Extent and Distribution of Anatoxin-a in the Klamath River: A Review of Toxin Monitoring and Benthic Cyanobacteria Observations

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Prepared by Aquatic Ecosystem Sciences LLC.
for the Klamath Tribal Water Quality
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Summary

Anatoxin-a, a potent neurotoxin, can be a public health concern in lakes, reservoirs, and rivers where cyanobacteria are present. Cyanobacteria taxa associated with the production of anatoxin-a have been documented in hydroelectric reservoirs on the Klamath River. Attached cyanobacteria genera associated with anatoxin-a production have also been documented in free flowing sections of the Klamath River. Concern about anatoxin-a in the Klamath River has spurred annual, albeit inconsistent sampling of anatoxin-a in the watershed. Prior to this report, these data have not been compiled and examined together across multiple years and agencies, leading to a more complete view of what is known about anatoxin-a in the Klamath River.

In this report, we compiled data from anatoxin-a samples in the Klamath River Watershed between the hydroelectric reach and the estuary collected from 2005 to 2019, as well as data from special studies that involved the deployment of Solid Phase Adsorption Toxin Tracking (SPATT) samplers. We include observations of attached cyanobacterial mats made during 2018 and 2019 surveys, as well as the results of toxin analysis from attached mats in 2019. We compiled anatoxin-a results from a total of 236 water samples, 43 SPATT samples, and seven algal mat samples. The majority (75%) of samples were collected from the flowing river below Iron Gate Dam, where 19 samples had detectable levels of anatoxin-a ($n = 214$). Four samples from the reservoirs ($n = 24$), four samples from the estuary ($n = 25$), and two samples from tributaries ($n = 22$) had anatoxin-a detections, indicating that anatoxin-a is widely distributed in diverse aquatic habitats in the Klamath Watershed. Toxin detections from the hydroelectric reservoirs, paired with observations of genera known to produce anatoxin-a in these reservoirs, including *Dolichospermum* (Buratti et al., 2017), suggest that reservoir cyanobacteria may be a source of anatoxin-a to the Klamath River.

In qualitative surveys in 2018 and 2019, attached cyanobacterial mats were observed throughout the Klamath River, with *Phormidium* being more common upriver from Happy Camp, while *Anabaena* was more often observed downriver from Happy Camp. All seven of the attached cyanobacterial mat samples collected from the Klamath and Salmon Rivers had detectable levels of anatoxin-a. This strongly suggests that attached algal mats are a source of anatoxin-a in the Klamath River.

Although the timing, spatial distribution, and sources are still poorly understood, the results of 14 years of anatoxin-a monitoring demonstrate that anatoxin-a is a public health concern in the Klamath River. Special studies of anatoxin-a and the toxin-producing cyanobacteria should be conducted to improve predictions of the distribution and timing of the toxin throughout the watershed. The conditions that promote these cyanobacteria should be studied so that restoration and management decisions will not further promote the proliferation of toxin-producing species. Finally, public health messaging should be updated to warn local residents and visitors of the risk associated with anatoxin-a, which is different than the well-messaged risk associated with the reservoir *Microcystis* blooms.

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1 Anatoxin-a Concerns in the Klamath River

Cyanobacteria, often referred to as blue-green algae, are widespread and commonly occur in freshwater environments. Cyanobacteria have the potential to produce a wide variety of toxins, creating problems for aquatic communities and public health when they proliferate in lakes, reservoirs, rivers and wetlands.

The Klamath River Watershed has regularly occurring, well-documented cyanobacterial blooms originating in lakes and reservoirs, which go on to affect downstream river reaches (Jacoby and Kann, 2007; Genzoli and Kann, 2016).In the Klamath River, both within and below the hydroelectric reservoirs, the liver-toxin microcystin has been widely and consistently monitored due to levels above public health thresholds occurring annually in the source reservoirs and receiving waters (Otten et al., 2015; Genzoli and Kann, 2017). Although *Microcystis aeruginosa*, the dominant planktonic cyanobacteria blooming in the hydroelectric reservoirs is not a likely producer of anatoxin-a, other species in these blooms are known producers of anatoxin-a (Harke et al., 2016; Buratti et al., 2017). These include species in the genus *Anabaena/Dolichospermum* and members of the family Ocillatoriaceae.

Due to the presence of these potential anatoxin-a producers and the public health risk associated with anatoxin-a, the Karuk and Yurok Tribes have been collecting and analyzing surface water samples for anatoxin-a in the Klamath River since 2008. Additionally, Pacific Corp has collected and analyzed samples from the reservoirs each year since 2014, generally following the detection of species known to produce anatoxin-a in the reservoirs.

Although the majority of water samples had toxin values below detection limits (which ranged from at least 0.05 to 10 μg/L throughout the sample period), there were also detections of anatoxin-a that exceeded public health thresholds. This information has left water quality managers knowing that anatoxin-a is a potential public health risk in the Klamath River, but the paucity of consistent and comparable long-term data provides limited information about the timing, spatial extent, or sources of anatoxin-a. Although planktonic cyanobacteria blooming in the hydroelectric reservoirs continues to be a potential source of anatoxin-a to the Klamath River, benthic cyanobacterial mats may be a previously unrecognized source of anatoxin-a. Recent studies and observations of benthic mat-forming cyanobacteria suggest that benthic cyanobacteria may be common in rivers, especially those that experience Mediterranean climates (Fetscher et al., 2015). Further, a 2016 study of anatoxin-a producing cyanobacteria in the Klamath River found that the gene responsible for anatoxin-a production during this study was likely associated with benthic, mat-forming members of the Oscillatoriaceae family (Otten, 2017). Toxin analysis was only conducted for 2 of the 55 water samples analyzed in the Klamath River below Iron Gate Dam in this study, so uncertainties remain about how the anatoxin-a synthesis gene relates to toxin concentrations in the Klamath River.

In this report, we compile what is currently known about anatoxin-a occurrence in the Klamath River. We present the results of water-column samples analyzed for anatoxin-a from 2005 to 2019, as well as results of other sampling efforts related to anatoxin-a. Observations of benthic cyanobacteria that were encountered in the Klamath River in 2018 and 2019 are presented and we

conclude with suggestions to improve public health monitoring and outreach. Suggestions for special studies that will improve our understanding of the distribution and public health risk of anatoxin-a in the Klamath River are also presented.

1.1 Background: Toxin Production in Benthic Cyanobacterial Mats

Anatoxin-a production has been widely documented in benthic cyanobacterial mats (Catherine et al., 2013). These toxins have been implicated in the death and illness of dogs, wildlife, and humans across the globe with numerous instances of dog deaths occurring in watersheds close to the Klamath River, including the South Umpqua River and Eel River (Backer et al., 2013; Carmichael, 2001; Farrer et al., 2015; Puschner et al., 2008). Benthic cyanobacteria show high variation in patterns of anatoxin-a production, bringing challenges to quantifying the public health risk from benthic cyanobacteria (Wood et al., 2010, 2012).

Phormidium, a common genus of benthic cyanobacteria in the Klamath River, can produce highly variable anatoxin-a concentrations. Unlike phytoplankton, which are more evenly distributed in the water column of rivers, benthic cyanobacterial mats are highly variable in their spatial distribution. Additionally, the algal mats themselves may contain many different genera and strains of cyanobacteria. A two-year study in seven New Zealand Rivers with *Phormidium* proliferations found anatoxin-a in mat material in six of the 10 sites (Wood et al., 2017). Cultured *Phormidium* taken from 1-cm^2 of *Phormidium* mats at three different riverbed locations contained 30 different strains of *Phormidium*. Of these strains, 18 had toxic variants, demonstrating the high level of variation in toxin-production capabilities at even very small spatial scales (Wood et al., 2012). Further, Wood and colleagues (2012) showed that within specific strains, not all individuals had the gene needed for toxin production, and those that produced anatoxin-a produced up to a 100-fold difference in the anatoxin-a concentrations.

In the Eel River, both *Phormidium* and *Anabaena*, another common genus of cyanobacteria, produce anatoxin-a, with toxin concentrations varying both among sites and through time (Bouma-Gregson et al., 2018, 2017). Samples of floating *Anabaena* mats collected weekly at one site from June through September had a range of 0–11,203 ng/g dry weight of anatoxin-a, showing that simply the presence of *Anabaena* does not always indicate toxic conditions (Bouma-Gregson et al., 2018). Samples of *Phormidium* and *Anabaena* throughout the Eel River showed similar variation with 69% and 82% of *Anabaena* and *Phormidium* mat samples testing positive for anatoxin-a. Despite sometimes high concentrations of anatoxin-a in mat material, water column anatoxin-a concentrations in the Eel River were below public health thresholds (Bouma-Gregson et al., 2018).

Anatoxin-a from benthic cyanobacteria may be highly variable throughout rivers. Patchy mat distribution, different strains within mats, and variation in toxin production genes within strains all contribute to challenges in predicting toxin concentrations in rivers where benthic cyanobacteria are the source of anatoxin-a. Further, environmental variables that promote toxin producing strains or toxin production within strains remains largely unknown, thus the tools to predict toxin levels based on observed environmental conditions are non-existent (Buratti et al., 2017). Research is needed to better understand the toxin production patterns and drivers in the Klamath River and in rivers worldwide.

1.2 Background: Patterns and Drivers of Benthic Cyanobacterial Mats

Relatively little is known about the distribution, environmental drivers, and toxin risk associated with benthic cyanobacteria. This gap in knowledge is in part due to fewer studies focused on benthic cyanobacteria than freshwater planktonic species, as well as unique and complex sets of conditions promoting the proliferation of cyanobacterial mats across time and space.

Most research on *Phormidium* comes from New Zealand, where benthic mat proliferations have increased in the past decade (McAllister et al., 2016). Here, high nitrogen concentrations leading to higher nitrogen-to-phosphorus ratios were associated with increased growth of *Phormidium*, a non-nitrogen-fixing genus (Wood, Susanna and Young, Rodger, 2011). Warmer stream temperatures and higher velocities promoted the growth of *Phormidium*, which was seasonally present in the studied New Zealand rivers from early summer through the Fall (Hart et al., 2013; Wood et al., 2017).

Benthic *Anabaena* mats are common in coastal California rivers, including the Eel River where proliferations of *Anabaena* have been most widespread when pools and backwaters are disconnected from main flows and groundwater, allowing some surface water to become stagnant and warm (Power et al., 2015). *Anabaena* can fix atmospheric nitrogen, giving it a competitive advantage in low nitrogen conditions. In the Klamath, we found *Anabaena* mats more often in backwaters and along river margins at sites downriver from Happy Camp where nitrogen levels are lower and other nitrogen fixing periphyton are more common than at sites upriver from Happy Camp (Gillett et al., 2016).

In order to protect public health from local exposures, it is important to not only understand where benthic cyanobacteria grow, but also where they become dispersed in the river. Studies in New Zealand rivers show that *Phormidium* mats tend to hang on to the substrate, and dislodge with flows many times higher than annual median flows, which was higher than flows needed to dislodge other periphyton communities (Hart et al., 2013; Wood et al., 2017). In the absence of high flow events, older stages of *Phormidium* mats can form and trap air bubbles, causing them to dislodge, rise to the surface, and be transported downstream (McAllister et al., 2016). *Anabaena* mats are also easily transported downstream due weak benthic attachment and bubble formation that causes them to float, with two fates that can further threaten public health. First, *Anabaena* mats may be transported and settle out in a new location where they re-establish, promoting additional cyanobacterial coverage. Second, river margins or protected backwaters can collect transported *Anabaena* that remains on or near the surface as it decays with the potential for high toxin concentrations becoming more accessible to people, pets, and wildlife (Bouma-Gregson et al., 2017).

2 Analysis of Anatoxin-a Samples in the Klamath River

2.1 Water Column Grab Samples

We compiled anatoxin-a data from samples collected by the Yurok Tribe Environmental Program (YTEP), the Karuk Department of Natural resources, Pacific Corp, and California Department of Health Services (CDHS). We cross-checked data

files and eliminated entries that were deemed to be the same sample repeated in multiple data files. We treated duplicate samples as independent samples when two samples were taken from the same time and location but were still independent samples. For data from CDHS in 2005 where four samples were clearly labeled as lab splits, we calculated the mean of the lab splits and used this as the observed anatoxin-a concentration.

Samples were collected as water-column grab samples, either from the main current in the upper 0.5 m of the water column or from river margins in the upper 0.1 m of the water column where algae are likely to accumulate, similar to base-line and public health sampling protocols for microcystin toxin (Klamath Blue Green Algae Working Group, 2009). Samples were analyzed for anatoxin-a by enzyme linked immunosorbent assay (ELISA, Bend Genetics) or by liquid chromatography/mass spectrometry (LC-MS/MS; Green Water Laboratory and California Department of Fish and Game's Fish and Wildlife Water Pollution Control Laboratory in Rancho Cordova, CA).

