FINAL
2008 Klamath River Blue-Green Algae Summary Report

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I. Introduction

This report summarizes the presence of toxicogenic cyanobacteria in the Klamath River within the Yurok Indian Reservation (YIR) boundaries in 2008. The Yurok Tribe Environmental Program (YTEP) and the Karuk Tribe collaborated to monitor water quality conditions from downstream of Iron Gate Dam to the Klamath River Estuary. The Karuk Tribe will be publishing a report that summarizes the conditions from Orleans to just downstream of Iron Gate Dam. Results from water samples collected in 2008 indicated that the water quality in the Klamath River was negatively impacted by levels of the cyanobacterium *Microcystis aeruginosa* (MSAE) and its resultant toxin, microcystin. MSAE and microcystin levels within the YIR boundaries did not exceed the State of California's recommended thresholds for recreational waters in 2008. However, MSAE and microcystin levels upstream of the YIR did exceed the State of CA's recommended thresholds for recreational waters in the Klamath River, Iron Gate Reservoir and Copco Lake.

Cyanobacteria, also known as blue-green algae (BGA), are commonly found in many freshwater systems across the world. The species of concern here are known as toxigenic species, since they have the potential to produce chemicals that are toxic to humans and animals. In general, the toxins produced by these algae can be divided into two groups, those which can cause liver damage (hepatotoxins) and those which can damage the central nervous system (neurotoxins), although other health effects are possible.

At least 46 species of cyanobacteria have been shown to be toxic to vertebrates (Chorus & Bartrum, 1999). Some of the more common toxin-producing genera include *Microcystis*, *Anabaena*, *Aphanizomenon*, *Lyngbya*, *Nodularia, Planktothrix*, *Nostoc* and *Cylindrospermopsis.* It should be noted that cyanobacteria likely produce toxins that have not been characterized.

Microcystis aeruginosa is a type of blue-green algae which releases the liver toxin microcystin when it dies and decomposes. Microcystin can cause rashes, skin irritation, conjunctivitis, nausea, vomiting, diarrhea, liver damage, tingling, numbness, paralysis, and death in humans and animals. Microcystin causes the most damage when it builds up in the liver; it also accumulates in other organs and in the muscle tissue of humans and animals. Microcystin is not excreted by humans and animals, so the dose can increase over time. When a large enough dose accumulates liver damage, increased liver size and death can result. Mortality in fish, domestic animals, and humans has been recorded following exposure to microcystin from a single-dose and from long-term exposure.

Exposures Pathways

The primary exposure pathway of concern for exposure to cyanotoxins is through ingestion of water. Skin irritation can result from exposure to the algae itself, however the cyanotoxins are not likely to cross the skin barrier and enter the bloodstream. Inhalation of microcystin is possible, especially during activities such as water skiing or splashing, where contaminated water is aerosolized.

Ingestion of contaminated water can occur through both incidental and intentional pathways. Incidental ingestion most commonly occurs during recreation especially in turbid or discolored lakes. The risk of incidental ingestion of the toxin is particularly high for children playing in near-shore areas where algal scum tends to accumulate. Because of their small body size, children are at greater risk from exposure—it takes a smaller dose to make them sick than it does to sicken an adult. Exposure levels can be broadly defined as high, moderate and low based on recreational activity (Table 1).

Table 1. Exposure level of recreational activity (modified from Queensland Health, 2001).

Level of Exposure	Recreational Activity
High	Swimming, diving, water skiing
Moderate	Canoeing, sailing, rowing
Low to none	Fishing, pleasure cruising, picnicking, hiking

At this time, there is insufficient information to determine the risk of consuming fish caught in waters with toxigenic cyanobacteria. Studies have shown that toxins mainly accumulate in the liver and other internal organs of fish, although microcystin has been detected in the fillet (Vasconcelos, 1999; de Magalhães et al., 2001). However, Fetcho 2006 reports that no microcystin was detected in salmon or steelhead filets collected from fish sampled at Weitchpec during the 2005 *Microcystis* bloom. At a minimum, the internal organs and skin should be removed and discarded prior to cooking fillets. Shellfish have been shown to accumulate cyanotoxins in edible tissue (Vasconcelos, 1999). It is recommended that people call the Department of Human Services for more information on fish consumption while advisories are in effect.

Detrimental Environmental Effects of *Microcystis aeruginosa*

In addition to causing many known, well-documented human and animal health effects, microcystins can have a detrimental effect on the food chain by limiting growth of beneficial phytoplankton species, discouraging zooplankton feeding and population growth, decreasing total dissolved oxygen in the water column, and ultimately lowering success of fish and other large organisms.

"Even low microcystins concentration at the base of the food web poses a threat to the upper food web because microcystins may bioaccumulate." The impact of *Microcystis* species on the quantity and quality of phytoplankton biomass available to the food web may be a greater threat to the food web than toxicity. Blooms can reduce growth of other phytoplankton species because of their buoyancy and ability to block light further down the water column, and their relative ability to out-compete species which cannot tolerate high light and temperatures at the surface. Dissolved microcystin in the water may also inhibit feeding by zooplankton (De Mott et al., 1991). In addition, high biomass produced by blooms and the associated decomposition can eventually impact fishery production through influence on dissolved oxygen concentration (Lehman et al., 2005).

Microcystin Toxin Information

WHO has established minimum tolerance levels for recreational contact with microcystin. Because of the time it takes to analyze water samples for the presence of microcystin, WHO recommends the use of cell counts per milliliter of water as a crude surrogate for concentrations of microcystin. However, because the toxin is released as the organism decomposes, the risk from microcystin presence in waters is at its greatest after the bloom has initially begun to decompose and increases until well after the last cells are observed in samples.

WHO has set the following thresholds for MSAE/microcystin concentrations in recreational waters:

	Microcystis cells/milliliter	Microcystin micrograms/liter
Low Risk:	20,000	4
Moderate Risk:	100,000	20
Severe Risk	10,000,000 *or* visible scum	200

The consumption limit for microcystin is set as 0.04 micrograms per kilogram of bodyweight per day. However, because even the consumption of relatively low doses of microcystin over time will damage the liver of animals, continued consumption of known contaminated food sources is not recommended.

The State of CA has set thresholds for posting waterbodies to minimize impacts to recreational users in a document titled "Cyanobacteria in California Recreational Water Bodies Providing Voluntary Guidance about Harmful Algal Blooms, Their Monitoring, and Public Notification".

The State of CA's has set the following thresholds for posting recreational waters:

- Scums present containing toxigenic* species
- MSAE or *Planktothrix* ≥ 40,000 cells/ml
- Population of potentially toxigenic BGA species ≥ 100,000 cells/ml
- Concentration of microcystin ≥ 8 ppb

*Potentially toxic blue-green algae that have been detected in California include those of the genera *Anabaena, Microcystis*, *Aphanizomenon, and Gloeotrichia.* Additional bluegreen algae that are known to be potentially toxic may be added to this list.

II. Methods

YTEP follows methods as specified in the USEPA approved "Lower Klamath River Nutrient, Periphyton, Phytoplankton and Algal Toxin Sampling Analysis Plan (SAP)". At each sample site, sample water was collected with a pre-rinsed churn splitter as specified in the grab sample protocol located in Appendix B. The churn was rinsed three times with distilled water followed by three rinses with site river water. Samples were drawn in a moving portion of the river in an attempt to collect water samples to represent the river as a whole. The churn splitter allowed for distribution of a homogenous water

mixture into sample bottles used for algal identification and enumeration and testing for microcystin.

The sample bottle for identification and enumeration of algal species contained Lugol's preservative and the toxin sample was preserved by freezing the bottle. Both of these samples were drawn from the same churn of water because they are complementary to one another. All samples were labeled with the following information: date, time, sampler, sample site, study name. The sample ID was comprised of a two or three digit site ID and the date (e.g. TG090108).

If a sampling crew member identified an area along the river that had scum lines, an additional sample was collected at this site. The sample was labeled appropriately and photographs of the sample area were taken. Additional quality control measures were included in the sampling. At one site per trip a replicate split sample was sent to the laboratory to assess laboratory performance and to gain improved confidence in the data.

Environmental information was also recorded at the time water samples were collected. The data included water temperature, pH, specific conductance, dissolved oxygen and other observational notes. Water samples were also collected to be analyzed for the concentration of nutrient analytes and sent to Aquatic Research Inc. in Seattle, Washington (WA). Chain-of-custody (COC) sheets were also filled out to document the handling of the samples from the time of collection to the time of laboratory analysis. This is a standard procedure for handling samples.

Water samples that were collected for algae speciation and enumeration were mailed overnight to Aquatic Analysts in White Salmon, WA for analysis. Microscope slides are prepared at the laboratory from each sample by filtering an appropriate aliquot of the sample through a 0.45 micrometer membrane filter (APHA Standard Methods, 1992, 10200.D.2; McNabb, 1960). A section is cut out and placed on a glass slide with immersion oil added to make the filter transparent, followed by placing a cover slip on top, with nail polish applied to the periphery for permanency. Most algae are identified by cross-referencing several taxonomic sources.

Algal units (defined as discrete particles - either cells, colonies, or filaments) are counted along a measured transect of the microscope slide with a Zeiss standard microscope (1000X, phase contrast). Algal units are measured accurately to 0.1 mm with a stage micrometer. The algal densities are calculated from the area observed (transect length times diameter of field of view), the effective filter area, and the volume of sample filtered. Only those algae that were believed to be alive at the time of collection (intact chloroplast) are counted. A minimum of 100 algal units are counted. (Standard Methods, 1992, 10200.F.2.c.). If toxic cyanobacteria are present in the 100 algal units count the taxonomist then counts 4 times that area but only for the toxic species. Average biovolume estimates of each species are obtained from calculations of microscopic measurements of each alga. The number of cells per colony, or the length of a filament, are recorded during sample analysis to arrive at biovolume per unit-alga. Average biovolumes for algae are stored in a computer, and measurements are verified for each sample analyzed.

Water samples that were collected for microcystin processing were stored in glass containers and mailed on ice overnight to USEPA Region 9 lab in Richmond, California for analysis using the enzyme linked immunosorbent assay (ELISA) method. These methods have been adapted to a commercial ELISA kit (Microcystin Plate Kit, EP-022) that is produced by Envirologix, Inc. (Portland Maine), which USEPA Region 9 lab in Richmond, CA employs and measures total microcystin. Additional samples were submitted to the California Department of Fish and Game's Fish and Wildlife Water Pollution Control Laboratory in Rancho Cordova for the analysis of microcystin variants and anatoxin-a using liquid chromatography duel mass spectrometry (LC-MS/MS).

III. Site Selection

In general, the various sampling locations were chosen in order to represent the average ambient water conditions throughout the water column. The sites listed below indicate established sampling locations for the collection of water samples for nutrient analysis and phytoplankton speciation and enumeration from May through October on a biweekly interval.

Once the presence of MSAE was detected at sampling sites upstream of the YIR additional samples were collected beginning on July 9, 2008 and continued through October to test for the presence of microcystin.

YTEP collected water samples for toxin and algae speciation analysis at the following mainstem Klamath River locations (river miles are approximate):

- **WE - Klamath River at Weitchpec (upstream of Trinity River) – RM 43.5**
- **TC – Klamath River Above Tully Creek – RM 38.5**
- **TG - Klamath River at Turwar Boat Ramp – RM 6**
- **LES - Lower Estuary Surface – RM 0.5**

YTEP collected water samples for toxin and speciation analysis at the following major tributary location:

- **TR - Trinity River near mouth (above Klamath River confluence) – RM 0.5**

YTEP did collect water samples in the Klamath River Estuary August 29, 2008 during the 2008 Tribal commercial fishing season to determine if elevated levels of toxicogenic cyanobacteria were present. Two samples were collected in addition to the routine sampling location in the Lower Estuary. One sample was collected at the upper extent of the Klamath River Estuary underneath the 101 Bridge to determine what was entering the estuary and one sample was collected in the center of the channel adjacent to YTEP's water level recorder in the estuary approximately 800 yards upstream of the mouth of the river. These results are provided in this report separately and are clearly identified as unique sampling locations.

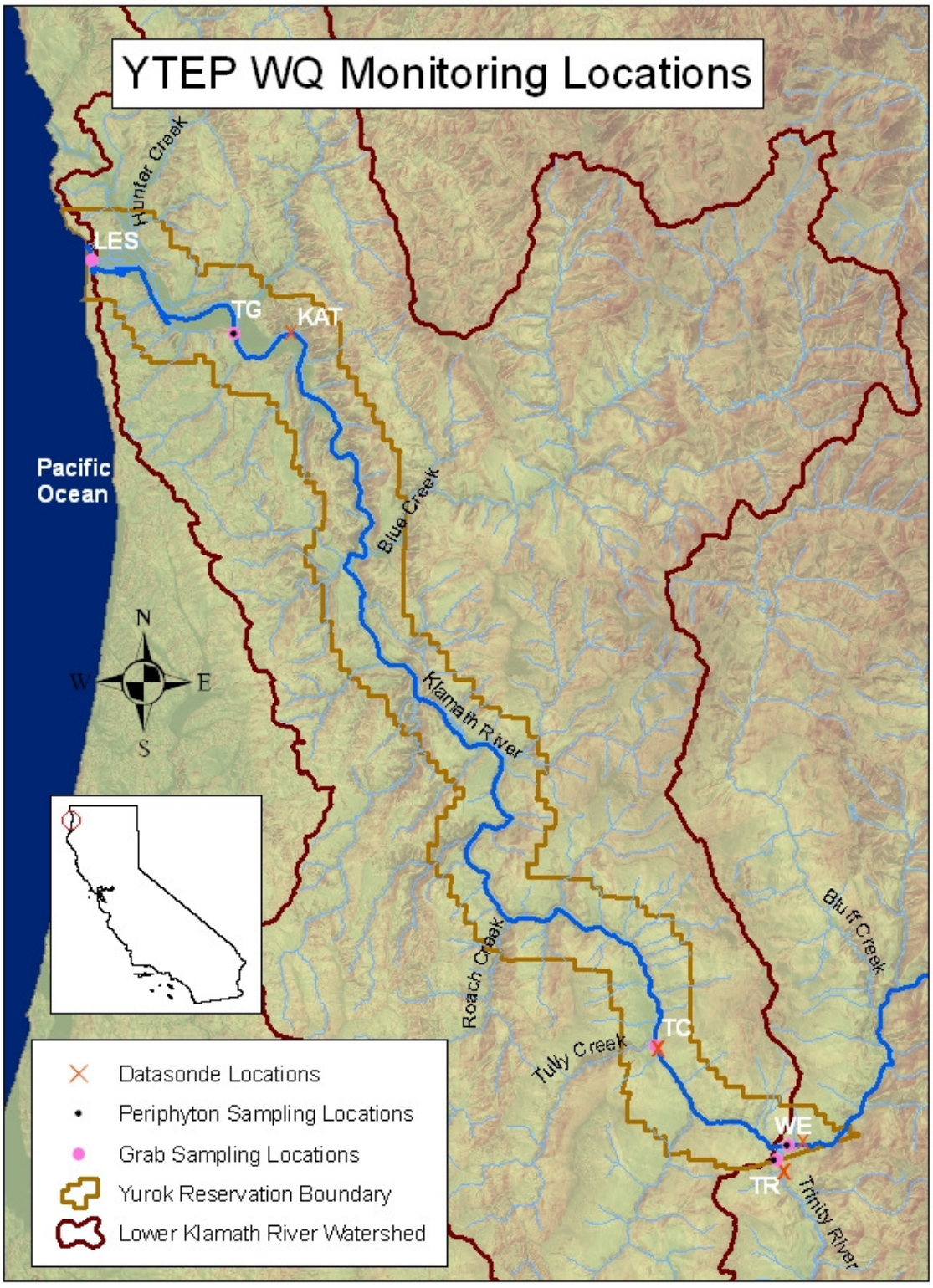

Figure 1. Map of phytoplankton and microcystin river monitoring locations, 2008.

IV. Quality Assurance

YTEP follows methods as specified in the "Lower Klamath River Nutrient, Periphyton, Phytoplankton and Algal Toxin Sampling Analysis Plan (SAP)" approved by the USEPA in June 2008. Quality assurance/Quality control (QA/QC) of the collection, preparation and analysis of water samples for microcystin and phytoplankton speciation and enumeration was achieved by following a standard water sample collection protocol using a churn sampler and submitting samples to labs that follow strict protocol that have QA/QC measures. All field personnel that were involved in collection of water samples have been trained appropriately by the Water Division Program Manager and are properly supervised to ensure proper protocol is followed consistently throughout the monitoring season. Field crews collecting samples ensured representativeness of samples by selecting free-flowing water from established sampling locations and using a churn splitter to mix sample water once collected. All samples were transported to the appropriate laboratories following chain of custody procedures to ensure proper handling of the samples.

The collection and analysis of field replicate samples were performed during each sampling event to determine the labs' precision of data. Since the Yurok and Karuk Tribes collaborated on this project it was necessary for each Tribe to alternate every other event to collect the QA/QC samples. YTEP collected QA/QC samples in the sampling event that was near the beginning of the month and the Karuk Tribe would collect the QA/QC samples near the end of the month. Field replicates were collected by splitting samples in the field using the churn splitter. One of the split samples was sent with its' associated split with a different ID code for analysis of both algae identification and enumeration and microcystin so as to not alert lab staff of the fact that the samples were replicates.

Data is thoroughly reviewed once received from the laboratory. YTEP is the primary organization responsible for data review, although the professional laboratories analyzing water quality samples will also note potential problems with outliers or other anomalies in sample results. Information regarding QA/QC procedures for the laboratory is available upon request. One hundred percent of laboratory-generated data was checked on receipt by the Project Manager for consistency and acceptability, including whether replicates are within specified targets and meet data quality objectives. Any unusual values outside the range of norm will be flagged and all aspects of field data sheets, shipping handling and laboratory handling and testing will be reviewed. Outliers will be identified and removed from the dataset if deemed necessary by the QA Officer. Water temperature, conductivity, pH and dissolved oxygen are measured in the field when samples are collected and values of these hand-held measurements can be used to check field conditions at the time of sampling.

The data manager visually inspects all entered data sets to check for inconsistencies with original field or laboratory data sheets. Where inconsistencies are encountered, data will be re-entered and re-inspected until the entered data is found to be satisfactory or results will be discarded. The Project Manager will maintain field datasheets and notebooks in

the event that the QA Officer needs to review any aspect of sampling for QA/QC purposes.

Phytoplankton

Not all of the phytoplankton replicate samples contained MSAE. QA samples collected in May, June, July, August and October did not contain MSAE, therefore, no relative percent differences were calculated for these months. The common algae in each replicate did, at times, change rank for most samples collected between May and October (see table 2). However, the same species generally make up the bulk of the samples. Furthermore, the abundances (whether density, biovolume, or Trophic State Index (TSI)) were similar for the replicates. Considering the biological variability in this river the replicate results are not significantly different. Discrepancy among replicates may also reflect how well the churn was able to split particulates in samples.

The September QA replicate sample and primary samples did contain low levels of MSAE and the RPD was 5.5%. The replicate and blank samples collected indicate that the phytoplankton samples are valid and acceptable.

Microcystin

All of the primary, replicate and blank samples submitted to the lab reported results less than the reporting limit of 1.8 micrograms/Liter. The QA samples collected by YTEP indicate that the toxin results are valid and acceptable.

V. Results:

Phytoplankton QA

Table 2. Phytoplankton results for the QA replicate samples collected by YTEP, 2008.

Lab Slide ID	Station ID	Date	TSI	Total Density	Total Biovolume	Sp1	Sp1%	Sp2	Sp2%	Sp3	Sp3%	Sp4	Sp4%	Sp5	Sp5%	#Spp	APF9 cells/ml	MSAE cells/ml	ABX9 cells/ml	Oscillatoria sp. cells/ml
LU02	TG	5/14/08	43.5	1,148	414,095	DTTN	53.5	ACMN	7.0	NZDS	5.3	NVCV	5.3	CMMN	3.5	24	0	0	0	0
LU04	TG rep	5/14/08	41.2	911	299,897	DTTN	46.5	NZDS	5.9	ACMN	5.9	RHCU	5.9	CMMN	5	26	0	0	0	0
LU17	TG	6/11/08	41.4	1,165	307,469	DTTN	37.9	ACMN	12.9	RHCU	5.6	NZDS	5.6	NZFR	4.8	24	0	0	0	0
LU21	TG rep	6/11/08	41.3	1,039	306,033	DTTN	36.8	NZDS	12.3	ACMN	11.3	COPC	7.5	RDMN	4.7	23	0	0	0	0
LU32	TG	7/9/08	59.5	2,751	3,794,286	MLGR	65.6	RDMN	6.6	DTTN	6.6	NVGR	2.5	EPSX	2.5	18	0	0	0	0
LU34	TG rep	7/9/08	52.1	781	1,367,900	MLGR	81.5	DTTN	3.7	GFSB	2.2	COPC	2.2	NVGR	1.5	15	0	0	0	0
LU53	TG	8/7/08	46.6	1,079	634,580	KMXX	15.5	EPSX	14.7	DTTN	12.9	RDMN	12.9	COPC	4.3	27	0	0	0	0
LU58	TG rep	8/7/08	49.2	2,131	913,912	RDMN	16.1	DTTN	13.4	EPSX	13.4	COPC	8.9	KMXX	6.2	28	0	0	0	0
LU71	TG	9/3/08	47.7	1,482	739,304	RDMN	25.8	COPC	14.2	CXER	8.9	SCQD	7.1	SNUL	6.2	27	158	4,615	0	0
LU73	TG rep	9/3/08	44.7	1,718	491,911	RDMN	27.1	SLMN	17.8	COPC	10.0	CHXX	6.4	EPSX	6.4	21	0	4,368	0	0
LU99	TG	10/1/08	46.4	715	623,770	EPSX	28.2	RHCU	18.4	COPC	16.5	SNUL	6.8	DTVL	4.9	22	0	0	0	0
LU97	TG REP	10/1/08	45.7	653	564,055	EPSX	27.1	COPC	15.9	RHCU	14.9	SNUL	8.4	DTVL	7.5	23	305	0	341	0

Key to Species Codes is located in Combined Species List located in Appendix A

APF9 = *Aphanizomenon flos-aquae MSAE = Microcystis aeruginosa ABX9= Anabaena sp.*

Phytoplankton

Table 3. Phytoplankton results for water samples collected in the Klamath River and mouth of Trinity River May - October 2008.

Site ID	Date	Trophic State Index	Total Density	Total Biovolume	Sp1	Sp1%	Sp2	Sp2%	Sp3	Sp3%	Sp4	Sp4%	Sp5	Sp5%	#Spp	APF9 cells/ml	MSAE cells/ml	ABX9 cells/ml	Oscillatoria sp. cells/ml
WE	5/14/08	47.0	1,951	674,398	DTTN	56.6	NZDS	11.3	ACMN	5.7	NZFR	3.8	COPC	3.8	17	0	0	0	0
TC	5/14/08	43.1	1,099	289,469	DTTN	59.6	ACMN	10.5	CMMN	2.6	RHCU	2.6	NZFR	2.6	19	0	0	0	0
TG	5/14/08	43.5	1,148	414,095	DTTN	53.5	ACMN	7.0	NZDS	5.3	NVCV	5.3	CMMN	3.5	24	0	0	0	0
LES	5/14/08	41.6	1,143	316,863	DTTN	59.6	ACMN	11.4	NZDS	4.4	NZFR	3.5	RHCU	2.6	19	0	0	0	0
TR	5/14/08	38.9	269	218,531	DTTN	24.3	CMMN	17.8	ACMN	12.1	FRCR	9.3	GFAN	7.5	18	0	0	0	0
WE	5/28/08	34.9	568	124,347	DTTN	25.8	ACMN	15.5	RDMN	10.3	NZDS	9.3	NZFR	5.2	23	0	0	0	0
TC	5/28/08	38.2	620	198,846	ACMN	20.0	DTTN	19.1	NZDS	11.8	RDMN	6.4	NZFR	5.5	29	0	0	0	0
TG	5/28/08	39.1	724	224,506	DTTN	23.7	ACMN	11.9	NZDS	11.0	RHCU	8.5	NZFR	5.9	26	0	0	0	0
LES	5/28/08	32.2	277	85,460	DTTN	31.7	ACMN	11.5	RHCU	8.7	NZDS	5.8	FRVA	3.8	29	0	0	0	0
TR	5/28/08	33.4	321	101,052	DTTN	39.6	ACMN	8.8	GFAN	8.8	AFPR	4.4	CMSN	4.4	23	0	0	0	0

Key to Species Codes is located in Combined Species List located in Appendix A

APF9 = *Aphanizomenon flos-aquae MSAE = Microcystis aeruginosa ABX9= Anabaena sp.*

Table 3(contd.) Phytoplankton results for water samples collected in the Klamath River and mouth of Trinity River May - October 2008.

Site ID	Date	Trophic State Index	Total Density	Total Biovolume	Sp1	Sp1%	Sp2	Sp2%	Sp3	Sp3%	Sp4	Sp4%	Sp5	Sp5%	#Spp	APF9 cells/ml	MSAE cells/ml	ABX9 cells/ml	Oscillatoria sp. cells/ml
WE	6/11/08	42.6	1,334	366,789	DTTN	33.6	ACMN	16.4	NZDS	8.2	RHCU	5.5	COPC	4.5	25	0	0	0	0
TC	6/11/08	41.2	1,143	302,324	DTTN	50.5	ACMN	8.1	NZDS	8.1	COPC	3.6	RHCU	2.7	24	0	0	0	0
TG	6/11/08	41.4	1,165	307,469	DTTN	37.9	ACMN	12.9	RHCU	5.6	NZDS	5.6	NZFR	4.8	24	0	0	0	0
LES	6/11/08	44.0	1,102	446,330	DTTN	48.2	ACMN	8.2	NZDS	6.4	STHN	4.5	RDMN	4.5	23	0	0	0	0
TR	6/11/08	37.7	528	184,773	DTTN	49.0	ACMN	13.5	GFAN	7.7	COPC	5.8	HNAR	3.8	20	0	0	0	0
WE	6/25/08	49.6	1,863	959,682	DTTN	39.5	MLGR	14.9	ACMN	9.6	RDMN	6.1	COPC	5.3	20	0	0	0	0
TC	6/25/08	48.3	1,748	810,681	DTTN	48.0	MLGR	9.0	ACMN	8.0	NZDS	7.0	RDMN	6.0	20	0	0	0	0
TG	6/25/08	48.7	1,954	847,379	DTTN	47.0	MLGR	10.3	ACMN	6.8	NZDS	6.8	RDMN	5.1	21	0	0	0	0
LES	6/25/08	48.2	1,725	794,646	DTTN	43.1	MLGR	12.8	ACMN	9.2	RDMN	7.3	STHN	6.4	19	0	0	0	0
TR	6/25/08	38.6	537	210,495	DTTN	59.4	COPC	6.6	ACMN	5.7	GFAN	4.7	CMAF	2.8	22	0	0	0	0
WE	7/9/08	56.3	1,691	2,455,783	MLGR	63.0	RDMN	14.8	COPC	5.2	KMXX	3.7	RHCU	2.2	17	0	0	0	0
TC	7/9/08	54.9	1,442	2,012,923	MLGR	52.8	DTTN	9.9	RDMN	9.2	COPC	5.6	ACMN	3.5	22	0	0	0	0
TG	7/9/08	59.5	2,751	3,794,286	MLGR	65.6	RDMN	6.6	DTTN	6.6	NVGR	2.5	EPSX	2.5	18	0	0	0	0
LES	7/9/08	54.4	1,033	1,890,004	MLGR	77.3	RDMN	7.7	KMXX	2.2	GFAN	1.7	COPC	1.7	21	0	0	0	0
TR	7/9/08	40.3	563	265,483	DTTN	54.0	COPC	8.0	ACMN	6.2	GFAN	3.5	HNAR	2.7	25	0	0	0	0
WE	7/23/08	40.9	423	286,969	COPC	35.8	EPSX	13.8	RDMN	6.5	DTTN	5.7	MLGR	5.7	23	0	0	0	0
TC	7/23/08	45.5	648	545,465	MLGR	25.2	COPC	11.9	RDMN	10.5	EPSX	7.0	KMXX	7.0	22	0	0	0	0
TG	7/23/08	42.5	870	360,760	DTTN	28.7	COPC	11.1	RDMN	11.1	EPSX	11.1	KMXX	5.6	25	0	0	0	0
LES	7/23/08	36.1	233	147,518	DTTN	27.0	COPC	10.1	RDMN	7.9	EPSX	7.9	ACMN	6.7	22	0	0	0	0
TR	7/23/08	36.5	340	157,253	DTTN	41.6	COPC	14.9	GFAN	4.0	SNUL	4.0	RDMN	3.0	26	0	0	0	0
WE	8/7/08	43.4	531	410,228	EPSX	27.9	COPC	27.9	KMXX	5.4	RDMN	5.4	DTVL	3.6	26	0	1,149	0	0
TC	8/7/08	42.4	604	355,947	EPSX	21.6	COPC	16.7	RDMN	7.8	KMXX	7.8	DTTN	6.9	28	0	0	0	0
TG	8/7/08	46.6	1,079	634,580	KMXX	15.5	EPSX	14.7	DTTN	12.9	RDMN	12.9	COPC	4.3	27	0	0	0	0
LES	8/7/08	39.3	465	231,791	KMXX	20.6	DTTN	17.6	RDMN	14.7	COPC	10.8	EPSX	9.8	19	0	0	0	0
TR	8/7/08	37.6	377	182,497	COPC	24.8	CHXX	7.6	CMAF	7.6	EPSX	7.6	RDMN	6.5	28	28	0	0	0

Key to Species Codes is located in Combined Species List located in Appendix A

APF9 = *Aphanizomenon flos-aquae MSAE = Microcystis aeruginosa ABX9= Anabaena sp.*

Table 3(contd.) Phytoplankton results for water samples collected in the Klamath River and mouth of Trinity River May - October 2008.

Site ID	Date	Trophic State Index	Total Density	Total Biovolume	Sp1	Sp1%	Sp2	Sp2%	Sp3	Sp3%	Sp4	Sp4%	Sp5	Sp5%	#Spp	APF9 cells/ml	MSAE cells/ml	ABX9 cells/ml	Oscillatoria sp. cells/ml
WE	8/20/08	47.8	994	750,382	EPSX	31.5	COPC	28.8	SNUL	5.4	RHCU	4.5	NZFR	4.5	24	0	739	0	22
TC	8/20/08	49.0	1,265	891,396	EPSX	25.2	COPC	22.6	SCQD	7.8	AKFL	7.0	RDMN	5.2	25	0	0	0	0
TG	8/20/08	43.1	925	394,155	RDMN	19.3	SCQD	14.3	COPC	11.8	EPSX	8.4	CHXX	5.9	26	0	0	0	0
LES	8/20/08	36.9	351	165,101	COPC	18.2	SCQD	13.9	EPSX	12.8	RDMN	9.6	CCMG	6.4	30	0	379	0	0
TR	8/20/08	33.2	181	98,052	COPC	20.6	CMAF	11.8	EPSX	8.8	RDMN	5.9	ACMN	5.9	31	0	0	0	0
WE	9/3/08	46.3	950	614,210	COPC	29.2	NZFR	10.7	EPSX	8.8	NVCR	7.8	RDMN	6.8	26	312	4,066	0	0
TC	9/3/08	46.8	876	657,264	COPC	27.1	EPSX	14.5	NZFR	8.7	NVCR	5.8	RHCU	4.8	29	176	3,777	0	85
TG	9/3/08	47.7	1,482	739,304	RDMN	25.8	COPC	14.2	CXER	8.9	SCQD	7.1	SNUL	6.2	27	158	4,615	0	0
LES	9/3/08	39.6	494	242,180	RDMN	36.9	COPC	12.6	NZFR	5.8	SCQD	5.8	DTTN	3.9	24	48	4,707	0	0
TR	9/3/08	34.2	230	113,351	COPC	27.6	EPSX	8.2	DTTN	8.2	CMAF	6.1	NVCV	6.1	29	0	0	0	0
WE	9/17/08	43.9	748	437,410	COPC	20.7	EPSX	14.8	NZFR	9.9	RHCU	6.9	NVCR	5.9	25	134	3,002	0	0
TC	9/17/08	43.0	706	386,020	COPC	28.5	SNMZ	11.6	EPSX	10.5	NZFR	8.4	NVCR	7.4	26	126	1,429	0	0
TG	9/17/08	44.8	877	493,858	COPC	17.3	EPSX	12.2	RDMN	11.2	DTTN	10.2	SNMZ	6.1	24	429	2,099	0	0
LES	9/17/08	39.0	475	223,100	RDMN	26.7	COPC	14.3	APF9	8.2	DTTN	6.7	CCMG	5.7	25	507	1,900	0	0
TR	9/17/08	34.7	221	122,087	COPC	15.7	EPSX	13.3	DTTN	9.6	CMAF	6.0	RDMN	6.0	27	0	0	0	0
WE	10/1/08	43.1	614	391,165	COPC	25.2	NZFR	14.6	SNUL	10.7	NVCR	9.7	RHCU	9.7	18	0	0	0	0
TC	10/1/08	43.2	521	396,081	COPC	23.0	NZFR	16.7	EPSX	11.5	SNUL	8.4	NVCR	5.2	23	130	0	0	0
TG	10/1/08	46.4	715	623,770	EPSX	28.2	RHCU	18.4	COPC	16.5	SNUL	6.8	DTVL	4.9	22	0	0	0	0
LES	10/1/08	33.9	137	108,278	COPC	41.4	EPSX	10.3	DTVL	8.0	DTTN	4.6	GFSB	3.4	25	63	95	0	0
TR	10/1/08	30.8	91	70,287	COPC	41.4	SNUL	15.7	EPSX	7.1	SCQD	5.7	CMAF	5.7	17	0	0	0	0
WE	10/15/08	44.0	974	442,898	COPC	41.3	NZFR	17.4	NVCV	5.8	NVCR	5.0	EPSX	4.1	23	0	0	0	0
TC	10/15/08	43.7	734	428,849	COPC	37.1	NZFR	21.0	RHCU	4.8	NVCR	3.8	SNUL	3.8	20	0	0	0	0
TG	10/15/08	45.7	899	561,211	RHCU	22.4	COPC	18.1	SNUL	7.5	NZFR	7.5	EPSX	5.3	22	181	0	0	0
LES	10/15/08	36.8	189	164,047	COPC	27.1	RDMN	17.1	EPSX	10.0	RHCU	10.0	SNUL	5.0	22	32	0	0	0
TR	10/15/08	35.5	221	135,448	NZFR	46.5	COPC	10.8	NVCV	8.7	ACMN	6.5	SPXX	5.4	19	0	0	0	0

Key to Species Codes is located in Combined Species List located in Appendix A

APF9 = *Aphanizomenon flos-aquae MSAE = Microcystis aeruginosa ABX9= Anabaena sp.*

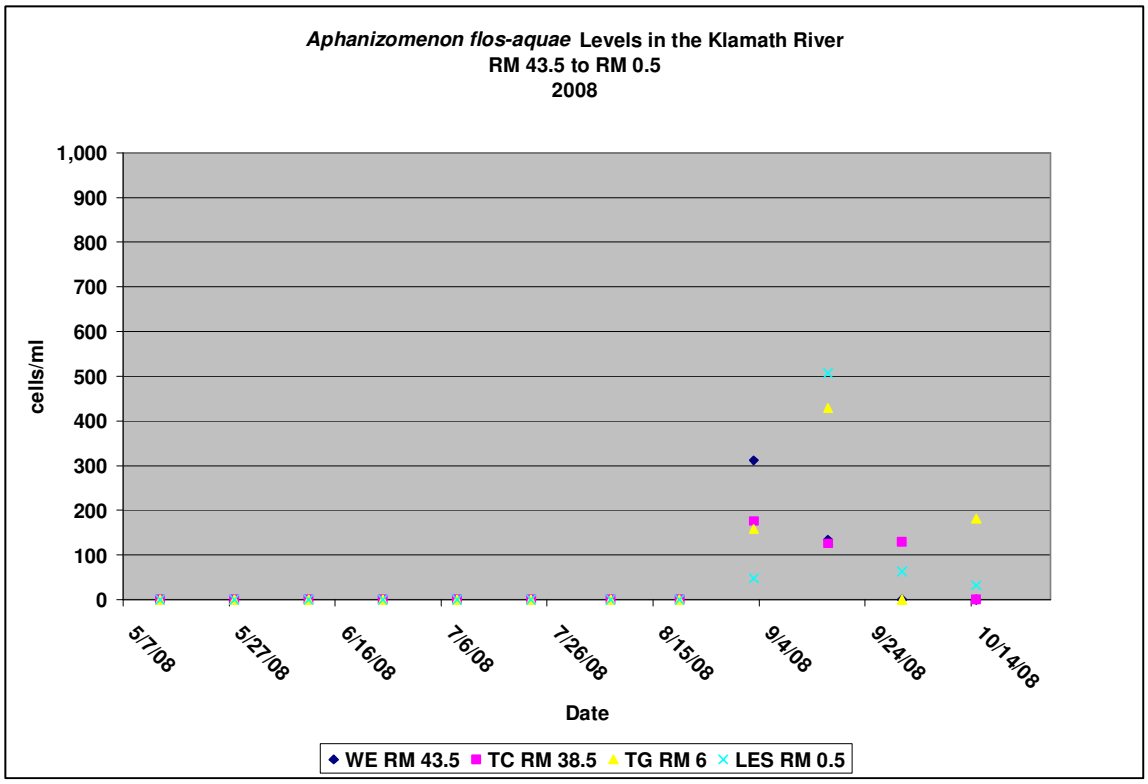

Figure 2. *Aphanizomenon flos-aquae* levels for water samples collected in the Klamath River from RM 43.5 to RM 0.5, May through October 2008.

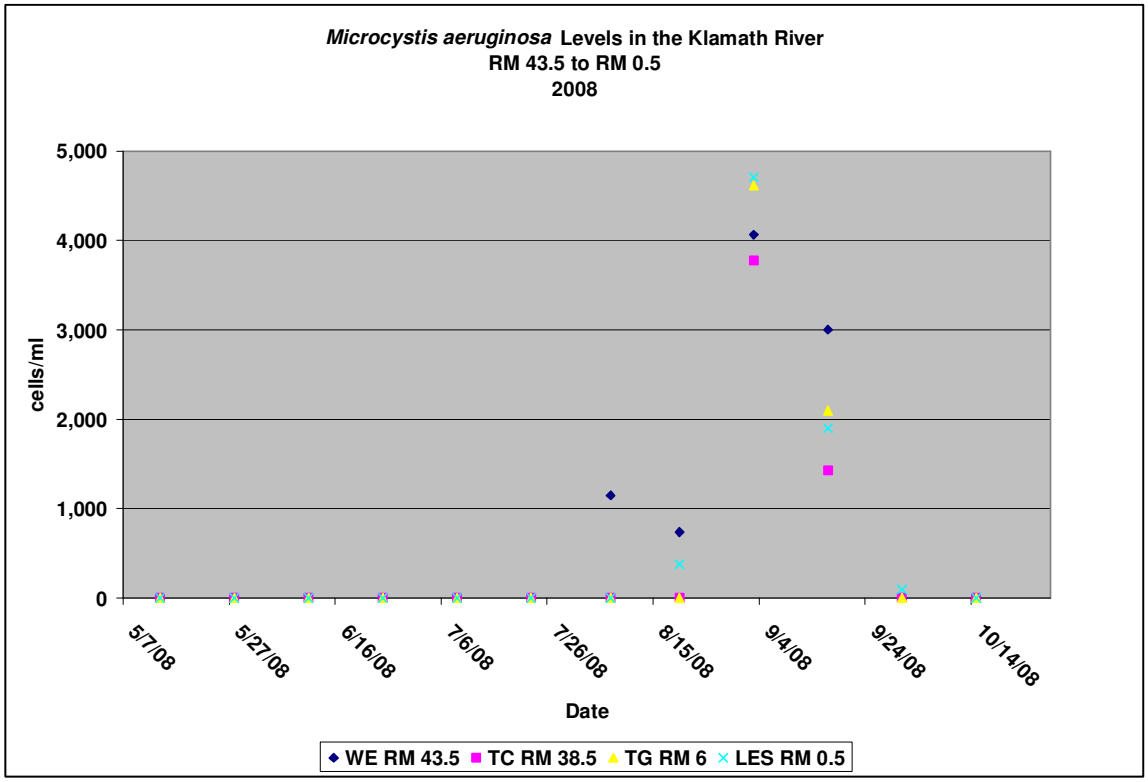

Figure 3. *Microcystis aeruginosa* levels for water samples collected in the Klamath River from RM 43.5 to RM 0.5, May through October 2008.

Table 4. *Microcystis aeruginosa* levels for water samples collected in the Klamath River Estuary, August 2008.

Site ID	Date	APF9 cells/ml	MSAE cells/ml	ABX9 cells/ml
LE 800	8/29/2008	81	1611	0
UE 101 BR	8/29/2008	384	1914	0

LE 800=Lower Estuary Surface mid channel 800meters upstream from the mouth
UE 101 BR=Upper Estuary Surface at 101 Bridge
APF9 = *Aphanizomenon flos-aquae MSAE = Microcystis aeruginosa ABX9= Anabaena sp.*

Cyanotoxins

Table 5. Microcystin results for water samples collected in the Klamath River and mouth of Trinity River, July to October 2008

Total Microcystin		Date							
units: µg/L	Site	7/9/2008	7/24/2008	8/7/2008	8/20/2008	8/29/2008	9/3/2008	9/17/2008	10/1/2008
USEPA Region 9 Lab ELISA reporting limit: 1.8 µg/L	WE	<1.8	<1.8	<1.8	<1.8	DNS	<1.8	<1.8	<1.8
	TC	<1.8	<1.8	<1.8	<1.8	DNS	<1.8	<1.8	<1.8
	TG	<1.8	<1.8	<1.8	<1.8	DNS	<1.8	<1.8	<1.8
	LES	<1.8	<1.8	<1.8	<1.8	DNS	<1.8	<1.8	<1.8
	LE 800	DNS	DNS	DNS	DNS	<1.8	DNS	DNS	DNS
	UE101BR	DNS	DNS	DNS	DNS	<1.8	DNS	DNS	DNS
	TR	<1.8	DNS	<1.8	<1.8	DNS	DNS	<1.8	<1.8

Table 6. Microcystin variants results for water samples collected in the Klamath River, July to October 2008.

Microcystin Variants								
units: µg/L	Sample Date	7/9/2008	7/24/2008	8/7/2008	8/20/2008	8/29/2008	8/29/2008	9/3/2008
CA Department of Fish and Game	Sample Site	WE	WE	WE	WE	LE800	UE101BR	WE
Water Pollution Control Laboratory	MC-RR	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
reporting limit 1.0 µg/L	MC-Demethyl-RR*	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
	MC-LR	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
	MC-Demethyl-LR*	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
	MC-YR	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
	MC-LA	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
	MC-LW	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
	MC-LF	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0

Table 7. Anatoxin-a results for water samples collected in the Klamath River, July to October 2008.

Anatoxin-a								
units: µg/L	Sample Date	7/9/2008	7/24/2008	8/7/2008	8/20/2008	8/29/2008	8/29/2008	9/3/2008
CA Department of Fish and Game	Sample Site	WE	WE	WE	WE	LE800	UE101BR	WE
Water Pollution Control Laboratory	Anatoxin-a	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
reporting limit: N/A								

VI. Discussion:

Aphanizomenon flos-aquae

Aphanizomenon flos-aquae (APF9) is a species of concern due to its ability to produce toxins and its abundance in the reservoirs managed by PacifiCorp located upstream of the YIR. To date the APF9 that is in the Klamath Basin is believed to be of a non-toxin producing strain. However, at times when the Klamath River turns bright green APF9 is present in the water column. APF9 was present in 13 of 48 samples collected in the Klamath River reach located within the YIR from RM 44 to RM 0.5 (WE,TC,TG,LES) and was detected in one of the water samples collected at the Trinity River monitoring site at low levels. Summary information of all algae species identified and enumerated in this river reach is presented in Table 3 and Appendix A. APF9 ranked as the 25th most dominant species out of 110 total species when looking at the average percent density (0.68%).

APF9 was first detected in the Klamath River on September 3, 2008 at the WE, TC, TG and LES monitoring sites. APF9 continued to be present at low levels in the Klamath River at multiple sampling sites until the end of the sampling season (October 15, 2008). The highest level of 507 cells/ml was recorded at the LES monitoring site. It is interesting to note that one sample out of twelve collected at the Trinity River monitoring site reported very low levels of APF9 at 28 cells/ml. APF9 was detected in the Trinity River on August 7, 2008. This is the first and only time that water samples collected in the Trinity River detected the presence of this species since phytoplankton sampling began in 2005. However, one periphyton sample collected at the Trinity River monitoring site in 2008 did detect APF9, so it has been documented as being present in the Trinity River. YTEP will continue to monitor phytoplankton trends over time in the Trinity River and will respond appropriately to any increase in toxicogenic cyanobacteria species.

Microcystis aeruginosa

MSAE was present in 12 of 48 samples collected in the Klamath River reach located within the YIR from RM 44 to RM 0.5 (WE,TC,TG,LES) and was not detected in any of the water samples collected at the Trinity River monitoring site. Summary information of all algae species identified and enumerated in this river reach is presented in Table 3 and Appendix A. MSAE ranked as the 34th most dominant species out of 110 total species when looking at the average percent density (0.46%). MSAE was first detected in the Klamath River on August 7, 2008 at the WE sampling site. MSAE continued to be present in the Klamath River at multiple monitoring sites through October 1, 2008. MSAE was not detected in the Klamath River on the last monitoring event of the season that took place on October 15, 2008. The highest density of MSAE occurred at the LES sampling site on September 3, 2008 and was measured at 4,707 cells/ml.

These results indicate that MSAE was present in the Klamath River within the YIR for over two months, with cell density and microcystin levels peaking at the middle of September. The timing is of significance because of the presence of adult salmon and steelhead migrating upstream during this time period. This is also a time of increased

cultural and recreational use of the Klamath River by both Tribal Members and sport fishermen.

Anabaena spp.* and *Oscillatoria spp.

Anabaena spp. and Oscillatoria spp. are also a species of concern due to their ability to produce toxins. *Anabaena spp.* was not detected in any of the primary water samples collected at the Klamath and Trinity River monitoring sites located within the YIR from RM 44 to RM 0.5 (WE,TC,TG,LES,LE800,UE101BR). However, *anabaena spp.* was detected in one of the replicate samples collected at TG on October 1, 2008 at 341 cells/ml (see table 2). Summary information of all algae species identified and enumerated in this river reach is presented in Table 3 and Appendix A.

Oscillatoria spp. was detected at the WE monitoring site on August 20, 2008 and at the TC monitoring site on September 3, 2008 at low levels. YTEP will continue to monitor phytoplankton trends over time in the Klamath River and will respond appropriately to any increase in toxicogenic cyanobacteria species.

Microcystin and Anatoxin-a

Microcystin was not present above the reporting limit of 1.8 micrograms/Liter (µg/L) in samples collected in the Klamath River reach located within the YIR from RM 44 to RM 0.5 and the mouth of the Trinity River (WE,TC,TG,LES LE800,UE101BR, TR) that were analyzed by Region 9 USEPA laboratory in Richmond, CA (see table 5). The samples submitted to USEPA's laboratory were analyzed by the ELISA method. Microcystin was not present above the reporting limit of 1.8 micrograms/Liter (µg/L) in samples collected in the Trinity River. Additional samples were submitted to CA Fish and Game's Water Pollution Control Laboratory in Rancho Cordova, CA to cross check the microcystin results from USEPA and to determine if anatoxin-a was present in the Klamath River. These water samples were analyzed by LC-MS/MS. Microcystin and anatoxin-a were not present above the reporting limit of 1 µg/L for samples that were submitted to the laboratory for testing (see tables 6 and 7).

Literature Cited

Chorus I and Bartrum J, Eds (1999). Toxic Cyanobacteria in Water: A Guide to their Public Health Consequences, Monitoring and Management. London: E & FN Spon (published on behalf of the World Health Organization).

de Magalhães VF, Soares RM and Azevedo S (2001). Microcystin contamination in fish from the Jacarepagua Lagoon (Rio de Janerio, Brazil): ecological implication and human health risk. Toxicon 39:1077-1085.

DeMott, W.R., Q. Zhang & W.W. Carmichael, *Effects of Toxic Cyanobacteria and Purified Toxins on the Survival and Feeding of a Copepod and Three Species of Daphnia*, Limnology and Oceanography, 36: 1346-1357, 1991

Fetcho, K. 2006. Klamath River Blue-Green Algae Bloom Report. Yurok Tribe Environmental Program, January 2006.
http://www.yuroktribe.org/departments/ytep/Water.htm

Lehman, P.W., G. Boyer, C. Hall, S. Waller, & K. Gehrts, *Distribution and Toxicity of a New Colonial Microcystis Aeruginosa Bloom in the San Francisco Bay Estuary, California*, Hydrobiologia 541:87-99, 2005

Vasconcelos VM (1999). Cyanobacterial toxins in Portugal: effects on aquatic animals and risk for human health. Brazilian Journal of Medical and Biological Research 32:249-254.

Appendix A

Table A-1. Combined Algae Species List for Klamath River Sites located at River Mile 44 to 0.5 (WE, TC, TG, and LES) May to October, 2008.

		48 samples Total		
#	Algae Species	Ave % Den	# samples	Code
1	Diatoma tenue	17.57	43	DTTN
2	Cocconeis placentula	13.80	46	COPC
3	Epithemia sorex	7.39	35	EPSX
4	Rhodomonas minuta	7.28	42	RDMN
5	Melosira granulata	7.24	19	MLGR
6	Achnanthes minutissima	4.92	40	ACMN
7	Nitzschia frustulum	4.70	45	NZFR
8	Rhoicosphenia curvata	4.12	45	RHCU
9	Nitzschia dissipata	2.45	24	NZDS
10	Synedra ulna	2.37	35	SNUL
11	Scenedesmus quadricauda	2.03	24	SCQD
12	Chromulina sp.	1.88	21	KMXX
13	Navicula cryptocephala	1.77	23	NVCR
14	Diatoma vulgare	1.69	29	DTVL
15	Navicula cryptocephala veneta	1.63	34	NVCV
16	Gomphonema angustatum	1.29	37	GFAN
17	Ankistrodesmus falcatus	0.96	20	AKFL
18	Cymbella minuta	0.92	23	CMMN
19	Chlamydomonas sp.	0.87	22	CHXX
20	Cymbella sinuata	0.83	26	CMSN
21	Nitzschia paleacea	0.81	24	NZPC
22	Navicula tripunctata	0.80	23	NVTP
23	Gomphonema subclavatum	0.71	25	GFSB
24	Stephanodiscus hantzschii	0.70	15	STHN
25	**Aphanizomenon flos-aquae**	**0.68**	**13**	**APF9**
26	Synedra mazamaensis	0.68	11	SNMZ
27	Achnanthes lanceolata	0.66	18	ACLC
28	Cyclotella meneghiniana	0.63	14	CCMG
29	Gomphonema ventricosum	0.61	26	GFVT
30	Cryptomonas erosa	0.55	17	CXER
31	Gomphoneis herculeana	0.54	18	GSHR
32	Cymbella affinis	0.53	19	CMAF
33	Nitzschia palea	0.47	19	NZPL
34	**Microcystis aeruginosa**	**0.46**	**12**	**MSAE**
35	Amphora perpusilla	0.40	16	AFPR
36	Gomphonema olivaceum	0.36	16	GFOM
37	Selenastrum minutum	0.33	10	SLMN
38	Navicula decussis	0.33	13	NVDC
39	Fragilaria vaucheriae	0.31	10	FRVA
40	Fragilaria construens venter	0.22	9	FRCV
41	Nitzschia communis	0.22	12	NZCM
42	Nitzschia microcephala	0.21	4	NZMC
43	Hannaea arcus	0.20	8	HNAR
44	Navicula gregaria	0.20	7	NVGR
45	Nitzschia acicularis	0.20	7	NZAC

Table A-1(contd.). Combined Algae Species List for Klamath River Sites located at River Mile 44 to 0.5 (WE,TC, TG, and LES) May to October, 2008.

		48 samples Total		
#	Algae Species	Ave % Den	# samples	Code
46	Fragilaria construens	0.15	8	FRCN
47	Sphaerocystis schroeteri	0.15	6	SFSR
48	Navicula sp.	0.15	7	NVXX
49	Achnanthes linearis	0.11	5	ACLN
50	Glenodinium sp.	0.08	3	GDXX
51	Cocconeis klamathensis	0.06	2	COKL
52	Unidentified microflagellate	0.06	2	SNUL
53	Melosira varians	0.06	3	MLVR
54	Nitzschia amphibia	0.06	3	NZAM
55	Synedra socia	0.06	2	SNSC
56	Gomphonema tenellum	0.06	3	GFTN
57	Nitzschia linearis	0.06	2	NZLN
58	Asterionella formosa	0.06	3	ASFO
59	Amphora ovalis	0.05	3	AFOV
60	Navicula graciloides	0.05	3	NVGC
61	Unidentified flagellate	0.05	3	MXFG
62	Dictyosphaerium ehrenbergianum	0.04	1	DCEH
63	Epithemia turgida	0.04	2	EPTR
64	Gomphonema truncatum	0.04	2	GFTR
65	Tetraedron minimum	0.04	1	TEMN
66	Scenedesmus acuminatus	0.04	2	SCAC
67	Pinnularia borealis	0.04	2	PLBO
68	Gloeocystis sp.	0.04	2	GLXX
69	Stephanodiscus astraea minutula	0.04	2	STAM
70	Tetraedron sp.	0.04	2	TEXX
71	Dinobryon sertularia	0.04	2	DBST
72	Pediastrum duplex	0.04	2	PSDP
73	Synedra rumpens	0.04	1	SNRM
74	Gomphonema acuminatum	0.04	2	GFAC
75	Navicula mutica	0.03	2	NVMT
76	Oscillatoria sp.	0.02	2	OSXX
77	Closteriopsis longissima	0.02	1	CBLG
78	Navicula pupula	0.02	1	NVPP
79	Rhopalodia gibba	0.02	1	RPGB
80	Gloeocystis ampla	0.02	1	GLAM
81	Synedra radians	0.02	1	SNRD
82	Scenedesmus denticulatus	0.02	1	SCDT
83	Nitzschia sp.	0.02	1	NZXX
84	Achnanthes recurvata	0.02	1	ACRC
85	Fragilaria virescens	0.02	1	FRVR
86	Gomphonema sp.	0.02	1	GFXX
87	Navicula minuscula	0.02	1	NVML
88	Synedra ulna contracta	0.02	1	SNUC
89	Fragilaria crotonensis	0.02	1	FRCR
90	Cyclotella stelligera	0.02	1	CCST

Table A-1(contd.). Combined Algae Species List for Klamath River Sites located at River Mile 44 to 0.5 (WE, TC, TG, and LES) May to October, 2008.

		48 samples Total		
#	Algae Species	Ave % Den	# samples	Code
91	Neidium affine	0.02	1	NDAF
92	Cymbella microcephala	0.02	1	CMMC
93	Gomphonema clevei	0.02	1	GFCL
94	Caloneis ventricosa	0.02	1	CAVT
95	Tabellaria fenestrata	0.02	1	TBFN
96	Fragilaria leptostauron	0.02	1	FRLP
97	Melosira italica	0.02	1	MLIT
98	Nitzschia volcanica	0.02	1	NZVL
99	Caloneis sp.	0.02	1	CAXX
100	Cymbella sp.	0.02	1	CMXX
101	Diatoma hiemale mesodon	0.02	1	DTHM
102	Coelastrum microporum	0.02	1	CUMC
103	Achnanthes exigua	0.02	1	ACEX
104	Gyrosigma spencerii	0.02	1	GYSP
105	Cocconeis pediculus	0.02	1	COPD
106	Denticula elegans	0.02	1	DNEL
107	Eunotia pectinalis	0.02	1	EUPC
108	Nitzschia fonticola	0.02	1	NZFT
109	Nitzschia capitellata	0.02	1	NZCP
110	Pinnularia sp.	0.01	1	PLXX

Table A-2 Combined Algae Species List for Mouth of Trinity River Site (TR) May to October, 2008.

		12 Samples Total		
#	Algae Species	Ave % Den	# samples	Code
1	Diatoma tenue	24.87	11	DTTN
2	Cocconeis placentula	15.40	12	COPC
3	Achnanthes minutissima	6.47	12	ACMN
4	Nitzschia frustulum	4.74	6	NZFR
5	Epithemia sorex	4.49	9	EPSX
6	Gomphonema angustatum	4.09	10	GFAN
7	Cymbella affinis	4.05	11	CMAF
8	Navicula cryptocephala veneta	2.66	9	NVCV
9	Rhodomonas minuta	2.57	11	RDMN
10	Synedra ulna	2.46	8	SNUL
11	Cymbella minuta	2.34	7	CMMN
12	Rhoicosphenia curvata	1.93	8	RHCU
13	Cymbella sinuata	1.56	9	CMSN
14	Chlamydomonas sp.	1.40	6	CHX1
15	Amphora perpusilla	1.36	8	AFPR
16	Scenedesmus quadricauda	1.34	5	SCQD
17	Gomphonema subclavatum	1.24	8	GFSB
18	Navicula tripunctata	1.01	6	NVTP
19	Fragilaria crotonensis	0.94	2	FRCR
20	Ankistrodesmus falcatus	0.88	6	AKFL
21	Hannaea arcus	0.87	6	HNAR
22	Selenastrum minutum	0.82	4	SLMN
23	Nitzschia paleacea	0.81	5	NZPC
24	Nitzschia palea	0.71	4	NZPL
25	Gomphonema olivaceum	0.68	6	GFOM
26	Navicula decussis	0.65	5	NVDC
27	Nitzschia dissipata	0.65	7	NZDS
28	Achnanthes lanceolata	0.57	6	ACLC
29	Spirogyra sp.	0.55	2	SPXX
30	Chromulina sp.	0.54	4	KMXX
31	Gomphonema ventricosum	0.44	3	GFVT
32	Melosira italica	0.40	5	MLIT
33	Achnanthes recurvata	0.39	3	ACRC
34	Navicula cryptocephala	0.35	3	NVCR
35	Achnanthes linearis	0.33	4	ACLN
36	Synedra mazamaensis	0.28	2	SNMZ
37	Fragilaria vaucheriae	0.27	1	FRVA
38	Cryptomonas erosa	0.26	3	CXER
39	Diatoma vulgare	0.26	3	DTVL
40	Navicula gregaria	0.25	3	NVGR
41	Melosira varians	0.25	1	MLVR
42	Gomphonema tenellum	0.25	3	GFTN
43	Gloeocystis ampla	0.20	1	GLAM
44	Nitzschia sp.	0.18	2	NZXX
45	Nitzschia acicularis	0.17	2	NZAC
46	Navicula sp.	0.17	2	NVXX
47	Navicula minuscula	0.17	1	NVML
48	Caloneis ventricosa minuta	0.16	2	CAVM
49	Synedra rumpens	0.16	2	SNRM
50	Sphaerocystis schroeteri	0.16	1	SFSR

Table A-2 (Contd.) Combined Algae Species List for Mouth of Trinity River Site (TR) May to October, 2008.

#	Algae Species	12 Samples Total Ave % Den	# samples	Code
51	Asterionella formosa	0.16	1	ASFO
52	Epithemia turgida	0.15	2	EPTR
53	Anabaena sp.	0.13	1	ABXX
54	Tetraedron minimum	0.12	1	TEMN
55	Gomphoneis herculeana	0.10	1	GSHR
56	Scenedesmus abundans	0.10	1	SCAB
57	Diatoma tenue elongatum	0.09	1	DTTE
58	Fragilaria capucina mesolepta	0.09	1	FRCM
59	Fragilaria leptostauron	0.09	1	FRLP
60	Navicula anglica	0.09	1	NVAG
61	Cymbella tumida	0.09	1	CMTM
62	Denticula elegans	0.09	1	DNEL
63	Navicula mutica	0.09	1	NVMT
64	Nitzschia amphibia	0.09	1	NZAM
65	Nitzschia recta	0.09	1	NZRC
66	Unidentified flagellate	0.09	1	MXFG
67	Diatoma hiemale mesodon	0.08	1	DTHM
68	Hemidinium sp.	0.08	1	HDXX
69	Caloneis sp.	0.08	1	CAXX
70	Cyclotella stelligera	0.08	1	CCST
71	Fragilaria construens venter	0.07	1	FRCV
72	Nitzschia fonticola	0.07	1	NZFT
73	Nitzschia linearis	0.07	1	NZLN
74	Aphanizomenon flos-aquae	0.06	1	APF9

Appendix B
Grab Sample Protocol

'Grab sampling' refers to water samples obtained by dipping a collection container into the upper layer of a body of water and collecting a water sample (USGS File Report -00213). For quality assurance/quality control (QA/QC) purposes replicate, and blank bottle sets will be prepared and collected for one site each sampling period. These additional bottle sets will be handled, prepared and filled following the same protocol used for regular bottle sets and samples. General water quality parameters will also be measured with a freshly calibrated portable multi-probe water quality instrument during grab samples and recorded onto data sheets.

Upon arrival at each site, the sampling churn will be rinsed three times with distilled water. The goal of rinsing is 'equipment decontamination – the removal from equipment, residues from construction and machining and the removal of substances adhering to equipment from previous exposure to environmental and other media' (USGS Open File Report 00213). After rinsing with D.I. water, the churn will be rinsed three times with stream water. The churn is then fully submerged into the stream and filled to the lid with sample water. Completely filling the churn allows for all samples to be filled from one churn; thereby minimizing differences in water properties and quality between samples.

Proper use of the churn guarantees the water is well mixed before the sample is collected. The churn should be stirred at a uniform rate by raising or lowering the splitter at approximately 9 inches per second (Bel-Art Products, 1993). This mixing must continue while the bottles are being filled. If filling is stopped for some reason, the stirring rate must be resumed before the next sample is drawn from the churn. As the volume of water in the churn decreases, the round trip frequency increases as the velocity of the churn splitter remains the same. Care must be taken to avoid breaking the surface of the water as the splitter rises toward the top of the water in the churn.

Sample bottles and chemical preservatives used were provided by associated laboratories and were considered sterile prior to field usage. Sample bottles without chemical preservatives were rinsed with stream water from the churn 2-3 times before filling with sample water. In the case of bottles that contained chemical preservatives, bottles were not rinsed before sample collection and care was taken to avoid over-spillage that would result in chemical preservative loss. Collected samples will be placed in coolers on ice or dry ice for transport to contracted laboratories for analysis.

QA/QC – Replicate bottle set

To ensure laboratory and sampling accuracy, one site every sampling period was randomly selected to receive one additional QA/QC bottle set. This bottle set contain replicate water samples. Replicate samples are obtained using the same process as regular samples. These are used to assure the laboratory maintains precision within results.

All bottle sets are then placed on ice and are transported to the associated laboratories. All grab samples were processed within 24 hours or within known laboratory holding periods.

Bibliography

Bel-Art Products. Churn Sample Splitter Instructions, 37805 Series. Pequannock, NJ, 1993.

Eaton, Andrew D., Lenore S. Clesceri, and Arnold E. Greenberg., ed. Standard Methods for the Examination of Water and Wastewater. 19th Edition. Washington D.C., 1995.

Lurry,D.L. and C.M. Kolbe. Interagency field manual for the collection of Water Quality Data. USGS Publication, Open File Report 00-213.