for Toxicogenic Cyanobacteria
Project #: Tahoe Keys POA
Reported: 10/20/2017 12:10

SAMPLE RESULTS

Sample ID	Method	Target	Result	Quantitation		Notes
				Limit	Units	
Cy-06-04	ELISA	Anatoxin-a	ND	0.15	µg/L	U
Cy-06-04	ELISA	Microcystin	ND	0.15	µg/L	U
Cy-06-04	QPCR	Anatoxin-a	160	100	copies/mL	
Cy-06-04	QPCR	Microcystin	584	100	copies/mL	
Cy-06-10	ELISA	Anatoxin-a	ND	0.15	µg/L	U
Cy-06-10	ELISA	Microcystin	ND	0.15	µg/L	U
Cy-06-10	QPCR	Anatoxin-a	373	100	copies/mL	
Cy-06-10	QPCR	Microcystin	542	100	copies/mL	
Cy-06-14	ELISA	Anatoxin-a	ND	0.15	µg/L	U
Cy-06-14	ELISA	Microcystin	0.10	0.15	µg/L	C1,J
Cy-06-14	QPCR	Anatoxin-a	289	100	copies/mL	
Cy-06-14	QPCR	Microcystin	1,243	100	copies/mL	
Cy-06-08	ELISA	Anatoxin-a	ND	0.15	µg/L	U
Cy-06-08	ELISA	Microcystin	0.12	0.15	µg/L	C1,J
Cy-06-08	QPCR	Anatoxin-a	ND	100	copies/mL	U
Cy-06-08	QPCR	Microcystin	231	100	copies/mL	
Cy-06-06	ELISA	Anatoxin-a	ND	0.15	µg/L	U
Cy-06-06	ELISA	Microcystin	0.11	0.15	µg/L	C1,J
Cy-06-06	QPCR	Anatoxin-a	ND	100	copies/mL	U
Cy-06-06	QPCR	Microcystin	670	100	copies/mL	
Cy-06-05	ELISA	Anatoxin-a	ND	0.15	µg/L	U
Cy-06-05	ELISA	Microcystin	ND	0.15	µg/L	U
Cy-06-05	QPCR	Anatoxin-a	ND	100	copies/mL	U
Cy-06-05	QPCR	Microcystin	ND	100	copies/mL	U
Cy-06-07	ELISA	Anatoxin-a	ND	0.15	µg/L	U
Cy-06-07	ELISA	Microcystin	ND	0.15	µg/L	U
Cy-06-07	QPCR	Anatoxin-a	ND	100	copies/mL	U
Cy-06-07	QPCR	Microcystin	ND	100	copies/mL	U



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Analysis for Toxigenic Cyanobacteria

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Reported: 10/20/2017 12:10

MICROSCOPY RESULTS - Identification of CyanoHABs

Sample ID	Dominant	Present	Present	Notes
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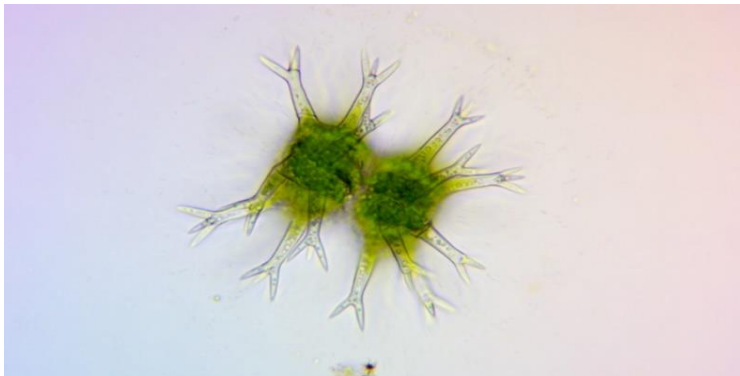
Cy-05-01 *Dolichospermum*



This sample contained a low amount of a large-celled morphology of *Dolichospermum* sp.; no other cyanobacteria were observed. The photomicrograph was taken under 400X magnification.

Sample ID	Dominant	Present	Present	Notes
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Cy-05-11 NA



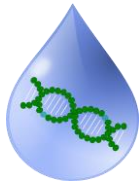
There was a low amount of eukaryotic algae in this sample, and no cyanobacteria were observed. The photomicrograph was taken under 400X magnification.

Sample ID	Dominant	Present	Present	Notes
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Cy-05-02 *Dolichospermum*



This sample contained a moderately low amount of a small-celled morphology of *Dolichospermum* sp.; no other cyanobacteria were observed. The photomicrograph was taken under 400X magnification.



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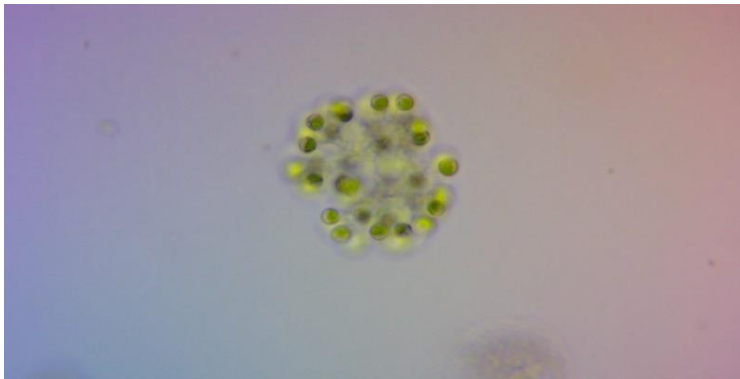
Project #: Tahoe Keys POA

Reported: 10/20/2017 12:10

MICROSCOPY RESULTS - Identification of CyanoHABs

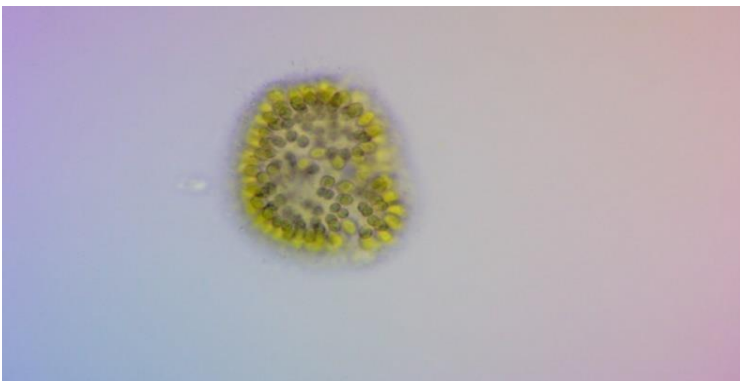
Sample ID	Dominant	Sub-dominant	Present	Notes
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Cy-05-10	<i>Snowella</i>	<i>Dolichospermum</i>	<i>Woronochinia</i>	This sample contained a moderate amount of <i>Snowella</i> sp. and low amounts of both <i>Dolichospermum</i> sp. and <i>Woronochinia</i> sp.; no other cyanobacteria were observed. The photomicrograph (<i>Snowella</i>) was taken under 400X magnification.
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Sample ID	Dominant	Sub-dominant	Present	Notes
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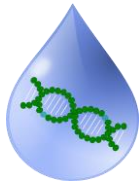
Cy-05-14	<i>Woronochinia</i>	<i>Dolichospermum</i>	<i>Snowella</i>	This sample contained a high concentration of <i>Woronochinia</i> sp. and moderate amounts of large and small-celled <i>Dolichospermum</i> sp. and <i>Snowella</i> sp.; no other cyanobacteria were observed. The photomicrograph (<i>Woronochinia</i>) was taken under 400X magnification.
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Sample ID	Dominant	Sub-dominant	Present	Notes
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Cy-05-06	<i>Woronochinia</i>	<i>Snowella</i>	<i>Dolichospermum</i>	This sample contained a moderate amount of <i>Woronochinia</i> sp. and <i>Snowella</i> sp., and a low amount of <i>Dolichospermum</i> sp.; no other cyanobacteria were observed. The photomicrograph (<i>Woronochinia</i>) was taken under 400X magnification.
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Analysis for Toxicogenic Cyanobacteria

