Standard Operating Procedures

Environmental Sampling of Cyanobacteria for Cell Enumeration, Identification and Toxin Analysis

Developed for the 2009 AIP Interim Measure 12
Water Quality Monitoring Activities
Klamath River

Prepared by the Klamath Blue Green Algae Working Group
Cyanobacteria Sampling SOP. V6
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Declaration of Agreement

This SOP was developed collaboratively with consensus by the Klamath Blue-Green Algae Work Group. There was input and participation from: US EPA Region 9; the CA State Water Resource Control Board; the North Coast Regional Water Quality Control Board; US DOI Bureau of Reclamation; Yurok Tribe; Karuk Tribe; PaciCorp; Klamath Watershed Institute; University of California - Berkeley, Aquatic Ecosystem Sciences; Water Course Engineering; and E&S Environmental Chemistry Inc. The US EPA Region 9, the North Coast Regional Water Quality Control Board, US DOI Bureau of Reclamation, PacifiCorp, the Yurok Tribe and the Karuk Tribe have agreed to abide by this SOP until such time as the group agrees to subsequent modifications or addendums. While parties have contributed to and agreed upon the technical rigor of the SOP, it has not undergone formal review by quality assurance officers at the participating entities. The work group anticipates formal quality assurance review for subsequent years. The data that is generated from the sampling entities following the methods stated in this Final SOP will be acceptable to all parties that concur with this agreement.

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List of Acronyms

AIP	Agreement in Principle
SOP	Standard Operating Procedures
BOD	biochemical oxygen demand
De-I	de-ionized water
DOC	dissolved organic carbon
GPS	global positioning system
MSDS	Material Safety Data Sheets
QA/QC	quality assurance/quality control

1.0 INTRODUCTION

The purpose of this SOP is to develop common sample collection methods for use by various river monitoring entities assessing the water quality of the Klamath River. This SOP covers cyanobacteria sampling of ambient river and reservoir waters, tissue sampling for cyanobacterial toxin analysis, and cyanobacterial/algal scum sampling.

These sampling methods / techniques support achieving the various data objectives defined in the AIP Interim Measure 12: Water Quality Monitoring Activities, Monitoring Year 2009 (referred to hereafter as the 2009 AIP Monitoring Plan). The AIP Monitoring Plan includes public health monitoring for blue-green algae (cyanobacteria) and base-line monitoring for the Klamath River. This SOP is intended to provide specifics on the methods to be used for the identified cyanobacteria sampling including: sampling technique, volume of sample needed for the identified analyses, and preservatives. Analytical methods and the number and kinds of quality assurance/quality control (QA/QC) samples will be presented in the 2009 AIP Monitoring Plan or the Quality Assurance Project Plan for the 2009 AIP Monitoring Plan (currently under development), as appropriate.

2.0 KLAMATH RIVER MONITORING PROGRAM OVERVIEW

The 2009 AIP Monitoring Plan includes monitoring stations located on the Klamath River (including reservoirs) from Link River in Oregon, to the estuary on the California coast. The 2009 AIP Monitoring Plan is being conducted as one of numerous monitoring and/or study efforts in the larger Klamath River Basin, including annual monitoring of tributaries above Upper Klamath Lake, Upper Klamath Lake, and tributaries to the Klamath River including the Lost River basin. The various efforts are being captured in a basinwide plan being developed by the Klamath Basin Water Quality Monitoring Coordination Group (KBWQMCG). The scope of the larger KBWQMCG basinwide monitoring effort (still in draft) (including monitoring activities to be done by other parties) provides the larger framework for the 2009 AIP Monitoring Plan effort.

2.1 DESCRIPTION OF PROGRAM AND ASSOCIATED TASKS

The purpose of this SOP is to provide a detailed description of the methodology to be used for cyanobacteria sampling in the water quality monitoring program, to improve consistency in the practices and procedures implemented by the various river monitoring entities.

Under the 2009 AIP Monitoring Plan, water quality samples and measurements will be taken at various sites on the Klamath River and its reservoirs, to support identification and evaluation of potential Public Health concerns associated with the presence of cyanobacteria, and to assess the water quality conditions. This sampling will also support the baseline Water Quality monitoring component of the 2009 AIP Monitoring Plan. Samples will be analyzed to identify and enumerate cyanobacteria (e.g., *Microcystis aeruginosa, Aphanizomenon flos-aquae*, and other species). The samples will also be analyzed for microcystin content using the ELISA immunoassay method and limited characterization by LC/MS/MS as indicated in the AIP Plan. The samples will be analyzed promptly for cyanobacteria and microcystin, allowing for Public Health assessment of conditions in sampled waters including the Klamath River and the Copco and Iron Gate reservoirs.

2.2 CYANOBACTERIAL MONITORING OBJECTIVES AND DESIGN

The 2009 AIP Monitoring Plan presents the program objectives and how the sampling design was developed to collect data needed to inform anticipated management decisions. This SOP is intended to support the collection of water and tissue samples for cyanobacteria toxin analyses, algal cell enumeration and identification, and biomass estimates, as applicable.

Sample locations include both shoreline and open water sites within the reservoir and along the Klamath River. Water and tissue samples will be collected at designated locations, and sent to analytical laboratories for algae species and enumeration, and microcystin concentrations by ELISA and/or LC/MS/MS.

2.3 OVERVIEW OF MONITORING ACTIVITIES AND SCHEDULE

The remainder of this SOP provides protocols for collecting and field-processing the following types of cyanobacteria samples:

- water samples for taxonomic identification
- water samples for determination of biomass
- water and tissue samples for estimation of microcystin toxins – both qualitative and quantitative (concentration)

To monitor public health risks, water samples should be collected at locations used for public access and recreation. Sampling should characterize concentrations associated with a reasonable maximum exposure (RME) scenario. Sampling locations may include: shoreline sites on Copco and Iron Gate reservoirs, and river sites stretching from Link River Dam to Turwar. Characterization of a RME scenario includes identifying the scenarios with the most sensitive receptors and exposure parameters. Exposure duration and frequency are parameters of an exposure scenario likely to be the most sensitive (to have significant impact on the exposure estimates) due to the degree of variability of the parameter values.

For cyanobacterial public health evaluations, the primary RME scenario is recreational exposure by children playing in shallow water at public access areas, where they could be exposed to cyanotoxins by incidental ingestion and inhalation, as well as by dermal contact. Children are considered to be a sensitive receptor - a result of high exposure (contact rate, duration and frequency), relatively lower body weight, and physical and neurological development. To characterize this RME scenario of recreational exposure by children, where possible, surface water samples should be collected in numbers adequate to statistically characterize the range of cyanotoxin concentrations in the area (e.g., to develop the 95^{th} or 90^{th} percentiles), however in blooms each sample event may disturb conditions for subsequent samples and this protocol is not designed to layout procedures for such statistically rigorous sampling methods. In the 2009 AIP Monitoring Plan, grab sampling is recommended to characterize cyanotoxin concentrations within public access areas for public health protection. In collecting these grab samples, field crews should consider all areas where a child might “explore” or from where concentrated scum might be transported, including areas beyond the range of likely exposure for the general population; and sampling should occur where maximum

concentrations of cyanobacteria and associated toxins are likely to be found (e.g., areas where higher levels of cyanobacteria are visible in the water column).

An additional cyanobacterial public health RME scenario to be considered is members of commercial, subsistence or recreational fishing communities, particularly children, exposed by ingestion of locally caught fish and mussels. To characterize this RME scenario of ingestion exposures by children, monitoring of tissue samples for public health evaluation may include sampling of fish (e.g., yellow perch) from Copco and Iron Gate reservoirs, and mussels or fish from the Klamath River below Iron Gate Dam. Mussel and fish sample collection will be conducted at locations known to be fishing/harvesting locations. Tissue sampling results will provide data to evaluate potential ingestion exposures related to recreational, commercial and subsistence fishing activities.

To characterize the period when cyanotoxins may pose a public health risk, mussel and tissue sampling is recommended to start before blooms occur or prior to the posting of health advisories for cyanobacteria and to conclude after the bloom has ended and conditions allow the removal of public health posting in the Klamath River (e.g., late October).

To the extent possible, both fish and mussel sample collection will be conducted on the same day as water quality sampling (public health and baseline monitoring).

3.0 SAMPLING PROCEDURES

3.1 Preparing for Sample and Data Collection

3.1.1 Safety

Exposure to cyanobacterial toxins, even at very low doses, can present potential risks including skin rashes (contact dermatitis), upper respiratory irritation, and other effects from recurring low level exposures (chronic exposures).

Samplers should have training for cyanobacterial sampling in accordance with federal, state and local requirements, and including (but not limited to) safety protocols and appropriate use of personal protective equipment (PPE) such as the selection and use of appropriate gloves to reduce skin exposure while sampling. Occupational exposure risk prevention is the responsibility of each river monitoring entity.

Samplers are encouraged to wear appropriate PPE including gloves throughout sample collection and processing. **Because cyanobacteria may be toxic, avoid having sample water contact the skin; always wear gloves and avoid splashing or other indirect contact with the face**.

3.1.2 Clean Sampling Procedures

Sampling protocols require the use of clean sampling procedures. These sampling procedures help to reduce (to the extent feasible given current resources) the amount of contamination introduced when collecting water-quality samples in the field. “Clean” sampling procedures involve (1) using equipment that is constructed of non-contaminating materials and that has been cleaned rigorously before field work and between field sites; (2) handling equipment in a manner that minimizes contamination; (3) collecting, processing, and handling samples in a manner that prevents contamination; and (4) routinely collecting quality-control (QC) samples.

The Appendix B and C describe some of these techniques in more detail.

3.1.3 Documentation of Field Conditions and Sampling

Field conditions and observations should be documented. Do not postpone making field notes to prevent incorrect or incomplete recollection of information. Additionally, all photos taken should be documented in the field notes.

Observations to be noted include:
- water color and the presence of any visible cyanobacteria or accumulations near the surface;
- the location and extent of surface accumulations;
- any odors that may be associated with cyanobacterial accumulations;
- description of the weather (sunny, percent cloud cover, wind speed, etc);
- evidence of unusual conditions or disturbances (e.g., recent fires, sediment loading);
- changes to water flow conditions (such as those from recent rainfall); and
- the dominant land use and land cover in the area surrounding the sampling locations.

Photo documentation is required to allow later evaluation and comparison of each location over time. At the initial sampling event, or before, establish consistent locations to be used for future photos. Create a photo record of the site by taking photos of the surroundings from several angles. Additionally, for each sampling event, photograph each sampling location to document the area around the sampling site and capture images of any algal distribution present at the time of sampling by photographing from a birdseye view from the boat looking down at the water. Note all photos taken, including time, location, camera, photoID number in field notes so that photos can later be labeled accordingly. Take additional photos of any significant change in the site area (e.g., burn areas erosion). Include an object or person in photos to show scale. Describe all photo points in the field notes. These photographs will provide documentation of physical influences and changes that could impact water quality.

Field measurements to be recorded should include:
- sampling time for each sample collected at a site, and each site;
- sample location coordinates. Use portable GPS where possible, and at a minimum provide location information that can be converted as accurately as practically feasible into degrees, minutes, seconds, and fractions of seconds of latitude and longitude;
- field-measured water quality parameters, where collected, including temperature, conductivity, dissolved oxygen, and pH;
- field workers present, and their roles.

3.2 Sampling Protocols

This section presents general information on cyanobacterial sampling, including sample containers to use, protocols for collection of samples, and information on sample handling (e.g., preservation) and documentation following collection.

Samples for cyanobacterial enumeration, toxin analyses and other water quality parameters should be collected from the same grab or composite sample. Therefore, the volume of the grab or composite sample must be sufficient for all planned samples at a given location. To avoid disturbing the water at each sampling location, shoreline and river grab samples should be obtained prior to other activities that would disturb the water.

Water sample collection for all cyanobacteria characterization should be conducted between 10 AM and 3 PM. Ahn, et. al. (2008), found cyanobacterial depth modulation resulted in optimal *Microcystis* sampling periods at 12 noon and 3 pm (highest biomass) and lowest biomass at the 9 AM and 5 PM sampling times. Because three hours (between noon and 3 pm) may not be adequate to collect all designated samples, the period between 10 AM and 3 PM is identified as the window for cyanobacterial sampling. Any variations from this should be recorded in field notes.

To maintain fully valid samples, immediately following collection, cyanobacterial and cyanotoxin samples should be labeled and placed in coolers with ice, to protect them from light and keep them chilled. Samples should be shipped as soon as possible, and within 24 to 48 hours. Days and timing of sample collection should be planned to assure samples can be appropriately chilled up to and throughout shipment, until received by the lab during the lab's business hours. When samples cannot be shipped within 24 to 48 hours, specific arrangements should be made with the receiving laboratory to identify requirements for sample preservation until they can be successfully shipped.

3.2.1 SAMPLE CONTAINERS

Sample bottles used for submitting samples to designated laboratories should meet the criteria listed below and presented in Table 3-1. These containers are not necessarily provided by the laboratories conducting the analyses.

- ELISA Cyanotoxin samples – Use small 4 oz. pre-cleaned glass thick-walled jars. Sample containers should be filled only 25% full; do not fill more than one-quarter full, to reduce the danger of breakage during chilling on ice or freezing.
- LC/MS/MS Cyanotoxin samples – Use 125 mL amber glass bottles with Teflon lid-liners. These sample bottles should be filled completely (e.g., to top of shoulder) to assure adequate sample volume for analytical needs.
- Cyanobacterial (phytoplankton) community composition (enumeration and identification) and biomass calculation – Use clean 125 mL amber glass or plastic bottles.
- Fish Tissue Samples - Individual fish should be wrapped in foil, placed in a clean zip-lock bag, and a unique sample name/number should be securely applied to the bag.

- Mussel tissue samples - Each sample (a minimum of 100 gms, or 3.5 oz) should be wrapped in foil, placed in a clean zip-lock bag, and a unique sample name/number should be applied to the bag.

Table 3-1. Sample bottle requirements for laboratory analysis of cyanobacteria parameters.

Parameters	**Sample container size**	**Container type**	**Preservation (in Field and for shipping)**
Microcystin in water by ELISA	125 mL	amber glass bottle	Chill to 6°C with double bagged wet ice or blue ice
Microcystin in water by LC/MS/MS	125 mL	amber glass bottles with Teflon lid-liners	Chill to 6°C with double bagged wet ice or blue ice
Water samples for cyanobacterial identification and enumeration	125 mL	amber glass or plastic bottles	Add 1 ml Lugol's solution to every 100 ml sample volume for minimum 1% Lugol's Iodine concentration.
Fish Tissue Samples for LC/MS/MS analysis	5 to 15 fish	wrapped in foil, and placed in a clean zip-lock bag	Double bagged wet or Dry Ice
Mussel tissue samples for LC/MS/MS analysis	minimum of 100 gms, (3.5 oz)	wrapped in foil, or glass bottle, and placed in a clean zip-lock bag	Double bagged wet or Dry Ice

All sample bottles and lids should be inspected for cleanliness and any defects before being used. Do not use a container or lid if defects are present or containers do not appear clean.

Assemble and prepare labels for all bottles for the identified different analytical sub-samples. At the time of sampling, complete the label to include the sample location name and number, date, time, type of sample (e.g., integrated photic zone), sample depth (e.g., 0 to 0.5 m), and type of analysis (e.g., cyanotoxins by ELISA).

3.2.2 SUB-DIVIDING SAMPLES

All samples to be collected at a given location (for all analyses and for Quality Assurance [QA] sample requirements), should be subdivided from a well mixed total sample volume. Protocols presented in Sections 3.2.3 and 3.2.4 for collecting various sample types, refer to these protocols for sub dividing samples.

Where the sample volume collected is not correct (either too much or too little) or needs to be sub-divided into two or more sample containers, the following protocols should be used. The use of a churn splitter (see 3.2.2.1) applies to all of the water collection methods described in subsequent sections of this SOP except surface water grab samples for which the sub-dividing method in 3.2.2.2 should be used.

3.2.2.1 Use of a Churn Splitter to create sub-samples

Proper use of the churn splitter optimizes the mixing of water before sub-samples are created.

- Prior to each use, the churn splitter (churn) should be rinsed three times with De-I or distilled water.
- For sampling, fill the churn splitter at least ¾ full (e.g., 10.5 liters in a 14 liter churn), so as to have enough water for all samples and to meet minimum volume requirements for proper churn operation. The churn should be filled as described in the sample collection protocols (identified below in Section 3.2.4). Alternatively, where specifically stated in the protocols below, the total sample volume may be collected directly into the churn (i.e. dipping the churn into the water to collect the sample).
- Churn the sample at a uniform rate of approximately 9 inches per second. The churning disc should touch the bottom of the tank on every stroke and the stroke length should be as long as possible without breaking the water surface. Inadequate stirring may result in non-representative sub-samples. Faster mixing can result in damage or deformation of algal cells. Avoid breaking the water surface at the top, as this introduces excessive air into the sample, and can alter the characteristics of the sample (e.g., for dissolved gases, bicarbonate, and pH).
- Churn the sample in the splitter for at least 10 strokes to assure uniform dispersion of the suspended material prior to taking the sub-samples. As sub-samples are withdrawn and the volume of sample in the churn decreases, maintain the churning rate of approximately 9 inches per second. If a break in churning is necessary, the stirring rate must be reestablished for at least 10 strokes before withdrawals are continued.
- While operating the churn, withdraw sample water to fill sample bottles for all parameters.
- Additional protocols are presented with the applicable sample collection protocol in Sections 3.2.3 and 3.2.4.

3.2.2.2 Creating sub-samples from the Sampling container

This protocol is to be used only for the collection of Public Health surface water samples (see Section 3.2.3) where total sample volume needed is small, and adequate sample volume is collected in a single container. Collect the sample volume as described in Section 3.2.3, below. Mix and sub-divide the sample volume as follows:

- Seal or close the sample collection container securely
- Mix by gently inverting the bottle 3 times. Do not shake, as this may damage or deform algal cells, or introduce excessive air into the sample.
- Promptly decant into sample bottles, to avoid separation or settling in the sample.
- Between each aliquot poured, repeat the mixing process, as described above.
- Additional protocols are presented with the applicable sample collection protocol in Sections 3.2.3 and 3.2.4.

3.2.3 GRAB SAMPLING FOR PUBLIC HEALTH ASSESSMENT

Water samples for public health assessment will be collected as grab samples, using the same sampling protocol at all locations. Use of this protocol is intended to maximize consistency of sample collection. In order to provide comparable and consistent sampling techniques for samples collected by various monitoring entities, the grab sampling technique described below will be used to collect water samples for cyanobacterial and cyanotoxin characterization for the purposes of public health assessments, from the designated water bodies.

For each sampling event and location, adequate volume should be collected as one sample, as described below, and mixed and subdivided (according to Section 3.2.2.2) to fill all samples containers for all specified analytical parameters (e.g. phytoplankton enumeration and cyanotoxin quantification).

In order to provide comparable and consistent sampling techniques for samples collected by various monitoring entities under the 2009 AIP Monitoring Plan, the grab sampling technique described below will be used to collect water samples for cyanobacterial and cyanotoxin characterization for the purposes of public health assessments from the designated reservoir and river shoreline sites, and its use is encouraged for any entity collecting separate cyanobacterial or cyanotoxin characterization samples in the Klamath Basin .

At each sampling location, samplers should conduct an initial visual survey of the public access area to identify where surface grab samples would be collected to represent a reasonable maximum exposure at that public access location (referred to hereafter as the RME location – see Section 2.3). Because cyanobacteria can accumulate and dissipate rapidly, depending on sun and wind conditions, a location having a greater presence of cyanobacteria (see Appendix D for photos of what blooms can look like) should be identified within each designated public access area, where the public is likely to come into contact with cyanotoxins. This requires subjective selection by the sampler, but should be limited to locations within the public access area (e.g., roughly 50 meters). When possible, sampling crew field trainings should be conducted before the sampling season begins, and involve comparing where different samplers subjectively select to sample in an effort to normalize the selection process.

For grab samples collected by hand-held methods, the sampler should boat or wade to where the sample will be collected, and that sample should be collected before other work is done at that location to minimize collection from a disturbed water column. Care must be taken to avoid collecting particulates that are re-suspended as the result of accessing the sampling location. Using the protocol described below, collect a grab sample from the upper 10 cm of the water column. If the sample is collected from a depth other than the upper 10 cm, this should be noted on the sample container and in the field notes with the reason for this deviation.

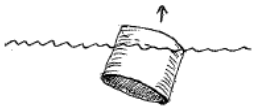
Grab samples will be collected using a clean sampling container.

Sampling at exactly the same GPS coordinates each time is not required; however, sampling should be within the public access point area (e.g. 50 meters of dock, boat launch, etc.). Following an initial visual survey of the public access area to identify the RME location where samples will be collected, collect surface grab samples as follows, using a wide-mouth jar that is turned on it's side and than submerged into the upper 10 cm of the water (see figure):

- Use a cleaned wide-mouth shallow jar (~8 cm diameter and 10 cm depth).
- Open jar. Tip opening of jar towards the water (at approximately a 45 angle) and slowly break water surface and begin to dip jar into the water. Turn the sampling container so that bottom side of jar is 8 cm below and horizontal to the surface. In other words, the jar will fully enter the water, but the top rim and side will not go below the surface (see figure). If in flowing water, when turning the bottle upright, turn it so that the opening faces upstream. The sampling bottle should not be moved along the surface to fill. Because of the wide mouth and shallow depth, it will be immediately filled.

- Tilt the full jar upright as it is slowly removed.

- Carefully raise the full jar from the water.
- Cap the container, tightening securely. Invert the jar gently three times, uncap the jar and pour to aliquot a portion into the first sample bottle, re-cap the jar and again gently invert the jar three times. Now uncap the jar and pour to aliquot a portion into the second sample bottle. The second sample bottle may be a replicate for the same lab, a different lab, or a non-replicate for different analyses at the same or a different lab. Any additional sub-dividing of the sample in the jar must be done by recapping and gently reinverting the collection jar three times.



- Clearly label the sample container, so that each sample is uniquely identified and includes the following information: the water body name, station location, date and time collected, sampler's name, type of sample (e.g. public health shoreline grab), sample depth (for example, 0 to 10 cm), and type of analysis (for example, cyanotoxin by ELISA).
- Place sample container in a zip-lock bag and seal. This is to prevent shipping ice from rendering sample label un-readable. If the sample bottle is glass, first wrap in protective bubble wrap or use foam sleeves to prevent breakage.
- Promptly place the labeled sample container in a cooler with ice to both protect from sunlight, and chill, until shipped. Double-bagged wet ice or blue ice is acceptable as long as the maximum threshold temperature (6°C) for samples is not exceeded. Block ice is discouraged to protect sample bottles from breaking during shipping.
- Replenish ice supply as often as needed to maintain samples at or below 6°C, and prior to preparing coolers for shipping to the appropriate laboratories.

3.2.4 SAMPLING FOR BASELINE MONITORING – OPEN WATER SAMPLING

This section describes the protocols for field collection of samples to be collected at open water locations in the reservoirs (3 sampling types in the reservoirs) and in the river:

- Reservoir - Surface/shallow grab samples taken from the upper 10 cm;
- Reservoir - Discrete depth grab samples at 0.5 meters (m) below the surface
- Reservoir - Depth-integrated vertical composite samples from the surface to a depth of 8 m (approximately the depth of the reservoir's photic zone)
- River – Surface/ shallow grab samples from the upper 0.5 meter depth in mixed, flowing portion of river.

The purpose and protocol for each of these is provided below. For each of these, samplers should navigate to the GPS coordinates of fixed sampling site locations, and maintain this position (e.g., with an anchor or by tying up to fixed objects) to the extent possible, throughout the sampling process.

3.2.4.1 Reservoir Surface-to-10 cm Grab Samples

Open-water samples will be collected from the reservoir at baseline monitoring sites, sampling from the surface to a depth of 10 cm. These surface/shallow grab samples will be collected at open water locations to continue characterization and monitoring of phytoplankton population dynamics and cyanotoxin levels. These samples will also be used to support the public health evaluation, as open-water activities such as water skiing are common in the reservoirs during the summer. Samples will be collected using the same protocol as the public health surface grab samples (see Section 3.2.3)

3.2.4.2 Open-Water Reservoir 0.5 m-Discrete Depth Grab Sampling

Open-water samples will be collected from the reservoir at baseline monitoring sites sampling from 0.5 meters below the surface, to continue characterization and monitoring of cyanobacteria present and cyanotoxin levels for general characterization of water quality consistent with time series data. Discrete depth samples are being taken to continue ongoing monitoring of cyanobacteria. Samples will be collected using a Van Dorn (or equivalent) sampler as follows:

To collect the discrete depth grab samples:

- Use a Van Dorn bottle, appropriately cleaned (see Appendix B)
- Check to ensure the closure mechanisms are working.
- Open the sampler ends
- Maintaining the sampler in the horizontal position, slowly lower the bottle to a depth of 0.5 meters.
- Trip the closure mechanism to seal the sample water into the sampler.
- Bring the sampler to the surface.
- Draw off water into a clean churn splitter by means of the nozzle in the lower stopper.
- Repeat the above steps until the total volume of water required for all indicated analyses has been collected.
- When adequate sample volume has been collected, operate the churn for mixing and sub-sampling, in accordance with Section 3.2.2.1.
- Clearly label any and all sample containers generated so that each sample is uniquely identified and includes information such as the water body name, station location, date and time collected, sampler's name, type of sample (e.g. public health shoreline grab), sample depth (for example, 0 to 10 cm), and type of analysis (for example, cyanotoxin by ELISA).
- Place sample containers in zip-lock bags and seal. This is to prevent shipping ice from rendering sample label un-readable. If the sample bottle is glass, first wrap in protective bubble wrap or a foam sleeve to prevent breakage.
- Promptly place the labeled sample containers in a cooler with ice to both protect from sunlight, and chill, until shipped. Double-bagged wet ice or blue ice is acceptable as long as the maximum threshold temperature (6°C) for samples is not exceeded. Block ice is discouraged to protect sample bottles from breaking during shipping.
- Replenish ice supply as often as needed to maintain samples at or below 6°C, and prior to preparing coolers for shipping to the appropriate laboratories.

3.2.4.3 Open-Water Reservoir 0–8 m Depth-integrated Composite Sampling

Depth-integrated samples (0 to 8 meters) will be used to represent overall reservoir conditions in the mixed or photic-zone. However, because of the generally irregular distribution of cyanobacterial communities across the photic zone, concentrations of toxins may be diluted when using this sample-composite method, and thus these samples are not appropriate for use in assessing public health risks, as cell densities and cyanotoxin level may appear falsely low. At open-water reservoir sites, an integrated vertical sample will be collected from the surface to a depth of 8 m (approximate depth of the photic zone in Iron Gate and Copco reservoirs), and obtained using an integrated hose sampler. The photic zone depth can be verified with secchi depth readings.

Depth-integrated samples at the open water reservoir sites will be collected using a hose sampler of 1.5 - 2 inch diameter, lowered in a vertical orientation. The hose sampler diameter is recommended to allow only one sample to adequately fill the churn splitter which will be used for subdividing into sample bottles (see Section 3.2.2.1).

- Navigate to the GPS coordinates of fixed sampling site.
- Use a cleaned hose sampler made of Teflon or clear, flexible laboratory-grade tubing, and approximately 10 meters long hose (to accommodate the 8 m of water column to be sampled, and have a portion of the hose accessible for handling and clamping above the water surface). The length of tubing should be weighted on one end (end to be lowered), marked with depth increments beginning at the weighted end, and with a plainly visible line at the 8 meter depth.
- Prior to sample collection, rinse the hose with native water by lowering the hose slowly, allowing it to fill with water until the bottom is at the bottom of the desired depth interval (8 meters), and pulling the hose back up to the surface. The hose must be open at both ends to allow air in the hose to be displaced by the water in the sampled interval.
- To collect a vertically integrated sample, with both ends of the hose open, lower the hose slowly allowing it to fill with water until the lower end is at the desired depth interval (8 meters). Retain a portion of the hose above the water surface for handling.
- Clamp the end of the hose above the surface to seal the hose and retain the water column sample in the hose.
- Carefully bring the weighted end of the full hose to the surface, draining the contents of the hose into a clean churn. The integrated sample should not exceed the recommended fill capacity of the churn.
- Repeat the above steps until the recommended fill capacity of the churn, and the necessary sample volume of water required for all analyses has been collected.
- When adequate sample volume has been collected, operate the churn for mixing and sub-sampling, in accordance with Section 3.2.2.1.
- Clearly label the sample containers so that each sample is uniquely identified and includes information such as the waterbody name, station location, date and time collected, sampler's name, type of sample (e.g. public health shoreline grab), sample depth (for example, 0 to 10 cm), and type of analysis (for example, cyanotoxin by ELISA).
- Place sample containers in ziplock bags and seal. This is to prevent shipping ice from rendering sample label un-readable. If the sample bottle is glass, first wrap in protective bubble wrap or a foam sleeve to prevent breakage.
- Promptly place the labeled sample containers in a cooler with ice to both protect from sunlight, and chill, until shipped. Double-bagged wet ice or blue ice is acceptable as long as the maximum threshold temperature (6°C) for samples is not exceeded. Block ice is discouraged to protect sample bottles from breaking during shipping.
- Replenish ice supply as often as needed to maintain samples at or below 6°C, and prior to preparing coolers for shipping to the appropriate laboratories.

3.2.4.4 OPEN-WATER RIVER SAMPLING

At river sites, a grab sample will be taken at each open-water location, in the current at approximately 0.5 meters (m) below the surface.

- Prior to initiating sampling, look upstream to identify the best mixed zone for sampling, noting any secondary channels to be sampled. Samples should be collected at a location in the moving portion of the river in an attempt to collect water samples to best represent the overall water quality. If the river is braided and the stream channels are accessible by wading or from a bridge, proportionate amounts of sample should be collected from each flow segment, to achieve a representative sample of the true mixed flow of the river.
- Use a cleaned sampling device (rinsed three times with distilled water or deionized); a sampling container or a churn splitter[1] may be used as the sampling container.

- **Wading to the sampling location:**

  i. Wade out to well-mixed area of river, staying down-stream of expected sampling location to avoid disturbing water and sediments.

  ii. With the sampling container oriented vertically with the mouth downward to retain air inside, push the contained into the water until the mouth is at the desired sampling depth, 0.5 m below the water surface.

  iii. Holding the mouth at the desired sampling depth and with the open end facing upstream, fully submerge the rest of the sampling container into the stream, allowing air to escape and the container to fill with water from the desired depth. The mouth of the container should at all times be upstream of the sample collector, sampling apparatus, and any disturbed sediments

  iv. Repeat the above steps until the necessary sample volume of water required for all analyses has been collected (not necessary if a churn is used as the sampling device). An integrated sample may be obtained by collecting and compositing several samples from different locations within the flow areas of interest.

- **Sampling from a remote location**: **(e.g., bridge):**

  i. Identify the location(s) in the main channel(s) to be sampled. If sample is to be collected from different channel(s), determine volume of water to be collected from each channel to proportionately collect sufficient volume to fill the churn splitter for subdividing into sample jars.

  ii. Use a cleaned Van Dorn sampler, and rinse with native water by lowering and submerging sampler in water.

  iii. Check to ensure the closure mechanisms are working.

  iv. Open both ends of the sampler.

  v. Maintaining the sampler in the horizontally position, slowly lower the bottle into the water and to 0.5 m below the water surface.

  vi. Trip the closure mechanism to seal the sample water into the sampler.

  vii. Carefully raise the full sampler to within reach.

  viii. Draw off water into the churn splitter by means of the nozzle in the lower stopper of the Van Dorn sampler.

[1] A churn splitter may be used as the sampling device, **with caution**; the health and safety of the sampler should at all times be the highest priority - churns full of water can be very heavy, threatening the sampler's health, safety and stability.

ix. Repeat the above steps until the necessary sample volume (at least ¾ of the churn volume) of water required for all analyses has been collected (not necessary if a churn is used as the sampling device). An integrated sample should be obtained by collecting and compositing several samples from different locations within the flow areas of interest.

- When adequate sample volume has been collected, operate the churn for mixing and sub-sampling, in accordance with Section 3.2.2.1.
- Clearly label the sample containers so that each sample is uniquely identified and includes information such as the waterbody name, station location, date and time collected, sampler's name, type of sample (e.g. public health shoreline grab), sample depth (for example, 0 to 10 cm), and type of analysis (for example, cyanotoxin by ELISA).
- Place sample containers in ziplock bags and seal. This is to prevent shipping ice from rendering sample label un-readable. If the sample bottle is glass, first wrap in protective bubble wrap or a foam sleeve to prevent breakage.
- Promptly place the labeled sample containers in a cooler with ice to both protect from sunlight, and chill, until shipped. Double-bagged wet ice or blue ice is acceptable as long as the maximum threshold temperature (at or below 6°C) for samples is not exceeded. Block ice is discouraged to protect sample bottles from breaking during shipping.
- Replenish ice supply as often as needed to maintain samples at of below 6°C, and prior to preparing coolers for shipping to the appropriate laboratories.

3.2.5 TISSUE SAMPLING

Fish and mussel collection may be conducted to support tissue cyanotoxins analysis (e.g., by LC/MS/MS) to address public health monitoring and evaluation.

3.2.5.1 Fish Sampling

Sufficient samples of the target fish species caught and consumed by fishers and their families from a water body should be collected and analyzed to adequately evaluate the potential human health risks from consumption of these tissues.

Individual fish will be processed for the analysis of filets and the analysis of livers will involve composite samples based on the following information. Composite samples will be done at the lab by qualified personnel utilizing non-contamination techniques since only whole fish will be sent to the laboratory**..**

To support analytical requirements for composited samples (i.e., the "75 percent rule") the length of the smallest fish collected in a sampling event at a given location should be at least 75 percent of the length of the largest fish in those sampled at that location and date. For example, if the largest fish s 200 mm, the smallest fish must be at least 150 mm. When possible, a narrower size range is preferred for the fish included in each set to be used for a composite sample (CalEPA 2005). Size should be measured as fork length (FL).

FISH SIZE
Fish that are sampled must meet any legal requirements for minimum and/or maximum

sizes established by the appropriate agencies in their current regulations(e.g., California Department of Fish and Game, DFG Sport Fishing Regulation). Additionally, fish without specific legal size requirements must be of "edible" size. Fish should be sampled from sizes that are typically caught and consumed by fishers so that measures of contaminant levels will be representative of consumer exposures. Sampling a range of fish that fishers catch will also provide a more representative estimate of their likely exposure. The same sources used to determine target species may also provide information on the sizes of fish fishers catch. Past sampling data can also be used to determine typical catch sizes (CalEPA 2005).

TIMING OF SAMPLING

Ideally, the timing of sampling programs should remain consistent between years (e.g., every summer). This is especially necessary when samples are collected from the same water body over several years to adequately evaluate the potential human health risks from consumption of these tissues. However, some fish species may only be available during certain times of the year, in which case, the ability to collect them must take priority. U.S. EPA (2000) recommends sampling fish during the period when they are most commonly harvested. Sampling periods should be used consistently when ongoing monitoring is conducted.

Selection of a sampling interval for ongoing monitoring depends on the objectives of the sampling program. For the purpose of this study fish tissue samples will be collected when cyanobacteria blooms are present.

FISH COLLECTION

Whenever possible, the Global Positioning System (GPS) will be used to locate the sampling location (e.g. latitude and longitude) during fish collection efforts. If GPS positions cannot be obtained, then sampling locations will be determined using USGS topographical maps and the latitude and longitude recorded for this site.

Sample integrity requires that fish be handled in a manner that prevents loss of contaminants already in the fish and prevents extraneous tissue contamination. Loss of contaminants already in the fish tissue will be prevented in the field by ensuring that the skin on fish specimens has not been lacerated by the sampling gear.

Target fish species will be collected for tissue analysis for cyanotoxins using gill netting, line fishing (aka angling), or other techniques as needed. Fish should be maintained in the field in a live-well until they can be frozen on wet or dry ice. Caught fish will only be placed on clean surfaces, such as aluminum foil. Ice chests will be cleaned prior to any sampling activities. Samples will be placed in waterproof plastic bags to avoid contamination from melting ice. Sampling equipment will be free from contaminants such as oils, grease and fuels. All utensils or equipment used directly in handling fish (e.g., such as fish hooks, measuring boards and scales) will be cleaned prior to each field sampling effort and transported in a manner that will maintain its cleanliness (wrapped in plastic bags or aluminum foil). The field collection team will clean this equipment between sampling sites by rinsing with distilled water and maintained in a clean environment.

After the fish has been collected follow the steps specified below:

1. Put on a fresh pair of gloves and prepare a clean work surface to process the sample. Keep hands, work surfaces, and wrapping materials clean and free of potential contaminants (mud, fuel, formalin, sun screen, insect repellant, etc.)

2. Examine the fish for any abnormal external conditions (e.g., lesion, parasites, etc) and take a photograph of the fish and record any abnormal external conditions.
3. Record the common name of the fish and measure the fork length (FL) to the nearest millimeter.
4. Weigh the fish to the nearest gram.
5. Wrap the fish in a single piece of heavy duty aluminum foil such that it is completely covered and enclosed. A second layer of foil may be necessary for a secure and uncompromised outer layer.
6. Place the sample in a self-sealing plastic bag, expel excess air, and seal the bag.
7. On the outside of the plastic bag apply a label (or write directly on the plastic bag with indelible ink) with the following information:

- Project Name
- Sample Location
- Field ID Number
- Date and Time
- Sampling Crew's Initials
- Fish Species Name

8. Place the labeled bag into a second self-sealing plastic bag, expel excess air and seal the bag. Check for leaks.
9. Immediately place the double-bagged sample into an insulated cooler with sufficient wet or dry ice.
10. Samples should be transported frozen, shipped by overnight delivery or driven within 24 hours of collection, with adequate amounts of dry or wet ice to keep the samples frozen until delivery and processing at the designated laboratory,.

The information listed below will be recorded in a field notebook or on a field datasheet:
Geographic location (latitude and longitude) using Global Positioning System
Species name
Date and time
Method of collection (e.g., gill net, trap, electrofish, etc.)
Station number
Sample identification number / numbers
Weather conditions (e.g., cloud cover, rain or shine, windy)
Water depth of capture (feet)
Sex of species, if possible
Evidence of hatchery markings (e.g.., fin clips, tags)(under "Comments")
Fork fish length (in metric units)
Total fish weight (in metric units to the nearest gram)
Sampling crew names
External marks or gross physiological abnormalities
(under "Comments")

3.2.5.2 Mussel Sampling

Sufficient samples of the target shellfish species caught and consumed by fishers and their families from a water body should be collected and analyzed to adequately evaluate the potential human health risks from consumption of these tissues. Dissection of the shellfish will be done in the designated laboratory using non-contaminating techniques.

SHELLFISH SIZE
Shellfish that are sampled should be approximately 100 grams (approximately 3.5 ounces) of mussel tissue, not including the weight of the shell. The size of the shells associated with these weight estimates will be based on experience from previous sample collection efforts.

SHELLFISH COLLECTION
Whenever possible, the Global Positioning System (GPS) will be used to locate the sampling location (e.g. latitude and longitude) during shellfish collection efforts. If GPS positions cannot be obtained, then sampling locations will be determined using USGS topographical maps and the latitude and longitude recorded for this site.

Sample integrity requires that shellfish be handled in a manner that prevents loss of contaminants already in the shellfish and prevents any tissue contamination from occurring. Caution should be taken to ensure that shells are not broken during sample handling and transportation activities.

Target shellfish species will be collected for tissue analysis for cyanotoxins by removing them from the substrate manually with bare or gloved hands. If it is difficult to remove the shellfish from the substrate a dull knife or spade may be used to extricate them from the river substrate. Shellfish should be maintained in the field in a live-well until they can be frozen on wet or dry ice. Shellfish will only be placed on clean surfaces, such as aluminum foil. Ice chests will be cleaned prior to any sampling activities. Samples will be placed in waterproof plastic bags to avoid contamination from melting ice. Sampling equipment will be free from contaminants such as oils, grease and fuels. All utensils or equipment used directly in handling shellfish (e.g., such as fish hooks, measuring boards and scales) will be cleaned prior to each field sampling effort and transported in a manner that will maintain its cleanliness (wrapped in plastic bags or aluminum foil). The field collection team will clean this equipment between sampling sites by rinsing with distilled water and maintained in a clean environment.

After the shellfish has been collected follow the steps specified below:

1. Put on a fresh pair of gloves and prepare a clean work surface to process the sample. Keep hands, work surfaces, and wrapping materials clean and free of potential contaminants (mud, fuel, formalin, sun screen, insect repellant, etc.)
2. If possible identify and record the common name of the shellfish and measure the total length (TL) to the nearest millimeter.
3. Wrap the shellfish in a single piece of heavy duty aluminum foil.
4. Place the sample in a self-sealing plastic bag, expel excess air and seal the bag.
5. On the outside of the plastic bag apply a label (or write directly on the plastic bag with indelible ink) with the following information:
    - Project Name
    - Sample Location
    - Field ID Number
    - Date and Time
    - Sampling Crew's Initials
    - Shellfish Species Name
6. Place the labeled bag into a second self-sealing plastic bag, expel excess air and seal the bag. Check for leaks.
7. Immediately place the double-bagged sample into an insulated cooler with wet or dry ice.

8. Samples should be transported frozen, shipped by overnight delivery or driven within 24 hours of collection, with adequate amounts of dry or wet ice to keep the samples frozen until delivery and processing at the designated laboratory,.

The information listed below will be recorded in a field notebook or on a field datasheet:
Geographic location (latitude and longitude) using Global Positioning System
Species name
Date and time
Station Location Name
Sample identification number / numbers
Weather conditions (e.g., cloud cover, rain or shine, windy)
Water depth of capture (feet)
Total length of each shellfish in a sample (in metric units)
Sampling crew names
External marks or gross physiological abnormalities
(under "Comments")

3.3 SAMPLE HANDLING AND CUSTODY REQUIREMENTS

3.3.1 Sample Labeling and Preservation

Documentation - Field samplers should clearly mark all sample containers so that each sample container is uniquely identified. All sample containers must be labeled with the following information at a minimum: date, time of collection, sampler, analysis (microcystin), and sample number, location or other identifiers. It is good practice to record sample time on the bottle immediately after sampling, because it provides a backup unique identifier. Sample collection times should not be pre-printed, to help ensure that bottles are not switched (e.g., grab wrong pre-labeled jar).

Once samples have been collected and labeled, clean the outside of the containers with paper towels or other absorbent materials to remove any spilled sample from the exterior of the container.

The chain-of-custody form must be reviewed to ensure that it matches the information on the sample bottles.

Sample Preservation – Samples must be kept chilled (on ice or refrigerated) and in the dark until shipped. Immediately upon collection, water samples should be chilled and maintained at 6 degrees C (or frozen as specified above) prior to and throughout shipping. Tissue samples should be frozen and shipped with dry ice or frozen and shipped with blue ice. Samples for algal cell enumeration and identification should be either immediately placed on wet ice or blue ice and within 8 hours preserved with a sufficient volume (1 mL/100 ml or 1% minimum) of Lugol's solution, or at the time of collection immediately preserved with Lugol's solution in the field and kept cool. Lugol's solution should be added until a drop of the preserved sample placed on a white writing paper turns the paper black almost immediately.

Samples for ELISA Microcystin analysis can be shipped frozen or with sufficient ice or blue ice to bring them to and keep them at 6 C or lower. Results for samples received above 6 C will be

flagged. If samples are frozen, care should be taken to fill bottles only about 1/4 full to prevent breakage. Only a very small amount, less than 1 mL, is actually used in the analysis. Samples must be shipped within 2 weeks of sampling to ensure that the lab has sufficient time to analyze them within the holding time.

3.3.2 Sample Documentation, Chain of Custody and Shipping

Sample Shipping - When ever possible, samples should be shipped on the same day they are collected, following the instructions provided by the analyzing laboratory. Sample collection should be planned accordingly. Should samples not be shipped promptly (same day), arrangements for sample custody, preservation and handling should be made with the laboratory, and samples shipped for receipt by the laboratory as soon as practicable, without compromising the sample validity. Communicate with the analytical laboratory to assure the lab will be open to receive shipped samples.

Samples are typically placed in a cooler, with double-bagged wet ice or blue ice, and shipped using priority overnight mail to arrive at the analyzing laboratory the next day/morning. For shipping, glass samples bottles should be protected from breakage ("bubble wrap" is recommended).

For samples to be analyzed for cyanotoxins by ELISA at the USEPA Region 9 lab, the Regional Sample Control Coordinator **must** be called at 510-412-2389 before samples are shipped to alert the lab to expect samples. If a message is left it should include: the project (i.e. Klamath River), the analyte (i.e. microcystin); the number of samples; shipping date; and tracking number.

Samples should be shipped for overnight delivery Monday to Thursday. Samples must **not** be shipped Friday, unless special arrangements have been made for the samples to be received and processed by the receiving lab, so that they do not sit in a warehouse and warm up before delivery on Monday.

Samples to be analyzed by the USEPA Region 9 lab must be shipped to:

Sample Custodian
US EPA Region 9 Lab
1337 S. 46th St., Bldg. 201
Richmond, CA 94804
510-412-2389

DO NOT ship to Andrew Lincoff; if so, they may languish on the warehouse shelf.

Samples to be analyzed by the CA Fish and Game lab in Rancho Cordova, CA should be mailed to:

Dr. Abdou Mekebri
Fish and Wildlife Water Pollution Control Lab
2005 Nimbus Road
Rancho Cordova, CA 95670
916-201-1770

Samples to be analyzed by Aquatic Analysts should be mailed to:

Jim Sweet
Aquatic Analysts
17063 SE Wiley Way
Milwaukie, OR 97267
503-869-5032

Chain of Custody (COC)

All samples collected in the field require Chain of Custody (COC). Chain-of-custody (COC) sheets will serve to document the handling of the samples from the time of collection through the time of laboratory analysis.

After collection, samples are in the custody of the sampling team; the COC forms should clearly document all the samples collected during that sampling day, associated sample identification name/numbers, and the date and time of collection for each sample. The COC form may be completed at the end of the day when sampling is finished. The COC form is shipped with the samples to the analytical laboratory.

Custody is transferred when coolers are shipped to the analytical laboratory or transferred to a courier. At that point the COC form is signed by the samplers (as surrendering the container) and the recipients (either the receiving laboratory or the courier, where the bill of lading serves as COC transfer until received by the laboratory). Appropriate sample documentation should be placed in a separate re-sealable plastic bag and attach to the inside lid of the shipping cooler.

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Appendix A - Sampling equipment

Sampling equipment should be assembled, checked, and calibrated just before a sampling trip. This includes assuring that every piece of equipment that might be needed is available, the equipment is in good working order, power supplies/batteries are fresh, and meters hold their calibrations. If meters or equipment are in questionable shape, bring along a replacement. An extensive checklist of sampling equipment and supplies is listed below. Always clean and/or decontaminate equipment before use. Consult with the applicable monitoring plan about cleaning procedures for various sampling protocols and their respective analytical accuracy requirements (e.g., parts per million or parts per billion). To avoid cross contamination during transport to the sampling site(s), wrap inorganic equipment in plastic and organic equipment in aluminum foil.

Suggested Equipment and Supplies

-
- Personal Protective Equipment
- Field datasheets or field notebook
- Clipboard
- List of multi-probes to place/service/retrieve
- List of sites to sample and parameters
- Sharpie pens and pencils
- Multi-probe profile unit
- 2 YSI field cables
- 2 YSI 650 MDS handheld units
- 1 Bucket
- Spare batteries - AA and C cell
- Tools to replace batteries
- Turbidity water sampler
- Turbidity meter
- Secchi disk
- Thermometer/Anemometer
- Keys
- Waders or wading sandals
- Camera – charged, and w/ extra memory cards
- Van Dorn
- Churn
- Distilled water and/or De-I
- Sample bottles
- Gallon zip-loc bags
- Shipping labels
- COC forms
- Leather and/or rubber gloves
- Cell phone, or other communication devices appropriate to the situation
- First Aid kit
- GPS unit

Field-folder checklist for water-quality stations

Possible Information to Record:

Station description
GSP location of nearby gaging station (if one is present)
GPS location of sampling sites: high and low streamflows
Hydrologic and geologic sections
Name of landowner, tenant, or other responsible party
Site access instructions (for example, call owner or site operator before arrival at site, obtain key to unlock security gate)
Photographs to document site conditions
Maps to site (State and local)
Profiles of cross section of stream channel at sampling locations(s)
Stream-bottom geometry
Physical and field measurements
Safety information
Nearest emergency facilities
Phone numbers (home) of emergency contacts
Traffic condition and traffic plan showing where to park, placement of flags and cones
Location of power lines
Environmental hazards, such as weather and animals
Sampling schedule
Laboratory analyses to be requested and associated codes
When to collect samples (high or low flow, time of day)
Bottle types needed for each analytical schedule
Sampling instructions
Cumulative discharge curves at about 10-, 50-, and 90-percent duration
Velocity cross sections at about 10-, 50-, and 90-percent duration
Equipment to use at various flows
Flow-duration curve
Discharge rating curves and (or) tables
Shipping instructions
Amount of ice to use
Mailing labels to and from laboratory
Location of nearest post office or shipping agent
Surface-water field form and an example of completed form
Special equipment needed to address site-specific conditions

Appendix B - Decontamination Procedures

Decontamination of Sampling Equipment and Supplies

Equipment decontamination is intended to remove residues from the environment, prior sampling, and handling or manufacturing activities adhering to equipment or other supplies that will come into contact with the sample. Equipment used for sampling (sample collection, processing, and handling) must be cleaned before being used. Sampling equipment must be cleaned before the first use each sampling day and re-cleaned before use at the next site to avoid cross contamination between sampling sites.

- Clean equipment. If the sampling equipment will not be reused during a field trip, after using triple rinse the sampler components thoroughly with clean water (tap, distilled or deionized water) before they dry and place the sampler in a plastic bag for transport to the office laboratory for cleaning. If the sampling equipment will be reused during the field trip, triple rinse the sampler components with distilled or deionized water before they dry. Field-clean the sampler at the next sampling site before use.

Generally the sequence for cleaning equipment for sampling cyanobacteria or cyanotoxins can be summarized as follows: detergent wash; tap/De-I rinse; De-I soak; and air dry.
The following detailed equipment cleaning procedures should be used:

- Use gloves, which are changed between each step.
- Scrub the equipment / tubing in tap or De-I water with a nonmetallic, non-colored brush to remove visible debris.
- Soak the equipment and tubing for 30 minutes in 0.2-percent Liquinox solution or another phosphate-free detergent
- Thoroughly rinse the equipment and tubing with tap water.
- Rinse the equipment three times with De-I water.
- Allow everything to air dry completely.

Avoid using samplers with plastic components, as the plastic may adsorb cyanotoxins and cross-contaminate samples. Do not forget to decontaminate equipment before use. Once the equipment is decontaminated, wrap inorganic equipment in plastic and organic equipment in aluminum foil for storage and transport.

Churn Splitter Cleaning and Rinsing

- For purposes of this section regarding Churn splitter cleaning and rinsing, churn splitter refers to churn splitter container, lid, and churning disk.
- At the beginning of each sampling day, triple rinse churn splitter with distilled water or de-I water. If the site is an open water baseline site (see section 3.2.4) then also rinse it one time with native (stream or reservoir) water. Do not rinse with native water before collecting public health surface grab samples (see section 3.2.3). For each rinse, water is run through the discharge spout. After collecting each sample, remove visible debris and triple rinse the churn again with distilled or de-I water.
- Rinsing with HCL is not necessary for sampling for cyanobacteria and cyanotoxins.

Appendix C - Quality-Assurance Plans and Quality-Control Samples

Assuring the validity of environmental data or QA is accomplished by collecting QC samples, reviewing and analyzing QC data, and making adjustments to data-collection procedures on the basis of the results. Because environmental sampling is done in a relatively uncontrolled manner subject to many variables, large sources of error may exist. Therefore, measures of control over the collection of water-quality samples are crucial for answering any questions regarding the validity of the data. QA plans should contain planned and systematic procedures necessary to provide confidence that the data will satisfy established requirements for quality. Detailed information about QA/QC samples can be found throughout the literature and within specific agencies responsible for water-quality data collection. QA sample requirements are addressed in the related sampling plan, the 2009 AIP Monitoring Plan.

This section gives a review of the types of QC samples commonly required in QA plans. Not all are specified by the 2009 AIP Monitoring Plan, however they may be required by future monitoring plans for projects using this SOP.

For quality assurance/ quality control (QA/QC) purposes duplicates and blank samples are prepared and collected each sampling period. These additional bottle sets are handled, prepared and filled following the same protocol used for regular samples, so as to be indistinguishable by the analyzing laboratory. .

Blank solutions or "blanks" are solutions (De-I or distilled water) having target constituent concentrations at concentrations less than the method detection limits. These solutions are used to develop specific types of blank samples, and may be laboratory certified.

Equipment blanks are blank solutions that are processed through sampling equipment used for collecting and processing an environmental sample (e.g., sampling container and churn splitter), prepared a samples and submitted for laboratory analysis. Once analyzed, the blanks indicate the effectiveness of cleaning of field equipment. Equipment blanks should be collected after sampling the station with the highest contamination.

Field blanks are containers of distilled or de-I water that are subjected to the same aspects of sample collection, field processing and preservation, transportation, and laboratory handling as environmental samples. Field blanks are used to check for contamination in the laboratory and for cross contamination during the collection and shipment of samples. Trip or travel blanks are containers of distilled or de-I water that are put in the same type of bottle as used for environmental samples and are kept with the set of sample bottles both before and after sample collection. These blanks are used to detect contamination that occurs during sample transport or storage or in the laboratory.

Duplicate samples are used to assess the performance in those parts of the procedure that are replicated. Duplicate samples are collected in a manner such that the samples are thought to be essentially identical in composition. Many types of duplicate samples are possible, and each might yield slightly different results in a dynamic hydrologic setting. Split duplicates sent to separate laboratories are used to ensure that results from the different laboratories are comparable. Split duplicates sent to the same laboratory are used to measure the variability of the laboratory.

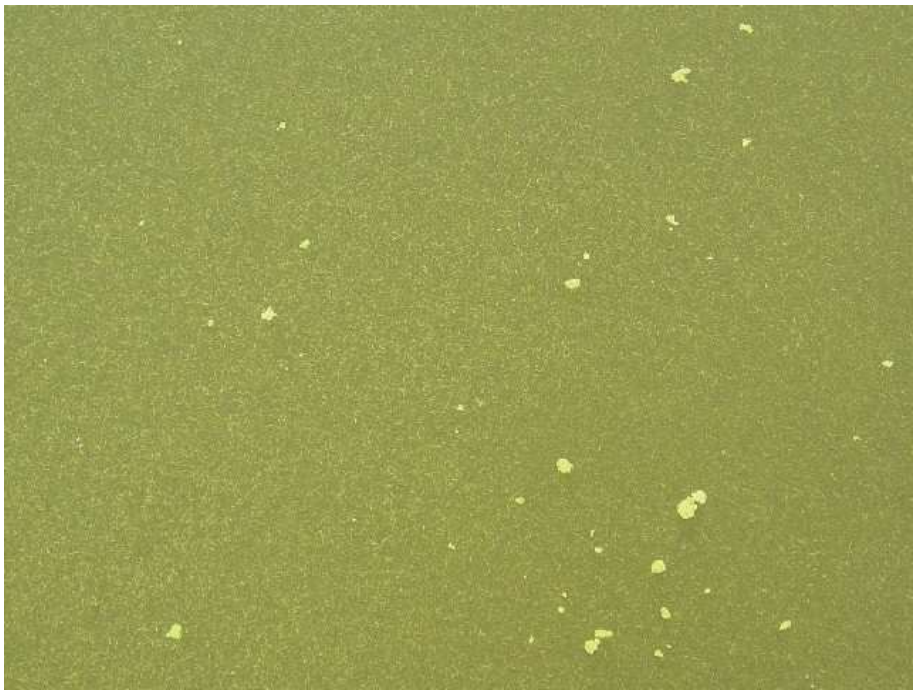
Concurrent or sequential duplicates are used to ensure that samples collected with different samplers or by different sampling methods are comparable.

Appendix D – Bloom Photos[2]

[2] Please note that photos are included to show what blooms have looked like and could look like in some of their many appearances. Photos are not intended to represent sampling techniques and should not be referred to as such.


AUG 8 2006


AUG 8 2006

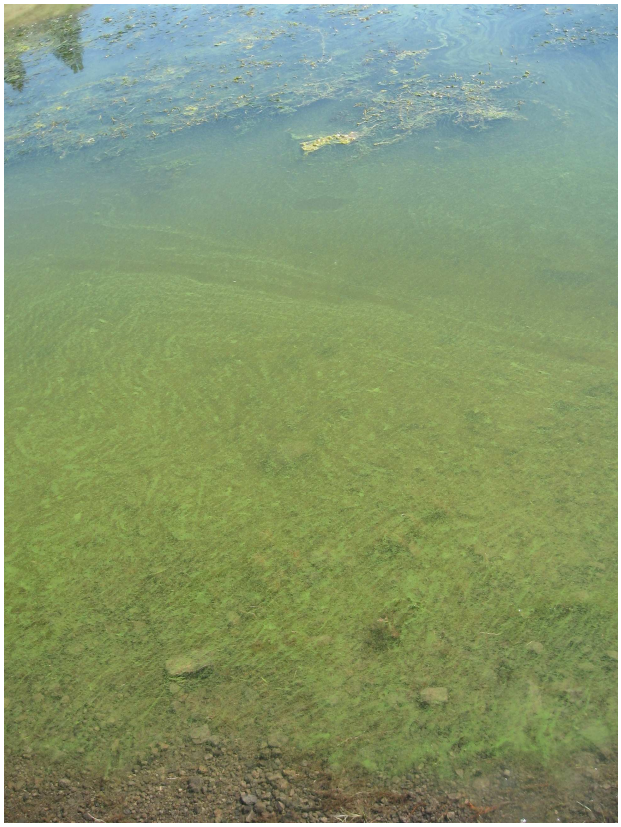



JUN 26 2007


JUN 27 2007


JUL 23 2008


JUL 23 2008



This is not a cyanobacteria bloom, but there are cyanobacteria mixed in: