

# **Bear Creek Post-Alameda Fire Benthic Algae Survey, 2022**



**Prepared by:**  
**Aquatic Ecosystem Sciences LLC**

**Prepared for:**  
**Rogue Valley Council of Governments**

**June 2024**

# **Bear Creek Post-Almeda Fire Benthic Algae Survey, 2022**

**Prepared by:  
Aquatic Ecosystem Sciences LLC**

**Prepared for:  
Rogue Valley Council of Governments**

**June 2024**

**Citation:**

Kann, J. 2024. Bear Creek Post-Almeda Fire Benthic Algae Survey, 2022. Tech Memo Prepared by Aquatic Ecosystem Sciences LLC, Ashland Oregon. Prepared for Rogue Valley Council of Governments, Central Point, Oregon. 20 p.

## Introduction

The Almeda Drive Fire occurred along the Bear Creek riparian corridor and in adjacent urban and suburban areas, destroying homes and businesses in Ashland, Talent, Phoenix, and Medford, Oregon on September 8<sup>th</sup>, 2020. In addition, the fire destroyed native and planted riparian vegetation that helped improve local quality along Bear Creek, a tributary to the Rogue River. In an effort to better understand the effect of this fire on the water quality of Bear Creek, the Rogue Valley Council of Governments and other local partners including the Oregon Department of Environmental Quality, Rogue River Watershed Council, and Rogue Valley Sewer Services, implemented a post-fire monitoring program through a grant from the Oregon Watershed Enhancement Board.

As part of the overall water quality monitoring plan, the partners included monitoring for potentially toxic cyanobacteria in Bear Creek. Given expected increased nutrient runoff to Bear Creek associated with burn zones, increased water temperature and water column light due to diminished riparian vegetation, and loss of riparian filtration capacity, the potential existed for increased growth of toxigenic cyanobacteria. Cyanobacteria, also known as blue-green algae, can produce and release toxins into the waters where they proliferate. Dense growths of cyanobacteria that impact water quality and public health are known as harmful algal blooms or HABs. Attached, or benthic, cyanobacteria are common in rivers and streams where the more commonly considered planktonic cyanobacteria are often absent. These types of benthic HABs are known to be very prevalent in wadable streams with similar characteristics to Bear Creek (e.g., Fetscher et al. 2015; Bouma-Gregson et al. 2028).

Benthic cyanobacteria have the capacity to produce many types of toxins, but anatoxin is of particular concern because it is a potent neurotoxin that can harm wildlife, pets, and people. In stream systems such as Bear Creek the greatest likelihood is for the HABs to occur as benthic algal mats attached to rocks and other substrate, as opposed to planktonic forms. These mats can take many forms, and generalized warnings are often used to alert the public (Figure 1). Since most waterbodies in Oregon are not monitored, the Oregon Health Authority recommends that the public becomes familiar with cyanobacteria bloom characteristics, whether planktonic or benthic<sup>1</sup>.

Since there were no previous monitoring data for benthic algae in Bear Creek within the Almeda fire zone between Ashland and Medford, the goal of the project was to conduct a reconnaissance level, qualitative survey in order to inform:

1. whether potentially toxigenic species are present in the system, and
2. the design of a more quantitative monitoring program in subsequent years, should that be desired.

---

<sup>1</sup><https://www.oregon.gov/oha/PH/HEALTHYENVIRONMENTS/RECREATION/HARMFULALGAE/BLOOMS/Pages/index.aspx>

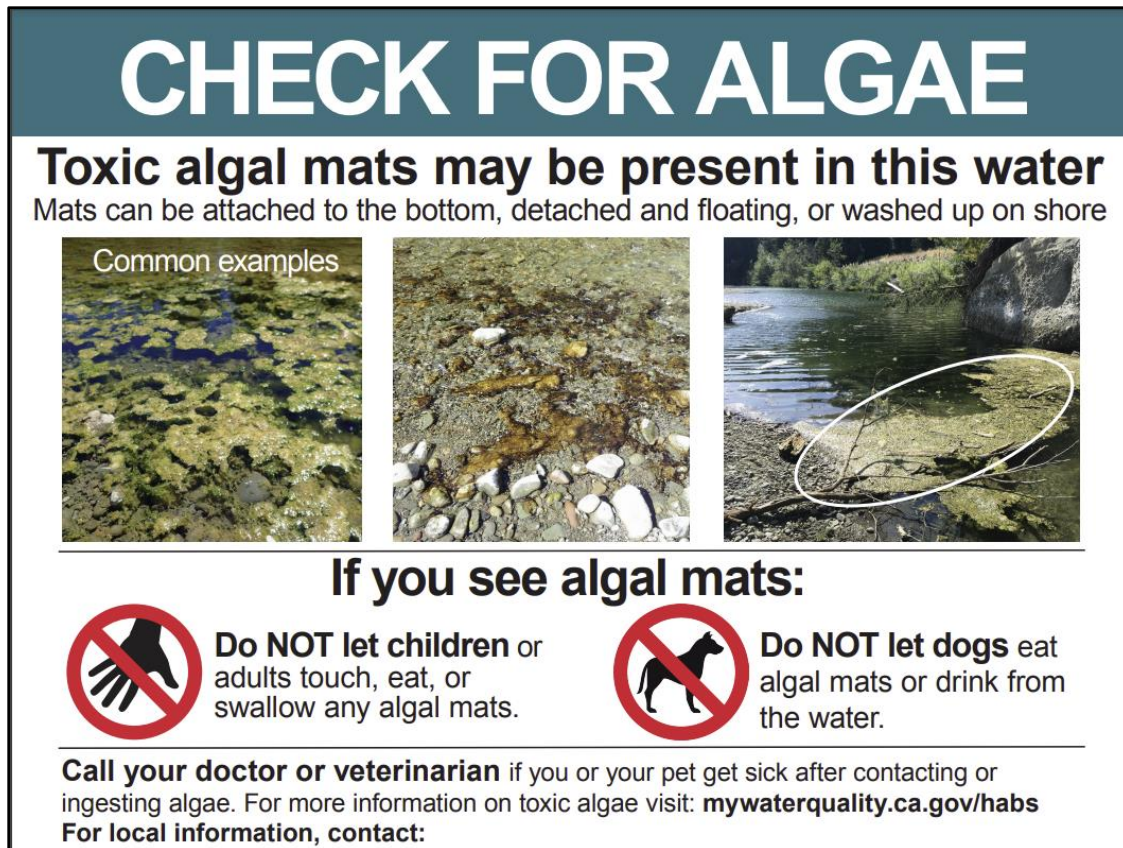


Figure 1. Example of benthic cyanobacteria warning sign for California. Inset photos show various benthic algal forms.

[https://mywaterquality.ca.gov/habs/resources/benthic\\_posting\\_guidance.html](https://mywaterquality.ca.gov/habs/resources/benthic_posting_guidance.html)

## Methods

The initial qualitative survey was to consist of a single longitudinal survey for benthic and other attached algal mats, with the most upstream survey starting point located above the fire zone at North Mountain Park in Ashland, and the most downstream station just below US Cellular Community Park in Medford. However, due to the lack of observable cyanobacteria during the initial survey, we performed two additional surveys during 2022. The surveys utilized the Bear Creek Greenway for bicycle access to sampling locations, and visual surveys for attached algae were made longitudinally at a series of access points targeting potential attached algae habitats. Once algal mats were observed, the location was recorded via GPS, and a subset of samples were collected for analysis. The sampling time-period targeted the primary seasonal increase in benthic algal mats, typically occurring between late July and mid-August.

Preliminary field identification was used to target cyanobacteria as opposed to other green algae or diatoms. If the coverage of attached algae was large, or multiple smaller patches existed, and microscopy revealed the presence of cyanobacteria, a composite sample was

collected and samples were shipped overnight to Bend Genetics in Sacramento, CA ([www.Bendgenetics.com](http://www.Bendgenetics.com)) for species identification and algal toxin analysis. The plan was to follow a tiered approach to decrease costs. If the initial microscopic algal identification showed that no potentially toxigenic species were present, then no further analysis for toxins occurred. If potentially toxigenic species were present, then further laboratory analysis was performed using two methods; QPCR<sup>2</sup> to estimate the number of toxin production genes (only those toxins that might be associated with the species present were analyzed for), and ELISA<sup>3</sup> for specific toxin concentration.

## Results/Discussion

Although the qualitative attached cyanobacteria survey for Bear Creek was originally intended to consist of only one survey with multiple samples taken longitudinally, due to the lack of observable cyanobacteria mats during the initial survey performed on July 7, 2022, we used that survey as a reconnaissance-level effort from which to determine a series of future sampling locations. Benthic cyanobacteria tend to have a distinct morphology (see Appendix I), allowing for targeted sampling once observed. Since the goal was to target visually observed cyanobacteria for toxin analysis, we opted to perform additional surveys rather than to send samples from the 7/7/2022 survey for laboratory analysis. This allowed us to perform multiple site visits and to assess potential cyanobacteria development subsequent to the initial survey date. For the initial 7/7/2022 survey date we<sup>4</sup> rode bicycles the entire length of the Bear Creek Greenway from North Mountain park in Ashland to the US Cellular Fields in south Medford. We visually assessed approximately one dozen locations with reasonable access from the Greenway to the Bear Creek channel. Based on access and potential algal substrate we ultimately chose seven locations for follow-up surveys (Figure 2).

The initial 7/7/2022 survey revealed a minimal amount of attached algal growth of any type, and both visual surveys and microscopic confirmation indicated a lack of cyanobacteria at all locations surveyed (Figure 3). Conditions on 7/7/2022 were fairly turbid, and fine sediment appeared to be smothering algal growth that had previously become established. The source of the turbidity, which would have contributed to light limitation of algal growth, appeared to be recent release of irrigation water from the upstream Emigrant Lake, which also may have contributed to scouring of existing algal growth (flows increased from ~ 3 cfs in late June to >20 cfs during the first week of July; Figure 4)<sup>5</sup>.

---

<sup>2</sup> quantitative real-time polymerase chain reaction (qPCR)-based molecular techniques.

<sup>3</sup> Enzyme-Linked Immunosorbent Assay

<sup>4</sup> assisted by Bill Meyers from DEQ, Laurel Genzoli from the University of Montana, and volunteer Scott Churchill.

<sup>5</sup> Discharge at Bear Cr. below Ashland Cr. (USGS gage <https://waterdata.usgs.gov/monitoring-location/14354200/#parameterCode=00065&period=P7D&showMedian=false>)