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MICROSCOPY RESULTS - Identification of CyanoHABs

Sample ID	Dominant	Present	Present	Notes
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Cy-05-05 *Dolichospermum*



This sample contained a low amount of the large-cell morphology of *Dolichospermum* sp.; no other cyanobacteria were observed. The photomicrograph was taken under 400X magnification.

Sample ID	Dominant	Present	Present	Notes
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Cy-06-03 *Dolichospermum*



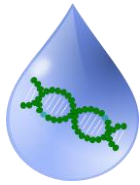
This sample contained a moderately high amount of large-celled *Dolichospermum* sp.; no other cyanobacteria were observed. The photomicrograph was taken under 40X magnification.

Sample ID	Dominant	Present	Present	Notes
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Cy-06-01 *Dolichospermum*



This sample contained a moderate amount of the large-celled morphology of *Dolichospermum* sp.; no other cyanobacteria were observed. The photomicrograph was taken under 400X magnification.



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Analysis for Toxigenic Cyanobacteria

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Reported: 10/20/2017 12:10

MICROSCOPY RESULTS - Identification of CyanoHABs

Sample ID	Dominant	Present	Present	Notes
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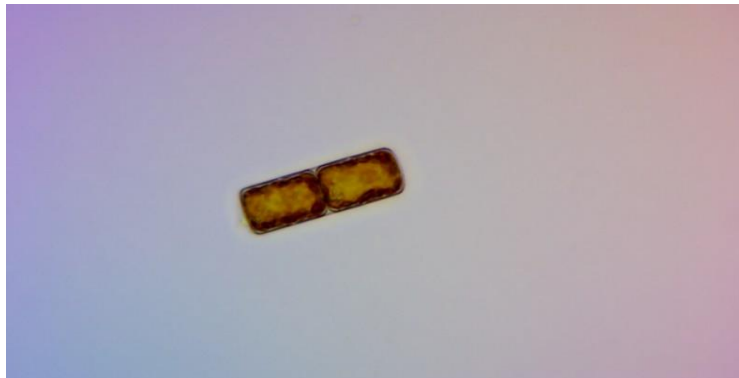
Cy-06-12 NA



Overall this sample contained a low amount of phytoplankton, no cyanobacteria were observed and there was only a low amount of diatoms present. The photomicrograph was taken under 400X magnification.

Sample ID	Dominant	Present	Present	Notes
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Cy-06-13 NA



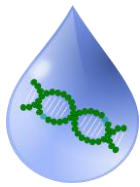
There was only a low amount of eukaryotic algae present in this sample, no cyanobacteria were observed. The photomicrograph was taken under 400X magnification.

Sample ID	Dominant	Present	Present	Notes
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Cy-06-11 NA



There was a low amount of the diatom *Asterionella* sp. in this sample, although no cyanobacteria were observed. The photomicrograph was taken under 400x magnification.



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MICROSCOPY RESULTS - Identification of CyanoHABs

Sample ID	Dominant	Present	Present	Notes
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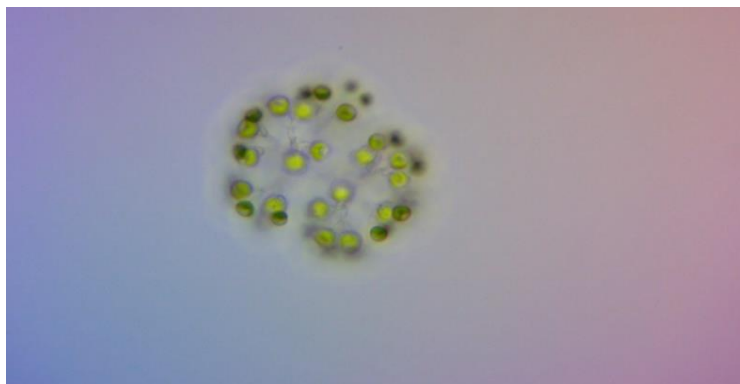
Cy-06-09 *Dolichospermum*



This sample contained a moderate amount of the small-celled morphology of *Dolichospermum* sp.; no other cyanobacteria were observed. The photomicrograph was taken under 400X magnification.

Sample ID	Dominant	Present	Present	Notes
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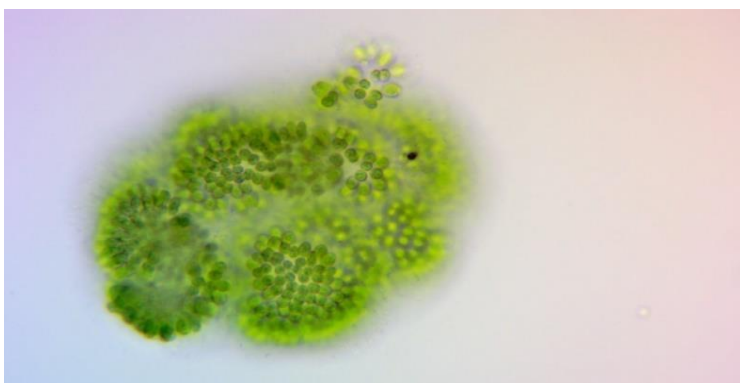
Cy-06-02 *Snowella* *Dolichospermum*



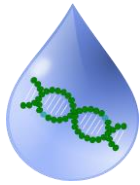
This sample contained a moderate amount of *Snowella* sp. and a low amount of the small-celled morphology *Dolichospermum* sp.; no other cyanobacteria were observed. The photomicrograph (*Snowella*) was taken under 400X magnification.

Sample ID	Dominant	Sub-dominant	Present	Notes
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Cy-06-04 *Woronochinia* *Snowella*



This sample contained a moderately high concentration of *Woronochinia* sp. and a moderate amount of *Snowella* sp.; no other cyanobacteria were observed. The photomicrograph (*Woronochinia*) was taken under 400X magnification.



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MICROSCOPY RESULTS - Identification of CyanoHABs

Sample ID	Dominant	Sub-dominant	Present	Notes
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Cy-06-10 *Dolichospermum* *Woronochinia*



This sample contained a moderate amount of the small-celled morphology *Dolichospermum* sp. and *Woronochinia* sp.; no other cyanobacteria were observed. The photomicrograph was taken under 400X magnification.

