Surface Water Ambient Monitoring Program (SWAMP)

Quality Assurance Project Plan

For

Harmful cyanobacteria blooms and their toxins in Clear Lake and the Delta (California)

10-058-150

Prepared for:

Central Valley Regional Water Quality Control Board

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STATE WATER RESOURCES CONTROL BOARD

Version 2.0b

April, 2011

GROUP A ELEMENTS: PROJECT MANAGEMENT

1. TITLE AND APPROVAL SHEETS

Quality Assurance Project Plan

For

Harmful cyanobacteria blooms and their toxins in Clear Lake and the Delta (California)

10-058-150

Version 2.0

April 2011

The Regents of the University of California, Santa Cruz

APPROVAL SIGNATURES

UNIVERSITY OF CALIFORNIA SANTA CRUZ:

Title:	Name:	Signature:	Date*:
Project Director and QA Officer	Cécile E. Mioni	On file	April 27, 2011
Project Director, co-PI and QA Officer	Raphael Kudela	On file	April 27, 2011
Project Manager	Kendra Hayashi	On file	April 27, 2011

UNIVERSITY OF CALIFORNIA DAVIS:

Subcontractor

Project Director, co-PI and QA Officer	Dolores Baxa	On file	April 25, 2011

CALIFORNIA STATE, DEPARTMENT OF WATER RESOURCES:

Collaborating agency (Environmental Monitoring Program)

Field Officer	Scott Waller	On file	April 27, 2011

LAKE COUNTY:

Collaborating agency

Field Officer	Tom Smythe	On file	April 26, 2011

STATE BOARD (SWRCB**):

Title:	Name:	Signature:	Date*:
Contract Manager	Meghan Sullivan		
SWAMP QA officer (SWRCB/SWAMP)	Beverly van Buuren		
Regional Board QA officer (CVRWQCB)	Leticia Valadez	On file	April 25, 2011

* This is a contractual document. The signature dates indicate the earliest date when the project can start.

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3. DISTRIBUTION LIST

The key personnel that will oversee the implementation of the QAPP are listed below.

Title:	Name (Affiliation):	Tel. No.:
Project Director and QA officer	Cécile Mioni (UCSC)	541-515-0425
Project Director and QA officer	Raphael Kudela (UCSC)	831 459-3290
Project Manager	Kendra Hayashi (UCSC)	831-459-4298
Project Director & QA officer	Dolores Baxa (UCD)	530-754-8020
Regional Board Contract Manager	Meghan Sullivan (CVRWQCB)*	916-464-4858
SWAMP Board QA Officer	Beverly van Buuren (SWAMP)*	206- 297-1378
Regional Board QA Officer	Leticia Valadez (CVRWQCB)*	916- 464-4634
Field Officer	Scott Waller (DWR/EMP)	916-651-0194
Field Officer	Tom Smythe (Lake County)	707-263-2344

4. PROJECT/TASK ORGANIZATION

4.1 Involved parties and roles.
This section of the QAPP identifies the management elements of the water quality study. It includes a description of the staff organization, the background and objectives of the research, the tasks involved in implementing the study, the data quality objectives and performance measures, and the requirements for documentation and reporting results. The Project QA managers that will advise on the project, but not participate in the execution of this program and delivery of the final report are listed with an asterisk in the distribution list and table 4-1.

Table 4-1. Personnel responsibilities

Name	***Organizational Affiliation***	***Title***	***Contact Information*** (Telephone, fax, e-mail address)
Meghan Sullivan*	CVRWQCB	Contract Manager	916-464-4858 (voice) msullivan@waterboards.ca.gov
Cécile Mioni	UCSC	Project Director and QA Officer	541-515-0425 (cell, voice) 831-582-4122 (fax) cmioni@ucsc.edu
Beverly van Buuren*	SWRCB/SWAMP	SWAMP QA officer	206- 297-1378 (voice) bvanbuuren@mlml.calstate.edu
Leticia Valadez*	CVRWQCB	Regional QA officer	916- 464-4634 lvaladez@waterboards.ca.gov
Raphael Kudela	UCSC	Project Director and QA Officer	831-459-3290 (voice) 831-459-4882 (fax) Kudela@ucsc.edu
Kendra Hayashi	UCSC	Project Manager	831-459-4298 (voice) khayashi@ucsc.edu
Dolores Baxa	UCD	Project Director and QA Officer	530-754-8020 (voice) 530-752-7690 (fax) dvbaxa@ucdavis.edu
Scott Waller	EMP/DWR	Field Officer	916-651-0194 (voice) swaller@water.ca.gov
Tom Smythe	Lake County	Field Officer	707-263-2344 (voice) 707-263-1965 (fax) tom_s@co.lake.ca.us

Meghan Sullivan (CVRWQCB) will serve as a contract manager. The contract manager will review, evaluate and approve study design and site locations, coordinate with other monitoring efforts in the study areas, and verify the completeness of all tasks.

Beverly van Buuren (SWRCB/SWAMP) and **Leticia Valadez** (CVRWQCB) will serve as regional QA officers. They will be responsible for verifying that the quality assurance and quality control procedures found in this QAPP meet the standards developed for Surface Water Ambient Monitoring Program (SWAMP) as set forth in the Electronic Template for EPA QAPP guidelines and the SWAMP Measurement Quality Objectives (MQOs).

Cécile Mioni is an assistant researcher at the University of California, Santa Cruz (UCSC). As the Project Director she will be the project administrator and will provide technical services as needed for contract completion. She will monitor, supervise and review all work performed and coordinate budgeting and scheduling to assure that the contract is completed within budget, on schedule and in accordance with approved procedures, applicable laws and regulations. The director will also manage sub-contracts to ensure delivery of work products according to contract scope, schedule and budget. She will ensure that contract requirements are met through completion of a final report and quarterly progress reports that she will submit to the Contract Manager. She will prepare and review QA reports as the QA officer and ensure the QAPP is properly followed. She will also prepare and execute a monitoring plan with the assistance of the Field officers and in accordance with State Water Bored SWAMP format and will submit this plan to the contract manager for peer review and approval.

Raphael Kudela is a professor at the University of California Santa Cruz (UCSC. As the Project Director for the UCSC component, he will be the project administrator and will oversee project coordination, purchases, budget analysis, LC-MS data management and analysis, and report writing. The director will review QA reports as the QA officer and ensure the QAPP is properly followed. The Project Director position includes responsibility for laboratory analyses (LC-MS) and will serve as primary supervisor for student assistants participating in the UCSC component.

Kendra Hayashi is the project manager and is primarily responsible for the preparation for and coordination of laboratory activities related to the monitoring program. The duties include overseeing the collection, inventory and storage of water samples, assisting in the implementation of field components of the QAPP and reviewing measurements to ensure QAPP guidelines are being met; assisting laboratory activities, sample processing, data analysis, and writing project reports. Kendra will produce QA reports for the Project Director (RK)'s review, and make requested corrective actions if data quality specified in the QAPP is not met.

Dolores Baxa is associate project scientist at UCD and will be subcontracting on this project. As the Project Director for the UCD component, she will be responsible for the molecular sample processing as well as their analysis and she will serve as primary supervisor for student assistants participating in the UCD component. She will also prepare and review QA reports as the QA officer for molecular samples and she will ensure that the QAPP is properly followed for her section.

Scott Waller (California Department of Water Resources) and **Tom Smythe** (Lake County) will provide access to sampling stations as well as to ancillary data. They will facilitate field collection and will provide oversight to ensure local and state regulations are met.

Student assistants under the supervision of project manager and director, student research assistants will assist with laboratory and field procedures. Responsibilities include routine analysis of water samples, washing and preparing sample bottles for fieldwork, helping to maintain the laboratory, and data entry.

4.2 Quality Assurance Officer role

The Quality Assurance Officers will be responsible for maintaining the QAPP and for ensuring that personnel have the most current approved version of the QAPP. Prior to conducting any sampling activities, the Quality Assurance Officer shall contact the individuals identified in the QAPP organization chart (Figure 1) to confirm that all parties have been trained to follow the most recent version of the approved QAPP. The QA officer will also generate quality control reports that will be reviewed by the contract manager.

4.3 Persons responsible for QAPP update and maintenance.

The project director (Cécile Mioni) in association with the other project co-PIs (Raphael Kudela and Dolores Baxa) and the contract manager will update the QAPP and be responsible for making changes to the QAPP. Cécile Mioni will be responsible for submitting drafts for review, submitting updates and/or changes to the RWQCB preparing a final copy, and submitting the final copy for signatures.

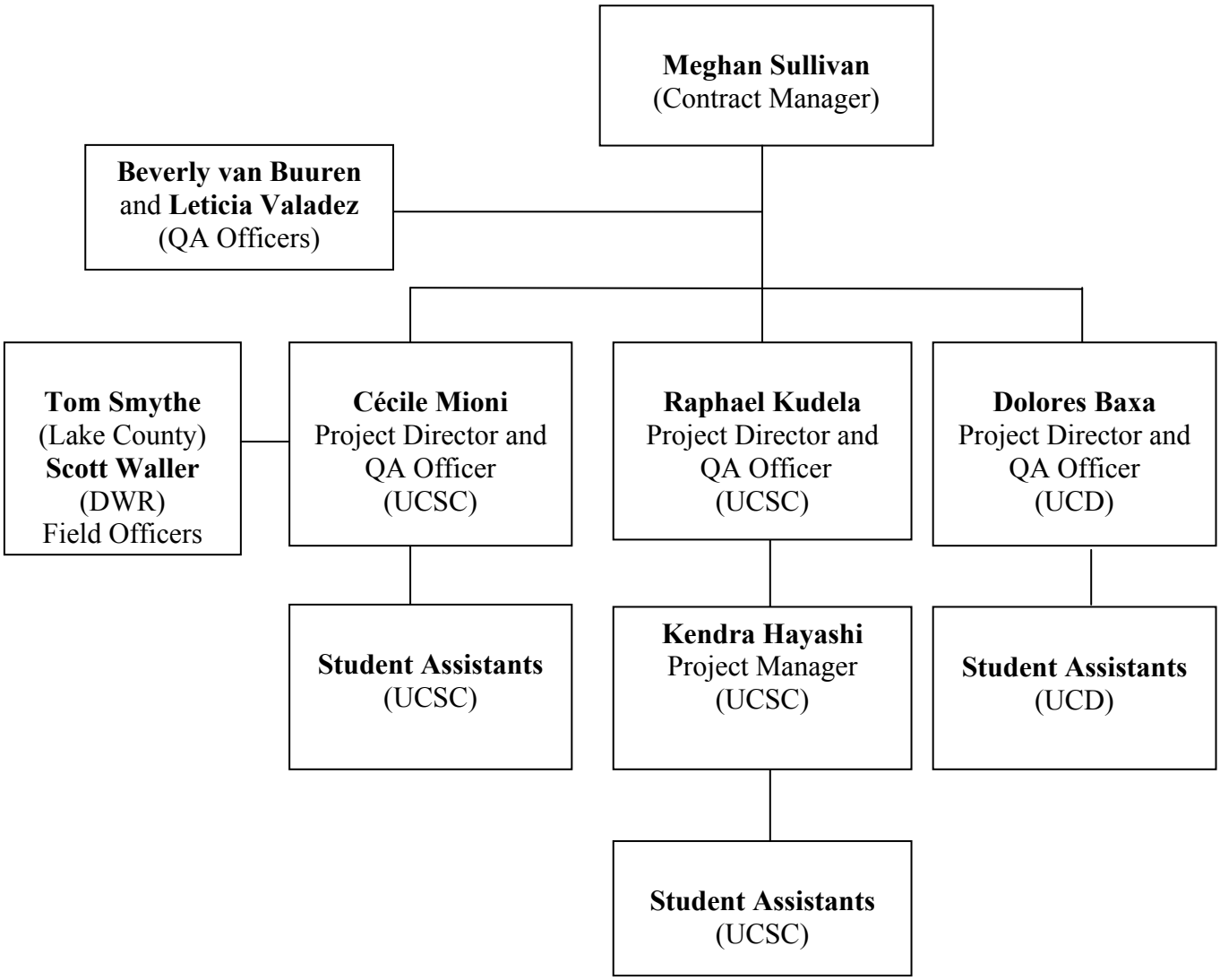
The Contract Manager (Meghan Sullivan) will make sure all personnel training and QAPP implementation is properly documented.

The Project QA managers that will advise on the project, but not participate in the execution of this program and delivery of the final report are listed with an asterisk in the distribution list as well as in table 4-1.

4.4 Organizational chart and responsibilities

Figure 4-1 shows the organization of staff participating in the Water Quality Monitoring Study.

Figure 4-1 Organizational chart



5. PROBLEM DEFINITION/BACKGROUND

5.1 Problem statement.

Section 305(b) of the Clean Water Act requires states to assess and report on the water quality status of waters within the states. In accordance with the Clean Water Act Section 303(d), all states must identify "impaired" bodies of water that are not meeting water quality standards and must develop monitoring and control plans for each stressors. In California, the State Water Resources Control Board (SWRCB) and Regional Water Quality Control Boards (RWQCBs) are responsible for meeting Section 303(d) requirements and to report this information on a nationwide basis. The integrated data reports are usually submitted to the U.S. Environmental Protection Agency (USEPA).

Harmful cyanobacteria (HC) and their toxins are growing contaminants of concern and USEPA recently (May 29, 2008) made the decision to add microcystin toxins as an additional cause of impairment for the Klamath River, CA. However, HC are some of the less studied causes of impairment in California water bodies and their distribution, abundance and dynamics, as well as the conditions promoting their proliferation and toxin production are not well characterized. HC affect both water quality and ecosystem health within urban, agricultural, and main-stem areas (e.g. dissolved oxygen sags, taste and odor problems in drinking water, toxins) and the efficiency of water diversion and treatment operations (clogging filters in water treatment plants, fish screens or channels). Noxious toxins produced by HC, collectively referred as cyanotoxins, reduce the water quality and may impact the supply of clean water for drinking as well as the water quality which directly impacts the livelihood of other species including several endangered species. For example, the coincident appearance of *Microcystis* (producer of the liver cancer promoting toxin called microcystin) and the decline of various pelagic organisms including the delta smelt (*Hypomesus transpacificus*) striped bass and threadfin shad (*Doromosa petenense*) and their copepod preys (*Eurytemora affinis* and *Pseudiaptomus forbesii*) in the freshwater sections of the Delta suggest that the presence of *Microcystis* is one of the factors responsible for the fishery decline since 2000 (IEP-POD 2007, Lehman et al. 2008, 2010). Indeed, a better understanding of the population and dynamics of HC and their toxins in the California water bodies is crucial for mitigating future impacts of HC blooms on water quality, assessing the risks to public health and estimating seasonal fluctuation in water quality parameters. Also, such information is also needed for enhancing existing resource management and for developing new tools and decision support systems that improve management effectiveness that will ensure low risk associated with HC blooms.

The goal of the work proposed here is to monitor the distribution of *Microcystis aeruginosa* as well as other HC of concern (e.g. *Aphanizomenon sp*., *Anabaena sp.* and *Lyngbya sp.* in Clear Lake*)* and their toxins in the surface waters of two Californian water bodies listed in the 303(d) that have been plagued by recurrent HC blooms: the Delta and Clear Lake. Our proposed research builds on previous work on HC in these water bodies.

In the Delta, the spatial and temporal dynamics of *M. aeruginosa* blooms have been identified along with their environmental covariates (Lehman et al., 2005, 2008). Analyses of toxin distributions have shown potential direct and indirect effects on fish (Lehman et al., 2008, 2010). These largely correlative results pave the way for a mechanistic analysis of the conditions that distinguish bloom periods and locations from non-bloom periods and locations, and that result in production of toxins. However, these findings also point to a need for a deeper and more comprehensive understanding of *Microcystis*-dominated blooms and toxin production. Concentrations of microcystin toxin and *Microcystis* cell densities are not strongly

correlated in the Delta (Lehman et al. 2008, Baxa et al. 2010, Mioni et al in prep). Different strains of *Microcystis* vary in their ability to produce toxins but cannot be distinguished by microscopy (Moisander et al., 2009). Preliminary research in the Delta also indicates that toxicity may not be due solely to *Microcystis,* but may also arise through the association of *Microcystis* with an unidentified filamentous cyanobacterium (Mioni et al *in prep*). The presence of other potentially HC has been documented. For example, the toxin-producing cyanobacterium *Cylindrospermopsis raciborskii* has been observed recently in the NSFE (Mueller-Solger, *pers. com*.). This cyanobacterium was originally thought to be a tropical or subtropical alga but has been recorded as rapidly expanding in some temperate regions and is regarded as an invasive species (Briand et al., 2004, Pearl and Huisman, 2008).

Clear Lake is naturally eutrophic and scum forming cyanobacteria (blue-green algae) usually bloom from spring to fall and can produce solid mats and noxious odors. Some of these cyanobacteria are known toxin producers and have been reported in the surface lake water every year during the Department of Water Resources (DWR) monitoring from 1969 to the mid 1990's (Richerson et al. 1994): *Microcystis*, *Aphanizomenon* (anatoxin-a and saxitoxin producer), *Anabaena* (anatoxin-a, Microcystins and Saxitoxin producer), *Oscillatoria* (microcystins and anatoxin producer), *Lyngbya* (saxitoxin and lyngbyatoxin-a producer), *Chroococcus* (microcystin producer). In summer 1990, very low levels of microcystin toxins were reported (CDHS, 1991). No HC monitoring or toxicology strudies have been conducted since the mid-1990's. Our preliminary data for the couple of years indicate a shift in the HC composition. Mat-forming blooms of *Lyngbya sp*., which was not a dominant species prior to the mid-1990's, have plagued the lake in summer 2009 and 2010. Our preliminary data for summer 2010 indicate that the *Lyngbya* bloom might be toxic (lyngbyatoxin-a). Our preliminary data also indicate that microcystin and anatoxin-a toxins were also present in the surface waters of the lake in August 2010. Total microcystin toxins concentration exceeded the World Health Organization advisory level for drinking water (1 µg/L) at three stations (2.3 – 3.2 µg/L) and Anatoxin-a was detected at two stations located in the lower arm (0.52 – 7.78 µg/L).

5.2 Decisions or outcomes.

We propose a bioassessment work plan that will combine monitoring and mapping of HC abundance and toxin concentrations as well as other environmental variables (temperature, electrical conductivity, pH, chl *a*, dissolved oxygen, nutrients, DOC, DIC) throughout the Delta and Clear Lake over a one-year period. The study period will be centered during HC bloom season (June – October). In order to describe the spatial and temporal distribution (occurrence and abundance) of HC and their toxins in the Delta we will work closely with preexisting monitoring programs such as CALFED funded monitoring program (PI: Cecile Mioni) and the DWR Environmental Monitoring Program (http://www.baydelta.water.ca.gov/emp). All these ancillary data will be available to this project at no cost. In Clear Lake, we will collaborate with Lake County Department of Water Resources, Department of Health Services and Vector Control. This project will provide a better understanding of the mechanisms underlying the source, occurrence and toxicity levels of HC in Clear Lake and the Delta.

5.3 Water Quality or Regulatory Criteria

Some species of cyanobacteria produce a diverse group of toxins with potentially severe human health effects, including acute hepatoenteritis and neurotoxicity; however, the most common complaints after recreational exposure to cyanobacteria and cyanotoxins are gastroenteritis and allergic reactions such as skin rashes, respiratory symptoms, and eye

irritation (Graham et al. 2009). Despite the widespread of occurance of cyanobacteria blooms in the US water bodies, including in California, and the potential health risks they present to humans and animals, there are currently no federal guidelines for monitoring of recreational hazards associated with cyanobacteria in the United States (Graham et al. 2009). Therefore the preliminary World Health Organization (WHO) guidelines for recreational activities serve as the foundation for monitoring programs (Graham et al. 2009). However, the WHO preliminary guidance values were developed specifically for exposure to the cyanobacterium *Microcystis sp.* and to one of its related cyanotoxin, microcystin-LR, in drinking water (1 µg/L) as well as during recreational activities (Chorus and Bartram 1999, Graham et al. 2009). There is currently no official guidelines for other cyanotoxins with potential adverse health effects (e.g. lyngbyatoxin-a, anatoxin-a). Recently, the State Water Resources Control Board (SWRCB) and the Office of Environmental Health Hazard Assessment (OEHHA) have compiled the information available in the literature regarding the adverse health effects of the six main cyanotoxins and have been working on developing health protective "action levels" to reduce algal toxin exposure (Kim Ward, *pers. com.*). Unfortunately, the report summarizing this research effort has not been published yet.

In California, the Environmental Protection Agency currently uses the following guidance values to make decisions about when to post health advisories or close recreational water bodies to limit recreational exposure to cyanobacteria and associated toxins (Graham et al. 2009):

- 40,000 to 100,000 cells/mL
- Microcystin $\geq$ 8 µg/L
- Scum associated with toxigenic species

The determination of "action levels" is furthermore complicated by the complex relationship between the presence/abundance of cyanobacteria and concentrations of cyanotoxins in the water. Cyanobacteria counts can overestimate the risk of cyanotoxin poisoning if cyanobacteria are present but not producing toxins either because the strain is not toxigenic or because the environmental drivers controlling toxin production differ from that controlling the growth of the cyanobacteria. Alternatively, cell counts can also underestimate the risk of cyanotoxin poisoning because cyanotoxins may persist in the water after a cyanobacterial bloom has subsided. Additionally, some species of cyanobacteria can produce more than one toxin and the individual toxins can be produced by more than one species of cyanobacteria. As a result, public health decisions require measured concentration of cyanotoxins in addition to cell counts.

This project will provide baseline information useful for the determination of dose levels that may result in adverse health effects in Clear Lake and the Delta watershed as well as for reducing the presence and toxicity of CHABs. Currently, the environmental drivers of cyanobacteria blooms and toxicity are not well understood and require further study. This project will also address this gap.

6. PROJECT/TASK DESCRIPTION

6.1 Work statement and produced products.

Water Characteristics –

The UCSC sampling crew will collect discrete (HC, toxins and environmental water characteristics) and continuous (toxins) samples during the HC bloom season (June – October). We will collect discrete samples to include water characteristics measurements (temperature, electrical conductivity, pH, chl a, dissolved oxygen, nutrients, DOC, DIC) as outlined in "SWAMP Bioassessment procedure 2009 - Standard Operating Procedures for Collecting Stream Algae Samples and Associated Physical Habitat and Chemical Data for Ambient Bioassessments in California" (June 2009) and in SWAMP Quality Assurance Program Plan (September 1, 2008).

- *In the Delta* - Surface water samples will be collected at discrete sampling stations located in critical habitats of the San Joaquin-Sacramento Delta in collaboration with preexisting water quality and phytoplankton monitoring programs (figure 6-1). Sampling will be done on board the DWR/USBR research vessels during the routine monitoring cruises at the discrete stations.
- *In Clear Lake* - Surface water samples will be collected at discrete sampling stations located in each arm of Clear Lake (see figure 6-2). Clear Lake Department of Water Resources will provide boat time and assistance with ancillary data measurements in the field.

Assessment of the toxicity of the HC growing in Clear Lake and the Delta –

Cyanotoxins will be monitored monthly at discrete stations and continuously at other stations (using Solid Phase Adsorption Toxins Tracking, aka "SPATT"). Cyanotoxins will be measured using commercially available ELISA kits (following manufacturer's instructions) and LC/MS (Doctor Kudela's lab). Doctor Kudela has previously run laboratory intercalibrations with an LC/MS/MS system operated by the California Water Pollution Control Lab and his (LC/MS) results are comparable (with LC/MS/MS). The estimated method detection limits and reporting limits are 0.02 μg/L for MC.

We will detect and determine the concentrations of the toxins (microcystins, lyngbyatoxins, anatoxin-A, cylindrospermopsin, nodularin, saxitoxins) present in the discrete surface water samples. High throughput toxicology testing will be performed with commercially available ELISA kits (following manufacturer's instructions). These kits provide quantitative analyses even at low concentrations and are highly sensitive to a given molecule. Samples tested positive for the targeted toxin or for toxin-producing strain(s) will be analyzed using LC/MS to validate the results and to identify the presence of isomers and congeners (confirmatory testing). Toxins such as saxitoxins and cylindrospermopsins will only be measured using ELISA kits (Abraxis) because they are not being measured routinely on LC/MS at this time. On the other hand, lyngbyatoxin-a will only be tested using LC/MS because no ELISA kit targeting this toxin are currently commercially available.

Because toxin concentrations varies greatly on a spatiotemporal scale in these environments (e.g. due to wind mixing or tidal mixing), we will also use the SPATT (Solid Phase Adsorption Toxin Tracking) methodology which is a modification of a method originally

developed for marine lipophilic toxins by Dr Kudela (UCSC) for continuous toxin tracking by passively absorbing dissolved toxins from the water column. SPATT devices will be attached at continuous monitoring stations (figures 6-1 & 6-2). Such devices will allow us to integrate the temporal fluctuations by concentrating the toxins over time (by opposition to discrete sampling technique) and will allow us to detect cyanotoxins at lower levels. Comparing the levels of targeted toxins between locations will provide us with important information to track the sources of toxic HC growth and toxin production as well as the impact on these toxins on living organisms. Furthermore, the SPATTs will help us determine the persistence and transport of microcystins away from these sources (i.e. stations distal from the bloom epicenter), and therefore the half-life of this toxin. SPATTs are currently being used to monitor the toxins levels in the Monterey Bay (Miller et al. 2010). We will extend existing SPATT methods to include cyanotoxins. Samples will be analyzed using ELISA kits and/or LC/MS as described above, based on protocols established through State of California Water Resources Control Board contract 07-120-250.

HC identification and enumeration using traditional microscopy and molecular methods –

Discrete water samples will be preserved in glutaraldehyde (2.5% v/v) for algal cell identification and enumeration using epifluorescence microscopy (UCSC) and an inverted microscope (EcoAnalysts, Inc.). EcoAnalysts, Inc. is currently analyzing the algal taxonomy samples for local agencies (e.g. DWR) and therefore, we will be able to standardize our data by using the same standard protocol in order to allow comparison between monitoring groups and monitored systems. The HC abundance will also be determined using epifluorescent microscopy (UCSC).

Because of the high degree of phenotypic plasticity exhibited in natural assemblages it is difficult to accurately and consistently identify HC species on microscopic observation alone, requiring a phylogenetic approach for identifying species and strains. At selected stations (based on microscopic analyses and toxicology results), we will characterize molecularly the types of HC that occur in the Delta and Clear Lake using 16S ribosomal RNA fingerprinting (Dolores Baxa, UCD). Using this approach, different strains within the same species can be differentiated. When applicable (i.e. when the toxin gene sequence has been published), we will determine molecularly the strains' ability to produce toxins (e.g. PCR amplification of *mcy* genes in ambient *Microcystis* strains).

Outreach –

We will work closely with the Lake County Department of Health to investigate possible algae-related symptoms by Lake county residents, domestic animals and wildlife (Dr Tait, Lake County Department of Health).

The project data will serve as a source of information that will direct and promote actions to improve water quality and enhance other monitoring programs. A better understanding of the population and dynamics of HC and their toxins is needed to enhance existing resource management and to develop new decision support systems that improve management effectiveness to ensure low risk associated with HC blooms. We will disseminate our results broadly (publications, presentations, reports) and provide a detailed list of recommendations

relevant for regulators, local governments, industries (e.g. water treatment plants) as well as environmental managers and policy makers.

Deliverable –

The PI will provide quarterly reports, including collected data as well as copies of SWAMP field sheets, during the life of the project. At the end of the project, we will provide a final report summarizing the data collected.

Sampling site –

Suggested sampling sites (table 1, Figures 6.1 & 6.2) and sampling frequency are based on historical data, our preliminary data and accessibility to the ship. The suggested sampling sites include the following locations but may be modified based on HC distribution. Indeed, because of temporal variations in the onset of HC abundance, we will use an adaptive monitoring strategy. For example, extra stations may be added if none of these stations coincide with the epicenter of a HC bloom in order to capture the full bloom progression and associated environmental drivers on a spatiotemporal scale. On the other hand, during the peak of the bloom season, high abundance of mat-forming HC might prevent the boat from accessing near-shore station(s) (especially in Clear Lake). In this case, due to safety concerns we might have to skip the station and attempt to collect near-shore samples from land (e.g. from a pier, provided permit or authorization).

Table 6-1: Station codes, Site names, types of station and locations

Station Codes	Station Name	Study Area	Type of Station
1	Lakeport	Clear Lake, CA	Discrete
CL-1	Upper Arm	Clear Lake, CA	Discrete
2	Horseshoe Bend	Clear Lake, CA	Discrete & Continuous
3	Clearlake (City)	Clear Lake, CA	Discrete & Continuous
CL-3	Lower Arm	Clear Lake, CA	Discrete
4	The Keys	Clear Lake, CA	Discrete & Continuous
CL-4	Oaks Arm	Clear Lake, CA	Discrete
D24A	Rio Vista (SAC)	Delta, CA	Continuous
D12	Antioch Ship Channel (SJR)	Delta, CA	Discrete
D12A	Antioch (SJR)	Delta, CA	Continuous
D19	Frank's Tract (SJR - flooded island)	Delta, CA	Discrete
D28A	Old River at Rancho del Rio	Delta, CA	Discrete & Continuous
D16	Twitchell Island (SJR)	Delta, CA	Discrete
D26	Potato Point (SJR)	Delta, CA	Discrete
D29	Prisoners Point (SJR)	Delta, CA	Continuous

SJR = San Joaquin River
SAC = Sacramento River

In the Delta, we will monitor the spatial and temporal distribution of harmful cyanobacteria and their toxins with a special focus on *Microcystis aeruginosa* (Fig. 6-1). Blooms of the cyanobacterium *Microcystis aeruginosa* have been recorded in the Delta during Summer (June – October) since 1999 and the presence of other potentially harmful cyanobacteria has been documented (Lehman et al. 2003, 2005, 2008, 2010). *Microcystis aeruginosa* can produce a variety of toxins collectively called microcystins which are associated with both acute and chronic liver damage (Carmichael 1995). Exposure to microcystins has been linked to cancer in humans and wildlife (Carmichael 1995, Codd 1995) and reduced feeding success in zooplankton (Ger et al. 2010, 2010b). These hepatotoxins have been detected in the Delta (Mioni et al. in prep, Lehman et al. 2005, 2008, 2010) and entered the foodweb (Lehman et al. 2010, Ger et al. 2010, 2010b). Also, the toxin-producing cyanobacterium *Cylindrospermopsis raciborskii* has been observed recently in the northern SF Estuary (Mueller-Solger, *pers. com*.). This cyanobacterium was originally thought to be a tropical or subtropical alga but has been recorded as rapidly expanding in some temperate geographical areas and is regarded as an invasive species (Briand et al. 2004). It is thought that its increased occurrence, rather than being just a recent invasion, is a combination of several factors such as improved water quality monitoring, availability of suitable habitat through climate warming.

Sampling will be conducted monthly by partnering with California Department of Water Resources (DWR) and more specifically the Environmental Monitoring Program (EMP) of the Interagency Ecological Program (http://www.water.ca.gov/iep/activities/emp.cfm). This program includes monthly monitoring of water quality variables (conductivity, pH, dissolved oxygen, turbidity, dissolved chloride, chlorophyll fluorescence, water temperature, air temperature, wind speed and direction, solar radiation) as well as biological characteristics, such as phytoplankton and zooplankton community composition and biomass in the Sacramento-San Joaquin Delta, as well as Suisun Bay, and San Pablo Bay. All these ancillary data will be available to this project at no cost. Sampling for this study will be done on board the DWR/USBR research vessels (RV/San Carlos and R/V Endeavor) during the monthly routine water quality monitoring cruises at the discrete stations located in the Central Delta (i.e. the epicenter of Microcystis bloom, table 6-1, fig. 6-1). We will compare our results (see below) with the EMP group data. Additionally, we are partnering with Dr. Alex Parker (Romberg Tiburon) to contribute to an ongoing seasonal sampling program during *Microcystis aeruginosa* blooming season (June-September). Variables measured during this companion study include nutrients concentration, DOC, temperature, salinity (Alex Parker, Romberg Toburon), zooplankton composition (Wim Kimmerer, Romberg Tiburon), algal taxonomy (Cécile Mioni, UCSC), and cyanotoxin levels (Raphael Kudela, UCSC). Using molecular methods, this companion program will also monitor the toxin-producing chemotypes of *Microcystis* the expression of the gene involved with toxin production (Hans Paerl, University of North Carolina). All these ancillary data will be available to this project at no extra cost.

Because toxins concentrations varies greatly on a spatiotemporal scale in a tidal environment, we will also use the SPATT (Solid Phase Adsorption Toxins Tracking, Dr Kudela UCSC) methodology which is a new technology designed for continuous toxin tracking by passively absorbing dissolved toxins from the water column (Miller et al. 2010). SPATT devices will be attached each month at four of the EMP/DWR real-time continuous monitoring stations located in the Delta (table 6-1, http://www.water.ca.gov/bdma/meta/continuous.cfm). All the ancillary data collected by the EMP/DWR group at these real-time monitoring stations will be available to this project at no extra cost.

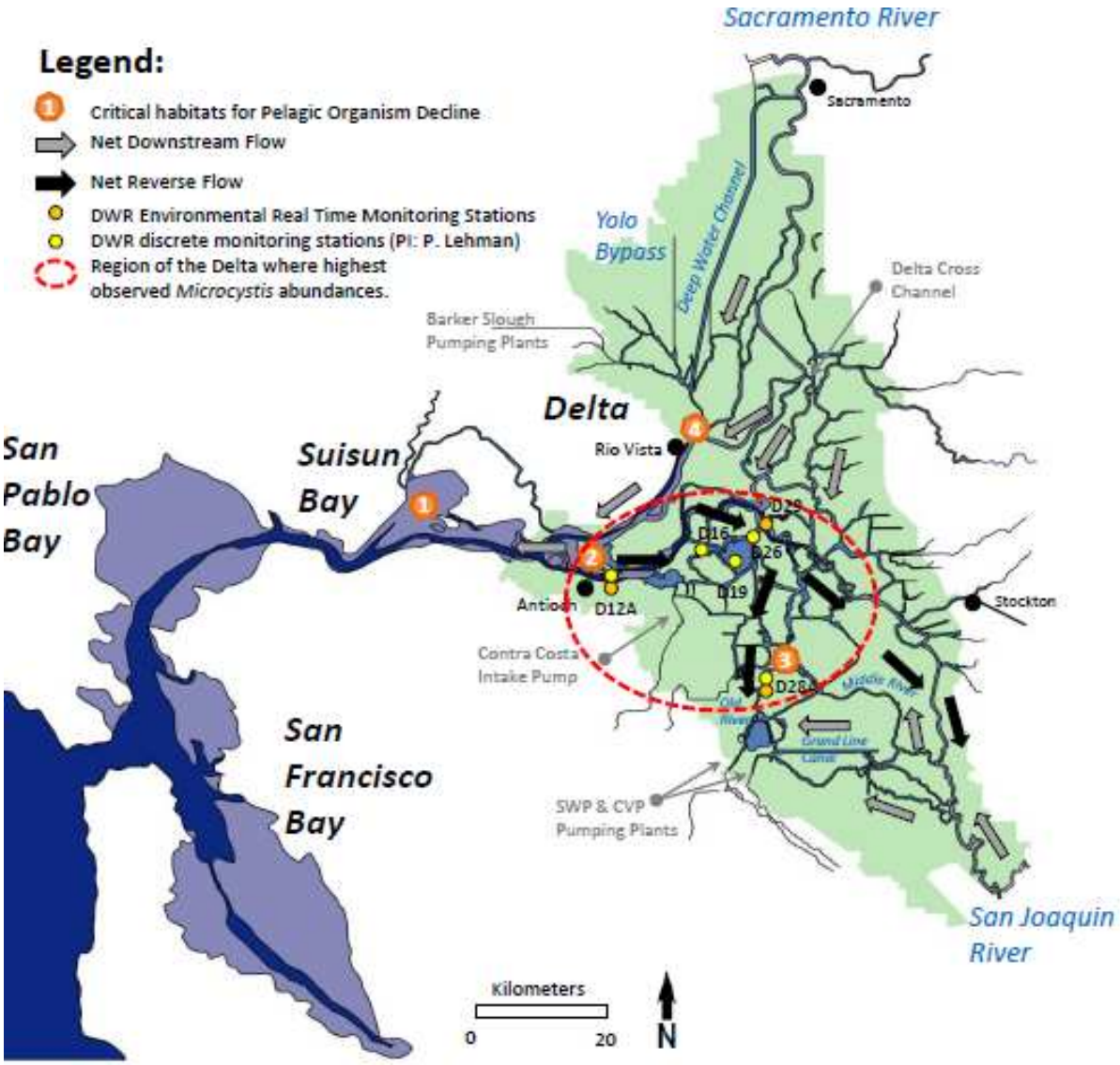


Figure 6-1 – Delta sampling locations

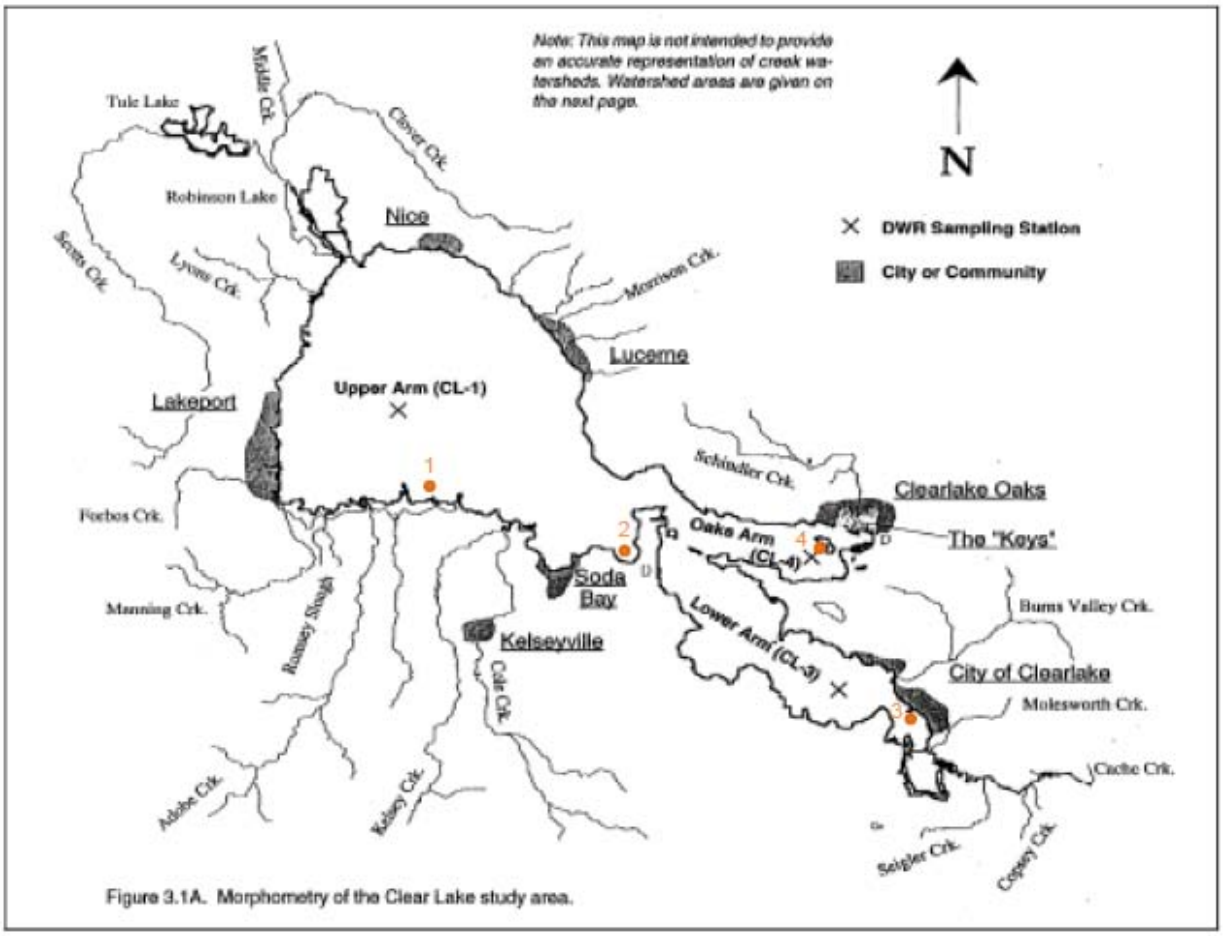


Figure 6-2 – Clear Lake sampling locations. CL-1, CL-2 and CL-3 stations are DWR monitoring stations. They were also used for a toxicology study performed in 1990.We will use these stations as discrete monitoring stations. The stations 1, 2, 3, 4 are located at coastal buoys (county owned) and will be our continuous stations for toxins and temperature (SPATTs and Hobos). We will also do discrete samples monthly at these stations.

Clear Lake is shallow, warm, naturally eutrophic system with three distinct regions ("arms") which behave like separate lakes in some respect (Richerson 1994). Historical records suggest that scum forming cyanobacteria have been blooming recurrently from Spring to Fall. The highest densities are usually observed in the eastern arms of the lake where prevailing winds can push the floating algae into solid mats that rot and release noxious odors (Richerson 1994). Some of these cyanobacteria are known toxin producers and have been reported in the surface lake water every year since the beginning of the DWR monitoring in 1969 (Richerson 1994): *Microcystis* (Microcystin producer), *Aphanizomenon* (anatoxin-a and paralytic shellfish poisoning producer), *Anabaena* (anatoxin-a, Microcystins and Saxitoxin producer), *Oscillatoria* (microcystins and anatoxin-a producer), *Lyngbya* (saxitoxin and lyngbyatoxin-a producer), *Chroococcus* (Microcystin).

Compiled literature evidence suggests that the success of cyanobacteria in Clear Lake is a result of complex and synergetic environmental factors rather than a single dominant variable (Richerson 1994). However, many of these factors are altered directly and indirectly by temperature. Some of these harmful cyanobacteria species (e.g. *Microcystis*) prefer water temperatures above 20°C and this threshold temperature is reached early in the summer season

(June). Many cyanobacteria, including *Microcystis*, produce intracellular gas vesicles that make cells buoyant. During stable thermal conditions, buoyant cyanobacteria can accumulate in surface forming a dense surface scum that shades underlying nonbuoyant phytoplankton and therefore suppressing their non-HC phytoplankton through light competition. Warm temperatures also stimulate nutrient (e.g. phosphorus) release at the sediment-water interface as well as the mineralization of organic matter which contributes to the accumulation of dissolved nutrients (and toxic metal such as Hg) under the surface. Buoyant cyanobacteria such as *Microcystis* can migrate vertically which allows these species to have access to nutrient pools that are otherwise low or depleted in surface.

Although cyanobacteria blooms occur every year in Clear Lake, two major blooms were recorded by DWR and Lake County since the beginning of their monitoring program (1969) and both blooms resulted in serious air quality problem and even restricted boat passage (Horse shoe Bend, Oaks Arm). Channels were too thickly clogged for small boats to navigate:

- September/ October 1990: *Microcystis* bloom (following a drought)
- Summer 2009: *Lyngbya* bloom

In 1990, twenty samples from Clear lake were analyzed by the California Department of Health Services for microcystin toxins assessments using the mouse bioassay method and all samples were below the World Health Organization drinking advisory limit of 1 µg/L. There was no toxicology report available since then.

During summer 2010 (June – September), we (Cécile Mioni and Raphael Kudela, UCSC, Tom Smythe, Lake County) monitored another major bloom event using the historical DWR discrete monitoring stations (Richerson 1996, figure 6-2). The bloom was composed of a mixed assemblage of potentially toxic cyanobacteria: *Microcystis, Anabaena, Lyngbya* and *Aphanizomenon*. The highest densities were observed in the Lower Arm, Horseshoe Bend (station 2) and Oaks Arm. We detected several cyanotoxins (microcystin and anatoxin-a) at concentrations exceeding the World Health Organization drinking advisory limit of 1 µg/L. We also suspect the presence of other cyanotoxins such as lyngbyatoxin-a. We have acquired the appropriate standard for this toxin and will monitor this toxin as well during this study.

In this study, sampling will be conducted monthly during the bloom season (June – October) by partnering with Lake County. Sampling will be conducted at the historical DWR monitoring stations (Richerson, 1996, fig 6-2) to allow comparison with historical records. Lake county will provide ship time as well as field sampling assistance. The sampling dates will be chosen based on the ship and crew availability (e.g. no overlap with Delta cruises). SPATT continuous toxin tracking devices will be deployed monthly at four stations (stations 1, 2, 3, and 4 – fig. 6-2) to monitor the dissolved toxin levels in between the monthly discrete sampling events.

6.2. Constituents to be monitored and measurement techniques.

Monitoring will consist of monthly field measurements for HC identification (microscopy and gene-based phylogeny), abundance and toxicity as well as for water characteristics (pH, temperature, electrical conductivity, chl *a*, secchi depth, dissolved oxygen, nutrients, DOC, DIC) as outlined in "SWAMP Bioassessment procedure 2009 – Standard Operating Procedures for Collecting Stream Algae Samples and Associated Physical Habitat and Chemical Data for Ambient Bioassessments in California" and in SWAMP Quality Assurance Program Plan (November 19, 2008).

Laboratory analyses of planktonic cyanobacterial samples will include the extraction and fluorometric determination of chlorophyll *a*. Phytoplankton samples will be preserved in glutaraldehyde and screened for cyanobacteria. Cyanobacteria enumeration will be performed on the inverted microscope at 40x after sedimentation by professional taxonomists (EcoAnalyst, Inc.). Samples dominated by buoyant HC (e.g. *Microcystis*) will be enumerated using epifluorescent microscopy at UCSC. For samples dominated with colonial *Microcystis*, samples will first be broken up using the colony disaggregation method (Bernard et al. 1994), filtered, mounted on slides and examined via epifluorescent microscope employing the natural unit method or "clump count" method where one organism is defined as any unicellular organism or a natural colony (SOP UCSCCM-002).

Nutrient concentrations, including nitrate+nitrate, ammonium, and orthophosphate will be determined using Hach water quality kits. The product is measured colorimetrically to determine the concentration of nutrients that reacted using a spectrophotometer (Shimadzu UV 1201). Certified QA/QC standards (e.g. SCP Science) will be included for all nutrient analytical runs. This methodology meets EPA guidelines.

More specifically, each nutrient will be determined using the following methods:

Nutrient	***Method***	***Range***	***Principle***
Nitrate -N	Hach 8192	0 – 0.5 mg/L	Cadmium reduction
Nitrite -N	Hach 8507	0 – 0.3 mg/L	Diazotization method
Ammonia-N	Hach 8038	0 – 2.5 mg/L	Nessler method
Ortho-Phosphate-P	Hach 8048	0 – 2.5 mg/L	Ascorbic acid reduction

Dissolved organic carbon (DOC), dissolved inorganic carbon (DIC), dissolved total nitrogen (DTN) and total nitrogen (TN) will be determined via high temperature catalytic combustion using a Shimadzu TOC-$V_{CPH/CPN}$ Analyzer (EPA 415.1).

Further details for the collection of samples, handling, and laboratory procedures are provided in Section 13.

6.3 Project schedule

Table 6-3 shows the major tasks that will be undertaken, and the anticipated time line for the performance of each task.

TASK	PRODUCT	DATE
1	Project Administration	
	1.1 Program Coordination	Ongoing
	1.2 Draft Final Report	January 31, 2012
	1.2 Final Report	March 30, 2012

	1.2 Monthly Reports	July 29, 2011, and monthly thereafter (during bloom season: June – October 2011)
2	Quality Assurance Project Plan	
	2.1 Draft QAPP	March 1, 2011
	2.2 Final QAPP	March 31, 2011
	2.3 Review and Revise	Ongoing
3	Monitoring Plan	
	3.1 Draft Monitoring Plan	March 1, 2011
	3.2 Final Monitoring Plan	March 31, 2011
	3.3 Review and Revise	Ongoing
4	Sample Collection	June 2011 – October 2011
	4.1 SWAMP Field Sheets	October 31, 2011
5	Sample Analysis	Completed by November 21, 2011
	5.5 Analytical Results	December 16, 2011

6.4 Geographical setting

The geographic scope of data collection for this project encompasses samples collected from Clear Lake and the San Joaquin Delta.

Clear Lake – The Clear Lake Watershed is located in Lake County, within the California Coast Ranges, 80 miles north of San Francisco Bay. The topography of the watershed is generally steep and rugged with elevations ranging from 1,318 - 4,840 feet above sea level (CLIWMP 2010). Clear Lake is the oldest, largest, natural freshwater lake in California, with 68 square miles of surface area. It is a relatively shallow lake, with an average depth of 27 feet (8.2 m) and a maximum of 60 ft (18m); and it is usually mixed from top to bottom due to the winds, gas vents and water springs. It is used for recreational activities (sport fishing, water contact sports) and drinking water source. Clear Lake dam regulates the water level of the lake since 1914. The lake is naturally eutrophic and scum-forming cyanobacteria have been blooming for the past century from spring to fall and can produce solid mats and noxious odors. Some of these cyanobacteria are known toxin producers and have been reported in the surface lake water every year since the beginning of the DWR monitoring in 1969: *Microcystis* (Microcystin producer), *Aphanizomenon* (Anatoxin and paralytic shellfish poisoning producer), *Anabaena* (Anatoxin, Microcystins and Saxitoxin producer), *Oscillatoria* (Microcystins and Anatoxin producer), *Lyngbya* (Saxitoxin and lyngbyatoxin producer), *Chroococcus* (Microcystin). Clear Lake was added to the federal Clean Water Act Section 303(d) list of impaired water bodies for nutrients in 1986. Although Clear Lake water clarity improved significantly beginning in 1992, widespread, noxious, and persistent HC blooms occurred during the summer of 2009 and 2010.

Phosphorus is thought to be one of the main drivers of cyanobacteria growth in Clear Lake. The N:P ratio is 7-15 mol:mol which favors cyanobacteria (Richerson, 1994; Tom Smythe confirmed that this values were still actual). Two scum forming cyanobacteria, *Aphanizomenon* and *Anabaena*, are also nitrogen fixers. In the 1970's, Home and colleague determined that ca. 40% of the total nitrogen budget to the lake was supplied by nitrogen fixation. He also noted that high ammonia concentrations could stimulate the irregular appearence of *Microcystis*. Enrichment incubations were conducted by Wurtsbaugh and Home (1983) and iron was shown to enhance nitrogen fixation while nitrate additions suppressed it. It has also been suggested that the Cache Creek Dam could have played a role in the increased occurrence of harmful cyanobacteria blooms. Physical factors appear to play an important role in controlling algal abundance. The scum forming cyanobacteria are usually in higher abundance in the eastern and lower arms of the lake where prevailing winds (westerly, northwestern winds) can push the floating algae into mats.

The proposed project is consistent with the 2010 Clear Lake Integrated Watershed Management Plan (CLIWMP 2010). The CLIWMP includes several activities that will be aided by this project including "coordinate a comprehensive Clear Lake watershed monitoring program", "improve understanding of Clear Lake limnology", "develop aquatic and invasive species treatment plan," "protect and restore Lake and Shoreline Wildlife Habitat," and "support and increase watershed education and outreach." By providing baseline and background estimates, and monitoring & bioassessment tools, this project will assist the Board in completing tasks outlined in the CLIWMP.

Delta –The San Francisco Bay-Delta is a highly urbanized estuary, with a population of approximately 7 million in the surrounding area. The watershed comprises approximately 40% of the area of California. It is one of the most anthropogenically altered estuaries in the United States (Nichols et al. 1986) and some of the world's largest ecosystem restoration efforts are currently underway to mitigate these alterations (Cloern & Jassby 2000, Kimmerer 2004). The Delta is a hydrodynamically complex system comprised of an intensely managed network of natural and human-made levees and lakes, diked agricultural fields, and relicts of tidal marshlands. The Delta ecosystem is comprised of two estuarine systems, the Sacramento and San Joaquin rivers, each with different hydrodynamic and hydrologic regimes (Conomos, 1985). The Delta is shallow (mean depth 6m), such that benthic suspension feeders may consume a substantial proportion of planktonic production (Cloern 1982, Jassby et al. 2002). Water column hydrography (e.g. stratification) is also an important physical factor that affects both the abundance and vertical distribution of plankton in this system (Rollwagen-Bollens et al. 2006). The important nutrient sources within the Delta are agricultural drains and wastewater inputs (Hager & Schemel 1992). Seasonal blooms of cyanobacteria (*Microcystis aeruginosa*) have been recorded since 1999 and the colonial form of *M. aeruginosa* is now present throughout 180 km of waterways from freshwater to brackish water environments (Mioni et al. *in prep* Lehman et al. 2005, 2008, 2010). The presence of other potentially harmful cyanobacteria has been documented. Microcystin toxins have been detected in the Delta and entered the foodweb (Lehman et al. 2010). The toxicity and widespread distribution of *M. aeruginosa* in NSFE demonstrated the potential of this organism to negatively impact many beneficial uses and implies that it would be desired to initiate active and long-term monitoring,

forecasting and alert programs. The potential adverse impact of this HAB on the estuary is large. Water from the northern region is used directly for drinking water and irrigation and the region is an important recreational area for sport fishing and water contact sports. The estuary is habitat for many anadromous, commercial and recreational fish including striped bass and Chinook salmon and is a feeding ground for marine mammals. The estuary also contains many threatened or endangered aquatic organisms including the Delta smelt and Chinook salmon and many of these endangered fish species are declining (Bennett & Moyle 1996, CABDA 2000). Some of these declines may be linked to the quantity and quality of the phytoplankton carbon available at the base of the food web. Indeed, *M. aeruginosa* blooms can reduce the growth of other phytoplankton impacting food quality and availability (Lehman et al. 2005, 2008).

This study will focus on enhancing preexisting monitoring programs rather than duplicating these programs. We have been coordinating with members of the Regional Water Quality Control Board to ensure that our proposal addresses needs of the Board, as well as requesting feedback from other Partner Agencies, such as the Lake County Department of Water Resources, Department of Health and Vector control, as well as the California Department of Water Resources (Environmental Monitoring Program). We will also coordinate our efforts with the Southern California Coastal Water Research Project, the city of Watsonville (Pinto Lake), California State University – Monterey Bay, the California Academy of Science and other relevant regional and local agencies. In collaboration with the above agencies and institutions, we will contribute to local planning and outreach efforts, and we've been involved in water quality improvement efforts in Elkhorn Slough.

6.5 Constraints

We have designed the work to minimize the constraints. Nevertheless, conditions and events could limit our success in meeting the completion dates. First, because of alternating high and low water years and resulting varying surface water flow through the watershed, conditions in Clear Lake maybe more or less conducive to the formation of HC Blooms and the presence of cyanotoxins. This may reduce the amount of data to inform cyanobacteria toxicity and abundance in relation to environmental conditions. Secondarily, Lake county in association with the California regional water control board and the Californa state water resources control board could take actions to change the conditions of Clear Lake to reduce the potential risk posed by HC and associated toxins. If lake treatment is undertaken it will compromise our ability to measure the environmental variables associated with the development and proliferation of HC and cyanotoxins. Also, because of temporal variations in the onset of HC abundance, an adaptive monitoring strategy will be necessary to capture the full bloom progression and associated environmental drivers on a spatiotemporal scale. For example, due to the patchiness of bloom events extra stations may be added if none of these stations coincide with the epicenter of the HC bloom. On the other hand, during the peak of the bloom season, high abundance of mat-forming HC might prevent the boat from accessing near-shore station(s) (especially in Clear Lake). In this case, due to safety concerns we might have to skip the station and attempt to collect near-shore samples from land (e.g. from a pier, provided permit or authorization). Finally, there is always the unlikely event that we have major personnel losses or changes. This may require new staff hired and training. Unless there are a series of losses from project leaders, these should be seen as minor inconveniences that will not affect the work schedule.

7. QUALITY OBJECTIVES AND CRITERIA FOR MEASUREMENT DATA

7.1. Data Quality Objectives

The Data Quality Objectives include Data Quality Indicators (DQIs) and Measurement Quality Objectives (MQOs). This QAPP deviates from the SWAMP QAPP checklist by renaming the template DQOs as DQIs and MQOs as appropriate.

Data acquisition activities will include both field measurements and laboratory analyses, and the quality objectives depend on the amount of error that can be tolerated. However, data collected for this project has the potential of being used for additional purposes in conjunction with other data sets collected in accordance with SWAMP requirements, and the quality objectives selected for the project have been refined to reflect this foresight. Table 7-1 lists the measurement or analyses type specific to this project, and specifies applicable DQIs. These quality objectives for field measurements are listed in Table 7-2; Table 7-3 shows the quality objectives for laboratory analyses of conventional constituents.

Five indicators will be used to assess data quality: precision, accuracy, representativeness, comparability, and completeness. A brief discussion of the objectives for each of these indicators is provided below.

Table 7-1 – Measurement or analyses type and applicable data quality indicators

Measurement or Analyses Type	***Applicable Data Quality Indicators***
Field Measurements:	
Dissolved Oxygen, pH, Specific Conductivity, Temperature, secchi depth	Accuracy, Precision, Completeness
Laboratory Testing:	
Nutrients, DOC, DIC	Accuracy, Precision, Completeness, Representativeness, Comparability
Laboratory Testing:	
chlorophyll a, cyanotoxins	Accuracy, Precision, Completeness
Laboratory Testing:	
cyanobacteria identification and enumeration, taxonomic molecular biologic analysis	Precision, Completeness, Representativeness

7.2 Data Quality Indicators

7.2.1 Precision

Precision measures how closely repeated measurements of a given sample agree with each other.

Nutrients, chlorophyll a, Dissolved Organic Carbon
The precision of the nutrient concentration, chlorophyll *a* concentration and cyanobacteria cell count measurements will be evaluated by replicate analysis of every sample. In addition, we

will collect a randomly selected duplicate sample will at each sampling event. Precision is either measured by calculating the relative percent difference (RPD), used when only two replicates are analyzed, or the relative standard deviation (RSD), used when two or more replicates are analyzed.

$RPD = (X_1 - X_2) / (X_1 + X_2) / 2 * 100$
where X_1 = the larger of two values and X_2 = the smaller of two values.

$RSD = (S / X_M)* 100$
where X_M is the mean and S is the standard deviation,
$S = [\sum (X - X_M)^2 / (n - 1)]^{1/2}$

The RPD or RSD must be less than 25% for a sample to be accepted. Precautions will be taken to keep the RPD and RSD to minimum (aka below 10%). If the RPD or RSD is greater than 10%, the sample may be shaken to ensure it is well mixed, or refiltered to remove interfering particles, and reanalyzed. Due to the colonial nature of these cyanobacteria assemblage, we might not be able to maintain the RPD or RSD below our target value of 10%, especially for the chlorophyll a samples. Therefore, we are using the more realistic RPD or 25% (SWAMP threshold value).

Toxins
The precision of toxin concentration measurements will be evaluated by replicate analysis of every sample. Precision is either measured by calculating the relative percent difference (RPD), used when only two replicates are analyzed, or the relative standard deviation (RSD), used when two or more replicates are analyzed, as described for nutrients. The RPD must be less than 25% to be accepted. If greater than 10% the sample will first be reanalyzed, and second (if still >10%) the raw sample will be reprocessed using an SPE cleanup step.

7.2.2 Accuracy

Accuracy (bias) measures the conformity between measured and true values.

Field measurements
To achieve accuracy in measurements of pH, dissolved oxygen, IC, pH and depth, the portable measuring device (e.g. YSI) is calibrated before every sampling event.

Laboratory Analyses
To determine the accuracy of nutrients, DOC, Chlorophyll *a* data and cyanotoxins, certified quality control (QC) references of known nutrient (e.g. SCP Science) and DOC concentrations respectively will be analyzed both at the beginning and at the end of every batch of field samples. The reference values for all of these QC samples must be within 80-120% of the true concentrations for the batch of samples to be immediately accepted.

When relevant (e.g. DOC and nutrient samples), field and laboratory blanks will be prepared and analyzed to demonstrate freedom of contamination.

In addition, one matrix spike will be analyzed with each run of samples. A matrix spike is prepared by splitting a routine sample, and adding a known concentration of each nutrient to

one of the sub-samples. Both the spiked and unspiked sub-samples are analyzed to calculate the percentage of added nutrients that is detected in the spiked sample. Percent recovery is calculated as:

$R= (Cs - C) / S * 100$

Where, R= percent recovery, Cs = spiked sample concentration, C = sample background concentration, and S= concentration of nutrient added to sample.

The percent recovery of nutrients in the spiked sample must be 80-120% for each sample run to be accepted.

The accuracy of toxins will be determined by performing analyses on standard reference materials obtained from various sources (documented with each set of samples). At least one sample in each set of field samples will also be spiked with standard reference material, with percent recovery (calculated as above) between 80-120% considered acceptable.

7.2.3 Comparability

Comparability describes the confidence with which one data set can be compared to another data set. To achieve comparability between nutrients, chlorophyll *a*, DOC, and toxin analyses data from different collection days, the same set of QA/QC references (e.g. SCP Science) will be used for 15 to 20 consecutive sample batches. A new set of calibration standards will be prepared for each nutrient analytical runs, a set of certified QA/QC references will be used to assess the accuracy of the calibration standards. Comparability between our data and those of other studies will be achieved by using standard methods for sampling, handling and analysis. Also, by analyzing temperature, DO, and pH at the same time that water samples are collected a complete assessment of field conditions is made.

7.2.4 Representativeness

Representativeness describes the degree to which the results of analyses represent the samples collected, and the samples in turn represent the environment from which they were taken. Achieving representativeness in conducting scientific studies or monitoring is important, because without adequate representativeness, it is not valid to extrapolate results of the study to generate conclusions about the system at large. A way to achieve representativeness is by sampling from several locations within the area of interest, and systematically sampling points throughout the sampling reach, so as to remove the personal bias of the sample collector, which would skew the results.

In this project, we strive to achieve representativeness of samples so that the data can then be used to formulate conclusions about the study system at large. Because shoreline sampling can miss early warning signs of bloom formation and because of the distributative effect of wind on cyanobacteria distribution (Kromkamp and Mur 1984; Oliver and Ganf 2000; Wallace et al. 2000) can result in biased results (Rogalus et al. 2008), both shoreline and offshore sampling will be performed. The wind conditions and other relevant meteorological conditions will be reported in the field log book.

For toxin analyses, we rely on SPATT to provide representative sampling based on time-integrated (typically 1 month) values, rather than relying only on grab samples.

7.2.5. Completeness

Completeness is the percentage of samples collected that will be analyzed. There are no statistical criteria that require a certain percentage of data. The completeness goal for this project is 100% but to include potential issues that could be encountered in the field or during transport, we have set a completeness goal to 90%. To assure completeness, established protocols for sample transportation and laboratory processing will be followed to minimize data loss following collection. Also, the excess water sample will be retained until completion of the laboratory analysis to allow for re-analysis if the sample is mishandled or if the nutrient concentration is beyond the detection limit laboratory analytic method.

7.2.6 Data Quality Objectives for this study

Field and Laboratory Measurements Data Quality Objectives for the project are provided in Tables 7-2 and 7-3.

Table 7-2 – Measurement Quality Objectives and Specifications for Field Measurements

Parameter	**Unit**	**Accuracy**	**Precision**	**Resolution (minimum)**	**Target Reporting Limit**	**Completeness**
Depth	m	NA	No SWAMP requirement	0.01	0.02	90%
Dissolved oxygen	mg/l	± 0.5 mg/L	No SWAMP requirement; will use ± 0.5 or 10%	0.2	0.2	90%
pH	pH	± 0.5 units	No SWAMP requirement – suggest ± 0.5 or 5%	0.1	NA	90%
Specific Conductivity	µS/cm	± 2 µS/cm	No SWAMP requirement	1	2	90%
Secchi Depth	cm	NA	No SWAMP requirement	0.01	0.02	90%
Temperature	^{o}C	± 0.5 ^{o}C	No SWAMP requirement – suggest ± 0.5 or 5%	0.5	NA	90%

Note: Specific conductivity is the value after correction for temperature, which is done by the instrument automatically.
NA – not applicable

Table 7-3 – Measurement Quality Objectives and Specifications for Laboratory Measurements

Group	Parameter	Accuracy	Precision	Recovery	Target Reporting Limits	Completeness
Conventional Constituents (Nutrients)	Nitrate	Standard Reference Materials (SRM, CRM, PT) within 95% CI stated by provider of material. If not available then with 80% to 120% of true value	Laboratory duplicate, Blind Field duplicate, and MS/MSD 25% RPD (target ± 10%)	Matrix spike 80% - 120% or control limits at ± 3 standard deviations based on actual lab data.	0.01 mg/L	90%
	Ammonium				0.05 mg/L	
	Orthophosphate				0.01 mg/L	
	Nitrite				0.005 mg/L	
DOC	DOC	CRM within the 95% CI stated by the provider. Laboratory Control Material (LCM) ± 20% to 25% of stated value. No accuracy criteria for grain size.	Replicates within ± 25% (target ± 10%)	Will use 80% - 120%	0.1 mg/L	90%
Cyanobacteria	Taxonomic Identification	N/A	No SWAMP requirement—suggest duplicate ±25% RPD	identification and counts	No SWAMP requirement, suggest 20,000 cells/mL	90%
	Chlorophyll a	No SWAMP requirement—suggest ±30% of standard reference material	No SWAMP requirement—suggest duplicate ±25% RPD	identification and counts – action limits are not applicable	No SWAMP requirement; suggest 10 µg/L	
	Cyanotoxin	No SWAMP requirement—suggest ±30% of standard reference material	No SWAMP requirement—suggest duplicate ± 25% RPD (target ± 10%)	identificaiton and counts – action limits are not applicable	No SWAMP requirement; suggest 1 µg/L for Microcystin	

* NA, Not Applicable
SRM, standard reporting method
CRM, conventional reporting method
PT, Proficiency testing
CI, confidence interval
MS, matrix spike
MSD, matrix spike duplicate
RPD, relative percent difference

8. SPECIAL TRAINING NEEDS/CERTIFICATION

8.1 Specialized training or certifications.

Personnel assigned to perform field sampling have prior field experience and training in water quality monitoring. However, no special certification is required for this task. Personnel conducting laboratory analysis also have prior laboratory experience and training in chemistry, but do not require special certification.

The QA Officer is responsible for overseeing the training of laboratory analysts and field operators, which will be performed by the laboratory and field managers, respectively. Each analyst must be trained and able to read and understand the SOP before they are permitted to perform the method. The responsibilities of the managers and the analyst/ operator are outlined in each SOP. An example of how analysts/ operators will be trained to perform procedures outlined in a SOP is described below.

- Laboratory analysts: it is the responsibility of analysts/technicians to;
  - Read and understand the SOP and follow it as written.
  - Produce quality data the meets all of the laboratory requirements.
  - Complete the required demonstration of proficiency before performing this procedure without supervision
  - Authorization for the analyst to perform each method will be documented by the laboratory manager on the SOP with a date indicating when the analyst can begin the procedure.
  - Repeat the required initial demonstration of proficiency each time a modification is made to the method.
- Laboratory managers: it is the responsibility of the laboratory manager to:
  - Ensure that all analysts have the technical ability and have the adequate training required to perform this procedure.
  - Ensure that all analysts have completed the required demonstration of proficiency before performing this procedure without supervision.
  - Produce quality data that meets all laboratory requirements.

Field training on cyanobacteria sampling has already occurred numerous times as all field crew has conducted field data collection for other projects and because the sampling is similar to previous phytoplankton collection. Additional cyanobacteria training specific to this project will occur the first week of data collection. Since most of the staff is employed on other grant projects, much of the training on the use of labs and general field and laboratory tasks has already occurred. Training in additional tasks will be provided whenever the sampling schedule allows for new field participant. Training is documented (Appendix A) and located in Building in EMS D436 at UCSC.

Table 8-1 Specialized personnel training or certification.

Specialized Training Course Title or Description	***Training Provider***	***Personnel Receiving Training/ Organizational Affiliation***	***Location of Records & Certificates****
Training of laboratory personnel in laboratory procedures (UCSC)	EH&S Staff	UCSC research staff and students	EMS D436, UCSC
Training of field personnel for conventional water quality constituents sampling procedures	Cécile Mioni	CSUMB research staff	Building 13, Rm 101, CSUMB
Training of field personnel for harmful algae and toxins	Cécile Mioni & Raphael Kudela	UCSC research staff and students	EMS D436, UCSC

9. DOCUMENTS AND RECORDS

9.1 Field and Laboratory Records

All field results will be recorded at the time of completion using standard field data sheets. Data sheets will be reviewed for obvious outliers and omissions before leaving the sample site. Upon return to CSUMB samples will be checked off on the field sheet or chain of custody forms will be completed for all samples to be sent to contract laboratories. Laboratories will keep records for sample receipt and storage, analyses, and reporting. Data sheets and chains of custody will be stored by CSUMB & UCSC in hard copy form for three years from the time the study is completed.

Each data generating activity—field collection and laboratory analysis—will be documented in accordance with the guidelines described below.

Field Activities: Field personnel will generate a log for each sampling day. The information to be recorded in the log includes:

- Project ID;
- Name(s) of individual(s) conducting sampling;
- Type of sampling;
- Date and time;
- Location of sampling;
- GPS coordinates
- Number and types of samples collected

- Concentration of dissolved oxygen, electrical conductivity, pH, water temperature, turbidity, secchi depth.
- QA/QC information (Location where field duplicate is taken and location where field blank is opened);
- Additional information that may affect the integrity of the samples;
- Name of individual receiving bottles in laboratory; and
- A check-box to mark each sample when received in laboratory.

The field data sheets will be indexed and stored for reference for a minimum of 3 years at the laboratory.

Each sample bottle will be labeled with the following information:

- Site code;
- Date and time;
- Type of sample;
- Number of samples collected (if more than 1).
- Storage conditions (ice, dry ice, etc.)

Laboratory Activities: The laboratory personnel will complete a hard-copy report every time samples are processed (see Appendix B). This report will include:

- Date that analysis was performed;
- Name of individual(s) who performed and reviewed the work;
- Method of analysis performed;
- Summary of analytical results;
- Summary of QA/QC data; and
- Name of computer file where data is stored.

The hard-copy report will be indexed and stored as backup for a minimum of 2 years in the applicable laboratory (UCSC, UCD, or EcoAnalyst, Inc.).

In addition, the laboratory personnel will maintain a log of maintenance activities for the Spectrophotometer and the Shimadzu TOC analyzers.

9.2 Report Format and Data Package

Data from this project will be summarized in the form of: 1) quaterly reports (monthly informal reports will be sent to the contract manager during the bloom season), 2) a draft and final report, and 3) a project database. All reports will be formatted as Microsoft Word documents or pdf file documents. The project database will be in Excel. The reports and the project's master database will be relinquished to the Grant Manager. Release of data will

include comprehensive documentation. This documentation will include database table structures (including table relationships) and lookup tables used to populate specific fields in specific tables. Releases to the public will also include QA classifications of the data (flags, as appropriate) and documentation of the methods by which the data were collected (metadata). Data will be released to the general public once a final report documenting the study has been prepared.

9.3 Procedures to protect data storage and retrieval

Maintaining digital data requires intentional and active backing up procedures. All data on this project are stored on university or PI's computers that have sophisticated back up systems on a regular basis (daily or weekly). In addition, we will perform manual backups of data and store digital data on optical media on a monthly basis. These media will be stored off-site to ensure redundancy.

9.4 QAPP Amendments and redistribution

Amendments to the QAPP will be made by Cécile Mioni, Raphael Kudela and Dolores Baxa, and distributed via electronic copy to all signatories and affected individuals.

GROUP B: DATA GENERATION AND ACQUISITION

This section of the QAPP describes in greater detail all aspects of data collection and analysis, including the sampling process, sample handling procedures, laboratory methods, analytical methods, quality control activities, equipment use and maintenance, and data management.

10. SAMPLING PROCESS DESIGN

10.1 Sampling Design Strategy

We will collect planktonic cyanobacteria and surface water samples, as well as data on physical characteristics from Clear Lake and the Delta in order to 1) characterize of the spatial and temporal cyanobacteria population dynamics; 2) determine cyanotoxin concentrations at the surface; 3) determine the environmental stressors conducive to HC blooms; 4) set a baseline necessary to the development of predictive models of HC blooms and toxin production; and 5) use monitoring data analysis to direct management measures for Clear Lake and the Delta watershed. Note: This study focuses on cyanobacteria and cyanotoxin monitoring, therefore, physic-chemical environmental variables are for informative purpose rather than the focus of this study.

10.2 Data collection type and frequency

Sampling will be done on a monthly basis and will be coordinated through CVRWQCB, UCSC and collaborating agencies: Lake county water department for Clear Lake and the California Department of Water Resources (Environmental Monitoring Program) for the Delta. Sample collection will follow the protocols outlined in "SWAMP Bioassessment procedure 2009 - Standard Operating Procedures for Collecting Stream Algae Samples and Associated Physical Habitat and Chemical Data for Ambient Bioassessments in California" (June 2009) and in SWAMP Quality Assurance Program Plan (November 19, 2008). We will collect 5 samples for each of the 12 discrete stations (one sample/month/station) between June and October 2011 and 5 continuous samples (SPATT) for each of the 7 continuous stations (one sample/month/station) between June and October 2011.

10.3 Project activity schedules

Monitoring of the HC bloom and cyanotoxins in Clear Lake and the Delta will occur on a monthly basis during the bloom season (June – October 2011).

In the Delta, the sampling schedule will be based on DWR water quality monitoring schedule. The schedule for the study period will be as follow:

- Stations D16, D26, MD10A, P8 will be sampled on 6/8/11, 7/7/11, 8/4/11, 9/1/11 (October: TBD)
- Stations D12, D19, D28A will be sampled on 6/9/11, 7/6/11, 8/3/11, 9/2/11 (October:TBD)
    - Continuous stations (D12A, D28A, D29): TBD (once monthly from June to October, when the DWR/EMP group collect chl*a* samples)

In Clear Lake, the monthly sampling schedule will be based on the availability of Lake county boat as well as on the Delta cruises schedule so that there is no overlap.

Data collected from previous years will be used for correlation with environmental stressors. The sampling protocol provides a balance of the trade-off between sufficient sampling, sampling effort as well as capturing the formation and maturation of HC bloom.

10.5 Reconciliation of natural variation with project information

As with any study that takes place in the environment, as opposed to controlled laboratory settings, natural variation will play into this project in many ways. Such variations can be problematic, as it can result in "noise" in the dataset, which makes it more difficult to pick up the true signals under investigation, and establish relationships between stated predictor and response variables of interest. Potential sources of natural variations that can affect the plankton community will be reported (e.g. wind, local management of the bloom), thus allowing the application of statistical means to normalize for parameters, as necessary, and minimize noise in the dataset.

10.6 Reduction of bias

Bias could affect the results of the work undertaken in this study. A potential source of bias relates to the selection of locations for sampling. Potential source of bias will be reduced by having field data collectors strictly adhere to the protocol, which provides an objective means, and thereby eliminates the potential for bias, and the non-representative data that would result from this bias.

11. SAMPLING METHODS

The purpose of this section is to detail how samples will be collected consistently between locations and by all sampling teams, with no contamination being introduced during the collection. Details of sampling methods employed in this monitoring project are summarized below. *Any issues (e.g. change in schedule) should be reported to the lead PI (Cécile Mioni) who will in turn report the problem to the contract manager*.

11.1 Collection of water-column samples

Sample collection will follow the protocols outlined in "SWAMP Bioassessment procedure 2009 - Standard Operating Procedures for Collecting Stream Algae Samples and Associated Physical Habitat and Chemical Data for Ambient Bioassessments in California" (June 2009) and in SWAMP Quality Assurance Program Plan (November 19, 2008). Prior to the beginning of sample collection at each site, GPS coordinates will be checked for accuracy. After collection, sample containers will be placed in ice chests with wet ice or dry ice. Proper precautions will be taken at all times in order to avoid transferring invasive organisms and pathogens between sites. Samples containers will be labeled with site identification code, collection and date time, and sampler's ID. After collection, samples will be delivered to the lab as soon as possible (e.g. same day) to meet all designated holding time requirements. The receipt of all samples will be logged in the sample logbook.

At each discrete stations, we will collect sub-surface grab samples for toxicity and algal identification and biomass assessment (chl *a*, enumeration, molecular analysis) along with GPS coordinates, notable field conditions (weather conditions, evidence of recent rainfall and fires, human influence and other habitat characteristics such as microalgae thickness, presence/absence of cyanobacterial mat), water chemistry measurements (temperature, secchi depth, specific conductance, pH, dissolved oxygen, nutrients, DOC). Additionally, photo documentation of the sampling site will be collected when relevant and archived. The sampling team will record all relevant information in the field log book and the chain of custody.

Hand-held quality water meter (YSI) and analytical instrument will be calibrated prior to sample analysis in accordance to the manufacturer's guidelines and to the SWAMP Quality Assurance Program Plan. For the determination of toxins, 50-mL subsamples will be collected in 60-mL glass jars (certified clean by Environmental Sampling Supply, Inc.), transported on dry ice, and stored frozen (- 20°C) until analysis. Algal samples will be collected in sterile 50-mL polypropylene tubes and fixed with buffered glutaraldehyde (final concentration 2.5%) immediately after collection. Algal samples will be transported in the dark and kept away from heat (e.g. in wet ice chests, cold room) and analyzed by EcoAnalysts, Inc. lab. For Chl *a*, 50 to 200-mL of sample water will be filtered onto Whatman glass fiber filters (GF/F) in the dark and transported on dry ice. Samples will be kept frozen (-20°C) in the dark until analysis. Chl *a* will detected using a Turner fluorometer. For molecular samples, 50-100 mL of water samples will be filtered onto a sterile 0.2-µm supor membrane filter and shipped on dry ice to UCD. Samples will be kept frozen (- 20/-80°C) until analysis. Ammonium, NOx, and PO4 will be detected using Hach kits following the manufacturer's protocols (USEPA certified). Certified QA/QC standards (e.g. SCP Science) will be included for all nutrient analytical runs. Samples for DOC analysis will be filtered through a sterile 0.2-µm filter and collected into acid washed and combusted 40 mL borosilicate glass scintillation vials with teflon lined screw caps. Samples will be acidified with HCl and purged to remove inorganic (and purgeable organic) carbon, kept cool (4°C) in the dark until analysis. Samples will be analyzed using a Shimadzu TOC analyzer (EPA method 415.1, American Public Health Association Method 5310 B).

SWAMP requires that some sample analysis be initiated within 48 hours of sample collection (e.g. nutrients, toxicity tests). UCSC will make every effort to initiate tests within 48 hours of sample collection; however, due to the intense sampling schedule of this project, a 48-h holding time may not be feasible (e.g. weekends and holidays). If UCSC is unable to initiate sample analysis within 48-hrs, the program manager will be informed and the CVRWQCB will be consulted. All processing times and dates will be recorded in our lab book and any delay in processing will be reported, data will appear with an asterisk in our excel spreadsheets. We will be using alternate preservation methods that have been successfully tested to expand the holding time of the toxins and nutrients samples.

- <u>*Cyanotoxins*</u>: Raphael Kudela's lab has performed experiments to determine the preservation of toxins by freezing samples. Results from these experiments demonstrated that freezing was a successful method for the preservation of cyanotoxin samples. Moreover, unlike some marine toxins (e.g. domoic acid), cyanotoxins can be extremely stable and resist common chemical breakdown such as hydrolysis or oxidation under conditions found in most natural water bodies. For microcystins, the half-life at typical ambient conditions is 10 weeks as they break down slowly in full sunlight especially when water-soluble pigments are present (EPIRAB – OEHHA 2009). Although cyanotoxins can be

broken down by some bacterial proteases, in many circumstances these bacteria are not present so the toxin persists for months or even years once released into cooler, dark, natural water bodies (EPIRAB – OEHHA 2009).

- *Nutrients*: Freezing is the main alternative to addition of preservatives (Nollet 2007). Freezing offers the advantage over poisoning in that the sample matrix is not altered (Nollet 2007). From the literature freezing appears to be the preferred method for storage of samples for phosphorus analysis (Worsfold et al. 2005). Sample filtration is required since freezing will rupture cells and release phosphorus into solution [please note that all the nutrient samples will be filtered in the field]. Frozen storage appears to be much more efficient that the other storage options offering sample preservation for months to years. For example, Avanzino and Kennedy (1993) reported suitable storage of stream water samples for 4 to 8 years with no significant changes in phosphorus, nitrate plus nitrite, and ammonia concentrations. None of the differences observed over long periods of frozen storage were more than twice the estimated standard deviation of the analytical methods used in the study. [note: most labs using this preservation method use a holding time of 90 days or less and we will process the samples within a week]. Several authors suggested that quick freezing is also preferable than slow freezing, increasing the precision of the results (Morse et al. 1982).

11.2 Collection of toxin data

Sampling generally follows the methods described in section 11.1. Whole water is collected from ~0.1 m depth by rinsing glass sample containers three times. Surface scums of algae will not be avoided, but are not deliberately collected to avoid biasing the water sample concentrations. SPATT bags are deployed at ~0.1 m depth using a nylon rope and Hoop-La plastic embroidery hoop.

11.3 Equipment and support facilities

Clear Lake – The sampling boat, YSI, secchi disk, hobos, battery and life vests will be procured from the Lake County Department of water resources.

Delta - Sampling will be conducted monthly by partnering with the California Department of Water Resources Environmental Monitoring Program (http://www.baydelta.water.ca.gov/emp/). This program includes regular monitoring of water quality variables (conductivity, pH, dissolved oxygen, turbidity, dissolved chloride, chlorophyll fluorescence, water temperature, air temperature, wind speed and direction, solar radiation) as well as biological characteristics, such as phytoplankton and zooplankton community composition and biomass in the Sacramento-San Joaquin Delta, Suisun Bay, and San Pablo Bay. For more details, see: http://www.baydelta.water.ca.gov/emp/metadata_index.html. All these ancillary data will be available to this project at no cost. Sampling will be done on board the DWR/USBR research vessels during the monthly routine monitoring cruises at the discrete stations. We will share our data with the EMP group to allow comparison and to enhance their preexisting program with data that are not currently being monitored.

12. SAMPLE HANDLING AND CUSTODY

12.1 Sample Collection and Initial Preservation

We will apply the recommended containers and preservation methods listed in appendix B of the SWAMP QAPP (November 19, 2008). The bottles that will be used for sample collection are detailed in Table 12-1. ALL samples, are immediately placed in a ice chest. Water samples for nitrate, ortho-phosphate, ammonium, and DOC will be hand-filtered on site using acid washed syringes and sterile 0.45µm-capsule filters (nutrients) or 0.2 µm-capsule filters (DOC). Water samples destined for chlorophyll *a* will be filtered on site using Whatman GFF glass fiber 0.7µm filter. Cyanobacteria samples for taxonomic ID and enumeration are preserved by adding glutaraldehyde (final concentration 2.5%) in 50ml aliquots. Cyanobacterial DNA will be collected through filtration of collected water through a sterile polycarbonate filter.

Holding Time: Table 12-1 summarizes the handling procedures and maximum holding times for samples. Samples may be disposed of when analysis is completed and all analytical quality assurance/ quality control procedures are reviewed and accepted. Samples will be archived for at least 90 days (nutrients) or 6 months (toxins, cell enumeration, DNA). Please refer to section 11.1 for alternate preservation methods that will be used to increase nutrients and toxins samples holding time in the event that the samples cannot be processed within 48 hours.

12.2 Transport to the Laboratory/Preservation

UCSC will make every effort to transport all samples to the lab within 4 hours after collection and to initiate tests within 48 hours of sample collection (please refer to section 11.1 for comments on holding time). All water chemistry samples will be filtered in the field. All nutrient samples, chlorophyll *a*, intracellular microcystins and DNA samples will be transported on dry ice (or flash frozen in liquid nitrogen for DNA samples) and kept frozen in the lab until analysis (-20ºC). The TOC will be acidified and kept in the refrigerator. Preserved cyanobacteria samples will be transported on wet ice and kept in the refrigerator until they are shipped to EcoAnalysts, Inc. for analysis. All these and additional details are outline in Table 12-1.

To ensure that no bottles are lost in transport, the laboratory personnel will check-off each sample bottle on the field log sheet (Appendix F) upon returning to the laboratory. Since all laboratory analyses are internal, checking the log sheet supplants the need for a chain-of-custody form. A chain of custody form will be used when sending taxonomic ID samples to contract laboratories (Appendix G).

Table 12-1: Collection containers, preservation, and holding times for samples.

Sample Type	Container	Volume	Initial Preservation	Filtering	Storage Time
Nutrients	Polyethylene bottle, pre-cleaned in lab using HCl	125 mL	Cool to 4°C and in the dark, dry ice after filtration	On site, within 4 hours	48 hours @4°C up to 90 days – frozen
DOC	40-mL glass vial with Teflon lined cap.	40 mL	Acidified, cool to 4°C and in the dark	On site, within 4 hours	28 days, 4 °C (acidified)
Chlorophyll *a*	Amber Polyethylene	50 – 200 mL	Cool to 4°C and in the dark, dry ice after filtration	On site, within 4 hours	28 days, frozen (filters)
Dissolved Microcystin	Certified glass jars	50 mL	Dry ice, dark	Within 4 hour	28 days frozen at -20°C, longer at -80°C
Cyanobacteria samples for taxonomic ID and enumeration	Falcon tube	50 mL	Glutaraldehyde added to final concentration of 2.5% v/v, 4°C, dark	NA	4°C, dark (indefinite)
Cyanobacterial DNA	Microcentrifuge tubes	50-100 mL	Dry ice, dark or liquid nitrogen	On site, within 4 hrs	28 days frozen at -20°C, longer at -80°C
Whole water toxin samples	Certified glass jars	2 x 50 mL	Dry ice, dark	NA	28 days frozen at -20°C, longer at -80°C
SPATT samplers	Glass bottle with Milli-Q water	125 mL	Dry ice, dark	NA	-20ºC for 28 days (indefinitely at -80° C)

13. ANALYTICAL METHODS AND FIELD MEASUREMENTS

13.1 Field Water Quality Measurements

Dissolved oxygen, pH, electrical conductivity, water temperature and sampling depth are determined using the YSI according to manufacturer's instructions. The range, resolution, and accuracy for each of these parameters are shown in below in Table 13-2. The Standard Operating Procedure has been included in Appendix H.

Table 13-1: Specifications for water quality parameters measured with the Hydrolab.

Parameter	*Range*	*Resolution*	*Accuracy*
DO (mg/L)	0 to 50 mg/L	0.01 mg/L	0 to 20mg/L: ± 0.1 mg/L or 1% of reading whichever is greater 20 to 50 mg/L: ±15% of reading
Conductivity (mS/cm)	0 to 100 mS/cm	0.001 to 0.1 mS/cm (range dependent)	±0.5% of reading + 0.001 mS/cm
Temperature (°C)	-5 to +50ºC	0.01ºC	± 0.15ºC
pH	0 to 14 untis	0.01 unit	±0.2 unit
Turbidity	0 to 1,000 NTU	0.1 NTU	±2% of reading or 0.3 NTU, whichever is greater

Except for the SPATT, there will be no deployment of in situ monitoring equipment. There will be no continuous monitoring data to be stored and maintained because the SPATT toxin measurements are integrated over the time period during which they are immersed.

13.2 Laboratory Analytical Methods

SOPs for all laboratory analyses are available in Appendix H.

SOP Document Control Number	SOP Description
UCSCCM-001	CLEANING/DECONTAMINATION PROCEDURES
UCSCCM-002	PROCEDURE FOR COLLECTION OF CYANOBACTERIA & CYANOTOXINS SAMPLES
UCSCCM-003	PROCEDURE FOR TAXONOMY ANALYSIS OF CYANOBACTERIA BY ECOANALYSTS, INC.
UCSCLM-002	DETERMINATION OF CHLOROPHYLL-a IN FRESHWATER PHYTOPLANKTON BY FLUORESCENCE
UCSCLM-011	DETERMINATION OF TOC WITH SHIMDZU ANALYZER

Nutrient

Nutrient concentrations, including nitrate, nitrate, ammonium, and orthophosphate will be determined using Hach water quality kits following the manufacturer's instructions (see 6.2). The product is measured colorimetrically to determine the concentration of nutrients that reacted. The kits have low detection limits, permitting small quantities of nutrients to be detected.

Each run of samples will be analyzed with reference materials and QCs; blank and calibration standards every 20 samples per analytical batch, at least 1 field blank and 1 field duplicate (5% of total sample count), and at least 1 spiked matrix sample (every 20 samples per analytical batch). The spiked matrix sample is prepared as in the following example:

E.g.: A sample with a NO_3-N concentration of 2.6mg L^{-1} is split into 15.0ml sub-samples for spiking. For example, a stock solution of 25mg N L^{-1} will be added to one sub-sample in order to increase the nutrient concentration without significantly changing the volume. Adding 0.6mL of the stock solution will increase the concentration by (0.6mL) (25 mg L^{-1}) / (15.0mL + 0.6mL) = 0.96 mg L^{-1}. The new concentration will be 0.96 mg L^{-1} + 2.6 mg L^{-1} = 3.56 mg L^{-1}.

All calibration standards, QCs, and samples will be analyzed with 2 or 3 replications. The range of nutrient concentrations that can be accurately detected differs for each method (see Table 13-1). If a sample contains concentrations above the calibration range, it will be diluted and reanalyzed.

DOC

Dissolved organic carbon (DOC) will be determined via high temperature catalytic combustion using a Shimadzu TOC Analyzer according to SOP UCSCLM-011. Total Organic Carbon includes the measurement of organic carbon in surface waters by injecting a sample into a reaction chamber, packed with a catalyst and held at a fixed temperature. The organic carbon is converted to carbon dioxide by the action of the catalyst and the elevated temperature. The concentration of carbon dioxide generated is directly proportional to the concentration of organic carbon in the sample. The sample is transported to a non-dispersive infrared analyzer sensitized to respond only to carbon dioxide and the concentration is displayed on the digital display meter. The analyzer measures total carbon which includes both organic and inorganic carbon.

Similarly, each run of samples will be analyzed with QCs, at least 1 field blank and field duplicate, and at least 1 analytical duplicate. All calibration standards, QCs, and samples will be analyzed with 3-5 replications. The range of nutrient concentrations that can be accurately detected differs for each method (see Table 13-1). If a sample contains concentrations above the calibration range, it will be diluted and reanalyzed.

Chlorophyll *a* Determination

Chlorophyll *a* samples will be processed according to SOP UCSCLM-002 located in appendix H. Chlorophyll *a* sample will be collected on filters will be extracted with acetone and sonification followed by centrifuge and analysis with fluorescence with a Turner Designs fluorometer (10AU). Chlorophyll *a* and correct for phaeophytins using the acidification calculations outlined by standard methods (AWWA 2005). We will include three check standards of known concentration with every batch to monitor the reliability of the method.

Toxins

Common cyanobacterial toxins (MCY-LR, MCY-RR, MCY-LA, MCY-YR, anatoxin-A, cylindrospermopsin, lyngbyatoxin-a, nodularin, saxitoxin) will be measured according to SOP UCSCCM-002 located in Appendix H.

UCSCCM-002 PROCEDURE FOR COLLECTION OF CYANOBACTERIA & CYANOTOXINS SAMPLES

Calibration standards of certified reference materials are included with each run, and the unknown (sample) concentrations are determined by areal integration of the peaks corresponding to known toxins. One of the concentrations is near the Method Detection Limit (MDL) for each parameter. Concentrations of the remaining standards correspond to the expected range of concentrations found in the samples analyzed. Calibration standards are prepared by utilizing secondary dilution standards and/or stock solutions. The instrument performs auto-tuning and sensitivity tests before each run.

Refer to Element 14 for additional Quality Control Procedures.

Refer to Element 15 for maintenance procedures and schedules of all equipment.

Table 13-2: Laboratory Analytical Methods

Analyte	Method	Detection Range*
Nitrate-N (NO_3^- and NO_2^-)	Hach Method 8192	0.01 – 0.5 mg L^{-1}
Ammonia-N (NH_3)	Hach Method 8038	0.02 – 2.5 mg L^{-1}
Nitrite-N (NO_2^-)	Hach Method 8307	0.002 – 0.3 mg L^{-1}
Orthophosphate (PO_4^-)	Hach Method 8048	0 – 2.5 mg L^{-1}
Total Organic Carbon	EPA 415.1	0.050 mg L^{-1}- 4000 mg L^{-1}
Chlorophyll *a*	EPA 445	0.05 µg L^{-1}
Enumeration/Identification	UCSCCM 002 UCSCCM 003	Presence/absence
Cyanobacterial toxins	Mekebri et al. 2008	0.5 ng/mL for all toxins

*Lower end of the detection range is the analytical detection limit, upper end is the highest calibration standard used. The data quality objective for this study is 10% .

Taxonomic Identification and Enumeration of Cyanobacteria Samples

Cyanobacteria samples will be collected according to the SOP UCSCCM-003 located in Appendix H.

UCSCCM-002 PROCEDURE FOR COLLECTION OF CYANOBACTERIA & CYANOTOXINS SAMPLES.

Cyanobacteria samples will be initially screened for the presence of cyanobacteria by professional taxonomists at EcoAnalyst, Inc using standard Uthermölh procedures (sedimentation chambers and inverted microscope, UNESCO 2010). More information can be found on the contractor's website (http://www.ecoanalysts.com) or by contacting their project coordinator: Shanda McGraw at smcgraw@ecoanalysts.com or by phone at (208) 882-2588 Ext. 30. For more detail, refer to the following SOP located in appendix H:

UCSCCM-003 PROCEDURE FOR TAXONOMY ANALYSIS OF CYANOBACTERIA BY ECOANALYSTS, INC.

Samples dominated by buoyant species such as the colonial Microcystis (demonstrating mucilaginous matrix between cells) will be also analyzed at UCSC using epifluorescence microscopy (UNESCO 2010, Rinta-Kanto et al. 2005). Samples will be first broken up using the colony disaggregation method (Bernard et al. 2004), filtered on black polycarbonate filters (Poretics), mounted on slides and examined via epifluorescent microscope. The Microcystis cells will be enumerated via the natural unit method (SOP UCSCCM-002). For more details, refer to the following SOP located in appendix H:

UCSCCM-002 PROCEDURE FOR COLLECTION OF CYANOBACTERIA & CYANOTOXINS SAMPLES

Molecular Cyanobacterial Toxin Gene Analysis

We will use polymerase chain reaction (PCR) to identify HC strains using molecular markers (e.g. 16S rRNA gene sequence) and to estimate potential toxicity based on the presence of genes associated with cyanotoxins (e.g. *mcy* gene for microcystin toxins). This molecular analysis will be performed by our subcontractors from UCD (Dolores Baxa) using their standard protocols (e.g. Baxa et al. 2010). In short, they will extract cyanobacterial DNA from the frozen samples retained on filters. Extracted DNA will be combined with PCR reagents in the thermocycler with appropriate settings for targeting toxin and taxa-specific genes. After cycling, each PCR reaction will be loaded for electrophoresis in ethidium bromide agarose gels with an appropriate DNA ladder. The resulting bands will be visualized, interpreted and photographed under UV illumination. Based on the outcome of the electrophoresis and availability of technology, some of the extracted cyanobacteria DNA will be sequenced for further analysis.

Turnaround Times/Failures in Laboratory Equipment

Laboratory turnaround times are not applicable for this project.

Failures in field and laboratory measurement systems involve, but are not limited to such things as, instrument malfunctions, failures in calibration, sample jar breakage, blank contamination, and quality control samples outside of the defined limits (Data Acceptability Criteria). In many cases, the field crew or lab analyst will be able to correct the problem. If the problem is resolvable by the field crew or lab analyst, then they will document the problem in their field notes or laboratory record and complete the analysis. If the problem is not resolvable, then it is conveyed to the respective supervisor, who will make the determination if the analytical system failure compromised the sample results and should not be reported.

Hazardous Waste Disposal

Used sample that has been processed with reagent is a regulated hazardous waste and disposed of according to UC Santa Cruz's Environmental Health and Safety Program. Each nutrient test or glutaraldehyde contaminated waste (cell taxonomy) has a separate waste bottle that is clearly labeled and stored within secondary containment in a hood. Bottles are kept for no longer than 3 months, and then picked up by the Environmental Health and Safety Officer.

14. QUALITY CONTROL

The quality control procedures employed in this study to ensure that the data quality objectives (DQOs) outlined in Section 7, are achieved are summarized in Tables 14-1 and 14-2. Failure to meet DQOs will result corrective actions to be taken to reconcile differences between collected data quality and requirements.

14.1 Field sampling

During field sampling events, field blank (1 per day or 10% site, whichever is the more frequent) and duplicate nutrient 1 per day or 5% of sites, whichever is more frequent)are collected at a randomly selected sampling site (to evaluate the precision of the sampling

technique and to assess short-term environmental variability at the sample site. As mentioned in Section 7, precision is measured by calculating the relative percent difference (RPD) between the duplicate samples as shown again below:

$$RPD = (X_1 - X_2) / (X_1 + X_2) / 2 * 100$$

where X_1 = the larger of two values and X_2 = the smaller of two values.

The difference or percent difference, as appropriate, will be compared against the precision criteria established for field measurements in section 7.

To assess potential nutrient contamination of bottles from the cleaning process, handling, transport, and environmental deposition, a travel blank is sent into the field for every sampling event. Travel blank preparation consists of filling a clean sample bottle with deionized water in the lab, transporting into the field and opening it briefly at a sampling location.

14.2 Laboratory

During laboratory analysis, each run of nutrient and chlorophyll a samples will be analyzed with QCs, at least 1 field blank and field duplicate, and at least 1 spiked matrix sample. Corrective actions will be taken if control limits are exceeded. Any issues will be documented in the log book and will be reported to the contract manager. Any suspicious sample or control will be rerun.

The precision of the laboratory measurements is measured by calculating the relative percent difference (RPD) between the duplicate samples as shown for the field duplicate samples above. The percent recovery for the matrix spike sample is calculated as:

$$R= (Cs - C) / S * 100$$

Where, R = percent recovery, Cs = spiked sample concentration, C = sample background concentration, and S = concentration of nutrient added to sample. The percent recovery of nutrients in the spiked sample must be 80-120% for each sample run to be accepted. All calibration standards, QCs, and samples will be analyzed with 2 or 3 replications and the average of all accepted values will be reported.

Table 14-1. (Element 14) Sampling (Field) QC.

Matrix: water
Sampling SOP: SWAMP QAMP Appx D
Analytical Parameter(s): Nitrate, Ammonium, Ortho-Phosphate, Dissolved Organic Carbon, temperature, conductivity, pH and DO.
Analytical Method/SOP Reference: SWRCB, SOP for field collection of water samples
Sample locations: As many as 300 throughout the central coast of California

Field QC	**Frequency/Number per sampling event**	**Acceptance Limits**
Temp, pH, conductivity, DO with YSI Multiprobe	1st measurement of day in triplicate, minimum of 1/20 measurements in triplicate.	No SWAMP criteria
Equipment Blanks	1 per 20 with at least one in every batch	No detectable amount of substance in blanks.
Field Blanks	1 per sampling event or 10% of samples (whichever is more frequent	No detectable amount of substance in blanks.
Trip Blanks	1 per sampling event	No detectable amount of nutrients.
Cooler Temperature	NA	4°C
Field Duplicate Pairs (triplicate for hydrolab measurements)	1 per sampling event or at least 5% of samples (whichever is more frequent)	80-120%
Collocated Samples	None	
Field Splits	None	
Field Matrix Spikes	None	
Other:	None	

Table 14-2. (Element 14) Analytical QC.

Matrix: water
Sampling SOP: SWAMP QAMP Appx D
Analytical Parameter(s): Nitrate, Ammonium, Nitrite, Ortho-Phosphate, Dissolved Organic Carbon, Chlorophyll *a*
Analytical Method/SOP Reference: Hach 8192, Hach 8038, Hach 8507, Hach 8048, EPA415.1, EPA 445
Sample locations : As many as 300 throughout the central coast of California

Laboratory QC	**Frequency/Number**	**Acceptance Limits**
Method Blank	1 per analysis run	Below detection limit
Reagent Blank	1 per analysis run	
Storage Blank	None	
Instrument Blank	1 per analysis run	No detectable amount of substance in blanks.
Lab. Duplicate	1/20 samples	80-120%
Lab. Matrix Spike	1/20 samples	80-120%
Matrix Spike Duplicate	1/20 samples for metals	80-120%
Lab. Control sample	None	
Surrogates	None	
Internal Standards	5 per run, for metals run with each sample	80%-120%, for metals samples are normalized against the internal standard
Others:		

14.3 Review and corrective action

Field and laboratory data collected during this practice effectiveness monitoring project will be reviewed at the point of collection with data quality objectives (DQOs) outlined in this QAPP. The Central Valley Regional Board project manager will review data quaterly to determine if the DQOs have been met and if data do not meet the project's specifications, the following actions will be taken. Firstly, the Regional Board project manager will review the errors and determine if the problem is equipment failure, calibration/ maintenance techniques, or monitoring/sampling techniques. They will suggest corrective action. If the problem cannot be corrected by training, revision of techniques, or replacement of supplies/equipment, then the Regional Board project manager will review the DQOs and determine if the DQOs are feasible. If the specific DQOs are not achievable, they will determine whether the specific DQO can be relaxed, or if the parameter should be eliminated from the monitoring program. Any revisions to DQOs will be appended to this QA plan with the revision date and the reason for modification. The appended QAPP will be sent to the quality assurance panel that approved and signed this plan. When the appended QAPP is approved, the water quality program manager will work with the data coordinator to ensure that all data meeting the new DQOs are entered into the database. Archived data can also be entered.

Any problem encountered during assessment may lead to the following responses:

- Equipment calibration prior to scheduled date
- Equipment repair
- Supplemental training for team members
- Discussion at meeting with involved parties
- Consultation with project leader
- Reevaluation of methods

15. INSTRUMENT/EQUIPMENT TESTING, INSPECTION, AND MAINTENANCE

YSI Schedule: The YSI multiprobes require maintenance on an as-needed basis. The probes should be inspected prior to each collection trip (at the same time the instrument is calibrated) to ensure that they are in working order. Table 15-1 details the maintenance requirements for each probe. It is the responsibility of DWR and Lake County to maintain their respective YSI and to store necessary spare parts.

Testing, inspection, and maintenance for all field equipment and instruments will be carried out by the field officers. Corrective actions will be taken as soon as problems are identified. In general, most corrective actions are minor maintenance issues and can be completed within an hour in the laboratory. If more intensive maintenance is required the field officer will contact the manufacturer to attempt additional corrective actions; if these fail, the probe will be repaired by the manufacturer and obtain a loaner. These activities are documented on a YSI calibration form stored in the laboratory and will be reported in the progress reports.

Table 15-1: Maintenance requirements for YSI components.

Component	***Maintenance Activities***	***Maintenance Schedule***
Multiprobe Body	Use the calibration or storage cup filled with tap water to protect the sensors from drying out. Rinse the multiprobe with soap and water after returning from sampling.	Every collection trip.
pH Sensor	Rinse the pH electrode with methanol, or if necessary soak in 0.1M HCl for 5 minutes and then rinse in buffer for 10 minutes. Replace the electrolyte around the reference electrode. Replace the porous junction of the electrolyte chamber when clogged.	When obviously dirty or when measurements equilibrate slowly.
Temperature Sensor		No maintenance required.
Conductivity Sensor	Remove the cell block and o-rings and polish electrodes with emery cloth. Reinstall the o-rings and cell block and soak in DI water overnight.	When obviously dirty or when measurements equilibrate slowly.
DO Sensor	Change the DO membrane and electrolyte. Store the sensor without electrolyte if it will not be used for 2 weeks or longer.	When calibration is difficult or membrane is dirty.

Laboratory Schedule:

The LC/MS have components that must be maintained daily as well as parts that must be cleaned or replaced on a monthly to yearly basis. The LC/MS is under service contract with Agilent and is serviced as required. The LC/MS will be maintained according to the manufacturer's instructions by Dr. Kudela's lab manager and spare part will be stored in Dr Kudela's lab.
Rob Franks, the laboratory manager of the Marine Analytical Laboratory 3rd Floor, Earth and Marine Sciences Building, University of California Santa Cruz, is responsible for the repair and maintenance of the lab equipement (shimadzu TOC analyzer, spectrophotometer, sonicator). Spare parts for the shimadzu TOC analyzer, spectrophotometer and sonicator are stored in the Marine Analytical Lab (http://ims.ucsc.edu/mal/).

Testing Equipment

As part of the calibration procedure all equipment is tested for appropriate responses and behavior prior to analysis. If equipment fails to behave properly as specified in the operating manual, the QA Officer will be notified immediately. Corrective action will be taken with either project staff or with the guidance of technical support from the manufacturer until the equipment runs properly. All problems and corrective actions will be noted in the Equipment Maintenance Log.

Individuals Responsible for Equipment Inspection and Maintenance

Cécile Mioni, Raphael Kudela and Kendra Hayashi will be responsible to inspect and maintain all equipment, check logs on a monthly basis, and determine scheduled maintenance requirements, and assign staff to perform this required maintenance. Subcontractors will be responsible for their own log and for the maintenance of their own equipments.

16. INSTRUMENT/EQUIPMENT CALIBRATION AND FREQUENCY

The laboratory manager will maintain all documentation and scheduling of equipment calibration determined by the frequency of use and specification of equipment.

The YSI calibration will be checked prior to each collection trip. Post sampling calibration checks will also be performed. If the YSI fails to calibrate, it will be sent to the manufacturer for repair. We will maintain a folder with hard copy calibration results and repairs made to the equipment.

The Shimadzu TOC will be calibrated each time samples are run and calibration standards will be run for every 10% of samples or 10 samples, whichever is more frequent. We will trouble shoot these instruments until calibration and check pass QA standards. This could involve working closely with the manufactures technical support. We will maintain a folder with hard copy calibration results and repairs made to the equipment.

The LC/MS will be calibrated each time samples are run and calibration verification will be run for every 35 samples, whichever is more frequent after the initial calibration. The linear regression for the calibration will exceed $r^2 = 0.995$ and the LC/MS will be calibrated and checked to pass QA standards.

Analytical balances are checked by placing in-house weights on the balance once per month. If a balance does not produce a reading within 0.1% of the labeled value of the weight, the balance is taken out of service for repair. Balances are cleaned and calibrated against NIST traceable weights once per year. Calibration results and repairs made to the equipment will be maintained in a folder within the marine analytical laboratory (Rob Franks).

Pipettes are serviced and calibrated annually by an external contractor and checked regularly, by delivering the exact amount and type of solvent corresponding to their planned use and weighing the amount of solvent delivered on a NIST-traceable balance. Any Pipette which cannot be adjusted to deliver a weight of a solvent within 2 % of the required weight is taken out of service for repair or replacement. Deficiencies should be documented for pipette calibration using pipette calibration sheet in the laboratory and will be checked monthly.

Table 16-1. (Element 16) Calibration of sampling equipment and analytical instruments.

Equipment / Instrument	***SOP reference***	***Calibration Description and Criteria***	***Frequency of Calibration***	***Responsible Person***
YSI Multiprobe	Manufacturer's Instructions	Calibration of pH and conductivity with two standards for each parameter. Recovery 85% - 115%.*	checked daily and calibrated when needed	Field Officers
Shimadzu TOC analyzer	Shimadzu TOC analyzer Operating Instructions	Calibration with 3 standards spanning the range of sample concentrations. Calibration verification with 1 standard every 35 samples after initial calibration. Recovery 80% - 120%.	Daily	Individual operating the instrument, and Rob Franks (Marine Analytical Lab)
LC/MS	LC/MS Operating Instructions	Calibration with 3-5 standards spanning the range of sample concentrations. Calibration verification with 4 standards every 35 samples after initial calibration, using standards from a different source than that used for initial calibration. Recovery 80% - 120%.	Daily	Individual operating the instrument, and Laboratory Officer at UCSC (Raphael Kudela)

Balances	SOP for calibrating & maintaining Balances and Pipettes	Calibration with NIST traceable weights. Monthly checks of calibration with certified in-house weights must be within ± 0.1%	annually	Laboratory Officers at UCSC (Rob Franks, Marine Analytical Lab)
Pipettes	SOP for calibrating & maintaining Balances and Pipettes	Automatic pipettes should be serviced and calibrated by and external contractor annually. Check calibration monthly by delivering a known amount of solvent on a NIST-traceable balance-weight of solvent within 2% of required weight	annually	Laboratory Officers at UCSC

*See appendix H for Hydrolab calibration logging sheet

17. INSPECTION/ACCEPTANCE OF SUPPLIES AND CONSUMABLES

Sample collection bottles will be washed, acid washed if applicable, and reused between collection days. Bottles and caps will be inspected for damage prior to sampling, and only sound bottles with intact threads will be used. Caps will be tested for tightness prior to transport of samples.

Critical supplies and consumables for field and laboratory are obtained from high quality scientific sources. In most cases, we order chemicals from Fisher Scientific, who supplies much of the university laboratories chemical and container needs in the state. All supplies have batch tracking and meet high internal quality control criteria. All chemical materials are properly stored in accordance with label requirements and county environmental health requirements. All sample bottles and storage containers are acid washed upon receipt. In contrast to metals and organic compounds, nutrient concentrations from the environment are usually an order of magnitude higher than most potential factory contamination issues.

All reagents (solid and liquid) will be dated when they are opened and will be discarded according to the manufacturer expiration dates. Furthermore, all laboratory prepared reagents have specific shelf lives that are strictly adhered to according to the SOPs for each method. At the time they are made they are labeled with this discard date.

If a chemical has been contaminated or incorrectly labeled it is easily determined with each analysis run, since our standard curves are nearly identical from one day to the next. The laboratory officer certifies each run by inspecting these curves (among other criteria). This

process ensures that samples are analyzed using the proper reagents and these reagents have not been compromised. Our QC procedures are documented during each run (Appendix B).

Table 17-1. Inspection/acceptance testing requirements for consumables and supplies.

Project-Related Supplies / Consumables	***Inspection / Testing Specifications***	***Acceptance Criteria***	***Frequency***	***Responsible Individuals***
Water and phytoplankton Sample Bottles	Inspected for cracks and stripped threads.	Only acid-washed (except for plankton falcon tubes), non-damaged bottles and lids with intact threads will be used	Prior to each sampling event.	Cécile Mioni Kendra Hayashi

18. NON-DIRECT MEASUREMENTS (EXISTING DATA)

No existing data will be used for this project.

19. DATA MANAGEMENT

The data management tasks include:

- Recording data in the field – Cécile Mioni, field officers
- Tracking samples in the lab – Cécile Mioni, Kendra Hayashi
- Management of data files – Cécile Mioni, Kendra Hayashi
- Data entry – Cécile Mioni, Kendra Hayashi
- Data QA Checks – Cécile Mioni, Raphael Kudela
- Upkeep of document control system – Cécile Mioni, Kendra Hayashi
- Hardware and software acceptability – Cécile Mioni, Raphael Kudela

19.1 Field and Laboratory Data

As stated in Section 9, field and laboratory data will be recorded on hard-copy logs or reports, and the information will also be maintained in electronic form. Calibration and accuracy check records for field instruments will be captured on the appropriate data sheet as shown in (Appendix D). The calibration and accuracy checks records on the data sheet will include the following:

- Date, Time, Reason (pre-event or post event)
- Instrument ID
- Standard Material (ID of Standard solution)
- 'True' Value of Standard Material
- Reading before any adjustments

- Adjustments and outcome
- Operator

These calibration and accuracy check records will be kept on file at UCSC.

The staff person who returns with the samples for that day will record incoming samples in the sample storage management log on return to the laboratory. Field data sheets are checked by the project directors, and entered into an electronic database by a staff person. Upon entering, the field data sheets will be signed and archived.

The laboratory assistant will check off nutrient samples to be analyzed on the field logging sheet. As mentioned in Section 13, nutrient concentrations are determined using a Hach kits. Following nutrient analysis, samples will be checked by the laboratory assistant before they are exported into a spreadsheet (MS Excel) to produce a hard-copy report. The information from this report will also be recorded in a database file. Upon entering, the laboratory data sheets will be signed and archived.

Following initial data entry, staff will review electronic data, comparing it to the original data sheets for a minimum of 20% of entries. All errors will be corrected and documented (AppendixD). If error rates exceed 5%, the proportion of entries for which electronic data are reviewed will be increased until error rates are less than 5%. After performing data checks, and ensuring that data quality objectives have been met, data analysis will be performed. As the Quality Assurance Officers, Cécile Mioni and Raphael Kudela will also review all the field and laboratory information to verify that the sampling and analysis procedures were conducted in accordance with the QAPP. The Quality Assurance Manager's approval will be required before the data is analyzed further.

The Access files are stored on a UNIX server and backed up daily to another campus location. The hard-copy report will be indexed and stored as backup for a minimum of 2 years at CSUMB.

19.2 Data Transfer to SWAMP

Migration of data from the in-house database described above to a SWAMP-compatible form will be done by querying the in-house database and exporting the data in a form compatible for upload to the SWAMP database (e.g., comma-delimited, Excel, etc.). The original data tables will be renamed if necessary and will be linked to tables that have been transformed to SWAMP compatible formats. Several preliminary steps will be taken to ease the transition of data from the in-house database to the SWAMP database. To the extent possible, and financially feasible, in-house database fields will include those noted in the SWAMP QAPP Appendix J for similar data types. In-house database fields indicating Station Code, Sample Date, and Sample Time will remain the same for all related field-generated samples, observations and results, in order to link information. In addition, every combination of Station Code, Event Type, Sample Date, Sample Time, Sample Type Code, Sample Replicate, Project ID, and Agency Code will ensure that each record in the sample table is unique.

19.3 SOP Document Control System

A SOP numbering system has been developed for all methods used in this project. The system used for generating document control numbers includes an initial 4 letter institution identifier- for example, SOP's performed at the University of California Santa Cruz begin with the abbreviation "UCSC". This abbreviation is followed by a two-letter abbreviation for the primary use of the document. These include:

FM – Field Method
LM – Laboratory Method
DM-Data Management

After the method identifier, a dash is entered followed by three digits which allows for 999 method SOPs. An example of this numbering system is UCSCFM-001, University of California Santa Cruz, Field Method– 001. Below the SOP ID is the Revision # (Rev#:). This allows the user to verify that the SOP in use is not a draft and allows the QA Officer to track the number of times a procedure has been materially changed. Next is the date the SOP was written. The current page and total number of pages must be listed to ensure that the SOP is complete. The document control information must be placed in the upper right hand section of the SOP and all SOPs are located at K:/Documents/Methods. These folders are read-only for external users (outside of QA), which provides control of the SOP system. Upon final review and acceptance, the QA Officer will assign either a new number or revision number from the sequence and transfer it to the final folder.

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19.4 Hardware and software acceptability

To ensure end project deliverables satisfy the requirements and expectations of the SWAMP data management team, the acceptability of hardware and software configurations will be evaluated using several criteria. All hardware and software will be classified according to its intended function and potential consequences of failure, as outlined below in Sections 19.1 and 19.2. Following classification, performance requirements will be evaluated. In the first phase of evaluation, intended uses for hardware/software will be compared with the operating limits of the hardware/software, as determined by testing and/or product specifications. For Classes A, B, and C, if the functional limits of the hardware/software can accommodate the intended usage and the hardware/software is compatible with existing configurations, the hardware/software will be deemed acceptable. For existing hardware and software in Classes A, B, and C, the above requirements are sufficient for approval. For acquired hardware/software, a second phase will be required in which performance will be monitored

under normal operating conditions for 30 days. If the system operates normally during those 30 days, the hardware/software will receive approval. For hardware/software in Class D, independent testing will be required, to demonstrate, as appropriate, that the hardware/software properly handles abnormal conditions and events, does not perform adverse or unintended functions, and does not degrade the system. The SWAMP information management team will be contacted in cases where Class E hardware/software is needed to determine acceptable evaluation procedures. Hardware and software evaluation forms are presented in Appendix E.

19.4.1 Software Classification.

- Class A
    - Proprietary software applications that is necessary to operate laboratory or field equipment. The software is distributed with the equipment or is available for download from the manufacturer or distributor of the equipment.
- Class B
    - Software applications that are necessary for day-to-day operations that are in widespread use for the designated application, and whose failure to operate on a given desktop or laptop computer will not result in project delays exceeding 3 months or adversely affect project deliverables.
- Class C
    - Software applications necessary for general day-to-day operations whose failure to operate on a given desktop or laptop computer will not result in project delays exceeding 3 months or adversely affect project deliverables and do not meet the criteria specified under Classes A or B above.
- Class D
    - Software application whose failure to operate on a given desktop or laptop computer may result in project delays exceeding 3 months or may adversely affect project deliverables.
- Class E
    - Software applications necessary to produce project deliverables that do not meet the above classification criteria.

19.4.2 Hardware Classification.

- Class A
    - Desktop or laptop computer and/or standard operating components necessary for general day-to-day activities whose failure to operate will not result in project delays exceeding 3 months or adversely affect project deliverables.
- Class B
    - Server and/or standard server components necessary for general day-to-day activities, including data storage and backup, whose failure to operate will not result in project delays exceeding 3 months or adversely affect project deliverables.
- Class C
    - Any desktop, laptop, server, or standard operating components necessary for general day-to-day activities, whose failure to operate may result in project

delays exceeding 3 months or may adversely affect project deliverables and do not meet the criteria specified in Classes A and B above.

- Class D
    - Any desktop, laptop, server, or standard operating components necessary for general day-to-day activities, including data storage and backup, whose failure to operate may result in project delays exceeding 3 months or may adversely affect project deliverables.
- Class E
    - Computer hardware necessary to produce project deliverables that do not meet the above classification criteria.

GROUP C: ASSESSMENT AND OVERSIGHT

20. ASSESSMENTS & RESPONSE ACTIONS

Monthly assessments will be performed as part of the project to ensure that the sampling and analysis activities were in accordance to the approved QAPP. The QA officer requires all staff to immediately report data anomalies and to discontinue all work until resolutions have been determined. Stop work orders will remain in effect until data meet DQOs as specified by this document. These will be conducted as soon as the QAPP is approved and until all collected samples have been analyzed and processed. Short written reports will be provided to the QA Officer by those conducting assessments, unless the assessment is conducted directly by the QA Officer. Assessments will include:

Surveillance of Sample Collection Activities. The Quality Assurance Manager will be responsible for oversight of sampling activities and will review field logs to verify that the samples were collected in accordance with QAPP requirements. The Quality Assurance Manager will also participate to field sampling event to monitor activities. If the Quality Assurance Manager finds any of the field activities to be in violation of QAPP requirements, locations will be re-sampled.

Data Quality Assessment. The laboratory technician will be responsible for providing a summary of QA/QC data to the Quality Assurance Manager to verify that the performance criteria of the QAPP were met. If it is determined that the precision and accuracy objectives were not met, all samples will be re-analyzed and the Quality Assurance Manager will review laboratory techniques to minimize errors.

Assessment of Data Entry. Once the performance criteria are met, data analysis can be conducted. The Quality Assurance Manager will review data files to ensure that errors are detected and corrected. Data entry will be reviewed by creating plots to identify outliers, and sorting the data to identify missing values.

Documentation of corrective actions. All corrective actions required over the reporting period will be summarized in logging sheets, calibration forms and maintenance records. All logging sheets, calibration forms, and maintenance records will be inspected to as part of this process and will be explicitly cited in the assessment document.

21. REPORTS TO MANAGEMENT

The Field and Laboratory Personnel will be responsible for reporting any QA/QC deficiencies to the Quality Assurance Manager. The Quality Assurance Manager will present quarterly reports to the CVRWQC Contract Manager for review and submittal to the RWQCB, including a discussion on QAPP compliance and any corrective actions undertaken during the sampling, analysis, and data entry activities. The QA managers will also submit a draft report and final report on the findings of the study to the Contract manager for submittal to the RWQCB.

Table 21-1. QA management reports.

TASK	PRODUCT	DATE
1	Project Administration	
	1.1 Program Coordination	Ongoing
	1.2 Draft Final Report	January 31, 2012
	1.2 Final Report	March 30, 2012
	1.2 Monthly Reports	July 29, 2011, and monthly thereafter (during bloom season: June – October 2011)
2	Quality Assurance Project Plan	
	2.1 Draft QAPP	March 1, 2011
	2.2 Final QAPP	March 31, 2011
	2.3 Review and Revise	Ongoing
3	Monitoring Plan	
	3.1 Draft Monitoring Plan	March 1, 2011
	3.2 Final Monitoring Plan	March 31, 2011
	3.3 Review and Revise	Ongoing
4	Sample Collection	June 2011 – October 2011
	4.1 SWAMP Field Sheets	October 31, 2011
5	Sample Analysis	Completed by November 21, 2011
	5.5 Analytical Results	December 16, 2011

GROUP D: DATA VALIDATION AND USABILITY

22. DATA REVIEW, VERIFICATION, AND VALIDATION REQUIREMENTS

This section of the QAPP addresses the quality assurance activities that occur following completion of sampling activities, including data review, verification and validation. Data generated by project activities will be reviewed against the data quality objectives cited in Element 7 and the quality assurance/quality control practices cited in Elements 14, 15, 16, and 17. Data will be separated into three categories: 1) data meeting all data quality objectives as specified in this document and those specified by SWAMP, 2) data failing precision or recovery criteria, and 3) data failing to meet accuracy criteria. Data meeting all data quality objectives, but with failures of quality assurance/quality control practices will be set aside until the impact of the failure on data quality is determined. Once determined, the data will be moved into either the first category or the last category. These data meet the criteria as specified in this document and where appropriate all SWAMP criteria.

As stated in previous sections, the Field and Lab Personnel as well as the Quality Assurance Manager will be responsible for verifying that the sample collection, handling, and analysis procedures were in accordance with the approved QAPP. In addition, the Quality Assurance Manager will be primarily responsible for reviewing the data. Data falling in the first category is considered usable by the project. Data falling in the last category is considered not usable. Data falling in the second category will have all aspects assessed. If sufficient evidence is found supporting data quality for use in this project, the data will be moved to the first category, but will be flagged with a "J" as per EPA specifications. Any data resulting from procedures in conflict with QAPP requirements will be rejected.

23. VERIFICATION AND VALIDATION METHODS

The Standard Operation Procedure for Verification and Validation is as follows:

Data verification is the process of evaluating the completeness, correctness, and conformance of the dataset against the method, procedural, or contractual requirements. The goal of data validation is to evaluate whether the data quality goals established during the planning phase have been achieved (USEPA 2002). Data quality indicators will be continuously monitored by the analyst producing the data (i.e., field and lab personnel), as well as the QA officer throughout the project to ensure that corrective actions are taken in a timely manner. Calculations determine data quality for verification and validation have been thoroughly discussed in Sections 13 and 14 and form the basis of determining data quality in the analysis phase. Following these sections and the required calculations we have developed data quality control charts using a computing environment R and the code can be provided upon request. As noted above, individual samples not meeting data quality objectives will be re-run or re-collected, unless evidence suggests that DQOs were not met due to natural variation outside the control of field or laboratory personnel (USEPA 2002). Instances where DQOs are not met will be documented, along with corrective actions taken (Appendices C and

F). At the completion of data collection, the Project Manager will gather the data, DQI records, and other appropriate records, to evaluate whether criteria described in the QAPP were met, overall. If any of the criteria are not met, whenever possible, corrective actions will be taken by the Project Manager after consultation with the RWQCB QA Program Manager. Software for data verification will not be required. The final outputs of the data verification process will be verified data and data verification records that narrate any non-compliance issues or shortcomings of the data produced in field and laboratory activities and a certification statement that the data have been verified by the Program Manager. This certification should be made available to data users to inform them that the data have been verified.

Data validation is an analyte- and sample-specific process that extends verification to determine the analytical quality of the dataset (USEPA 2002). Laboratory and field personnel responsible for conducting QA analysis will be responsible for documenting when data do not meet measurement quality objectives as determined by data quality indicators. Records in the database for which data do not meet data quality objectives will be flagged with a coded or narrative note. These flags will be carried through to the final data to ensure that data users are aware of any validation issues. Following data verification at the completion of data collection, data will also be validated (USEPA 2002) by the Program Manager. Field activities will be validated by 1) evaluating the field records for consistency, 2) reviewing QC information, 3) summarizing deviations and determining the impact on data quality, 4) summarizing the samples collected, and 5) preparing a field data validation report. Laboratory data will be validated by 1) assembling planning documents and data, 2) reviewing verified, reported sample results collectively for the dataset as a whole, including laboratory qualifiers, 3) summarizing data and QC deficiencies and evaluating the impact on overall data quality, 4) assigning data validation qualifiers as necessary, and 5) preparing an analytical data validation report (USEPA 2002).

Results of data verification and data validation will also be summarized in the final report to notify data users of potential deficiencies in the data. The laboratory officer will be responsible for resolving these issues.

Data verification and validation for sample collection and handling activities will consist of the following tasks:

- Verification that the sampling activities, sample locations, number of samples collected, and type of analysis performed is in accordance with QAPP requirements;
- Documentation of any field changes or discrepancies;
- Verification that the field activities (including sample location, sample type, sample date and time, name of field personnel. etc) were properly documented.
- Verification of proper completion of sample labels and secure storage of samples.
- Verification that all samples recorded in the field log were received by the laboratory.

Data verification and validation for the sample analysis activities will consist of the following tasks:

- Appropriate methodology has been followed;
- Instrument calibrations are correct;
- Nutrient concentration peaks are integrated correctly;
- QC samples meet performance criteria;

- Analytical results are complete and correct;
- Documentation is complete.

Verification and validation of data entry includes:

- Creating histograms of data by date and by site to identify outliers;
- Sorting data to identify missing or mistyped (too large or too small) values;
- Double-checking all typed values.

24. RECONCILIATION WITH USER REQUIREMENTS

The data quality will be evaluated according to this document, with respect to sampling design, sampling method, field and laboratory analysis, quality control, and maintenance. Data will be evaluated using Sections 7-23. By properly following the guidelines in each of these sections, the data quality will be validated—if samples fail to meet these guidelines, the data quality will be questioned and flagged. Flagged data will be carefully scrutinized to determine if the data can be included in the final analysis.

The data quality objectives for this Study were created with the requirements of the data users in mind. The protocols and methods used here are standard and the objectives set for precision and accuracy are similar to those used by other research groups. Data that do not meet these data quality objectives will be discarded from analysis. Data that is not rejected outright but is qualified in some way will be documented in final reports. For example, if data for a stream does not meet the completeness objective because the stream dried up, the data may still be used, but the duration of its flow will be noted.

Data limitations will be reported to data users through a combination of flags using codes or narratives, and metadata files that accompany the data files. Flag formatting will follow, to the degree reasonably possible, SWAMP database protocols (see SWAMP QAPP Appendix J). For example, following SWAMP recommendations, data that lie between the method detection limit and the target reporting limit will be reported as the actual measured value (not negative), with the flag "DNQ," indicating that the analyte was detected, but not quantifiable. Metadata files will include information such as the methods used, method detection limits, scope of the project, etc. Additionally, fields will be included in the data that indicate information pertinent to data users, such as the method used, method detection limits, units, etc.

Adequate and accurate monitoring and assessment are the cornerstones to preserving, enhancing, and restoring water quality. The information gathered in this project is critical to protect the beneficial uses of water, to develop water quality objectives, conduct federal Clean Water Act assessments, and to determine the effects of pollution and of pollution protection programs. These data will help ensure that water quality is comprehensively measured

- to protect beneficial uses, and to evaluate protection and restoration efforts;
- to develop and implement a set of monitoring indicators (and assessment thresholds), which can be used to track the status and trends of water quality and

to evaluate the effectiveness of management actions to improve water quality in California;
- to develop and implement a progressive quality assurance program using a systems-based approach to the generation and storage of application-appropriate data/metadata;
- to make credible ambient monitoring data available to all stakeholders in a timely manner;
- to provide a consistent science-based framework for the evaluation of monitoring data relative to state and regional standards and the protection of beneficial uses and for tracking the effectiveness of management actions;
- to report all collected data as information and in a timely and publicly accessible manner;
- to provide the support needed to implement a coordinated and comprehensive monitoring and assessment program.

Overall, these data will provide a dataset for SWAMP that can be used to evaluate the regional Basin Plan, how waters meet the Beneficial Uses, and with follow up studies might be used as a baseline study of algae community composition in central coast streams.

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APPENDIX A- TRAINING LOG SHEET

UCSC Water Quality Laboratory and Field Training Documentation

Name:_____ Position:_____

Date of Hire:_____

Procedure	SOP read (trainee initials)	SOP satisfactorily performed (trainer initials)
Acid Dishwashing		
Acid Bath Maintenance		
Using Balances, Pipettes, and Glassware		
Handling Hazardous Materials		
Benchtop pH Calibration and Use		
Sampling—Lab Preparation		
Sampling—Lab Processing		
Printing Labels		
Preparing and Using Matrix Spike Solution		
Operating the spectrophotometer for NO3, NH4, Ortho-P		
Sampling—Field Procedures		
Data logs		

APPENDIX B- NUTRIENT ANALYSIS DATA SHEET

Nutrient Analysis Data Coversheet				
Date:				Sample Description:
Analyzer:				Lachat File(s):
Reprocessor:				Excel/Text File(s):
QA/QC Summary:				
Accuracy		Ortho-P (%)	Ammonium (%)	Nitrate (%)
beginning	QC1			
	QC2			
	QC3			
end	QC1			
	QC2			
	QC3			
Drift		Ortho-P (ppm)	Ammonium (ppm)	Nitrate (ppm)
	blank			
	QC1			
	QC2			
	QC3			
Percent Recovery		Ortho-P (PR)	Ammonium (PR)	Nitrate (PR)
Matrix Spike:				
Precision		Ortho-P (RSD)	Ammonium (RSD)	Nitrate (RSD)
Analytical Duplicate:				
Representativeness		Ortho-P (RSD)	Ammonium (RSD)	Nitrate (RSD)
Field Duplicate	Location:			
Contamination		Ortho-P (ppm)	Ammonium (ppm)	Nitrate (ppm)
Travel Blank	Location:			
beginning	Carrier			
end	Carrier			

*Nutrient analysis data sheet for QA/QC checks.

APPENDIX C- FIELD SAMPLING METHODS

*Modified from Appendix D of the SWAMP QAMP (SWAMP 2002) and "SWAMP Bioassessment Procedures - Standard Operating Procedures for collecting stream algae samples and associated physical habitat and chemical data for Ambient Bioassessments in California (2009)

Water Sample Collection – Water chemistry and bacteriological samples, as requested, are collected at the same location. *Water samples are best collected before any other work is done at the site*. If other work (i.e., sediment sample collection, flow measurement or biological/habitat sample collection or assessment) is done prior to the collection of water samples, it might be difficult to collect representative samples for water chemistry and bacteriology from the disturbed stream. Care must be taken, though, to not disturb sediment collection sites when taking water samples.

The following general information applies to all types of water samples, unless noted otherwise:

Sample Collection Depth

Sub-Surface Grab Sample: Samples are collected at 0.1m below the water surface. Containers should be opened and re-capped under water in most cases.

Depth-integrated Sample: If a depth-integrated sample is taken, the sample is taken from discrete intervals within the entire water column.

Surface Grab Sample: Samples are collected at the surface when water depth is <0.1m.

Where to Collect Samples

Water samples are collected from a location in the stream where the stream visually appears to be completely mixed. Ideally this would be at the centroid of the flow (*Centroid* is defined as the midpoint of that portion of the stream width, which contains 50% of the total flow), but depth and flow etc. do not always allow centroid collection. For stream samples, the sampling spot must be accessible for sampling physicochemical parameters, either by bridge, boat or wading. Sampling from the shoreline of any water body (meaning standing on shore and sampling from there) is the least acceptable method, but in some cases is necessary.

In reservoirs, lakes, rivers, and coastal bays, samples are collected from boats at designated locations provided by RWQCB's.

Sampling Order if Multiple Media are Requested to be Collected

The order of events at every site has to be carefully planned. For example, if sediment is to be taken, the substrate can not be disturbed by stepping over or on it; water samples can not be taken where disturbed sediment would lead to a higher content of suspended matter in the sample. *For the most part, water samples are best collected before any other work is done at the site*. This information pertains to walk-in sampling.

APPENDIX D- DATA REVIEW CHECKLIST

DATA REVIEW CHECKLIST

Sample Description: _____ Lachat/ Excel File Names: _____
Parameter: _____ Date of Review: _____

* If error rates exceed 5%, the proportion of entries for which the electronic data are reviewed will be increased until the error rates are less than 5%.

Analysis Overview		
Data Entry		
	Review date	**Comments/ Corrective actions taken**
Raw Data Complete		
Results Entered into database correctly (*requires that 20% of entries are checked*)*		
Check below:		
Site/ Date/ Time (field logs c.f. lab results)		
Measurement ranges for each parameter per sampling event		
Field QC entered		
Quality Control		
Holding Times not exceeded		
Preservation Checked		
Digestion Verified		
Initial Instrument Performance Checks Verified		
Calibration Verification Checked		
Sample-Specific QC (Internal Standards or Analytical Spikes) verified		
Final Check		
Technical Review Done		
Narrative Complete		

Analyst: _____ Peer Reviewer: _____
Date: _____ Date: _____
Comments Attached? (Y/N): _____

APPENDIX E- HARDWARE AND SOFTWARE EVALUATION FORMS

Hardware/Software Evaluation Form

Evaluator:

Evaluation (circle one): Pass / Fail / Requires Independent Testing*

Name/Description of Product:

Existing or Acquired? If acquired, include acquisition date and supplier.

Is it likely that failure of this hardware/software to operate properly could result in project delays exceeding 3 months or adversely affect project deliverables? Explain.

Class (circle one): A B C D E

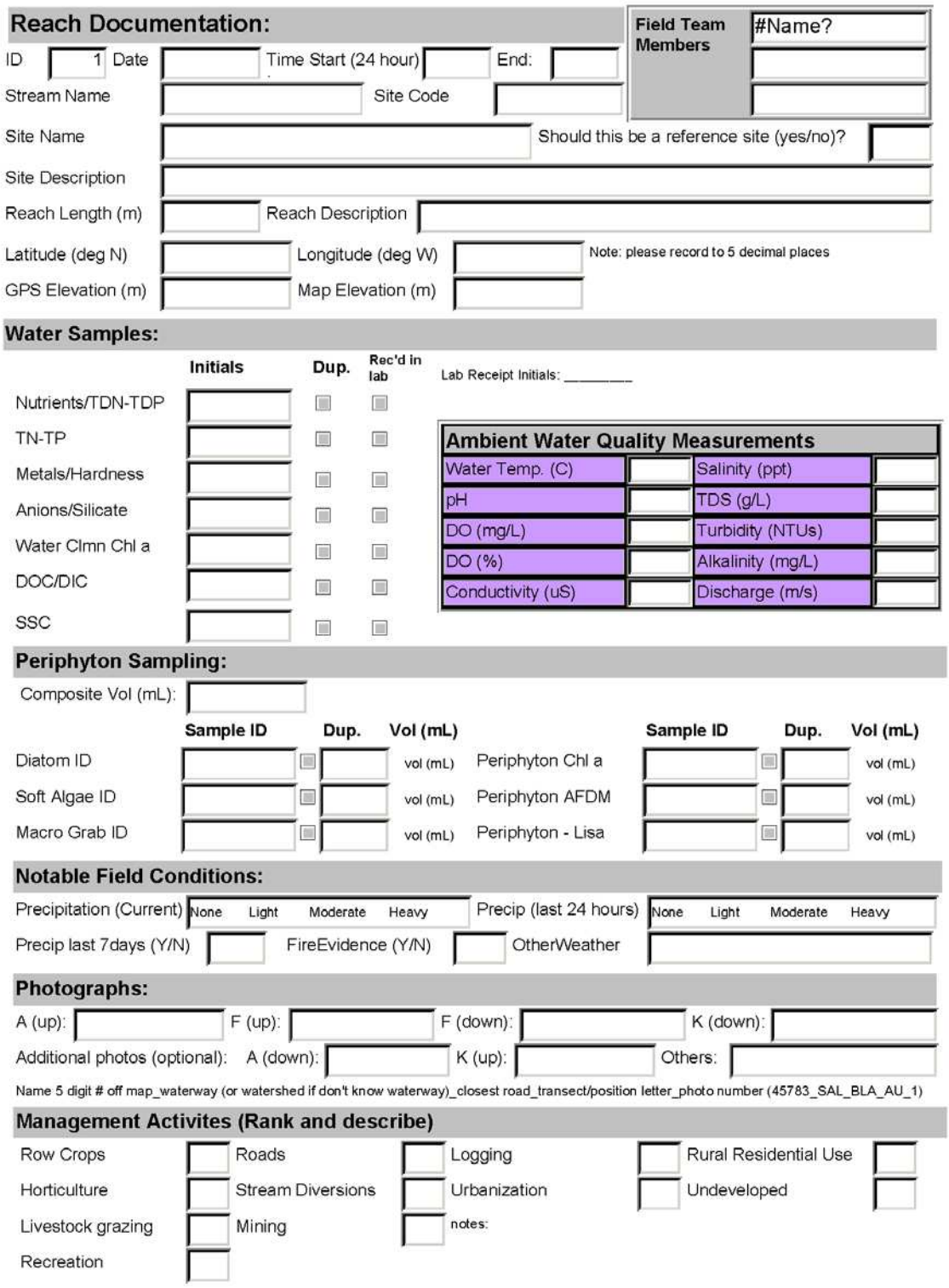
Description of Intended Use:

System Requirements:

Describe situations where intended use may not be accommodated by the hardware/software:

***Attach testing report if independent testing was required.**

APPENDIX F –FIELD DATA COLLECTION SHEET



Reach Documentation:

ID 1 Date Time Start (24 hour) End:

Stream Name Site Code

Site Name Should this be a reference site (yes/no)?

Site Description

Reach Length (m) Reach Description

Latitude (deg N) Longitude (deg W) Note: please record to 5 decimal places

GPS Elevation (m) Map Elevation (m)

Field Team Members #Name?

Water Samples:

	Initials	Dup.	Rec'd in lab
Nutrients/TDN-TDP			
TN-TP			
Metals/Hardness			
Anions/Silicate			
Water Clmn Chl a			
DOC/DIC			
SSC			

Lab Receipt Initials: _____

Ambient Water Quality Measurements

Water Temp. (C)		Salinity (ppt)	
pH		TDS (g/L)	
DO (mg/L)		Turbidity (NTUs)	
DO (%)		Alkalinity (mg/L)	
Conductivity (uS)		Discharge (m/s)	

Periphyton Sampling:

Composite Vol (mL):

	Sample ID	Dup.	Vol (mL)		Sample ID	Dup.	Vol (mL)
Diatom ID			vol (mL)	Periphyton Chl a			vol (mL)
Soft Algae ID			vol (mL)	Periphyton AFDM			vol (mL)
Macro Grab ID			vol (mL)	Periphyton - Lisa			vol (mL)

Notable Field Conditions:

Precipitation (Current) None Light Moderate Heavy Precip (last 24 hours) None Light Moderate Heavy

Precip last 7days (Y/N) FireEvidence (Y/N) OtherWeather

Photographs:

A (up): F (up): F (down): K (down):

Additional photos (optional): A (down): K (up): Others:

Name 5 digit # off map_waterway (or watershed if don't know waterway)_closest road_transect/position letter_photo number (45783_SAL_BLA_AU_1)

Management Activites (Rank and describe)

Row Crops		Roads		Logging		Rural Residential Use	
Horticulture		Stream Diversions		Urbanization		Undeveloped	
Livestock grazing		Mining		notes:			
Recreation							

Site Evaluation: Stream Geomorphology and Riparian Integrity

Riparian Cover		4 = > 95% 3 = 85 - 94% 2 = 75 - 84% 1 = < 75%
Livestock Herbivory		4 = 0 - 5% 3 = 5 - 25% 2 = 25 - 50% 1 = > 50%
% Bank Binding Root Mass		4 = > 85% 3 = 65 - 85% 2 = 35 - 64% 1 = < 35%
Erosional Deposition		4 = None 3 = Some in specific, limited locales 2 = Obvious signs 1 = Mass wasting
Stream Incisement		4 = No incisement 3 = Old incisement 2 = Deep incisement;new floodplain development 1 = Deep incisement; active downcutting
% bank Lateral Incisement		4 = < 5% 3 = 5 - 15% 2 = 15 - 35% 1 = > 35%
Stream Slope (%)		if <2% must use scope to measure
Water Color		Black Grey Brown Clear Dark Green Light Green Yellow
Water Clarity		4 = Clear 3 = Slightly turbid 2 = Turbid 1 = Very turbid

Rapid Assessment: Estimated % Substrate Size

(insert best guess in % for each category, without discussing. 1st person fill out, then ask other person for their numbers)

Technician Name	Bedrock/Concrete (>car)	Boulder (0.25-4 m)	Cobble (64-250 mm)	Coarse gravel (16-64 mm)	Fine gravel (2-16 mm)	Sand (0.06-2 mm)	Fines (<0.06 mm)	% macro algal cover

Discharge Measurement

first measurement = left bank when looking downstream, depth = 0, velocity = n/

Stage: ft m Flow Tracker File name:

Velocity Area Method (preferred) Transect Width (m):

	Dist (m)	Depth (m)	Velocity (m/s)		Dist (m)	Depth (m)	Velocity (m/s)	Notes
1				11				
2				12				
3				13				
4				14				
5				15				
6				16				
7				17				
8				18				
9				19				
10				20				

APPENDIX G – CHAIN OF CUSTODY FORM: CYANO SAMPLES

CHAIN OF CUSTODY FORM: MOLECULAR CYANOBACTERIA SAMPLES (for UCD)

Sampling Agency: UCSC	*Project Name:* Clear Lake
Address/Phone of Algae Sampling Lab:	*Crew Members (Sign and Date):*

Sample # Sample ID Site Name Volume Date Collected

Relinquished By (Sign and Date): Received By (Sign and Date): Sample Location:

APPENDIX H - SOP LIST AND SOPS

SOP Document Control Number	SOP Description
UCSCCM-001	CLEANING/DECONTAMINATION PROCEDURES
UCSCCM-002	PROCEDURE FOR COLLECTION OF CYANOBACTERIA & CYANOTOXINS SAMPLES
UCSCCM-003	PROCEDURE FOR TAXONOMY ANALYSIS OF CYANOBACTERIA BY ECOANALYSTS, INC.
UCSCLM-002	DETERMINATION OF CHLOROPHYLL-a IN FRESHWATER PHYTOPLANKTON BY FLUORESCENCE
UCSCLM-011	DETERMINATION OF TOC WITH SHIMDZU ANALYZER

CLEANING/DECONTAMINATION PROCEDURES

STANDARD OPERATING PROCEDURE FOR CLEANING/DECONTAMINATION

By Cécile Mioni, Institute of Marine Sciences, UCSC.

1.0 PERFORMANCE CRITERIA

1.1 The cleaning/decontamination procedures must ensure that all equipment that contacts a sample during sample collection is free from the analytes of interest and constituents that would interfere with the analytes of interest.

1.2 The detergents and other cleaning supplies cannot contribute analytes of interest or interfering constituents unless these are effectively removed during a subsequent step in the cleaning procedure.

1.3 The effectiveness of any cleaning procedure (including all cleaning reagents) must be supported by equipment blanks with reported non-detected values.

The cleaning procedures outlined in this SOP are designed to meet the above-mentioned performance criteria. Alternative cleaning reagents or procedures may be used. However, the organization must be prepared to demonstrate through documentation (i.e., company-written protocols and analytical records) and historical data (i.e., absence of analytes of interest in equipment blanks) that it consistently meets these performance criteria. Field quality control measures must support the use of alternative reagents or procedures.

2.0 CLEANING REAGENTS

2.1 Types and grades of reagent

Recommendations for the types and grades of various cleaning supplies are outlined below. The recommended reagent types or grades must be selected to ensure that the cleaned equipment is free from any detectable contamination.

2.1.1. DETERGENTS:

Use Luminox (or a non-phosphate solvent based equivalent), Liqui-Nox (or a non-phosphate equivalent) or Alconox (or equivalent). EPA recommends Luminox (or equivalent) since solvent rinses can be eliminated from the cleaning process. Liquinox (or equivalent) may be substituted (solvent rinses, when applicable, must be performed), and Alconox (or equivalent) may be substituted if the sampling equipment will not be used to collect phosphorus or phosphorus-containing compounds.

2.1.2. SOLVENTS

Note: If the detergent Luminox (or equivalent) is used, solvent rinses are not required.

2.1.2.1. Use pesticide grade isopropanol as the rinse solvent in routine equipment cleaning procedures. This grade of alcohol must be purchased from a laboratory supply vendor.

2.1.2.2. Other solvents, such as acetone or methanol, may be used as the final rinse solvent. However, methanol is more toxic to the environment and acetone may be an analyte of interest for volatile organics. **Do not use** acetone if volatile organics are of interest.

2.1.2.3. Properly dispose of all wastes according to applicable regulations. Containerize all solvents (including rinsates) for on-site remediation or off-site disposal, as required.

2.1.2.4. Pre-clean equipment that is heavily contaminated with organic analytes with reagent grade acetone and hexane or other suitable solvents.

2.1.2.5. Store all solvents away from potential sources of contamination (gas, copier supplies,...).

2.1.3. ANALYTE-FREE WATER SOURCES

2.1.3.1 Analyte-free water is water in which all analytes of interest and all interferences are below method detection limits.

2.1.3.2 Maintain documentation (such as results from equipment blanks) to demonstrate the reliability and purity of analyte-free water source(s).

2.1.3.3 The source of the water must meet the requirements of the analytical method and must be free from the analytes of interest. In general, the following water types are associated with specific analyte groups:

- Milli-Q (or equivalent polished water): suitable for all analyses.
- Organic-free: suitable for volatile and extractable organics.
- Deionized water: not suitable for volatile and extractable organics if the analytes of interest are present in concentrations that affect the result.
- Distilled water: not suitable for volatile and extractable organics, metals or ultra-trace metals.

2.1.3.4 Use analyte-free water (Milli-Q) for blank preparation and the final decontamination water rinse.

2.1.3.5 In order to minimize long-term storage and potential leaching problems, obtain or purchase analyte-free water just prior to the sampling event. If obtained from a source (such as a laboratory), fill the transport containers and use the contents for a single sampling event. Empty the transport container(s) at the end of the sampling event.

2.1.3.6 Discard any analyte-free water that is transferred to a dispensing container (such as a wash bottle) at the end of each sampling day.

2.1.4. ACIDS

2.1.4.1. Reagent Grade Nitric Acid: 10 - 15% (one volume concentrated nitric acid and five volumes Milli-Q).

Use for the acid rinse unless nitrogen components (e.g., nitrate, nitrite, etc.) are to be sampled.

2.1.4.2. Reagent Grade Hydrochloric Acid: 10% hydrochloric acid (one volume concentrated hydrochloric and three volumes Milli-Q).

Use when nitrogen components are to be sampled (e.g. nutrient containers).

2.1.4.3. If samples for the nitrogen-containing components are collected with the equipment, use the hydrochloric acid rinse.

2.1.4.4. If sampling for ultra trace levels of metals, use an ultra-pure grade acid.

2.1.4.5. Freshly prepared acid solutions may be recycled during the cleaning process. Dispose appropriately at the end of the cleaning process or if acid is discolored or appears otherwise contaminated (e.g., floating particulates).

2.1.4.6. Dispose of any acid waste following UCSC EH&S protocols.

2.2 Storage of cleaning reagents

The contents of all containers must be clearly marked.

2.2.1. DETERGENTS: Store in the original container or in a high density polyethylene (HDPE) or polypropylene (PP) container.

2.2.2. SOLVENTS

Store solvents to be used for cleaning or decontamination in the original container until use. If transferred to another container for field use, the container must be either glass or Teflon.

Use dispensing containers constructed of glass or Teflon.

2.2.3. ANALYTE-FREE WATER:

2.2.3.1. Transport in containers appropriate to the type of water to be stored. If the water is commercially purchased, use the original containers when transporting the water to the field.

2.2.3.2. Containers made of glass, Teflon, polypropylene, or Polyethylene (PE) are acceptable.

2.2.3.3. Use glass, Teflon, polypropylene or PE to transport organic-free sources of water on-site.

2.2.3.4. Dispense water from containers made of glass, Teflon, PE or polypropylene.

2.2.3.5. Do not store water in transport containers for more than 3 days before beginning a sampling event.

2.2.3.6. Store and dispense acids using containers made of glass, Teflon, PE or polypropylene.

3.0 GENERAL CLEANING REQUIREMENTS

3.1. Before using any equipment, clean/decontaminate all sampling equipment (net, bucket, tubing, lanyards, etc.) that are exposed to the sample.

3.2. Clean all equipment in a designated area having a controlled environment (lab, or base of field operations) and transport to the field precleaned and ready to use, unless otherwise justified.

3.3. Rinse all equipment with water after use, even if it is to be field-cleaned for other sites. Rinse equipment used at contaminated sites immediately with water.

3.4. Whenever possible, transport sufficient clean equipment to the field so that an entire sampling event can be conducted without the need for cleaning equipment in the field.

3.5. Segregate equipment that is only used once from clean equipment and return to the in-house cleaning facility to be cleaned in a controlled environment.

3.6. Protect decontaminated field equipment from environmental contamination by securely wrapping and sealing with one of the following:

3.6.1. Aluminum foil (commercial grade is acceptable);

3.6.2. Clean, untreated, disposable plastic bags (e.g. Ziploc bag). Plastic bags may be used:

- For all analyte groups except volatile and extractable organics;
- For volatile and extractable organics, if the equipment is first wrapped in foil or if the equipment is completely dry.

3.7. Containerize all solvent rinsing wastes, detergent wastes and other chemical wastes requiring off-site or regulated disposal. Dispose of all wastes in conformance with applicable regulations.

4.0 CLEANING SAMPLE COLLECTION EQUIPMENT

4.1 On-site/In-Field Cleaning

4.1.1. Cleaning equipment on-site is not recommended because:

- Environmental conditions cannot be controlled.
- Wastes (solvents and acids) must be containerized for proper disposal.

4.1.2. If performed, follow the appropriate cleaning procedure.

Note: Properly dispose of all solvents and acids.

4.1.3. Rinse all equipment with water after use, even if it is to be field-cleaned for other sites.

4.1.4. Rinse equipment used at contaminated sites immediately with water.

4.2. Heavily contaminated equipment

In order to avoid contaminating other samples, isolate heavily contaminated equipment from other equipment and thoroughly decontaminate the equipment before further use. Equipment is considered heavily contaminated if it has been used to collect samples from a source known to contain significantly higher levels than background.

Note: Cleaning heavily contaminated equipment in the field is not recommended.

4.2.1. ON-SITE PROCEDURES

Protect all other equipment, personnel and samples from exposure by isolating the equipment immediately after use.

4.2.1.1. At a minimum, place the equipment in a sealed untreated plastic bag (e.g. trash bag).

4.2.1.2. Do not store or ship the contaminated equipment next to clean, decontaminated equipment, unused sample containers, or filled sample containers.

4.2.1.3. Transport the equipment back to the base of operations for thorough decontamination.

4.2.1.4. If cleaning must occur in the field, and in order to document the effectiveness of the procedure, collect and analyze blanks on the cleaned equipment.

4.2.3. CLEANING PROCEDURES

4.2.3.1. If organic contamination cannot be readily removed with scrubbing and a detergent solution, prerinse equipment by thoroughly rinsing or soaking the equipment in acetone.

- Do not use solvent soaks or rinses if the material is clear acrylic.
- Use hexane only if preceded and followed by acetone.

4.2.3.2. After the solvent rinses, use the appropriate cleaning procedure.

4.2.3.3. Scrub, rather than soak all equipment with sudsy water.

4.2.3.4. If the field equipment cannot be cleaned utilizing these procedures, discard unless further cleaning with stronger solvents and/or oxidizing solutions is effective as evidenced by visual observation and blanks.

4.2.3.5. Clearly mark or disable all discarded equipment to discourage use.

4.3 General Cleaning

Follow these procedures when cleaning equipment under controlled conditions. Check manufacturer's instructions for cleaning restrictions and/or recommendations.

4.3.1. Procedure for Teflon and Glass Sampling Equipment

This procedure must be used when sampling for **ALL** analyte groups or if a single decontamination protocol is desired to clean all Teflon and glass equipment.

1. Rinse equipment with hot tap water.
2. Soak equipment in a hot, sudsy water solution (Liqui-Nox or equivalent).
3. If necessary, use a brush to remove particulate matter or surface film.
4. Rinse thoroughly with hot tap water.
5. If samples for volatile or extractable organics will be collected, rinse with appropriate solvent. Wet equipment surfaces thoroughly with free-flowing solvent.
6. If samples for DOC, soak overnight in an 10% HCl bath.
7. Rinse thoroughly with analyte-free water (Milli-Q).
8. Allow to air dry.
9. If samples for DOC, bake the clean glass vials in an oven at 450°C for at least 4 hours.
10. Wrap and seal clean sampling equipment according to section 3.6 as soon as the equipment is air-dried.

4.3.2. General Cleaning Procedure for Plastic Sampling Equipment

1. Rinse equipment with hot tap water.
2. Soak equipment in a hot, sudsy water solution (Liqui-Nox or equivalent).
3. If necessary, use a brush to remove particulate matter or surface film.
4. Rinse thoroughly with hot tap water.
5. Thoroughly rinse (wet all surfaces) with the appropriate acid solution (10% HCl). Check manufacturer's instructions for cleaning restrictions and/or recommendations.
6. Rinse thoroughly with Milli-Q water. Use enough water to ensure that all equipment surfaces are thoroughly flushed with water.
7. Allow to air dry as long as possible.
8. Wrap clean sampling equipment according to the procedure described in section 3.6.

4.3.3. Cleaning Procedure by Analyte Group

4.3.3.1. REUSABLE GLASS COMPOSITE SAMPLE CONTAINERS

1. Wash containers following the procedure outlined in section 4.3.1. End with a final solvent rinse if organics are to be sampled.
2. Invert containers to drain and air dry for at least 24 hours.
3. Cap with aluminum foil.

4. After use, rinse with water in the field, seal with aluminum foil to keep the interior of the container wet, and return to the lab or base of operations.
5. Do not reuse the vials used to collect cyanotoxin compounds or if a discoloration (or film) is observed after cleaning procedure. Such containers must be properly disposed of at the conclusion of the sampling and processing activities.

4.3.3.2. REUSABLE PLASTIC COMPOSITE SAMPLE CONTAINERS

1. Wash containers following the procedure outlined in section 4.3.2.
2 . Inspect the containers to determine if they can be reuse by the criteria in section 4.3.3.1.5 above.

4.3.3.3. FILTRATION UNITS

1. Wash Filtration units following the procedure outlined in section 4.3.
2. Drive rinsing solution (Milli-Q water/acid) through the porous filter holder between samples.
3. Seal the filtration unit in a Ziploc bag for storage to prevent contamination if the unit will not be used to sample organics.

4.3.3.4. SYRINGES

1. Wash syringes following the procedure outlined in section 4.3.2.
2. Use a different clean syringe at every site to prevent contamination. Separate the used syringes from the clean equipment by placing them in a plastic bag after use.
3. Seal the syringes in a Ziploc bag for storage to prevent contamination.

4.3.3.5. ANALYTE-FREE WATER CONTAINERS

1. Wash new containers following the procedure outlined in section 4.3.2.
2. For reused container. Rinse the exterior and interior with Milli-Q water, soak in a 10% HCl bath overnight, Rinse thoroughly with Milli-Q water. Invert and allow to air-dry.

4.3.3.6. ICE CHESTS AND SHIPPING CONTAINERS

1. Wash the exterior and interior of all ice chests with laboratory detergent after each use.
2. Rinse with tap water and air dry before storing.

4.3.3.7. FIELD INSTRUMENTS (E.G. YSI, PLANKTON NET)

Follow manufacturer's recommendations for cleaning instruments. At a minimum:
1. Rinse and wipe down equipment body, probes, and cables with Milli-Q water.
2. Store equipment according to the manufacturer's recommendation or seal in a untreated plastic bag to eliminate environmental contamination.

5.0 PRECLEANED SAMPLE CONTAINERS

5.1 Obtaining clean sample containers

Obtain clean sample containers in one of two ways:

5.1.1. From commercial vendors as precleaned containers. The cleaning grades must meet EPA analyte specific requirements. Keep all records for these containers (lot numbers, certification statements, date of receipt, etc.) and document the container's intended uses;

Organizations that order precleaned containers must retain the packing slips, and lot numbers of each shipment, any certification statements provided by the vendor and the vendor cleaning procedures.

5.1.2. From internal groups within the organization that are responsible for cleaning and maintaining containers according to the procedures outlined in this SOP.

The contractor must verify that the laboratory follows the container cleaning procedures outlined in this SOP.

If the laboratory cleaning procedures are different, the contractor must require that the laboratory use the following cleaning procedures or provide documentation and historical records (e.g. equipment blank) to show that their in-house procedure produces containers that are free from the analytes of interest.

CYANOBACTERIA & CYANOTOXINS

STANDARD OPERATING PROCEDURE FOR COLLECTION OF CYANOBACTERIA & CYANOTOXINS SAMPLES

By Cécile Mioni, Institute of Marine Sciences, UCSC.

1 SCOPE AND APPLICATION

1.1 This method provides a procedure for the collection of aqueous or cyanobacteria samples for toxin analysis, algal enumeration and identification, or biomass estimates. This SOP does not address sampling design issues which can be site or project specific. Care should be taken to ensure the sampling design will support the types of management decisions that the resulting data are intended to support.

1.2 Use the following SOPs & documentation procedures in conjunction with this SOP:

UCSCCM-001	CLEANING/DECONTAMINATION PROCEDURES
UCSCCM-003	PROCEDURE FOR TAXONOMY ANALYSIS OF CYANOBACTERIA BY ECOANALYSTS, INC.
APPENDIX C	FIELD SAMPLING METHODS
APPENDIX F	FIELD DATA AND COLLECTION SHEET
APPENDIX G	CHAIN OF CUSTODY FORM: CYANO SAMPLES

2 SUMMARY OF METHOD

2.1 This method is modified from the sampling and processing protocols outlined in "SWAMP Bioassessment procedure 2009 - Standard Operating Procedures for Collecting Stream Algae Samples and Associated Physical Habitat and Chemical Data for Ambient Bioassessments in California" (June 2009) as well as the manual "Microscopic and molecular methods for quantitative phytoplankton analysis" (the Intergovernmental Oceanographic Commission of ©UNESCO 2010). Our procedure was adapted to cyanobacteria assemblages.

2.2 During all phases of algae sampling and processing, in order to preserve specimen integrity, every attempt should be made to keep the sample material out of the sun to protect the algae from heat and desiccation as much as possible. In addition, before sampling at any given site, the sampling device (bucket, graduated cylinders, syringe, etc.) that will collect the sampling material must be scrubbed with a stiff-bristled brush or scouring pad and thoroughly rinsed with water so that no algal materials is carried over from the previous site contaminate the current sample.

2.3 The identification/enumeration samples are each aliquoted into sterile 50-mL centrifuge tubes (e.g. falcon tubes) and chemically fixed immediately following collection. Samples are stored in the dark on wet ice during transport and stored in the dark in a cold room until analysis.

2.4 Samples for molecular analysis 1–50 mL of the water sample are filtered onto a sterile membrane filter (e.g. Supor Pall Gelman), placed in a sterile centrifuge tube (e.g. eppendorf), and flash frozen in liquid nitrogen and stored frozen until analysis.

1.1 Water samples for toxin analysis are transferred to glass jar (e.g. glass jars certified clean by Environmental Sampling Supply, Inc.) to minimize loss of MC due to

adherence to the container wall (Utkilen et al., 1999). Samples are stored in the dark on dry ice during transport and kept frozen in the dark until analysis.

2.0 DEFINITIONS

2.1 Field duplicates- Two separate samples collected at the same time and place under identical circumstances and treated exactly the same throughout field and laboratory procedures. Provide measure of the precision associated with sample collection, preservation and storage, as well as with laboratory procedures.

2.2 Quality Control Sample (QCs) - It is a solution of known concentration obtained from a source external to the laboratory to check laboratory performance.

3.0 INTERFERENCES

3.1 All photosynthetic pigments and some toxins are light and temperature sensitive. Work must be performed in subdued light and all standards, QC materials and filter samples must be stored in the dark at -20^{o}C to prevent degradation.

3.2 Some fixatives can give false positive results for toxin analysis. Precautions should be taken to avoid contamination of toxin samples with the fixatives used for cyanobacteria identification/enumeration.

4.0 HEALTH AND SAFETY

4.1 Cyanobacteria bloom sampling requires specialized training, safety protocols and personal protective equipment (PPE). The degree and type of safety measures and PPE required depends on the unique characteristics of the bloom to be sampled. Typical short-term acute risks include, but are not limited to, contact dermatitis and upper respiratory irritation. Long-term risks are unknown; however certain cyanobacteria are known to produce toxins that are tumor promoters, even at very low doses. Samplers should use appropriate PPE to reduce occupational exposure to these toxins.

4.1.1. MINIMUM SAFETY PLANNING AND PROCEDURES: It is beyond the scope of this field sampling SOP to describe safety protocols for every contingency. Follow the listed minimum safety procedures below, where applicable:

4.1.1.1. Produce a written sampling plan and review with all personnel.

4.1.1.2. Ensure that all personnel are appropriately trained.

4.1.1.3. Ensure that sampling personnel meet employer medical requirements.

4.1.1.4. Conduct site reconnaissance and identify hazards.

4.1.1.5. Provide appropriate PPE and sampling equipment to all samplers.

4.1.1.6. Establish site control (exclusion zones, access corridors, etc.).

4.1.1.7. Establish decontamination protocols for samplers.

4.1.1.8. Prepare for emergencies (backup personnel, spill containment, fire equipment, first aid, evacuations, etc.)

4.2 Lab safety- Safety glasses are required for all laboratory analysis. Use gloves to avoid skin irritation from contact with toxins or reagents (e.g. fixatives); work under the fume hood when possible. Please refer to the Material Data Safety Sheets (MSDS) file for any other information about personnel protective equipment and other safety considerations.

4.3 Chemical hygiene- Please refer to the MSDS file for any questions concerning a chemical's toxicity and the necessary safety precautions.
4.4 Waste Disposal- Dispose of waste in the appropriate collection bottle. This is disposed of by UCSC Environmental Health and Safety (EHS) officers on request.

5.0 PERSONNEL/ TRAINING/ RESPONSIBILITIES

5.1 General Responsibilities- This method is restricted to use by or under the supervision of the analyst experienced in the method. Each analyst must be trained and able to read and understand the SOP.
5.2 Laboratory analysts: it is the responsibility of analysts/technicians to;
- 5.2.1 Read and understand the SOP and follow it as written.
- 5.2.2 Produce quality data the meets all of the laboratory requirements.
- 5.2.3 Complete the required demonstration of proficiency before performing this procedure without supervision
- 5.2.4 Repeat the required initial demonstration of proficiency each time a modification is made to the method.

5.3 Laboratory managers: it is the responsibility of the laboratory manager to:
- 5.3.1 Ensure that all analysts have the technical ability and have the adequate training required to perform this procedure.
- 5.3.2 Ensure that all analysts have completed the required demonstration of proficiency before performing this procedure without supervision.
- 5.3.3 Produce quality data that meets all laboratory requirements.

6.0 EQUIPMENT AND SUPPLIES- All reusable lab ware that comes in contact with sample should be clean and acid free. Dishwashing should include soaking in laboratory grade detergent and water, rinsing with tap water then rinsing with milli-Q water.

6.1 Collect samples for algal toxin analysis in clean wide mouth glass jar with caps having a Teflon® liner.
6.2 Collect samples for algal enumeration and identification in sterile plastic 50-mL centrifuge tubes (e.g. falcon tubes).
6.3 Visually inspect containers and caps for defects. Do not use if defects are present or containers do not appear clean.

7.0 REAGENTS AND STANDARDS

7.1 Glutaraldehyde
7.2 Cyanotoxins standards. May be obtained from a commercial supplier (e.g. provided with ELISA kits).
7.3 Milli-Q water

8.0 SAMPLE COLLECTION. PRESERVATION AND STORAGE

8.1 Grab samples

In order to provide consistency between sampling agencies and programs, it is recommended that all samples be collected as grab samples (Appendix C). Collect separate, discrete samples of the water column and of the scum layer. These samples should be analyzed and interpreted separately.

8.1.1. Clearly mark the sample container (sterile 50-mL centrifuge tube) so that each sample is uniquely identified. Information, such as the water body name, station location, date and time collected, sampler's name, is important to help identify the individual sample.

8.1.2. Collect individual grab samples a clean bucket at the specified location.

8.1.2.1 Slowly submerge the bucket, opening first, into the water. If an algal scum layer is present, attempt to avoid submerging the sampling device through the algal scum layer as this may introduce algal scum into the sample and may not be representative of the water column.

8.1.2.2. Invert the sampler at the desired sampling depth so the opening is upright and pointing towards the direction of flow (if applicable). Allow water to enter the container until it is almost full.

8.1.2.3. Return the filled sampler (bucket) quickly to the surface.

8.1.2.4. Collect sample directly into the appropriate container (sterile 50-mL centrifuge tube). Pour out a small volume of sample, if needed, and add the necessary amount of glutaraldehyde (final: 2.5% v/v).

8.1.3 Quickly cap the container.

8.1.4 Store the sample in cooler filled with wet ice.

8.1.4 Decontaminate the sampler (bucket) by scrubbing with a brush.

8.1.5 Equipment blanks will need to be collected to document that the decontamination process was successful and subsequent samples were not contaminated by the equipment. Equipment blanks should be made at locations where the cyanobacteria cell density is very high (e.g. scum).

8.2 Water column sample

8.2.1 *For informative purpose only:* This sample is not quantitative and therefore cannot be used for enumeration but it will be helpful for taxonomy identification of the cyanobacteria assemblage, especially if the cyanobacteria density is low.

8.2.2 Collect subsurface sample within the top 0.5 m of the water column using a clean 20-µm mesh vertical net to concentrate the scum forming cyanobacteria. If the sample is to be collected from another depth, this should be noted on the sample container and sample submittal form. *Do not collect the sample by skimming the surface.*

8.2.3 Slowly submerge the net to the desired depth. Allow water to enter the container until it is full.

8.2.5 Return the net quickly to the surface.

8.2.6 Collect sample directly into the appropriate container (sterile 50-mL centrifuge tube). Pour out a small volume of sample, if needed, and add the necessary amount of glutaraldehyde (final: 2.5% v/v).

8.2.7 Quickly cap the container and tighten securely.

8.2.8 Store the sample in wet ice cooler.

8.2.9 Decontaminate the net by holding the net upside down while rinsing the OUTSIDE of the net. *Do not scrub the net as it might damage the mesh*. *Do not*

rinse the interior of the plankton net with site water as it will contaminate the mesh. Once in the lab, soak the net in milli-Q water overnight and rinse the outside of the net three times with milli-Q water while holding the net upside down. Do not use acid as it would dissolved the mesh…

8.2.10 Equipment blanks will need to be collected to document that the decontamination process was successful and subsequent samples were not contaminated by the equipment.

8.3 Scum Sample Core

8.3.1 If conditions permit, a scum sample core can be taken using a wide mouth polycarbonate jar having a 3/8" diameter hole in the bottom and a line marking a known volume (see diagram below).

8.3.2. The jar is inverted and gently forced through the scum layer until the surface of the scum reaches the line on the jar. The hole in the bottom of the jar allows air to escape while sampling.

8.3.3. With the jar still in place, the lid is screwed onto the jar securely before removing the jar from the water.

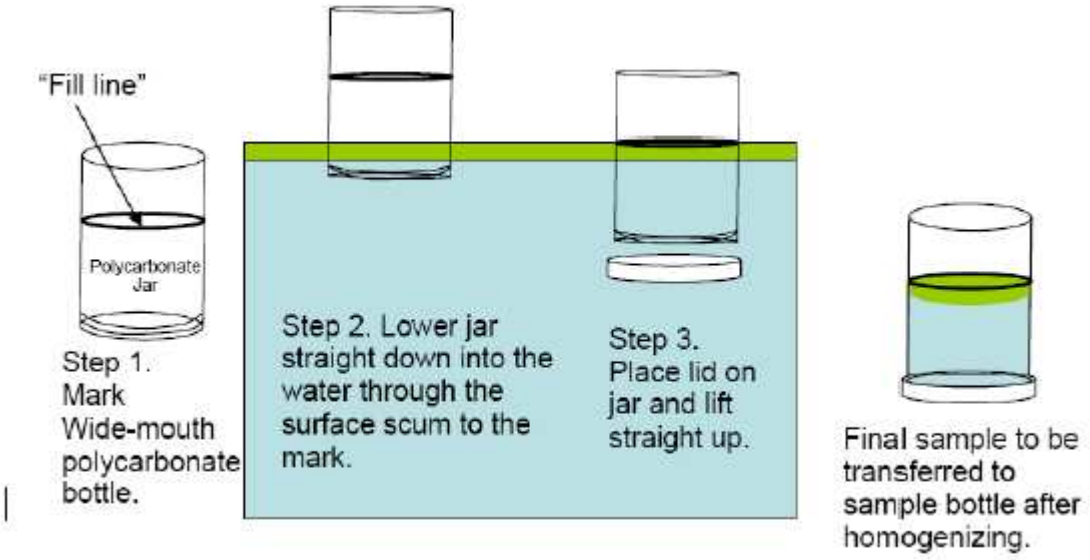
8.3.4. Once the jar is free of the water, a finger is placed over the hole in the bottom and the jar is mixed by gently inverting for 15-20 seconds in order to homogenize the scum layer with the underlying water.

8.3.5. The homogenized sample is then quickly transferred to a clean wide mouth amber bottle for transport.

8.3.6. Label the sample container.

8.3.7. A clean coring device must be used for each sample or the device must be decontaminated after each use.

8.3.8. If decontamination is to be performed in the field, equipment blanks will need to be collected to document that the decontamination process was successful and subsequent samples were not contaminated by the equipment.



9.0 TRANSPORT AND HANDLING OF SAMPLES

9.1 After the samples have been collected and containerized, clean the outside of the containers with water, paper towels or other absorbent materials to remove any spilled sample from the exterior of the container.
9.2 Protect glass containers from breakage ("bubble wrap" is recommended).
9.3 Samples need to be shipped to the laboratory in time to meet the prescribed holding time for the analyses being performed. Samples for cyanobacteria identification/enumeration will be stored for 6 months, except for the samples processed by EcoAnalysts, Inc., these samples are stored for 90 days. Since there is limited data available to establish appropriate holding times for cyanobacteria toxin analyses, initial holding times may be quite conservative until additional studies are completed. For this study, we will store the cyanobacteria toxin samples for 6 months.

10.0 PRESERVATION

10.1 Samples for cyanobacteria toxin analysis should immediately be placed in a cooler on wet ice.
10.2 Samples for algal enumeration and identification should be either immediately placed on wet ice and then preserved with a sufficient volume of Glutaraldehyde immediately in the field or within 4 days of collection time. Samples preserved with glutaraldehyde in the field should be kept cool (wet ice). The exact volume of preservative needed will be dependent upon the density of the algae and the size of the sampling container.
10.3 For long-term storage
10.3.1. Sample containers for enumeration/taxonomy should be stored in the dark. Add gluteraldehyde to achieve a minimum of 2.5% final concentration.
10.3.2 Samples for cyanobacteria toxin analysis should immediately be placed in a cooler on dry ice.
10.3.3 Samples for cyanobacteria molecular analysis should be immediately flash frozen in liquid nitrogen. Samples will be processed using preexisting methods (Baxa et al. 2010).

11.0 DOCUMENTATION

11.1 Label each bottle with an appropriate field ID number.
11.2 Complete field records (Appendix F).
11.3 Make notes on the transmittal form and in field records about any relevant observations or problems (Appendix F & G).

12.0 PROCEDURE

12.1 Taxonomy/Enumeration using Uthermohl method

Refer to SOP "UCSCCM-003 PROCEDURE FOR TAXONOMY ANALYSIS OF CYANOBACTERIA BY ECOANALYSTS, INC."

12.2 Enumeration

Samples dominated by buoyant HC (e.g. *Microcystis*) will be enumerated using epifluorescent microscopy at UCSC. For samples dominated with colonial *Microcystis*, samples will first be broken up using the colony disaggregation method (Bernard et al. 2004), filtered, mounted on slides and examined via epifluorescent microscope employing the natural unit method or "clump count" method where one organism is defined as any unicellular organism or a natural colony. These generated data can be compared with data from other sampling sites in the SWAMP program, the IEP program and throughout the United States.

Surface water samples (50-mL each) for cyanobacteria enumeration will be analyzed using a modified version of the filtering - quantitative phytoplankton analysis method for epifluorescence microscopy (UNESCO 2010, Rinta-Kanto et al. 2005). Enumeration of cyanobacteria using epifluorescence microscopy do not require calcofluor staining. We will be using epifluorescence microscopy to visualize the cyanobacteria cells based on their pigment properties (presence of autofluorescing phycoerythrin in cyanobacteria only).

12.2.1 Grab samples will be fixed with 2.5% (v/v) glutaraldehyde.

12.2.2 If the sample is dominated by colonial cyanobacteria-strain, such as colonial *Microcystis*, samples will first be broken up using the colony disaggregation method (Bernard et al. 2004)

12.2.3 A 10-50 mL of fixed sample will be filtered under gentle vacuum through 1–2-mm pore size, 25-mm diameter, black polycarbonate membranes (GE Osmonics). This polycarbonate membranes are specifically designed for epifluorescence microscopy and present the advantage to have zero background autofluorescence which permits cyanobacteria to be more visible. The polycarbonate membrane is mounted carefully (filter side up) on a drop of drop ofimmersion oil (Type FF) (R. G. Chargille Laboratories, Inc.,Cedar Grove, NJ) on a properly labeled microscope slide, another drop of paraffin oil is added on top of the filter before to put on the cover slip (24 x 24mm).

12.2.4 The abundance of autofluorescing phycoerythrin containing cells (cyanobacteria) will be determined on a Zeiss Axioplan epifluorescence microscope equipped with an ocular grid using green excitation (Zeiss filter set 20, excitation 546-nm bandpass, and emission 575–640-nm bandpass filters) and a 40x objective.

12.2.5 For samples dominated with colonial Microcystis, samples will be examined via epifluorescent microscope employing the natural unit method or "clump count" method where one organism is defined as any unicellular organism or a natural colony. An algal "unit" is defined as a natural counting unit such as a colony or filament. An algal cell is defined as the individual cells within a colony or filament. Unicellular algal specimens, such as diatoms or flagellates, are considered as one cell and one unit.
Both unit and cell counts are recorded for enumeration samples (qualitative and quantitative). The decision on which counting method to use depends on the project and its particular objectives.

- *For the natural unit count*, all units that extend into the grid a distance of at least 1/4 of the grid width (or length) should be counted. Of the algae which extend into the grid a distance of less than 1/4 of the grid width, those crossing the top or left border are counted as 'in.' Those crossing the bottom and right boundaries of the field are not counted. Use the laboratory counter to keep a running total of the number of fields observed.

- *For the cell count*, everything within the grid should be counted. Cells of blue-green filaments are defined as 10µm increments of the filament. Only blue-green algal filaments are counted as 10 µm representing one cell. Other filaments, such as green algal filaments, lend themselves to counting actual cells because of the structure of the filament. Individual cells of all colonies should be counted. If a cell lies only partially in the field, those crossing the top or left border are counted as 'in'. Those crossing the bottom and right boundaries of the field are not counted.
- For BLOOM-net and scum core samples, there is no count performed. Simply scan the sample to determine the dominant taxon (or taxa). Taxa are recorded on the benchsheet as being dominant, co-dominant, or present. It is also noted if the sample is not an algae.

12.2.6 Slides will be stored at -20°C.

12.2.7 Calculation of cell concentrations (cell mL-1):

The conversion factor (*CF*):

$$CF = \frac{B_a}{B_c}$$

Where *Ba* = Area of the filter (mm^2).
Bc = Area of the part of the filter counted (mm^2).

The concentration of the species *C* (cells mL-1) is then:

$$C = N * \frac{CF}{V}$$

Where *V* = Volume of sample concentrated on the filter (mL).
N = Number of cells counted for the species of interest.

1.1.1 *Quality assurance*

To ensure high quality results all steps of the method must be validated. Steps in the method to validate are

• homogenisation of sample
• distribution on filter (should be homogeny)
• repeatability and reproducibility

Ultimately the quality of the result from this method is dependent on the skill of the analyst. The variation of parallel samples counted by the same analyst and the variation in parallel samples counted by different analysts are two of the most important considerations in quality assurance (Willén 1976). For this reason, only one analyst will perform the cell enumeration and the results will be compared to EcoAnalysts, Inc. Please note that due to the natural buoyancy of some cyanobacteria species, the filter method will give more accurate quantitative results than the Uthermöhl (sedimentation) method. However, the two methods are complementary for this study as the filtration method may result in cell distortion and therefore limit a precise taxonomic identification. Uthermöhl will therefore provide more detailed qualitative information (taxonomy).

1.2 Toxin Analysis

12.3.1 ELISA kits.

Toxin levels including microcystins, cylindrospermopsin and saxitoxins will be determined using tested commercially available enzyme linked immunosorbent assay (ELISA) kits. These kits provide quantitative analyses even at low concentrations and are highly sensitive to a given molecule.

12.3.1.1. To detect microcystins, commercially available MCs-ELISA test kits (Envirologix) will be used. The detection limit for this assay based on MC-LR is 0.15 ppb (µg/L). The assay range is between 0.16 and 2.5 ppb. Coefficients of variation (CVs) for standards are <10%. The assay exhibits very good cross-reactivity with all cyanobacterial cyclic peptide toxin congeners tested to date. The evaluation of the ELISA can be performed using commercial ELISA evaluation programs: 4-parameters (preferred) or alternatively Logit/Log or point to point. For more details see http://www.rapidmethods.com/pdf/ep022spec.pdf

12.3.1.2. Cylindrospermopsin ELISA test kit is available commercially from Abraxis and will be used for quantitative cylindrospermopsin determination. The detection limit for this assay is 0.04 ppb (µg/L). The assay range is between 0.05 and 2 ppb. Coefficients of variation (CVs) for standards are <10% and for samples <15%. The assay exhibits good cross-reactivity with cylindrospermopsin and not with other non-related algal toxins tested to date. The kit, a 96-well microtiter plate format with ready to use, color coded reagents, enables simultaneous measurement of multiple samples.The kit accuracy and reliability were tested against HPLC and results validated. QC and QA standards are available and are ran with the samples. For more information see:
http://www.abraxiskits.com/moreinfo/PN522011USER.pdf

12.3.1.3. Saxitoxin (paralytic shellfish poisons, PSP) ELISA test kit is available commercially from Abraxis and will be used for quantitative saxitoxin determination. The ELISA kit detects saxitoxins in water samples at the parts per trillion (ppt) levels. The antibody binds Saxitoxin and other related PSP toxins with varying degrees and does not cross-react with other non-related toxins or compounds. The assay range is between 0.02 ppb and 0.4 ppb in water. Coefficients of variation (CVs) for standards are <10% and for samples <15%. The kit, a 96-well microtiter plate format with ready to use, color coded reagents, enables simultaneous measurement of multiple samples. As for the ASP kit accuracy and reliability were tested, results validated and QC and QA standards are available and are analyzed with samples. For more information see: http://www.abraxiskits.com/uploads/products/docfiles/58_PN52255BUSER.pdf

12.3.2 LC/MS analysis

Discrete samples will be analyzed to measure the concentration of targeted (cyanotoxins microcystins, lyngbyatoxin-a, anatoxin-a, nodularins) and to identify the presence of isomers and congeners. Samples for toxins will be analyzed using an Agilent 1200 liquid chromatograph (LC) connected to a 6130 single quad MS, using Selected Ion Monitoring (SIM) following a modification of the protocol reported by Mekebri et al. (2009). The following microcystin ions (m/z) will be monitored: 519.8 RR; 105.6 YR, 995.7 LR, 981.7 demethyl-LR, 910.6 LA, are monitored using $[M+H]^+$ in SIM mode. Full scan will also be collected over the

range 100-1100 Da. The SIM windows were established for microcystins using the daughter ions, which are the Adda fragments of m/z 135.2 and m/z 213 produced by the transition of the protonated parent ions. We have previously run laboratory intercalibrations with an LC/MS/MS system operated by the California Water Pollution Control Lab, and our (LC/MS) results are comparable (with LC/MS/MS). The estimated method detection limits and reporting limits are 0.02 µg/L for MC. Samples will be prepared by sonication with 10% methanol of filtered biomass, and direct injection of filtered whole water, thereby providing particulate, dissolved, and total MC, as necessary, we will use standard SPE cleanup if direct injection proves difficult to interpret. For MC analyses, data are reported relative to calibration standards. For toxins for which no standards are commercially available, we will follow published protocols for LC/MS analysis of these compounds and will report values based on identification of compounds based on mass and soft-ion fractionation of daughter ions.

We will use exhisting SPATT methods (Miller et al. 2010) to include microcystins and other cyanotoxins. Samples will be analyzed using ELISA kits and/or LC/MS as described above, based on protocols established through State of California Water Resources Control Board contract 07-120-250.

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ECOANALYSTS, INC. TAXONOMY

PROCEDURE FOR TAXONOMY ANALYSIS OF CYANOBACTERIA BY ECOANALYSTS, INC.

By EcoAnalysts, Inc (http://www.ecoanalysts.com/).

1. EcoAnalysts, Inc. receives samples preserved with 2% Glutaraldehyde's solution in sterile 50-mL bottles.
2. Samples are processed using the Utermöhl microscope method (Utermöhl 1958). Phytoplankton are identified to the lowest taxonomic level practical (genus or species preferred).
3. Subsampling: count at least 400 total algal units and 100 units of the dominant taxon or taxa (genus or species level). The count of the major taxon will count towards the total units, which must be at least 400 total.
4. Assign each counted algal unit a group code (i=individual cell, f=filament, c=colony, n/p=unknown).
5. Identify all taxa regardless of size. Make a note on the data sheet for each organism that cannot be identified, and why each organism cannot be identified.
6. Provide a photographic documentation of taxon (species or genus) identification when they are first encountered. For each organism that cannot be identified, provide photographic documentation in case the organism(s) are identified at a later date. For each photograph, note the type of microscope and camera, and at what magnification the photograph was taken.
7. Store all samples for 90 days, and then dump.

Data and will be delivered within 90 days of receipt of samples. Digital reference collection will be returned after all samples for the year have been processed.

Reference

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CHLOROPHYLL

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STANDARD OPERATING PROCEDURE FOR IN VITRO DETERMINATION OF CHLOROPHYLL-a IN FRESHWATER PHYTOPLANKTON BY FLUORESCENCE

By Kristy Morris, Shennan Lab, Environmental Studies Department, UCSC.

1.0 SCOPE AND APPLICATION

1.1 This method provides a procedure for the fluorometric determination of chlorophyll *a* and its magnesium-free derivative, pheophytin *a* in freshwater phytoplankton.

1.2 This method is modified from the US EPA Method 445.0 and the APHA Standard Methods for the Examination of Water and Wastewater, 20th Edition, Method 10200H "Chlorophyll". The major difference between the method described here and the US EPA Method 445.0 is that the filters are not macerated.

2.0 SUMMARY OF METHOD

2.1 Chlorophyll-containing phytoplankton in a measured volume of sample water are concentrated by filtering at low vacuum through a glass fiber filter. The pigments are extracted from the phytoplankton in 90% acetone and allowed to steep overnight, but not to exceed 24hrs, to ensure thorough extraction of chlorophyll a. The fluorescence of the sample is measured before and after acidification with 0.1M HCl

3.0 DEFINITIONS

3.1 Stock Standard Solution (SSS)- A solution prepared in the laboratory using reference materials purchased from a reputable commercial source.

3.2 Laboratory reagent blank (LRB)- An aliquot of reagent water (Milli-Q) or other blank matrices that are treated exactly the same as the sample including exposure to all glassware, equipment, solvents, reagents, internal standards and surrogates that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, reagents or apparatus.

3.3 Field duplicates- Two separate samples collected at the same time and place under identical circumstances and treated exactly the same throughout field and laboratory procedures. Provide measure of the precision associated with sample collection, preservation and storage, as well as with laboratory procedures.

3.4 Quality Control Sample (QCs) - It is a solution of known concentration obtained from a source external to the laboratory to check laboratory performance.

4.0 INTERFERENCES

4.1 Any substance extracted from the filter or acquired from laboratory contamination that fluoresces in the red region of the spectrum may interfere in the accurate measurement of both chlorophyll a and pheophytin a

4.2 Spectral interferences resulting from the fluorescence of the accessory pigment chlorophyll b, and the chlorophyll a degradation product pheophytin a, can result in

the overestimation of chlorophyll a concentrations. However, highly selective optical filters used in this method minimize these interferences.

4.3 Quenching effects are observed in highly concentrated solutions or in the presence of high concentrations of other chlorophylls or carotenoids. Samples should be diluted.

4.4 Fluorescence is temperature dependent with higher sensitivity occurring at lower temperatures. Samples, Standards, LRBs and QCs must be at the same temperature to prevent errors/ low precision. Analysis of samples at ambient temperatures is recommended in this method.

4.5 All photosynthetic pigments are light and temperature sensitive. Work must be performed in subdued light and all standards, QC materials and filter samples must be stored in the dark at -20^{o}C to prevent degradation.

5.0 HEALTH AND SAFETY

5.1 Lab safety- Safety glasses are required for all laboratory analysis. Use gloves to avoid skin irritation from contact with acetone; work under the fume hood when possible. Please refer to the Material Data Safety Sheets (MSDS) file for any other information about personnel protective equipment and other safety considerations

5.2 Chemical hygiene- Hazards of the chemicals used in this method were discussed in the previous section. Please refer to the MSDS file for any further questions concerning a chemical's toxicity and the necessary safety precautions.

5.3 Waste Disposal- Dispose of waste in the acetone collection bottle. This is disposed of by UCSC Environmental Health and Safety (EHS) officers on request.

6.0 PERSONNEL/ TRAINING/ RESPONSIBILITIES

6.1 General Responsibilities- This method is restricted to use by or under the supervision of the analyst experienced in the method. Each analyst must be trained and able to read and understand the SOP.

6.2 Laboratory analysts: it is the responsibility of analysts/technicians to;

- 6.2.1 Read and understand the SOP and follow it as written.
- 6.2.2 Produce quality data the meets all of the laboratory requirements.
- 6.2.3 Complete the required demonstration of proficiency before performing this procedure without supervision
- 6.2.4 Repeat the required initial demonstration of proficiency each time a modification is made to the method.

6.3 Laboratory managers: it is the responsibility of the laboratory manager to:

- 6.3.1 Ensure that all analysts have the technical ability and have the adequate training required to perform this procedure.
- 6.3.2 Ensure that all analysts have completed the required demonstration of proficiency before performing this procedure without supervision.
- 6.3.3 Produce quality data that meets all laboratory requirements.

7.0 RELATED DOCUMENTS

7.1 SOP for the handling of hazardous materials

7.2 SOP for using Balances, Pipettes and Glassware

7.3 SOP for preparing Standards and QCs

8.0 APPARATUS AND MATERIALS- All reusable lab ware that comes in contact with chlorophyll solutions should be clean and acid free. Dishwashing should include soaking in laboratory grade detergent and water, rinsing with tap water then rinsing with deionized water.

8.1 Turner Designs TD-700 Fluorometer (Analytical Marine Laboratory)
8.2 10ml Borosilicate glass tubes with caps
8.3 Whatmann glass micro fiber filters GF/F 0.7um retention 47mm
8.4 Millipore glass filtration unit with vacuum with 47-mm fritted glass disk base
8.5 Tweezers or flat tipped forceps
8.6 Assorted Class A calibrated pipettes
8.7 50ml, 100ml and 1-L class A volumetric flask

9.0 REAGENTS AND STANDARDS

9.1 Acetone, HPLC grade
9.2 Hydrochloric Acid
9.3 Chlorophyll a free of chlorophyll b. May be obtained from a commercial supplier.
9.4 Milli-Q water
9.5 0.1M HCl solution- Add 0.85 ml of concentrated HCl to approximately 50ml of water and diluted to 100ml.
9.6 Aqueous Acetone Solution- 90% acetone/ 10% Milli-Q water. Carefully measure 100ml of water into a volumetric flask and fill to the line. Transfer to a 1-L flask. Fill flask to the line with Acetone. Mix, Label and Store in Amber bottle in Flammables Cabinet.
9.7 Chlorophyll Standard Stock Solution (SSS)- Chlorophyll *a* from Sigma is shipped in an amber glass ampoule. This should be stored in the freezer until use. Tap the ampoule until all of the dried chlorophyll has settled on the bottom. Working in a darkened room, carefully break the tip off the ampoule and transfer contents into a 50 ml volumetric flask and dilute to volume with 90% acetone. Transfer to a darkened bottle or wrap the flask in foil to protect from the light. The concentration of the solution must be determined spectrophotometrically using a multiwavelength spectrophotometer. Label bottle including the chlorophyll lot number. When stored in an airtight container at room temperature, the SSS is stable for at least six months.
9.8 Chlorophyll a Primary Dilution Standard (PDS) - add 1 ml of the SSS (section 9.7) to a 100ml volumetric flask and dilute to volume with aqueous acetone solution (section 9.6). If exactly 1mg of pure chlorophyll was used to prepare the SSS, the concentration of the PDS is 200 ug/L. Prepare fresh prior to use and label flask and wrap in foil.
9.9 Quality Control Sample (QCs)- Since there are no commercially available QCs- QCs are prepared from the PDS at the following concentrations:

- QC1 = 5 ug/L
- QC2 = 20 ug/L
- QC3 = 50ug/L

10.0 SAMPLE COLLECTION. PRESERVATION AND STORAGE

10.1 Healthy phytoplankton are generally obtained from the photic zone (the depth at which the illumination level is 1% of the surface illumination). Obtain a 250ml grab sample in a darkened HDPE Azlan bottle. Store on ice until delivery to the laboratory. Unfiltered samples can be stored in the refrigerator at 4^{o}C for 24-48hr. **Samples must be filtered within 48 hours and filters may be frozen (-20^{o}C) for up to 28 days**.

11.0 PROCEDURE

11.1 Extraction

11.1.1 Thoroughly but gently agitate the 250ml sample bottle to suspend particles. Mixing the sample well improves the replication greatly.

11.1.2 Conduct filtration in an area with subdued light. Gently filter a predetermined quantity of sample- filter vacuum should not exceed 20kPa). If you don't know the volume of sample to extract, start by measuring 50 ml of sample into a graduated cylinder and poor into the vacuum tower. For sample volumes less than 50ml use a calibrated pipette to dispense the sample into the vacuum tower. Continue adding sample in increments until color is visible on the filter.

11.1.3 After filtration, fold each filter in half, using tweezers and a spatula and place inside a clean falcon tube. Repeat again to obtain 2-3 replicate samples from each sample and ensure that the sample bottle is agitated between every replicate. Perform a LRB (100mL Milli-Q) for every 10 samples.

11.1.4 Freeze samples for up to 28 days before extraction

11.1.5 To extract chlorophyll, add 25ml of aqueous acetone solution to the falcon tubes with filters in them. Cap the tubes and invert several times to mix, then sonicate gently as to not disturb the filter paper and steep in the refrigerator in the dark extract the chlorophyll for 2 hours.

11.2 Sample Analysis

11.2.1 Remove samples from the refrigerator and allow both the sample and QCs to come to room temperature.

11.2.2 Turn on the Fluorometer (back left side) and allow 10 minutes for warm-up countdown to complete. Use the 90% acetone solution to zero the instrument on the sensitivity setting that will be used for sample analysis.

11.2.2.1 Press "0" then press "1" to start blanking.

11.2.3 Read and record the values for the QCs prior to sample analysis

11.2.4 Remove the filters from the samples with clean tweezers and record the fluorescence of the sample. If the chlorophyll concentration of the sample is 90% of the upper limit of the LDR (LDR=150 ug/L. therefore 135ug/L) it will be necessary to perform a 1:100 dilution (Add 50uL of extract to 4.95 ml of aqueous acetone solution).

11.2.5 Remove the glass tube from the instrument and add 2 drops of 0.1M HCl. Thoroughly mix the sample and allow 90 seconds before measuring the fluorescence of the sample again.

12.0 DATA ANALYSIS AND CALCULATIONS

12.1 For 'corrected chlorophyll a', calculate the chlorophyll *a* in the water sample as follows:

$$\textbf{Chlorophyll } a \textbf{ (ug/L)} = F_s\,(r/(r-1))^*\,(R_b-R_a)^*(V_e/V_s)$$

Where:

F_s = the conversion factor of the sensitivity setting (=1 in our laboratory)

r/ r-1 = 2.096, or r = 1.9124 ($r = R_b/R_a$, as determined with pure chlorophyll *a* for the instrument).

R_b = reading before acidification

R_a = reading after acidification

V_e = volume of extract, and

V_s = volume of sample.

13.0 QC/ QA CRITERIA

13.1 An LRB is performed for every 10 samples. The LRB should be less than the calculated lower detection limit for the analysis

13.2 The QCs should be within ± 10% of the known value

13.3 Check the instrument calibration using the solid standard-it should read 132.7 ug/L.

13.4 The RSD of the replicate measurements should be within ± 15%

STANDARD OPERATING PROCEDURE FOR THE DETERMINATION OF TOTAL ORGANIC CARBON WITH SHIMADZU TOC ANALYZER

By Kristy Morris, Shennan Lab, Environmental Studies Department, UCSC.

1.0 SCOPE AND APPLICATION

1.1 This method provides a procedure for the determination of total organic carbon in surface waters, and domestic and industrial wastes. In this method the sample is filtered through a 0.45 um pore size filter.

1.2 The method is based on combustion of organic carbon to inorganic carbon dioxide.

This method is modified from the EPA approved SM 1998 5310.

1.2 SUMMARY OF METHOD

1.2.1 The TOC analyzer efficiently oxidizes low molecular weight organic compounds, hard-to-decompose insoluble, and macromolecular organic compounds. The 680°C combustion catalytic oxidation method can efficiently analyze all organic compounds. The technique has been recognized for its ability to analyze samples with particulates, refractory compounds, and high salt matrices in wastewater and other difficult matrices. The oxidized carbon dioxide is measured using an NDIR detector in the gas phase.

2.0 DEFINITIONS

2.1 Stock Standard Solution (SSS)- A solution prepared in the laboratory using reference materials purchased from a reputable commercial source.

2.2 Laboratory reagent blank (LRB)- An aliquot of reagent water (Milli-Q) or other blank matrices that are treated exactly the same as the sample including exposure to all glassware, equipment, solvents, reagents, internal standards and surrogates that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, reagents or apparatus.

2.3 Field duplicates- Two separate samples collected at the same time and place under identical circumstances and treated exactly the same throughout field and laboratory procedures. Provide measure of the precision associated with sample collection, preservation and storage, as well as with laboratory procedures.

2.4 Quality Control Sample (QCs) - It is a solution of known concentration obtained from a source external to the laboratory to check laboratory performance.

2.5 Matrix Spike samples- Matrix spike samples are samples to which known quantities of a solution with one or more well-established analyte concentrations have been added. These samples are analyzed to determine the extent of matrix interference or degradation on the analyte concentration during sample processing and analysis.

3.0 INTERFERENCES

3.1 There are no appreciable interference compounds

4.0 HEALTH AND SAFETY

4.1 Lab safety- Safety glasses are required for all laboratory analysis. Use gloves to avoid potential contamination of sample from personnel sources or when filling purge acid source. Please refer to the Material Data Safety Sheets (MSDS) file for any other information about personnel protective equipment and other safety considerations. In particular, the following chemicals have the potential to be highly toxic or hazardous, for detailed explanation consult the MSDS.

- 4.1.1 Sulfuric Acid
- 4.1.2 He Carrier Gas

4.2 Chemical hygiene- Hazards of the chemicals used in this method were discussed in the previous section. Please refer to the MSDS file for any further questions concerning a chemical's toxicity and the necessary safety precautions.

4.3 Waste Disposal-Excess reagents, samples and method process wastes should be characterized and disposed of in an acceptable manner and in accordance with the UCSC EHS hazardous waste identification checklist. Laboratory analysts should consult the Laboratory Manager before disposing of potentially hazardous wastes.

5.0 PERSONNEL/ TRAINING/ RESPONSIBILITIES

5.1 General Responsibilities- This method is restricted to use by or under the supervision of the analyst experienced in the method. Each analyst must be trained and able to read and understand the SOP.

5.2 Laboratory analysts: it is the responsibility of analysts/technicians to;

- 5.2.1 Read and understand the SOP and follow it as written.
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- 5.3.2 Ensure that all analysts have completed the required demonstration of proficiency before performing this procedure without supervision.
- 5.3.3 Produce quality data that meets all laboratory requirements.

6.0 RELATED DOCUMENTS

6.1 SOP for the handling of hazardous materials

6.2 SOP for preparing Standards and QCs

7.0 APPARATUS AND MATERIALS

7.1 Shimadzu TOC-VCSH Analyzer

- 7.1.1 Shimadzu ASI-V Autosampler

7.1.2 Instrument Controller
7.1.3 Data Collection Software
7.1.4 High Sensitivity Catalyst
7.1.5 CO_2 Absorber (soda lime)

7.2 "Zero Air" compressed air and regulator
7.3 40 mL borosilicate vials and septum caps
7.4 Laboratory glassware and pipettes
7.5 Balance

8.0 REAGENTS AND STANDARDS

8.1 Preparation of Standards
8.1.1 *Calibration Standards:*
Standards are prepared by of single element standards purchased from vendors that provide traceability to NIST standards. For this study KHP (Potassium hydrogen phthalate)nwill be used as the calibration standard. Working standards for calibration are prepared at concentrations stated below.

Standard #	mg C/L
1	0
2	5
3	50
4	100

9.0 SAMPLE COLLECTION. PRESERVATION AND STORAGE

9.1 TOC can react after collection. The samples should be stored on ice on return to the laboratory, filtered refrigerated and analyzed within 48 hours.

10.0 PROCEDURE

10.1 Shimadzu TOC Instrument Operating Parameters

Compressed Air Pressure:	
Supply pressure @ tank regulator	550 kPa
Carrier Gas Pressure	200 kPa
Carrier Gas Flow Rate	150 mL/min
Sample Volume	500 uL
Acid Addition	1.5 % v/v 2N HCl
Analysis	NPOC
Calibration Method	Linear Regression
Catalyst	High Sensitivity
Sparge Time	4 minutes
Washes	2
Combustion Temperature	680ºC

10.2 Procedure
10.2.1 Follow manufacturers instructions for calibration, analysis and data processing. Refer to instrument manual.

10.2.2 After use, sample vials and septum are rinsed well with DI water and soaked overnight in 0.5 N HCl, rinsed well again and soaked overnight in DI water and air dried. The vials are baked at 550ºC for at least one hour, cooled, and stored in a seal container until use.

10.3 System Notes

10.3.1 Yearly preventative maintenance as suggested by the manufacturer is required for optimum performance.

10.3.2 Catalytic tube must be maintained and replaced as needed.

11.0 DATA ANALYSIS AND CALCULATIONS

11.1 The instrument software prepares a standard curve by plotting standard response against known concentration. Sample response is compared to the standard curve to determine concentration.

11.2 Organic carbon in DI water must be corrected for in determination of sample concentration. The calibration curve is shifted to pass through zero and the sample concentration determined from this corrected curve. Instrumental noise, which also is a contributor to the background signal, has been determined to be negligible with this instrument for the method currently used.

11.3 Samples with concentration greater than the highest calibration standard are diluted and reanalyzed.

12.0 QC/ QA CRITERIA

12.1An LRB is performed at the beginning and end of each run or for 5% of samples, whichever is more frequent. The LRB should be less than the detection limit for this method (DL = 0.008 mg C/L)

12.2QCs are performed at the beginning and end of each run or for 5% of samples, whichever is more frequent. QC samples should be within ± 10% of the known value.

12.3The RSD of the replicate measurements should be within ± 15%.

12.4A set of duplicate samples are analyzed for each run and duplicate measurements should be within ± 15%.

12.5A set of field duplicate samples are analyzed for each run and duplicate measurements should be within ± 15%.

12.6A matrix spike solution is performed for every run and the percent recovery of the matrix spike should be 100± 15%.

SOP Training Documentation

Employee Name	**Employee** training has been received (initials/date)	**Trainer** employee has been trained and procedure performed successfully (initials/date)	**Comment** MT- More training is required EA- employee is authorized to perform the SOP

APPENDIX I - SIGNATURES

APPROVAL SIGNATURES

UNIVERSITY OF CALIFORNIA SANTA CRUZ:

Title:	Name:	Signature:	Date*:
Project Director and QA Officer	Cécile E. Mioni		
Project Director, co-PI and QA Officer	Raphael Kudela		
Project Manager	Kendra Hayashi		

UNIVERSITY OF CALIFORNIA DAVIS:

Subcontractor

Project Director, co-PI and QA Officer	Dolores Baxa		

CALIFORNIA STATE, DEPARTMENT OF WATER RESOURCES:

Collaborating agency (Environmental Monitoring Program)

Field Officer	Scott Waller		

LAKE COUNTY:

Collaborating agency

Field Officer	Tom Smythe		

STATE BOARD (SWRCB**):

Title:	Name:	Signature:	Date*:
Contract Manager	Meghan Sullivan		
SWAMP QA officer (SWRCB/SWAMP)	Beverly van Buuren		04/25/11
Regional Board QA officer (CVRWQCB)	Leticia Valadez		

* This is a contractual document. The signature dates indicate the earliest date when the project can start.

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Project Director and QA Officer	Cécile E. Mioni		
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Project Manager	Kendra Hayashi		

UNIVERSITY OF CALIFORNIA DAVIS:

Subcontractor

Project Director, co-PI and QA Officer	Dolores Baxa		April 25, 2011

CALIFORNIA STATE, DEPARTMENT OF WATER RESOURCES:

Collaborating agency (Environmental Monitoring Program)

Field Officer	Scott Waller		

LAKE COUNTY:

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CALIFORNIA STATE, DEPARTMENT OF WATER RESOURCES:

Collaborating agency (Environmental Monitoring Program)

Field Officer	Scott Waller		

LAKE COUNTY:

Collaborating agency

Field Officer	Tom Smythe	Thomas R Smythe	Apr 26, '11

STATE BOARD (SWRCB**):

Title:	Name:	Signature:	Date*:
Contract Manager	Meghan Sullivan		
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UNIVERSITY OF CALIFORNIA SANTA CRUZ:

Title:	Name:	Signature:	Date*:
Project Director and QA Officer	Cécile E. Mioni	On file	April 27, 2011
Project Director, co-PI and QA Officer	Raphael Kudela	On file	April 27, 2011
Project Manager	Kendra Hayashi	On file	April 27, 2011

UNIVERSITY OF CALIFORNIA DAVIS:

Subcontractor

Project Director, co-PI and QA Officer	Dolores Baxa	On file	April 25, 2011

CALIFORNIA STATE, DEPARTMENT OF WATER RESOURCES:

Collaborating agency (Environmental Monitoring Program)

Field Officer	Scott Waller	On file M. Scott Waller	April 27, 2011

LAKE COUNTY:

Collaborating agency

Field Officer	Tom Smythe	On file	April 26, 2011

STATE BOARD (SWRCB**):

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Project Director, co-PI and QA Officer	Raphael Kudela		27-Apr-1-2011
Project Manager	Kendra Hayashi		27 Apr 11

UNIVERSITY OF CALIFORNIA DAVIS:

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