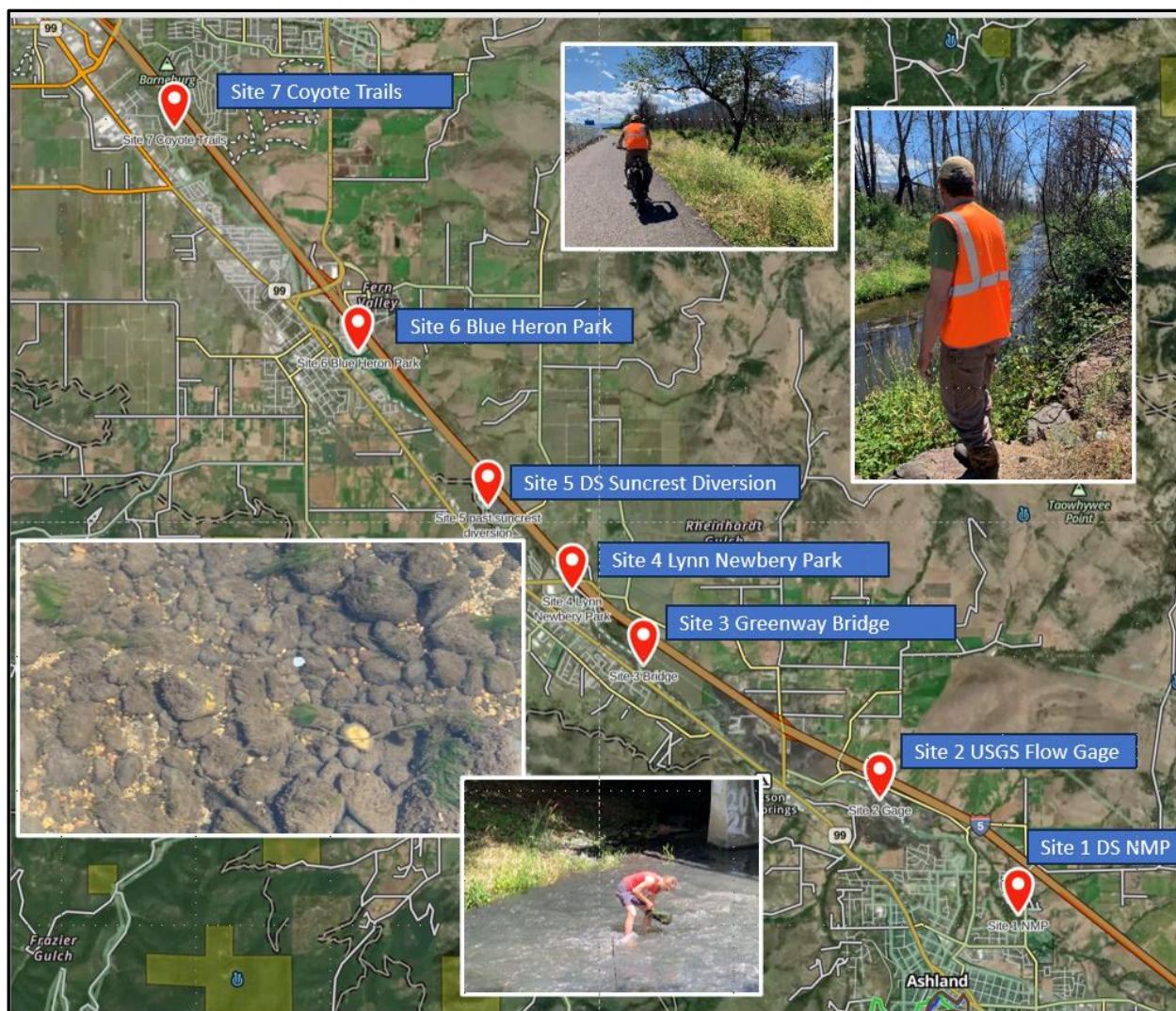


Figure 2. Map showing seven chosen Bear Creek attached algae survey locations.

| Site Name                    | Description                      | Latitude (W) | Longitude (N) |
|------------------------------|----------------------------------|--------------|---------------|
| Site 1 DS NMP                | Downstream of North Mtn. Park    | 122.70366    | 42.20474      |
| Site 2 USGS Flow Gage        | Gage along Greenway near Ashland | 122.72462    | 42.21837      |
| Site 3 Greenway Bridge       | Talent Bike Path Bridge          | 122.76379    | 42.23513      |
| Site 4 Lynn Newbury Park     | Talent Lynn Newbury Park         | 122.77061    | 42.24478      |
| Site 5 DS Suncrest Diversion | Downstream of Suncrest Diversion | 122.78874    | 42.25403      |
| Site 6. Blue Heron Park      | Phoenix Blue Heron Park          | 122.81082    | 42.27285      |
| Site 7 Coyote Trails         | Coyote Trails Nature Center      | 122.83969    | 42.29945      |

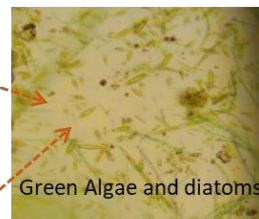
## Initial Site Reconnaissance: July 7, 2022

12 potential sites assessed based on access above, within, and below fire zone.

North Mountain Park



Blue Heron Park



Near US Cellular Fields



- No indication of cyanobacteria
- TID recently began release from Emigrant Lake causing high turbidity
- Evidence that previously established attached algae was scoured due to higher flow releases.
- Decision made to survey again in August

Figure 3. Select locations showing high turbidity conditions and low quantity of attached algae during the 7/7/2022 Bear Creek attached algal survey.

# Bear Creek Below Ashland Creek at Ashland, OR - 14354200

May 31, 2022 - October 31, 2022  
Discharge, cubic feet per second

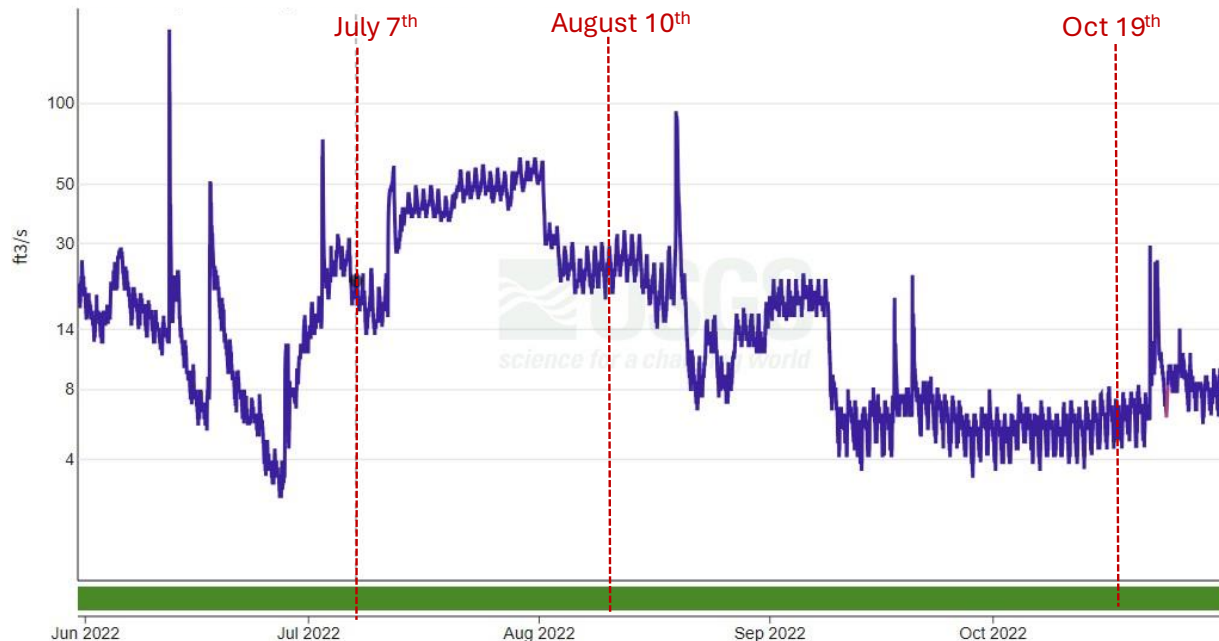


Figure 4. Discharge at Bear Creek below Ashland Creek showing 2022 benthic algal sampling dates. USGS gage: Bear Creek Below Ashland Creek at Ashland, OR - 14354200

The seven sites established during the initial survey were next surveyed on August 10, 2022. At most locations on 8/10/2022 conditions were similar to those on 7/7/2022, with evidence of high siltation and turbidity (flows entering the upper reach of Bear Creek remained elevated at > 20 cfs; Figure 4), and little evidence of benthic cyanobacteria. With the exception of the Coyote Trails site, microscopic analysis showed various diatoms, most of which were in a state of decomposition (Figure 5). For confirmation, we collected samples of attached algae from two locations that did not appear to be cyanobacteria— the Ashland BP Gage (Site 2) and Talent BP Bridge (Site 3). Although mostly similar to other stations in terms of benthic algal coverage, we observed several small patches on bedrock substrate at the Coyote trails location (Site 7) that appeared to be cyanobacteria. We collected a composite sample from several areas in the Site 7 cross-section, a bedrock sample, and a cobble sample for both microscopy and toxin analysis. Initial microscopy performed by Aquatic Ecosystem Sciences confirmed the presence of cyanobacteria in the bedrock sample at Site 7 (Figure 5; bottom row) and that algae at the Ashland BP Gage (Site 2) and Talent BP Bridge (Site 3) were not cyanobacteria.

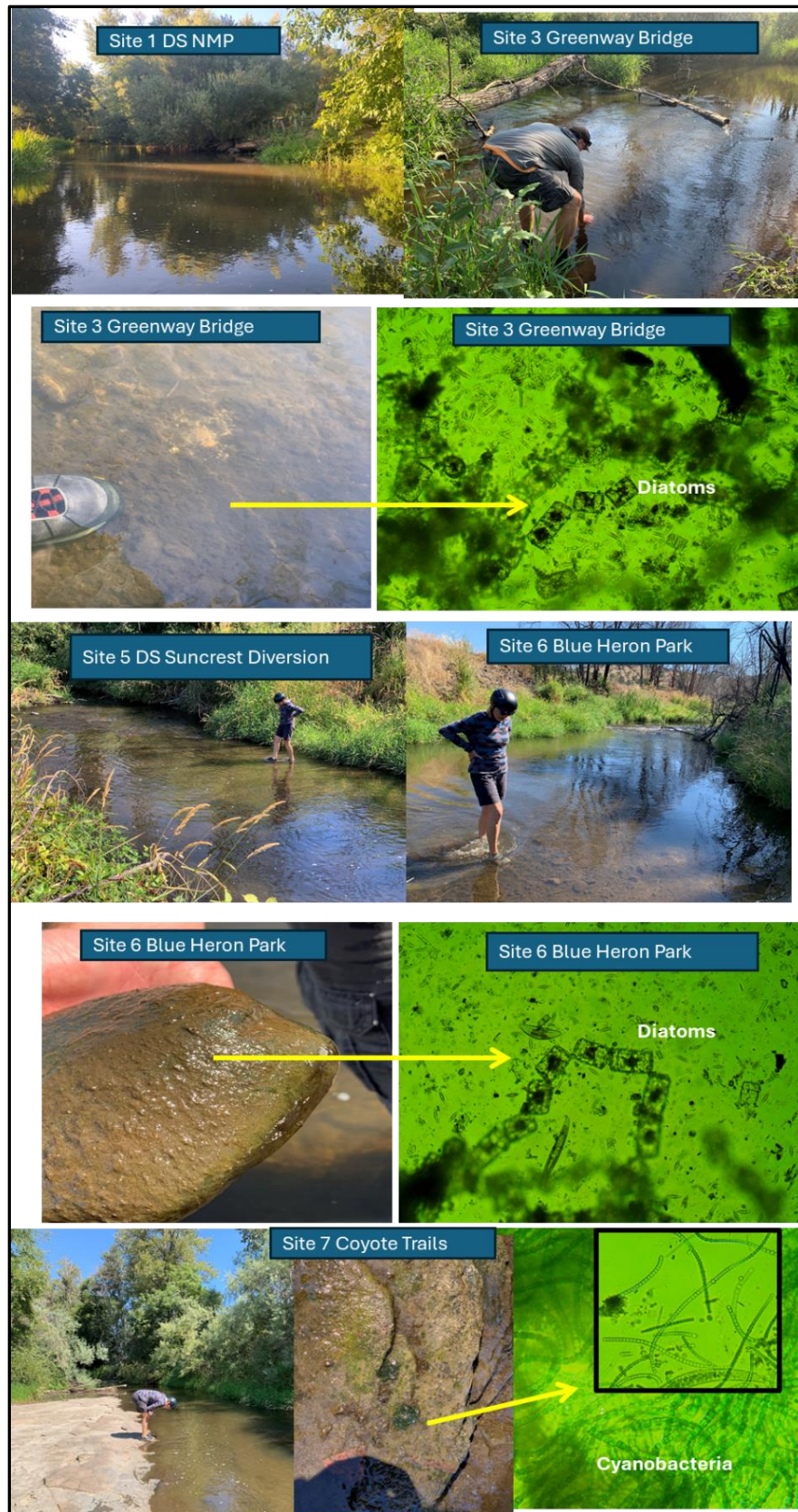


Figure 5. Bear Creek benthic cyanobacteria longitudinal survey, August 10, 2022.

Likewise, microscopic analysis by Bend Genetics confirmed that the Coyote Trails (Site 7) bedrock algae was comprised of two different cyanobacteria genera (*Anabaena* and *Cylindrospermum*) based on microscopy, and QPCR analysis revealed that both the bedrock and cobble sample contained the genes that produce the cyanobacterial toxin microcystin (Figure 6; see Appendix II for full lab report). As suspected based on the visual survey, QPCR analysis confirmed that toxin producing genes were not present in either of the other two samples (Sites 2 and 3) where the attached algae did not visually appear to be cyanobacteria (Figure 6).

For the bedrock sample with a positive QPCR result<sup>6</sup>, the ELISA test for toxin concentration showed a very low level of microcystin (just above the quantitation limit at 0.18 µg/L). Although the cobble sample had double the gene copies for microcystin as the bedrock sample (414 copies per/ml vs. 198 copies per/ml), ELISA was not performed on the cobble sample. However, even with a doubling in toxin concentration, the microcystin value for the cobble sample would be 0.32 µg/L, a value well below any public health risk. No other algal toxins such as anatoxin were detected.

A final visual survey was conducted on October 19, 2022, and again there was no visible growth of benthic cyanobacteria (Figure 7). Although the water had better clarity, in general, than the earlier surveys (flows declined to approximately 7 cfs; Figure 4), in many locations there was still a fair amount of silt covering the Bear Creek substrate. In addition, where substrate was less silted, the predominant algal genera were non-toxic diatoms and green algae. Shorter daily light duration and cooler temperatures are also likely contributing to low benthic algal growth during the October 19th survey.

## Conclusions

Bear Creek in general showed very poor primary production in terms of benthic attached algae during the three surveys performed in 2022. Conditions of high siltation and turbidity likely compromised available substrate, and both potentially toxic cyanobacteria and other algae such as diatoms were not prevalent either above, within, or below the Alameda fire zone. Thus, although increased light and temperature, and increased nutrients due to fire effects were possible (also note that the survey was undertaken approximately two years after the fire), it appears that other factors such as flow, sedimentation, and turbidity may have limited attached algal growth in the reach between North Mountain Park and the US Cellular Fields. The finding of very low levels of toxin at the Coyote Trails site does illustrate the potential for toxin producing cyanobacteria in Bear Creek, and if growths similar to those in Appendix I are observed in the future, users of the waterway should exercise caution.

---

<sup>6</sup> QPCR indicates the copies/ml of toxin producing genes, which is related to toxin concentration, but does not directly measure the concentration of toxin present. Enzyme Linked Immunosorbent Assay (ELISA) directly measures toxin concentration.

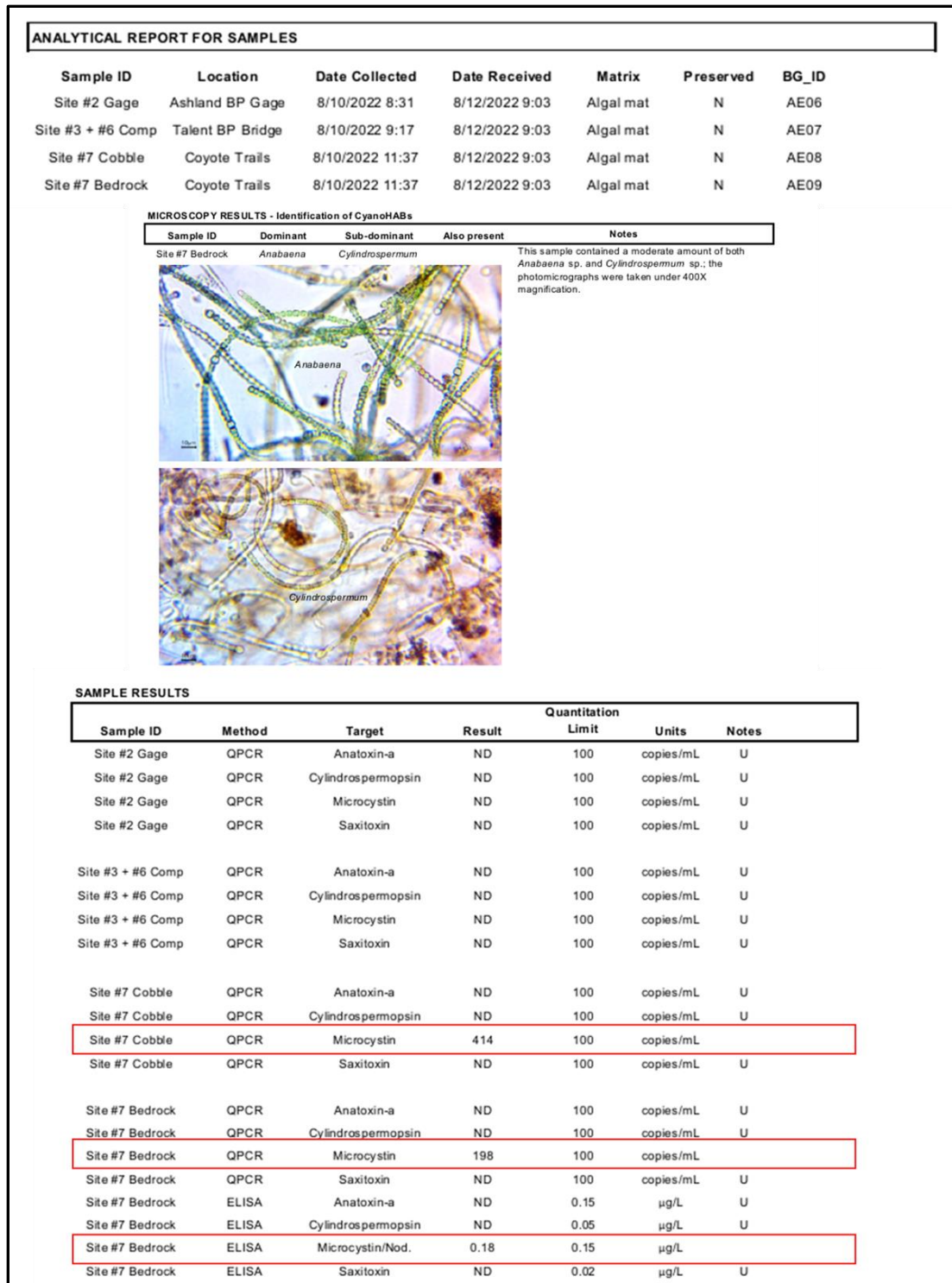


Figure 6. Bend Genetics lab results for the Bear Creek 8/10/2022 benthic cyanobacteria survey.



Figure 7. Bear Creek benthic cyanobacteria longitudinal survey, October 19, 2022.

## Literature Cited

Bouma-Gregson, K., R. M. Kudela, and M. E. Power. 2018. Widespread anatoxin-a detection in benthic cyanobacterial mats throughout a river network. PLOS ONE 13:e0197669.

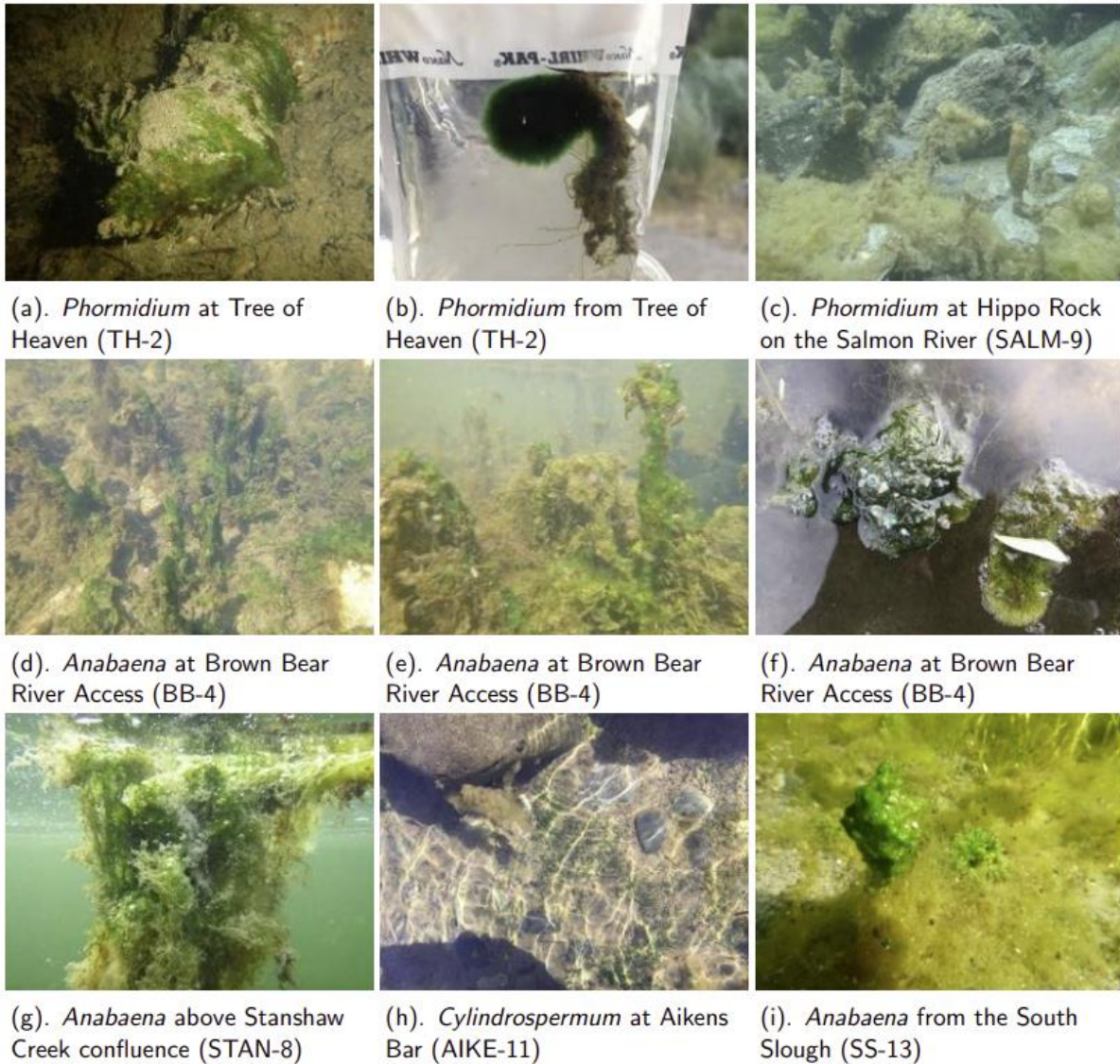
Fetscher, A. E., M. D. Howard, R. Stancheva, R. M. Kudela, E. D. Stein, M. A. Sutula, L. B. Busse, and R. G. Sheath. 2015. Wadable streams as widespread sources of benthic cyanotoxins in California, USA. Harmful Algae 49:105–116.

Genzoli, L and J. Kann. 2020. Extent and Distribution of Anatoxin-a in the Klamath River: A Review of Toxin Monitoring and Benthic Cyanobacteria Observations. Prepared by Aqua-c Ecosystem Sciences LLC for the Klamath Tribal Water Quality Consortium. 24 p. + Appendices. April 2020. [https://klamathwaterquality.com/documents/KlamathAnatoxin\\_2020\\_04\\_30\\_small.pdf](https://klamathwaterquality.com/documents/KlamathAnatoxin_2020_04_30_small.pdf)

## Acknowledgements

We gratefully acknowledge assistance for the field surveys by Bill Meyers from Oregon DEQ, Laurel Genzoli from the University of Montana, and volunteer Scott Churchill from Ashland, Oregon.

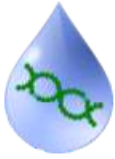
## Appendix I: Visual Examples of Benthic Cyanobacteria



Reproduced from Figure 5 in:

Genzoli, L and J. Kann. 2020. Extent and Distribution of Anatoxin-a in the Klamath River: A Review of Toxin Monitoring and Benthic Cyanobacteria Observations. Prepared by Aqua-c Ecosystem Sciences LLC for the Klamath Tribal Water Quality Consortium. 24 p. + Appendices. April 2020. [https://klamathwaterquality.com/documents/KlamathAnatoxin\\_2020\\_04\\_30\\_small.pdf](https://klamathwaterquality.com/documents/KlamathAnatoxin_2020_04_30_small.pdf)

## Appendix II



**Bend Genetics, LLC**  
107 Scripps Drive, Ste. 210  
Sacramento, CA 95825  
Tel: (916) 550-1048

**Date:** 8/19/2022  
**Subject:** Cyanobacteria testing results – Bear Creek  
**From:** Tim Otten, Laboratory Director  
**To:** Jacob Kann  
Aquatic Ecosystem Sciences

Testing results are attached for microscopy, QPCR and ELISA analyses conducted on four algal mat samples collected on 8/10/2022. All data have been reviewed and are considered final.

**Analyses included in this report:**

- Quantification of total cyanobacterial toxins (anatoxin-a, cylindrospermopsin, microcystin/nodularin and saxitoxin) by enzyme linked immunosorbent assay (ELISA).
- Quantification of total toxin producing cyanobacteria by toxin class using real-time quantitative polymerase chain reaction (QPCR) methods.
- Microscope photos and identification of potentially toxigenic (PTOX) cyanobacteria.

**Bend Genetics, LLC**

107 Scripps Drive, Ste. 210  
Sacramento, CA 95825  
Tel: (916) 550-1048

**Customer:** Aquatic Ecosystem Sciences

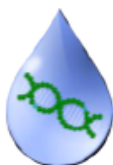
Analysis for Toxic Cyanobacteria

**Project:** Bear Creek

**Reported:** 8/19/2022 16:35

**ANALYTICAL REPORT FOR SAMPLES**

| Sample ID         | Location         | Date Collected  | Date Received  | Matrix    | Preserved | BG_ID |
|-------------------|------------------|-----------------|----------------|-----------|-----------|-------|
| Site #2 Gage      | Ashland BP Gage  | 8/10/2022 8:31  | 8/12/2022 9:03 | Algal mat | N         | AE06  |
| Site #3 + #6 Comp | Talent BP Bridge | 8/10/2022 9:17  | 8/12/2022 9:03 | Algal mat | N         | AE07  |
| Site #7 Cobble    | Coyote Trails    | 8/10/2022 11:37 | 8/12/2022 9:03 | Algal mat | N         | AE08  |
| Site #7 Bedrock   | Coyote Trails    | 8/10/2022 11:37 | 8/12/2022 9:03 | Algal mat | N         | AE09  |

**Bend Genetics, LLC**

107 Scripps Drive, Ste. 210

Sacramento, CA 95825

Tel: (916) 550-1048

**Customer:** Aquatic Ecosystem Sciences

Analysis for Toxic Cyanobacteria

**Project:** Bear Creek**Reported:** 8/19/2022 16:35**SAMPLE RESULTS**

| Sample ID         | Method | Target             | Result | Quantitation |           | Notes |
|-------------------|--------|--------------------|--------|--------------|-----------|-------|
|                   |        |                    |        | Limit        | Units     |       |
| Site #2 Gage      | QPCR   | Anatoxin-a         | ND     | 100          | copies/mL | U     |
| Site #2 Gage      | QPCR   | Cylindrospermopsin | ND     | 100          | copies/mL | U     |
| Site #2 Gage      | QPCR   | Microcystin        | ND     | 100          | copies/mL | U     |
| Site #2 Gage      | QPCR   | Saxitoxin          | ND     | 100          | copies/mL | U     |
|                   |        |                    |        |              |           |       |
| Site #3 + #6 Comp | QPCR   | Anatoxin-a         | ND     | 100          | copies/mL | U     |
| Site #3 + #6 Comp | QPCR   | Cylindrospermopsin | ND     | 100          | copies/mL | U     |
| Site #3 + #6 Comp | QPCR   | Microcystin        | ND     | 100          | copies/mL | U     |
| Site #3 + #6 Comp | QPCR   | Saxitoxin          | ND     | 100          | copies/mL | U     |
|                   |        |                    |        |              |           |       |
| Site #7 Cobble    | QPCR   | Anatoxin-a         | ND     | 100          | copies/mL | U     |
| Site #7 Cobble    | QPCR   | Cylindrospermopsin | ND     | 100          | copies/mL | U     |
| Site #7 Cobble    | QPCR   | Microcystin        | 414    | 100          | copies/mL |       |
| Site #7 Cobble    | QPCR   | Saxitoxin          | ND     | 100          | copies/mL | U     |
|                   |        |                    |        |              |           |       |
| Site #7 Bedrock   | QPCR   | Anatoxin-a         | ND     | 100          | copies/mL | U     |
| Site #7 Bedrock   | QPCR   | Cylindrospermopsin | ND     | 100          | copies/mL | U     |
| Site #7 Bedrock   | QPCR   | Microcystin        | 198    | 100          | copies/mL |       |
| Site #7 Bedrock   | QPCR   | Saxitoxin          | ND     | 100          | copies/mL | U     |
| Site #7 Bedrock   | ELISA  | Anatoxin-a         | ND     | 0.15         | µg/L      | U     |
| Site #7 Bedrock   | ELISA  | Cylindrospermopsin | ND     | 0.05         | µg/L      | U     |
| Site #7 Bedrock   | ELISA  | Microcystin/Nod.   | 0.18   | 0.15         | µg/L      |       |
| Site #7 Bedrock   | ELISA  | Saxitoxin          | ND     | 0.02         | µg/L      | U     |



**Bend Genetics, LLC**  
107 Scripps Drive, Ste. 210  
Sacramento, CA 95825  
Tel: (916) 550-1048

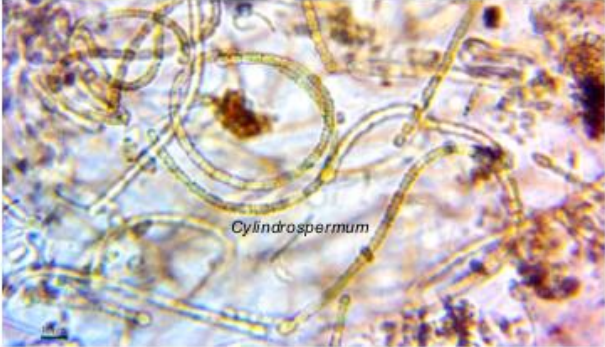
**Customer:** Aquatic Ecosystem Sciences  
Analysis for Toxic Cyanobacteria  
**Project:** Bear Creek  
**Reported:** 8/19/2022 16:35

**MICROSCOPY RESULTS - Identification of CyanoHABs**

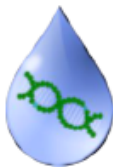
| Sample ID       | Dominant        | Sub-dominant           | Also present | Notes                                                                                                                                                         |
|-----------------|-----------------|------------------------|--------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Site #7 Bedrock | <i>Anabaena</i> | <i>Cylindrospermum</i> |              | This sample contained a moderate amount of both <i>Anabaena</i> sp. and <i>Cylindrospermum</i> sp.; the photomicrographs were taken under 400X magnification. |



*Anabaena*



*Cylindrospermum*


**Bend Genetics, LLC**

107 Scripps Drive, Ste. 210  
Sacramento, CA 95825  
Tel: (916) 550-1048

**Customer:** Aquatic Ecosystem Sciences

Analysis for Toxic Cyanobacteria

**Project:** Bear Creek

**Reported:** 8/19/2022 16:35

**QUALITY CONTROL**

| Method | Analyte         | Result | Qualifiers / Comments | Units     | Spike Level | %REC  | % REC Limits |
|--------|-----------------|--------|-----------------------|-----------|-------------|-------|--------------|
| QPCR   | anaC - Blank    | ND     | U                     | copies/mL | 0           |       |              |
| QPCR   | cyrA - Blank    | ND     | U                     | copies/mL | 0           |       |              |
| QPCR   | mcyE - Blank    | ND     | U                     | copies/mL | 0           |       |              |
| QPCR   | mcyE - Spike    | 48,081 |                       | copies/mL | 50,000      | 96.2  | 70-130       |
| QPCR   | sxtA - Blank    | ND     | U                     | copies/mL | 0           |       |              |
| ELISA  | ATX - Blank     | ND     | U                     | µg/L      | 0           |       |              |
| ELISA  | ATX - Positive  | 0.63   |                       | µg/L      | 0.75        | 84.5  | 70-130       |
| ELISA  | ATX - Matrix Sp | 1.34   |                       | µg/L      | 1.25        | 107.0 | 70-130       |
| ELISA  | CYN - Blank     | ND     | U                     | µg/L      | 0           |       |              |
| ELISA  | CYN - Positive  | 0.76   |                       | µg/L      | 0.75        | 100.7 | 70-130       |
| ELISA  | CYN - Matrix Sp | 1.12   |                       | µg/L      | 1.00        | 112.5 | 70-130       |
| ELISA  | MC - Blank      | ND     | U                     | µg/L      | 0           |       |              |
| ELISA  | MC - Positive   | 0.72   |                       | µg/L      | 0.75        | 96.5  | 70-130       |
| ELISA  | MC - Matrix Sp  | 0.83   |                       | µg/L      | 1.00        | 83.1  | 70-130       |
| ELISA  | STX - Blank     | ND     | U                     | µg/L      | 0           |       |              |
| ELISA  | STX - Positive  | 0.072  |                       | µg/L      | 0.075       | 95.9  | 70-130       |
| ELISA  | STX - Matrix Sp | 0.197  |                       | µg/L      | 0.200       | 98.3  | 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                                                                                    |