Sample ID	Dominant	Sub-dominant	Present	Notes
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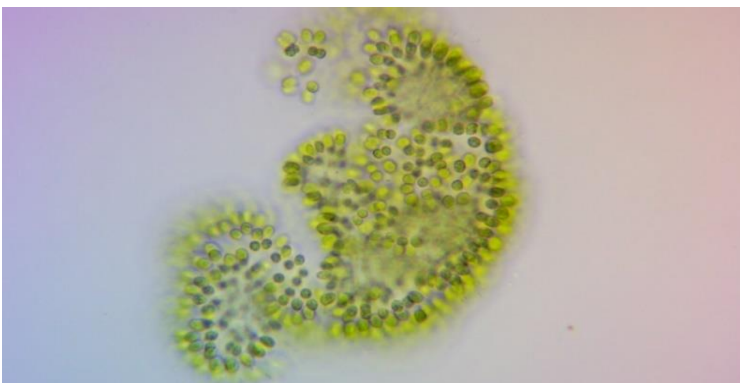
Cy-06-14 *Dolichospermum* *Woronochinia*



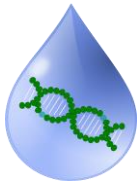
This sample contained a relatively high amount of small-celled *Dolichospermum* sp. and a moderate amount of *Woronochinia* sp.; no other cyanobacteria were observed. The photomicrograph was taken under 400X magnification.

Sample ID	Dominant	Sub-dominant	Present	Notes
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Cy-06-08 *Woronochinia* *Dolichospermum*



This sample contained a moderately high amount of *Woronochinia* sp. and a moderate amount of *Dolichospermum* sp.; no other cyanobacteria were observed. The photomicrograph was taken under 400X magnification.



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Analysis for Toxigenic Cyanobacteria

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Reported: 10/20/2017 12:10

MICROSCOPY RESULTS - Identification of CyanoHABs

Sample ID	Dominant	Present	Present	Notes
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Cy-06-06 *Dolichospermum* *Woronochinia*



This sample contained a moderate amount of the small-celled *Dolichospermum* sp. and a moderately low amount of *Woronochinia* sp.; no other cyanobacteria were observed. The photomicrograph was taken under 400X magnification.

Sample ID	Dominant	Present	Present	Notes
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Cy-06-05 *Dolichospermum*



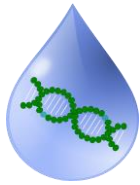
This sample contained a low amount of the large-celled *Dolichospermum* sp. morphology; no other cyanobacteria were observed. The photomicrograph was taken under 400X magnification.

Sample ID	Dominant	Present	Present	Notes
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Cy-06-07 NA



This sample contained a relatively high concentration of a motile eukaryotic algae; however, no cyanobacteria were observed. The photomicrograph was taken under 400x magnification.



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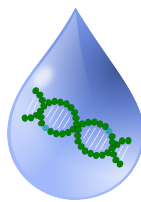
Project: SWAMP_FHAB_2017
Analysis for Toxigenic Cyanobacteria
Project #: Tahoe Keys POA
Reported: 10/20/2017 12:10

QUALITY CONTROL

Method	Analyte	Result	Qualifiers / Comments	Units	Spike Level	%REC	%REC Limits
ELISA	ATX - Blank	ND	U	µg/L	0		
ELISA	ATX - Positive	0.71		µg/L	0.75	95.2	70-130
ELISA	ATX - Matrix Sp	1.27		µg/L	1.25	101.2	70-130
ELISA	MC - Blank	ND	U	µg/L	0		
ELISA	MC - Positive	0.61		µg/L	0.75	81.4	70-130
ELISA	MC - Matrix Sp	0.70		µg/L	0.75	93.4	70-130
QPCR	anaC - Blank	ND	U	copies/mL	0		
QPCR	anaC - Matrix Sp	45,046		copies/mL	50,000	90.1	70-130
QPCR	mcyE - Blank	ND	U	copies/mL	0		

QUALIFIERS/COMMENTS/NOTES

- C1 The reported concentration for this analyte is below the quantification limit.
- C2 The reported concentration for this analyte is above the calibration range of the instrument.
- J The reported result for this analyte should be considered an estimated value.
- U Undetected



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Date: 9/1/2017

Subject: Cyanobacteria testing results

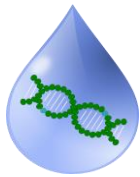
From: Tim Otten, Laboratory Director

To: Kristen Hunter
Sierra Ecosystem Associates

Attached are the results of microscopy, toxin and DNA analyses conducted on samples collected from the Tahoe Keys on 8/28/2017. These data have been reviewed and are considered final.

Analyses included in this report:

- Quantification of total cyanobacteria (16S rDNA) by real-time quantitative polymerase chain reaction (QPCR) method.
- Quantification of total cyanobacterial toxins (anatoxin-a, cylindrospermopsin, microcystin/nodularin and saxitoxin) by enzyme linked immunosorbent assay (ELISA) method.
- Identification of potentially toxigenic cyanobacteria (PTOX) via microscopy.

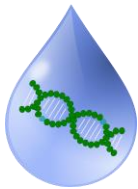


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Project: Sierra Ecosystem Associates
Analysis for Toxic Cyanobacteria
Agreement #: Tahoe Keys POA
Reported: 9/1/2017 16:30

ANALYTICAL REPORT FOR SAMPLES

Sample ID	BG_ID	Date Collected	Date Received	Matrix	Preserved
CY-02-01A	SE07	8/28/2017 10:10	8/28/2017 16:30	Water	N
CY-02-01B	SE08	8/28/2017 10:10	8/28/2017 16:30	Water	N
CY-02-02A	SE09	8/28/2017 11:15	8/28/2017 16:30	Water	N
CY-02-02B	SE10	8/28/2017 11:15	8/28/2017 16:30	Water	N
CY-02-05A	SE11	8/28/2017 13:18	8/28/2017 16:30	Water	N
CY-02-05B	SE12	8/28/2017 13:18	8/28/2017 16:30	Water	N
CY-02-06A	SE13	8/28/2017 12:31	8/28/2017 16:30	Water	N
CY-02-06B	SE14	8/28/2017 12:31	8/28/2017 16:30	Water	N
CY-02-10A	SE15	8/28/2017 11:37	8/28/2017 16:30	Water	N
CY-02-10B	SE16	8/28/2017 11:37	8/28/2017 16:30	Water	N
CY-02-11A	SE17	8/28/2017 10:51	8/28/2017 16:30	Water	N
CY-02-11B	SE18	8/28/2017 10:51	8/28/2017 16:30	Water	N
CY-02-14A	SE19	8/28/2017 12:07	8/28/2017 16:30	Water	N
CY-02-14B	SE20	8/28/2017 12:07	8/28/2017 16:30	Water	N

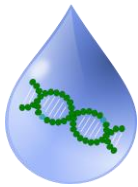


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 Analysis for Toxic Cyanobacteria
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Reported: 9/1/2017 16:30

