Draft

Mid-Klamath River Nutrient, Periphyton, Phytoplankton and Algal Toxin Sampling Analysis Plan (SAP)

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February 2009

Karuk Tribe Water Quality Program
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Karuk Water Quality Project Manager _____

Karuk Water Quality QA Officer _____

For EPA use:

Approved by EPA Project Manager: Date:

_____ _____

Expedited Review? □ Yes □ No

Received by QA Office: Date:

_____ _____

Reviewed by: Date:

_____ _____

Approved: Date:

_____ _____

Region 9 Quality Assurance Manager

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Note: The section numbering system used herein corresponds to U.S. EPA's 2000 *SAP Guidance and Template* version 1. Sections that do not apply are generally not included here, resulting in some gaps in the numbering system.

1.0 INTRODUCTION

The Karuk Tribe is the second largest Tribe in California with more than 3,500 members. Its Ancestral Territory covered over 70 miles of the Klamath River and included a major tributary, the Salmon River, and numerous minor tributaries (Figure 1). The Karuk Tribe Water Quality Program (KTWQP) monitors and assesses the conditions and trends of surface water and groundwater upriver, within, and upslope of Karuk Aboriginal Territory (KAT). The mission of the Karuk Tribe is to (Karuk 2008):

> "protect, promote, and preserve the cultural, resources, natural resources, and ecological processes upon which the Karuk People depend. This mission requires the protection and improvement of the quality and quantity of water flowing through Karuk Ancestral Territory and Tribal trust lands."

The Klamath River in California is listed as an impaired water body on the Clean Water Act (CWA) Section 303(d) list for temperature, nutrients and dissolved oxygen (CSWRCB, 2005). A major beneficial use that concerns all Klamath River Tribes is the salmon that have sustained them for thousands of years and that can be profoundly impacted by water pollution. Klamath River pollution from toxic algae species has now also been recognized in Klamath Hydroelectric Project reservoirs (Kann and Corum, 2006) and downstream to the estuary (YTEP, 2005). This Sampling Analysis Plan (SAP) applies to collection of data on nutrients, phytoplankton, periphyton and algal toxins within and upstream of the KAT. Understanding the range and patterns in data will inform the Karuk Tribe so that appropriate standards can be set to prevent water pollution and protect beneficial uses. Data may ultimately be used for nutrient budgets, nutrient cycling and spiraling analysis, and tracking the abundance of toxic algae and associated algal toxins.

Although this SAP covers only covers several KTWQP sampling sites, it is part of a basin-wide effort. KTWQP will be coordinating sampling with the Yurok Tribe, which will collect data at additional Klamath River locations downstream on their reservation. The Yurok Tribe Environmental Program (YTEP) will follow identical protocols and methods as detailed below, but will file a separate SAP for its sampling because of the separate chain of custody.

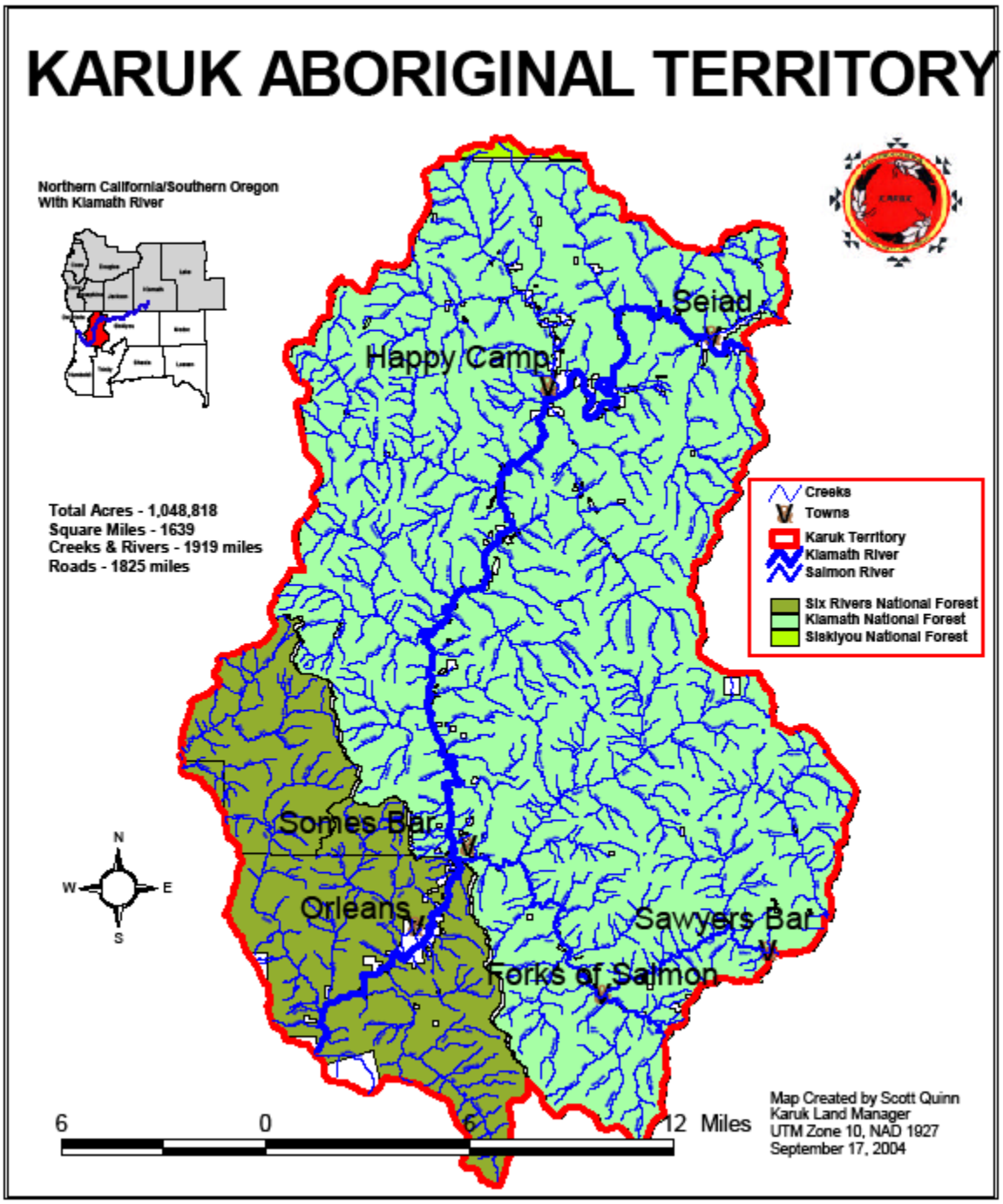

Figure 1. Map of Karuk Aboriginal Territory, including towns, counties and where it is relative to the State of California and Oregon. Map from Karuk Tribe.

1.1 Site Names

The Klamath River may be sampled at Orleans (OR), downriver of Seiad Valley (SV), at Walker Bridge (WA) below Iron Gate Dam (KRBI), and above Copco Reservoir (KRAC). Tributaries that may be sampled include the Salmon River near the mouth (SA), the Scott River at Johnson's Bar (SC), and the Shasta River near the mouth (SH). The following sites overlap with US Geological Survey (USGS) gaging stations: OR, SA, SV, SH, and KRBI. Additional sites may be sampled within the reservoirs including both open water stations within Iron Gate (IR01 and Copco (CR01) and shoreline stations at Iron Gate at Jay Williams Boat Dock (IRJW), Iron Gate at Camp Creek Recreation Area (IRCC), Iron Gate at Spring Hill (IRSH), Copco at Copco Cove (CRCC), and Copco at Mallard Cove (CRMC).

1.2 Sampling Locations

The KTWQP sampling sites for grab sampling of nutrients, phytoplankton and algal toxins are on the Klamath River at Orleans (OR) (RM 59), Seiad Valley (SV) (RM 129), Walker Bridge (WA) (RM 156), below Iron Gate at the hatchery bridge (KRBI) (RM 189), and above Copco (KRAC) (RM 206), the Salmon River approximately 1 mile up from the mouth (SA), the Scott River at Johnson's Bar (SC), and the Shasta River approx 300 yards up from the mouth (SH).

Periphyton sample sites are at four locations along the mainstem Klamath below Iron Gate, 1) Orleans (OR), 2) Seiad Valley (SV), 3) Walker Bridge (WA), and at 4) below Iron Gate (KRBI). As described below, these samples are taken by defined areas of stream substrate, usually cobble sized rocks. Periphyton sampling focuses on the River below Iron Gate, so no sampling will occur above the reservoirs.

1.3 Responsible Agency

The Karuk Tribe Water Quality Program will be responsible for collecting all samples above and within KAT and insuring that sampling and handling protocols are followed. KTWQP will properly pack samples and expedite shipping to appropriate water quality laboratories for analysis.

1.4 Project Organization

Table 1 lists key players and contractors, including those collecting samples, contractors that will process samples and KTWQP staff that will oversee quality control (QC) procedures.. Laboratories that will process samples are 1) Aquatic Research Inc. in Seattle, Washington, 2) Aquatic Analysts Inc. in White Salmon, Washington, 3) the U.S. Environmental Protection

Agency Region IX Laboratory in Richmond, California, and 4) the California Department of Fish and Game's Fish and Wildlife Water Pollution Control Laboratory in Rancho Cordova.

Table 1. All parties participating in collection, shipping and handling, analysis of Klamath River nutrient, phytoplankton and algae generated toxics data on the YIR and those responsible for implementation of QA/QC procedures.

Title/Responsibility	Staff/Contractor	Phone Number
EPA Project Manager	Loretta Vanegas	(415) 972-3433
Project Manager	Susan Corum	(530) 469-3456
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KTWQP Staff	Luana Hillman	(530) 469-3258
Quality Assurance Officer	Susan Corum	(530) 469-3456
Contractor, Aquatic Ecosystems	Jacob Kann	(541) 482-1575
Contractor, Aquatic Research Inc.	Steve Lazoff	(206) 632-2715
Contractor, Aquatic Analysts	Jim Sweet	(509) 493-8222
USEPA Region 9 Lab	Andy Lincoff	(510) 412-2330
CA Fish and Game Lab	Dave Crane	(916) 358-4395

1.5 Statement of the Specific Problem

The Klamath River is listed as an impaired water body under Clean Water Act (CWA) section 303(d) in both California and Oregon (CSWRCB, 2005; ODEQ, 2006). Total Maximum Daily Load (TMDL) studies related to pollution abatement are complete for Upper Klamath Lake and its tributaries in Oregon (ODEQ, 2002) but in progress for the Lower Klamath (Link River and Keno Reservoir to the ocean) (St. John, 2005). Nutrient pollution in the Lower Klamath River can be traced to several sources: agricultural activities, the nitrogen fixing blue-green algae species *Aphanizomenon flos-aquae* that flourishes in Upper Klamath Lake and Klamath Hydroelectric Project reservoirs, and from the Lost River and Lower Klamath Lake basin via direct winter pumping and the Straits Drain (Kier Associates, 2007).

Nutrient pollution in the Lower Klamath River causes elevated pH and dissolved ammonia and depressed dissolved oxygen. Recent studies related to Klamath Hydroelectric Project (KHP) relicensing have brought to light linkages between nutrient pollution in the Lower Klamath River and fish health (Karuk, 2006). Algae beds and deposits of benthic organic matter in the Klamath River just below Iron Gate Dam provide ideal habitat for a polychaete worm that plays host to one of the Klamath River's most deadly fish diseases, the protozoan *Ceratomyxa shasta* (Stocking and Bartholomew, 2004; Stocking, 2006). The combination of direct stress to fish from water pollution in combination with increased abundance of pathogens has lead to more than 40% of downstream migrant juvenile Chinook salmon dying before they reach the ocean in some years (Foott et al., 2003; Nichols and Foott, 2005).

The recent discovery of toxic algae species, such as *Microcystis aeruginosa* (MSAE), in KHP reservoirs (Kann and Corum, 2006; Kann and Corum, 2007; Kann, 2007) and the Klamath River (YTEP, 2005), now pose risks to human health in late summer and fall from recreational or cultural-use contact. Data collected under this SAP will help better understand the complex nature of Klamath River nutrient pollution and the prevalence of algal toxins upstream and within KAT.

2.0 BACKGROUND

The Klamath River system drains much of northwestern California and south-central Oregon (Figure 2). The KHP and diversion projects have altered natural flow regimes (Hardy and Addley, 2001) and algal and nutrient dynamics (Kann and Asarian, 2005; Kann and Asarian, 2006; Kann and Asarian, 2007). Copco and Iron Gate reservoirs, the lowest in the KHP, are often dominated by the nitrogen fixing blue-green algal species such as *Aphanizomenon flos-aquae* (Kann and Asarian, 2006; Kann and Asarian, 2007). The Klamath River is more often limited by nitrogen than phosphorus (NRC, 2004; Hoopa TEPA 2008). Nutrient concentrations in reservoir outflows are periodically substantially higher than in reservoir inflows, making nutrients available for downstream growth of algae and macrophytes (Kann and Asarian, 2005), although patterns vary by year (Kann and Asarian, 2007).

Photosynthetic activity in algae beds and by periphyton in downstream locations elevates pH during daylight hours and plant respiration at night contributes to depressed dissolved oxygen (D.O). High pH in combination with water temperatures of 25^{o} C, which are common on the Klamath River in summer, cause a conversion of ammonium ions to dissolved ammonia (Goldman and Horne, 1983) that is toxic to salmonids at low levels (Heisler, 1990). Nutrient

concentrations generally decline with increasing distance downstream of Iron Gate Dam due to dilution and natural river nutrient retention processes (assimilation into periphyton, denitrification, and/or settling)(Asarian and Kann 2006); however, there are still water quality problems on the KAT and other downstream reaches.

Severe nutrient-related water quality problems are apparent just upstream of the YIR boundary (RM 43.5); consequently, concern over impacts on the YIR require further study. For example, the average daily maximum pH at Orleans (RM 66) in August 2004 was 8.5, which exceeds NCRWQCB (2005) *Basin Plan* standards, and created stressful conditions for salmonids (Wilkie and Wood, 1995). NCRWQCB samples for dissolved ammonia at Ikes Falls (RM 70) in June 1996 were as high as 0.050 mg/l, which is recognized as lethal for salmonids (Heisler, 1990). In August of 1997, U.S. Fish and Wildlife Service (USFWS) Arcata Field Office (Halstead, 1997) measured D.O. as low as 3.4 mg/l at Big Bar (RM 50), which was causing mortality of hearty, warmwater-adapted fish species such as suckers and dace, as well as salmonids.

A preliminary nutrient budget by reach for the Klamath River (Asarian and Kann, 2006) found insufficient quantity and quality of data to fully understand nutrient dynamics in the Klamath River. Problems included laboratory detection limits for nitrogen forms that were too high, insufficient temporal and spatial resolution of samples, and lack of periphyton/macrophyte data. Due to lower nutrient concentrations, detection limit issues were particularly important in the lower reaches of the Klamath River such as on the YIR.

Kann (2005) detected high concentrations of a toxic blue-green algae species MSAE in a fall 2004 reconnaissance sample. The Karuk Tribe followed up with more sampling of Iron Gate and Copco reservoirs and found the widespread presence of high concentrations of *Microcystis* in both Copco and Iron Gate Reservoirs in 2005-2007 (Kann and Corum, 2006; Kann and Corum, 2007; Kann 2007). A *Microcystis* bloom was documented in the Klamath River within the YIR boundaries in August and September 2005 (YTEP, 2006b). The timing is significant 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 fisherman.

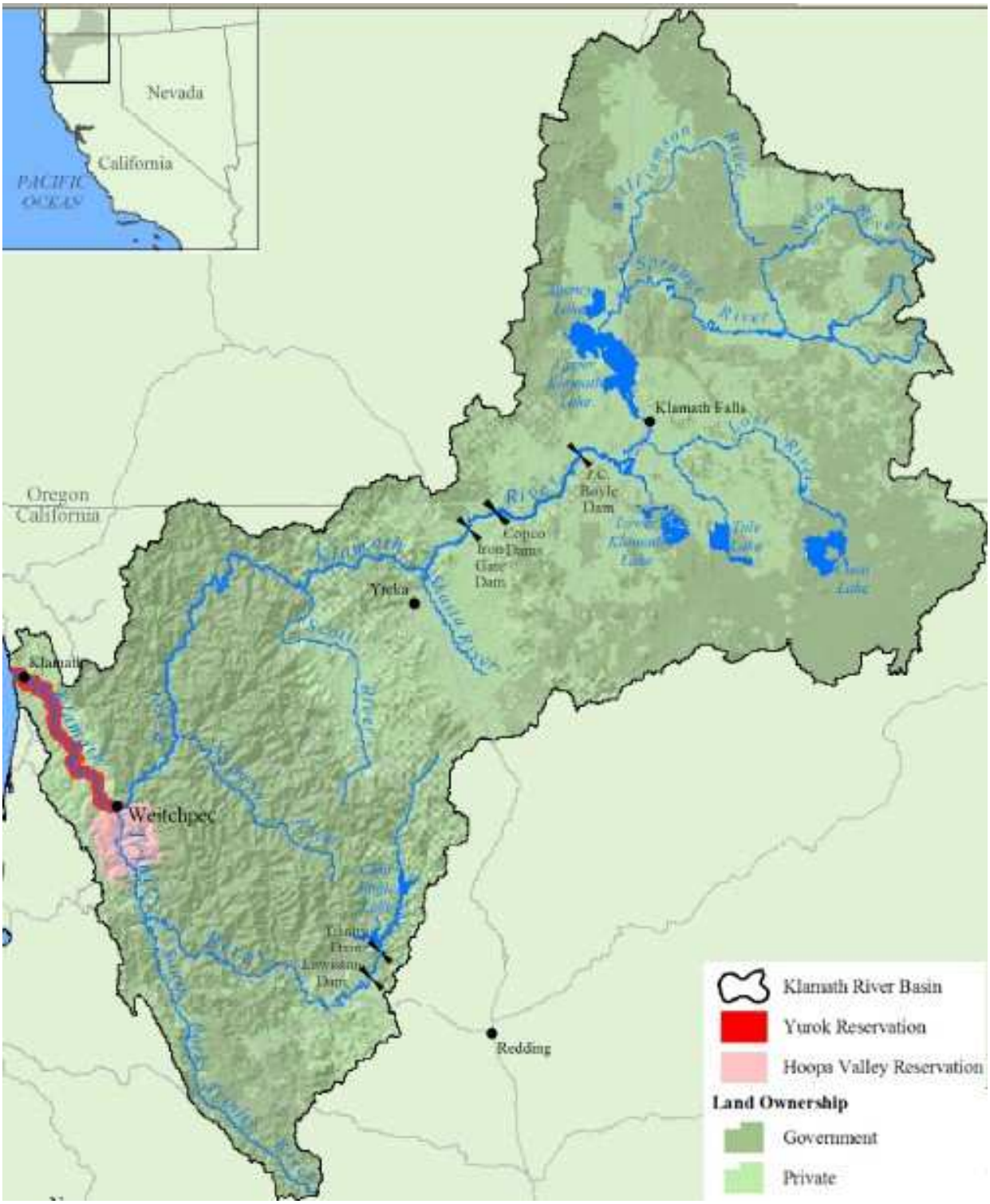

Figure 2.1 Klamath River Basin in California and Oregon, including the Trinity River sub-basin showing the Yurok and Hoopa Reservations and the location of KHP dams and reservoirs.

Coordination between the Karuk and Yurok Tribe will allow KTWQP to anticipate when MSAE levels may be high so that samples can be analyzed for microcystin by the United States Environmental Protection Agency (U.S. EPA) Region 9 Laboratory in Richmond, California. Samples in 2007 found toxic blue-green algae species other than MSAE and tests for these and related toxins will also be conducted in 2008 (YTEP, 2008) at the California Department of Fish and Game's Fish and Wildlife Water Pollution Control Laboratory in Rancho Cordova.

The Karuk and Yurok Tribes have been collecting water quality samples throughout the Klamath River Basin for nutrient and algae analysis since 2001 (KTDNR 2008; YTEP, 2004a; 2004b; 2005). Both Tribes initially cooperated with the United States Fish and Wildlife Service (USFWS) between 2001-2005 and collected samples according to USFWS' previously formulated SAP. Current development of this SAP is necessary because the Tribes no longer coordinate with USFWS for sample collection and analysis. YTEP samples downstream reaches of the Klamath River and major tributaries in YIR and has already filed a separate but similar SAP for nutrient, phytoplankton, periphyton and algal toxins because they have a separate chain of custody and quality assurance chain of command.

2.1 Site or Sampling Area Description

Table 2 lists the KTWQP sampling sites for nutrients, phytoplankton, periphyton and algal toxins, including site codes, spatial coordinates, general location and a specific description of access. The sampling area includes 147 river miles of the mainstem Klamath River upstream and within KAT and the Salmon, Scott, and Shasta Rivers above their convergence with the Klamath River. The Salmon River is within KAT, whereas the Scott and Shasta Rivers are upstream of KAT. Although the Klamath River is bordered mostly by forests and wildlands, nutrient pollution and now toxic algae are creating water quality problems in KAT. A map of specific locations of the sampling sites is shown in Figure 2.2. Grab samples for nutrients, phytoplankton and algal toxins will be collected at eight sampling sites. Periphyton samples may be collected at four locations (OR, SV, WA, KRBI), and public health sampling of just phytoplankton and algal toxins may occur at six locations within the reservoirs (IR01, IRJW, IRCC, CR01, CRCC, CRMC).

Table 2. Site codes and locations of KTWQP sampling stations for nutrients, phytoplankton, periphyton and algal toxins. Grab indicates collecting nutrients, phytoplankton, and algal toxins. Periphyton means just collecting periphyton samples, and public health designates just sampling for phytoplankton and algal toxins.

Code	Latitude	Longitude	Grab	Periphyton	Public Health	Location
OR	N 41 18.336	W 123 31.895	X	X		Klamath River at Orleans
SA	N 41 22.617	W 123 28.633	X			Salmon River at USGS Gage
SV	N 41 50.561	W 123 13.132	X	X		Klamath River downstream of Seiad Valley
SC	N 41 46.100	W 123 01.567	X			Scott River at Johnson's Bar
WA	N 41 50.242	W 122 51.895	X	X		Klamath River at Walker Bridge
SH	N 41 49.390	W 122 35.700	X			Shasta River at USGS Gage
KRBI	N 41 55.865	W 122 26.532	X	X		Klamath River below Hatchery Bridge
KRAC	N 41 58.345	W 122 12.101	X			Klamath River above Copco Reservoir
IR01	N 41 56.330	W 122 25.930			X	Iron Gate open water near log booms
IRJW	N 41 57.721	W 122 26.425			X	Iron Gate Jay Williams Boat Dock
IRCC	N 41 58.368	W 122 26.114			X	Iron Gate Camp Creek Recreation Area
CR01	N 41 58.932	W 122 19.694			X	Copco open water
CRCC	N 41 59.035	W 122 19.802			X	Copco Reservoir Copco Cove
CRMC	N 41 58.441	W 122 17.869			X	Copco Mallard Cove

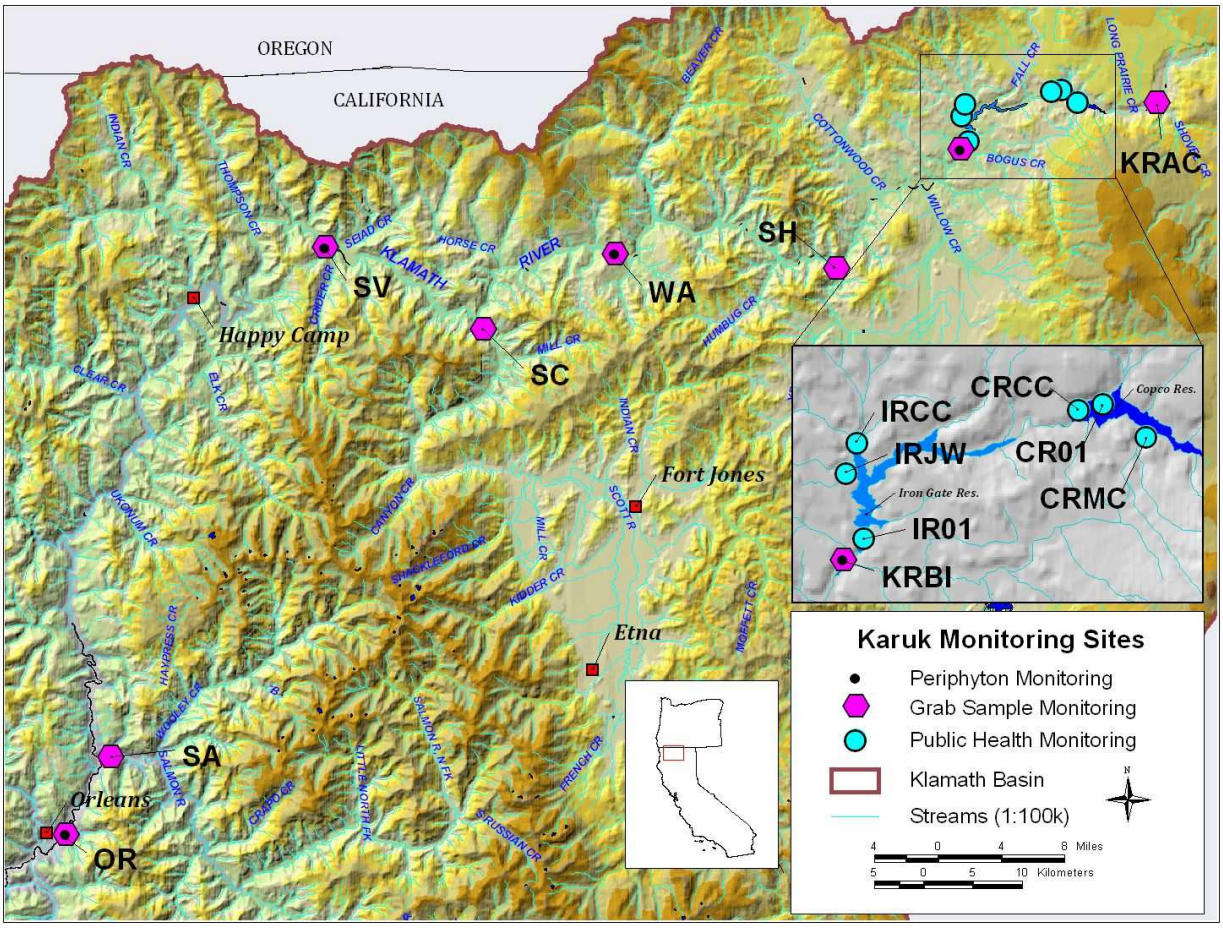

Figure 2.2: Location of periphyton, grab sample, and public health monitoring sites upstream and within KAT.

2.2 Operational History

Klamath River nutrient pollution has been widely recognized since the 1950's (Phinney and Peak, 1962; CH2M Hill, 1985; Kier Associates, 1991). The adult salmon kill in September 2002 (CDFG, 2003; Guillen, 2003), chronic high mortality of juvenile salmon (Nichols and Foott, 2005) and discovery of problems with toxic algae in KHP reservoirs (Kann and Corum, 2006) all point to a water quality crisis. As noted above, sources of pollution include upstream agricultural operations and nitrogen fixing algae in Upper Klamath Lake, Lost River, Lower Klamath Lake and KHP reservoirs. The lowest two KHP reservoirs are also recognized as fostering toxic algae species as well. The extent of nutrient pollution and problems with algal toxins above and within KAT are not well studied and create a need for more information and the sampling regime discussed herein.

2.3 Previous Investigations/Regulatory Involvement

The Klamath River Water Quality Monitoring Coordination Workgroup that includes Tribes and State and federal agencies was formed after the September 2002 adult salmon kill and coordinated water quality sampling subsequently increased. Asarian and Kann (2006) used existing nutrient data to construct a nutrient budget by reach for the Klamath River and their study lists all nutrient related water quality samples collected between 1996-2004. They pointed out data gaps for nutrient sampling using adequate laboratory detection limits and the need for more periphyton samples. The Hoopa Tribal Environmental Protection Agency (TEPA) (2008) used existing data to characterize Klamath River nutrient pollution and to set limits on their Reservation waters just upstream of Weitchpec. Figure 2.3 is adapted from Hoopa TEPA (2008) and shows all sampling sites in the years 2000-2004 by type for the lower and mid-Klamath River (note: no site was sampled for every parameter in every year).

In 1989 the Karuk Tribe formed the Department of Natural Resources which primarily focused on fisheries work. About ten years later, the KTWQP was started. Water quality data was collected in coordination with USGS and USFWS and generally focused on the KAT but also occurred upstream of the KAT. In 1995, USFWS monitored Klamath River water quality as linkages between water pollution and fish health became more apparent. Data have included grab samples for nutrients and those derived from continuous recording data probes that capture

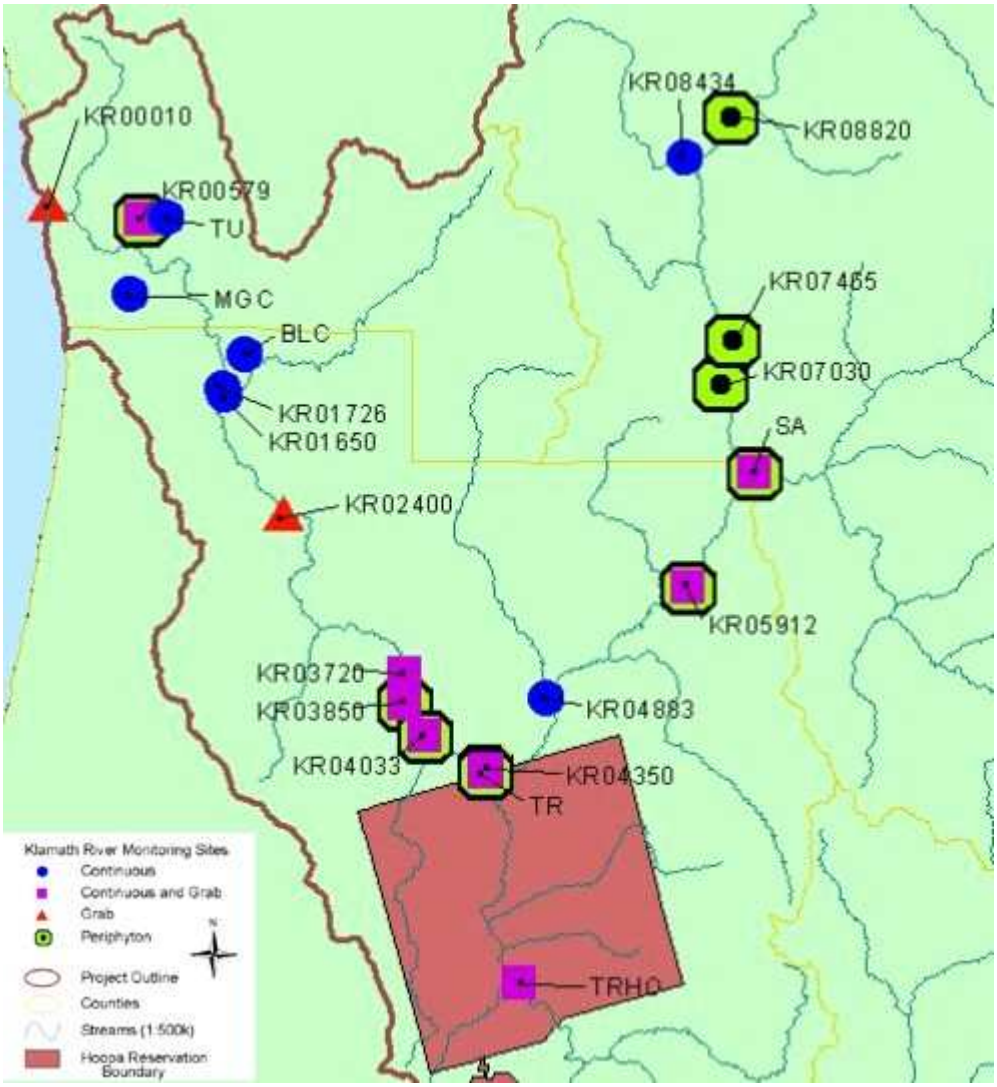

Figure 2.3. This map is taken from Hoopa TEPA (2008) (Figure 9) and shows all sites where nutrient related data were collected on the lower and mid-Klamath River by sample type from 2000-2004.

parameters such as pH, D.O., temperature and conductivity. In 2004, the Yurok Tribe, NCRWQCB, and PacifiCorp conducted a Klamath River periphyton study that included sites above and within the KAT, with results summarized by Eilers (2005) and Hoopa TEPA (2008).

The Karuk Tribe began cooperative water quality sampling, including nutrients, with USFWS in 2001. KTWQP has operated continuous water quality datasondes at several locations above and within KAT since that time for temperature, D.O., pH, and conductivity. Monitoring for toxic algae species began in 2005 and continued through 2008. Periphyton sampling occurred in 2008.

The KTWQP has been responsible for all of its sample collection, transportation to applicable laboratories, data storage, and data analysis related to nutrients since 2007. The KTWQP has been assisted by Aquatic Ecosystems for analysis of phytoplankton and toxic algae data. Nutrient data collected from 2001-2006 by KTWQP underwent extensive QA/QC examination. Data from 2007 and 2008 are currently being integrated into the Yurok Environmental Data Storage System (YEDSS). This innovative database is able to update the U.S. EPA's STORET system. Data will also be added to the comprehensive TMDL database, which is shared and augmented by the Klamath River Water Quality Monitoring Coordination Workgroup and used by the U.S. EPA and NCRWQCB for the Klamath River TMDL.

2.5 Environmental and/or Human Impact

Nutrient and toxic algae pollution in the Klamath River is causing stressful conditions for Pacific salmon species and their juveniles and providing an environment that fosters an increase in disease organisms (YTEP, 2006c). Reduced salmon production and loss of access to salmon as a food resource has had major health consequences on the health of Native Americans in the Klamath River basin (Norgaard, 2005).

Although MSAE may also be contributing to fish health problems, it also has the capacity to directly affect human health. As MSAE cells die and decay the hepatotoxin microcystin is released, which can cause a range of reactions in humans and/or animals: rash, irritation, conjunctivitis, nausea, vomiting, diarrhea, liver damage, tingling, numbness, paralysis, and death (Chorus and Bartram 1999; Chorus 2001). Once ingested, microcystin is not excreted and instead bioaccumulates and can cause liver damage, decreased liver function and eventually mortality (WHO, 1998). Mortality in fish, domestic animals, and humans has been recorded following from both single-dose events and long-term exposure to microcystin (Carmichael 1994).

Trace amounts of microcystin were measured in the liver of a half-pounder steelhead from the Lower Klamath River (YTEP, 2006b), giving rise to concern for fish health and for the health of those who consume the fish. Sampling by the State Water Resources Control Board (SWRCB) showed microcystin in mussels, yellow perch, and hatchery Chinook (Kann, 2008). Phytoplankton samples in 2007 also detected other toxin producing blue-green algae species and toxins other than those produced by MSAE have been detected in KHP reservoirs (YTEP, 2008). The presence, prevalence and effects on people and fish of these other toxins needs further exploration both on the KAT and upstream reaches.

3.0 PROJECT DATA QUALITY OBJECTIVES

3.1 Project Task and Problem Definition

The study area is the Klamath River and tributaries above and within the KAT, although the YTEP will be conducting identical sampling in downstream reaches and tributaries. This project will help understand the extent of nutrient pollution and the prevalence of algal toxins and the risk both pose to fish and human health. The KAT is directly impacted by effects from the KHP and land management occurring within and upstream of KAT.

Specific questions this study should answer include:

1) Are there nutrient levels indicative of pollution in the Klamath River, including reaches within the KAT?
2) Do periphyton samples show a density of chlorophyll *a* indicative of nutrient pollution?
3) Are there dangerous levels of MSAE and microcystin toxin in the Klamath River, including reaches within the KAT?
4) Are there other potentially toxic blue-green species present in the Klamath River and algal toxins other than the most common microcystin variant?

KTWQP investigations occur within and above KAT. YTEP will provide data to answer the same questions for downstream reaches. In the longer term, theses samples will show pollution variation between water years and provide a basis to judge effectiveness of short-term and long-term management and regulatory actions taken to abate pollution throughout the Klamath River Basin. This will also allow participation of Tribes as resource co-managers and as full partners in adaptive management. Within the KAT specifically, the data may be used as justification for improvement of standards needed to protect Tribal members, the public and other beneficial uses.

Evidence gathered will help regulating agencies make informed decisions off of the 401 certification of the KHP and Klamath TMDL and prompt further action on non-point source pollution from agriculture through mechanisms such as the Klamath River and Lost River TMDL implementation. In the short term, action will be taken immediately to inform appropriate agencies and the public in the event that potentially dangerous levels of blue-green algae cell counts or toxins are discovered.

3.2 Data Quality Objectives (DQOs)

Data quality objectives (DQOs) are quantitative and qualitative criteria that establish the level of uncertainty associated with a set of data. DQO protocols (U.S. EPA, 2006) must be followed in water quality studies funded by or in partnership with the U.S. EPA. Meeting DQOs assures that data produced accurately reflects concentrations of contaminants and can be used to judge compliance or non-compliance with water quality standards. In order to minimize uncertainty and provide information suitable for decision support, sampling methods will follow standard protocols defined below known to produce trustable results, and strict QA/QC procedures will be implemented. The QA Officer will work with the Project Manager to examine all aspects of sampling, shipping and chain of custody and laboratory results and correct any problems immediately.

KTWQP is cooperating with other agencies and Tribes to help understand patterns of pollution generated by nutrients and algal toxins in the Klamath River. Previous studies, such as Asarian and Kann (2006), prescribed the level of accuracy of nutrient data, such as nitrogen, needed for a fuller understanding of Klamath River nutrient pollution. Nutrient grab samples will be collected bi-weekly (every two weeks) between May and October at KTWQP sampling locations and analyzed for the following parameters:

- Total Phosphorus
- Ortho-Phosphorus
- Total Nitrogen
- Nitrate and Nitrite
- Ammonia
- Chlorophyll *a*/Phaeophytin *a*
- Total Organic Carbon
- Total Suspended Solids
- Total Dissolved Solids
- Alkalinity
- Calcium
- Magnesium

Additional analytes may be added or omitted from the sample matrix based on funding or input from the Klamath River Water Quality Monitoring Coordination Workgroup. KTWQP is using one of the most well recognized laboratories in the West for nutrient analysis, Aquatic Research, Inc. in Seattle, Washington.

Periphyton data collection will follow protocols of previous studies (Eilers, 2005) that are consistent with widely recognized standards (Porter et al., 1995; U.S. EPA, 2002). Samples will be preserved with lugol's iodine and shipped o Aquatic Analysts in White Salmon, Washington which will determine species composition. An additional sample will be packed on ice and shipped overnight delivery to Aquatic Research, Inc. in Seattle, WA to determine levels of chlorophyll *a*.

Phytoplankton sample analysis will include species composition and cell counts determined by Aquatic Analysts. Very low detection levels are being set for microcystin and other toxins because of the risk posed to human health; therefore, only laboratories specializing in detection of these substances are being used. The analysis for microcystin toxin using the enzyme linked immunosorbent assay (ELISA) method will be performed by the U.S. EPA Region IX Laboratory in Richmond, California, similar to cooperative efforts of 2007 and 2008.

The California Department of Fish and Game's Fish and Wildlife Water Pollution Control Laboratory in Rancho Cordova will perform the analysis of microcystin variants and anatoxin-a using liquid chromatography/mass spectrometry (LC-MS/MS). MSAE cell counts may not directly relate to toxin levels and high counts may lead to low levels of toxin or vice versa. YIR 2007 sampling results reported that toxicogenic cyanobacteria species other than MSAE were present, including *Aphanizomenon, Anabaena* and *Oscillatoria* (YTEP, 2008). Samples destined for the U.S. EPA lab and ELISA testing will be split and a duplicate sent to the California Department of Fish and Game's Fish and Wildlife Water Pollution Control Laboratory in Rancho Cordova for LC-MS/MS testing. This will allow KTWQP and cooperators to answer questions as to whether toxic algae pollution is restricted to microcystin-LR or if other forms (LA, YR, RR, LF, LW) or if another toxin, anatoxin-a, is also present.

3.3 Data Quality Indicators (DQIs)

Data quality indicators (DQI) relate to accuracy, precision, representativeness, comparability, completeness and methods detection limits. The quality control criteria established by KTWQP for data gathering, sampling, and analysis activities assures that important data gaps regarding Klamath River nutrient and toxic algae pollution can be filled with scientifically accurate data.

Hoopa TEPA (2008) found that nitrogen in the Klamath River is correlated with maximum pH, diel pH fluctuation, and minimum D.O.; therefore, nitrogen is an important index of nutrient pollution. KTWQP will adopt reference levels for key nutrients nitrogen, phosphorous and total

inorganic nitrogen similar and MSAE and microcystin (Table 3.3) to those chosen as standards for the Klamath River on the Hoopa Valley Indian Reservation (Hoopa TEPA, 2008), which intersects with the river just above Weitchpec. An indication of high quality data will be sufficient resolution and accuracy to support comparison with these objectives. Similarly, Hoopa TEPA (2008) recognize that periphyton chlorophyll *a* levels can be used as an index of pollution, and recommended a maximum annual peak biomass limit of 150 mg/m^2 to protect water quality and fisheries.

Table 3.3 Limits of pollution for various nutrient parameters, MSAE and microcystin toxins.

Water Quality Parameter	**Recognized Pollution Level**
Total Nitrogen (TN) (mg/L)	0.2 mg/l
Total Phosphorus (TP) (mg/L)	0.035 mg/l
Periphyton Chlorophyll *a* (mg/m^2)	150 mg/m^2
Microcystis aeruginosa cell count	40,000 cells/ml
Microcystin Toxin	1 µg/l

Microcystin is a newly studied issue in California, but a consortium of State agencies has set provisional standards for hazardous conditions for recreational water bodies (CSWRCB, CDPH, and OEHHA, 2007). The standards for public health protection and limits of pollution levels are 40,000 cells of MSAE and/or 8 µg/L to trigger posting of a water body to close for recreational contact. This is consistent with Oregon's standards (ODHS 2005) limits for MSAE and microcystin. KTWQP and YTEP will issue warnings and communicate with all appropriate agencies should Klamath River samples exceed these thresholds.

The primary DQI specific to this project is whether uncertainty associated with each measurement is low enough to provide sufficient resolution to determine values relative to the above references.

Accuracy: The degree of agreement between a measurement and the true or expected value is the definition of accuracy. Nutrient data accuracy will be checked by the laboratories through the use of spikes, samples with known concentrations of analytes that are prepared by a certified provider to test laboratory results for accuracy. Spiked samples should have a percent recovery of + or - 20%.

Precision: Precision of results will be tested using duplicate samples, usually taken as field splits, with a target of less than 20% relative percent difference (RPD).

Comparability: Samples will be taken with comparable methods across the universe of samples in 2008 on the Klamath River and its tributaries so will be comparable within the year. Methods are also consistent with previous samples that make up baseline and trend data for nutrients, phytoplankton, periphyton and algal toxins.

Completeness: Given the high quality of past samples taken by KTWQP, completeness on this project is expected to be over 90%, which is highly desirable because samples will only be taken bi-weekly (every two weeks) in 2008.

Representativeness: This is the expression of the degree to which data accurately and precisely represent a characteristic of an environmental condition or a population. Field crews collecting samples will ensure representativeness of samples by selecting free-flowing water from established sampling locations and using a churn splitter to mix sample water once collected (Lurry and Kolbe, 2000) and by following protocols (Eilers, 2005; U.S. EPA, 2002) for periphyton.

See Table 3.31 for comparability measures and detection limits for nutrient samples, including U.S. EPA or American Public Health Association (APHA) (Eaton et al., 1995) approved sampling methods.

3.4 Data Review and Validation

All KTWQP field personnel have been thoroughly trained in the protocols of data collection for nutrients, phytoplankton, periphyton and algal toxins. Results they have collected over the last several years have been of high quality. Each field visit requires that staff fill out field data sheets, a field notebook with standard entries and label samples appropriately in the field (Appendix B). Sampling is always conducted by at least two staff for safety reasons and to maintain consistency. KTWQP 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 laboratories, other than U.S. EPA Region IX, are attached as Appendices F, G and H. One hundred percent

of laboratory-generated data will be checked on receipt by the Project Manager for consistency, including whether blanks and duplicates are within specified targets

Table 3.31 Nutrient grab sample analytical methods and reporting limits (Aquatic Research Inc. 2007).

Parameter	Method	Reporting Limit (mg/L)	MDL (mg/L)
Total Phosphorus	EPA 365.1	0.002	0.002
Ortho Phosphorus	EPA 365.1	0.001	0.001
Total Nitrogen	SM204500N	0.100	0.045
Nitrate + Nitrite	EPA 353.2	0.010	0.005
Ammonia	EPA 350.1	0.010	0.006
Chlorophyll *a* / Phaeophytin *a*	SM1810200	0.0001	0.0001
Total Organic Carbon	EPA 415.2	0.250	0.095
Total Suspended Solids	EPA 160.2	0.050	0.10
Total Dissolved Solids	EPA 160.1	5.00	1.00
Alkalinity	EPA 310.1	1.00	0.20
Calcium	EPA 200.7	0.100	0.008
Magnesium	EPA 200.7	0.100	0.011

and meet DQOs. Once data are merged or entered into a database, charting tools will be used to further check for data anomalies or errors. Outliers will be defined as in U.S. BOR (2005)

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. 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.

3.5 Data Management

The Project Manager will use the following information to evaluate data quality:

- Sample chain of custody documentation is complete and correct
- Sample preparation information is complete and correct
- Sample integrity has been maintained
- Instrument performance criteria have been met
- Calibration criteria have been met
- Holding times, sample preservation, and sample storage criteria have been met
- Analyte identification and quantification are correct

- QC samples and method blanks are within control limits
- Documentation (including the case narrative) is complete and correct.

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.

The Yurok Tribe received a grant under the Environmental Information Exchange Network Program and used it to develop the Yurok Tribe Environmental Data Storage System (YEDSS). This system has been shared with the Klamath Basin Tribal Water Quality Workgroup, including the Karuk Tribe. Nutrient data covered by this SAP will be captured in YEDSS, which has automatic QA/QC screening so that data entries that fall outside excepted ranges are automatically flagged. Raw data and data that have under-gone further QA/QC are automatically archived separately and metadata associated with each data type are also stored within the system and can be easily accessed when questions arise. Phytoplankton, periphyton and algal toxin data will be entered into Excel spreadsheets that are checked for accuracy by the Project Manager and backed up onto the KTWQP network and an external hard drive system that is maintained offsite.

3.6 Assessment Oversight

The Field Manager will check field forms, equipment calibration reports, and results of laboratory analysis every two weeks. Any discovery of problems with logistics of sampling or data will be documented and corrected as soon as discovered. The Field Manager will be encouraged to bring problems to the attention of the QA Officer/Project Manager before routine monthly meetings when problems with QA/QC procedures are suspected. The QA Officer/Project Manager is also the KTWWQP Coordinator and has the authority to make any necessary changes to maintain or improve quality of field sampling or laboratory results and will take immediate action as decided through meetings with the Field Manager and in consultation with the U.S. EPA.

Data quality will be assessed by looking at how samples compare to the existing universe of Klamath River data and recognized ranges of expected values from the literature. If data that do not fall within expected ranges cannot be corrected or validated through cross-checking, it will not be used in any analysis, but maintained with an associated metadata file describing why those data did not meet QA/QC standards.

4.0 SAMPLING RATIONALE

Background information above showed the need for samples of nutrients, periphyton, phytoplankton and algal toxins on and above the KAT.

4.3 Water Sampling

Nutrient pollution and toxic blue-green algae blooms have been detected in the mainstem Klamath River on and above the KAT posing a risk to fish health and human health. KTWQP has been monitoring water quality in the mainstem Klamath River since 1998.

Monitoring Locations: Sampling sites (Table 2) were selected based on the following criteria:

- OR (Klamath River at Orleans) – Conditions of the Klamath River at the downriver end of the KAT. Just upstream of a USGS gage.
- SA (Salmon River near mouth) – Conditions of the Salmon River, an important tributary that enters the Klamath River near the center of the world for the Karuk Tribe. Site of a USGS gage. Major tributary that provides habitat for all Tribal Trust fish species.
- SV (Klamath River below Seiad Valley) – This site is just downstream of Seiad Valley but upstream of the USGS gage. This is near the upstream end of the KAT thereby indicative of water quality conditions entering the KAT.
- SC (Scott River at Johnson's Bar) – This site is about one mile up from the confluence of the Scott and Klamath Rivers. It represents water quality conditions coming out of the lower canyon reach of the Scott River.
- WA (Klamath River at Walker Bridge) – This site is located between two major tributaries, the Scott and Shasta Rivers and is downriver of the town of Klamath River. This site provides water quality information after the effects of the KHP have been reduced but before entering the KAT where more minor tributaries enter the River.
- SH (Shasta River at USGS Gage) – This site is located at the USGS gage and is upstream of the confluence about 300 meters.
- KRBI (Klamath River below Iron Gate) – This site is located immediately downstream of Iron Gate dam and upstream of the USGS gage. It is the start of the free-flowing River below the KHP.
- KRAC (Klamath River above Copco) – This site is located upstream of Copco Reservoir at the access bridge upstream of Shovel Creek. This is the end of the

peaking reach just before the Klamath River enters the lower 3 reservoirs for the KHP.

- IR01 (Iron Gate Reservoir Open Water) – This site is located in Iron Gate at the center of the log booms in the lower portion of the reservoir. It represents open water conditions for toxic algae blooms.
- IRJW (Iron Gate Reservoir Jay Williams Boat Dock) – This site is located near the Jay Williams boat ramp. It is a common access point for recreators on Iron Gate and is a shoreline grab sample station.
- IRCC (Iron Gate Reservoir Camp Creek Recreation Area) – This site is located near the Camp Creek boat ramp. It is a common camping spot on Iron Gate and is a shoreline grab sample station.
- CR01 (Copco Reservoir Open Water) – This site is located in Copco Reservoir in the open water in the lower portion of the reservoir. It represents open water conditions for toxic algae blooms.
- CRCC (Copco Reservoir Copco Cove) – This site is located near the Copco Cove boat ramp. It is one of two public boat ramps on the reservoir and is a shoreline grab sample station.
- CRMC (Copco Reservoir Mallard Cove) – This site is located near the Mallard Cove boat ramp. It is a commonly used camping and recreation area on the reservoir and is a grab sample station.

Periphyton sampling only takes place at the four locations in the mainstem Klamath below the KHP. Sites may be expanded to include the major tributaries if funding is available in the future. Also, sites may be reduced if funding is not available to continue periphyton sampling.

Decisions regarding adding or removing sites for sampling will consider the following criteria:

- Are there substantial differences in observed water quality between two adjacent sampling sites? If there are differences, adding a site between the two may be necessary to understand what is occurring (e.g. the presence of some nutrient sink/source).
- Identification of new threat/issues. For example, when the Karuk Tribe became aware several years ago that the toxic algal species MSAE is present in the Klamath River, KTWQP began a phytoplankton and algal toxin monitoring program in the Copco and Iron Gate Reservoirs and the Klamath River to assess spatial and temporal variations in the toxic algae blooms.

- Trade-offs between spatial and temporal sampling intensity. Would dropping unnecessary sites free up resources to allow for more frequent sampling at other more important sites?

Timing of Samples: KTWQP will collect bi-weekly (every other week) or monthly samples between May and October. This time period was selected because it is when nutrients impair water quality in the mainstem Klamath River and when toxic algae blooms may occur. Although biweekly sampling is preferred to understand nutrient dynamics of the Klamath River (Asarian and Kann, 2006; Kann and Asarian, 2007) and assess public health threats from algae toxins, funding availability may limit sampling in certain years to a monthly interval.

Late spring through fall are important times for juvenile salmonids (Chinook, Coho, steelhead) migration, adult spring and fall Chinook migration into the Klamath basin, and migration and rearing of lamprey and green sturgeon, which are all of great importance to the Karuk People. Water quality conditions may impact these species of importance and may also impact the use of the river for subsistence fishing, ceremonial use, other cultural use, and recreation. MSAE blooms and those of other toxic algae species occur in late summer and early fall, when fishing for subsistence and ceremonial use is at its peak. Detection of nutrient pollution and toxic algae in the Klamath River above and within the KAT has caused KTWQP to create a long-term monitoring dataset.

Justification for Analytes

Grab Samples: The following parameters from grab samples were selected based on the following criteria or concerns:

- Total Phosphorus – This parameter is an indicator of runoff from agriculture and is typically found at very low concentrations in unpolluted waters. This parameter is of interest since the availability of phosphorus is key in stimulating algae blooms. The Upper Klamath Basin has extensive agricultural land use and algae blooms occur regularly throughout the summer and early fall months in the Upper Basin and persist downriver as well.
- Ortho-Phosphorus – This parameter is the dissolved form of phosphorus that is readily available for utilization by plants and algae. KTWQP is interested in this parameter since algae blooms occur regularly in the Klamath Basin and may be affected by Ortho-Phosphorus levels.

- Total Nitrogen – Total Nitrogen is a common indicator of water quality and the relative supply of both nitrogen and phosphorus and their concentrations can be indicative of human impacts on a water body. This parameter was chosen because the Klamath River is listed as impaired for nutrients.
- Nitrate + Nitrite – Nitrate and Nitrite are both highly soluble in water and are commonly used as indicators of water quality. Nitrate is a component of fertilizers, sewage, and manure. This parameter was chosen because the Klamath River is listed as impaired for nutrients and agricultural land use in the Upper Basin, Scott River and Shasta River contribute to nutrient pollution.
- Ammonia – Ammonia is highly soluble in water and is commonly used as an indicator of water quality. Ammonia is a component of fertilizers, sewage, and manure and; therefore, an index of nutrient loading from agricultural activities upstream. High pH in combination with high water temperature converts ammonium ions to dissolved ammonia, which is highly toxic to fish at relatively low levels. Having pH, water temperature and ammonia levels will allow calculation of dissolved ammonia.
- Chlorophyll *a* / Phaeophytin *a* – Chlorophyll *a* and Phaeophytin *a* (a breakdown product of chlorophyll *a*) are indirect measurements of algal biomass. This parameter was chosen because it is a well recognized index of nutrient pollution (U.S. EPA, 2000). This is a concentration (mass per unit volume) measurement based on water grab samples, as opposed to mass/unit area for periphyton samples (see below).
- Total Organic Carbon – Elevated levels of total organic carbon can cause an increase in biological oxygen demand, decreasing D.O. in the water column resulting in unfavorable conditions for aquatic life. Total organic carbon is affected by climate, flow, and the amount of vegetation within or contributing to detritus in the water column. KTWQP chose this parameter because flows in the Klamath River drop substantially during summer months and there is a high accumulation of algae and aquatic vegetation, which could result in high total organic carbon levels.
- Total Suspended Solids – Total Suspended Solids in the water column can impact aquatic life by clogging fish gills, decreasing foraging success, and ultimately can result in decreasing growth rates of fish inhabiting water with high levels of suspended solids. High concentrations of suspended solids can also decrease light penetration through the water column, which indirectly affects other parameters

such as temperature and dissolved oxygen concentrations (by decreasing photosynthesis). KTWQP chose this parameter because it will allow tracking of the concentration of organic and inorganic particles in the water throughout the sampling season.

- Total Dissolved Solids – Total Dissolved Solids is a measure of inorganic salts and dissolved organic matter. KTWQP chose this parameter because it will allow tracking of the concentration of organic and inorganic salts in the water throughout the sampling season.
- Alkalinity – Alkalinity is the total measure of substances in water that have an 'acid neutralizing' ability. Results from alkalinity analyses will indicate the Klamath River's ability to react with acidity and 'buffer' pH levels. KTWQP chose this parameter because pH levels in the Klamath River are elevated, which may cause stressful conditions for aquatic life.
- Calcium and Magnesium – Calcium and magnesium both contribute to water hardness and may provide a buffer that moderates pH fluctuation. The ratio of these two minerals may provide insight into what is driving harmfully high alkalinity in the Klamath River.
- MSAE and Toxic Blue-Green Algae Cell Counts: Health warnings by the WHO (1998) and the State of California (CSWRCB, CDPH, and OEHHA, 2007) for potentially toxic blue-green algae species are based on cell counts.
- Microcystin and Other Algal Toxins: Microcystin-LR is the most common toxin generated by blue-green algae species, but the potential for other forms of microcystin (LF, LW, LA, YR, RR) or other types of toxins like anatoxin-a are also being explored to understand all potential human health risks.

Periphyton Samples: Periphyton sample analytes are justified as follows:

- Chlorophyll *a:* This is a standard parameter for understanding nutrient pollution and is measured in units of mass per unit area based on rock scrapings (see below).
- Algae Species Enumeration and Composition: Species collected in periphyton samples also are useful in understanding whether the community structure reflects nutrient polluted conditions.

Decisions regarding adding or removing sites for sampling will consider the following criteria:

- Water quality models may require collection of data regarding new parameters. For example, models may require measuring sediment oxygen demand in the Klamath River.
- Specific research questions. For example, bettering understanding of how changes in nutrient loading would affect the Klamath River Estuary may require collected detailed information on the quantities and types of organic matter entering the River from the KHP (e.g. ratio of particulate vs. dissolved organic matter, and particle-size distribution of particulate organic matter).

5.0 REQUEST FOR ANALYSES

5.1 Analysis Narrative

The U.S. EPA worked cooperatively with KTWQP in 2006 and ran ELISA tests for the blue-green algae generated toxin microcystin-LR at their Region IX Laboratory in Richmond, California. This SAP anticipates the same working relationship and same analytes in the future as long as the agency desires. U.S. EPA has been concerned about potential health effects of microcystin and partnership with the Karuk Tribe is the most cost-effective way to acquire data needed to manage risk. Because the U.S. EPA Region 9 Lab will only be processing microcystin-LR using the ELISA method and samples do not require fixing with chemicals; therefore, there is no need for a Request for Analytical Service Matrix table here.

Detailed shipping and handling of samples and QA/QC requirements will be met by using KTWQP Chain of Custody form (Appendix C). Samples from all the sampling stations (Table 2.1) will be shipped bi-weekly (every two weeks) from May through October. In addition, a complete set of duplicates will be collected from one sampling location during each sampling event to evaluate the field crew's and lab's performance.

YTEP (2008) noted that 2007 phytoplankton data showed the highest levels of *MSAE* since 2005, but there were very low microcystin-LR toxin levels. Also, in addition to MSAE other toxic blue-green algae species were discovered. KTWQP needs to test the hypothesis that toxins other than microcystin-LR may be present. Consequently, a split sample will be sent to the California Department of Fish and Game's Fish and Wildlife Water Pollution Control Laboratory in Rancho Cordova to use LC-MS/MS to look for microcystin variants (LF, LW, LR, LA, YR, RR) and other algal toxins (i.e. anatoxin-a).

Field water quality samples taken contemporaneously with nutrient grabs, phytoplankton, periphyton and toxic algae surveys are collected using a YSI datasonde. Calibration methods of

the YSI datasonde are attached as Appendix E. Parameters measured include water temperature, dissolved oxygen, conductivity, and pH. Data will be recorded into a field notebook. The Project Manager will check calibration logs to ensure that QA/QC procedures are followed increasing chances that spot data collected during sampling accurately reflect ambient water quality conditions. As discussed above, these data can be used to resolve questions that may arise with regard to sampling anomalies or outliers.

5.2 Analytical Laboratories Other Than U.S. EPA

Table 5 lists all parameters that will be measured and to which laboratory each will be shipped for processing. The other laboratories participating in sampling analysis under this plan are Aquatic Research, Inc. (AR), Aquatic Analysts, Inc. (AA) and California Department of Fish and Game's Fish and Wildlife Water Pollution Control Laboratory in Rancho Cordova.

Aquatic Research Inc. (AR) has processed Karuk Tribe samples for the reservoir studies from 2005-2007 (Kann and Corum, 2006) and provided reliable services for the Klamath Tribes in Oregon since 1990. AR has some of the lowest reporting limits for nitrogen related parameters on the West coast and has certified lab status from the states of Washington and California.

Aquatic Analysts Inc. (AA) has been identifying Klamath River algae samples since 2004. Jim Sweet is the owner and expert taxonomist and has assisted with Upper Klamath algae studies for the Klamath Tribes of Oregon.

The California Department of Fish and Game's Fish and Wildlife Water Pollution Control Laboratory in Rancho Cordova will process algal toxin sample splits from two Klamath River sampling sites for microcystin variants (LF, LW, LR, LA, YR, and RR) and anatoxin-a using the LC-MS/MS method. The California Department of Fish and Game's Fish and Wildlife Water Pollution Control Laboratory in Rancho Cordova has highly trained staff and state-of-the-art equipment. Further information regarding laboratory QA/QC procedures is included as Appendices F, G and H.

6.0 FIELD METHODS AND PROCEDURES

KTWQP follows standard water quality grab sample procedures for nutrients, phytoplankton, and algal toxins using a churn to mix samples and following an appropriate regimen of blanks, duplicates and other steps to assure quality. Periphyton samples follow procedures as defined by

the U.S. EPA (2002) and USGS (Porter et al., 1995) as previously used on the Klamath River by Eilers (2005).

6.1 Field Equipment

Standard methods will be used for collecting nutrient, phytoplankton, periphyton and algal generated toxics with specific equipment and steps for use described below. All samples are shipped to the laboratory on ice the same day samples are collected (see Section 7.0).

6.1.1 Field Equipment List

Field equipment for nutrient, phytoplankton and toxin samples, include a churn splitter and bottles provided by laboratories. A YSI datasonde is used to capture ambient water quality (temperature, pH, D.O. and conductivity). The churn splitter requires cleaning with distilled water in the field (see Churn Cleaning SOP, Appendix A).

The following are the items on the KTWQP grab sampling check list that staff refer to before going into the field to collect nutrient, phytoplankton or algal toxin data:

1. Portable Water Quality instrument = YSI instrument
2. Ice (in bottles or packs)
3. Sample Bottles (from laboratory)
4. Camera
5. Extra labels for sample bottles
6. Coolers
7. Churn splitter
8. Van dorn sampler
9. Clip board
    a. Data sheet
    b. Pencils
    c. Permanent markers
    d. Field notebook
    e. Chain of Custody forms
    f. Protocol Instructions
    g. Shipping forms
10. Nitrile Gloves
11. Watch
12. Waders and boots

13. Distilled Water- 5+ gallons

Table 5. Nutrient, phytoplankton, periphyton an algal toxin parameters and the laboratory to which each will be shipped for analysis.

Parameter	**Laboratory**	**Method**	**Reporting Limit (mg/L)**	**MDL (mg/L)**
Total Phosphorus	AR	EPA 365.1	0.002	0.002
Ortho Phosphorus	AR	EPA 365.1	0.001	0.001
Total Nitrogen	AR	EPA 351.1	0.100	0.045
Nitrate + Nitrite	AR	EPA 353.2	0.010	0.005
Ammonia	AR	EPA 350.1	0.010	0.006
Chlorophyll *a* / Phaeophytin *a*	AR	APHA Standards (10200H)	0.0001	0.0001
Phytoplankton speciation and enumeration	AA	APHA Standards	NA	
Total Organic Carbon	AR	EPA 415.2	0.250	0.095
TSS	AR	EPA 160.2	0.050	0.10
TDS	AR	EPA 160.1	5.00	1.00
Alkalinity	AR	EPA 310.1	1.00	0.20
Calcium	AR	EPA 200.7	0.100	0.008
Magnesium	AR	EPA 200.7	0.100	0.011
Microcystin-LR	US EPA	ELISA	1.8 µg/l	1.8 µg/l
Microcystin (LR,LA,YR,RR,LF,LW) Anatoxin-a	CA Fish and Game	LC-MS/MS	1.0 µg/l	1.0 µg/l
Periphyton Chlorophyll-a	AR	APHA Standards (10200.H.3)	1 mg/m^2	1 mg/m^2
Periphyton speciation and enumeration	AA	APHA Standards	NA	NA

The following equipment is needed to follow the methods of Eilers (2005), U.S. EPA (2002) and USGS (Porter et al., 1995) for collection of periphyton samples:

1) Flow meter
2) Measuring tape

3) Measuring staff/yard stick for water depth
4) Grid (1.5 square feet) used to determine algae cover at sample sites
5) Tub for keeping rocks selected for sampling submerged to carry to sampling site.
6) Microscope slides (1 " by 3") to judge sampling area and for sample application
7) Scraping tools such toothbrushes, scrapers, razor blades and spatulas
8) Tray or pan used for working surface
9) Jars for capturing sample scrapings
10) Coolers with ice for shipping samples to labs
11) Sample jars with Lugol's solution for periphyton speciation and enumeration (from Aquatic Analysts)
12) Sample jars with chemical preservative (MgCO3) for fixing chlorophyll *a* (from Aquatic Research)

The Karuk Tribe has multiple YSI datasondes and flow meters to provide replacement equipment, in the event of any equipment malfunction.

6.1.2 Calibration of Field Equipment

The KTWQP YSI multi-channel datasondes are very reliable, if properly calibrated. KTWQP staff calibrate the YSI datasonde before use in the field daily following YSI instructions and other standard procedures for calibration (U.S. EPA, 2001) that are attached as Appendix E. Every winter the YSI recorders are sent back to the factory and any defective sensors replaced.

6.2 Field Screening

Field screening is not appropriate for the sampling regime proposed under this SAP.

6.5 Water Sampling

6.5.1 Surface Water Sampling

Grab samples will be collected within one to two days at all locations using standard techniques from USGS (Lurry and Kolb, 2000). Timing of samples will be bi-weekly (every two weeks) between May and October. General water quality parameters (temperature, dissolved oxygen, conductivity, and pH) will also be measured simultaneously with a YSI datasonde that has been calibrated (using procedures in Appendix E) and data recorded onto the grab sample datasheet.

At the locations previously selected (Section 1.1), water samples will be collected with a churn splitter to ensure that the sample is homogeneously mixed before the sample bottles are filled

(Figure 6.5.1). Depending on location, two collection methods may be used. For most sites, the churn is fully submerged into the stream and filled to the lid with flowing water, not stagnant water. For sites from a bridge (WA and KRAC), a Van dorn sampler is used to collect 3 samples from across the channel. The samples are poured into the churn and treated the same as all other sites. Prior to filling the churn for nutrient, phytoplankton and algal toxin sampling, the churn will be rinsed three times with distilled water. The goal of rinsing is to remove substances adhering to equipment from previous exposure to environmental and other media (Lurry and Kolb, 2000). After rinsing with distilled water, the churn is rinsed three times with stream water. Samples are collected from uniformly mixed water by wading out into the water channel from the bank and the churn is fully submerged into the stream and filled to the lid with sample water. Completely filling the churn allows for all sample bottles to be filled from one churn; thereby minimizing differences in water properties and quality between samples.

Figure 6.5.1. KTWQP staff collects Klamath River water using a Van dorn sampler and then deposits in churn to ensure representativeness of sample. Photo taken in 2006 at Klamath River above Copco Reservoir.

Proper use of the churn guarantees that the water is well mixed before the sample is collected. The churn should be stirred at a uniform rate by raising and lowering the splitter at approximately 9 inches per second while bottles are being filled (Bel-Art Products, 1993). If filling is stopped for some reason, the stiffing 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 will be provided by the associated laboratories and are considered sterile prior to field usage. Sample bottles without chemical preservatives will be rinsed with stream water from the churn three times before filling with sample water. In the case of bottles that contain chemical preservatives, bottles are not rinsed before sample collection and care is taken to avoid over-spillage that would result in chemical preservative loss. Collected samples are placed in coolers on ice for transport to contracted laboratories for analysis.

For quality assurance/ quality control (QA/QC) purposes duplicate and blank bottle sets are prepared and collected for one site each sampling period. These additional bottle sets are handled, prepared and filled following the same protocol used for regular bottle sets and samples.

6.6 Biological Sampling

Periphyton samples will be collected at the four mainstem Klamath River sampling sites below the KHP at the same time as the water quality grab samples. Periphyton sampling techniques employed are those recommended by U.S. EPA (2002) and USGS (Porter et al., 1995) and previously applied on the Klamath River by Eilers (2005). This section discusses samples of periphyton that will be analyzed for species diversity, while parallel samples are also collected at the same time for chemical analysis (chlorophyll *a*)(see section 6.6.1 below), which is a measure of weight per unit area (mg/m^2) of streambed. Site selection is not random, but rather chosen to represent periphyton communities in exposed sites that are probably most prevalent because of the Klamath Rivers width, as opposed to very-near shore or deep water assemblages, which are less extensive and less likely to affect water quality.

1. Select five representative cobbles from the stream bed at each sampling location. Rocks selected should not include extremes of algal cover. The specific stream bottom area sampled should meet the following criteria:

- Depth: 1 to 2 feet (use current meter staff)
- Velocity: 1 to 2 feet per second (current meter)
- Exposure: Clear solar path (i.e., no serious topographic or riparian shading)

2. Record the stream velocity, water depth, distance from the shore and the stream width for the location in which rocks will be removed for sampling on the datasheet.

3. Place 1.5 square foot grid on stream bed where cobbles are to be collected and make note of percent cover of algae within the total grid area (Figure 6.6.1).

4. Record any general observations that may be useful such as weather conditions and/or any drastic change in stream flow that could influence the periphyton community (i.e., recent rain event that caused increase in flow or scheduled flow releases or reductions).

5. Place cobbles selected for sampling in a tub containing water of sufficient depth to keep them submerged and transport to a convenient sample-processing area.

6. Select an area the size of a 1 inch by 3 inch microscope slide on an area of the clast that is representative and can be easily scraped (Figure 6.6.2). Two samples per location are collected for species identification and enumeration and also for chemical samples.

7. Scrape area of selected cobbles into sample jars (Figure 6.2) that contain Lugol's solution for cell preservation to aid species identification. The tray over which the sample has been processed is then carefully poured into the sample jar.

8. Label sample jars.

9. Pack labeled jars in cooler and complete field datasheets.

Although biological samples for species diversity do not require rush shipping, they are shipped the same day as collected along with chlorophyll *a* samples that do require 48 hour delivery.

Grid estimation of periphyton cover helps to gauge changes from month to month. Grid data are recorded on a separate datasheet (Appendix D). Effort is made to select an area that has not been disturbed by the sampling crew but still meets the same depths and velocities of location where the rock samples were taken. Use view finder of camera used for field documentation to visually inspect the amount of periphyton or macrophyte in each quadrant and record. Two samples should be taken, if one is not sufficiently representative.

Figure 6.6.1 This photo shows the 1.5 ft^2 grid for field estimation of periphyton cover in the vicinity of sample collection. Photo courtesy of YTEP.

Figure 6.6.2 Sample area equivalent to a 1" X 3" microscope slide is selected prior to scraping. Photo was taken by KTWQP staff at Klamath River Orleans site in June 2006.

6.6.1 Biological Sampling for Chemical Analysis

Periphyton collection for chlorophyll *a* is identical to steps described above for species diversity sampling with the following noted exceptions. Distilled water may be used in washing contents of trays over which samples have been processed. These samples also require immediate refrigeration and so are placed in coolers with ice that have been brought into the field and which will be used for shipping samples to the laboratory. Samples are shipped via overnight carrier in a sealed cooler packed with blue ice so that lab analysis is conducted within 48 hours. The wet ice will be double bagged to prevent leakage. The laboratories have specified they prefer blue ice over double bagged wet ice when we ship overnight to their labs. Grab samples for phytoplankton are also analyzed by Aquatic Research for chlorophyll-a and phaeophytin-a using a spectrophotometer, but sampling protocols do not vary from standard collection methods for nutrients, algal toxins or phytoplankton cell counts.

7.0 SAMPLE CONTAINERS, PRESERVATION AND STORAGE

As described above (Section 5.2), all samples collected are destined for one of four laboratories with which KTWQP is working. These laboratories provide containers for each sample type, including appropriate preservatives. For example, the periphyton chlorophyll *a* sample jars have a preservative of saturated solution of $MgCO_3$ prepared by Aquatic Research. Lugol's is added to periphyton and phytoplankton grab samples to preserve cell structure. Nutrient samples do not require fixing. If there are no agents for fixing samples in sampling jars, they are rinsed three times with river water prior to being filled with sample. Labs also supply coolers suitable for secure shipping along with blue ice, and KTWQP packs sufficient ice in them to maintain cold conditions conducive to sample preservation.

As mentioned above, special care will be taken in the cleaning of the sampling churn that is used to ensure the representativeness of nutrient, phytoplankton and algal toxic samples. It will be rinsed with distilled water three times and river water three times, before being submerged in the river for sampling. Churn cleaning SOP is attached as Appendix A.

8.0 DISPOSAL OF RESIDUAL MATERIALS

This section does not apply to the type of sampling conducted under this SAP.

9.0 SAMPLE DOCUMENTATION AND SHIPMENT

All samples will be fully documented and complete notes will accompany every sampling event, including photo monitoring.

9.1 Field Notes and Logbooks

Sampling from each day of data collection will be recorded in the field notebook, which includes:

1. Survey crew identification
2. Date and time
3. Ambient water quality measurements (temperature, pH, D.O., conductivity)
4. Number of bottles collected of each sample type (nutrients, phytoplankton, periphyton, and toxins)
5. Note fields for recording site conditions

As noted above, grid information on the percent cover of the stream bottom by periphyton is also recorded on the Grid Data Sheet (Appendix D). All water quality information is recorded with a YSI datasonde that is calibrated before going into the field every day samples are collected. Since this is the only source of field-recorded water quality data, YSI instrument calibration is not noted on sampling data sheets.

9.1.2 Photographs

Photographs will be taken at each sampling location during each sampling event. They will serve to verify information entered in the field logbook. For each photograph taken, the following information will be written in the logbook:

- Time, date, location, and weather conditions
- Description of the subject photographed
- Name of person taking the photograph

9.2 Labeling

All samples collected will be labeled in a clear and precise way for proper identification in the field and for tracking in the laboratory. At a minimum, the sample labels will contain the following information: station location, date of collection, analytical parameter(s), and method of preservation. Every sample, including samples collected from a single location but going to separate laboratories, will be assigned a unique sample number. Labels will be taped to all sample bottles with packing tape and label also serving as a security seal while samples are in transit.

9.3 Chain of Custody

All sample shipments for analyses will be accompanied by a KTWQP Nutrient, Phytoplankton and Periphyton, or Algal Toxin Chain of Custody Form (Appendix B). These forms will be completed and sent with each sample for each laboratory and each shipment (i.e., each day). If multiple coolers are sent to a single laboratory on a single day, duplicate forms will be completed and sent in each cooler.

Until the samples are shipped, the custody of the samples will be the responsibility of KTWQP staff assigned to collection and shipment of samples and the Project Manager. The chain of custody form includes date and time of transfer to carrier and carrier shipping number. Each laboratory listed above will be responsible for chain of custody once they have received from the shipping company. As noted above, seals on sample bottles help maintain security during shipment.

9.4 Packaging and Shipment

Sturdy coolers suitable for secure sample transit are provided by the laboratories and KTWQP staff makes sure that packing materials and ice are supplemented to protect samples in transit. The KTWQP Algal Toxin Chain of Custody Form supplies U.S. EPA staff at the Region 9 Richmond Laboratory with a Regional Analytical Program (RAP) number. Shipment of samples will not include a copy of the KTWQP field notebook, so that labs cannot introduce bias because locations are unknown to them.

10.0 QUALITY CONTROL

KTWQP will implement a fully coordinated QA/QC program including field QC samples, confirmation samples, background samples, laboratory QC samples, and split samples. Locations of QA/QC samples will vary between the sampling sites on the Klamath River and major tributaries. KTWQP and YTEP will share all QA/QC sample information as it comes back from the lab so that QA/QC is constantly analyzed by both staffs. Most QA/QC samples will be sent to the laboratory blind, while laboratory QC samples will be identified and additional sample collected, if necessary (e.g., a double volume). One blank and duplicate sample will be collected every sampling event.

10.1 Field Quality Control Samples

Field quality control samples will be taken for assessment of field contamination and assessment of sampling variability. Duplicate and blank samples are disguised with unique sample site IDs

and times so the lab does not know the difference between QA/QC samples and the primaries samples that have been submitted for analysis. For QA/QC purposes duplicate and blank bottle sets are prepared and collected for one site each sampling period with coordination between the KTWQP and YTEP. These additional bottle sets are handled, prepared and filled following the same protocol used for regular bottle sets and samples.

10.1.1 Assessment of Field Contamination (Blanks)

Blank samples are utilized to assess accuracy of the analysis and to verify that the handling, transportation or laboratory sample handling do not introduce error. Some blanks for nutrient samples may be filled in the KTWQP lab, while others may be filled in the field (see below). Distilled water is used for all blanks.

10.1.1.1 Equipment Blanks

Sometimes blanks will be drawn from the churn with water from the third rinse with distilled water in the field. This will check the effectiveness of previous churn cleanings as per Churn SOPs (Appendix A).

10.1.1.2 Field Blanks

Field blank samples will be obtained by pouring distilled water into a sampling container at the sampling point instead of river water. This will allow assessment of environmental contamination from the field and laboratories.

10.1.2 Assessment of Sample Variability (Field Duplicate or Co-located Samples)

Duplicate samples are obtained using the same process as regular samples. These are used to ensure the laboratory analytical precision. Standard levels of duplicate sampling are 10% and the program of QA/QC offered for this SAP would be for one location of the 11 being cooperatively sampled per sampling event, except for algal toxins which the QA procedure calls for 10% duplicate samples.

The site numbers for locations will proceed upstream from the more downriver end of KAT and proceed upriver. YTEP has the first five site numbers that correspond with sites from near the Pacific Ocean to the upstream end of the YIR. Thus, locations covered under this SAP for the Karuk Tribe are as follows for QA/QC testing purposes:

6. Klamath at Orleans (OR)

7. Salmon River at USGS gage (SA)
8. Klamath River downstream of Seiad Valley (SV)
9. Scott River at Johnson's Bar (SC)
10. Klamath River at Walker Bridge (WA)
11. Shasta River at USGS gage (SH)
12. Klamath River below Iron Gate dam (KRBI)
13. Klamath River above Copco dam (KRAC)
14. Iron Gate open water (IR01)
15. Iron Gate Jay Williams Boat Dock (IRJW)
16. Iron Gate Camp Creek (IRCC)
17. Copco open water (CR01)
18. Copco Copco Cove (CRCC)
19. Copco Mallard Cove (CRMC)

The target is that duplicates will have less than 20% RPD for most parameters (U.S. BOR, 2005). The exception is cell counts for *MSAE* and other blue-green algae species, which are recognized as highly variable. Consequently, a 50% overlap between sample splits would be acceptable (Appendix F).

10.3 Field Screening, Confirmation, and Split Samples

Field screening and confirmation samples do not apply to the type of sampling conducted under this SAP; split samples are discussed on Section 10.3.3.

10.3.3 Split Samples

As noted above, samples destined for the U.S. EPA lab and ELISA testing will be split and duplicates sent to the U.C. Davis for LC-MS/MS testing. This will allow KTWQP and cooperators to answer questions as to whether toxic algae pollution is restricted to microcystin-LR or if there are combinations of blue-green algae generated toxins, such as microcystin LF, LW, LA, YR and RR or anatoxin-a. California Department of Fish and Game's Fish and Wildlife Water Pollution Control Laboratory in Rancho Cordova measurement of microcystin-LR, will also be used for cross confirming the U.S. EPA R 9 Lab ELISA results.

10.4 Laboratory Quality Control Samples

To determine accuracy blind samples with known concentrations of different analytes known as spikes are done by Aquatic Research, Inc., which will be analyzing nutrient samples. Specific details of laboratory accuracy can be found in Appendix F.

Laboratories with which KTWQP is contracting have long records of high quality data provision and information regarding their procedures are attached as Appendices F, G, H.

11.0 FIELD VARIANCES

As conditions in the field may vary, it may become necessary to implement minor modifications to sampling as presented in this plan. When appropriate, the QA Office will be notified and a verbal approval will be obtained before implementing the changes. Modifications to the approved plan will be documented in the sampling project report. Sample periodicity and QA/QC sampling levels may vary in future years depending on the level of funding and commitment to cooperative sampling with the U.S. EPA.

12.0 FIELD HEALTH AND SAFETY PROCEDURES

Water samples may be hazardous when MSAE or other toxic blue-green algae species are present, otherwise field hazards are low. KTWQP staff conducting phytoplankton and toxic samples will be advised to minimize contact with the water in that season and be careful not to ingest any and to wash thoroughly after returning from the field.

Some sampling sites are in remote locations and steep hillsides adjacent pose risks to staff. Sampling will always be done at least in pairs for safety reasons and KTWQP staff will be advised to use caution when working on slippery substrate characteristic of the Klamath River margin. Poison oak is a common occupational hazard and washing after return from the field is also encouraged for that reason.

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APPENDICES