A total of 236 individual anatoxin-a samples were compiled from 21 sites spanning the Mid and Lower Klamath Watersheds. Most samples (82%; 193 total samples) were collected from the mainstem of the Klamath River from Below Iron Gate Dam to above the estuary. Two sites were regularly sampled in the Klamath River Estuary, South Slough and Lower Estuary, comprising 10% of the samples (23 total samples). The remaining samples were taken from Copco and Iron Gate reservoirs (18 total samples, 8%) and 2 samples were collected from tributaries, with one sample from the Shasta River and one from the Trinity River (Figure 1).

The number of water column samples collected each year for anatoxin-a analysis has been highly variable. One sampling effort was conducted by CDHS in 2005, followed by two years without anatoxin-a sampling. Since 2008, limited and highly variable sampling has coccured each year. The most samples in a year were collected in 2009, with 64 samples, while only three samples were collected in 2011. From 2011 to 2016, there was an increase in the number of samples collected each year, followed by a decrease in samples after 2016 (Figure 2).

Samples were collected from late spring to autumn (Figure 2). The distribution of sample timing within a year was generally well-aligned with when we would expect higher rates of algae growth both in reservoirs and on the river substrate. June through October, when most samples were collected, is also the period of higher water contact in the Klamath River during recreational and Tribal ceremonial use.

Of the 236 water column samples of anatoxin-a collected between Copco Reservoir and the Klamath Estuary from 2005 to 2019, 15 samples had toxin concentrations above the laboratory detection limits (Table 1). These positive detections ranged from >2.1 μg/L to >525 μg/L. Sites with positive detections were distributed throughout the Klamath River, from Iron Gate Reservoir to the South Slough of the Klamath Estuary. Of the 18 reservoir samples, grab sample detections of anatoxin-a were limited to data collected by CDHS in 2005, with no detections of anatoxin-a reported in the reservoirs by Pacific Corp (Klamath Water Quality Data, https://www.pacificorp.com/energy/hydro/klamath-river/water-quality.html, Table 1). Although detection limits were not regularly reported for the anatoxin-a data, variation in the detection limits between at least 0.05 and 10 μg/L would have caused moderate levels (>10 μg/L) of anatoxin-a to be reported as non-detectable when detection

limits were high.

Of the 15 samples with anatoxin-a above reporting limits, all occurred in years with higher numbers of total samples taken, with the exception of the single sampling effort conducted in 2005. This suggests that taking only a few samples in a year is not adequate to capture periods of elevated anatoxin-a. The lack of standardized protocols may have also reduced the detection of anatoxin-a where holding times, exposure conditions, detection limits, and analytical methods have varied.

Although there was a relatively low percentage of samples with detectable anatoxin-a concentrations, some patterns were evident. First, four of the samples with positive detections were accompanied by a duplicate sample with a positive detection. The concentrations of these paired duplicates ranged from very similar (84.3 and 86.3 μg/L at IG on 2010-07-21) to highly variable concentrations (>2.1 μg/L and 14.9 μg/L at IB on 2015-07-22). Second, four samples (excluding a duplicate) collected at different sites over a nine day period (2016-09-04 to 2016-09-13) on the Lower Klamath River had very similar concentrations of toxins, indicating that toxins were well mixed in the water column throughout the Lower Klamath River on those dates. Although it is possible that the samples were by chance all collected near similar anatoxin-a producing benthic sources, this finding is more likely indicative of well-mixed anatoxin-a coming from an upstream source. More targeted sampling protocols and special studies are needed to identify sources of anatoxin-a.

2.2 SPATT Samples

In addition to water column grab samples for anatoxin-a, the Karuk Tribe Department of Natural Resources and YTEP deployed Solid Phase Adsorption Toxin Tracking (SPATT) samplers in the Klamath River and tributaries in 2015 and 2016. SPATT samplers were deployed in the field for approximately 30 days before retrieval and extraction. SPATT samplers were deployed in the Klamath River above Copco Reservoir, in Iron Gate and Copco reservoirs, in the mainstem of the Klamath River below the reservoirs, in the estuary, and in 19 tributary streams on the Yurok Reservation (Tables 2 and 3). In tributary streams, SPATT samplers were placed above the confluence with the Klamath River, but no more than 0.25 miles upriver from the confluence. SPATT sampler construction and deployment followed methods described in Kudela (2011), and samples were analyzed by the Kudela Lab of Biological Oceanography at the University of California, Santa Cruz.

Seven of the 43 SPATT samplers accumulated anatoxin-a over the deployment period. Five SPATT samplers deployed by the Karuk Tribe Department of Natural resources in 2015 showed detectable levels of anatoxin-a, including one from above Copco Reservoir, two from Iron Gate Reservoir, and two from the Klamath River below Iron Gate Reservoir. The highest accumulated anatoxin-a concentration from these samples was from Jay Williams Boat Ramp access in Iron Gate Reservoir (18.4 ng/g, Table 2). Two SPATT samplers deployed by YTEP in 2016 showed detectable levels of anatoxin-a, including the Lower Estuary site (6.09 ng/g) and Wautec Creek (0.74 ng/g, Table 3). The frequency of positive accumulation of anatoxin-a in this study was lower than from studies conducted in the Eel River where 54% of SPATT samples showed anatoxin-a accumulation, compared to 16% in this study (Bouma-Gregson et al., 2018).

The deployment of the SPATT samplers confirmed that anatoxin-a is widely distributed

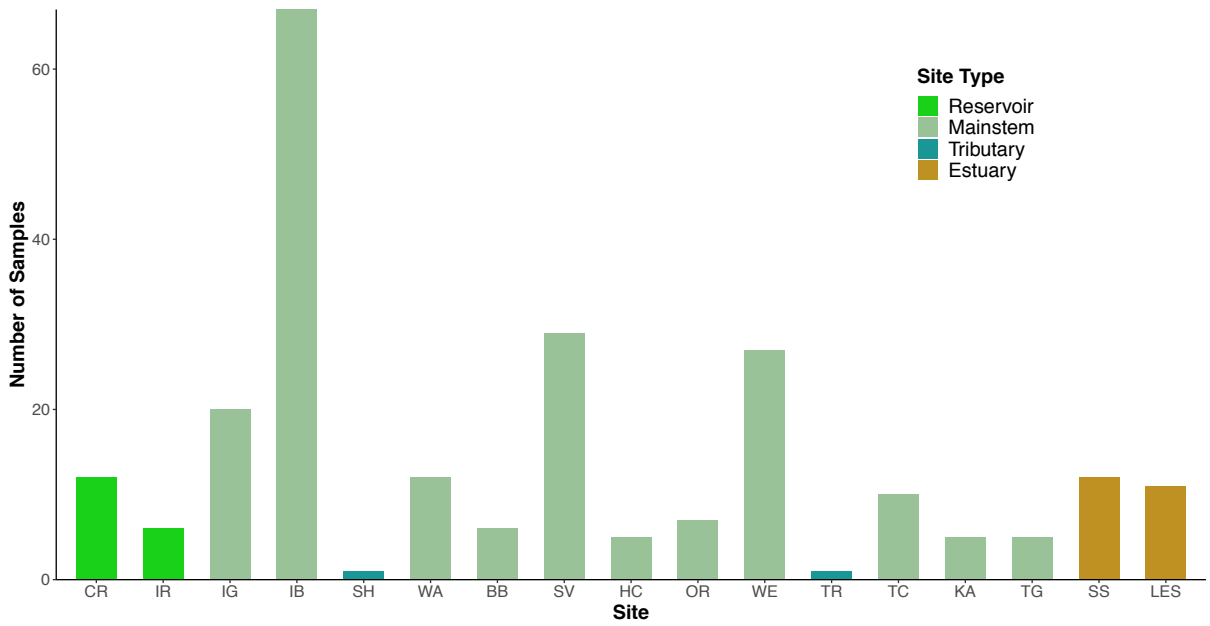


Figure 1. Number of water column anatoxin-a samples collected in the Klamath River, hydroelectric reservoirs (Iron Gate and Copco reservoirs), tributaries (Trinity and Shasta Rivers), and the Klamath River Estuary by site. Sites are ordered from upriver to downriver from left to right.

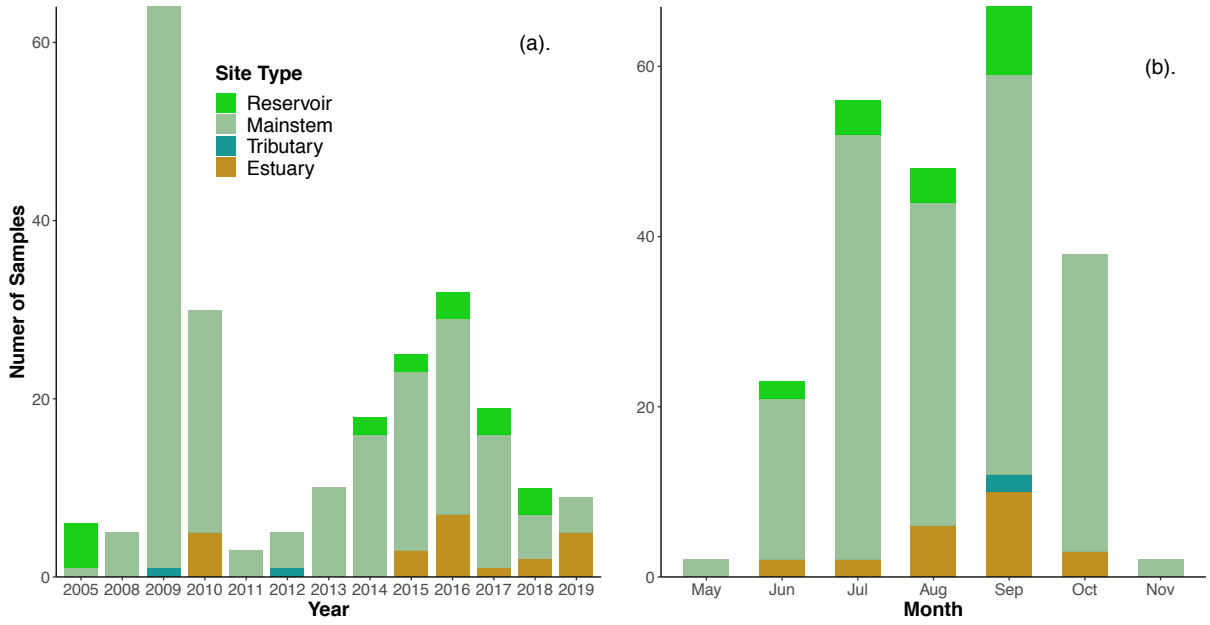


Figure 2. Number of water column anatoxin-a samples collected in the Klamath River, hydroelectric reservoirs (Iron Gate and Copco reservoirs), tributaries (Trinity and Shasta Rivers), and the Klamath River Estuary by year (panel a) and by month (panel b).

Table 1. Positive detections from anatoxin-a (μg/L) samples from the Klamath River, Klamath River Estuary, tributaries, and hydroelectric reservoirs from 2005 to 2019.

Sampling Agency	Date	Time	Site	Anatoxin-a (μg/L)	Sample Environment
CDHS	2005-09-03		IGOP-S	32.3	Iron Gate Reservoir
CDHS	2005-09-03		IGOP-N	23.7	Iron Gate Reservoir
Karuk	2010-07-21		IG	84.3	Klamath River
Karuk	2010-07-21		IG	86.3	Klamath River
Karuk	2015-07-08	13:14	IB	>525	Klamath River
Karuk	2015-07-08	13:14	IB	128	Klamath River
Karuk	2015-07-22	13:00	IB	>2.1	Klamath River
Karuk	2015-07-22	13:00	IB	14.9	Klamath River
Karuk	2015-08-05	11:46	WA	20.4	Klamath River
Karuk	2015-08-05	12:29	IB	19.1	Klamath River
Yurok	2016-09-13	12:41	LES	4.4	Estuary
Yurok	2016-09-13	13:16	TG	4.4	Klamath River
Yurok	2016-09-13	13:26	TG	4.6	Klamath River
Yurok	2016-09-13	14:00	SS	6.6	Estuary
Yurok	2016-09-14	11:31	WE	4.7	Klamath River

throughout the mid and Lower Klamath River Basin in a range of habitats. Despite the relatively low occurrence of anatoxin-a at sampled Klamath River and tributary sites, additional sampling is warranted to be able to better understand anatoxin-a dynamics in the Klamath Watershed, including the possibility of benthic algal mats in reservoir margins contributing to anatoxin-a in the Klamath River system. Prior to this study, very little sampling had occurred in tributary streams along the Klamath River (Figure 1). The detection of anatoxin-a at Wautec Creek, as well as detections of anatoxin-a from other nearby rivers (Bouma-Gregson et al., 2018; Fetscher et al., 2015; Asarian and Higgins, 2018), suggest that cyanotoxins may come from more sources than only the upstream reservoirs and lakes, where cyanotoxins (primarily very high levels of microcystin) have been documented for many years (Otten et al., 2015; Genzoli and Kann, 2017). Visual observations of benthic cyanobacterial mats in tributary streams, outlined below, further indicate the potential for anatoxin-a to occur in these tributaries, which are used for cultural practices, recreation, and as drinking water sources.

