# **Spatial and temporal patterns of benthic cyanobacteria proliferations and anatoxin in the Klamath River Watershed, summer 2021**

**Laurel Genzoli** Consulting Aquatic Ecologist laurel.genzoli@gmail.com

**Jacob Kann** Aquatic Ecosystem Sciences, LLC. 295 East Main St., Suite 7 Ashland, Oregon 97520 jacob@aquatic-ecosciences.com

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## **Risk from benthic cyanobacteria**

Toxin-producing cyanobacteria are an emerging public health threat in lakes and rivers (Testai et al., 2016). Although cyanobacteria are a natural component of algal biofilms, proliferations of cyanobacteria-dominated mats appear to be increasing or are more widespread than previously expected (McAllister et al., 2016; Bouma-Gregson et al., 2018; Fetscher et al., 2015). Cyanobacteria can produce toxins that when ingested, can cause illness and death to humans, pets, and wildlife (Chorus and Welker, 2021; Wood, 2016). Increasingly, dog deaths following visits to rivers have been associated with anatoxin, a potent neurotoxin produced by cyanobacteria (Puschner et al., 2008; Backer et al., 2013; Catherine et al., 2013). Our understanding of the drivers and impacts of toxin-producing cyanobacterial blooms has relied largely on research of planktonic blooms dominated by taxa with different toxin-production dynamics and habitat requirements than benthic cyanobacteria proliferations (Wood et al., 2020; Scott and Marcarelli, 2012). Understanding of benthic cyanobacteria occurrence, toxin-production dynamics, and ecology lags public health concerns that these toxins pose, in part due to challenges associated with observing and documenting benthic algae.

The heterogeneous, patchy nature of benthic algae at the reach scale poses challenges to documenting their distribution and toxin dynamics in rivers and streams where they proliferate (Ledger et al., 2008). Even in small patches that appear homogeneous at a macroscopic scale, benthic algal mats can be dominated by different taxa and strains, resulting in differential toxin-production within very small patches (Wood et al., 2010, 2012). Sampling recommendations include collecting composite samples from algae mats across observed patches to capture some of the variation (Wood et al., 2020), but the extreme heterogeneity of benthic algae and inability of surveyors to collect samples from inaccessible river habitats may result in misrepresented toxin levels.

While sampling benthic material poses challenges to adequately capturing algal mat variation in a reach, water column grab samples are known to under-represent the biomass of benthic algae by orders of magnitude (Tekwani et al., 2013). Further, anatoxin degrades rapidly when exposed to sunlight, especially in warm temperatures and alkaline waters (Colas et al., 2021), reducing the likelihood of detecting anatoxin in the water column. Such under-representation translates to underestimates or even a total lack of detection of possible toxin exposure when benthic algae are the source of toxins. Still, water column grab sample methods developed for plankton are currently the sampling norm in many rivers dominated by benthic algae, and public health thresholds are based on water column concentrations of cyanotoxins (USEPA, 2019). Sloughed algal material

may provide a useful indicator of reach-scale toxin production and export from benthic cyanobacteria (Bouma-Gregson et al., 2017; Perry and Perry, 1991). Thus, due to the highly patchy nature of benthic algae and surveyor limitations in rivers, nets capturing transported coarse algal material may provide a sampling strategy to monitor water quality and public health risk associated with benthic cyanobacteria mats.

## **Benthic cyanobacteria in the Klamath River and tributaries**

Limited sampling of anatoxin from ambient water column grab samples in the Klamath River and tributaries has generally resulted in non-detectable levels of the toxin, with detection only found in 15 of 236 samples (6%)(Genzoli and Kann, 2020). Although the sample size is much lower, recent targeted samples of cyanobacterial mats in 2019 confirmed benthic cyanobacteria proliferations along the length of the Klamath River, as well as in tributaries. All eight samples of these benthic algal mats analyzed for anatoxin resulted in detectable levels of anatoxin, indicating the potential for benthic algal mats to be a source of anatoxin in the Klamath River and its tributaries (Genzoli and Kann, 2020).

Similar sampling in the Russian River has also shown higher concentration of anatoxin in cyanobacterial mats, compared to both Solid Phase Adsorption Toxin Tracking (SPATT) and grab samples, which both reflect concentrations of toxins in the water column (Fadness, R. unpublished data). Although the 2019 data suggests that benthic cyanobacteria mats are a source of anatoxin in the Klamath River, we know little about the extent of mats and toxins, how to efficiently monitor anatoxin for public health, or how prevalent cyanobacteria are within the algal community.

## **Study objectives**

The objectives of the summer 2021 benthic cyanobacteria study were to sample the distribution and extent of benthic cyanobacteria and associated anatoxins to gain baseline information about the public health risk that benthic cyanobacteria may pose in the Klamath River and tributaries. This sampling effort represents the first extensive sampling of benthic cyanobacteria and associated anatoxin over a large geographical area in the Klamath Basin. The specific goals of the sampling were to address the following questions:

- 1. What is the spatial and temporal distribution of benthic cyanobacteria and anatoxin in the Middle and Lower Klamath River Watershed?
- 2. What sampling and analysis methods are appropriate for regular public health monitoring?

Table 1. Klamath River and tributary sites, with site code, location, and indication ("o") of which samples were collected in each of three sampling efforts (July, August, and September).

	<b>Site Name</b>	Longitude Code Latitude				July				August				September			
					Composite-mat	Disturbance	Thalweg	Net-capture	Composite-mat	Disturbance	Thalweg	Net-capture	Composite-mat	Disturbance	Thalweg	Net-capture	
Sites <b>River</b> Klamath Sites Tributary	15 at Screw Trap	15	41.863138	$-122.56472$	O	O	O	O	O	O	O	$\mathbf{o}$	O	O	O	O	
	Tree of Heaven	<b>TH</b>	41.863040	$-122.56465$	$\Omega$	$\Omega$	$\mathbf{o}$	$\Omega$	O				O				
	<b>Brown Bear</b>	<b>BB</b>	41.823420	$-122.96181$		O	O	O	O	O	O	O	O	O	O	O	
	Seiad Valley at Sluice Box	<b>SV</b>	41.842464	$-123.22012$	$\mathbf{o}$	$\mathbf{o}$	$\mathbf{o}$	O	O	O			O	O	O	O	
	Happy Camp	HC	41.793273	$-123.36821$	$\mathbf{o}$	$\mathbf{o}$	$\mathbf{o}$	O	O	O	O	O	O	O	O	O	
	Orleans	<b>OR</b>	41.302890	$-123.53452$	O	$\mathbf{o}$	O	O	O	O	O	O	O	O	0	O	
	Weitchpec	<b>WE</b>	41.186310	$-123.69920$	O	O	O	O	O	O	O	$\mathbf{o}$		O	O	O	
	Klamath at Terwer	<b>KAT</b>	41.510010	$-123.97944$	$\Omega$	$\Omega$	$\mathbf{o}$	O									
	Scott River Jones Beach	<b>SRJB</b>	41.639660	$-123.05978$		$\Omega$	O		O	O	O	O	O	$\Omega$	O	O	
	Scott River Indian Scotty	<b>SRIS</b>	41.635610	$-123.07732$	$\mathbf{o}$				O	O			$\Omega$	O			
	Scott River nr Klamath	<b>SRMO</b>	41.775195	$-123.03501$		$\mathbf{o}$	$\mathbf{o}$										
	<b>Dillon Creek</b>	<b>DILL</b>	41.576099	$-123.53879$	$\mathbf{o}$				O				O				
	Salmon River Hippo Rock	<b>SALM</b>	41.378490	$-123.47554$	$\Omega$	$\Omega$	O	O	O	O	O	$\mathbf{o}$	$\mathbf{o}$	O	O	$\Omega$	
	<b>Trinity River Tish Tang</b>	<b>TRTT</b>	41.021284	$-123.63491$					O	O	O			O	0		
	<b>Trinity River Near Mouth</b>	<b>TRMO</b>	41.184620	$-123.70662$		O	O	O	O	O	O	O	O	O	O	0	

## **Study Sites**

We sampled cyanobacteria at 15 sites in the Klamath River Watershed, including eight sites along the mainstem of the Klamath River between Iron Gate Dam and the Klamath River Estuary, and seven sites on tributaries, including the Scott, Salmon, and Trinity Rivers and Dillon Creek (Table 1). To the extent possible, we chose mainstem sites that were evenly spaced. In addition, we prioritized sites utilized by the public and that are established water quality monitoring sites by the Karuk, Yurok, Hoopa and Quartz Valley Tribes, including sites with long-term water quality sondes when possible.

## **Methods**

In order to document patterns of cyanobacteria and anatoxin production throughout the summer, and at sites across the Middle and Lower Klamath Watershed, we assessed benthic cyanobacteria and anatoxin using multiple sample collection methods targeting benthic mats, river water, and transported algal material during the summer of 2021. Samples were analyzed for a suite of parameters to compare methods with respect to

feasibility and identification of methods most protective of public health.

#### **Field Methods**

#### *Targeted benthic mat sampling*

We conducted semi-quantitative surveys of wadeable river margins and collected composite samples from conspicuous algal mats at 10 - 14 sites monthly in July, August and September of 2021. From each river access point we surveyed an area of shoreline that was accessible by wading, and where benthic algae were visible given current water clarity conditions. Surveys consisted of walking either along the shoreline or in shallow river margins for 10 minutes. We documented the width and length of each survey area and composited up to 20 pinches of cyanobacterial mat material into a 60 mL amber glass jar to 40% full. Upon completion of the survey and mat sample collection effort we filled the jar with water to 80% full and placed the samples on ice. In addition, we qualitatively estimated conspicuous cyanobacteria mat cover using a scale of 0-3, where "0" indicated no conspicuous cyanobacteria mats, "1" indicated few isolated patches totaling less than 0.4 m<sup>2</sup>, "2" indicated moderate cover between 0.4–2 m<sup>2</sup>, and "3" indicated >2 m<sup>2</sup> of conspicuous cyanobacteria mat cover. We also recorded a qualitative description of the cyanobacteria conditions present at the site, including suspected taxa, associations with non-cyanobacteria taxa, river habitat occupied.

#### *Disturbance sampling*

Disturbance samples were taken to mimic increased water column levels of suspended algal material created when people wade into the river and dislodge benthic algal mats. We first agitated the algal material with our feet in an area of dense algae, and then collected a grab sample of dislodged, suspended materials in a 125 mL amber glass jar. At most sites we identified conspicuous cyanobacteria mats, and thus collected disturbance samples where these mats were present. At the few sites where we did not identify conspicuous mats, we collected disturbance samples from other types of dense algae to see if cyanobacteria cells and anatoxins were present in the absence of conspicuous cyanobacterial mats.



Figure 1. Algal drift net capturing coarse algal particles suspended in the water column

#### *Water column and algal drift sampling*

To estimate transported algal material, anatoxin, and the proportion of toxin-producing cyanobacteria, we sampled ambient water column conditions both instantaneously and over time via grab sampling and by employing drift nets at each site. We collected water column grab samples for indicators of cyanotoxin production from a well-mixed area of the water column in a 250 mL amber glass jars, and immediately transferred samples to a dark cooler.

To assess algal drift as a source of toxin export, we placed an invertebrate drift net in the current and secured the net with rebar stakes (Fig. 1). The net was deployed until enough drifting algal material was collected for analysis of both anatoxin and dry mass, which ranged from a few minutes to >30 minutes depending on quantity of algae suspended in the water column. We repeated the net deployment three times at a new location in the river during each deployment and composited the coarse algal material from the three deployments into a single jar, which was placed in a dark cooler until processing. Measurements of dry mass, paired with net deployment time and incoming velocity, which was recorded at three points in front of the net, will be used in subsequent analyses to calculate coarse particulate algal loads and anatoxins associated with those loads.

#### **Lab Methods**

We sent all sample types (net capture, composite mat, water column, and disturbance) to Bend Genetics for analysis of toxins, toxin production genes, and qualitative microscopy. Prior to sending the net samples for analysis of anatoxin and dry mass, we partitioned the samples after homogenizing the captured coarse particulates by cutting coarse material (primarily macrophytes and filamentous algae) into small pieces ( 1 cm) and then utilizing an immersion blender to further homogenize the sample. For each sample, we measured the total volume, partitioned 40 mL into an amber glass jar for toxin analysis, and reserved the remainder for analysis of dry mass. All samples were analyzed via QPCR for the anatoxin-production gene (AnaC gene), and were examined under a microscope for qualitative identification of dominant and subdominant taxa. Net samples, mat samples, and any other samples with QPCR results >10,000 AnaC gene copies/mL were also analysed for anatoxin concentration by ELISA.



(a). Box plots show median and ranges of AnaC (b). Count of composite mat samples for each gene copies by month for the four sample types sampling event with colors showing the collected

dominant taxa in each sample by month

Figure 2. AnaC gene copies in four sample types and dominant species in benthic cyanobacteria mat samples by month in the Klamath Watershed

### **Results**

#### **Spatial and Temporal Patterns**

Of the 141 benthic cyanobacteria samples from 15 sites and three sampling efforts in the summer of 2021, cyanobacteria and associated anatoxins were present at all sites and months, with increasing coverage and toxin indicators later in the summer (Data Appendix, Fig. 2). Although we encountered conspicuous cyanobacteria mats during the majority of surveys, the extent of mat coverage and toxin indicators were higher in August and September than in July. Toxin-production genes were highest in August in all sample types, except the net samples, which were slightly higher in September (Fig. 2a). All mat samples collected in the field contained cyanobacteria, confirming that we were successful in field-identifying cyanobacteria. *Microcoleus* and *Anabaena* were the most common taxa in benthic mats, but five other taxa were at times found to be dominant in mat samples (Fig. 2b). *Microcystis* was the dominant cyanobacteria in one mat sample in August, but this was due to large numbers of planktonic cells settling out of the water column onto cyanobacteria mats where benthic species were sub-dominant in the sample.

Spatial patterns of dominant cyanobacteria taxa and toxin production were generally weak. *Microcoleus* and *Anabaena* were commonly dominant in both the mainstem and tributary sites, although *Anabaena* was more commonly dominant at mainstem sites. *Ocillatoria* was only dominant at tributary sites, whereas *Microcystis* was only dominant at Table 2. Anatoxin by ELISA ( $\mu$ g/L) from composite cyanobacteria mat samples at Klamath River and tributary sites. Mat samples in July were only analyzed by QPCR. Sites with two samples (TRMO and TRTT) were of different taxa observed at the same site. "ns" indicates that no mats were observed and sampled.



one mainstem site, associated with upstream transport and cell settling (Fig. 3a). In the mainstem of the Klamath River, *Microcoleus* was always the dominant taxa at the most upstream site, and was dominant at most sites during September. *Anabaena* was more common at sites lower on the river, but only in the July and August samples (Fig. 3b). Little is known about conditions favoring specific cyanobacteria taxa or toxin production, but the patterns observed in the Klamath provide opportunities for further study aimed at identifying the conditions that favor specific taxa and associated toxin-production dynamics.

As with dominant taxa, patterns in toxin indicators across the watershed were generally weak or overshadowed by temporal variability. AnaC gene copies were overall slightly higher in tributary than mainstem mat samples (Fig. 4a), but there was substantial overlap in gene copies between site types. Of the six samples with the highest anatoxin and AnaC gene copies, two were in the mainstem and four were in tributaries, including the Salmon, Trinity and Scott rivers. High toxins in tributary streams show that cyanotoxins are a risk not only in the mainstem of the Klamath River where most river users are aware of concerns associated with reservoir-sourced cyanotoxins (Table 2).



(a). Count of composite mat samples with colors indicating dominant taxa from the mainstem of the Klamath River and from tributaries.

(b). Dominant taxa by month and site in the Klamath River. KAT was only sampled in July, and white rectangles at BB and WE indicate no mats found during surveys.

Figure 3. Dominant taxa from composite mat samples for each sampling event

Similar to toxin production between mainstem and tributary sites, the Klamath River showed weak longitudinal patterns in toxin-production genes. Mat samples showed a decrease in AnaC gene copies in the most upriver three sites, but mid-river sites had a large range in gene copies and no trend in median gene copy concentrations (Fig. 4b). Gene copies from net, disturbance and thalweg samples did not show longitudinal trends, indicating that cyanobacteria growth and toxin-production dynamics may be tied to local conditions. Research investigating the drivers that promote cyanobacteria, specific taxa, and toxin production is needed to better predict where cyanobacteria and associated toxins will present water quality and public health concerns.



(a). AnaC gene copies from mainstem and tributary sites. (b). AnaC gene copies from mainstem sites, shown from upriver to downriver

Figure 4. Box plots show median and ranges of AnaC gene copies

#### **Informing Monitoring Methods**

We compared field sampling techniques and lab analytical methods to consider what types of samples are logistically feasible and protective of public health. QPCR methods, which quantify the number of toxin-production genes in a sample and thus should represent the number cyanobacteria cells with toxin production capacity, were representative of toxin concretions measured by ELISA (Fig. 5). The adjusted  $r^2$  of the slope of the log-log relationship was 0.70 considering all sample types, and 0.82 when only assessing the relation among the composite mat samples. Assessing this relationship on a log scale requires acknowledging a large range in absolute toxin and gene copy concentrations at high concentrations. However, due to the high level of heterogeneity of riverbed algae, toxin production, and environmental conditions that promote, dilute, and degrade toxins, both ELISA and QPCR results from benthic cyanobacteria should be viewed as indicators of risk, rather than absolute quantities. The relatively strong relationship between QPCR and toxin by ELISA indicates that the more affordable QPCR lab method can be used to inform toxicity of benthic mats. The increased affordability allows for greater spatial and temporal coverage of sampling, as well as the use of plastic bottles instead of the amber glass that is required in toxin samples.



Figure 5. The log of anatoxin concentrations by the log of AnaC gene copies from a sample; colors indicate sample type (disturbance, composite mat, and net-capture).

#### In comparing

four types of field samples, composite samples of conspicuous cyanobacteria mat material were the most protective of public health (i.e., they typically showed the highest concentrations) and were logistically feasible to collect. Although public health thresholds exist for toxin concentrations in water column samples, we found that water from the well-mixed water column was always low in anatoxin production genes (AnaC gene copies mean  $= 923$ , max  $= 4833$ ), confirming that the well-mixed water generally presents a low risk of anatoxin exposure to river users, unless cyanobacterial material is suspended in the water column.

The drift net samples captured floating algal material in the water column, but by design were set to capture substantially more material than a river user or pet would be exposed to. However, these samples can be scaled and used to predict the likelihood that a swimmer would encounter toxins from suspended cyanobacteria. Additionally, drift net samples capture a mixed sample of what is floating in the water both near the site and from upstream, thus capturing material from a much larger area than what can be surveyed from shore. Of multiple sites sampled where no benthic mats were visible, we found toxins to be present in the drift nets, indicating upstream presence of mats. Drift nets may be especially useful early in the season to indicate the onset of benthic proliferations. Because drift nets need to be set in an area of flowing water and to sit for up to 30 minutes, they may be more challenging to deploy as part of currently scheduled site visits.

Mat samples consistently had the highest toxin concentrations and were reflective of paired disturbance samples taken at the same sampling location (Fig. 6). Mat samples represent a realistic "worst-case" exposure because they are present along shorelines, including in calm pools where parents may be comfortable allowing children to play and where dogs wade and drink, allowing both of these vulnerable groups access to play with and possibly ingest these anatoxin-containing mats. Understanding the worst-case



Figure 6. Disturbance samples with paired mat samples from from the same site and sampling day.

scenario and communicating this risk to the public will help mitigate the health risk associated with anatoxin exposure.

### **Conclusions**

Benthic cyanobacteria and associated anatoxins were widespread in the Klamath River Watershed in summer 2021. We collected 141 samples from 15 sites during three sampling efforts on the Klamath River and tributaries. Benthic cyanobacteria and indicators of anatoxin were found at all sites and sampling efforts. Cyanobacteria extent and toxin indicators were higher in August and September than in July, but the extent of mat coverage, toxin-production genes, toxin concentrations, and patterns in dominant taxa were variable among sites, making predicting occurrence of toxins difficult. The Salmon and Trinity Rivers, generally considered to have good water quality compared to the Klamath River, had high anatoxins, indicating that future sampling and public health messaging should include tributary streams of high water quality.

Continued surveying and sampling for benthic cyanobacteria will add to current understanding of their extent and the risk they pose to public health. Selecting sampling methods most likely to result in positive detections will help efficiently use sampling resources. Despite a history of sampling the water column for anatoxin, anatoxin in the water column was always low compared to other sample types and thus not the most protective indicator of public health risk. Drift nets, set to capture coarse particles of sloughed algae transported in the water column, required more survey effort but reflected toxins from sloughed algae from an area upstream which could not easily be surveyed otherwise. These drift net samples could be used early in the season to trigger more thorough search efforts once anatoxin detections rise in these samples. Although disturbance samples reflected benthic cyanobacteria mats samples at a site, they were always lower due to not being concentrated. Thus, composite samples of conspicuous cyanobacteria mats, which can easily be eaten by a dog or accessed by children, provided the most protective measure of public health risk at a site. Quantitative PCR methods, which assess the number of toxin-production genes in a sample, were reflective of toxin concentrations and are less costly to process, suggesting that QPCR lab analysis is a good option when sampling resources need to be extended to more sites.

Although benthic cyanobacteria is a naturally occurring part of the algal community, they can become a problem for water quality and public health when proliferations become extensive and produce anatoxin. Due to the widespread documentation of benthic cyanobacteria and anatoxin in the Klamath River in the summer of 2021, more sampling should be conducted to inform public health messaging, to better understand the extent of toxin-producing proliferations, and to understand the conditions driving cyanobacteria and toxin production in the Klamath River and tributaries.

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**Data Appendix: 2021 Klamath Benthic Anatoxin Survey**





**Data Appendix: 2021 Klamath Benthic Anatoxin Survey**



**Data Appendix: 2021 Klamath Benthic Anatoxin Survey**