SAMPLE RESULTS

Sample ID	Method	Target	Result	Quantitation		Notes
				Limit	Units	
CY-02-01A	QPCR	Total Cyano (16S)	4,270,059	100	copies/mL	
CY-02-01B	ELISA	Anatoxin-a	ND	0.15	µg/L	U
CY-02-01B	ELISA	Cylindrospermopsin	ND	0.05	µg/L	U
CY-02-01B	ELISA	Microcystin/Nod.	ND	0.15	µg/L	U
CY-02-01B	ELISA	Saxitoxin	ND	0.02	µg/L	U
CY-02-02A	QPCR	Total Cyano (16S)	7,257,632	100	copies/mL	
CY-02-02B	ELISA	Anatoxin-a	ND	0.15	µg/L	U
CY-02-02B	ELISA	Cylindrospermopsin	ND	0.05	µg/L	U
CY-02-02B	ELISA	Microcystin/Nod.	ND	0.15	µg/L	U
CY-02-02B	ELISA	Saxitoxin	ND	0.02	µg/L	U
CY-02-05A	QPCR	Total Cyano (16S)	797,486	100	copies/mL	
CY-02-05B	ELISA	Anatoxin-a	0.87	0.15	µg/L	
CY-02-05B	ELISA	Cylindrospermopsin	ND	0.05	µg/L	U
CY-02-05B	ELISA	Microcystin/Nod.	ND	0.15	µg/L	U
CY-02-05B	ELISA	Saxitoxin	ND	0.02	µg/L	U
CY-02-06A	QPCR	Total Cyano (16S)	15,413,918	100	copies/mL	
CY-02-06B	ELISA	Anatoxin-a	4.27	0.15	µg/L	
CY-02-06B	ELISA	Cylindrospermopsin	ND	0.05	µg/L	U
CY-02-06B	ELISA	Microcystin/Nod.	0.18	0.15	µg/L	
CY-02-06B	ELISA	Saxitoxin	ND	0.02	µg/L	U
CY-02-10A	QPCR	Total Cyano (16S)	18,215,143	100	copies/mL	
CY-02-10B	ELISA	Anatoxin-a	0.21	0.15	µg/L	
CY-02-10B	ELISA	Cylindrospermopsin	ND	0.05	µg/L	U
CY-02-10B	ELISA	Microcystin/Nod.	ND	0.15	µg/L	U
CY-02-10B	ELISA	Saxitoxin	ND	0.02	µg/L	U
CY-02-11A	QPCR	Total Cyano (16S)	13,577	100	copies/mL	
CY-02-11B	ELISA	Anatoxin-a	ND	0.15	µg/L	U
CY-02-11B	ELISA	Cylindrospermopsin	ND	0.05	µg/L	U
CY-02-11B	ELISA	Microcystin/Nod.	ND	0.15	µg/L	U
CY-02-11B	ELISA	Saxitoxin	ND	0.02	µg/L	U
CY-02-14A	QPCR	Total Cyano (16S)	31,411,377	100	copies/mL	
CY-02-14B	ELISA	Anatoxin-a	14.5	1.50	µg/L	
CY-02-14B	ELISA	Cylindrospermopsin	ND	0.05	µg/L	U
CY-02-14B	ELISA	Microcystin/Nod.	0.86	0.15	µg/L	
CY-02-14B	ELISA	Saxitoxin	ND	0.02	µg/L	U




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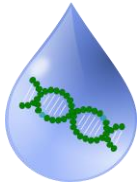
Microscope evaluation of potentially toxigenic cyanobacteria (PTOX)

Sample ID	Dominant	Sub-dominant	Present	Notes
CY-02-01A	<i>Dolichospermum</i>			There was a moderate amount of <i>Dolichospermum</i> sp. in this sample, no other cyanobacteria were observed. The photomicrograph was taken under 40X magnification.



Sample ID	Dominant	Sub-dominant	Present	Notes
CY-02-02A	<i>Dolichospermum</i>			This sample contained a moderately high amount of <i>Dolichospermum</i> sp., some of the filaments contained akinetes and most possessed heterocysts. No other cyanobacteria were observed. The photomicrograph was taken under 400X magnification.





Bend Genetics, LLC
87 Scripps Drive, Ste. 108
Sacramento, CA 95825
Tel: (916) 550-1048

Project: Sierra Ecosystem Associates
Analysis for Toxic Cyanobacteria
Agreement #: Tahoe Keys POA
Reported: 9/1/2017 16:30

Microscope evaluation of potentially toxigenic cyanobacteria (PTOX)

Sample ID	Dominant	Sub-dominant	Present	Notes
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CY-02-05A *Dolichospermum* *Woronochinia*

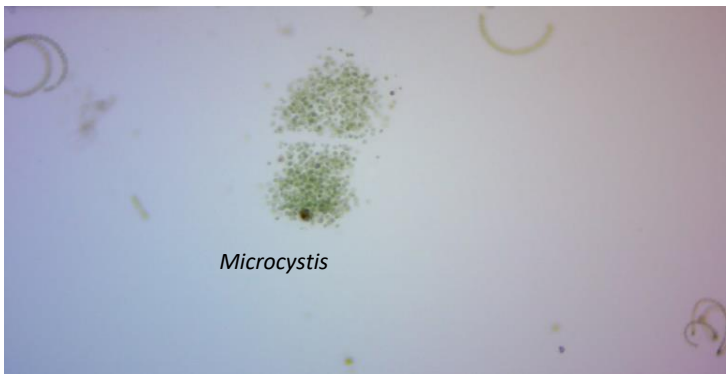
This sample contained mostly *Dolichospermum* sp., with a low amount of *Woronochinia* sp. also present. No other cyanobacteria were observed. The photomicrograph was taken under 400X magnification.

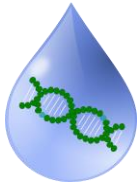


Sample ID	Dominant	Sub-dominant	Present	Notes
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CY-02-06A *Dolichospermum* *Microcystis*

There was a significant amount of *Dolichospermum* sp. and a moderate amount of *Microcystis* sp. in this sample. No other Cyanobacteria were observed. The photomicrograph was taken under 40X magnification.





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Project: Sierra Ecosystem Associates
Analysis for Toxic Cyanobacteria
Agreement #: Tahoe Keys POA
Reported: 9/1/2017 16:30

Microscope evaluation of potentially toxigenic cyanobacteria (PTOX)

Sample ID	Dominant	Sub-dominant	Present	Notes
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CY-02-10A *Dolichospermum*



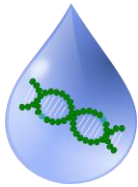
This sample contained a relatively high amount of *Dolichospermum* sp., no other cyanobacteria were observed. The top photomicrograph was taken under 40X magnification and the bottom under 400X magnification.

Sample ID	Dominant	Sub-dominant	Present	Notes
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CY-02-11A

NA

No cyanobacteria were observed.