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

|                                    |  |                                                 |  |                                             |  |                                       |  |                               |  |
|------------------------------------|--|-------------------------------------------------|--|---------------------------------------------|--|---------------------------------------|--|-------------------------------|--|
| Group: Bear Creek                  |  | Project Code: Bear Creek                        |  | Project Lead: Jacob Kuan                    |  | 541-487-1575                          |  | Brenda@aquatic-ecosystems.com |  |
| Fiscal Year: 2022                  |  | Sampling Procedure Used: Grab Sample            |  | Field Sampling Lead: Jacob Kuan             |  |                                       |  |                               |  |
| PD: Aquatic Ecosystem Sciences LLC |  | Sampling Agency: Aquatic Ecosystem Sciences LLC |  | Field Crew: J. Kuan, L. Gonzoli, Bill Meyer |  | Brenda Genetics Lab Contact: Tim Chen |  | (916) 550-1048                |  |
| Event Code: WQ                     |  |                                                 |  |                                             |  |                                       |  | gill@brendagenetics.com       |  |

| Sample ID                                                          | Sampling Location/<br>Station Name        | Sample<br>Date | Collection<br>Time | Sample<br>Volume | Total<br>Preservation         | Sample<br>Type Code | Sample<br>Container | Remarks | Water Analysis Authorization                           |                                      |                     |
|--------------------------------------------------------------------|-------------------------------------------|----------------|--------------------|------------------|-------------------------------|---------------------|---------------------|---------|--------------------------------------------------------|--------------------------------------|---------------------|
|                                                                    |                                           |                |                    |                  |                               |                     |                     |         | *Circled/boxed requested analysis, see comments below* | One bottle per sampling location/lot |                     |
| Site #2 Grage                                                      | Autoland Blue Pads Grage                  | 8/10/2022      | 8:31 AM            | 60 ml            | 10% ethanol/<br>Stored 2-10°C | Glass/<br>PETG      | Glass/<br>PETG      |         | Toxin Analysis<br>1 2 3 4                              | QPCR Analysis<br>3 6 7 8 9 10        | Microscopy<br>11 12 |
| Site #3 - 46 Composite                                             | Tucker Bule Pad Bridge + Blue<br>Horn Pad | 8/10/2022      | 9:17 - 10:36 AM    | 60 ml            | 10% ethanol/<br>Stored 2-10°C | Glass/<br>PETG      | Glass/<br>PETG      |         | Toxin Analysis<br>1 2 3 4                              | QPCR Analysis<br>3 6 7 8 9 10        | Microscopy<br>11 12 |
| Site #7 Cobble                                                     | Coyote Trails Cobble                      | 8/10/2022      | 11:37 AM           | 60 ml            | 10% ethanol/<br>Stored 2-10°C | Glass/<br>PETG      | Glass/<br>PETG      |         | Toxin Analysis<br>1 2 3 4                              | QPCR Analysis<br>3 6 7 8 9 10        | Microscopy<br>11 12 |
| Site #7 Bedrock B/C                                                | Coyote Trails Bed Rock                    | 8/10/2022      | 11:37 AM           | 60 ml            | 10% ethanol/<br>Stored 2-10°C | Glass/<br>PETG      | Glass/<br>PETG      |         | Toxin Analysis<br>1 2 3 4                              | QPCR Analysis<br>3 6 7 8 9 10        | Microscopy<br>11 12 |
| <p><b>Note:</b> Ambient 22.2 deg C - samples should be cooling</p> |                                           |                |                    |                  |                               |                     |                     |         |                                                        |                                      |                     |
|                                                                    |                                           |                |                    |                  | 10% ethanol/<br>Stored 2-10°C | Glass/<br>PETG      | Glass/<br>PETG      |         | Toxin Analysis<br>1 2 3 4                              | QPCR Analysis<br>5 6 7 8 9 10        | Microscopy<br>11 12 |
|                                                                    |                                           |                |                    |                  | 10% ethanol/<br>Stored 2-10°C | Glass/<br>PETG      | Glass/<br>PETG      |         | Toxin Analysis<br>1 2 3 4                              | QPCR Analysis<br>5 6 7 8 9 10        | Microscopy<br>11 12 |
|                                                                    |                                           |                |                    |                  | 10% ethanol/<br>Stored 2-10°C | Glass/<br>PETG      | Glass/<br>PETG      |         | Toxin Analysis<br>1 2 3 4                              | QPCR Analysis<br>5 6 7 8 9 10        | Microscopy<br>11 12 |
|                                                                    |                                           |                |                    |                  | 10% ethanol/<br>Stored 2-10°C | Glass/<br>PETG      | Glass/<br>PETG      |         | Toxin Analysis<br>1 2 3 4                              | QPCR Analysis<br>5 6 7 8 9 10        | Microscopy<br>11 12 |
|                                                                    |                                           |                |                    |                  | 10% ethanol/<br>Stored 2-10°C | Glass/<br>PETG      | Glass/<br>PETG      |         | Toxin Analysis<br>1 2 3 4                              | QPCR Analysis<br>5 6 7 8 9 10        | Microscopy<br>11 12 |
|                                                                    |                                           |                |                    |                  | 10% ethanol/<br>Stored 2-10°C | Glass/<br>PETG      | Glass/<br>PETG      |         | Toxin Analysis<br>1 2 3 4                              | QPCR Analysis<br>5 6 7 8 9 10        | Microscopy<br>11 12 |
|                                                                    |                                           |                |                    |                  | 10% ethanol/<br>Stored 2-10°C | Glass/<br>PETG      | Glass/<br>PETG      |         | Toxin Analysis<br>1 2 3 4                              | QPCR Analysis<br>5 6 7 8 9 10        | Microscopy<br>11 12 |
|                                                                    |                                           |                |                    |                  | 10% ethanol/<br>Stored 2-10°C | Glass/<br>PETG      | Glass/<br>PETG      |         | Toxin Analysis<br>1 2 3 4                              | QPCR Analysis<br>5 6 7 8 9 10        | Microscopy<br>11 12 |

**Total # subsamples: 1**

**Comments:** For each row above analysis corresponding to # 1 - 12 below

|                                                                           |                                                                                    |                                       |
|---------------------------------------------------------------------------|------------------------------------------------------------------------------------|---------------------------------------|
| 1 Microscopy + Nodularin by ELISA, Total fraction measured (no filtering) | 3 Microscopy in grain; lab analysis includes concentration + extraction per method | 11 Photograph & ID PTOX Cyanobacteria |
| 2 Analysis - 4 by ELISA, Total fraction measured (no filtering)           | 6 Anatoxin-a; lab analysis includes concentration + extraction per method          | 12 Cyanobacteria ID & enumeration     |
| 3 Cytodiagnostics by ELISA, Total fraction measured (no filtering)        | 7 Cytodiagnostics; lab analysis includes concentration + extraction per method     |                                       |
| 4 Section by ELISA, Total fraction measured (no filtering)                | 8 Section; lab analysis includes concentration + extraction per method             |                                       |
|                                                                           | 9 Nodularin gene; lab analysis includes concentration + extraction per method      |                                       |
|                                                                           | 10 Total Cyanobacteria; quantifies "cell equivalents/mL" by qPCR                   |                                       |

Samples Relinquished by: Jacob Kuan

Samples Received by: Jessica Harrison

Date & Time: 8/10/2022 1:10

Date & Time: 8/12/22 1:03

Name (Print and Sign):

Name (Print and Sign):

**Note: Bill to Aquatic Ecosystem Sciences**

Please mail samples with following day delivery (by 10:30 AM):

Brenda Genetics, LLC  
87 Scripps Drive Ste 301  
Sacramento, CA 95825  
Tel: (916) 550-1048

Brenda Genetics - COC