FINAL
2007 Klamath River Blue-Green Algae Summary Report

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Acknowledgements

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I. Introduction

This report summarizes the occurrence and extent of the blue-green algae bloom on the Klamath River within the Yurok Indian Reservation (YIR) boundaries in 2007. The Karuk Tribe will be publishing a summary report that details the results for the algae and nutrients conditions in Copco and Iron Gate Reservoirs. The Yurok Tribe Environmental Program (YTEP), the Karuk Tribe, BOR and PacifiCorp collected water samples in 2007 indicating that the water quality in the Klamath River was negatively impacted by levels of the cyanobacterium *Microcystis aeruginosa* (MSAE) and its resultant toxin, microcystin; with some levels exceeding World Health Organization (WHO) risk guidelines in all reaches of the Klamath River from downstream of Upper Klamath Lake to the Klamath River Estuary. Water quality sampling performed in 2005 and 2006 detected MSAE and microcystin in the Klamath River below Iron Gate Dam. The Karuk Tribe and YTEP were integral in collecting this data and sharing results with the water quality regulators and the local community.

Information on Microcystis aeruginosa

MSAE is a type of blue-green algae which, upon death and decomposition, releases the hepatoxin microcystin, which can cause any of the following reactions in humans and/or animals: rash, irritation, conjunctivitis, nausea, vomiting, diarrhea, liver damage, tingling, numbness, paralysis, and death. Microcystin bioaccumulates in the liver, organs, and to a certain degree the muscle mass of living animals. Microcystin is not excreted by animals, and dosage over time will eventually cause liver damage, decreased liver function and increased liver size, and eventually death. Mortality in fish, domestic animals, and humans has been recorded following exposure to microcystin resultant from both single-dose events and long-term exposure.

The toxin microcystin can produce negative health effects from contact with impaired waters, from incidental nasal/oral ingestion of impaired waters, and, most seriously, from swallowing or drinking impaired waters. Microcystin can be found in the organs and muscle meat of fish who feed in impaired waters. Continued exposure to even low levels of microcystin can produce harmful cumulative response in humans and animals. Because the timing for likely bloom occurrence coincides with the annual salmon runs along the Klamath River, there is significant cause for concern; Tribal subsistence fishers not only have increased contact with impaired waters due to subsistence fishing methods, they are also more frequently visiting impaired waters than recreational or sport users.

Initial bloom characteristics are green, green-blue, yellow, or whitish waters; blooms of increasing severity can form algal mats or visible surface scum, initially in nearshore areas and backwaters or where winds cause surface detritus to accumulate, and steadily progressing to cover open areas of still or slow-moving waters. Odor and taste changes may become noticeable at any time as decomposition of organisms sets in, but should not be used as an indicator for cellular mortality.

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.

II. Methods

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 determination 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. TG090107).

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.). 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 (CA) 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.

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 (July 10, 2007) additional samples were collected beginning on July 24, 2007 and continued through October to test for the presence of microcystin. The Trinity River monitoring site was sampled for nutrient analysis and algae speciation and enumeration on a monthly basis in order to save resources. Biweekly sampling did not occur in the Trinity River

because the routine algae speciation results showed that no toxicogenic cyanobacteria were present. However, algae speciation and enumeration and toxin sampling event did occur in the Trinity River on a monthly interval to maintain a long-term dataset and to verify the hypothesis that toxicogenic cyanobacteria sources are from locations on the Klamath River upstream of the YIR boundaries.

The Klamath River monitoring site below the confluence with the Trinity River (KBW) was relocated downstream approximately four miles to the Klamath River above Tully Creek monitoring site (TC) on September 5, 2007. This monitoring site was relocated due to YTEP's access being denied by the landowner. The data from both of these sites are considered comparable and representative of the conditions downstream of the Klamath and Trinity River confluence.

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**
- **KBW - Klamath River below Weitchpec RM 42.5**
- **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 edge water habitats in the Klamath River Estuary at various times during the 2007 monitoring season to determine if elevated levels of toxicogenic cyanobacteria were present. These results are provided in this report separately and are clearly identified as unique sampling locations (see figure 2).

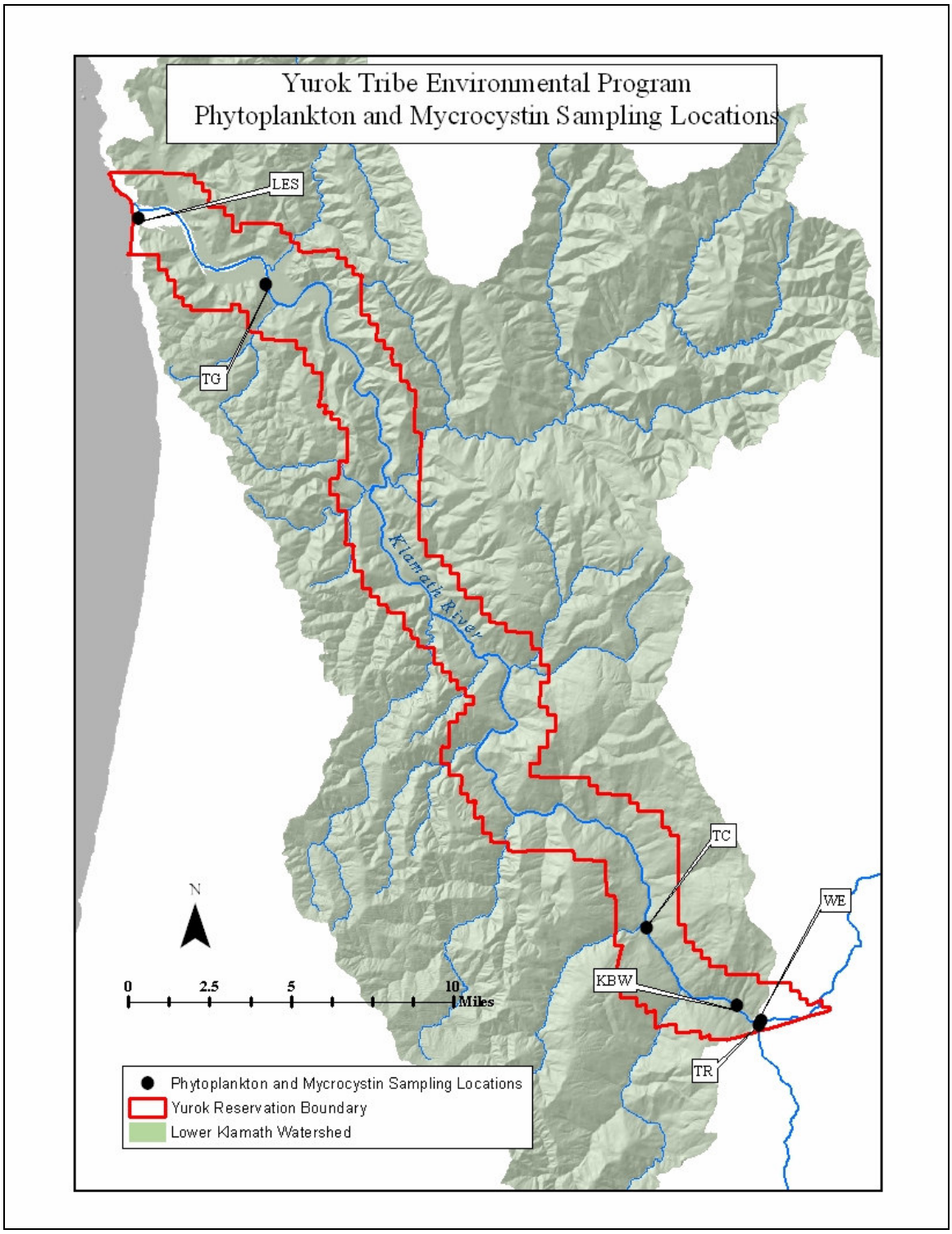

Figure 1. Map of phytoplankton and microcystin monitoring locations, 2007.

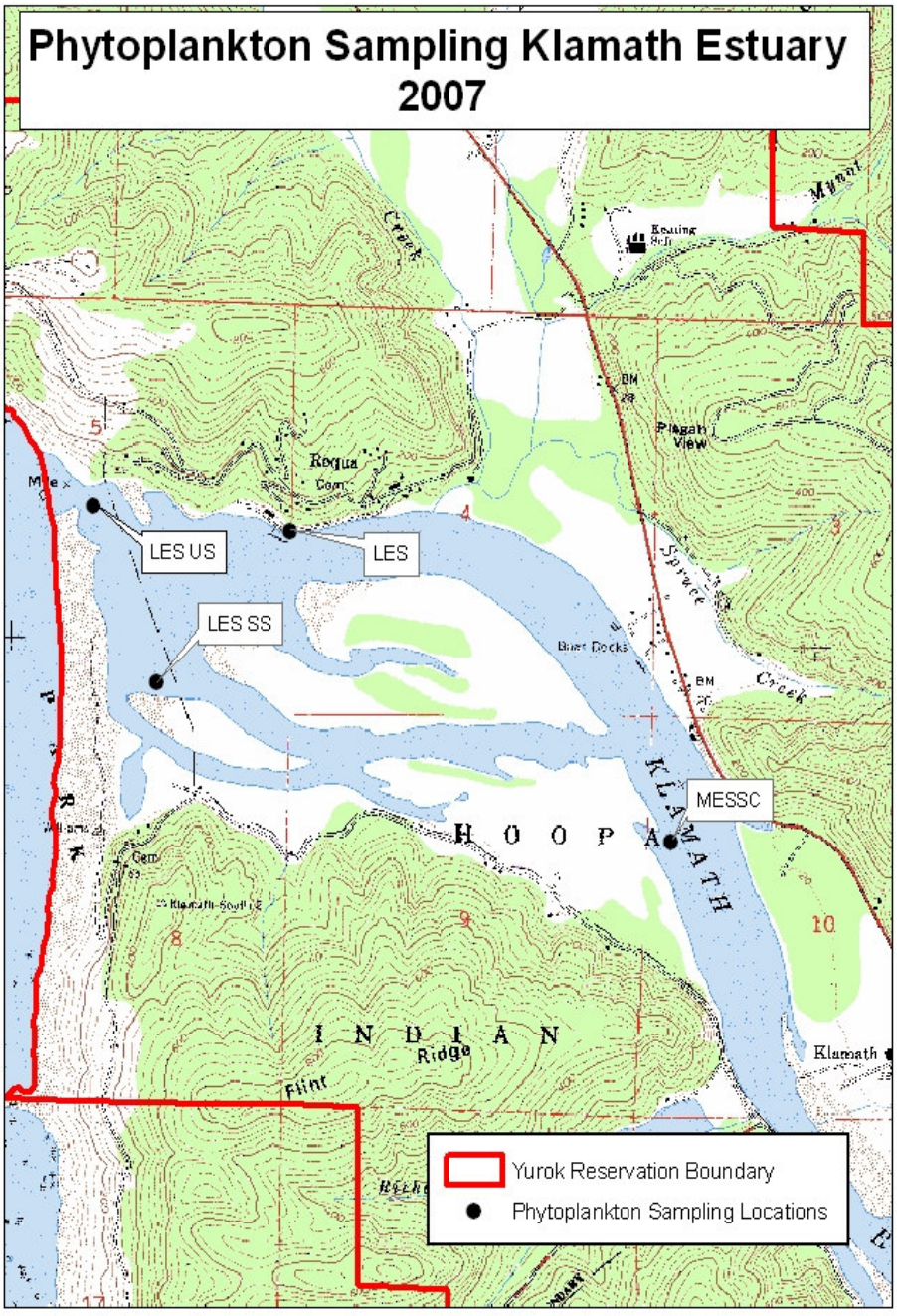

Figure 2. Map of phytoplankton monitoring locations including edge water habitats in the Klamath River Estuary, 2007.

IV. Quality Assurance

YTEP performs all surface water quality monitoring activities consistent with its Quality Assurance Program Plan that was approved by the USEPA in April 2001. 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 on a monthly basis to determine the labs' precision of data. 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 will visually inspect 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. Samples collected in May, June and October did not contain MSAE, therefore, no relative percent differences were calculated for these months. The common algae in each replicate did change rank for most samples collected between May and October (see table 3). 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 July QA replicate sample did contain low levels of MSAE (1,101 cells/ml) but the primary sample did not report the presence of MSAE, therefore a RPD was not calculated. The August QA replicate did contain MSAE, the primary sample reported 8,054 cells/ml and the replicate sample was 21,867 cells/ml, the RPD was 92.3%. The September samples contained the highest levels of MSAE reported for the 2007 season. The September primary sample was 90,764 cells/ml and the replicate sample was 34,133 cells/ml, the RPD was 90.7%.

The replicate results indicate that when MSAE is significantly present in water samples the variability in replicate sample results increases. The variability of the results needs to be viewed in light of the main objective of the phytoplankton sampling, which is to inform the public if water contact poses a health risk. The August results have a high RPD (92.3%) but both the primary and the replicate sample results are below the State of CA recommended threshold to post a waterbody when MSAE exceeds 40,000 cells/ml. The September results do pose a question in regards to deciding on whether to post a waterbody or not.

The September results from additional sites located up-river from the site in which replicate samples were collected (LES) in September aided YTEP in making the decision to post the Klamath River within YIR boundaries following the guidance provided by the State of California. In this case it was apparent that posting should occur because the most upstream monitoring site on the YIR also exhibited high levels of MSAE. The Klamath River site at Weitchpec that is above the Trinity River confluence had MSAE cell densities of 80,016 cells/ml. YTEP was justified in posting due to the fact that two sites on the reservation exhibited cell densities above the posting threshold and the fact that samples collected upstream of the YIR by the Karuk Tribe reported levels at three mainstem Klamath River monitoring sites ranging from 42,281 to 90,054 cells/ml indicating that a significant risk was present to those who would have had contact with the Klamath River downstream of Iron Gate Dam and could continue for some time.

Microcystin

QA replicate samples collected by both the Karuk Tribe and YTEP indicate that the toxin results are valid and acceptable. All of the replicate samples submitted to the lab generated RPD's less than 25% (see table 2). These results are delivered after YTEP

receives phytoplankton results, therefore, the toxin results were not used as the primary source of information for determination of the need to post.

V. Results:

Phytoplankton QA

Table 1. Phytoplankton results for the QA replicate samples collected by Karuk Tribe and YTEP, 2007.

Lab Slide ID	Station ID	Date	Total Density	Total Biovolume	TSI	Sp1	Sp1%	Sp2	Sp2%	Sp3	Sp3%	Sp4	Sp4%	Sp5	Sp5%	#Spp	APF9 cells/ml	MSAE cells/ml
KN03	TG	5/30/07	1,147	401,260	43.3	DTTN	21.7	NZFR	15.7	ACMN	13.0	RHCU	8.7	COPC	7.8	26	0	0
KN02	TG rep	5/30/07	1,028	324,550	41.7	DTTN	29.1	NZFR	14.6	ACMN	11.7	NZDS	5.8	NVCV	5.8	21	0	0
KP58P	SC	6/26/07	3,750	477,457	44.5	ACMN	83.9	CMMN	5.6	CMAF	2.8	DTTN	1.4	NVCR	1.4	11	0	0
KP55P	SC rep	6/26/07	3,769	478,245	44.5	ACMN	82	CMMN	7.4	CMSN	2.5	CMAF	1.6	NVCR	1.6	11	0	0
KN22	LES	7/24/07	706	70,594	31	KMXX	50.8	RDMN	22.6	SLMN	5.6	COPC	4.0	AKFL	3.2	15	0	0
KN24	LES rep	7/24/07	156	68,304	30.6	COPC	12.3	RDMN	12.3	EPSX	7.7	MSAE	7.7	SCQD	6.2	26	0	1,101
LA38P	KRBI	8/21/07	5,450	7,040,486	63.9	APF9	93.6	RDMN	1.5	GFVT	1	COPC	1	MSAE	1	9	107,113	8,054
LA39P	KRBI rep	8/21/07	5,631	5,532,622	62.2	APF9	82.5	NZPL	5.3	MSAE	3.9	CHXX	1.5	CXER	1.5	13	78,993	21,867
KN46	LES	9/18/07	1,738	1,074,461	50	RDMN	53.2	MSAE	12.6	CXER	4.5	COPC	4.5	NZFR	4.5	22	0	90,764
KN45	LES rep	9/18/07	1,321	537,601	45.4	RDMN	52.6	EPSX	7.8	MSAE	7.8	NZFR	5.2	SCQD	5.2	20	0	34,133
LF29P	KRBI	10/16/07	237	219,195	38.9	RDMN	32.5	CXER	26.3	MLGR	10.5	COPC	9.6	GFAN	4.4	20	0	0
LF30P	KRBI rep	10/16/07	505	206,473	38.5	RDMN	66.4	CXER	15.3	MLGR	3.6	COPC	2.9	GFAN	1.5	19	0	0

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

Microcystin QA

Table 2. Microcystin results for the QA replicate samples collected by Karuk Tribe and YTEP, 2007.

site ID	primary result	duplicate result	difference	RPD
LES072407	<1.8	<1.8	0.0	0.0
KRBI082107	5.4	4.7	0.7	13.9
LES091807	<1.8	<1.8	0.0	0.0
KRBI 101607	<0.18	<0.18	0.0	0.0

Phytoplankton

Table 3. Phytoplankton results for water samples collected in the Klamath River and mouth of Trinity River May - October 2007.

Site ID	Date	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	5/30/07	1,579	445,222	NZFR	20.5	ACMN	18.8	RHCU	11.6	DTTN	8.9	COPC	7.1	21	0	0	0	0
KBW	5/30/07	910	308,706	DTTN	16.8	ACMN	12.4	NZFR	9.7	RHCU	8.0	CMMN	6.2	29	0	0	0	0
TG	5/30/07	1,147	401,260	DTTN	21.7	NZFR	15.7	ACMN	13.0	RHCU	8.7	COPC	7.8	26	0	0	0	0
LES	5/30/07	1,039	281,086	DTTN	27.1	ACMN	17.8	NZFR	9.3	NZDS	5.4	STHN	5.4	27	0	0	0	0
TR	5/30/07	665	189,570	DTTN	58.8	ACMN	14.7	EPSX	4.9	NVCV	2.0	ACLC	2.0	20	0	0	0	0
WE	6/12/07	1,010	376,105	ACMN	20.4	COPC	16.5	RHCU	9.7	NZFR	8.7	CMAF	7.8	24	0	0	0	0
KBW	6/12/07	814	264,242	RDMN	15.8	NZFR	15.8	ACMN	12.3	COPC	8.8	DTTN	8.8	26	0	0	0	0
TG	6/12/07	2,372	1,037,347	DTTN	26.7	NZFR	17.8	ACMN	10.9	COPC	9.9	CMAF	8.9	16	0	0	0	0
LES	6/12/07	7,517	3,100,700	DTTN	24.0	NZFR	13.0	CMAF	12.0	RDMN	11.0	COPC	10.0	23	0	0	0	0
WE	6/26/07	631	223,815	COPC	30.9	ACMN	14.9	NZFR	9.6	RHCU	8.5	NVCV	4.3	22	0	0	0	0
KBW	6/26/07	844	404,883	COPC	29.0	ACMN	13.1	CMAF	8.4	RHCU	8.4	NZFR	6.5	27	0	0	0	0
TG	6/26/07	668	312,561	COPC	19.2	NZFR	12.1	DTTN	7.1	CMAF	7.1	ACMN	4.0	29	0	0	0	0
LES	6/26/07	374	111,803	COPC	23.3	NZFR	7.0	ACMN	7.0	CHXX	7.0	DTTN	7.0	24	0	0	0	0
TR	6/26/07	524	362,106	SNUL	20.0	COPC	19.0	DTTN	11.0	ACMN	8.0	GFAN	7.0	26	0	0	0	0
WE	7/10/07	395	205,770	COPC	42.1	SCQD	6.6	EPSX	6.6	SNUL	4.1	CMAF	4.1	22	0	0	0	0
KBW	7/10/07	413	266,618	COPC	30.9	CMAF	9.3	EPSX	7.2	SLMN	5.2	RHCU	5.2	30	0	0	0	0
TG	7/10/07	606	291,771	COPC	10.9	CMAF	9.8	ACMN	9.8	EPSX	8.7	SCQD	7.6	31	0	0	0	0
LES	7/10/07	262	133,065	COPC	15.1	ACMN	11.8	EPSX	9.7	CMAF	7.5	NZFR	6.5	25	0	0	0	0
WE	7/24/07	916	513,052	COPC	20.5	SCQD	15.4	EPSX	11.1	NZPL	5.1	SCAC	4.3	32	0	3,124	0	0
KBW	7/24/07	655	367,253	COPC	26.4	EPSX	16.0	SCQD	10.4	SNUL	5.7	NZFR	5.7	24	0	0	0	0
TG	7/24/07	929	509,862	SCQD	14.7	DTTN	9.8	RDMN	8.8	COPC	8.8	EPSX	5.9	32	0	0	0	0
LES	7/24/07	706	70,594	KMXX	50.8	RDMN	22.6	SLMN	5.6	COPC	4.0	AKFL	3.2	15	0	0	0	0
TR	7/24/07	264	133,177	COPC	34.2	EPSX	7.0	NZFR	6.1	CMAF	5.3	ACLC	4.4	29	0	0	46	0
WE	8/7/07	1,293	1,102,795	EPSX	43.4	COPC	10.1	NZFR	8.5	SNUL	6.2	APF9	6.2	20	1,473	6,013	0	0
KBW	8/7/07	1,062	751,149	EPSX	32.3	COPC	15.1	NVCR	6.5	NZFR	6.5	APF9	6.5	20	822	4,567	0	0
TG	8/7/07	741	771,305	EPSX	33.7	SNUL	8.9	COPC	6.9	NZFR	5.9	NZPC	5.9	27	0	0	0	0
LES	8/7/07	303	225,405	EPSX	15.7	APF9	12.4	RDMN	10.1	SNUL	6.7	AKFL	5.6	29	562	0	34	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 2007.

Site ID	Date	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	8/21/07	2,176	1,517,892	EPSX	20.0	NZFR	17.3	APF9	12.7	NVCR	6.4	SNUL	5.5	25	4,154	9,890	0	0
KBW	8/21/07	1,303	1,030,585	EPSX	32.7	NZFR	16.3	APF9	10.6	MSAE	7.7	COPC	7.7	18	2,067	18,040	0	0
TG	8/21/07	2,328	1,852,435	EPSX	27.0	NZFR	12.6	SNUL	8.1	SCQD	6.3	DTTN	5.4	25	629	4,195	210	0
LES	8/21/07	409	470,390	EPSX	25.0	DTTN	13.0	APF9	10.9	COPC	6.5	RDMN	6.5	26	712	4,003	0	0
TR	8/21/07	165	67,648	COPC	25.0	DTTN	13.1	SLMN	8.3	EPSX	8.3	NVCV	7.1	27	0	0	0	0
WE	9/5/07	1,811	1,099,073	EPSX	24.5	NZFR	18.9	COPC	7.5	RDMN	4.7	SNUL	3.8	25	0	7,517	0	0
TC	9/5/07	1,148	918,871	EPSX	27.3	COPC	9.1	SCQD	7.3	MSAE	6.4	NZFR	6.4	27	0	16,443	0	0
TG	9/5/07	2,875	2,459,548	EPSX	30.6	NZFR	10.5	SNUL	7.7	SCQD	5.7	NVCR	5.7	30	0	18,405	0	0
LES	9/5/07	847	404,360	RDMN	25.2	DTTN	11.3	CCMG	11.3	SCQD	10.4	EPSX	7.0	26	0	11,791	0	0
WE	9/18/07	1,358	1,294,346	COPC	22.3	EPSX	17.9	MSAE	13.4	NZFR	10.7	NVCR	6.3	25	0	80,016	0	0
TC	9/18/07	1,465	824,823	EPSX	20.0	COPC	19.1	NZFR	13.0	NVCV	7.8	MSAE	7.0	25	0	19,773	0	0
TG	9/18/07	2,405	1,003,685	NZFR	18.8	EPSX	17.0	RHCU	11.6	SCQD	6.3	MSAE	6.3	26	0	21,498	0	0
LES	9/18/07	1,738	1,074,461	RDMN	53.2	MSAE	12.6	CXER	4.5	COPC	4.5	NZFR	4.5	22	0	90,764	0	0
TR	9/18/07	98	35,960	DTTN	20.3	RDMN	10.9	EPSX	10.9	ACMN	7.8	COPC	7.8	24	0	0	0	0
WE	10/2/07	1,948	1,410,656	COPC	26.9	EPSX	20.4	NVCR	8.3	NVCV	6.5	NZFR	6.5	26	0	21,648	0	0
TC	10/2/07	1,337	920,406	COPC	27.0	EPSX	21.6	NZFR	9.0	NVCR	7.2	SNUL	5.4	22	0	7,228	0	0
TG	10/2/07	999	586,928	RDMN	26.7	COPC	15.2	EPSX	13.3	NZFR	9.5	DTTN	7.6	21	0	21,884	0	0
LES	10/2/07	395	259,235	COPC	14.5	DTTN	12.0	EPSX	10.8	NZFR	9.6	MSAE	9.6	27	0	13,777	0	0
WE	10/15/07	1,489	1,640,785	SNUL	22.8	COPC	14.9	NZFR	11.9	NVCR	9.9	DTVL	7.9	23	0	0	0	147
TC	10/15/07	838	679,823	SNUL	23.9	COPC	16.8	NVCR	10.6	DTTN	9.7	NZFR	7.1	21	0	0	0	0
TG	10/15/07	1,006	701,086	DTTN	25.3	COPC	17.2	EPSX	11.1	SNUL	10.1	DTVL	5.1	25	0	0	0	0
LES	10/15/07	272	196,572	DTTN	27.8	COPC	22.2	SNUL	12.2	EPSX	7.8	SNMZ	4.4	19	0	0	0	0
TR	10/15/07	261	117,991	DTTN	52.0	COPC	9.2	EPSX	8.2	SNUL	6.1	NZPC	5.1	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.*

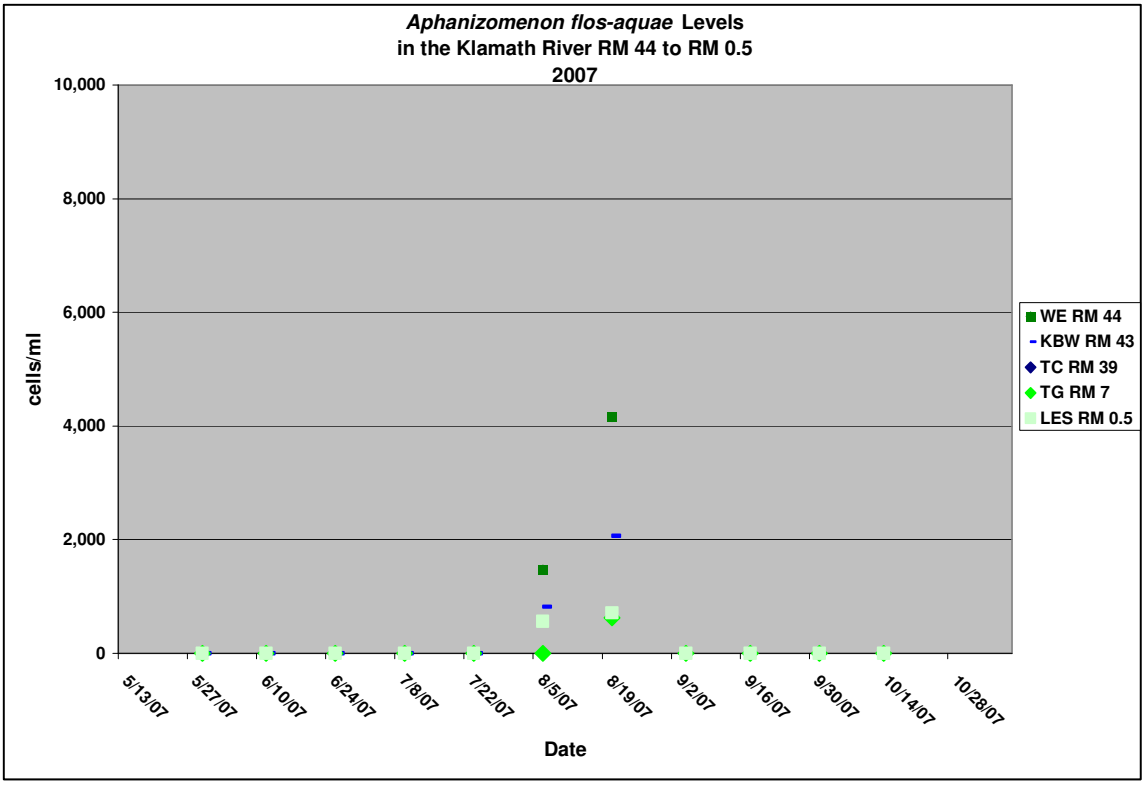

Figure 3. *Aphanizomenon flos-aquae* levels for water samples collected in the Klamath River from RM 44 to RM 0.5, May through October 2007.

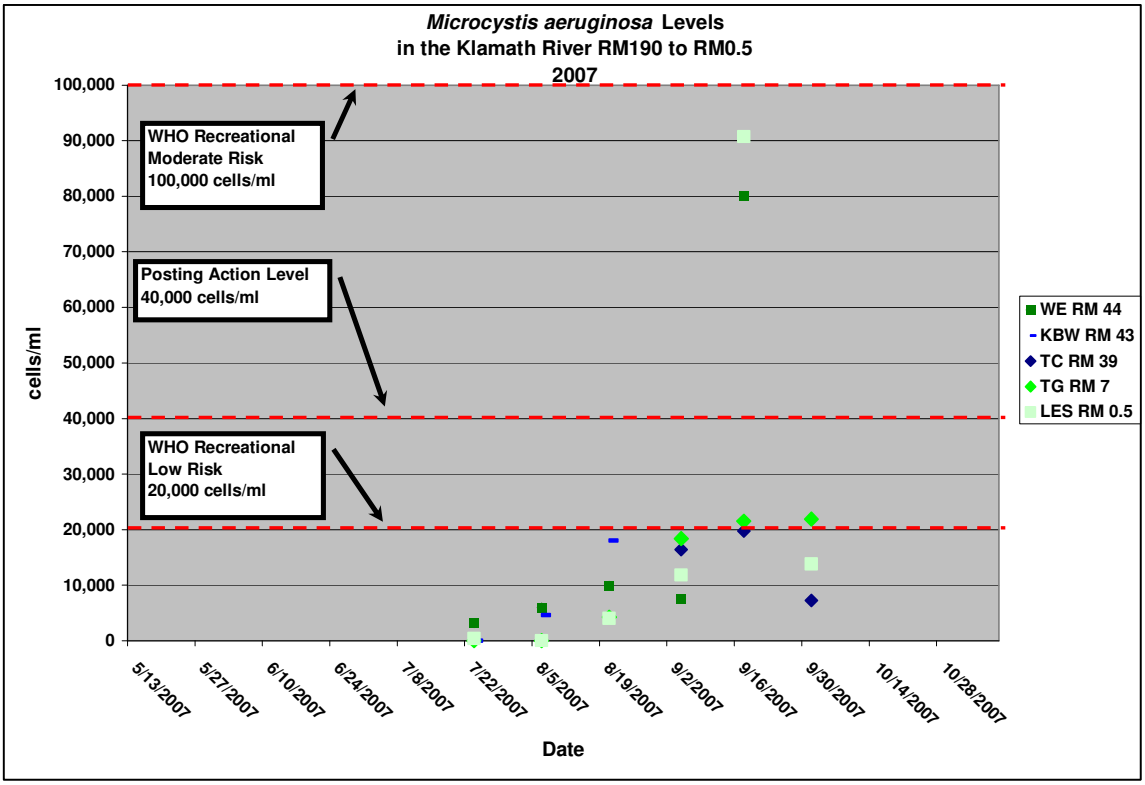

Figure 4. *Microcystis aeruginosa* levels for water samples collected in the Klamath River from RM 44 to RM 0.5, May through October 2007.

Table 4. *Microcystis aeruginosa* levels for water samples collected in edgewater habitats located in the Klamath River Estuary , May through September 2007.

Site ID	Date	APF9 cells/ml	MSAE cells/ml	ABX9 cells/ml
MES SC	5/21/2007	0	0	0
LES SS	7/30/2007	0	0	101
MES SC	9/12/2007	0	3,373,154*	410,000*
LES US Chute	9/12/2007	0	20,231	0

* Note: *Microcystis* counts calculated from observing an area 12 times greater than the rest of the sample.
APF9 = *Aphanizomenon flos-aquae* MSAE = *Microcystis aeruginosa* ABX9= *Anabaena sp.*
MES SC=Middle Estuary Surface Side Channel
LES SS=Lower Estuary Surface outlet of South Slough
LES US Chute=Lower Estuary Surface Upstream of Chute

Microcystin

Table 5. Microcystin results for water samples collected in the Klamath River from RM 44 to RM 0.5, July to October 2007.

Total Microcystin
units: µg/L
USEPA Region 9 Lab ELISA
quanitiation limit: 1.8 µg/L

	Date						
Site	7/24/2007	8/7/2007	8/21/2007	9/5/2007	9/18/2007	10/2/2007	10/15/2007
WE	<1.8	<1.8	1.8	<1.8	<1.8	<1.8	DNS
KBW	SL	<1.8	2.0	DNS	DNS	DNS	DNS
TC	DNS	DNS	DNS	<1.8	<1.8	<1.8	DNS
TG	<1.8	<1.8	<1.8	<1.8	<1.8	<1.8	DNS
LES	<1.8	<1.8	<1.8	<1.8	<1.8	<1.8	DNS
TR	<1.8	DNS	<1.8	DNS	<1.8	DNS	DNS

DNS = Did Not Sample

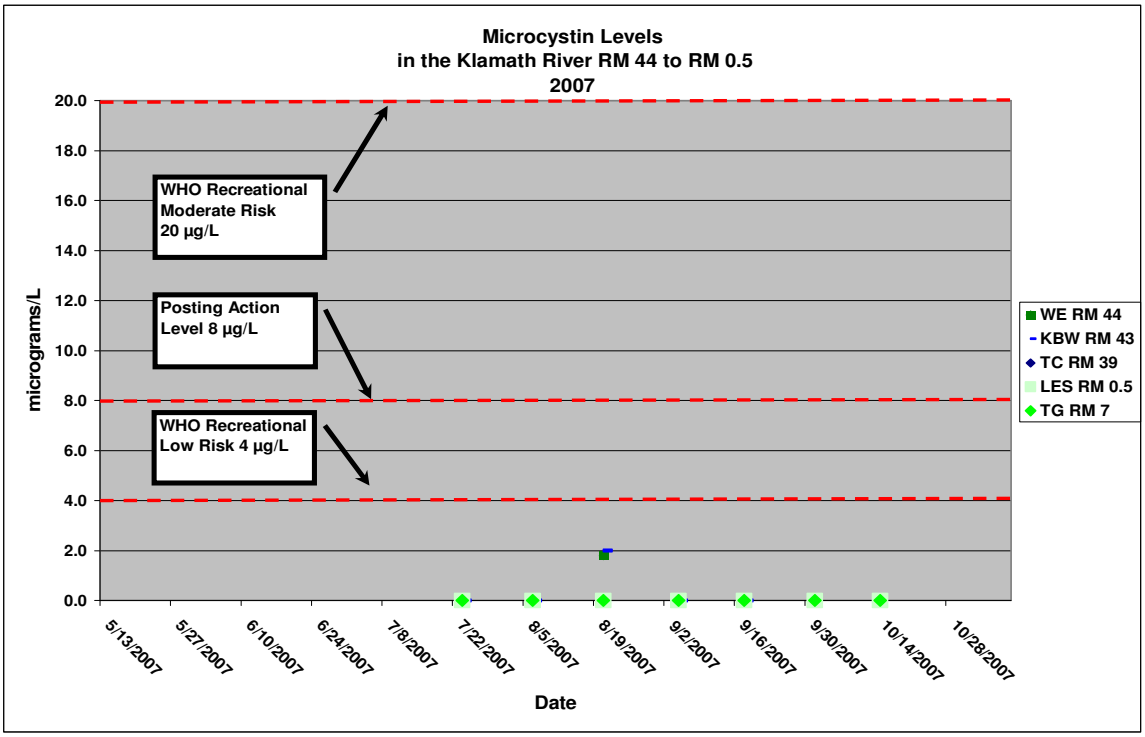

Figure 5. Microcystin levels for water samples collected in the Klamath River from RM 44 to RM 0.5, July through October 2007.

VI. Discussion:

Phytoplankton

Aphanizomenon flos-aquae

Aphanizomenon flos-aquae 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. *Aphanizomenon flos-aquae* was present in 7 of 50 samples collected in the Klamath River reach located within the YIR from RM 44 to RM 0.5 (WE,KBW,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 Appendix A. *Aphanizomenon flos-aquae* ranked as the 17th most dominant species out of 105 total species when looking at the average percent density (1.2%). *Aphanizomenon flos-aquae* was first detected on August 7, 2007 at the WE, KBW and LES monitoring sites. *Aphanizomenon flos-aquae* continued to be present in the Klamath River at multiple sampling sites on 8/21/07 and reached its highest level of 4,154 cells/ml at the WE monitoring site. *Aphanizomenon flos-aquae* was not detected in the Klamath River within the YIR during subsequent sampling events.

Microcystis aeruginosa

MSAE was present in 19 of 50 samples collected in the Klamath River reach located within the YIR from RM 44 to RM 0.5 (WE,KBW,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 Appendix A. MSAE ranked as the 13th most dominant species when looking at the average percent density (2.1%). MSAE was first detected on July 24th, 2007 at the WE sampling site. MSAE continued to be present in the Klamath River at multiple monitoring sites through October 2, 2007. MSAE was not detected in the Klamath River on the last monitoring event of the season that took place on October 15, 2007.

The highest density of MSAE occurred at the LES sampling site on September 18, 2007 and was measured at 90,764 cells/ml. This was the sampling event in which multiple sites in the Klamath River within the YIR exceeded the cell density posting action level of 40,000 cells/ml. These results triggered a response by YTEP to post notices informing the public to avoid contact with the Klamath River. This posting took place in conjunction with the State of CA and Humboldt County posting the river upstream of the YIR.

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 near 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.

Estuary Edge Water Habitats

Supplemental water samples were collected in the Klamath River Estuary in May, July and September to determine if toxicogencic cyanobacteria species were thriving in edge water habitats (see figure 2 and table 5). Two samples were collected (May 21 and September 12) in the middle estuary side channel that is located across from the Klamath River Jet Boat Tour's boat docks. One sample was collected at the outlet of the South Slough in the lower estuary on July 30, 2007 after YTEP staff observed filamentous algae growth and some surface accumulations that were in close proximity to macrophytes. Another sample was collected in the lower estuary just upstream of the chute on September 12, 2007 after the mouth had constricted and was causing the estuary water level to increase.

These additional water samples have indicated that the edge water habitats contain elevated levels of toxicogenic cyanobacteria when species in the free flowing river are present. High levels of *Anabaena spp*. and *MSAE*, 410,000 and 3,373,154 cells/ml respectively, were detected in the middle estuary when the routine river monitoring sites were reporting elevated levels. It is important to note that for this particular sample (MES SC 091207), it was important to have a reliable estimate of the Microcystis density, but not the other algae present in the sample. The taxonomist analyzed a lot more of the sample (12 times as much), but only counted Microcystis in that extra analysis. The MSAE counts were back-calculated (divided by 12) to adjust for the extra area observed on the microscope slide. These levels are the highest ever reported in the Klamath River within the YIR. No toxin samples were collected during these additional sampling events.

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 present in 4 of 50 samples collected in the Klamath River reach located within the YIR from RM 44 to RM 0.5 (WE,KBW,TC,TG,LES). Summary information of all algae species identified and enumerated in this river reach is presented in Appendix A. *Anabaena spp* was detected at low levels (highest level was 210 cells/ml at TG) at the routine monitoring sites during the scheduled events. The highest recorded level of *Anabaena spp.* was 410,000 cells/ml which occurred on September 12, 2007 when a sample was collected in edge water habitat located in a middle estuary side channel.

It is interesting to note that one sample out of five collected at the Trinity River monitoring site reported very low levels of *Anabaena spp.* at 46 cells/ml. This detection potentially may have been a result of wildlife transporting cells from the Klamath River due to the monitoring site location's close proximity the Trinity River at the confluence. This detection potentially may also have been a result of cross contamination at the lab or by field personnel. YTEP's blank QA samples in the past have showed very low presence or no presence of algae cells. YTEP will continue to monitor phytoplankton trends over time in the Trinity River and will respond appropriately to any increase in toxicogenic cyanobacteria species.

Oscillatoria spp. was detected at the WE monitoring site on October 15, 2007 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

Microcystin was present above the quantitation limit of 1.8 micrograms/Liter (µg/L) in 2 samples collected in the Klamath River reach located within the YIR from RM 44 to RM 0.5 (WE,KBW,TC,TG,LES). Microcystin was first detected in this river reach beginning on August 21, 2007 at the WE and KBW sampling sites. Microcystin was not detected in water samples that were collected in September or October. The highest reported microcystin level in this river reach occurred at the WE sampling site on August 21, 2007 and was measured at 2.0 µg/L.

These results are interesting because microcystin levels are lower in 2007 when compared to microcystin levels measured in 2006 even though samples collected in 2007 reported higher levels of MSAE. MSAE was also present later in the season in 2007 when compared to 2006 and different environmental variables were present, lower water temperatures, less intense sunlight, shorter days of sunlight, etc. Past experimental research has shown that that increases in surface temperature coupled with nutrient loading could initiate a shift in dominance within the Microcystis population, causing toxic cells to comprise a greater percentage of the total population (Davis and Gobler 2007). This type of information could add to the list of probable reasons why microcystin measured with the ELISA kit were lower in 2007 when compared to 2006. Another reason why the ELISA results were lower in 2007 was that other microcystin congeners may have been present that the ELISA test kit were unable to detect. There are over 50 known microcystin congeners and the ELISA test is designed to cross-react with some congeners but not all that are known to exist.

Literature Cited

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Appendix A

Table A-1. Combined Algae Species List for Klamath River Sites located at River Mile 44 to 0.5 (WE, KBW, TC, TG, and LES) May to October, 2007.

		50 samples total		
#	Algae Species	Ave % Den	# samples	Code
1	Cocconeis placentula	15.4	50	COPC
2	Epithemia sorex	11.8	44	EPSX
3	Diatoma tenue	8.3	43	DTTN
4	Nitzschia frustulum	8.1	47	NZFR
5	Rhodomonas minuta	5.4	37	RDMN
6	Achnanthes minutissima	4.7	41	ACMN
7	Synedra ulna	4.1	39	SNUL
8	Rhoicosphenia curvata	3.1	42	RHCU
9	Scenedesmus quadricauda	2.9	36	SCQD
10	Navicula cryptocephala	2.7	44	NVCR
11	Cymbella affinis	2.4	28	CMAF
12	Navicula cryptocephala veneta	2.1	37	NVCV
13	Microcystis aeruginosa	2.1	19	MSAE
14	Cyclotella meneghiniana	1.6	27	CCMG
15	Cymbella sinuata	1.3	34	CMSN
16	Nitzschia paleacea	1.3	29	NZPC
17	Aphanizomenon flos-aquae	1.2	7	APF9
18	Diatoma vulgare	1.2	28	DTVL
19	Nitzschia palea	1.2	26	NZPL
20	Gomphonema angustatum	1.1	28	GFAN
21	Chromulina sp.	1.1	4	KMXX
22	Cymbella minuta	1.1	27	CMMN
23	Selenastrum minutum	1.1	19	SLMN
24	Ankistrodesmus falcatus	0.9	25	AKFL
25	Stephanodiscus hantzschii	0.8	14	STHN
26	Achnanthes lanceolata	0.8	21	ACLC
27	Nitzschia dissipata	0.8	20	NZDS
28	Fragilaria construens venter	0.6	14	FRCV
29	Gomphonema subclavatum	0.6	19	GFSB
30	Amphora perpusilla	0.6	21	AFPR
31	Chlamydomonas sp.	0.6	16	CHXX
32	Synedra mazamaensis	0.6	15	SNMZ
33	Gomphonema ventricosum	0.5	20	GFVT
34	Cryptomonas erosa	0.5	15	CXER
35	Nitzschia communis	0.5	17	NZCM
36	Navicula tripunctata	0.4	18	NVTP
37	Nitzschia acicularis	0.4	12	NZAC
38	Synedra tenera	0.4	4	SNTN
39	Nitzschia amphibia	0.4	14	NZAM
40	Fragilaria vaucheria	0.3	13	FRVA
41	Navicula decussis	0.3	13	NVDC
42	Nitzschia innominata	0.3	11	NZIN
43	Melosira granulata	0.3	10	MLGR
44	Gomphoneis herculeana	0.2	9	GSHR
45	Cyclotella pseudostelligera	0.2	3	CCPS

Table A-1(contd.). Combined Algae Species List for Klamath River Sites located at River Mile 44 to 0.5 (WE, KBW, TC, TG, and LES) May to October, 2007.

		50 samples total		
#	Algae Species	Ave % Den	# samples	Code
46	Gomphonema olivaceum	0.2	10	GFOM
47	Melosira varians	0.2	8	MLVR
48	Nitzschia sp.	0.2	8	NZXX
49	Scenedesmus acuminatus	0.2	6	SCAC
50	Sphaerocystis schroeteri	0.2	7	SFSR
51	Navicula gregaria	0.2	5	NVGR
52	Scenedesmus denticulatus	0.2	6	SCDT
53	Unidentified flagellate	0.1	3	MXFG
54	Cocconeis pediculus	0.1	3	COPD
55	Achnanthes linearis	0.1	5	ACLN
56	Nitzschia linearis	0.1	6	NZLN
57	Navicula sp.	0.1	5	NVXX
58	Navicula pupula	0.1	4	NVPP
59	Navicula graciloides	0.1	4	NVGC
60	Scenedesmus abundans	0.1	5	SCAB
61	Fragilaria crotonensis	0.1	5	FRCR
62	Fragilaria construens	0.1	4	FRCN
63	Cocconeis disculus	0.1	2	CODS
64	Gomphonema clevei	0.1	4	GFCL
65	Coelastrum microporum	0.1	3	CUMC
66	Anabaena sp.	0.1	4	ABX9
67	Tetraedron minimum	0.1	3	TEMN
68	Ulothrix sp.	0.1	3	ULXX
69	Gyrosigma spencerii	0.1	3	GYSP
70	Gomphonema tenellum	0.0	2	GFTN
71	Amphora ovalis	0.0	2	AFOV
72	Navicula viridula	0.0	2	NVVR
73	Synedra parasitica	0.0	2	SNPR
74	Cymbella mexicana	0.0	2	CMMX
75	Cladophora sp.	0.0	2	CFXX
76	Epithemia turgida	0.0	2	EPTR
77	Glenodinium sp.	0.0	1	GDXX
78	Pediastrum boryanum	0.0	2	PSBR
79	Navicula capitata	0.0	2	NVCP
80	Denticula elegans	0.0	2	DNEL
81	Fragilaria pinnata	0.0	2	FRPN
82	Cymbella microcephala	0.0	1	CMMC
83	Gloeocystis ampla	0.0	1	GLAM
84	Hantzschia amphioxys	0.0	1	HZAM
85	Pinnularia sp.	0.0	1	PLXX
86	Cymbella tumida	0.0	1	CMTM
87	Rhopalodia gibba	0.0	1	RPGB
88	Gomphonema sp.	0.0	1	GFXX
89	Fragilaria leptostauron	0.0	1	FRLP
90	Diatoma hiemale mesodon	0.0	1	DTHM

Table A-1(contd.). Combined Algae Species List for Klamath River Sites located at River Mile 44 to 0.5 (WE, KBW, TC, TG, and LES) May to October, 2007.

#	Algae Species	50 samples total Ave % Den	# samples	Code
91	Nitzschia capitellata	0.0	1	NZCP
92	Kephyrion sp.	0.0	1	KFXX
93	Navicula radiosa	0.0	1	NVRD
94	Cyclotella ocellata	0.0	1	CCOC
95	Oscillatoria sp.	0.0	1	OSX9
96	Nitzschia microcephala	0.0	1	NZMC
97	Anomoeoneis vitrea	0.0	1	AOVT
98	Diploneis elliptica	0.0	1	DPEL
99	Melosira ambigua	0.0	1	MLAM
100	Rhopalodia musculus	0.0	1	RPMS
101	Synedra cyclopum	0.0	1	SNCY
102	Synedra socia	0.0	1	SNSC
103	Navicula menisculus upsaliensis	0.0	1	NVMU
104	Amphipleura pellucida	0.0	1	AMPL
105	Nitzschia volcanica	0.0	1	NZVL

Table A-2 Combined Algae Species List for Mouth of Major Tributary Site (TR) May to October, 2007.

#	Algae Species	5 samples total Ave % Den	# samples	Code
1	Diatoma tenue	20.0	5	DTTN
2	Cocconeis placentula	19.0	5	COPC
3	Epithemia sorex	7.9	5	EPSX
4	Synedra ulna	6.1	5	SNUL
5	Achnanthes minutissima	4.8	5	ACMN
6	Navicula cryptocephala veneta	3.4	4	NVCV
7	Nitzschia frustulum	3.0	5	NZFR
8	Gomphonema angustatum	2.8	4	GFAN
9	Rhodomonas minuta	2.6	3	RDMN
10	Achnanthes lanceolata	2.2	3	ACLC
11	Nitzschia paleacea	2.1	5	NZPC
12	Cymbella sinuata	2.1	4	CMSN
13	Selenastrum minutum	1.8	2	SLMN
14	Rhoicosphenia curvata	1.8	4	RHCU
15	Cymbella affinis	1.5	3	CMAF
16	Nitzschia acicularis	1.4	3	NZAC
17	Cymbella minuta	1.3	3	CMMN
18	Navicula cryptocephala	1.1	3	NVCR
19	Nitzschia palea	1.1	4	NZPL
20	Navicula decussis	1.0	3	NVDC
21	Gomphonema subclavatum	1.0	2	GFSB
22	Nitzschia dissipata	1.0	2	NZDS
23	Ankistrodesmus falcatus	0.9	4	AKFL
24	Scenedesmus quadricauda	0.9	2	SCQD
25	Melosira varians	0.8	3	MLVR
26	Amphora perpusilla	0.7	3	AFPR
27	Gomphonema ventricosum	0.6	2	GFVT
28	Chlamydomonas sp.	0.4	2	CHXX
29	Gomphonema tenellum	0.4	2	GFTN
30	Diatoma vulgare	0.4	2	DTVL
31	Scenedesmus denticulatus	0.4	1	SCDT
32	Gomphonema olivaceum	0.4	2	GFOM
33	Achnanthes linearis	0.4	2	ACLN
34	Cocconeis pediculus	0.4	1	COPD
35	Nitzschia communis	0.3	1	NZCM
36	Cymbella microcephala	0.3	1	CMMC
37	Cryptomonas erosa	0.3	1	CXER
38	Nitzschia linearis	0.3	1	NZLN
39	Synedra mazamaensis	0.3	1	SNMZ
40	Gloeocystis ampla	0.3	1	GLAM
41	Tetraedron minimum	0.2	1	TEMN
42	Nitzschia sp.	0.2	1	NZXX
43	Navicula tripunctata	0.2	1	NVTP
44	Navicula pupula	0.2	1	NVPP
45	Ulothrix sp.	0.2	1	ULXX
46	Fragilaria leptostauron	0.2	1	FRLP
47	Pediastrum boryanum	0.2	1	PSBR
48	Nitzschia innominata	0.2	1	NZIN
49	Gomphonema clevei	0.2	1	GFCL
50	Amphipleura pellucida	0.2	1	AMPL
51	Anabaena sp.	0.2	1	ABX9

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 deionized (D.I.) 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.

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