3 Field Observations of Benthic Cyanobacterial Mats

Benthic cyanobacterial mats were encountered in the Klamath River and tributaries in the summers of 2018 and 2019. These observations were primarily made while conducting surveys of benthic algae and aquatic plants, scouting sites, preparing field methods, and maintaining dissolved oxygen sensors. Observations were made from river mar-

Table 2. Anatoxin-a detected from SPATT samplers deployed in the Mid Klamath River from above Copco Reservoir to Orleans in 2015. River mile is miles from the mouth of the river moving upstream. Positive detections for anatoxin-a are indicated by bold type. Date indicates the date that the SPATT sampler was retrieved following a 30-day deployment.

Site Code	River Mile	Site Name	Date	Anatoxin-a (ng/g)	Sample Environment
KRAC	206.4	Above Copco	2015-07-01	0.0	Above Reservoirs
CRCC	200	Copco Cove	2015-07-01	0.0	Reservoir
IRJW	**192.8**	**Jay Williams**	**2015-07-01**	**0.6**	**Reservoir**
KRBI	189.7	Below Iron Gate	2015-07-01	0.0	Klamath River
KROR	59.1	Orleans	2015-06-30	0.0	Klamath River
KIS			2015-07-01	0.0	
KRAC	206.4	Above Copco	2015-07-29	0.0	Above Reservoirs
CRCC	200	Copco Cove	2015-07-29	0.0	Reservoir
IRJW	192.8	Jay Williams	2015-08-03	0.0	Reservoir
KRBI	189.7	Below Iron Gate	2015-07-29	0.0	Klamath River
KRIB	176	I5 Bridge	2015-08-03	0.0	Klamath River
KRSV	128.5	Seiad Valley	2015-07-29	0.0	Klamath River
KROR	59.1	Orleans	2015-07-30	0.0	Klamath River
KRAC	**206.4**	**Above Copco**	**2015-09-02**	**4.4**	**Above Reservoirs**
CRCC	200	Copco Cove	2015-09-02	0.0	Reservoir
IRJW	**192.8**	**Jay Williams**	**2015-09-02**	**18.4**	**Reservoir**
KRBI	**189.7**	**Below Iron Gate**	**2015-09-02**	**1.4**	**Klamath River**
KRIB	**176**	**I5 Bridge**	**2015-09-02**	**0.7**	**Klamath River**
KRSV	128.5	Seiad Valley	2015-09-03	0.0	Klamath River
KROR	59.1	Orleans	2015-09-03	0.0	Klamath River

Table 3. Anatoxin-a detected from SPATT samplers deployed in the Klamath River and tributaries along the Yurok Reservation in 2016. River mile is miles from the mouth of the river moving upstream, and river mile for tributary creeks indicate the location that the creek enters the Klamath River. Positive detections for anatoxin-a are indicated by bold type. All SPATT samplers were collected in October of 2016 after a 30-day deployment.

Site Code	River Mile	Site Name	Anatoxin-a (ng/g)	Sample Environment
WE	42.6	Weitchpec	0.00	Klamath River
ROY	6	Roy Rook	0.00	Klamath River
LES	**0.5**	**Lower Estuary**	**6.09**	**Estuary**
SS	0.2	South Slough	0.00	Estuary
GIS	41.8	Gist Creek	0.00	Tributary Creek
BEN	41.5	Ben's Creek	0.00	Tributary Creek
PIN	39.6	Pine Creek	0.00	Tributary Creek
OWL	37.8	Owl Creek	0.00	Tributary Creek
KEN	36.8	Kennick Creek	0.00	Tributary Creek
MIN	35	Miners Creek	0.00	Tributary Creek
MAW	34.5	Mawah Creek	0.00	Tributary Creek
MAR	34.3	Mareep Creek	0.00	Tributary Creek
CAP	32.2	Cappell Creek	0.00	Tributary Creek
ROA	30.6	Roach Creek	0.00	Tributary Creek
PEC	24.5	Pecwan Creek	0.00	Tributary Creek
JOH	23.7	Johnson Creek	0.00	Tributary Creek
TEC	21.5	Tectah Creek	0.00	Tributary Creek
BLU	16.1	Blue Creek	0.00	Tributary Creek
MCG	6.8	McGarvey Creek	0.00	Tributary Creek
TUR	5.5	Turwer Creek	0.00	Tributary Creek
WAU	**3.6**	**Wautec Creek**	**0.75**	**Tributary Creek**
RIC	2.75	Richardson Creek	0.00	Tributary Creek
SAL	1.1	Salt Creek	0.00	Tributary Creek

gins while wading, from a small river kayak, and while snorkeling. All observations were qualitative; we did not attempt to estimate percent cover in a quantitative manner, or identify individuals via microscopy beyond occasional microscopic confirmation of the dominant mat-forming genera we encountered.

3.1 2018 Observations

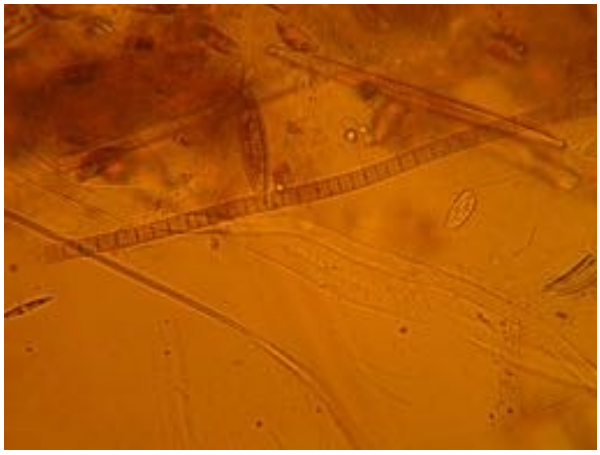
In 2018, we encountered benthic cyanobacteria from late June through the end of September in the Klamath River. On June 29^{th} we visited sites above I5 Bridge, at Ash Creek Bridge, and at Tree of Heaven. We encountered *Phormidium* at all three sites, growing in patches a few inches in diameter on rooted aquatic plants and on cobble substrate. On July 8^{th} we visited sites near Stanshaw Creek and Aikens Bar, where *Anabaena* was found growing at both sites. At Aikens bar, a large backwater above the gravel bar rapid was heavily covered in *Anabaena* (Figure 3). During a visit to some of these sites on September 30^{th}, *Phormidium* coverage had increased at the Tree of Heaven and Brown Bear Access points, to cover over 50% of the substrate in some near shore areas, while the *Anabaena* mats at Aikens Bar were no longer present (some of these sites are the same as sites surveyed more regularly in 2019, see table 4).

3.2 2019 Observations

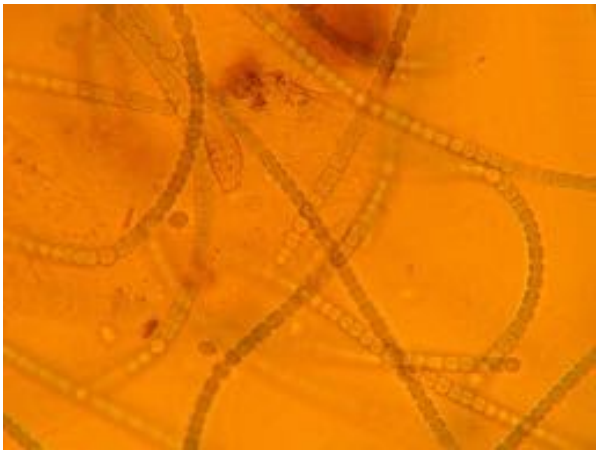
In the Summer and Fall of 2019, we documented observations of benthic cyanobacteria while conducting aquatic vegetation surveys and while maintaining dissolved oxygen sensors which were placed at seven sites on the Klamath River. These sensors were generally cleaned every two weeks from mid-June through the beginning of October, providing opportunities to search for benthic cyanobacteria regularly during these visits. Because all seven sensors were located across the river from the river access points, we were able to observe algae growing on the shore near access points, as well as on the far side of the river at these seven locations. In addition to the dissolved oxygen sensor sites, benthic algal observations were conducted 1) at locations where they were observed in 2018, 2) where river habitat was similar to locations where benthic cyanobacteria were previously observed, and 3) at popular swimming holes. Figure 4 shows locations where we encountered benthic cyanobacterial mats, and tables 4 and 5 list details about the location, habitat, and timing of cyanobacterial mats.

3.3 General Habitat Associations of Benthic Cyanobacteria in the Klamath River

Based on observations of benthic cyanobacterial mats in the Klamath River and tributaries in 2018 and 2019, we found general habitat associations for *Anabaena* and *Phormidium* that may help target future monitoring and research efforts. *Anabaena* tended to grow in stagnant or slow moving parts of the river, and was observed in shallow river margins and deeper backwaters. *Anabaena* was nearly always found growing on established filamentous green algae such as *Cladophora*. Sites where *Anabaena* was observed include the Klamath River above the mouth of Stanshaw Creek in the main channel, above the mouth of Dillon Creek in a backwater, and in the Klamath Estuary at the South Slough, among other sites (Tables 4 and 5). *Anabaena* was more commonly found at sites below Happy Camp. As a nitrogen-fixing cyanobacteria, low concentrations of nitrogen in



(a). Microscopic image of *Phormidium*; note chain of squared or rectangular cells within a long sheath



(b). Microscopic image of *Anabaena*; note chain of rounded cells and occasional larger cells, often described as looking like a string of beads

(c). Macroscopic image of *Phormidium*

(d). Macroscopic imagae of *Anabaena*

(e). *Phormidium* was found growing near the water's surface on rooted aquatic plants in slowed eddy currents under the Ash Creek Bridge in 2018

(f). *Anabaena* was found in the calm backwater pool above the gravel bar rapid on Aikens Bar in 2018; the *Anabaena* was covering a low mat of *Cladophora*

Figure 3. *Phormidium* (panels a, c, and e) and *Anabaena* (panels b, d, and f) encountered in the Klamath River in 2018, and the locations they were found.

Table 4. Location and details of benthic cyanobacterial observations from the Klamath River (between Iron Gate Dam and Dillon Creek) during the Summer and early Fall of 2019

Site Name	**Description of Benthic Cyanobacteria Observation**
Above I5 Bridge (I5-1)	*Phormidium* was found approximately 1 mile above I5-Bridge near the USFWS screw trap site from 03 August until the last site visit on 01 October. The *Phormidium* occurred as dark green, slimy patches of cyanobacteria growing on rooted aquatic plants, filamentous algae, and submerged willow branches on river margins. Patches were also free-floating in the water column. *Phormidium* was most conspicuous during the first observation in August, with fewer patches present during later observations.
Tree of Heaven (TH-2)	*Phormidium* was found at the Tree of Heaven river access, both at the boat ramp, and in the area upstream. We first observed *Phormidium* on 03 August, and continued to observe increasing *Phormidium* coverage through our last visit on 02 October. The *Phormidium* occurred as dark green, slimy patches of cyanobacteria growing on rooted aquatic plants, filamentous algae, submerged willow branches, decaying wood, and sand. By September, coverage was high at the margins of the boat ramp pool and in river margins upstream from the ramp.
Fisher's RV (FISH-3)	*Phormidium* was growing in patches along river margins on both sides of the river in and around boulders and bedrock upstream of the boat launch at Fisher's RV Park. We first observed *Phormidium* here in August and continued to encounter it through our final sampling day on 02 October.
Brown Bear Access (BB-4)	Multiple genera of cyanobacteria were observed at the Brown Bear river access in shallow, stagnant water downstream of the gravel bar boat launch beginning on 21 July. Decaying *Cladophora* was covered with bright green, gelatinous cyanobacteria, with some dried material at the water surface displaying teal colored phycocyanin pigment. Similar conditions were observed on 04 August when a sample was taken for toxin analysis, but no conspicuous mats were observed at follow-up visits on 30 August and 02 October. Although we identified the benthic cyanobacteria as *Anabaena*, Bend Genetics identified the sample collected for toxin analysis on 04 August as *Dolichospermum* (Table 6).
Curely Jack Campground (CJ-5)	Small amounts of unidentified cyanobacteria were growing at the water's edge on the boulder-bedrock lined shore across from the campground boat launch. Cyanobacteria was growing among green filamentous algae, and was identified by small spots of phycocyanin pigment on dried patches of algae on the river margin on 04 August. Benthic cyanobacteria was not observed on other site visits.
Above Dillon Creek (ADC-6)	*Anabaena* was found growing on *Cladophora* filaments in the backwater above the riffle at the Dillon Creek Confluence during one visit on 27 July. *Anabaena* filled the stagnant backwater, creating towers over 4 feet high. Smaller patches of *Anabaena* were present moving into the main channel as habitat become more shallow and swift moving toward the riffle, until the current became too swift for *Anabaena* to be maintained in the river.

Table 5. Location and details of benthic cyanobacterial observations from the Klamath River (between Dillon Creek and the Klamath River Estuary) during the Summer and early Fall of 2019

Site Name	**Description of Benthic Cyanobacteria Observation**
Dillon Creek (DILL-7)	A small patch of *Anabaena* was found growing within filamentous green algae on the far side of the large river-bar pool on 27 July. On 2 September, larger patches of *Anabaena* were found growing within filamentous green algae near the popular swimming beach on the Dillon Creek Pool. *Phormidium* was also found in these mats.
Above Stanshaw Creek (STAN-8)	*Anabaena* was found growing on *Cladophora* filaments in slow flowing water up to 6 feet deep in the main river channel near the confluence of Stanshaw Creek. *Anabaena* was also growing along river margins downstream of the creek mouth, and additional material was detaching and being transported downstream in the main river current. *Anabaena* was first observed on 22 July, and the extent of the *Anabaena* had noticeably extended 4 days later. By 4 August, much of the *Anabaena* was gone, apparently detached as it became too heavy for the decaying *Cladophora* to hold. Attached *Anabaena* was no longer noticeable from shore or kayak on a visit on 30 August, but *Anabaena* mat material was visible in the water column, likely floating into this section of river from an undocumented upstream source.
Salmon River (SALM-9)	On 4 August, we observed *Phormidium* growing in a slow pool in water 1-3 feet deep along the river right bank at the Hippo Rock river access. *Phormidium* was growing on older filamentous green algae. This *Phormidium* was a pink to brown color and growing as a thin film over decaying algae, in contrast to the dark green, thick mats of *Phormidium* observed in the Klamath River. We also observed a thin film of *Phormidium* on bedrock, boulders, cobble and sticks while surveying between Oak Bottom and Hippo Rock on 10 July, but at minimal coverage.
Big Bar River Access (BIG-10)	*Anabaena* was growing in the stagnant backwater above the Big Bar riffle in water that was 1 - 3 feet deep on 5 August. This was the only visit we made to Big Bar to look for benthic cyanobacteria.
Aiken's Bar (AIK-11)	*Cylindrospermum* was found as a thin layer covering the sand substrate in the backwater above the Aikens Bar riffle on 5 August 2019. No Benthic cyanobacterial mats were found during surveys on 9 July when this backwater was examined. This was the site of *Anabaena* coverage in 2018, which was growing on old *Cladophora* filaments in the backwater in 2018, suggested that *Anabaena* may be predicted in part by the presence of *Cladophora* for substrate.
Weitchpec (WE-12)	*Anabaena* was observed during multiple visits from 8 August to 11 September in stagnant or near stagnant water in a small edgewater cut off from the well mixed river just above the confluence of the Klamath and Trinity Rivers. Dead toad metamorphs were spotted at that site on 27 August and 3 September in water less than a foot deep.
South Slough (SS-13)	*Anabaena* was found in the South Slough of the Klamath Estuary near the kayak boat ramp off Klamath Beach Road during visits on 31 July and 05 August. *Anabaena* was growing among and on filamentous green algae and aquatic plants along the margin of the South Slough where daily tides are present. *Anabaena* was easier to spot from the shore during moderate to low tides.

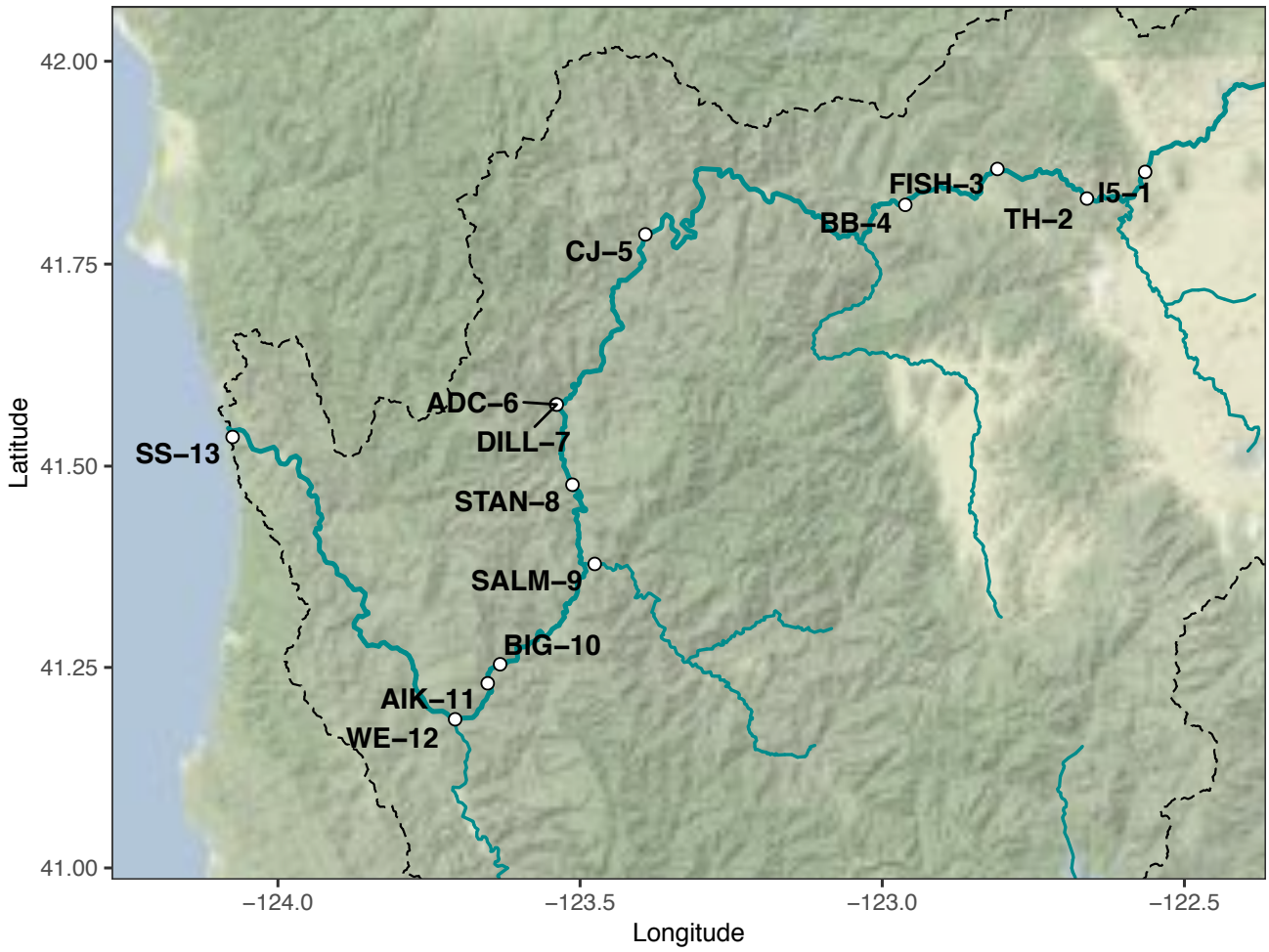
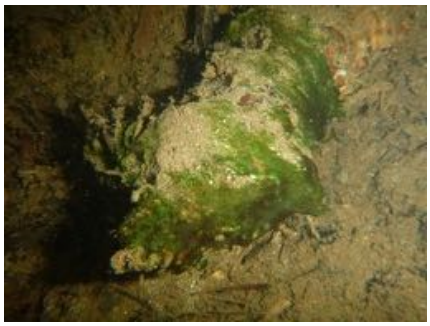


Figure 4. The Mid and Lower Klamath Watershed, showing locations where benthic cyanobacterial mats were found during the Summer and Fall of 2019. Site codes correspond to descriptions in tables 4 and 5.

the lower reaches of the Klamath River may favor *Anabaena* and other nitrogen fixing algaes (Gillett et al., 2016).

Phormidium was observed upriver, at sites near the I5 Rest Area and Tree of Heaven Campground, as well as in the Salmon River, among other sites. Although *Anabaena* appears to need calm, near stagnant water to proliferate, *Phormidium* was found in both still and swift moving areas of the river. It was found growing on cobbles, boulders, and bedrock, rooted aquatic plants, branches, and fine substrate. Growth forms were highly variable in color and texture, possibly indicating diverse species in this genus, which might be partially responsible for variable toxin levels associated with *Phormidium* in the Klamath River. Both *Phormidium* and *Anabaena* are prone to being dislodged and carried as suspended material in the water column. We observed these suspended clumps of cyanobacteria at sites both where we observed benthic species growing, and where we



(a). *Phormidium* at Tree of Heaven (TH-2)

(b). *Phormidium* from Tree of Heaven (TH-2)

(c). *Phormidium* at Hippo Rock on the Salmon River (SALM-9)

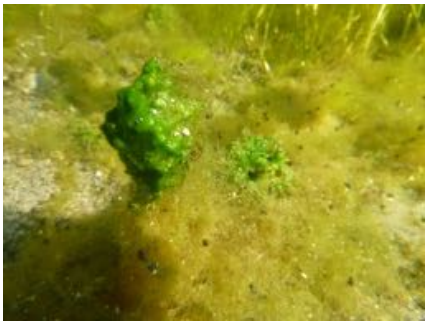
(d). *Anabaena* at Brown Bear River Access (BB-4)

(e). *Anabaena* at Brown Bear River Access (BB-4)

(f). *Anabaena* at Brown Bear River Access (BB-4)

(g). *Anabaena* above Stanshaw Creek confluence (STAN-8)

(h). *Cylindrospermum* at Aikens Bar (AIKE-11)



(i). *Anabaena* from the South Slough (SS-13)

Figure 5. Benthic cyanobacteria observed in the Klamath River (panels a, b, d, e, f, g, h, i) and Salmon River (panel c) during 2019 observations. Site codes correspond to locations in figure 4.

were not able to observe benthic algae, which may be due to our inability to adequately survey the river bottom or may be indicative of mats transported from upstream locations. Although the distribution of benthic cyanobacteria is very patchy in both space and time, observations on distribution combined with the recent toxin confirmation suggest that benthic cyanobacterial mats are a widespread (albeit patchy) source of anatoxin-a in the Klamath River.

4 Toxicity of Benthic Mats

From August 3^{rd} to 5^{th} 2019, we collected six samples of benthic cyanobacteria from sites along the Klamath River, spanning from above the I5 Bridge to the Klamath River Estuary (Table 6). An additional sample was collected from the Salmon River on August 13^{th} 2019. Samples of benthic cyanobacteria were collected directly from mats visually identified in the field as cyanobacteria. We confirmed mat material as containing high levels of cyanobacteria through microscopy, and sent samples to Bend Genetics for confirmation of genus identification, toxin analysis, and QPCR of the anatoxin-a production gene.

All seven samples were confirmed by Bend Genetics to contain high amounts of cyanobacteria. Anatoxin-a and anatoxin-a genes were present in all samples, but the concentration of toxins and gene copies were variable. The highest anatoxin-a concentration and anatoxin-a genes were present in *Phormidium* collected from above the I5 Bridge (359.5 μg/L), followed by *Phormidium* collected at Tree of Heaven (7.77 μg/L). Samples dominated by *Anabaena*, *Dolichospermum*, and *Cylindrospermum* at the remaining sites had low anatoxin-a concentrations ($<$1 μg/L), with the exception of the sample from the Salmon River, which had 2.08 μg/L of anatoxin-a. This sample from the Salmon River had *Phormidium* as a secondarily dominant cyanobacteria in the sample.

These samples were collected specifically to test benthic cyanobacterial mats as sources of anatoxin-a, and thus do not directly relate to the public health warning level established for ambient water in the state of California (20 μg/L). Although it is less likely for a person to ingest large amounts of algal mat material than ambient water, the high level of anatoxin-a observed in *Phormidium* mats above the I-5 Bridge, which was 18-times higher than the public health warning threshold for ambient water, warrants additional sampling and outreach to identify and communicate the extent of public health risk associated with benthic algal mats in the Klamath River.

Table 6. Anatoxin-a determined by enzyme linked immunosorbent assays (ELISA, μg/L), quantitative polymerase chain reaction (QPCR, gene copies/mL), and dominant cyanobacteria genera as determined by microscopy for cyanobacterial mat material. River mile is miles upstream from the mouth of the Klamath River and Salmon River, respectively.

Site Description	River Mile	Date	Anatoxin-a (μg/L)	QPCR (gene copies/mL)	Dominant Genera
Above I5 Bridge	179	03-Aug-2019	359.5	39,353,004	*Phormidium*
Tree of Heaven	172	03-Aug-2019	7.77	734,024	*Phormidium*
Brown Bear Access	150	04-Aug-2019	0.70	32,604	*Dolichospermum*
Big Bar Access	50	05-Aug-2019	0.77	27,848	*Anabaena*
Aikens Bar Access	48	05-Aug-2019	0.31	62,534	*Cylindrospermum*
South Slough	0.5	05-Aug-2019	0.15	37,914	*Anabaena*
Salmon River	1	13-Aug-2019	2.08	1,415,557	*Anabaena*

5 Recommendations for Monitoring and Special Studies

5.1 Public Health Monitoring

Thirteen years of anatoxin-a sampling in the Klamath Basin has shown that anatoxin-a is widespread in the Klamath River Watershed, but the current inconsistent and low-frequency sampling regime does not adequately capture the public health risk associated with anatoxin-a. In recent years, resources were allocated for only eight regular public health monitoring samples for anatoxin-a on the Mid and Lower Klamath River. This is insufficient to characterize the public health threat or to cover the toxin variation spatially and temporally. Additionally, little sampling has occurred in the hydroelectric reservoirs and mainstem tributaries, despite finding anatoxin-a at both of these locations. Meanwhile, current water column sampling protocols may lead to underestimating anatoxin-a exposure risk since benthic algal mats are also a source of anatoxin-a. Finally, anatoxin-a samples generally occurred at public health monitoring locations that were established to monitor risk of microcystin toxin, which has different dynamics than those of anatoxin-a in the Klamath River.

We recommend that public health monitoring plans for anatoxin-a in the Klamath River be revised for the summer 2020 water quality sampling season, and that resources be allocated to conduct more comprehensive and coordinated monitoring. Such monitoring should be designed to answer questions around the distribution, timing and sources of anatoxin-a in the Klamath River that will lead to improved public health messaging and management decisions that reduce the public health risk associated with cyanotoxins. Some of the major questions that remain surrounding anatoxin-a risk in the Klamath include:

1. When is anatoxin-a the greatest risk in the Klamath River?

2. What are the sources of anatoxin-a in the Klamath River, and how does the source in-

fluence how anatoxin-a is distributed and encountered in the Klamath River?

3. Are there locations in the Klamath River where anatoxin-a exposure is of greater risk, and if so, where are these locations and what is the scale that these exposure risks vary on?

4. To what extent is anatoxin-a a risk in tributary streams in the Klamath Watershed?

5. Can we improve the field and laboratory methods currently being used to more accurately assess anatoxin-a concentrations in the Klamath River?

With unanswered questions surrounding anatoxin-a in the Klamath River, resources should be put toward studies that fulfill goals of both public health monitoring and coordinated research that will bring insight into the anatoxin-a dynamics in the Klamath River. Below is a list of suggested studies that will help address questions related to anatoxin-a dynamics in the Klamath River, with the ultimate goal of improving public health for basin residents and visitors.

5.1.1 Timing of Anatoxin-a

To identify when anatoxin-a is present in the Klamath River, higher frequency toxin samples should be taken during the period that cyanobacteria are present in moderate densities. Due to inherent spatial variation, it will be impossible to characterize temporal variation at all sites, so representative sites for various habitats should be selected for temporal studies. At a minimum, one site should be selected from Iron Gate Reservoir and one site in the Klamath River below the reservoir, where the many of the positive detections for anatoxin-a have occurred (Table 1). Sample timing should be coordinated so that reservoir and river samples can be compared. SPATT samplers may better characterize temporal trends, but would need to be deployed for shorter periods of time than in past studies so that distinct temporal periods can be identified and to reduce the risk of anatoxin-a breaking down during SPATT deployment. Weekly SPATTs or grab samples paired with grab samples for algal species identification, QPCR, and benthic cyanobacteria searches conducted from June through October would bring new insight to the timing of anatoxin-a in the Klamath River Watershed.

5.1.2 Anatoxin-a Sources

Based on the limited available evidence, there appear to be two likely sources of anatoxin-a in the Mid and Lower Klamath River. Planktonic cyanobacterial blooms in the reservoir can produce anatoxin-a, which can be transported downstream. Second, benthic cyanobacteria both in the reservoir margins or in the mainstem river and tributaries can produce anatoxin-a. Genetic studies can help link toxin levels to probable genera (Otten, 2017), as can coordinating the timing of samples from the reservoirs and the mainstem Klamath River below the reservoirs. Thus, coordinating the temporal samples suggested above, and incorporating QPCR methods, will likely help show when anatoxin-a originates from reservoir planktonic blooms, vs. in the river.

Anatoxin-a from upstream reservoirs would be expected to result in well-mixed anatoxin-a levels in the river downstream of these reservoirs, whereas anatoxin-a from within-river benthic mat sources is not well understood and is likely to be highly patchy. To address this, special studies should be conducted that test (for antoxin-a) ben-

thic cyanobacterial mat material, water adjacent to these mats, as well as water from the well-mixed river at the access point that the mat was identified and sampled. This monitoring will help identify the extent to which algal mat material vs. water that has contacted the mat poses a public health risk. Cyanobacterial mat samples should be sampled for toxin concentration per dry weight of mat material. A minimum of 10 sampling events encompassing the three sample types should be conducted, with an emphasis on *Phormidium*-dominated mats.

5.1.3 Locations of Elevated Anatoxin-a

Understanding the risk to public health will be improved by knowing where anatoxin-a poses the greatest risk in the Klamath Watershed, as well as the spatial scale that anatoxin-a varies on. Because anatoxin-a can be associated with benthic cyanobacterial mats, identifying the locations of large or prolific mats, as well as locations that these mats are transported to, will help identify locations of greater risk. This will require natural resource personnel from a variety of agencies who work on the Klamath River to record and compile observations in a standardized format. Training in field identification and basic microscopic identification should be conducted so that natural resource personnel can field identify benthic cyanobacteria (S2). This training will facilitate natural resources staff to survey benthic cyanobacteria at established sampling sites while in the field for other water quality or fisheries related work. Field staff should note the presence or absence of conspicuous benthic cyanobacterial mats, basic habitat characteristics, and any locations where benthic mat material has accumulated (S3).

Because large particles of mat material may become dislodged and carried in the water column, these floating pieces of cyanobacterial mats may pose a pubic health risk to those swimming in the water, even when no benthic algal mats are present. To test the potential for mat material suspended in the water column as a source of anatoxin-a, water column algal material concentrated using a phytoplankton net should be analyzed for anatoxin-a per dry weight of organic material. A paired anatoxin-a sample of unfiltered water would show the range of toxin exposure risk between accidental ingestion of water from the well mixed water column and accidental ingestion of larger suspended particles.

5.1.4 Anatoxin-a Risk in Tributary Streams

Many tributaries of the Klamath River are considered to have good water quality, and are used for swimming, ceremonies, and drinking water. Due to the identification of cyanobacteria species and confirmed anatoxin-a in some of these tributaries, more surveys and sampling should be conducted in tributary streams to identify the possible risk in these streams. Identification of benthic algal mats should be the first step, followed by testing mat material for anatoxin-a. In streams used for drinking water, deployment of SPATT samplers would be an appropriate method for identifying anatoxin-a risk, as it is possible to miss areas of benthic cyanobacterial mats that may be located upstream of survey sites, or are challenging to identify.

6 Public Outreach Considerations

Warning the public of the risk of anatoxin-a in the Klamath River and tributaries should be tailored to the context of the Klamath River. Specifically, messaging should consider that local residents and

visitors are already accustomed to cyanobacterial warnings directed at the well-documented *Microcystis aeruginosa* bloom and associated microcystin toxin originating in the hydroelectric reservoirs. The risk associated with anatoxin-a, which is likely in part associated with benthic cyanobacteria, can differ. First, anatoxin-a is a potent neurotoxin which is more often associated with dog deaths, whereas microcystin, a persistent liver toxin, is more often attributed to cumulative damage, with the exception of very high doses (Buratti et al., 2017; Dreher et al., 2019). Thus, effects of exposure to these toxins can differ, in terms of both acute and long-term effects.

In addition to different types of toxins posing a public health risk, where and how people, pets, livestock, and wildlife are exposed to these toxins can also differ. *Microcystis aeruginosa* and associated microcystin toxins are relatively evenly distributed in the water column below the reservoirs, thus exposure risk tends to be similar throughout the Klamath River when toxin is present (Genzoli and Kann, 2017). Exposure risk to anatoxin-a likely differs when benthic cyanobacteria are the primary source of anatoxin-a because distribution of the toxin in the water column is not evenly distributed. This is because the benthic cyanobacteria which produce anatoxin-a grow in patches on the river bottom leading to uneven distributions throughout the river. Although the toxin can enter the water column (extracellular toxins), toxins are also associated with these unevenly distributed algal cells (as intracellular toxins). Further, the cyanobacteria patches sporadically dislodge, and are transported into the water column and down stream, remaining in clumps, and slowly breaking down into smaller pieces.

Although the primary exposure risk to cyanotoxins in the Mid and Lower Klamath River has been due to *Microcystis* blooms associated with the hydroelectric reservoirs, observations of anatoxin-a and benthic cyanobacteria producers necessitate that additional observations and targeted toxin samples continue for anatoxin-a. In consideration of differences between the planktonic *Microcystis* bloom and other toxin-producing cyanobacteria, the following are recommendations to communicate the potential health and exposure risk for anatoxin-a to the public.

1. Benthic cyanobacteria and anatoxin-a are patchy throughout the river, and regular testing for planktonic *Microcystis* and microcystin toxin does not capture this additional risk.

2. The timing and distribution of anatoxin-a producing species is poorly known, but they are present in the Klamath River and tributaries, with higher risk in late summer and fall.

3. The highest concentration of anatoxin-a may be in cyanobacterial [algal] mats, thus a high risk of anatoxin-a poisoning may be from accidentally ingesting algal mat material.

4. Do not let children enter the water where algal mats are present, and do not swim if there is visible algal mat material in the water column, even in clean, clear appearing water.

5. Dogs are particularly at risk because they drink directly from the rivers, and may even be attracted to the smell and taste of some toxin-producing algae. Do not let dogs drink from any water with viable algal mats.If algal mat material gets on their fur, immediately wash it off before they have a chance to groom themselves.

7 Conclusions

Anatoxin-a has been widely documented in the Klamath River and in some tributaries, although the timing and spatial extent are poorly documented. Anatoxin-a is produced by cyanobacteria, with benthic species in the free-flowing river being one source of anatoxin-a. Detection of anatoxin-a in hydroelectric reservoirs suggest that these habitats also promote anatoxin-a producing species, but for both reservoir and river sources, it is unknown how far down river or for how long the toxin may persist and be transported. Because sampling has been limited and inconsistent between years, it is not known if anatoxin-a presents a similar risk in all years, or if some environmental conditions promote more growth and toxin risk under certain conditions. To improve public health warnings and ultimately inform river management that could lead to reduced public health risk associated with anatoxin-a, consistent public health monitoring and studies identifying toxin dynamics should be implemented. With the removal of the four hydroelectric dams, currently scheduled for demolition in 2022, studies should begin in summer 2020 to establish baseline conditions to compare with post dam removal. These studies will help show how dam removal changes conditions for anatoxin-a production, and will ultimately help identify next steps for managing the toxin.

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Supplementary Materials

S1: Klamath Benthic Cyanobacteria Identification Guide

S2: Klamath Benthic Cyanobacteria Documentation Data Sheet

S3: Raw Data From Klamath River Watershed Water Column Grab Samples, 2005 - 2019

S4: Lab Reports For 2019 Klamath River Benthic Cyanobacteria Mat Samples

Klamath River

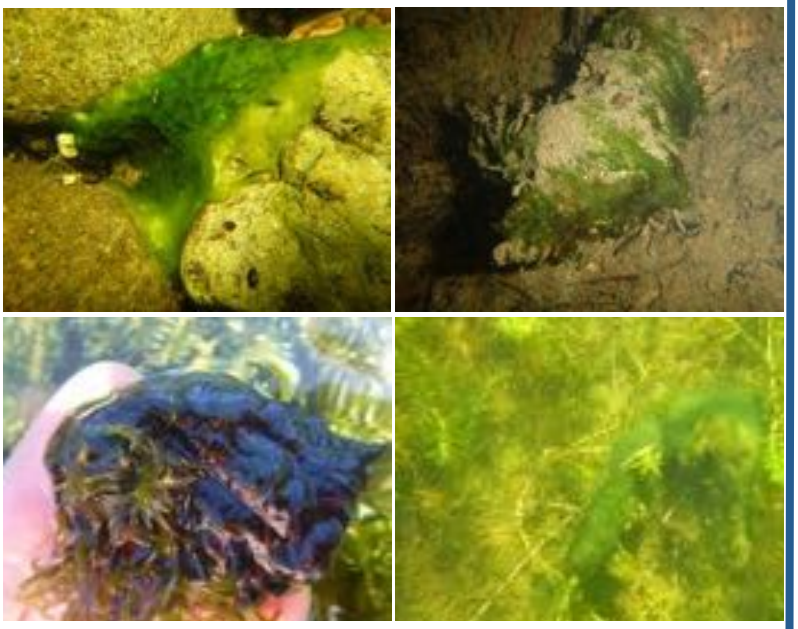
Benthic Cyanobacteria Identification Guide

Attached cyanobacteria such as *Phormidium* and *Anabaena* can produce neurotoxins. These genera were recently documented in the Klamath River. Understanding the extent, distribution, and seasonality of these potentially-toxic genera will guide targeted monitoring and outreach to protect public health. This guide is intended to assist natural resource field staff in identifying and documenting *Phormidium* and *Anabaena* in the Klamath River.

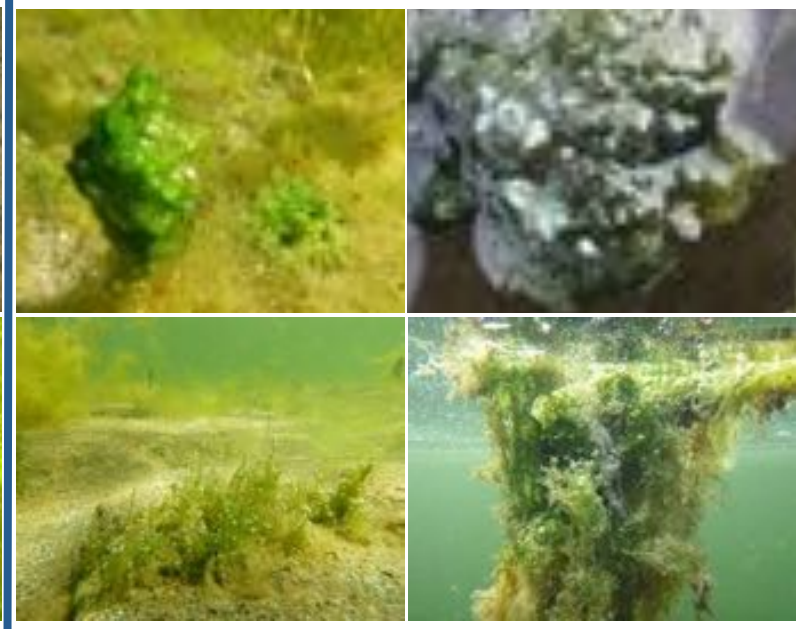
Phormidium

Anabaena

Macroscopic appearance in the Klamath River



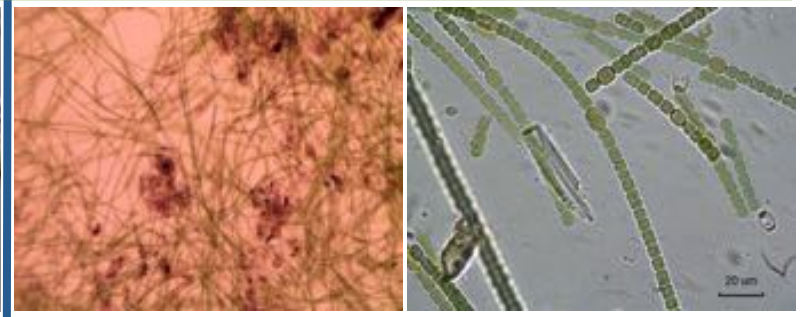
Phormidium grows in fast current or stagnant water. It grows on rocks, submerged branches, filamentous algae, and aquatic plants. In the Klamath, *Phormidium* tends to be a deep green color and has a slimy, robust texture. *Phormidium* floats on the water surface when dislodged.



Anabaena is delicate and tends to grow on filamentous algae in stagnant or slow moving water. *Anabaena* forms bubbles that cause mats to float toward the surface, sometimes detaching and floating downstream. When decaying, *Anabaena* and *Phormidium* can form blue specks.

Microscopic appearance in the Klamath River

Phormidium forms smooth, un-branched chains of cylindrical cells that are similar along each filament (no heterocystes). The terminal or end cells are often tapered to a rounded tip.



Anabaena looks like strings of beads with occasional larger cells along the string. These heterocystes are used for nitrogen fixation in low nitrogen conditions.

Benthic Cyanobacteria Documentation

Site Name:	Observer:	
Date: _____ /_____ /_____	Time: _____ : _____	
Site Description:		
Primary purpose of field visit:		
Description of cyanobacteria (growth form, substrate, attached, floating, percent coverage, etc.):		
Depth:	Velocity:	Water Temp:
Toxin Sample: Y / N	Photo ID (file name)*:	Microscope Confirmation: Y / N
Notes:		

***If filling this document out electronically, paste file directly into document.**

Follow-up visits

Site Name:	Observer:	
Date: _____ /_____ /_____	Time: _____ : _____	
Primary purpose of field visit:		
Description of cyanobacteria (growth form, substrate, attached, floating, percent coverage, etc.):		
Depth:	Velocity:	Water Temp:
Toxin Sample: Y / N	Photo ID (file name)*:	Microscope Confirmation: Y / N
Notes:		

***If filling this document out electronically, paste file directly into document.**

S3: Raw Data From Klamath River Watershed Water Column Grab Samples, 2005 - 2019

Raw data for water column samples analyzed for anatoxin-a in the Klamath River Watershed. Data is reported as obtained from each sampling agency. Anatoxin-a concentrations are reported as ND (no-detect), $<$ (less than a laboratory detection limit as indicated in the table), $>$ (more than the indicated number), or a numerical value, in μg/L. ND and 0 indicate no-detect, but with unspecified detection limits. Sample type was occasionally indicated, with SG indicating surface grabs, the typical sampling type for public health surveys, and OC indicating open-channel grabs, typical of baseline sampling protocols. Sample location indicates the general habitat category of the sample site.

Sampling Agency	Date	Time	Site	Anatoxin-a (µg/L)	Sample Type	Sample Location
Yurok	2010-08-25		LES	<0.10	OC	Estuary
Yurok	2010-09-08		LES	<0.10	OC	Estuary
Yurok	2010-09-22		LES	<0.10	OC	Estuary
Yurok	2010-10-06		LES	<0.10	OC	Estuary
Yurok	2010-10-20		LES	<2.0	OC	Estuary
Yurok	2015-06-23		SS	<10		Estuary
Yurok	2015-07-21		SS	<10		Estuary
Yurok	2015-09-22		SS	<10		Estuary
Yurok	2016-08-23	13:20	SS	ND		Estuary
Yurok	2016-08-23	11:03	LES	<0.05		Estuary
Yurok	2016-08-30	13:30	SS	ND		Estuary
Yurok	2016-09-13	12:41	LES	4.4		Estuary
Yurok	2016-09-13	14:00	SS	6.6		Estuary
Yurok	2016-09-27	12:23	LES	BRL		Estuary
Yurok	2016-09-27	13:19	SS	ND		Estuary
Yurok	2017-06-20	12:14	LES	ND		Estuary
Yurok	2018-08-21	12:21	LES	ND	OC	Estuary
Yurok	2018-09-25	11:36	LES	ND	OC	Estuary
Yurok	2019-07-09	11:58	SS	ND	SG	Estuary
Yurok	2019-08-06	10:53	SS	ND	SG	Estuary
Yurok	2019-09-10	11:47	SS	ND		Estuary
Yurok	2019-09-10	12:05	SS	ND	SG	Estuary
Yurok	2019-10-08	12:30	SS	ND	SG	Estuary
CDHS	2005-09-04		IG	0	SG	Mainstem
Yurok	2008-07-09		WE	<1	OC	Mainstem
Yurok	2008-07-24		WE	<1	OC	Mainstem
Yurok	2008-08-07		WE	<1	OC	Mainstem
Yurok	2008-08-20		WE	<1	OC	Mainstem
Yurok	2008-09-03		WE	<1	OC	Mainstem
Yurok	2009-05-28	10:22	TC	<5.0	OC	Mainstem
Karuk	2009-06-11	10:05	SV	ND		Mainstem
Yurok	2009-06-25	10:38	TC	<5.0	OC	Mainstem
Karuk	2009-06-25	11:20	SV	ND		Mainstem
Karuk	2009-07-09		IG	ND		Mainstem
Karuk	2009-07-09		SV	ND		Mainstem
Karuk	2009-07-09		SV	ND		Mainstem
Yurok	2009-07-10		WE	<5.0	OC	Mainstem
Yurok	2009-07-20		KA	ND	OC	Mainstem
Karuk	2009-07-22		BB	ND		Mainstem
Karuk	2009-07-22		HC	ND		Mainstem
Karuk	2009-07-22		IB	ND		Mainstem
Karuk	2009-07-22		OR	ND		Mainstem
Karuk	2009-07-22		SV	ND		Mainstem
Karuk	2009-07-23		SV	ND		Mainstem
Yurok	2009-07-23		TC	<5.0	OC	Mainstem
Karuk	2009-08-06		IG	ND		Mainstem
Karuk	2009-08-06		SV	ND		Mainstem
Yurok	2009-08-06	11:35	WE	<5.0	OC	Mainstem
Yurok	2009-08-18		KA	ND	OC	Mainstem
Karuk	2009-08-19		BB	ND		Mainstem
Karuk	2009-08-19		HC	ND		Mainstem
Karuk	2009-08-19		IB	ND		Mainstem
Karuk	2009-08-19		OR	ND		Mainstem
Karuk	2009-08-19		SV	ND		Mainstem
Karuk	2009-08-20		SV	ND		Mainstem
Karuk	2009-08-20		SV	ND		Mainstem
Karuk	2009-08-20		SV	ND		Mainstem
Yurok	2009-08-20	10:52	TC	<5.0	OC	Mainstem
Karuk	2009-09-03		IB	ND		Mainstem

Karuk	2009-09-03		IG	ND		Mainstem
Karuk	2009-09-03		IG	ND		Mainstem
Karuk	2009-09-03		SV	ND		Mainstem
Yurok	2009-09-03	10:34	TC	<5.0	OC	Mainstem
Yurok	2009-09-15		KA	ND	OC	Mainstem
Karuk	2009-09-16		BB	ND		Mainstem
Karuk	2009-09-16		BB	ND		Mainstem
Karuk	2009-09-16		HC	ND		Mainstem
Karuk	2009-09-16		IB	ND		Mainstem
Karuk	2009-09-16		OR	ND		Mainstem
Karuk	2009-09-16		SV	ND		Mainstem
Karuk	2009-09-17		SV	ND		Mainstem
Karuk	2009-09-17		SV	ND		Mainstem
Yurok	2009-09-17	10:58	TC	<5.0	OC	Mainstem
Karuk	2009-10-01		SV	ND		Mainstem
Karuk	2009-10-01		SV	ND		Mainstem
Yurok	2009-10-01	10:58	WE	<5.0	OC	Mainstem
Yurok	2009-10-01		IG	ND	OC	Mainstem
Yurok	2009-10-12		KA	ND	OC	Mainstem
Yurok	2009-10-12		KA	ND	OC	Mainstem
Yurok	2009-10-15	10:51	TC	<5.0	OC	Mainstem
Karuk	2009-10-15	10:30	SV	ND		Mainstem
Karuk	2009-10-26		BB	ND		Mainstem
Karuk	2009-10-26		HC	ND		Mainstem
Karuk	2009-10-26		IB	ND		Mainstem
Karuk	2009-10-26		OR	ND		Mainstem
Karuk	2009-10-26		SV	ND		Mainstem
Karuk	2009-10-26		SV	ND		Mainstem
Yurok	2009-10-29	10:16	TC	<5.0	OC	Mainstem
Yurok	2009-10-29		WE	<5.0	OC	Mainstem
Yurok	2009-10-29		TC	ND	OC	Mainstem
Yurok	2009-11-12	11:16	TC	<5.0	OC	Mainstem
Yurok	2009-11-12		WE	<5.0	OC	Mainstem
Yurok	2010-05-12		WE	<5.0	OC	Mainstem
Karuk	2010-06-09		SV	ND		Mainstem
Yurok	2010-06-09		WE	<5.0	OC	Mainstem
Karuk	2010-06-23		IB	ND		Mainstem
Yurok	2010-07-07		WE	<5.0	OC	Mainstem
Karuk	2010-07-08		SV	ND		Mainstem
Karuk	2010-07-21		IG	84.3		Mainstem
Karuk	2010-07-21		IG	86.3		Mainstem
Karuk	2010-07-21		IG	ND		Mainstem
Karuk	2010-07-21		SV	ND		Mainstem
Karuk	2010-08-11	14:37	IB	ND		Mainstem
Karuk	2010-08-11	10:42	SV	ND		Mainstem
Yurok	2010-08-11		WE	<5.0	OC	Mainstem
Karuk	2010-08-25		IB	ND		Mainstem
Karuk	2010-08-25		IB	ND		Mainstem
Karuk	2010-09-08		SV	ND		Mainstem
Karuk	2010-09-08		SV	ND		Mainstem
Yurok	2010-09-08		WE	<0.10	OC	Mainstem
Karuk	2010-09-22		BB	ND		Mainstem
Karuk	2010-09-22		SV	ND		Mainstem
Karuk	2010-10-06		HC	ND		Mainstem
Karuk	2010-10-06		SV	ND		Mainstem
Yurok	2010-10-06		WE	<0.10	OC	Mainstem
Karuk	2010-10-20		IG	ND		Mainstem
Karuk	2010-10-20		SV	ND		Mainstem
Karuk	2011-07-06	13:56	IB	ND		Mainstem
Karuk	2011-07-20	13:55	IB	ND		Mainstem
Karuk	2011-07-21	13:27	IG	ND		Mainstem

Karuk	2012-08-22	15:59	IB	ND	Mainstem
Karuk	2012-09-05	13:23	IB	ND	Mainstem
Karuk	2012-09-19	13:54	IB	ND	Mainstem
Karuk	2012-09-26	11:09	IB	ND	Mainstem
Karuk	2013-07-24	13:53	IB	ND	Mainstem
Karuk	2013-07-24	13:53	IB	ND	Mainstem
Karuk	2013-07-31	13:52	IB	ND	Mainstem
Karuk	2013-07-31	13:52	IB	ND	Mainstem
Karuk	2013-08-21	11:54	IB	ND	Mainstem
Karuk	2013-09-04	11:36	IB	ND	Mainstem
Karuk	2013-09-11	07:53	OR	ND	Mainstem
Karuk	2013-09-25	12:49	IB	ND	Mainstem
Karuk	2013-09-25	08:02	OR	ND	Mainstem
Karuk	2013-10-09	07:52	OR	ND	Mainstem
Karuk	2014-06-11	12:34	IB	ND	Mainstem
Karuk	2014-06-25	12:32	IB	ND	Mainstem
Karuk	2014-06-25	12:56	IG	ND	Mainstem
Karuk	2014-07-09	13:45	IB	ND	Mainstem
Karuk	2014-07-09	12:24	WA	ND	Mainstem
Karuk	2014-07-23	12:22	IB	ND	Mainstem
Karuk	2014-07-23	13:22	IG	ND	Mainstem
Karuk	2014-07-23	12:30	IB	ND	Mainstem
Karuk	2014-07-23	12:25	IB	ND	Mainstem
Karuk	2014-08-06	12:15	WA	ND	Mainstem
Karuk	2014-08-13	11:12	IB	ND	Mainstem
Karuk	2014-08-20	13:35	IB	ND	Mainstem
Karuk	2014-08-20	12:58	IG	ND	Mainstem
Karuk	2014-09-10	12:30	IB	ND	Mainstem
Karuk	2014-09-10	12:57	IG	ND	Mainstem
Karuk	2014-10-15	11:32	IB	ND	Mainstem
Karuk	2015-06-10	13:00	IB	ND	Mainstem
Karuk	2015-06-24	13:44	IB	ND	Mainstem
Karuk	2015-07-08	13:14	IB	>525	Mainstem
Karuk	2015-07-08	13:14	IB	128	Mainstem
Karuk	2015-07-08	11:54	WA	ND	Mainstem
Yurok	2015-07-08		WE	<10	Mainstem
Karuk	2015-07-22	12:55	IB	ND	Mainstem
Karuk	2015-07-22	13:00	IB	>2.1	Mainstem
Karuk	2015-07-22	13:00	IB	14.9	Mainstem
Karuk	2015-08-05	11:46	WA	20.4	Mainstem
Karuk	2015-08-05	12:29	IB	19.1	Mainstem
Yurok	2015-08-05		WE	<10	Mainstem
Karuk	2015-08-19		IB	ND	Mainstem
Karuk	2015-09-09	12:48	IB	ND	Mainstem
Karuk	2015-09-09	11:42	WA	ND	Mainstem
Yurok	2015-09-09		WE	<10	Mainstem
Karuk	2015-09-23	13:18	IG	ND	Mainstem
Karuk	2015-10-07	13:29	IB	ND	Mainstem
Karuk	2015-10-07	12:16	WA	ND	Mainstem
Yurok	2015-10-07		WE	<10	Mainstem
Karuk	2016-06-08		IB	ND	Mainstem
Karuk	2016-06-22		IB	ND	Mainstem
Karuk	2016-06-22		IG	ND	Mainstem
Karuk	2016-07-13		IB	ND	Mainstem
Karuk	2016-07-13		WA	ND	Mainstem
Yurok	2016-07-13	10:30	WE	<0.05	Mainstem
Karuk	2016-07-27		IB	ND	Mainstem
Karuk	2016-07-27		IB	ND	Mainstem
Karuk	2016-08-10		IB	ND	Mainstem
Yurok	2016-08-10	10:26	WE	<0.05	Mainstem
Karuk	2016-08-24		IB	ND	Mainstem

Yurok	2016-09-13	13:16	TG	4.4		Mainstem
Yurok	2016-09-13	13:26	TG	4.6		Mainstem
Karuk	2016-09-14		IB	ND		Mainstem
Karuk	2016-09-14		WA	ND		Mainstem
Yurok	2016-09-14	11:31	WE	4.7		Mainstem
Yurok	2016-09-27	13:59	TG	ND		Mainstem
Karuk	2016-09-28		IB	ND		Mainstem
Karuk	2016-10-12		IB	ND		Mainstem
Karuk	2016-10-12		WA	ND		Mainstem
Yurok	2016-10-12	09:37	WE	<0.05		Mainstem
Karuk	2016-10-19		IB	ND		Mainstem
Karuk	2017-06-12	13:11	IB	ND		Mainstem
Karuk	2017-06-12	12:46	IG	ND		Mainstem
Karuk	2017-07-12	12:50	IB	ND		Mainstem
Karuk	2017-07-12	11:42	WA	ND		Mainstem
Karuk	2017-08-09		IB	ND		Mainstem
Yurok	2017-08-09	11:29	WE	ND		Mainstem
Karuk	2017-08-23		IB	ND		Mainstem
Karuk	2017-09-13	11:58	IB	ND		Mainstem
Yurok	2017-09-13	11:37	WE	ND		Mainstem
Karuk	2017-09-27	13:34	IB	ND		Mainstem
Karuk	2017-09-27	13:10	IG	ND		Mainstem
Karuk	2017-10-11	12:30	IB	ND		Mainstem
Karuk	2017-10-11	11:50	WA	ND		Mainstem
Yurok	2017-10-11	11:16	WE	ND		Mainstem
Karuk	2017-10-18	11:13	IB	ND		Mainstem
Karuk	2018-06-06	12:07	IB	ND		Mainstem
Karuk	2018-07-25	12:36	IB	ND		Mainstem
Karuk	2018-08-08	11:11	IB	ND		Mainstem
Yurok	2018-08-21	11:02	TG	ND	OC	Mainstem
Yurok	2018-09-25	12:49	TG	ND	OC	Mainstem
Karuk	2019-06-05	11:58	IB	ND		Mainstem
Karuk	2019-06-19	12:52	IG	ND		Mainstem
Karuk	2019-07-10	12:14	WA	ND		Mainstem
Karuk	2019-07-10	12:57	IB	ND		Mainstem
CDHS	2005-09-03		IGOP-S	0	SG	Reservoir
CDHS	2005-09-03		IGOP-N	0	SG	Reservoir
CDHS	2005-09-03		CRCC	0	SG	Reservoir
CDHS	2005-09-03		IGOP-S	32.3	SG	Reservoir
CDHS	2005-09-03		IGOP-N	23.7	SG	Reservoir
PacifiCorp	2014-06-09		CRCC	ND		Reservoir
PacifiCorp	2014-06-24		CRCC	ND		Reservoir
PacifiCorp	2015-07-08	14:40	CRMC	ND		Reservoir
PacifiCorp	2015-08-05	10:55	IGCC	ND		Reservoir
PacifiCorp	2016-08-11	08:00	CRCC	<0.05		Reservoir
PacifiCorp	2016-09-06	18:20	CRCC	<0.05		Reservoir
PacifiCorp	2016-09-26	16:25	CRCC	<0.05		Reservoir
PacifiCorp	2017-07-23	10:40	CRCC	ND		Reservoir
PacifiCorp	2017-07-23	09:55	IRJW	ND		Reservoir
PacifiCorp	2017-08-27	10:40	CRCC	ND		Reservoir
PacifiCorp	2018-07-28	12:45	CRCC	<0.05		Reservoir
PacifiCorp	2018-08-11	12:15	CRCC	<0.05		Reservoir
PacifiCorp	2018-09-17	17:50	CRMC	<0.1		Reservoir
Yurok	2009-09-14		TR	ND	OC	Tributary
Karuk	2012-09-25		SH	ND		Tributary

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

Date: 8/12/2019

Subject: Cyanobacterial testing results – Agreement# 19-001-270

From: Tim Otten, Laboratory Director

To: Keith Bouma-Gregson, Freshwater HABS Program Manager
State Water Resources Control Board - Information Management & Quality Assurance

Testing results are attached for microscopy, QPCR and ELISA analyses conducted on six algal mat samples collected from the Klamath River (RB1; c/o Laurel Genzoli) from 8/3/19 – 8/5/19. All data have been reviewed and are considered final. An EDD report summarizing these data will be submitted to the State Water Board within 40 days of receipt of the data template.

Analyses included in this report:

- Quantification of total anatoxin-a by enzyme linked immunosorbent assay (ELISA).
- Quantification of total anatoxin-a producing cyanobacteria using real-time quantitative polymerase chain reaction (QPCR) methods.
- Microscope photos and identification of potentially toxigenic (PTOX) cyanobacteria.

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Project: RWB1_CYANO_2019
Analysis for Toxigenic Cyanobacteria
Project #: 19-001-270
Reported: 8/12/2019 16:00

ANALYTICAL REPORT FOR SAMPLES

Sample ID	Location	Date Collected	Date Received	Matrix	Preserved	BG_ID
SS-ANAB	South Slough	8/5/19 12:00	8/6/2019 9:15	Algal Mat	N	WB600
TH-PHOR	Tree of Heaven	8/3/19 20:00	8/6/2019 9:15	Algal Mat	N	WB601
BB-ANAB	Brown Bear	8/4/19 9:00	8/6/2019 9:15	Algal Mat	N	WB602
AB-ANAB	Aiken's Bar	8/5/19 9:00	8/6/2019 9:15	Algal Mat	N	WB603
I5-PHOR	I-5 @ trap	8/3/19 18:00	8/6/2019 9:15	Algal Mat	N	WB604
BIG-ANAB	Big Bar	8/5/19 8:00	8/6/2019 9:15	Algal Mat	N	WB605

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Project: RWB1_CYANO_2019
Analysis for Toxigenic Cyanobacteria
Project #: 19-001-270
Reported: 8/12/2019 16:00

SAMPLE RESULTS

Sample ID	Method	Target	Result	Quantitation Limit	Units	Notes
SS-ANAB	ELISA	Anatoxin-a	0.15	0.15	µg/L	
SS-ANAB	QPCR	Anatoxin-a	37,914	100	copies/mL	
TH-PHOR	ELISA	Anatoxin-a	7.77	0.30	µg/L	
TH-PHOR	QPCR	Anatoxin-a	734,024	100	copies/mL	
BB-ANAB	ELISA	Anatoxin-a	0.70	0.15	µg/L	
BB-ANAB	QPCR	Anatoxin-a	32,604	100	copies/mL	
AB-ANAB	ELISA	Anatoxin-a	0.31	0.15	µg/L	
AB-ANAB	QPCR	Anatoxin-a	62,534	100	copies/mL	
I5-PHOR	ELISA	Anatoxin-a	359.5	30.0	µg/L	
I5-PHOR	QPCR	Anatoxin-a	39,353,004	100	copies/mL	
BIG-ANAB	ELISA	Anatoxin-a	0.77	0.15	µg/L	
BIG-ANAB	QPCR	Anatoxin-a	27,848	100	copies/mL	

Bend Genetics, LLC
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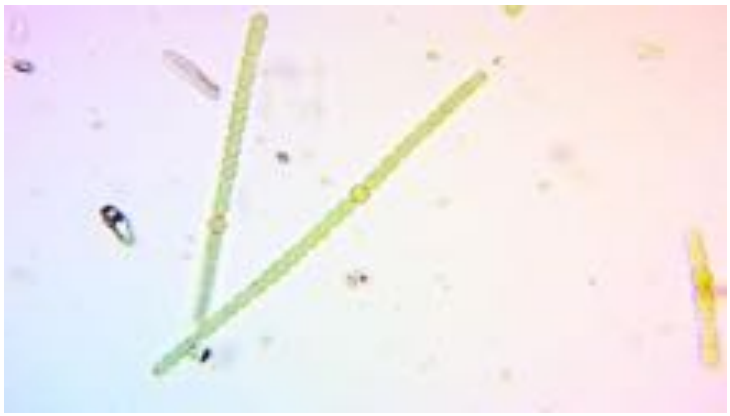
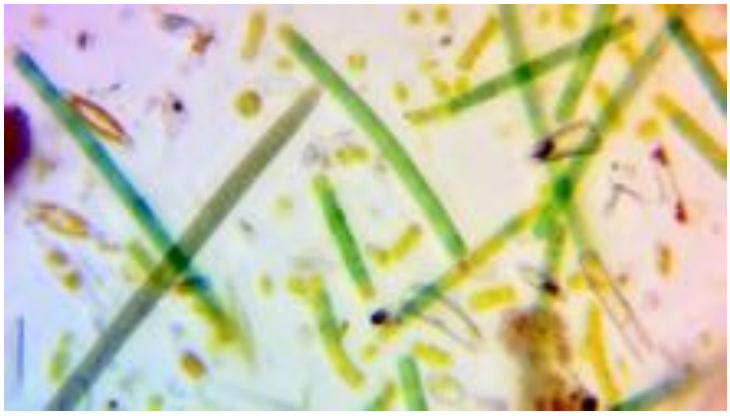
Project: RWB1_CYANO_2019
Analysis for Toxigenic Cyanobacteria
Project #: 19-001-270
Reported: 8/12/2019 16:00

MICROSCOPY RESULTS - Identification of CyanoHABs

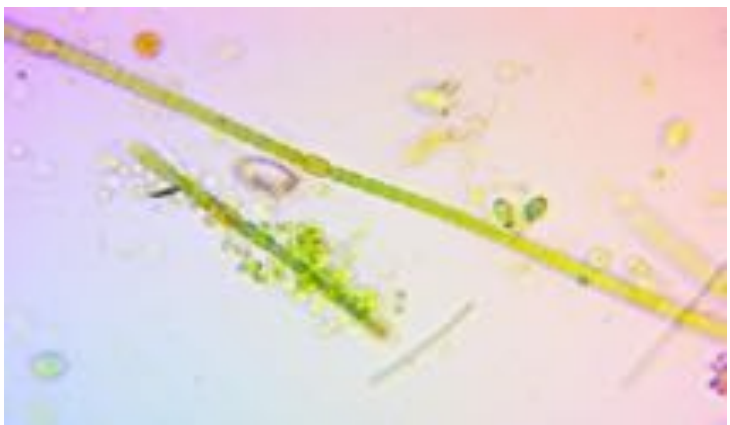
Sample ID	Dominant	Sub-dominant	Also present	Notes
SS-ANAB	*Anabaena*			This sample contained a high amount of *Anabaena* sp.; no other cyanobacteria were observed. The photomicrograph was taken under 400X magnification.



Sample ID	Dominant	Sub-dominant	Also present	Notes
TH-PHOR	*Phormidium*	*Lyngbya*		This sample contained a high amount of *Phormidium* sp. and a low amount of *Lyngbya* sp.; no other cyanobacteria were observed. The photomicrograph was taken under 400X magnification.



Sample ID	Dominant	Sub-dominant	Also present	Notes
BB-ANAB	*Dolichospermum*	*Microcystis*	*Geitlerinema*	This sample contained a high amount of *Dolichospermum* sp. and moderate amounts of both *Microcystis* sp. and *Geitlerinema* sp.; no other cyanobacteria were observed. The photomicrograph was taken under 400X magnification.



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Project: RWB1_CYANO_2019
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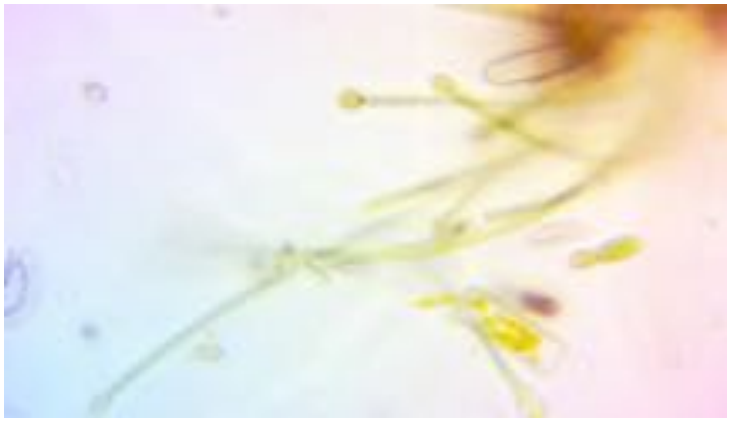
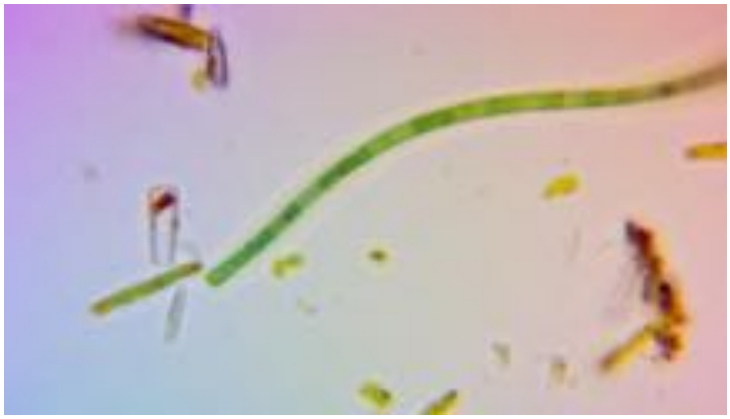
Project #: 19-001-270
Reported: 8/12/2019 16:00

MICROSCOPY RESULTS - Identification of CyanoHABs

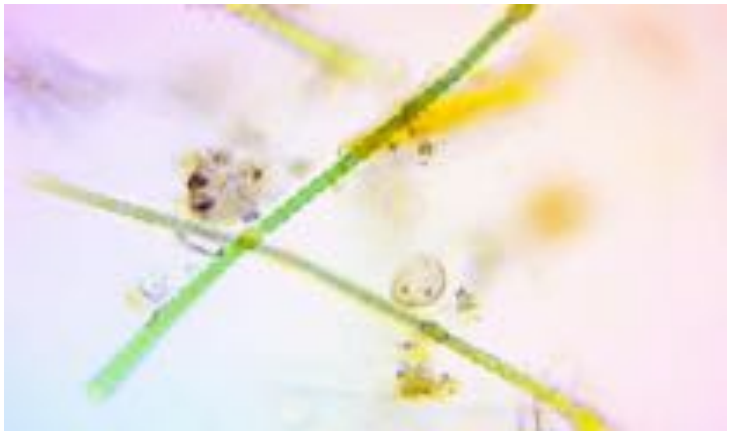
Sample ID	Dominant	Sub-dominant	Also present	Notes
AB-ANAB	*Cylindrospermum*			This sample contained a high amount of *Cylindrospermum* sp.; no other cyanobacteria were observed. The photomicrograph was taken under 400X magnification.



Sample ID	Dominant	Sub-dominant	Also present	Notes
I5-PHOR	*Phormidium*	*Lyngbya*		This sample contained a high amount of *Phormidium* sp. and a low amount of *Lyngbya* sp.; no other cyanobacteria were observed. The photomicrograph was taken under 400X magnification.



Sample ID	Dominant	Sub-dominant	Also present	Notes
BIG-ANAB	*Anabaena*	*Microcystis*		This sample contained a high amount of *Anabaena* sp. and a moderate amount of *Microcystis* sp.; no other cyanobacteria were observed. The photomicrograph was taken under 400X magnification.



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Project: RWB1_CYANO_2019
Analysis for Toxigenic Cyanobacteria
Project #: 19-001-270
Reported: 8/12/2019 16:00

QUALITY CONTROL

Method	Analyte	Result	Qualifiers / Comments	Units	Spike Level	%REC	%REC Limits
ELISA	ATX - Blank	ND	U	µg/L	0		
ELISA	ATX - Positive	0.73		µg/L	0.75	97.3	70-130
ELISA	ATX - Matrix Sp	1.26		µg/L	1.25	100.8	70-130
QPCR	anaC - Blank	ND	U	copies/mL	0		
QPCR	anaC - Spike	53,839		copies/mL	50,000	107.7	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

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Date: 8/20/2019

Subject: Cyanobacterial testing results – Agreement# 19-001-270

From: Tim Otten, Laboratory Director

To: Keith Bouma-Gregson, Freshwater HABS Program Manager
State Water Resources Control Board - Information Management & Quality Assurance

Testing results are attached for microscopy, QPCR and ELISA analyses conducted on one algal mat sample collected from the Salmon River (RB1; c/o Carry Alameda) on 8/13/19. All data have been reviewed and are considered final. An EDD report summarizing these data will be submitted to the State Water Board within 40 days of receipt of the data template.

Analyses included in this report:

- Quantification of total anatoxin-a by enzyme linked immunosorbent assay (ELISA) method.
- Quantification of total anatoxin-a producing cyanobacteria by real-time quantitative polymerase chain reaction (QPCR) method.
- Microscope photos and identification of potentially toxigenic (PTOX) cyanobacteria.

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Project: SWAMP_FHAB_2019_RB1
Analysis for Toxigenic Cyanobacteria
Project #: 19-001-270
Reported: 8/20/2019 10:45

ANALYTICAL REPORT FOR SAMPLES

Sample ID	Location	Date Collected	Date Received	Matrix	Preserved	BG_ID
SA081319-P	Salmon River	8/13/19 16:40	8/15/2019 10:05	Algal mat	N	WB645

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Project: SWAMP_FHAB_2019_RB1
Analysis for Toxigenic Cyanobacteria
Project #: 19-001-270
Reported: 8/20/2019 10:45

SAMPLE RESULTS

Sample ID	Method	Target	Result	Quantitation Limit	Units	Notes
SA081319-P	ELISA	Anatoxin-a	2.08	0.45	µg/L	
SA081319-P	QPCR	Anatoxin-a	1,415,557	100	copies/mL	

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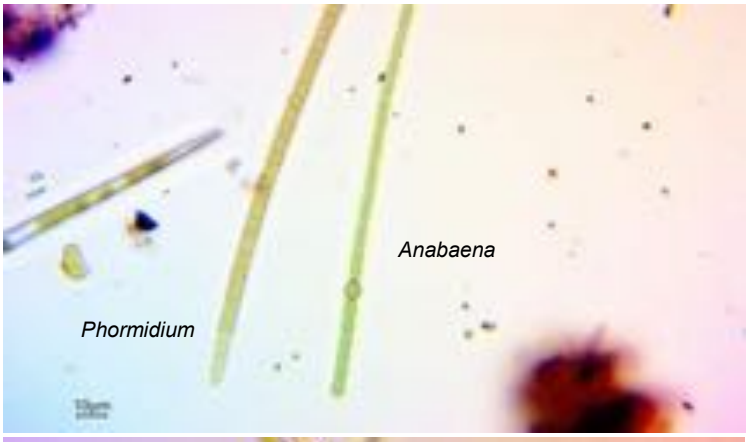
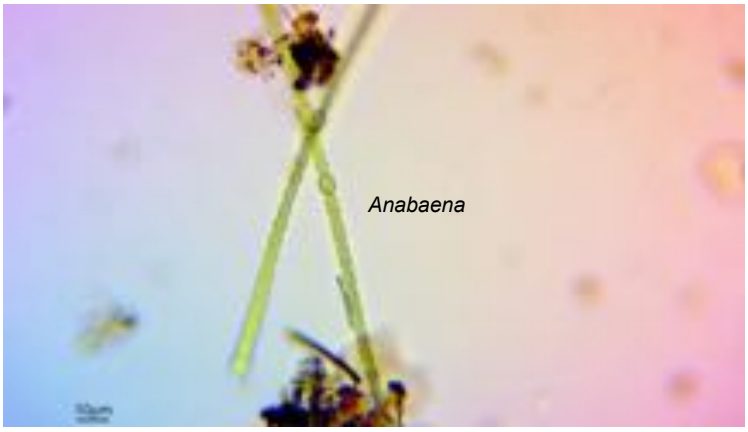
Project: SWAMP_FHAB_2019_RB1
Analysis for Toxigenic Cyanobacteria
Project #: 19-001-270
Reported: 8/20/2019 10:45

MICROSCOPY RESULTS - Identification of CyanoHABs

Sample ID	Dominant	Sub-dominant	Also present	Notes
SA081319-P	*Anabaena*	*Phormidium*		This sample contained a moderate amount of *Anabaena* sp. and a moderately low amount of *Phormidium/Microcoleus* sp.; no other cyanobacteria were observed. The photomicrographs were taken under 400X magnification.





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Project: SWAMP_FHAB_2019_RB1
Analysis for Toxigenic Cyanobacteria
Project #: 19-001-270
Reported: 8/20/2019 10:45

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	95.1	70-130
ELISA	ATX - Matrix Sp	1.28		µg/L	1.25	102.1	70-130
QPCR	anaC - Blank	ND	U	copies/mL	0		
QPCR	anaC - Spike	57,738		copies/mL	55,000	105.0	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