Bend Genetics, LLC
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Sacramento, CA 95825
Tel: (916) 550-1048

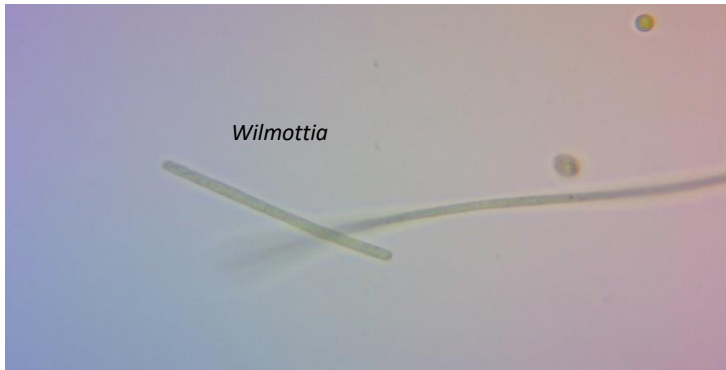
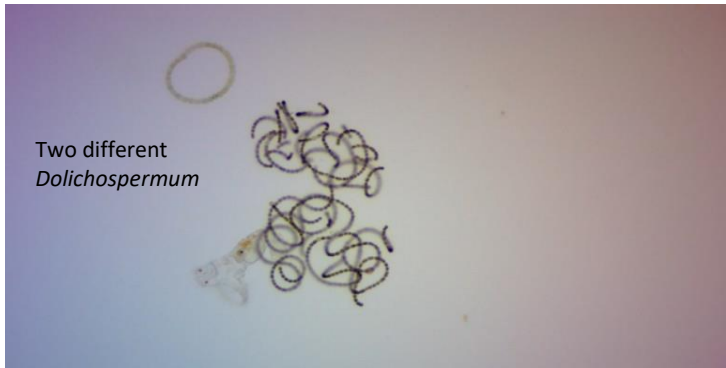
Project: Sierra Ecosystem Associates
Analysis for Toxic Cyanobacteria
Agreement #: Tahoe Keys POA
Reported: 9/1/2017 16:30

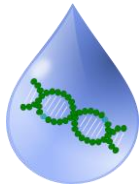
Microscope evaluation of potentially toxigenic cyanobacteria (PTOX)

Sample ID	Dominant	Present	Present	Notes
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CY-02-14A	<i>Dolichospermum</i>	<i>Woronochinia</i>	<i>Wilmottia</i>
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There were two different morphologies of *Dolichospermum* sp. present in this sample, one consisting of loosely coiled large diameter cells and the other of small diameter darkly pigmented cells. There was also a low amount of *Woronochinia* sp. and *Wilmottia* sp. observed. The top photomicrograph was taken under 40X magnification and the lower two under 400X magnification.





Bend Genetics, LLC
 87 Scripps Drive, Ste. 108
 Sacramento, CA 95825
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Project: Sierra Ecosystem Associates
 Analysis for Toxic Cyanobacteria
Agreement #: Tahoe Keys POA
Reported: 9/1/2017 16:30

QUALITY CONTROL

Method	Analyte	Result	Qualifiers / Comments	Units	Expected Value	%REC	%REC Limits
QPCR	Cy16S - Blank	ND	U	copies/mL	0		
QPCR	Cy16S - Matrix Sp	5,271,470		copies/mL	5,500,000	95.8	70-130
ELISA	ATX - Blank	ND	U	µg/L	0		
ELISA	ATX - Positive	0.68		µg/L	0.75	91.0	70-130
ELISA	ATX - Matrix Sp	1.09		µg/L	1.25	86.8	70-130
ELISA	CYN - Blank	ND	U	µg/L	0		
ELISA	CYN - Positive	0.68		µg/L	0.75	90.8	70-130
ELISA	CYN - Matrix Sp	0.89		µg/L	1.00	89.5	70-130
ELISA	MC - Blank	ND	U	µg/L	0		
ELISA	MC - Positive	0.71		µg/L	0.75	95.3	70-130
ELISA	MC - Matrix Sp	0.92		µg/L	1.00	92.0	70-130
ELISA	STX - Blank	ND	U	µg/L	0		
ELISA	STX - Positive	0.079		µg/L	0.075	104.8	70-130
ELISA	STX - Matrix Sp	0.201		µg/L	0.20	100.4	70-130

QUALIFIERS/COMMENTS/NOTES

- C1 The reported concentration for this analyte is below the quantification limit.
- C2 The reported concentration for this analyte is above the calibration range of the instrument.
- J The reported result for this analyte should be considered an estimated value.
- U Undetected

Appendix C

Chain of Custody Forms

Index:

July 3, 2017

August 28, 2017

October 20, 2017

**Freshwater Harmful Algal Bloom Monitoring
Request for Analysis and Chain of Custody Record**


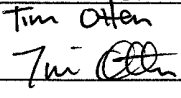
Group: TKPOA		Project Code:		Project Lead: Kristen Hunter	
Fiscal Year: 1718		Sampling Procedures Used:		Field Sampling Lead: Kristen Hunter	
PO:		Sampling Agency:		Bend Genetics Lab Contact: Tim Otten (916) 550-1048 ottentim@bendgenetics.com	
EventCode: WQ		Field Crew:			

SampleID	Sampling Location/ Station Name	Sample Date	Collection Time	Sample Volume	Circle / Select			Remarks	Water Analysis Authorization *Circle/select requested analysis, see comments below*		
					Field Preservation	Sample Type Code	Sample Container		One bottle per sampling location/site		
CY-01-01	site 1	7/3/17	12:35	250ML	10% diluent / Stored 2-10°C	Grab / Integrated	Glass / PETG		Toxin Analysis 1 2 3 4	QPCR Analysis 5 6 7 8 9	Microscope ID Y N
CY-01-02	site 2	7/3/17	12:10	250ML	10% diluent / Stored 2-10°C	Grab / Integrated	Glass / PETG		Toxin Analysis 1 2 3 4	QPCR Analysis 5 6 7 8 9	Microscope ID Y N
CY-01-05	site 5	7/3/17	10:26	250ML	10% diluent / Stored 2-10°C	Grab / Integrated	Glass / PETG		Toxin Analysis 1 2 3 4	QPCR Analysis 5 6 7 8 9	Microscope ID Y N
CY-01-06	site 6	7/3/17	11:25	250ML	10% diluent / Stored 2-10°C	Grab / Integrated	Glass / PETG		Toxin Analysis 1 2 3 4	QPCR Analysis 5 6 7 8 9	Microscope ID Y N
CY-01-10	site 10	7/3/17	11:50	250ML	10% diluent / Stored 2-10°C	Grab / Integrated	Glass / PETG		Toxin Analysis 1 2 3 4	QPCR Analysis 5 6 7 8 9	Microscope ID Y N
CY-01-11	site 11	7/3/17	12:20	250ML	10% diluent / Stored 2-10°C	Grab / Integrated	Glass / PETG		Toxin Analysis 1 2 3 4	QPCR Analysis 5 6 7 8 9	Microscope ID Y N
					10% diluent / Stored 2-10°C	Grab / Integrated	Glass / PETG		Toxin Analysis 1 2 3 4	QPCR Analysis 5 6 7 8 9	Microscope ID Y N
					10% diluent / Stored 2-10°C	Grab / Integrated	Glass / PETG		Toxin Analysis 1 2 3 4	QPCR Analysis 5 6 7 8 9	Microscope ID Y N
					10% diluent / Stored 2-10°C	Grab / Integrated	Glass / PETG		Toxin Analysis 1 2 3 4	QPCR Analysis 5 6 7 8 9	Microscope ID Y N
					10% diluent / Stored 2-10°C	Grab / Integrated	Glass / PETG		Toxin Analysis 1 2 3 4	QPCR Analysis 5 6 7 8 9	Microscope ID Y N
					10% diluent / Stored 2-10°C	Grab / Integrated	Glass / PETG		Toxin Analysis 1 2 3 4	QPCR Analysis 5 6 7 8 9	Microscope ID Y N
					10% diluent / Stored 2-10°C	Grab / Integrated	Glass / PETG		Toxin Analysis 1 2 3 4	QPCR Analysis 5 6 7 8 9	Microscope ID Y N

Total # sites/bottles:

Comments: For each row choose analysis corresponding to # 1 - 9 below; For anatoxin-a or saxitoxin add 10X preservative diluent (1:10 dilution) to water sample.

1 Microcystins + Nodularin by ELISA, Total fraction measured (no filtering)	5 Microcystin gene; lab analysis includes concentration + extraction per method
2 Anatoxin-a by ELISA, Total fraction measured (no filtering)	6 Anatoxin-a gene; lab analysis includes concentration + extraction per method
3 Cylindrospermopsin by ELISA, Total fraction measured (no filtering)	7 Cylindrospermopsin gene; lab analysis includes concentration + extraction per method
4 Saxitoxin by ELISA, Total fraction measured (no filtering)	8 Saxitoxin gene; lab analysis includes concentration + extraction per method
	9 Total cyanobacteria; quantifies "cell/mL", by qPCR

Samples Relinquished by: Justin Stang Name (Print and Sign)  Date & Time: 7/3/17 13:00	Samples Received by: Tim Otten Name (Print and Sign)  Date & Time: 7/3/17 3:50 PM	**Attention Lab - If sample arrives >10 °C do not analyze** Distribution of COC form: Original accompanies shipment, Electronic copy emailed customer_service@bendgenetics.com
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Please mail samples with following day delivery (by 10:30 AM):

Bend Genetics, LLC
87 Scripps Drive Ste 108
Sacramento, CA 95825
Tel: (916) 550-1048

**Freshwater Harmful Algal Bloom Monitoring
Request for Analysis and Chain of Custody Record**

Group: Fiscal Year: 17/18	Project Code: Sampling Procedures Used:	Project Lead: Field Sampling Lead:	
PO: EventCode: WQ	Sampling Agency: Field Crew:	Bend Genetics Lab Contact: Tim Otten	(916) 550-1048 ottentim@bendgenetics.com

SampleID	Sampling Location/ Station Name	Sample Date	Collection Time	Sample Volume	Circle / Select			Remarks	Water Analysis Authorization *Circle/select requested analysis, see comments below*		
					Field Preservation	Sample Type Code	Sample Container		One bottle per sampling location/site		
CY-02-01A	Site 01	8/28/17	10:10 AM	250 ML	10% diluent / Stored 2-10°C	Grab / Integrated	Glass / PETG		Toxin Analysis 1 2 3 4	QPCR Analysis 5 6 7 8 9	Microscope ID Y N
CY-02-01B	Site 01	8/28/17	10:10 AM	250 ML	10% diluent / Stored 2-10°C	Grab / Integrated	Glass / PETG		Toxin Analysis 1 2 3 4	QPCR Analysis 5 6 7 8 9	Microscope ID Y N
CY-02-02A	Site 02	8/28/17	11:15 AM	250 ML	10% diluent / Stored 2-10°C	Grab / Integrated	Glass / PETG		Toxin Analysis 1 2 3 4	QPCR Analysis 5 6 7 8 9	Microscope ID Y N
CY-02-02B	Site 02	8/28/17	11:15 AM	250 ML	10% diluent / Stored 2-10°C	Grab / Integrated	Glass / PETG		Toxin Analysis 1 2 3 4	QPCR Analysis 5 6 7 8 9	Microscope ID Y N
CY-02-05A	Site 05	8/28/17	13:18	250 ML	10% diluent / Stored 2-10°C	Grab / Integrated	Glass / PETG		Toxin Analysis 1 2 3 4	QPCR Analysis 5 6 7 8 9	Microscope ID Y N
CY-02-05B	Site 05	8/28/17	13:18	250 ML	10% diluent / Stored 2-10°C	Grab / Integrated	Glass / PETG		Toxin Analysis 1 2 3 4	QPCR Analysis 5 6 7 8 9	Microscope ID Y N
CY-02-06A	Site 06	8/28/17	12:31	250 ML	10% diluent / Stored 2-10°C	Grab / Integrated	Glass / PETG		Toxin Analysis 1 2 3 4	QPCR Analysis 5 6 7 8 9	Microscope ID Y N
CY-02-06B	Site 06	8/28/17	12:31	250 ML	10% diluent / Stored 2-10°C	Grab / Integrated	Glass / PETG		Toxin Analysis 1 2 3 4	QPCR Analysis 5 6 7 8 9	Microscope ID Y N
CY-02-10A	Site 10	8/28/17	11:37	250 ML	10% diluent / Stored 2-10°C	Grab / Integrated	Glass / PETG		Toxin Analysis 1 2 3 4	QPCR Analysis 5 6 7 8 9	Microscope ID Y N
CY-02-10B	Site 10	8/28/17	11:37	250 ML	10% diluent / Stored 2-10°C	Grab / Integrated	Glass / PETG		Toxin Analysis 1 2 3 4	QPCR Analysis 5 6 7 8 9	Microscope ID Y N
CY-02-11A	Site 11	8/28/17	10:51 AM	250 ML	10% diluent / Stored 2-10°C	Grab / Integrated	Glass / PETG		Toxin Analysis 1 2 3 4	QPCR Analysis 5 6 7 8 9	Microscope ID Y N
CY-02-11B	Site 11	8/28/17	10:51 AM	250 ML	10% diluent / Stored 2-10°C	Grab / Integrated	Glass / PETG		Toxin Analysis 1 2 3 4	QPCR Analysis 5 6 7 8 9	Microscope ID Y N

Total # sites/bottles:

Comments: For each row choose analysis corresponding to # 1 - 9 below; For anatoxin-a or saxitoxin add 10X preservative diluent (1:10 dilution) to water sample.

- | | |
|---|--|
| 1 Microcystins + Nodularin by ELISA, Total fraction measured (no filtering) | 5 Microcystin gene; lab analysis includes concentration + extraction per method |
| 2 Anatoxin-a by ELISA, Total fraction measured (no filtering) | 6 Anatoxin-a gene; lab analysis includes concentration + extraction per method |
| 3 Cylindrospermopsin by ELISA, Total fraction measured (no filtering) | 7 Cylindrospermopsin gene; lab analysis includes concentration + extraction per method |
| 4 Saxitoxin by ELISA, Total fraction measured (no filtering) | 8 Saxitoxin gene; lab analysis includes concentration + extraction per method |
| | 9 Total cyanobacteria; quantifies "cell equivalents/mL", by qPCR |

Samples Relinquished by: Name (Print and Sign) <i>Justin Stang</i>	Samples Received by: Name (Print and Sign) <i>Gabrie Gutierrez</i>	Distribution of COC form: Original accompanies shipment, Electronic copy emailed customer_service@bendgenetics.com
Date & Time: 8/28/17	Date & Time: 08-28-17	

Please mail samples with following day delivery (by 10:30 AM):
Bend Genetics, LLC
 87 Scripps Drive Ste 108
 Sacramento, CA 95825
 Tel: (916) 550-1048

SWAMP HAB
REQUEST FOR ANALYSIS AND CHAIN OF CUSTODY (COC) RECORD

SWAMP (Surface Water Ambient Monitoring Program)
is administered by the State Water Resources Control Board

Group: Freshwater HABs	SWAMP ProjectCode: SWAMP_FHAB_2017	Field Sampling Lead: <u>Kristen Hunter</u>	
Fiscal Year: 17/18	Sampling Procedures Used: SWAMP Aug17	SWAMP HAB QA/DM:	
Regional Board: RB6, Lahonton	Sampling Agency: TKPOA	Freshwater HAB Project:	
EventCode: WQ	Field Crew: <u>Kevin Schoonmaker & Kristen Hunter</u>		

SampleID	Sampling Location/ Station Name	Sample Date	Collection Time	Sample Volume	Field Preservation	Circle / Select		Remarks	Water Analysis Authorization *Circle/select requested analysis, see comments below*		
						Sample Type Code	Sample Container		Invoice TKPOA		
CY-05-01	TKPOA	10/16/17	9:30	<u>125</u> 250 mL	Cooled 2-6 °C Only	<input checked="" type="radio"/> Grab	<input checked="" type="radio"/> PETG	use of Preservative	Analysis: Toxins <u>1,2,3</u> only	Analysis: QPCR <u>5,6,7,8,9</u>	Analysis: <u>Microscope ID</u>
CY-05-11	TKPOA	10/16/17	10:30	<u>125</u> 250 mL	Cooled 2-6 °C Only	<input checked="" type="radio"/> Grab	<input checked="" type="radio"/> PETG	use of preservative	Analysis: Toxins <u>1,2,3</u> only	Analysis: QPCR <u>5,6,7,8,9</u>	Analysis: <u>Microscope ID</u>
CY-05-02	TKPOA	10/16/17	11:25	<u>125</u> 250 mL	Cooled 2-6 °C Only	<input checked="" type="radio"/> Grab	<input checked="" type="radio"/> PETG	Preservative	Analysis: Toxins <u>1,2,3</u> only	Analysis: QPCR <u>5,6,7,8,9</u>	Analysis: <u>Microscope ID</u>
CY-05-10	TKPOA	10/16/17	12:18	<u>125</u> 250 mL	Cooled 2-6 °C Only	<input checked="" type="radio"/> Grab	<input checked="" type="radio"/> PETG	Preservative	Analysis: Toxins <u>1,2,3</u> only	Analysis: QPCR <u>5,6,7,8,9</u>	Analysis: <u>Microscope ID</u>
CY-05-14	TKPOA	10/16/17	12:50	<u>125</u> 250 mL	Cooled 2-6 °C Only	<input checked="" type="radio"/> Grab	<input checked="" type="radio"/> PETG	Preservative	Analysis: Toxins <u>1,2,3</u> only	Analysis: QPCR <u>5,6,7,8,9</u>	Analysis: <u>Microscope ID</u>
CY-05-06	TKPOA	10/16/17	13:38	<u>125</u> 250 mL	Cooled 2-6 °C Only	<input checked="" type="radio"/> Grab	<input checked="" type="radio"/> PETG	Preservative	Analysis: Toxins <u>1,2,3</u> only	Analysis: QPCR <u>5,6,7,8,9</u>	Analysis: <u>Microscope ID</u>
CY-05-05	TKPOA	10/16/17	14:25	<u>125</u> 250 mL	Cooled 2-6 °C Only	<input checked="" type="radio"/> Grab	<input checked="" type="radio"/> PETG	Preservative	Analysis: Toxins <u>1,2,3</u> only	Analysis: QPCR <u>5,6,7,8,9</u>	Analysis: <u>Microscope ID</u>
				250 mL	Cooled 2-6 °C Only	<input type="radio"/> Grab	<input type="radio"/> PETG		Analysis: Toxins <u>1,2,3</u> only	Analysis: QPCR <u>5,6,7,8,9</u>	Analysis: Microscope ID
				250 mL	Cooled 2-6 °C Only	<input type="radio"/> Grab	<input type="radio"/> PETG		Analysis: Toxins <u>1,2,3</u> only	Analysis: QPCR <u>5,6,7,8,9</u>	Analysis: Microscope ID
				250 mL	Cooled 2-6 °C Only	<input type="radio"/> Grab	<input type="radio"/> PETG		Analysis: Toxins <u>1,2,3</u> only	Analysis: QPCR <u>5,6,7,8,9</u>	Analysis: Microscope ID

Total # sites/bottles: _____

Comments: For each row choose analysis corresponding to # 1 - 9 below; Sample volume = 250 mL; If testing for anatoxin add buffer solution after collection (1 part buffer to 9 parts sample).

- | | |
|---|--|
| <ul style="list-style-type: none"> 1 microcystins + nodularin by ELISA, Total fraction measured (no field filtering) 2 Anatoxin-a by ELISA, Total fraction measured (no field filtering) 3 Cylindrospermopsin by ELISA, Total fraction measured (no field filtering) 4 Saxitoxin by ELISA, Total fraction measured (no field filtering) | <ul style="list-style-type: none"> 5 Microcystin gene; lab analysis includes concentration + extraction per method 6 Anatoxin-a gene; lab analysis includes concentration + extraction per method 7 Cylindrospermopsin gene; lab analysis includes concentration + extraction per method 8 Saxitoxin marker; lab analysis includes concentration + extraction per method 9 Total cyanobacteria; quantifies "cell/mL", by qPCR |
|---|--|

Samples Relinquished by: Name (Print and Sign) _____ Date & Time _____	Samples Received by: **Attention Lab - If sample arrives >8 °C do not analyze** Name (Print and Sign) _____ Date & Time _____	Distribution of COC form: Original accompanies shipment, Electronic copy emailed customer_service@bendgenetics.com CyanoHab.Reports@waterboards.ca.gov
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**SWAMP HAB
Field Data Sheet**

SWAMP (Surface Water Ambient Monitoring Program)
is administered by the State Water Resources Control Board

Sample Date: <u>10-16-17</u>		Sample Time (first sample): <u>9:30</u>			Station Code: <i>optional</i>								
Waterbody Name: Tahoe				Person & Email/Phone: <u>Greg Hoover</u> <u>GHoover@tahoekeyspoa.org</u>									
Sampling Site Name: <u>Tahoe Keys POA</u>				<u>530-542-6444</u>									
SAMPLING LOCATION													
LOCATION: Bank, thalweg, midchannel, <u>open water</u> , other _____					STARTING BANK (Facing Downstream): LB / RB / <u>NA</u>								
SAMPLE LOCATION: Shore, beachline, wade, <u>boat</u> , dock, bridge, other _____							Datum: NAD83, WGS84						
GPS DEVICE:			GPS/DGPS: Lat (dd.ddddd):		Long (- dd.ddddd):								
Location description: (include landmarks)													
SAMPLES TAKEN FOR LABORATORY ANALYSIS													
SAMPLE TYPE: Grab / Integrated		COLLECTION DEVICE: <u>indiv. bottle w/gloved hand</u> , Individ. bottle w/pole, Bucket, Teflon tubing, Kemmerer, Van Dorn, Other: _____											
Sample ID	Position	Collect Depth(m):	Sample Volume	Micro cystins	Anatoxin-a	Cylindropermopsin	Saxatoxin	Organism ID	qPCR	Other: _____	Other: _____	Other: _____	
<u>Cy-05-01</u>	<u>Surface/</u> Mid/ Bottom	<u>0.1016</u>	<u>125ml</u>										
<u>Cy-05-11</u>	<u>Surface/</u> Mid/ Bottom	<u>0.1016</u>	<u>125ml</u>										
<u>Cy-05-02</u>	<u>Surface/</u> Mid/ Bottom	<u>0.1016</u>	<u>125ml</u>										
FIELD MEASUREMENTS (Optional)													
Position	Collect Depth(m):	Collection Device	Calib. Date	Phycocyanin	Chlorophyll a	Turbidity (ntu)	pH	Water Temp (°C)	Air Temp (°C)	O2 (mg/L)	O2 (%)	Specific Conductivity (uS/cm)	Other: _____
<u>Surface/</u> <u>Mid/Bottom</u>	1.52 <u>2.7432</u>	<u>YSI cy-05-01</u>	<u>10-12-17</u>			<u>0.8</u>	<u>7.70</u>	<u>10.6</u>		<u>13.15</u>		<u>96.6</u>	
<u>Surface/</u> <u>Mid/Bottom</u>	<u>1.52</u>	<u>YSI cy-05-11</u>	<u>10-12-17</u>			<u>0.1</u>	<u>8.22</u>	<u>12.4</u>		<u>13.17</u>		<u>93.2</u>	
<u>Surface/</u> <u>Mid/Bottom</u>	<u>2.1336</u>	<u>YSI cy-05-02</u>	<u>10-12-17</u>			<u>2.5</u>	<u>7.37</u>	<u>11.0</u>		<u>11.75</u>		<u>109.9</u>	
Notes:													

**SWAMP HAB
Field Data Sheet**

SWAMP (Surface Water Ambient Monitoring Program)
is administered by the State Water Resources Control Board

Sample Date: 10-16-17	Sample Time (first sample): 12:18	Station Code: <i>optional</i>
Waterbody Name: TKPOA	Person & Email/Phone: Greg Hoover@tahoekeysboa.org (Greg Hoover) 530-542-6444	
Sampling Site Name: TKPOA		

SAMPLING LOCATION	
LOCATION: Bank, thalweg, midchannel, <u>open water</u> , other _____	STARTING BANK (Facing Downstream): LB / RB / <u>NA</u>
SAMPLE LOCATION: Shore, beachline, wade, <u>boat</u> , dock, bridge, other _____	Datum: NAD83, WGS84
GPS DEVICE: _____	GPS/DGPS: Lat (dd.ddddd): _____ Long (- dd.ddddd): _____
Location description: (include landmarks)	

SAMPLES TAKEN FOR LABORATORY ANALYSIS												
SAMPLE TYPE: Grab / Integrated	COLLECTION DEVICE: <u>Indiv. bottle w/gloved hand</u> , Indiv. bottle w/pole, Bucket, Teflon tubing, Kemmerer, Van Dorn, Other: _____											
Sample ID	Position	Collect Depth(m):	Sample Volume	Micro cystins	Anatoxin-a	Cylindro spermopsin	Saxatoxin	Organism ID	qPCR	Other: _____	Other: _____	Other: _____
cy-05-10	<u>Surface</u> Mid/ Bottom	0.1016	125ml									
cy-05-14	<u>Surface</u> Mid/ Bottom	0.1016	125ml									
cy-05-06	<u>Surface</u> Mid/ Bottom	0.1016	125ml									

FIELD MEASUREMENTS (Optional)													
Position	Collect Depth(m):	Collection Device	Calib. Date	Phycocyanin	Chlorophyll a	Turbidity (ntu)	pH	Water Temp (°C)	Air Temp (°C)	O2 (mg/L)	O2 (%)	Specific Conductivity (uS/cm)	Other: _____
<u>Surface</u> Mid/ Bottom	1.82	YSI cy-05-10	10/17/17			2.2	7.39	10.5		10.86		123.8	
<u>Surface</u> Mid/ Bottom	1.82	YSI cy-05-14	10/12/17			4.0	7.61	10.3		12.74		135.5	
<u>Surface</u> Mid/ Bottom	2.13	YSI cy-05-06	10/12/17			2.8	7.76	10.7		12.76		136.6	

Notes:

**SWAMP HAB
Field Data Sheet**

SWAMP (Surface Water Ambient Monitoring Program)
is administered by the State Water Resources Control Board

Sample Date: <u>10-16-17</u>	Sample Time (first sample): <u>14:25</u>	Station Code: <i>optional</i>
Waterbody Name: <u>TKPOA</u>	Person & Email/Phone: <u>Greg Hoover</u> <u>GHoover@tahoekeyspoa.org</u>	
Sampling Site Name: <u>TKPOA</u>	<u>530-542-6444</u>	

SAMPLING LOCATION

LOCATION: Bank, thalweg, midchannel, <u>open water</u> , other _____	STARTING BANK (Facing Downstream): LB / RB / <u>(NA)</u>
SAMPLE LOCATION: Shore, beachline, wade, <u>boat</u> , dock, bridge, other _____	Datum: NAD83, WGS84
GPS DEVICE: _____	GPS/DGPS: Lat (dd.ddddd): _____ Long (- dd.ddddd): _____
Location description: (include landmarks)	

SAMPLES TAKEN FOR LABORATORY ANALYSIS

SAMPLE TYPE: Grab / Integrated	COLLECTION DEVICE: <u>Indiv. bottle w/gloved hand</u>	Indiv. bottle w/pole	Bucket	Teflon tubing	Kemmerer	Van Dorn	Other: _____					
Sample ID	Position	Collect Depth(m):	Sample Volume	Micro cystins	Anatoxin-a	Cylindro spermopsin	Saxatoxin	Organism ID	qPCR	Other: _____	Other: _____	Other: _____
<u>Cy-05-05</u>	<u>Surface/Mid/Bottom</u>	<u>0.1016</u>	<u>125ml</u>									
	Surface/Mid/Bottom											
	Surface/Mid/Bottom											

FIELD MEASUREMENTS (Optional)

Position	Collect Depth(m):	Collection Device	Calib. Date	Phycocyanin	Chlorophyll a	Turbidity (ntu)	pH	Water Temp (°C)	Air Temp (°C)	O2 (mg/L)	O2 (%)	Specific Conductivity (uS/cm)	Other: _____
<u>Surface/Mid/Bottom</u>	<u>3.048</u>	<u>YSI cy-05-05</u>	<u>10-12-17</u>			<u>1.8</u>	<u>6.93</u>	<u>10</u>		<u>7.46</u>		<u>264.1</u>	
Surface/Mid/Bottom													
Surface/Mid/Bottom													

Notes: