AGRICULTURAL RESEARCH FOUNDATION
FINAL REPORT
FUNDING CYCLE 2018 – 2020

TITLE: Assessment of the entry of toxic cyanobacteria from lake blooms into irrigation waters

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EXECUTIVE SUMMARY: Sampling during 2018 (summer/early fall) made it clear that cyanobacterial blooms present at the outlet of Upper Klamath Lake (Link River) become widely distributed in the Klamath Irrigation District. Fortunately, during both 2018 and 2019, the blooms at the southern end of Upper Klamath Lake (UKL), which were at risk of entering the irrigation system, were composed of *Aphanizomenon flos-aquae (AFA)*, and for a brief period, *Anabaena/Dolichospermum flos-aquae*, both of which are non-toxic. *Microcystis* blooms, which are typically toxic, did not appear in Link River and only traces were seen in the irrigation system. Our results show that cyanobacterial blooms present in Link River will be distributed throughout the irrigation system to points of usage. Distribution of toxic *Microcystis* blooms is expected to result in aerosolization of toxin, presenting a risk to farm and perhaps other rural personnel. Fortunately, in 2018 and 2019, *Microcystis* blooms did not develop in the southern end of UKL and Link River. If *Microcystis* blooms do occur in future years, their effect will be decreased if they occur after August, when irrigation demand is lower. The risk of distributing cyanobacterial toxins appears to be low in most years, but the Klamath Irrigation District should monitor the status of blooms in Link River and develop a plan for responding when *Microcystis* is present when active irrigation is occurring. The Irrigation District should also be aware that large amounts of *Aphanizomenon flos-aquae* (AFA) become aerosolized during regular irrigation practices and should be alert to any changes in the health risk advice for exposure to this non-toxic cyanobacterium. Finally, limited sampling of the irrigation intake supplying the Willow Creek valley downstream of Heppner does not raise concerns regarding exposure cyanobacterial blooms occurring in Willow Creek Reservoir. Water is drawn relatively deep from Willow Creek Reservoir, avoiding the transfer of most blooms downstream. However, as in the Klamath Irrigation District, Willow Creek irrigation officials should be aware of the potential for cyanobacterial blooms entering the irrigation system; it would be prudent to at least visually inspect intake water when blooms occur in the reservoir.

OBJECTIVES:

1. Collect water samples downstream from major cyanobacterial blooms (UKL, Willow Creek Reservoir) at key irrigation canal diversions to assess the extent to which blooms (cyanobacterial cells) and associated compounds constituting potential health hazards (toxins, other bioactive compounds) are drawn into the irrigation water supply.

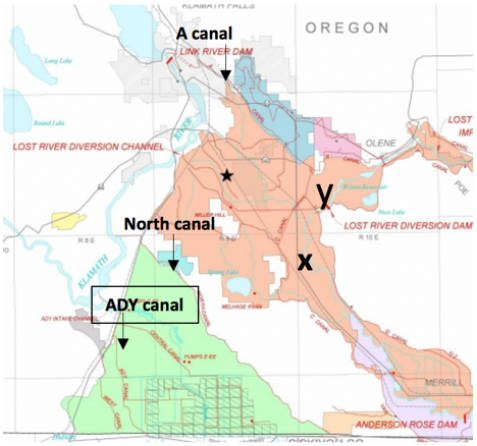
2. Analyze samples to assess the potential for disseminating health risks: measure the concentrations of microcystin (the toxin of concern in both reservoirs) and cyanobacteria, and

determine the genetic potential for production of bioactive compounds by genome analysis of the cyanobacteria present.

PROCEDURES:

1. Sample collections: Sampling sites for collection were selected in consultation with local irrigation experts. In the case of the ***Klamath Irrigation District***, the initial sampling was done together with Brian Charlton, Potato Faculty Scholar at the OSU Klamath Basin Research and Extension Center. The sampling sites were:

Pelican Marina, near the outlet of UKL (42.2390 N 121.8097 W)
Near the beginning of ***North Canal*** (initially 42.1222 N 121.8289 W and subsequently 42.101354 121.796169 W)
Near the beginning of ***ADY Canal*** (initially 42.0808 N 121.8456 W and subsequently 42.079572 N 121.844983 W)



G Canal near Merrill (42.0536 N 121.6006 W) deep inside the irrigation system (x in the figure)
Lost River (initially 42.0536 N 121.6006 W and subsequently 42.140954 N 121.678518 W) representing water derived from the Lost River and its Clear Lake and Gerber Reservoir sources (y in figure), which have suffered blooms in the past.

At Willow Creek Reservoir (Morrow County), Kevin Payne (Morrow County Soil and Water Conservation District) and Sarah Burnet/Frank Wilhelm (Univ. of Idaho) assisted in assessing the status and sampling blooms in Willow Creek Reservoir and in identifying the inlet for a new irrigation distributor as the most appropriate site to monitor bloom entrainment into irrigation waters. The sampling sites were:

Willow Creek Reservoir NW corner (45.347288 N 119.542713 W)
Willow Creek Reservoir boat dock (45.343754 N 119.539072 W)
Willow Creek irrigation intake, 2.0 miles downstream of the dam (45.368545 N 119.568530 W)

Sample collections were made in the Klamath irrigation district on 27 July, 11 August, 17 September, 2018, and on 16 July and 27 August, 2018 from Willow Creek. In 2019, we were alert for any *Microcystis* bloom developing at the southern end of UKL, but as in 2018 that did not eventuate. Samples were taken only on 2 June, 2019, when no significant bloom was present at the southern end of UKL or in the Irrigation District. *Anabaena/ Dolichospermum* blooms were present on 2 June, 2019 at Agency Lake (adjacent to northern end of UKL and in Howard Bay on the SW side of UKL); these blooms were sampled for genome sequencing.

Willow Creek samples were collected on 8/27/2018 and 7/2/2019. Only a minor bloom was present on 8/27/2018, while a strong bloom dominated by *Gloeotrichia* and *Oscillatoria* was present on 7/2/2019. This bloom was also sampled on 6/28/2019 and 8/25/2019 with a view to obtaining *Oscillatoria* samples for genome sequencing; *Oscillatoria* is frequently toxic, but it is

unusual in Oregon and its toxicity status is unknown. On both the 8/27/2018 and 7/2/2019 sampling dates, there was an insignificant amount of cyanobacteria in the irrigation intake.

Samples were brought to the Corvallis lab in a chilled state and were documented microscopically and processed to archive samples for genetic and toxin analysis. Samples were aliquoted for microcystin (cyanotoxin) analysis (2 x 1 ml). For DNA analysis, cyanobacteria were collected onto duplicate 1.2 µm glass fiber filters by filtration (volumes of 100 – 400 ml, as determined by volume needed to nearly clog filters). All sub-samples were archived at -80°C until use.

2. Microcystin analysis: Microcystin concentrations were determined using a microtiter plate ADDA ELISA kit #520011 (Abraxis) according to the manufacturer's instructions, conducting duplicate assays for each sample (1 determination for each aliquot).

3. Genetic analysis: DNA was extracted from material collected on glass fiber filters by use of a DNeasy PowerBiofilm extraction kit (Qiagen) involving grinding with a microfuge tube pestle and bead-beating (10 minutes at full speed on vortexer). To represent the number of AFA cells in samples, a qPCR assay developed specifically for quantitation of UKL AFA (Caldwell-Eldridge et al., 2016) was adapted to droplet digital PCR (ddPCR). The primers used were AFA_cpcA_F (TTAACCGCTAAAGCTCAACA) and AFA_cpcA_R (CATCGGATGCGTATTGATTA) targeting the AFA *cpc*A gene. ddPCR assays were conducted using the Bio-Rad QX200 AutoDG Droplet Digital PCR system (Oregon State University Center for Genome Research and Biocomputing), using EvaGreen dye-binding detection. Amplifications used BioRad QX200 ddPCR EvaGreen Supermix, 900 nM primers and 3 units of *Hin*dIII restriction enzyme per 20 µL reaction. Annealing was at 54°C and amplification used the default cycling conditions. Triplicate amplifications were performed on all samples, resulting in duplicate triplicates for each sampling.

Genome sequencing was conducted on DNA that was similarly isolated, except that bead-beating was limited to 2 mins. DNA was processed for SMRT cell sequencing on the Pacific Biosciences Sequel or Sequel 2 instrument as recommended by the manufacturer. Sequencing reads were assembled using the PacBio HGAP 4 assembler, with polishing and analysis using Geneious software. Gene annotation was conducted using the Prokka program.

SIGNIFICANT ACCOMPLISHMENTS:

1. Blooms from the southern-most part of Upper Klamath Lake are transported throughout the Klamath Irrigation District. Three sampling events in the Klamath Irrigation District occurred during dense *Aphanizomenon flos-aquae* (AFA) blooms (Fig. 1) in the outlet region of UKL (7/28, 8/11 and 9/17 during 2018), with no *Microcystis* evident microscopically. High concentrations of AFA were visually obvious at all sampling sites influenced by water originating from Link River. Some AFA was present in the Link River sampling site on 7/18 but not later in the season. The levels of AFA were quantified by using ddPCR to estimate the number of gene copies of *cpc*A, the gene for the cyanobacteria-specific phycocyanin-A gene (Table 1). The analyses confirm the presence of high levels of AFA within the distribution canals of the irrigation district, even at the relatively distant G canal sampling site near Merrill. AFA concentrations were at times higher in the canal samples, perhaps reflecting higher levels at

the southern end of UKL (i.e., Pelican Point) at the time the sampled water entered the irrigation district. Cyanobacterial blooms can also be very patchy, concentrated at the surface by buoyancy and driven into thick scums by persistent wind or currents.

Table 1 AFA *cpc*BA gene copy number in samples from Klamath Irrigation District (000's/mL)

Date sampled	Pelican Marina	North Canal	ADY Canal	G Canal	Lost River
7/28/18	73.0	149.4	168.8	160.1	60.1
8/11/18	34.9	10.3	3.8	95.0	
9/17/18	80.0	15.9	35.4	11.7	

Data are the averages of triplicate assays on each of two samples

The important conclusion here is that AFA originating in UKL and Link River become widely distributed in the irrigation district. These blooms are not cleared by unfavorable conditions in the sometimes rapidly flowing canals. There are many precedents for cyanobacterial blooms being swept down rivers, sometimes very long distances (Graham et al., 2012; Otten et al., 2015).

Fig. 1 Dense *Aphanizomenon flos-aquae* (AFA) bloom from UKL (Pelican Marina, 9/17/2018): the typical "grass clippings" appearance of AFA is evident when looking directly into the lake (left), at a sample bottle, or under the microscope (right, 100x).

Our results indicate that monitoring the state of cyanobacterial blooms at the very southern end of UKL, in Link River and the nearby upper reaches of the Klamath River provides a good reflection of the risk of blooms being transported throughout the irrigation system. We did not observe significant blooms entering via Lost River, but when that does occur, these can equally be expected to become distributed in the irrigation district. The connection between bloom status in irrigation canals and at the intake site is important because cyanobacterial blooms will not develop in flowing canals, although they may do so if canal flows are interrupted or in stagnant pools (see below).

2. *When AFA or Dolichospermum cyanobacteria predominate and no significant Microcystis is evident by microscopy, microcystin toxicity is unlikely to be a concern.* It is known that AFA from UKL does not produce the cyanotoxin microcystin, so that we would not expect microcystin toxicity to be a concern even when high levels of AFA occur in irrigation waters. To assess this presumption, we analyzed for microcystin presence in the same samples analyzed

for AFA in Table 1; we also analyzed samples from an additional sample run (6/2/2019) when AFA predominated (Table 2). Microcystin values in bulk canal water and in UKL were all below 2 µg/L, well below the 8 µg/L "recreational use value" that Oregon Health Authority employs to assess recreational exposure (i.e., non-alimentary) toxicity risk.

Table 2 Microcystin concentrations in samples from Klamath Irrigation District (µg/L)

Date sampled	Pelican Marina	North Canal	ADY Canal	G Canal	Lost River
7/28/18	0.174	0.136	0.158	0.173	0.159
8/11/18	0.155	0.156	0.168	0.282	0.151
9/17/18	1.762	0.664	1.046	0.631	0.270
6/2/19	0.124	0.152	0.163	0.117	

Data are averages of assays on duplicate samples.

Slow-flowing water can occur at some points along irrigation canals. At one such point immediately upstream of the weir at the North Canal sampling site (42.101354 121.796169 W) on 9/17/2018, a small *Microcystis* scum was observed in a region of still water (Fig. 2). A sample of this scum had 15.5 µg/L microcystin, issuing a caution that toxic *Microcystis* can occur in isolated patches even when AFA is overwhelmingly predominant inside the irrigation system.

Fig. 2 Bright green strands indicative of a toxic *Microcystis* scum at the North Canal sampling site, 9/17/2018.

Occasionally, typically during early spring, *Dolichospermum/Anabaena* is the dominant cyanobacterium in UKL. One such episode occurred on 6/2/2019, when such blooms occurred in Howard Bay (SW region of UKL) and Agency Lake (adjacent to the northern end of UKL). Genome sequencing conducted on these samples indicated that *Dolichospermum* sp. UKL201 and *Dolichospermum* sp. UKL202, the consensus cyanobacteria present in Agency Lake and Howard Bay, respectively, did not harbor cyanotoxin genes. ***Thus, toxicity concerns in the Klamath Irrigation District are associated with microcystin originating from Microcystis; AFA and Dolichospermum do not present known cyanotoxin risks in this system.***

We have determined the complete genomes of *Dolichospermum* sp. UKL201 and *Dolichospermum* sp. UKL202. This provides information for designing molecular probes for the detection of UKL *Dolichospermum,* and this information will be valuable for future research on these cyanobacteria. We are in the process of determining full genome sequences for AFA and *Microcystis* from UKL, sampled during and before this study. This information will likewise be useful. Although these cyanobacteria do not produce cyanotoxins (i.e., microcystin, nodularin

anatoxin-a, cylinderospermopsin or saxitoxin), they do have genes for the production of other bioactive compounds, such as anabaenopeptin, aeruginoside, cyanopeptolin, and anacyclamide; in the future, we may discover that exposure to these compounds is unhealthy.

3. *There appears to be low risk of cyanotoxin transfer into irrigation waters drawn from Willow Creek, Heppner.* We were not able to sample Willow Creek Reservoir during a major bloom in 2018, but we did sample during a *Gloeotrichia*/*Oscillatoria* bloom in 2019. For teh two samplings made (one during the 2019 bloom), the irrigation water intake contained almost no cyanobacteria as determined by microscopy. In view of the obvious lack of bloom transfer into the irrigation system, no analyses were conducted. However, since *Oscillatoria* is uncommon in Oregon and its toxicity status is unknown, we have submitted a 8/25/2019 sample for genome sequencing (analysis still under way). The result will help to assess the toxicity risk in the Willow Creek Irrigation District.

Microcystin analyses were conducted on limited samples from Willow Creek Reservoir (Table 3). No elevated levels of microcystin were detected, although Willow Creek Reservoir has been known to have concerning levels of cyanotoxins at time (for instance, 354 µg/L microcystin was present in Willow Creek Reservoir in October, 2015; OHA, 2015). In each case, only very low levels of microcystin were detected in the irrigation water intake.

Table 3 Microcystin concentrations in samples from Willow Creek Irrigation District (µg/L)

Date sampled	Willow Creek Reservoir	Willow Creek Irrigation Intake
8/27/2018	0.435	0.214
7/2/2019	1.302	0.150

Data are averages of assays on duplicate samples.

Sampling in the Willow Creek system was too sparse to allow a full assessment of the risk of cyanotoxins appearing in the irrigation water, which is taken from Willow Creek two miles below the dam. Nevertheless, the irrigation intake samples were devoid of visible cyanobacterial colonies or filaments, and the microcystin concentration on 7/2/2019 was at background levels at a time when there was 1.3 µg/L in the reservoir. The entrainment of cyanobacterial blooms downriver may be minimized in view of the water outlet being located at elevation 2037 ft, which is often considerably below the water level and blooms that mostly are near the surface. The spillway crest is at 2,113 ft, and the normal summer level begins around 2076 ft, but can fall to as low as 2047 ft (pers. comm., Kevin Mcallister, USACE). Note that not all cyanobacteria are concentrated at the surface; *Oscillatoria*, which was prominent in Willow Creek reservoir on 7/2/2019, can prefer the lower light levels of the sub-surface; no *Oscillatoria* was observed microscopically in the irrigation intake sample.

Since the toxicigenicity of *Oscillatoria* from Willow Creek Reservoir is not known, we are in the process of determining the complete genome sequence, which will reveal the presence or absence of cyanotoxin genes.

BENEFITS & IMPACT: A warning to be alert to toxin aerosolization via spray irrigation. By observing the transport of dense AFA blooms from Link River throughout the Klamath Irrigation District, this study serves as a warning that under appropriate circumstances toxic blooms could become aerosolized over a wide region. Toxic *Microcystis* blooms occur annually in UKL and sometimes spread to Link River. Such blooms would be expected to become distributed in the same manner as we have observed for AFA blooms, delivered to the spray irrigators that will produce aerosols. ***These aerosols would present a risk to personnel on the farm and perhaps in neighboring areas.*** Irrigators use water directly from canals, without any steps to remove cyanobacterial mass. Even with water containing cyanobacterial colonies that appear dense to the naked eye the colonies do not form mats and are unlikely to cause back-pressure problems in irrigation nozzles.

It is recommended that the Klamath Irrigation System develop a protocol for monitoring the presence of Microcystis blooms in Link River, as these can be expected to be toxic. The Klamath Basin Blue Green Algal Monitoring Program (ref) conducts routine monitoring that includes microcystin analyses. The Klamath Irrigation System should track these results to identify periods that present a risk of toxin distribution. Serious thought should be given to developing a response plan when such conditions occur.

The risk of distributing toxins in the Willow Creek Irrigation system appears to be low, but this issue should be watched.

Factors possibly mitigating the risk of toxin dispersal in irrigation systems. In the Klamath Irrigation System, irrigation usage falls off during August after most crop growth has occurred. *Microcystis* blooms have historically been most prevalent in UKL after August, though blooms have been found in certain cases earlier. ***Thus, low water usage late in the season favors avoidance of exposure to toxins.*** This factor may mitigate the impact of toxic blooms on irrigation practices.

In the Willow Creek irrigation area, the blooms that are common in the reservoir can be localized and may not be transferred into the irrigation system because of the position of the outlet (depth and location) relative to the blooms. This mitigating factor has been discussed above.

ADDITIONAL FUNDING RECEIVED DURING PROJECT TERM: $0

FUTURE FUNDING POSSIBILITIES: N/A

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