The City of Watsonville

Pinto Lake Restoration Project

AGREEMENT NO. 14-424-253 with CCRWQCB

Quality Assurance Program Plan

Version 2.0

Originated by:
The City of Watsonville

(September 23, 2015)
Edited March 21, 2016

Group A: Project Management

Element A1: Title and Approval Sheet

Project Title	Pinto Lake Restoration
Lead Organization	City of Watsonville
	500 Clearwater Lane
	Watsonville, CA 95076
Primary Contact	Jackie McCloud, Project Manager
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	Email address: jackie.mccloud@cityofwatsonville.org
Effective Date	September 21, 2015

3/24/14

Jackie McCloud, Project Manager **Date**
City of Watsonville

23 March 2016

Shanta Keeling, Grant Manager **Date**
Central Coast Regional Water Quality Board Region 3

23 March 2016

Karen Worcester, Quality Assurance Officer **Date**
Central Coast Regional Water Quality Board Region 3

3/24/16

Barbara Pierson, Laboratory Manager **Date**
City of Watsonville

3/24/16

Emma Pickering, Quality Assurance Officer **Date**
City of Watsonville

Element A2: Table of Contents

Element A3: Distribution List

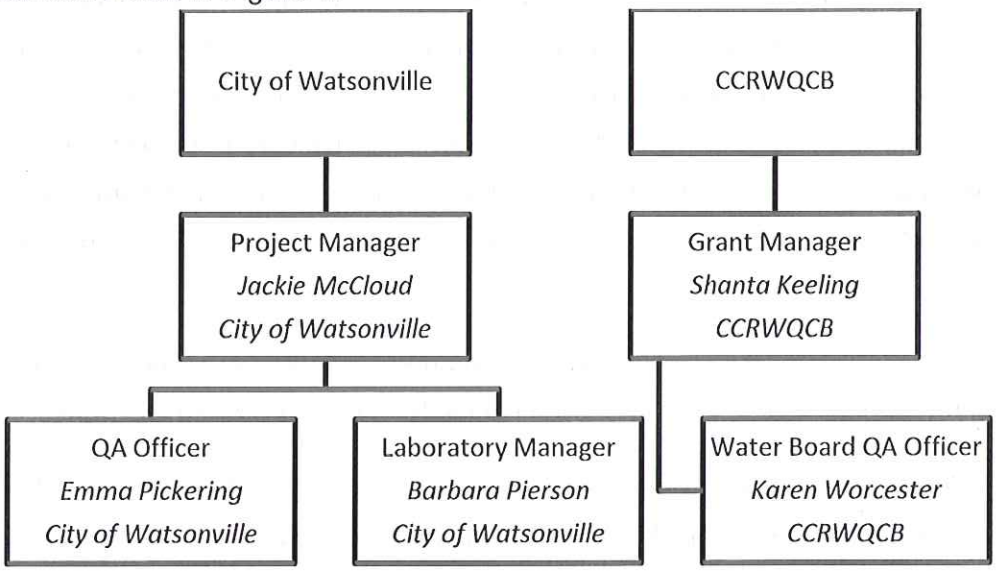
Each person listed in Table 1 will receive a copy of this QAPP and future revisions. Individuals taking part in the project may request additional copies of the QAPP from the Project Manager.

Table 1: Distribution List

Contact Information	Organization's Mailing Address
Jackie McCloud Project Manager 831-768-3172 jackie.mccloud@cityofwatsonville.org Barbara Pierson Laboratory Manager 831-768-3179 barbara.pierson@cityofwatsonville.org Emma Pickering Quality Assurance Officer 831-768-3170 emma.pickering@cityofwatsonville.org	City of Watsonville Water Resources Center 500 Clearwater Lane Watsonville, CA 95076
Shanta Keeling Grant Manager 805-549-3464 shanta.keeling@waterboards.ca.gov Karen Worcester Quality Assurance Officer 805-549-3333 karen.worcester@waterboards.ca.gov	Central Coast Regional Water Quality Board Region 3 895 Aerovista Place, Suite 101 San Luis Obispo, CA 93401

Element A4: Project Organization

Personnel involved in project implementation are listed in Table 1, and shown as an organization chart in Figure 1.



The City of Watsonville Project Manager will be responsible for the following activities:

- Develop and maintain approved QAPP
- Amend QAPP (when necessary)
- Data and sample collection efforts
- Compile and analyze data
- Issue quarterly reports to the Central Coast Regional Water Quality Control Board (CCRWQCB)

The City of Watsonville Quality Assurance (QA) Officer will be responsible for the following activities:

- Initial and ongoing review of the QAPP
- Review laboratory data for compliance with QA/Quality Control (QC) criteria
- Review all laboratory data for suitability in determining program objectives

The City of Watsonville Laboratory Manager will be responsible for the following activities:

- Sample receipt and analysis
- Ensure laboratory data compliance with QA/QC criteria

The City of Watsonville Project Reviewer will be responsible for the following activities:

- Initial and ongoing review of the QAPP
- Project oversight and technical assistance

Element A5: Problem Background

Pinto Lake is a heavily impaired waterbody in the Pajaro River watershed. Every year, the lake experiences massive cyanobacteria blooms. The primary cause of these toxic algal blooms is the presence of elevated nutrients in the lake sediments and nutrient-rich runoff from the surrounding watershed. These blooms produce cyanotoxins, which include dozens of very potent hepatotoxins and neurotoxins. The lake's toxin levels are typically at or above the State of California health limit (0.8 PPB) year-round. Toxin levels increase in the late summer and fall, often reaching levels over 10,000 PPB. One sample exceeded 2,893,000 PPB – over three million times the health limit.

This project targets the Pajaro River Nutrient TMDL and addresses the Non-Point Source (NPS) Grant Program Preference, as identified in Section I of the NPS Grant Program Guidelines.

Pinto Lake, a tributary of the Lower Pajaro River, was placed on the 303(d) list in 2008-2010 due to impairments from toxic algal blooms resulting from excessive nutrient loadings. The United States Environmental Protection Agency (US EPA) approved a Total Maximum Daily Load (TMDL) for nitrate in the Pajaro River in 2006. TMDLs for nutrients in the Pajaro River have been adopted, and TMDLs for the Pinto Lake Watershed are currently under development. Nutrient TMDLs in the Pinto Lake subwatershed seek to address biostimulation resulting from high nutrient levels that can cause excessive algal growth, including harmful algal blooms observed episodically in Pinto Lake.

The Pinto Lake Restoration Project is based on the findings of the Pinto Lake 319(h) Planning and Assessment project, which was completed in 2013. The main objectives of this project are:

- Treat internal nutrient loadings that drive cyanobacteria blooms using environmentally safe and proven technologies including polymers/coagulants such as aluminum sulfate (alum) or similar.
- Treat nutrient loadings from the tributaries (which flow seasonally into the lake) using best management practices, including but not limited to sediment basin, etc.
- Collect and analyze water quality data verifying reduction of nutrients in-lake and from the watershed as a result of treatment efforts.
- Coordinate with watershed stakeholders to gain participation in implementation efforts that reduce loadings to Pinto Lake.

Element A6: Project/Task Description

Sampling and Pre-treatment Nutrient Analysis

Nutrient loading data will be obtained from past sampling of lake sediments and runoff in the tributary creeks. This data will be used to determine the type and amount of polymer/coagulant for in-lake treatment and the designs for tributary treatment.

Geographic Focus

Pinto Lake is a shallow, hypereutrophic, 120-acre lake located in the Pajaro River watershed in the City of Watsonville in southern Santa Cruz County. It has an average depth of six feet and a maximum depth of 25 feet. Pinto Creek continues to flow seasonally to the lake. The lake is also supplied by springs, from shallow, non-potable groundwater. Pinto Lake discharges to Corralitos Creek.

The project is located in and directly benefits the Economically Disadvantaged Community of Watsonville.

In Lake Treatment

Polymer/coagulant will be applied by purpose-built barge that *uses a* GPS-linked computer system for boat guidance and integrated, real-time polymer/coagulant dosing control. Coagulant/polymer will take place before the cyanobacteria bloom season (March or April). Based on historical application practices on other lakes, the treatment will occur when the lake has a well-defined thermocline. The coagulant/polymer treatment will require one application, over multiple days.

Tributary Best Management Practices

Best management practices, including but not limited to sediment basins etc., will be constructed on the CCC and Amesti creeks to treat water runoff to the lake.

Project Summary and Work Schedule

Phase 1

- **Pre-Treatment Nutrient Analysis – In-Lake and Watershed Tributaries.** An initial study determined nutrient loadings from the lake's sediments (internal loadings) and nutrient loadings in the tributary sediments (external loadings). This data will be used to

quantify the level of treatment needed to control these sources. Water column data will be sampled in the lake to compare to post-treatment data so that percent reduction can be determined.

- **Design of Treatment Systems – In-Lake and Watershed Tributaries.** Treatment systems to meet nutrient control needs (and address any site constraints) will be designed for both in-lake and watershed tributaries. Design of the treatment systems will occur immediately following the completion of the loading analysis and notification of grant award and contracting.
- **Environmental Compliance.** Complete California Environmental Quality Act (CEQA) environmental compliance document, projected to be a Mitigated Negative Declaration.
- **Project Management.** City of Watsonville staff will manage the project including securing consultant and contract services, managing schedules and budgets, and coordinating the overall team efforts. Project Management services will continue through Phases 1 and 2.
- **Grant Administration.** The Resource Conservation District of Santa Cruz County (RCDSCC) will be responsible for overall grant administration activities, including grant invoicing and reporting. Grant Administration services will continue through Phases 1 and 2.

Phase 2

- **Implement In-Lake Treatment System(s) identified in Phase 1.** Based on the results of the Pinto Lake TMDL Planning and Assessment Project and verified in Phase 1, polymer/coagulants will be used to significantly reduce release of nutrients from lake sediments (internal loading).
- **Implement Tributary treatment System(s) identified in Phase 1.** The tributaries will be treated with best management practices, including but not limited to sediment basins etc.
- **Post-Treatment Nutrient Loading/Analysis.** In-lake and tributary water quality data will be collected and analyzed to determine the reduction in nutrients and cyanotoxin concentrations. The tributaries will be monitored upstream and downstream of the implemented best management practice. Included in the Monitoring Plan is the sample collection design.

- **Adaptively Manage Treatment Systems (in-lake and tributaries).** Additional treatment or different treatment locations may be required and implemented through this task.
- **Public Outreach.** The RCDSCC will provide bilingual outreach to farmers and residents in the Pinto Lake Watershed. Outreach to farmers will include technical and BMP assistance. The RCDSCC will also provide bilingual brochures regarding septic tank management to local residents.
- **Final Report.** The City of Watsonville will prepare a Final Report documenting all project task activities including pre and post monitoring results, design recommendations, project adaptations, costs and relevant project information. The report will be presented to maximize the transferability to other agencies and organizations faced with similar challenges.

This project's major tasks and timeline are outlined in the table below.

Table 2: Schedule of Major Project Tasks

Task Name	Task Description	End Date
Pre-Treatment Nutrient Analysis	A study to determine nutrient loadings from the lake's sediments and nutrient loadings in the tributaries.	October 2014
Design of Treatment Systems	Treatment systems to meet nutrient control needs (and address any site constraints) will be designed for both in-lake and watershed tributaries.	4/30/2016
Environmental Compliance	Complete CEQA environmental compliance document.	7/1/2016
Project Management	City of Watsonville staff will manage the project including securing consultant and contractor services, managing schedules and budgets, and coordinating the overall team efforts.	Ongoing
Grant Administration	RCDSCC will be responsible for overall grant administration activities, including grant invoicing and reporting.	Ongoing
Pre-treatment Water Quality data	The City will collect in-lake samples based on the Monitoring Plan sample design	5/16; 11/16; 1/17
Implement In-Lake Treatment Systems	Polymer/coagulants will be used to control internal nutrient loadings.	7/1/2017
Implement Tributary treatment Systems	The tributaries will be treated with best management practices, including but not limited to sediment basins, etc.	7/1/2017
Post-Treatment Nutrient Loading/Analysis	In-lake and tributary water quality data will be collected and analyzed to determine the reduction in nutrients and cyanotoxin concentrations.	5/17; 11/17; 1/18
Adaptively Manage Treatment Systems	Additional treatment or different treatment locations/technologies may be required and implemented through this task.	2/1/2018
Public Outreach	RCDSCC will provide bilingual outreach to farmers and	3/31/2018

	residents in the Pinto Lake Watershed.	
Final Report	The City of Watsonville will prepare a Final Report documenting all project task activities including pre- and post-monitoring results design recommendations, project adaptations, costs and relevant project information.	5/31/2018

Resources and Time Constraints

The project has two significant potential constraints:

- Securing necessary permits
- Sampling and treatment of the lake and tributaries is weather dependent. El Nino conditions could result in delays.

Element A7: Quality Objectives and Criteria

Detailed Performance Measures

The following methods and milestones will be utilized to monitor and verify the performance of the Project:

- Implementation of lake treatment and design and construction of tributary best management practices, including but not limited to sediment basins, etc.
- In- lake pre- and post-treatment analysis of water quality to show control of nutrients and cyanobacteria blooms pre and post treatment. The tributaries will be monitored upstream and downstream to show nutrient load reductions. Included in the Monitoring Plan is the sample collection design.
- Conduct, a minimum of four, watershed stakeholder meeting that will result in educating farmers and residents about runoff that contains sediments and nutrients.

The effectiveness of the polymer/coagulant treatment and the best management practices will be assessed by measuring nutrient and cyanotoxin reductions and a reduction in cyanobacteria blooms.

Quality Objectives

Data quality objectives for this project will consist of the following:

- Accuracy – Accuracy measures how close the measurement by an instrument is to the known value of the analyte being measured. It is determined by analysis of Certified Reference Material (CRM) or Laboratory Control Material (LCM) standards of known analyte concentration from a different source than the standards used for instrument calibration. Samples of this type will be included with every analytical batch or every ten samples, whichever is more frequent, and results will be compared to control charts that plot upper and lower control limits. The control limits are calculated as the mean percent recovery +/- 3 standard deviations of the most recent 20-30 CRM or LCM standards. Alternatively, some analytical methods may specify fixed control limits for accuracy standards.
- Precision – Precision measures how close multiple measurements of a sample are to one another. It is determined by analyzing a sample multiple times and determining how close the results are to one another.

- Completeness – Completeness is a relationship of how much of the data is available for use compared to the total potential data. Ideally, 100% of the data would be available. However, the possibility of unavailable data due to accidents, insufficient sample volume, broken bottles or lost samples, etc. is to be expected. Therefore, it will be a general goal of the project that 95% data completion is achieved.
- Recovery – Recovery measurements will be determined by laboratory spiking of a replicate sample with a known concentration of the constituent. The target level of addition is at least twice the original sample concentration. A laboratory-fortified sample matrix (a matrix spike, or MS) and a laboratory fortified sample matrix duplicate (a matrix spike duplicate, or MSD) will be used both to evaluate the effect of the sample matrix on the recovery of the compounds of interest and to provide an estimate of analytical precision. A minimum of 10% of the total number of samples submitted to the laboratory in a given year will be selected at random for analysis as matrix spikes and matrix spike duplicates. Every analytical batch will include MS/MSD analysis. Recovery data for the fortified compounds ultimately will provide a basis for determining the prevalence of matrix effects in the samples analyzed during the project. Analysis of the MS/MSD is also useful for assessing laboratory precision.
- Bias – A systematic error due to the experimental method that causes the measured values to deviate from the true value.
- Representativeness – Representativeness is the extent to which a sample has the same characteristics as the larger volume of water from which it is taken. Sample representativeness is ensured by proper collection and handling procedures. Minimizing sample degradation by use of preservatives, cooling and adherence to hold times will ensure representativeness.

Element A8: Special Training

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. Additionally, personnel conducting cyanobacteria identification must have training in algal species taxonomy. Personnel working with cyanobacteria will need to have appropriate safety training.

The Laboratory Manager is responsible for overseeing the training of laboratory analysts. Each analyst must be trained and able to read and understand the method's Standard Operating Procedure (SOP) before they are permitted to perform the method. Additionally, it is the responsibility of the laboratory manager to:

1. Ensure that all analysts have the technical ability and adequate training required to perform the procedure.
2. Ensure that all analysts have completed the required demonstration of proficiency before performing the procedure without supervision.
3. Produce quality data that meets all laboratory and QAPP requirements.

The Project Manager is responsible for ensuring that all personnel involved with data generation (including state personnel, contractors, and partners) have the necessary QA and data management training to successfully complete their tasks and functions.

Element A9: Documents and Records

Report Format

The format for all data reporting packages will be consistent with the requirements and procedures used for data validation and data assessment described in this QAPP.

Document/Record Control

The recording media for the project will be both paper and electronic. Hand-recorded data records will be taken with indelible ink, and changes to such data records will be made by drawing a single line through the error with the date and an initial by the responsible person. The Project Manager will have ultimate responsibility for any and all changes to records and documents. Similar controls will be put in place for electronic records. These controls include having the laboratory manager review all data before the QA Officer can enter data into CEDEN.

City of Watsonville's Project Manager shall retain all updated versions of the QAPP and be responsible for distribution of the current version of the QAPP. The City of Watsonville's Project Manager will approve annual updates. The Project Manager will retain copies of all management reports, memoranda, and all correspondence between the City of Watsonville and all project personnel.

Other Records and Documents

Other records and documents that will be produced in conjunction with this project include:

- Outreach materials, including workbook, fact sheets, brochures, etc.
- Amended QAPP
- Quarterly and annual progress reports
- Project final report

Storage of Project Information

Paper documents such as field data sheets, site inspection checklists, laboratory bench sheets, laboratory calibration logs and laboratory temperature logs will be stored at the City of Watsonville Water Resources Center for a minimum of 7 years or until completion of the project.

Backup of Electronic Files

Electronic files will be backed up daily and stored on secure, power-protected servers in accordance with the City of Watsonville Information Services Division's data protection policies.

Group B: Data Generation and Acquisition

Element B1: Sampling Process Design

The City of Watsonville shall conduct water quality sampling and monitoring from in-lake and tributary sources. The four sites selected for in-lake monitoring were based on previous sediment data. The two sites selected for tributary monitoring were determined based on winter flows and accessibility. Please refer to the City's updated monitoring plan for GPS locations for sampling sites.

To evaluate nutrient load reductions at the tributary implementation site(s) we will monitor upstream and downstream of the project the following parameters during at least 3 storm events:

- Total suspended solids (TSS)
- Total phosphorus (TP)
- Nitrate
- Total Ammonia
- Total Kjeldahl nitrogen (TKN)

We will estimate the volume of runoff passing through the project using a runoff model that is currently under development by Dr. Andy Fisher at UCSC which will be completed and available to assess project effectiveness after construction. This model will estimate what fraction of precipitation for a given area becomes runoff vs. infiltration based on a number of parameters including land cover, vegetation characteristics, and soil characteristics. To calculate runoff volume we will take the amount of precipitation during a storm multiplied by the drainage area times the runoff fraction estimated by the model. This runoff volume multiplied by the sediment and nutrient concentrations monitored during the storm at the monitoring locations upstream and downstream of the project will represent the nutrient load going in and coming out of the system, providing an evaluation of load reductions.

In-lake water quality parameters to be measured are nitrate, orthophosphate, TKN, TP, pH, dissolved oxygen, total ammonia, cyanobacteria species composition and cyanotoxins concentrations. At least three sets each of pre- and post-treatment water quality data will be taken at the identified monitoring sites in-lake to determine a percent reduction in nutrients. These will be collected in early spring, or fall when blooms are not underway, to reduce sample variability associated with nutrient uptake by algal growth. Timing for sample collection will be similar for pre- and post-treatment sampling.

All TSS, nitrate, orthophosphate, and total ammonia samples will be taken to the City of Watsonville Laboratory for analysis. Dissolved oxygen and pH measurements will be taken in the field by the City of Watsonville.

All TP and TKN samples will be taken to Monterey Bay Analytical Services laboratory for analysis.

The City of Watsonville will utilize University of California – Santa Cruz (UCSC) to determine the cyanotoxin concentrations pre-and post-treatment. California State University – San Marcos (CSUSM) will determine the cyanobacteria species composition. All cyanotoxin samples will be taken to UCSC's laboratory for toxin analysis. Sampling will occur weekly from the Pinto Lake boat dock, including grab samples for dissolved and total toxins, and Solid Phase Adsorption Toxin Tracking (SPATT) integrated samples. Cyanobacteria species composition will be collected simultaneously with SPATT samples when blooms are evident. An equal number of samples (from 8 to 12) will be collected pre- and post-treatment.

All cyanobacteria identification samples will be taken to CSUSM for species composition analysis. Three separate stream samples are collected in field and delivered to the lab.

Analytical water quality results will be received by the Project Manager and the QA Officer for review. Review of the data results will include acceptability of the data for input into the database. If revisions to the sampling or analytical methodologies are warranted, the Project Manager will present the proposed changes to the Project Management team for discussion and approval.

Element B2: Sampling Methods

In lake water quality data will be collected and analyzed to determine the reduction in nutrients and cyanotoxin concentrations.

The in lake sites will be sampled before alum treatment 3 times using a Niskin sampler. The City will sample when historically there has been a flux in nutrient concentrations, but when no evidence of blooms are present. These fluctuations typically occur during early spring and fall.

The City will collect 4 depth samples per site: 1 at the surface, 2 mid water column, 1 a foot above the sediment interface. These depths will be determined based on the surface elevation of the lake at time of sampling.

Water samples, for nitrate, phosphate, TKN, and TP analyses, will be collected as grabs, by filling sample container directly.

Dissolved oxygen and pH measurements will be taken in the field by the City of Watsonville.

UCSC will use SPATT to monitor cyanobacteria toxin levels in Pinto Lake on a weekly basis. Whole water is collected from about 0.1 m depth. Surface scums of algae will not be avoided, but are not deliberately collected to avoid biasing the water sample concentrations. Samples for taxa enumeration will be collected concurrent with toxin samples, beginning when blooms are evident and continuing weekly thereafter as analytical funds are available. An equal number of samplings (8 – 12) will be sampled in the pre- and post- treatment periods.
CSUSM will use the Surface Water Ambient Monitoring Program's (SWAMP) Laboratory Processing, Identification, and Enumeration of Stream Algae SOP to analyze the species composition in Pinto Lake. Analysis is conducted from two types of samples collected from each

sample site — fresh qualitative and preserved quantitative, resulting in a comprehensive taxa list with corresponding biovolume of algal taxa recorded in the quantitative sample.

The tributaries water quality data will be collected and analyzed for a reduction in nutrient load, both above and below the treatment. The tributaries will be monitored during the storm season, for a minimum of 3 storms. Samples will be collected for TSS, TP, TKN, nitrate, and total ammonia. These samples will be collected as grabs, by filling the containers directly.

Element B3: Sample Handling and Custody

In the field, all samples will be kept in a cooler with ice. All caps and lids will be checked for tightness prior to shipping. All samples will be handled, prepared, transported and stored in a manner so as to minimize bulk loss, analyte loss, contamination, or biological degradation. Sample containers will be clearly labeled with an indelible marker. Water samples will be kept in polyethylene bottles and kept cool at a temperature of 4°C until analyzed. Maximum holding times for specific analyses are listed in Table 4. Samples are placed in the ice chest with enough ice and appropriate packing material to completely fill the ice chest.

Because of the importance of program samples and analytical data, sample COC must be controlled and documented in the laboratory. Sample custody and document control procedures function to identify and document tracking and handling of samples and documents. COC procedures require that possession of samples be traceable from the time the samples are collected until completion and submittal of analytical results. A complete COC form is to accompany the transfer of samples to the analyzing laboratory.

The receiving laboratory has a sample custodian who examines the samples for correct documentation, proper preservation and holding times during the sample login process. It is the responsibility of the personnel of each analytical laboratory to ensure that all applicable regulations are followed in the disposal of samples or related chemicals.

Table 3: Sampling Guide

Analysis	Analytical Method	Container	Preservative	Hold time
TSS	Standards Method 2540 D.	1000mL Poly	Storage at 4°C	72 hours
Nitrate	EPA Method 300.0	125mL Poly	Storage at 4°C	48 hours

Orthophosphate	EPA Method 300.0	125mL Poly	Storage at 4°C	48 hours
TKN	Standard Methods 4500-NH3 BCE	500mL Poly	H2SO4 to pH<2; Storage at 4°C	28 days
TP	Hach 8190	500mL Poly	H2SO4 to pH<2;	28 days
pH	Standards Methods 4500-H+B	None, taken in field		
Dissolved Oxygen	Hach Method 10360	None, taken in field.		
Cyanotoxins: Microcystin LR, Microcystin RR, Microcystin YR, Microcystin LA	Mekebri et al. 2008	Amber glass bottle with deionized water	4°C, dark	indefinitely at -80° C
Identification (qualitative) and Enumeration (quantitative) Analysis of Algae	SWAMP Laboratory Processing, Identification, and Enumeration of Stream Algae	100mL Whirl-Pak® bag (qualitative)	Storage at 4°C	14 days
		50mL centrifuge tab (quantitative)	Storage at 4°C	Samples must be preserved with 2% glutaraldehyde within 4-days of collection

Element B4: Analytical Methods

Samples will be analyzed by the City of Watsonville Public Utilities Laboratory, Monterey Bay Analytical Service, Raphael Kudela of UCSC, and CSUSM. SOP's for each method are included in Appendix A.

TSS will be analyzed by comparing the weight of a filter, before and after a sample has filtered through it. Nitrate and phosphate will be analyzed through ion chromatography (IC). TKN and TP will be analyzed through colorimetric methods. Dissolved oxygen and pH will be measured using the HACH HQ40d portable multiprobe water meter. UCSC's preferred method of analysis, for cyanotoxins, is LCMS, following the protocols of Mekebri et al. (2008). CSUSM will follow SWAMP's protocol for Laboratory Processing, Identification and Enumeration of Stream Algae to determine the cyanobacteria species composition.

Analysis	Analytical Method	Reporting Limits
TSS	Standards Method 2540 D.	1 mg/L
Nitrate	EPA Method 300.0	0.02 mg/L as Nitrogen
Orthophosphate	EPA Method 300.0	0.02 mg/L as P
TKN	Standard Methods 4500-NH3 BCE	0.50 – 100 mg/L for a 100 mL sample
TP	Hach 8190	0.03 mg/L
pH	Standards Methods 4500-H+B	0 - 14
Dissolved Oxygen	Hach Method 10360	0.5 mg/L
Cyanotoxins: Microcystin LR, Microcystin RR, Microcystin YR, Microcystin LA	Mekebri et al. 2008	Grab samples: 0.005 ng/g (ppb) SPATT: about 1 ng/g resin.[1]
Identification (qualitative) and Enumeration (quantitative) Analysis of Algae	SWAMP Laboratory Processing, Identification, and Enumeration of Stream Algae	Not Applicable

Element B5: Quality Control

SWAMP Quality Control: Conventional Parameters in Fresh and Marine Water Laboratory Quality Control

Laboratory Quality Control	Frequency of Analysis	Measurement Quality Objective

[1] UCSC generally doesn't directly relate SPATT concentrations to grab samples, as they track each other but aren't directly comparable.

Calibration Standard	Per analytical method or manufacturer's specifications	Per analytical method or manufacturer's specifications
Calibration Verification	Per 10 analytical runs	80-120% recovery
Laboratory Blank	Per 20 samples or per analytical batch, whichever is more frequent	<RL for target analyte
Reference Material	Per 20 samples or per analytical batch, whichever is more frequent	80-120% recovery
Matrix Spike	Per 20 samples or per analytical batch, whichever is more frequent (n/a for chlorophyll a and pheophytin a)	80-120% recovery
Matrix Spike Duplicate	Per 20 samples or per analytical batch, whichever is more frequent (n/a for chlorophyll a and pheophytin a)	80-120% recovery; RPD<25% for duplicates
Laboratory Duplicate	Per 20 samples or per analytical batch, whichever is more frequent (chlorophyll a/pheophytin a: per method)	RPD<25% (n/a if native concentration of either sample<RL)
Internal Standard	Accompanying every analytical run as method appropriate	Per method
Field Quality Control	**Frequency of Analysis**	**Measurement Quality**

		Objective
Field Duplicate	5% of total project sample count	RPD<25% (n/a if native concentration of either sample<RL)
Field Blank, Travel Blank, Equipment Blank	Per method	<RL for target analyte

* Unless method specifies more stringent requirements

*Field duplicate relative percent differences are not calculated for chlorophyll a analyses for bioassessment

Participating laboratories will undertake the following specific steps to measure or estimate the effect of data errors, consistent with the QA Programs of each participating laboratory:

- Method Blank (MB, Prep Blank) – A sample of matrix similar to the batch of associated samples (when available) or deionized water that is free from the analytes of interest and is processed simultaneously with and under the same conditions as the routine samples through all steps of the analytical method and in which no target analyte or interferences are present at concentrations that impact the analytical results for sample analysis. The MB uses all of the same reagents and analytical processes as a routine sample. It is used to assess how background and laboratory contamination during preparation and analysis affect sample results. MB evaluation is typically performed once per analytical batch. MB evaluation assists to determine the acceptability of the data generated for that batch of samples. If the MB fails acceptance criteria, analysis should be halted and corrections shall be taken to resolve the problem.
- Instrument Blank – A clean unprocessed blank sample (e.g. distilled water, dilution solution) processed through the instrumental steps of the measurement process. Also used as a continuing calibration blank. It is used to determine instrument contamination or to decrease carryover from samples having high analyte concentration. When used as a CCB, the blank must meet acceptance criteria defined in the method. Corrections must be taken to correct the problem when the acceptance criteria are not met.
- Calibration Blanks (CCB) – An aqueous solution as free of the measured analyte as possible. It is diluted with reagents in the same manner as the calibration standards and is used as the zero base line in instrumental analyses. It is typically analyzed at the

beginning of an analysis and often at intervals during the course of prolonged analysis to monitor for instrument drift.

- Reagent Blank (LRB) – A sample consisting of reagent(s) without the target analyte or matrix, introduced into the analytical procedure at the appropriate point and carried through all subsequent steps; used to determine the contribution of the reagents and of the involved analytical steps. In some analyses, equivalent to the method blank.
- Laboratory Fortified Blank (LFB) – An aliquot of the same solution as the reagent blank to which a volume of known concentration of the method analyte is added. The analyte recovery is calculated to assess if the method is in control and if the laboratory is capable of making accurate measurements. May also be equivalent to the LCS.
- Laboratory Control Sample (LCS) – A reference sample prepared from an independent (secondary) standard (a standard other than that used for the calibration standards) containing the analytes of interest. It is used to ensure that the calibration is accurate and that the laboratory is in control while samples are analyzed. LCS samples are run at varying intervals, typically, at least once per analysis or once every 10-20 samples. May also be equivalent to the LFB.
- Instrument Performance Check (IPC) – A solution of method analytes used to verify the instrument performance periodically with respect to a defined set of method criteria. It is prepared from the same standard stock solutions used to prepare the calibrators and is matrix matched to the calibrators.
- Calibration Standards (CAL) – A solution prepared from the dilution of stock standard solutions. These solutions are used to calibrate the instrument response with respect to analyte concentration.
- Calibration Check Standard (CC)/ Initial Calibration Verification (ICV)/ Continuing Calibration Verification Standard (CCV) – A standard that is analyzed before continuing analysis and sometimes during the continuing course of the analysis to ensure that an existing calibration is in control.
- Matrix Spike (MS)/ Laboratory Fortified Matrix (LFM) – A volume of known analyte concentration is added to an aliquot of the sample and analyte recovery is determined. This spiked sample is carried through the entire analytical process including extraction, concentration, or digestion procedures. Spikes are utilized for recovery determinations.
- Laboratory Duplicate (DUP, D) – A QC element designed to measure analytical precision by analyzing a second aliquot of a sample.

- Matrix Spike Duplicate (MSD) / Lab Fortified Matrix Duplicate (LFM-D) – A second analyzed aliquot of a sample that has been intentionally spiked with a measurable concentration of analyte. A laboratory duplicate is used to determine the precision of the laboratory procedure.
- Proficiency Testing (PT) / Proficiency Evaluation (PE) Sample – A double-blind sample provided by an independent contractor for the purpose of demonstrating that the laboratory can successfully analyze the sample both qualitatively and quantitatively within specified acceptance limits. Participation in PT Studies allows the laboratory the opportunity for inter-laboratory comparisons.

Data Anomalies

Procedures for handling data anomalies (such as outliers and missing data) will be handled based on guidance prepared in the project-specific statistical methodology.

Element B6: Instrument/Equipment Inspection, Testing, and Maintenance

All laboratories providing analytical support for chemical or biological analyses will have the appropriate facilities to store, prepare, and process samples. Moreover, appropriate instrumentation and staff are necessary to provide data of the required quality within the schedule required by the program. Laboratory operations must include the following procedures:

- A program of scheduled maintenance of analytical balances, microscopes, laboratory equipment, field equipment and instrumentation.
- Routine checking of analytical balances using a set of standard reference weights (American Society of Testing and Materials (ASTM) Class 3, NIST Class S-1, or equivalents).
- Recording all analytical data in bound (where possible) logbooks, with all entries in ink, or electronic format.
- Monitoring and documenting the temperatures of cold storage areas and freezer units daily.
- Labeling all containers used in the laboratory with date prepared, contents, initials of the individual who prepared the contents, and other information, as appropriate.
- Having QAPP, SOPs, analytical methods manuals, and safety plans readily available to staff.

- Having raw analytical data, such as chromatograms, accessible so that they are available upon request.

Element B7: Instrument/Equipment Calibration and Frequency

Field instruments for measuring pH will be calibrated and checked against standards, immediately prior to sampling and verified post sampling with a check standard to verify accuracy. The DO probe will be checked pre-and post-monitoring for reading saturated air. All project laboratories maintain calibration practices as part of the method SOPs. Individual laboratory technicians are responsible for ensuring that calibration practices are performed as required by SOPs. Records of all calibration measurements will be maintained by each individual laboratory. Any equipment deficiencies that occur will be corrected immediately by trained personnel. Impairments of samples due to equipment problems will be reported to the Laboratory Manager as soon as possible and solutions agreed upon.

Element B8: Inspection/Acceptance for Supplies and Consumables

The procurement of supplies, equipment, and services must be controlled to ensure that specifications are met for the high quality and reliability required for each field and laboratory function. All equipment and material specifications used by City of Watsonville and UCSC are outlined in the respective laboratories' operating procedures and policies. Equipment and materials are purchased independently by each laboratory and field sampling teams. It is the responsibility of each staff person doing the ordering to inspect the equipment and materials for quality. Upon receipt of materials or equipment, a designated employee receives and signs for the materials. The items are reviewed to ensure the shipment is complete and they are then delivered to the proper storage location. All chemicals are dated upon receipt. All supplies are stored appropriately and are discarded upon expiration date.

Element B9: Non-Direct Measurements

The City of Watsonville will utilize tributary data collected in coordination with the Santa Cruz County Resource Conservation District and County of Santa Cruz Environmental Health. This data will be used to inform tributary design and capacity.

In lake the City will utilize sediment data collected pre-grant to determine nutrient concentrations.

Element B10: Data Management

As part of this project, the City of Watsonville will develop a data management strategy, and amend the QAPP based upon the strategy. The Project Manager is responsible for ensuring that that strategy is developed and that the QAPP is amended to reflect that strategy. The strategy will be consistent with the existing City of Watsonville's Quality Management Plan. Once amended, this QAPP section on data management will provide information on the following issues:

- Data management scheme, from field to final use and storage
- Standard recordkeeping and tracking practices, and document control system (citing relevant agency documentation)
- Data handling equipment/procedures that will be used to process, compile, analyze, and transmit data reliably and accurately
- Individuals responsible for elements of the data management scheme
- Process for CEDEN data upload through the Moss Landing Data Center. The City will have to register as an approved agency and identify stations for data collections and upload water quality data.

Group C: Assessment/Oversight

Element C1: Assessments and Response Actions

The Project Manager and QA Officer will ensure that qualified personnel are employed in all phases of project implementation and that all personnel receive appropriate training to complete assigned tasks. Results will be reviewed with laboratory staff and corrective action recommended and implemented where necessary.

If an audit of any field sampling or laboratory operation discovers any discrepancy, the Project Manager will discuss the observed discrepancy with the appropriate person responsible for the activity (see organization chart). The discussion will begin with whether the information collected is accurate, what were the cause(s) leading to the deviation, how the deviation might impact data quality, and what corrective actions might be considered. The Program Manager and QA

Officer have the power to halt all sampling and analytical work if the deviation(s) noted are considered detrimental to data quality.

Element C2: Reports to Management

Progress reports will note the status of project activities and identify whether any QA problems were encountered (and, if so, how they were handled). The project final report will analyze and interpret data, present observations, draw conclusions, identify data gaps, and describe any limitations in the way the data should be used.

Table 4: Project QA Status Reports

Type of Report	Frequency	Preparer	Recipients
Amended QAPP	Once, before data collection begins and as needed thereafter.	Jackie McCloud *Project Manager* *City of Watsonville*	Amended QAPP
Monitoring Reports	Quarterly	Jackie McCloud	Monitoring Reports
Progress Reports	Quarterly, following the calendar quarter. Due the 20th	Ari Rettinger *Grant Administration* *Resource Conservation District of Santa Cruz County*	CCRWQCB Project Manager
Annual Progress Summaries	Annually, by 9/30	Ari Rettinger	CCRWQCB Project Manager
Annual Project Report	Once, due 5/31/2018	Jackie McCloud	Annual Project Report

Group D: Data Validation and Usability

Element D1: Data Review, Verification, and Validation

This QAPP shall govern the operation of the project at all times. Each responsible party listed in Section A4 shall adhere to the procedural requirements of the QAPP and ensure that subordinate personnel do likewise.

This QAPP shall be reviewed at least once to ensure that the project will achieve all intended purposes. All the responsible persons listed in Section A4 shall participate in the review of the QAPP. The Project Manager and the QA Officer are responsible for determining that data are of adequate quality to support this project. The project will be modified as directed by the Project Manager. The Project Manager shall be responsible for the implementation of changes to the project and shall document the effective date of all changes made.

It is expected that unexpected changes may need to be made to the project. The Project Manager shall authorize all changes or deviations in the operation of the project. Any significant changes will be noted in the next report to CCRWQCB, and shall be considered an amendment to the QAPP. All verification and validation methods will be noted in the analysis provided in the final project report.

Element D2: Verification and Validation Methods

Each Laboratory Manager will be responsible for performing checks for all data per laboratory QA procedures prior to submission to the Project Manager. Once received by the QA Officer, all data records will be checked visually and recorded as checked by initials and dates. Issues will be noted. Reconciliation and correction will be done by the Project Manager, the project QA Officer, and the respective laboratory's manager.

The Laboratory Manager will review the case narratives and look at surrogate recoveries, spiked blank recoveries, MS recoveries, lab duplicates, and lab blanks to ensure they are acceptable as defined by project method quality objectives. The QA Officer will determine if the data are reasonable and consistent. The QA Officer will ensure that any anomalies in the data are appropriately documented.

Element D3: Evaluating Data in Terms of User Needs

Meeting and Reporting Needs of the Project

In-lake and tributary water quality data will be collected and analyzed to determine the reduction in nutrients and cyanotoxin concentrations.

Approach to Managing Unusable Data

A data quality assessment will be conducted by the Project Manager to include:

- Reviewing the criteria for measurement data, sampling design, and data collection documentation for consistency.
- Reviewing the case narratives, calculating basic statistics, and generating graphs of the data to learn about the structure of the data and identify patterns, relationships, or potential anomalies.

- Selecting the most appropriate procedures for summarizing and analyzing the data, based on sampling design, data review, and intended use of the data.
- Drawing conclusions from the data and evaluating the performance of the sampling design.

If the data quality indicators do not meet project requirements outlined in this QAPP, the data may be discarded. The project manager will evaluate the cause of the failure and make the decision to discard the data or re-sample if possible. If the failure is tied to the analysis, calibration, and maintenance, techniques will be reassessed as identified by the appropriate lab personnel.

Appendix A: Standard Operating Procedures

DETERMINATION OF INORGANIC ANIONS BY ION CHROMATOGRAPHY

EPA Method 300.0

City of Watsonville Environmental Laboratory (1/26/05)

Updated 1-26-12

Scope and Application

This document describes a procedure for the determination of bromide, chloride, fluoride, nitrate-N, nitrite-N, ortho-phosphate-P, and sulfate in drinking water, surface water, ground water, reagent water, leachate, domestic and industrial wastewater, industrial cooling water and solids extract.

Summary of Method

A small volume of sample, typically 25µL, is injected into the Dionex ICS-2000 chromatography system. The sample passes through a guard column and then a hydroxide selective anion-exchange analytical column where the anions of interest are retained. The instrument electrochemically creates and purifies an isocratic or gradient potassium hydroxide eluent solution. The anions are eluted from the column at characteristic times based on their individual affinity for the analytical column relative to that of the hydroxide eluent. Post-column, the eluent conductivity is suppressed electrochemically and the anions are detected at a conductivity cell. Conductivity vs. retention time data is collected and processed by Dionex Chromeleon software. Conductivity peaks on the sample chromatograms are compared to the peaks on the known standard chromatograms to quantify the anions of interest.

Interferences

1. Substances that have retention times that overlap with the anion of interest may cause interferences. If this is suspected, an alternate program with a different eluent concentration may resolve the interfering peak.
2. Very high concentrations of a particular anion can cause broadening of the peak to the point of interference with an adjacent peak. Sample dilution can be used to solve this problem.
3. Carry-over from high-concentration samples may lead to positive bias in subsequent samples. High-concentration samples or standards should not be run in advance of low-level samples without sufficient intervening blanks. Sample and standard sequences should be designed to avoid this problem.
4. Method interferences may be caused by contamination of reagent water, reagents, glassware and sample processing apparatus, leading to discrete artifacts or elevated baseline. Care must be taken in storing reagents and apparatus to protect them from contamination. Scrupulous cleaning methods must be used for glassware and acid washing should not be used.
5. Particulate contamination can damage instrument columns and flow systems and degrade analytical performance. All samples must be filtered through a 0.45µm or smaller filter before analysis. Reagent solutions may also require filtration.
6. Non-retained or slightly retained anions like low-molecular weight organic acids that elute early in the chromatographic run may interfere with fluoride analysis. The AS-18 column is designed to minimize this interference but reanalysis with an alternate eluent concentration program may be required to resolve the peaks.

Equipment and Supplies

- Analytical balance capable of weighing accurately to 0.0001g.

- Dionex ICS-2000 Ion Chromatography system Including: AG-18 guard column, AS-18 analytical column, ASRS Ultra II suppressor, heated conductivity cell, EluGen eluent generator, AS40 autosampler, and Chromeleon Software.
- Class-A volumetric pipettes and flasks.
- Dionex PolyVials and filter caps.
- Whatman Anotop-IC or Millipore IC Millex - LG syringe filters and 10ml luer-lock syringes.

Instrument Operating Conditions (typical)
Sample loop: 25µL
Flow rate: 1.0mL/min.
Column temperature: 30°C
Conductivity cell temperature: 35°C
Eluent concentration program: 19-40 mM KOH from 8-9 min.
Detection: Suppressed conductivity, AutoSuppression Recycle Mode, 109mA current
System backpressure: ~2000 PSI
Run Time: 14 min.

Reagents
Reagent water: Distilled or deionized water free of the anions of interest and particles larger than 0.2 µm. For example, use Fisher Scientific HPLC grade water.
Eluent solution: Reagent water is supplied to the eluent reservoir. Potassium hydroxide is supplied electrochemically by a Dionex EluGen cartridge.
Stock standard solutions 1000 mg/L: Purchase certified solutions, for example, use Absolute Standards Inc. single component calibration standards. Separate single anion stock standard solutions should be used. This will allow for the preparation of multi-anion calibration standards that contain different concentrations of the individual anions and will allow for the elimination of anions that are not being quantified or will interfere with the quantification of other anions. Alternatively, prepare stock standards from ACS reagent grade materials (dried at 105°C for 30 min.) as below.

- Bromide (Br^-) 1000 mg/L: Dissolve 1.2876 g sodium bromide (NaBr, CASRN 7647-15-6) in reagent water and dilute to 1 L.
- Chloride (Cl^-) l000 mg/L: Dissolve 1.6485 g sodium chloride (NaCl, CASRN 7647-l4-5) in reagent water and dilute to 1 L.
- Fluoride (F^-) 1000 mg/L: Dissolve 2.2100g sodium fluoride (NaF, CASRN 7681-49-4) in reagent water and dilute to 1 L.
- Nitrate (NO_3^--N) 1000 mg/L: Dissolve 6.0679 g sodium nitrate ($NaNO_3$, CASRN 7631-99-4) in reagent water and dilute to 1 L.
- Nitrite (NO_2^--N) 1000 mg/L: Dissolve 4.9257 g sodium nitrite ($NaNO_2$, CASRN 7632-00-0) in reagent water and dilute to 1 L.
- Phosphate (PO_4^{3-}-P) 1000 mg/L: Dissolve 4.3937 g potassium phosphate (KH_2PO_4, CASRN 7778-77-0) in reagent water and dilute to 1 L.
- Sulfate (SO_4^-) 1000 mg/L: Dissolve 1.8141 g potassium sulfate (K_2SO_4, CASRN 7778-80-5) in reagent water and dilute to 1 L.

Note: Stability of standards: Stock standards are stable for at least one month when stored at 4°C. Dilute working calibration standards should be prepared weekly, except those that contain nitrate, nitrite or phosphate should be prepared fresh daily.
Quality control standards: For blank spike and matrix spike applications. Purchase certified solutions from a different source than the stock standard solutions. For example, use Inorganic Ventures 300.0 Laboratory Fortification Solution.
Instrument Performance Check Solution: A mid-calibration-range check standard used to assess instrument calibration stability. For example, use Absolute Standards Inc. #59011 seven-anion standard.

Sample Collection, Preservation and Storage
Collect samples in 125ml polyethethylene bottles. Use new, certified-clean bottles or bottles thoroughly cleaned and rinsed with reagent water.
Sample preservation and hold times for the anions that can be determined by this method are as follows:

Analyte	Preservation	Hold Time
Bromide	None	28 days
Chloride	None	28 days
Fluoride	None	28 days
Nitrate-N	4°C	48 hours
Nitrite-N	4°C	48 hours
O-phosphate-P	4°C	48 hours
Sulfate	4°C	28 days

Multiple anions are often determined for a single sample. In this case, observe the shortest hold time and apply the most preservation.

Sample Preparation

Prepare a dilution of samples that are expected to have excessive anion concentrations or use an appropriate Dionex OnGuard column to strip interfering anions that won't be quantified or other interfering substances. Wastewater and seawater will usually require such treatment while drinking water will usually not. Samples with high levels of neutral organic compounds (wastewater) should be cleaned up with a hydrophobic SPE cartridge to prevent fouling of the stationary phase. Filter all environmental samples and method blanks with a Whatman Anotop-IC 0.2μm or Millipore IC Millex – LG syringe filter.

Quality Control

Linear Calibration Range: Established for all anions initially and then every 6 months or whenever there is a significant change in the background or instrument response. The initial demonstration of linearity must use sufficient standards to define the linear range of the calibration curve.

Method Detection Limits: Established for all anions initially and then checked every 6 months, when a new analyst begins work on the instrument, or whenever there is a significant change in the background or instrument response. Reagent water is fortified at a concentration 3-5 times the estimated instrument detection limit. Seven aliquots are processed as a sample would be. MDL = 3.14 X standard deviation of the measured concentration of the 7 replicates (in mg/L).

Method Blank (or Laboratory Reagent Blank): Treated as unknown samples, including filtration. Analyze at least one method blank with each batch of samples. The method blank to be analyzed must be different than the calibration blank used for background subtraction, which will always have a flat line as its chromatogram. Calculate accuracy as percent recovery (% recovery = measured blank spike concentration – measured background concentration / spiked concentration * 100). If any analyte values exceed the MDL then reagent contamination or unacceptable carry-over may be suspected and corrective actions must be taken before continuing with analysis.

Blank Spike (or Laboratory Fortified Blank): Analyze at least one blank spike with each batch of samples. Calculate accuracy as percent recovery as above. If recovery falls out of the control limits of 90-110% then corrective actions must be taken before continuing with analysis. When sufficient performance data

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=is collected (at least 20 to 30 analyses), control limits may be established at mean percent recovery +/- 3 standard deviations.

Matrix Spike (or Laboratory Fortified Sample Matrix): At least every tenth sample, analyze an aliquot of a randomly selected sample spiked with a known quantity of analyte. The spike concentration should be no less than five times the MDL and sufficiently high to be detected above the original sample. Recovery should be within the limits of 80-120% or corrective action is required. Performance based control limits may be established as with the blank spike.

Duplicates: Every tenth sample, analyze a duplicate aliquot of a randomly selected sample. Matrix spikes may be used for Duplicate analysis. The percent difference between the duplicates should not be more than 10% for samples whose concentration is more than 20 times the MDL, and not more than 25% for samples whose concentration is less than 20 times the MDL. Samples whose concentration is less than 5 times the MDL should not be evaluated for % difference. Performance based control limits may be established as with the blank spike.

Instrument Performance Check: A mid-calibration-range instrument performance check standard and a calibration blank is run after calibration, after every tenth sample, and at the end of the batch. The control

limits of +/- 10% must be met throughout the batch or recalibration and reanalysis of samples bracketed by at least one out-of-control instrument performance check standard must be performed.

Instrument Power-Up

The chromatography system and computer will normally be continually powered up. All components but the monitor are connected to an uninterruptible power supply that will allow the system to run for several minutes during a power failure. Short power interruptions should not affect the analysis.

1. Turn on the autosampler and the ICS-2000. The switches are on the right rear of the instruments.
2. Turn on the computer and monitor.

Software Start-Up

The Chromeleon server will automatically start up when the computer is powered up and can be monitored by double clicking on the 'Server monitor' desktop icon. The server must be running for Chromeleon software to control the ICS-2000.

1. Double click on the 'Chromeleon' desktop icon to start the software. The browser will open automatically.
2. Use the browser to open the control panel configured for our system. The file is called ICS-2000_Traditional_System_AS40.pan and is located in the control panel folder.

Eluent

Check the eluent bottle level, indicated on the control panel and the bottle itself. Refill with deionized water to 2L if the eluent bottle is low, especially if running a large sample set. Two liters will give about 33 hours of runtime at a 1ml/min flow rate. Set the eluent level on the control panel to match what is actually in the bottle by directly entering the level in the box or sliding the arrow with the mouse.

Regenerating the Suppressor

The membranes in the ASRS Ultra II suppressor may become depleted of hydronium ion (H_3O^+) if eluent is allowed to run through it while the power to it is off or insufficient for the eluent concentration. The depletion can also occur if the instrument is not operated for a period of a few weeks. The result is insufficient suppression of eluent conductivity and small peak area. If calibration standards are run at the beginning of a batch and the suppressor membranes are slowly regenerated throughout the batch, then the same standards would appear to increase in concentration if run at the end of the batch. This effect has been seen with this instrument with apparent concentration increases of up to 20% over the course of dozens of samples.

If the suppressor may be depleted for any reason, see section 4.2 in the ASRS Ultra II manual and/or follow these procedures:

1. Prepare a 200mN H_2SO_4 solution by diluting stock 1N acid 5X with deionized water and degassing.
2. Disconnect the line from the analytical column to the 'Eluent In' port at the suppressor.
3. Disconnect the line from the 'Eluent Out' port to the 'Cell In' port at the 'Eluent Out' port.
4. Disconnect the line from the 'Cell Out' port to the 'Regen In' port at the 'Cell Out' port and connect to the 'Eluent In' port on the suppressor.
5. Connect a line with a luer-lock fitting to the 'Eluent Out' port.
6. Use a 10ml syringe to push 5ml of 200mN H_2SO_4 into the 'Eluent Out' port. It will flow through all of the compartments of the suppressor and through the 'Regen Out' port to waste.
7. Follow with 5ml of degassed, deionized water from a new syringe.
8. Restore the plumbing to the original configuration.

.Priming the Pump

1. Open the waste valve on the secondary (left) pump head by turning the valve one half turn counterclockwise.
2. Click the 'Prime' button on the control panel.
3. Click the 'Yes' button in the window that opens to confirm that you opened the priming valve.
4. Watch the pump waste line and continue priming until no air bubbles are exiting. 10 to 15 seconds will usually be sufficient.
5. Click the 'Off' button on the control panel.

6. Close the waste valve by turning clockwise. Do not over-tighten.

Purging the Lines Pre-Column

1. Disconnect the lines that connect the injection valve to the guard column from the injection valve.
2. Connect a piece of spare tubing to the valve and place the loose end in a beaker.
3. Click the 'On' button on the control panel.
4. Allow the pump to run for about a minute until all air and standing eluent has been flushed from the lines then click the 'Off' button on the control panel.
5. Remove the spare line and reconnect the lines between the injection valve and the guard column.

Equilibration

1. Click the 'Equilibration' button on the control panel.
2. Make sure the Eluent Generator concentration setting is at 33mM and is toggled on (system pressure must be above 100PSI for this to happen) and that the suppressor is also on.
3. Select 'Acquisition on' from the 'Control' pull-down menu.
4. Check the 'ECD_1' and 'Channel_Pressure' boxes and click the 'OK' button.
5. Monitor the pressure and conductivity for at least 15 minutes.
6. Select 'Acquisition off" from the 'Control' pull-down menu. This must be done in order to start a batch of samples
7. Start the sample batch while the equilibration program is still running.

Equilibration should be done just prior to starting the sample run. Small sample sets can be prepared during equilibration but large sample sets should be prepared before equilibration. During equilibration the pressure will ramp up and stabilize and may be rising slightly when the run is started. A couple of large conductivity peaks may elute early in the equilibration and then the conductivity should drop to a level baseline before starting the batch.

Sample Order Guidelines

Proper sequencing of the samples and the use of blanks will minimize carry-over from high concentration samples to low concentration samples. The batch should start with at least 4 blanks. The final blank is the baseline subtraction blank (optional) and its chromatogram may be subtracted from all other chromatograms. This will smooth the baseline and eliminate background conductivity ramps that are created when gradient eluent concentrations are used. Then the standards are run starting with the lowest concentration and moving up and ending with at least two rinse blanks. A calibration blank and instrument performance check are run after the calibration standards, after every ten samples and then at the end of the batch. One blank spike and a method blank are included with each batch. A matrix spike and duplicate matrix spike are run at least after every ten samples. When a calibration blank or a method blank is run after a higher concentration spiked sample, a rinse blank should be run in between.

Example sample loading list:

1. rinse blank
2. rinse blank
3. rinse blank
4. rinse blank
5. baseline subtraction blank
6. calibration blank
7. low level calibration standard
8. mid level calibration standard
9. high level calibration standard
10. rinse blank
11. rinse blank
12. calibration blank
13. instrument performance check
14. rinse blank
15. blank spike
16. rinse blank
17. method blank
18. unknown1
19. unknown2
20. unknown3
21. unknown4
22. unknown5
23. unknown6
24. unknown7
25. unknown8
26. unknown9
27. unknown10
28. matrix spike (unknown4)
29. duplicate matrix spike (unknown 4)
30. rinse blank
31. calibration blank
32. instrument performance check
33. rinse blank
34. unknown11
35. unknown12
36. unknown13
37. unknown14
38. unknown15
39. unknown16
40. matrix spike (unknown 12)
41. duplicate matrix spike (unknown 12)
42. rinse blank
43. calibration blank
44. instrument performance check
45. rinse blank
46. rinse blank

Loading the Sample Vials

1. Wearing gloves, load the required number of Dionex 5ml PolyVials into the autosampler cassettes.
2. Label the vials with the sequence number or sample name.
3. Draw 10ml of sample into a 10ml luer-lock plastic syringe (for samples requiring filtration otherwise skip to step 6)
4. Attach a Whatman Anotop-IC 0.2µm filter to the syringe.
5. Dispense the first 2 ml to waste.
6. Dispense into the vial, filling to a level between the two lines on the cassette.
7. Insert a filter cap into the vial.
8. Use the cap tool to push the cap into the vial.
9. Press the 'Hold/Run' button on the autosampler until it toggles to 'Hold'.
10. Slide the cassette pusher toward the back of the autosampler
11. Place the cassettes (up to 11) into the tray with the black dots facing the right.
12. Press the 'Hold/Run' button until it toggles to 'Run'. The first vial will move into position.

If the cap is pushed into the vial so it is flush with the lip, using the flat side of the tool, the vial will be treated as a sample vial. If the tip of the cap is left protruding from the vial, using the indented side of the tool, the vial will be treated as a rinse vial. This rinse will only rinse the autosampler line and the injection loop and not the other plumbing and the column. A rinse blank run is the preferred rinse because all the lines in the ICS-2000 will be rinsed and a chromatogram will be produced showing the effectiveness of the rinse.

Autosampler Settings

When all of the cassettes are loaded and before starting the analytical batch, the autosampler settings should be as follows:

- Vial Type 5ml, Sample (automatically detected)
- Tray Ready (automatically detected)
- Inj TypeLoop (toggle button)
- Inj Mode Prop (toggle button)
- Bleed Off (toggle button)
- Inj/Vial 1 (toggle button)
- Sampler Ready (automatically detected)
- Injection First (automatically detected)
- Operation Lcl (toggle button)
- Hold/Run Run (toggle button)

The Hold/Run toggle button will be in the 'Hold' position until all of the cassettes are loaded. Toggling to 'Run' will move the first cassette into position and the autosampler will be ready to accept a load command from the ICS-2000.

Open Software Files and Start a Batch

In Chromeleon software, a sequence file represents every analytical batch. The sequence file serves a sample list and as a folder that will hold one or more program and method files. A new sequence file is created for each run. A program file is a list of operating instructions for the ICS-2000. The same program file will usually be used for multiple analyses. A method file contains instructions for identifying and quantifying peaks. Method files are usually copied from previous similar analyses, renamed and modified slightly. The browser operates like windows explorer and is used to access sequence, program, and method files.

1. Open the browser.
2. Select 'New' from the 'File' pull-down menu.
3. Click on 'Sequence (using Wizard)' and click 'OK'.
4. Click the 'Next' button to pass the intro window.
5. The fields should read as follows, then click 'Next':
   Timebase: Anion,

Computer: LAB-IC
Protocol: My Computer

6. At the 'Unknown Samples' window Click 'Use Template' from the first pull-down box.
7. For the 'Template for sample name' box, click 'Sample#n'.
8. Enter the total number of vials including blanks, samples, QC checks, and standards but not rinse vials. This number can be adjusted later.
9. Enter '1' for both 'Injections per Vial' and 'Start Position'.
10. Click the 'Apply' button and then the 'Next' button to go to the 'Standard Samples' window.
11. Enter '0' in the 'Number of Vials' box and click 'Next', the standard samples will be identified later from the browser.
12. At the 'Methods and Reporting' Window Select the program file and the method file to be used for the sequence. Use the pull-down box or the browse function to search the 'Programs' and 'Methods' folders. Programs and Methods can also be copied from other sequences and from the Dionex 'Templates' folder. The program and method files from the last run of similar samples might be good choices here.
13. Under 'Preferred Reporting Options' select the Dionex 'default' report as the preferred report and 'ECD_1' as the preferred channel. Click 'Next'.
14. At 'Saving the Sequence', enter the sequence name and the title, which may be the same. It's a good idea to identify the date of the run in the name. The data source should be 'LAB-IC_local'. Enter the name of the folder where the sequence will be stored. 'Anion' is the top folder for this purpose. Click 'Next'.
15. The sequence is now created and it can be viewed and changed from the browser.
16. Enter the names of the samples by clicking on the sample name and typing.
17. Change the entry in the 'Type' column to 'Standard' for the calibration standards.
18. Use the right click button to insert, delete or append a sample to the list. Make sure the numbers in the position column are sequential.
19. Rename the method file. From the browser right-click on the method file (.qnt) and select rename. Save changes made to the method file if prompted. Confirm that the new name will be changed where it is used in the sequence.
20. Add a stop program to the sequence to stop the instrument from running after the last sample is finished in the middle of the night. Save changes and click on a previous sequence that contains a stop program. Right click on the 'Stop.pgm' icon and select 'Copy'. Open the new sequence in the browser and paste the stop program in the box that has the program and method files. Append a sample to the sequence called blank or stop and change the 'Program' column entry to 'Stop'. A blank vial should be placed in the cassette for this sample.
21. The batch is ready to be run. When the instrument is equilibrated, turn the acquisition off. Set the autosampler 'Hold/Run' toggle button to 'Run'. On the menu bar click 'Batch' and then 'Start...'. Select the sequence to be run and click the 'Start' button.

Set up the Quantification Method

1. From the browser, click on one of the samples in the sequence. Then click on the 'QNT-Editor' button.
2. Click the 'General' tab near the bottom of the screen. Enter 'mg/L' in the 'Dimension of amounts' box. Select 'Total' for the calibration mode. If using blank subtraction, select 'Subtract a fixed sample' and use the browse function to select the blank that will be subtracted from each sample. This blank should have minimal anion peaks and will have had several blanks run before it.
3. Click the 'Detection' tab. Double click the parameter value field and a window will open. Starting at 0.000 retention time set the minimum peak area to 0.001 signal*min and click 'OK'.
4. Click the 'Peak Table' tab. Use the chromatogram scroll button on the tool bar near the top of the screen (a vial with a left or right arrow on it) to scroll to the chromatogram for the mid range standard. Right-click anywhere on the peak table and select auto-generate peak table. Select 'Enumerate peaks of the current chromatogram' and check 'Apply to peaks with Area greater than 0.01'. Click 'OK'
5. Use the Dionex AS-18 column application note to identify the peaks on the chromatogram of the mid-range standard. Double click on each 'peak name' box (notice the corresponding peak on the chromatogram is highlighted) and change the peak name to the name of the anion. A

carbonate peak from dissolved atmospheric carbon dioxide may be identified along with the anions of the standard. This can be labeled as carbonate or the line can be deleted from the peak table.

6. The retention times are automatically entered for you. For each peak, enter a peak window time of 3% of the retention time (Ret. Time x 0.03). Integration type, calibration type, and peak type should be set to Area, XLin, and Auto respectively.
7. Click the 'Amount Table' tab. Right-click anywhere on the table and select 'Columns...' and then select 'Edit Amount Columns...'. If an 'Unassigned Standards Detected' warning comes up click 'OK'. Check 'Assign standards on the basis of name'. Click the 'Auto-Generate' button and then select 'Generate a separate amount column for EACH standard' and click the 'Apply' button and then 'OK'.
8. Enter the individual anion concentrations for each standard. The F9 key can be used to fill down if the concentrations of each anion in a standard are the same.
9. Click the 'Calibration' tab. Standards can be disabled entirely or for individual anions at this tab. This may be necessary for a calibration curve where the high concentration standard is deviating significantly from linearity. To delete standards for an individual anion, first select the anion by clicking and highlighting its peak on the chromatogram, then click the peak 'Enabled' box for the standard to be deleted. Select 'Disable this standard for the selected peak'. The anion to be deleted will be indicated in parentheses. The 'Peak Tracking', 'Spectra Library Screening', and 'SST' tabs are not used for this method.

Inspect the Chromatograms and Confirm Peak Identification

Complex samples may result in chromatograms that contain peaks that are misidentified or incorrectly integrated by the software's algorithms. It is necessary, therefore, to confirm the peak identification and integration manually. It is important to make corrections to these errors but do so with consistency and a concern for the potential introduction of bias. Consult with a more experienced chromatographer if necessary.

1. From the Browser double-click on the first sample in the sequence. The integration window should open with the chromatogram for that sample. Click on the individual peaks on the chromatogram to highlight them for inspection. Use the Zoom tool to define the zoom area around the individual peak. The cancel zoom button resets the view. Click on the 'Next Chromatogram' or 'Previous Chromatogram' button to switch between chromatograms (the zoom level will be retained).
2. Look for peaks that have retention times that differ significantly from the known anion peaks in the standards but may be identified and labeled as the known anion. If such a misidentified peak is found in an unknown sample chromatogram it must be deleted or otherwise labeled. Right-click on the peak and select 'Delete Peak'. To change the label assigned to a peak right-click the peak and select 'Peak Properties'. Select the correct anion from the component list and press the thumbtack button to apply. Two peaks cannot be labeled as the same anion. If an attempt is made to switch to another chromatogram after making changes, the software will ask if the changes should be saved. Click 'Yes' if appropriate.
3. Look for double peaks that are integrated as a single peak. The peak that is retained most closely to the peak in the chromatogram of a known standard of similar concentration must be separated from the interfering peak. Right-click on the peak and select 'Split Peak' or highlight the interfering peak and delete it. Some cases will require manually dropping a delimiter line from the valley between the two peaks to the baseline. The peak delimiter tool is used to make these adjustments.
4. Look for baseline (shown in red) misidentification. Comparing the chromatogram to that of a blank may be helpful for this. Use the baseline tool to make these adjustments. Errors made during this process or when manually delimiting peaks can be eliminated by selecting 'Undo' from the 'Edit' menu or by switching chromatograms without saving changes.

Viewing and Printing results

1. Results can be viewed from the integration window by clicking on the 'Integration' button on the toolbar. The 'Show Report' button should be automatically toggled to the 'On' position. This will

cause the report to be shown below the chromatogram. Use the tabs at the bottom of the integration window to switch between 'Integration', 'Calibration', and 'Summary'. Calculated amounts are shown on the 'Summary' sheet. Only one anion is shown at a time. Click the peak on the chromatogram for a particular anion to show the summary for that anion for all samples. The summary can be printed from here but more printing options are available from the 'Printer Layout' window.

2. Click on the 'Printer Layout' button on the toolbar.
3. Select the 'Calibration (Current Peak)' tab. A calibration plot is shown with calibration information including correlation coefficient. Press the print button to print. Right click on the plot and select 'Calibration Plot Properties' to select a different anion or change the scale of the plot.
4. Select the 'Integration' tab. The individual chromatograms for each sample including peak information and calculated anion concentrations can be viewed and printed here. Press the 'Previous Chromatogram' or 'Next Chromatogram' buttons on the tool bar to scroll between samples.
5. Select the 'Summary' tab. The summary for a single anion is shown including retention time, area, and amount. To switch to another anion, click on the 'Integration' button and click on the peak of the anion of interest to select that anion and then click the 'Printer Layout' button to return to the summary page for the selected anion. Print the sheet or highlight and copy data and then paste to an excel spreadsheet for further data processing like calculation of spike recovery.

System Maintenance

The following routine maintenance procedures are to be performed by the laboratory analysts. Consult the instrument operator's manual or contact the Dionex technical call center for additional help at (800) 346-6390.

Daily

- Check the component mounting panel for any leaks or spills. Isolate and repair any leaks(operator's manual section 4.2)

Weekly

- In weeks when the instrument is not used, exercise the instrument by running the equilibration program (eluent concentration 33mM) for 20 minutes. Be sure to prime the pump and purge all gas from the lines before running the program.
- Check lines for crimping or discoloration. Relocate any pinched lines and replace damaged lines.
- Check the junctions between the pump heads and the pump casting for evidence of liquid leaks. Check the drain tubes at the rear of the pump heads for evidence of moisture. Normal friction and wear may gradually result in small liquid leaks around the piston seal. If unchecked, these leaks can gradually contaminate the piston housing, causing the pump to operate poorly. If leaks occur, replace the piston seals (section 5.6)

Every Six Months

- Calibrate the conductivity cell (section 5.1.3).
- Calibrate the vacuum degas assembly (section 5.1.5).
- Replace the pump piston rinse seals and piston seals (section 5.6).

Yearly

- Rebuild the injection valve (section 5.4). Order the Rebuild Kit (P/N 057896).
- Replace the AS40 Automated Sampler tip and tubing. The AS40/ASM Maintenance Kit (P/N 05564) contains all of the components required instructions for the operation.
- Clean the column according to the column care document at the end of the AS-18 column operator's manual. Always position the guard column after the analytical column during cleaning procedures. The column may require more frequent cleaning after analysis of samples with high concentrations of metals or hydrophobic compounds. Decreasing retention times and tailing peak shapes may indicate the need for cleaning.

References

Method 300.0 "Determination of inorganic anions by ion chromatography", Revision 2.1. Environmental Monitoring Systems Laboratory, Office of Research and Development, United States Environmental Protection Agency (1993)

Standard Methods for the Examination of Water and Wastewater, Method 4110B, "Anions by Ion Chromatography", 18th Edition (1992)
"Determination of inorganic anions in environmental waters using a hydroxide-selective column", Application Note 154, Dionex Corp., Sunnyvale, CA (2003)
ICS-2000 Ion Chromatography System Operator's Manual, Dionex Corp., Sunnyvale, CA (2003)
Chromeleon Chromatography Management System Tutorial and User Manual, Version 6.50, Dionex Corp., Sunnyvale, CA (2002)

TOTAL KJELDAHL NITROGEN

SM 4500-NH3 BCE Kjeldahl Nitrogen

Monterey Bay Analytical Services

Updated 11-17-2014

Scope and Applications:

Nitrogen plays a central role in a variety of chemical compounds and thus is an essential nutrient. The Kjeldahl method determines nitrogen in the trinegative state. It fails to account for nitrogen in the form of azide, azine, azo, hydrazone, nitrate, nitrite, nitro, nitroso, oxime, and semi-carbazone. If ammonia nitrogen is not removed in the initial phase of the procedure, the term "kjeldahl nitrogen" is applied to the result. Kjeldahl nitrogen minus the ammonia nitrogen is referred to as organic nitrogen. Many wastewater treatment facilities are required to monitor for total nitrogen discharged, this is calculated by adding the kjeldahl nitrogen to the nitrate and nitrite nitrogen.

Principle:

In the presence of sulfuric acid, potassium sulfate and copper sulfate catalyst, amino nitrogen of many organic materials is converted to ammonium sulfate. Free ammonia and ammonium nitrogen are also converted to ammonium sulfate. Sodium hydroxide is added in excess, neutralizing the acid and making the solution alkaline. The alkalinity releases the ammonia. Steam is introduced into the mixture which drives the ammonia out of solution. The distillate is collected in boric acid. The ammonia forms ammonium borate, a weak base. The borate is titrated with 0.02 N sulfuric acid and the nitrogen is calculated.

Reporting Limits:

0.50 mg/l for a 100 ml sample. Report levels below this as Not Detected. Adjust the PQL depending on the sample size.

Precision and Bias:

A QC sample tested seven times averaged 5.28 mg/l N with a standard deviation of 0.19 mg/l, a RSD of 3.6% and a negative error of 0.32 mg/l or 6%.

Working range:

Nitrogen levels of 0.50 to 100 mg/l can be analyzed. Range can be extended by dilution.

Interferences:

Nitrate in excess of 10mg/l as N can cause a negative interference or nitrate in the presence of reduced organic matter can be converted to ammonia and cause a positive bias. Residual chlorine reacts with ammonia; remove by sample pretreatment with sodium thiosulfate. High levels of organic matter can react with H2SO4 during digestion leading to an incomplete reduction of organic nitrogen to ammonia. To avoid this, add extra digestion reagent to samples believed to contain high levels of organic matter. For saline samples, standards and blanks should be prepared in synthetic ocean water.

Sample collection, Preservation and Holding times:

If an immediate analysis is not possible, preserve with sulfuric acid to pH 1.5 to 2. Usually 3 ml 50% sulfuric solution is added per liter. Refrigerate. Holding time can be up to 28 days, but sample should be tested within one week. A minimum of 100 ml of sample is required.

Reagents:

2.0%Boric acid Receiver solution: (Prepare monthly) 20 grams of boric acid (Fisher CAS# 10045-35-3) diluted to <1 liter with DI water. Add ~2 mL of mixed solution of Bromcresol Green-Methyl Red indicator solution (Hach CAT# 2329232), bring up to 1 liter.

Digestion Reagent: Combine 134 g Potassium Sulfate (K2SO4) (Fisher CAS# 7778-80-5) and 7.3 g of Copper Sulfate (Fisher CAS# 7758-99-8)(CuSO4) in 650 ml of water in a 1L beaker with a stir bar. Add 200 ml concentrated Sulfuric Acid (Fisher CAS# 7664-93-9)(H2SO4), CAUTION! Reaction becomes hot on addition of Sulfuric Acid. Dilute to 1 L with DI water, keep reaction stirring till it is cool enough to pour in to 1L plastic container and store @ 35°C. Sodium Hydroxide 30% Dissolve 300 g (NaOH) Sodium Hydroxide (Fisher CAS# 1310-73-2) in water and dilute to 1L in a plastic container. CAUTION reaction extremely hot! When solution is cool pour in to distillation unit container labeled 30% Sodium Hydroxide.

Always wear proper PPE and work under the fume hood when preparing these reagents.

Set up Procedure:

1. Set up 20 Kjeldahl Digestion flasks in holding apparatus from Buchi K-437 Digestion unit. Space for 16 samples, 1 blank, 1 QC and 2 Matrix Spikes.
2. Find 16 samples, determine amount of sample needed, up to 100 mL. (When possible, check NO3-concentrations and dilute samples over 10 mg/L NO3-N.) If there are not enough samples to fillevery digestion flask, replace unused flasks with Buchi glass plugs.
3. Pour 100 mL DI Water in to first two flasks.
4. Pour each sample in to flasks 2-18. If less than 100mL sample used dilute to 100mL with DI Water.
5. In flasks 19 and 20 put sample chosen for matrix spike. (Use sample not needing dilution and with NO3- concentrations < 10mg/L NO3-N if possible).
6. To flasks 2, 19 and 20 add 0.5mL Absolute Grade NIST Traceable Organic Nutrients TKN as N (part # 54152), (0.5mL of 1000mg/L spike creates a 5ppm spiking solution).
7. Add approximately 25mL Digestion Reagent and several boiling chips to each flask.

Digestion Procedure:

1. Turn on Buchi K-437 digestion unit and set temperature to 250°C.
2. Turn on water circulation baths (Chillers).
3. Wash top of unit with water.
4. Place flasks in the holding apparatus on to the digestion unit, place top on the flasks.
5. Attach tube that goes to Buchi B414 Scrubber. Verify all seals are tight.
6. Digest samples till most flasks are full of fumes (~3 hours).
7. Reset temperature to 380°C, when unit reaches temperature set timer for 30 minutes. Take note not to let the digestion block exceed 380°C. Excess heat can result in loss of nitrogen by formation of nitrogen oxides.
8. At end of 30 minutes take flask holding apparatus off Buchi K-437 and allow cooling before distillation. To avoid shocking the glassware, set down holding apparatus such that the bottoms of the digestion tubes do not make contact with the countertop.
9. While still warm, add ~10mL DI to flasks to avoid crystallization.

Distillation Procedure:

1. Set up Buchi K350 Distillation Unit.
2. Distill ~3 blanks before distilling samples to clean the unit.
3. Attach one flask at a time to left side behind cover of Buchi K350 Distillation unit, make sure to close cover, reaction will get hot. Start with flask 1 .

4. On right side of unit place Buchi glass beaker with 50mL of 2.0% Boric Acid receiver solution (be sure end of tube is submerged into solution in beaker).
5. When flask and beaker are in place press Reagent button to add NaOH to flask until a color change is observed.
6. When instrument is finished dispensing, 4:00 should be on the screen in top right corner, at this time press start to begin distillation (Procedure takes 4 minutes).
7. Dispose of contents of digestion flask in to acid/base bucket in sink, rinse flask with DI water and return it to holding apparatus.
8. Repeat for all flasks, control, samples and matrix spikes.

Titration Procedure:

1. For Titration use Metrohm 877 Titrino Plus (Serial # 1869001004148).
2. Be sure the 0.02N H2SO4 burette is loaded. If the burette requires changing, perform a prep function to ensure the lines are free of air bubbles (Menu > Manual Controls > Dosing > Prep).
3. Calibrate to pH electrode according to the pH SOP.
4. From the home screen on the Metrohm 877 Titrino Plus, select Method > Low Alk.
5. Titrate a low alkalinity ICV to ensure the autotitrator is working properly.
6. When distillation procedure is completed take beaker from right side of Buchi K350 Distillation unit, place in front the Metrohm autotitrator with the probe submerged in the liquid.
7. From home menu select Method > TKN. Titrate to a pH endpoint of 4.9.When titration is completed, record ppm and titrant volume on the worksheet. Dispose of contents of beaker in acid/base bucket in sink and wash out with DI water.

Helpful Hints:

1. Sometimes after digestion, the remaining salts in the digestion flasks can crystallize. When this happens, add ~10mL DI and reheat on the digestion block, stirring occasionally. USE CAUTION!!! Sometimes the solution can bump, expelling boiling sulfuric acid out of the digestion flask. Always stir in the fume hood with the opening of the flask pointed in a safe direction.
2. Measuring the pH of the blank to ensure the pH is slightly >4.9 (the titration endpoint) can help to determine the source of contamination when blank values are high.
3. Always check the pH after each titration. If the autotitrator passed the endpoint, the samples can be redistilled. Pour the distillate backing into an empty distillation flask, add sufficient NaOH until a color change is observed, and redistill into new Boric Acid.
4. Try to use fresh DI in the distillation unit. DI sitting for extended periods of time can absorb atmospheric ammonia.

Calculations:

$$(X-Y)Z=Results$$

X = Result given in ppm
Y = result of blank given in ppm
Z = dilution factor

$$\text{Liquid samples: mg NH3-N/L} = ((A-B) \times 280)/C$$
$$\text{Sludge or sediment samples: mg NH3-N/kg} = ((A-B) \times 280)/C$$

A = volume of acid titrated for sample, mL and
B = volume of acid titrated for blank.
C= sample volume

% Recovery and % Difference
a) LCS %Recovery: (Measured Value/Theoretical Value)*100%
b) MS %Recovery: (Spiked Sample Measured Value –Sample Measured Value)*100%
c) MS %Difference: (|MS – MSD|/(MS+MSD)/2)*100%

Quality Control:

Run a blank and a duplicate matrix spike and a method standard with set of analyses
Spike recoveries should be 80 to 120 % according to standard methods, however recoveries between 90 to 110% are desired.
Recovery of method standard must be 80 to 120 % according to standard methods, however recoveries between 90 to 110% are desired.
Each quarter an external reference sample must be analyzed. If results are not within acceptable range, perform corrective action and document.

Reporting

Review: Always have the lead chemist review all data before reporting. When possible, check to insure that TKN results are ≥NH3 results.

Reporting Units: The reporting units are mg NH3-N /L

Reporting Limits: The values are valid over a range from 0.5 to 100 mg NH3-N /L. Range can be extended by dilution.

Significant Figures and Reporting Values Below Detection Limit: Report to three significant figures, and to the nearest 0.1 mg/L. Results less than 0.5 mg NH3- N/ L are reported as "ND" ("Not Detected"). If samples were diluted, multiply the PQL by the dilution factor.

QC Data: Report all QC data (LCB, LCS, MS % Rec, MSD % Diff) in QC folder located in Q:\TKN.

References
Standard Methods for the Examination of Water and Wastewater, 20th Edition, 1998
Nitrogen Determination according to Kjeldahl, Training Papers from BUCHI 1998 #97765

Written By:__Sarah McGinnis_____Title:_Chemist____ Date:11/17/2014

Approved By:__David Holland_____Title: Laboratory Director Date: 2/15/2015

TOTAL PHOSPHORUS / COLORIMETRIC PHOSPHATE

Hach 8190

Monterey Bay Analytical Service

Updated 12-10-124

Scope and Applications:

This method applies to drinking water, ground water, reagent water, and wastewater.

Principle:

Total phosphorus consists of reactive phosphorus and acid-hydrolyzable phosphorus. Reactive phosphorus is a measure of phosphates that readily react with the color reagent without digestion and consist primarily of orthophosphate. Therefore, non-digested samples that are reacted with the color reagent are reported in terms of PO4-P. Samples for total phosphorus determination are acidified and digested with an oxidizer reagent before being neutralized and allowed to react with the color reagent. Absorbance of the reacted samples is measured at 890 nm.

Detection Limits:

The detection level for reporting is 0.03 mg/L P or PO4-P

Acceptance Criteria:
Low Level Calibration Verification (LCSL): ± 50%
Instrument Calibration Verification, Continuing Calibration Verification (ICV, CCV): ± 20%
Matrix Spike Recovery: ± 30%
Duplicate RPD%: < 10%
Second Source Quality Control Sample (QCS): ± 20%
Calibration r2: ≥ 0.9995

Working range:

The working range is 0.03-1.0 mg/L P. This range can be extended if the sample is diluted.

Interferences:

- Alkaline or highly buffered samples may require additional acid during the acid addition in order to bring the pH to < 1.
- Highly turbid samples may interfere.
- Very high levels of metals may interfere. Dilute as necessary.

Preservation and Holding Times:

If samples cannot be analyzed immediately, acidify to pH < 2 with H2SO4 and store at 4 °C.

Samples requiring dissolved phosphate or dissolved total phosphorus are to be filtered through a rinsed 0.45 □m filter upon receipt and then aci

Acidified samples must be analyzed within 28 days.

Instrumentation/Equipment:

Shimadzu UV-1800 UV Spectrophotometer

Safety Precautions:

- The Test 'N Tube vials contain acidic and corrosive solution. Avoid contact
- NaOH are both corrosive. Avoid contact.
- Potassium Persulfate reagent is an oxidizer. Avoid contact.

Reagents:

- 1.54N NaOH
- Potassium Persulfate for Phosphonate (Permachem Reagent) pillow packets (Hach cat#2084766 50/pack)
- PhosVer3 Phosphate Reagent (Permachem Reagent) pillow packets (Hach cat#32106069 100/pack, cat#2106046 50/pack)
- Test 'N Tube vials (Hach cat#2743645 50/pack; tubes and reagents)
- Absolute Organic Nutrients 1000ug/mL Total Phosphorus as P (cat#24152)
- Absolute Inorganic Nutrients 1000ug/mL O-Phosphate as P (cat#54153)
- Absolute 1000ug/mL Phosphate as P (cat#54105)

Calibration Standards:

Dilute the 1000 [illegible] stock solution to 100 □g/mL P by a solution to 9.0 mL deionized water. Use this 100 □g/mL P solutio

OC-CAL Concentration (mg/L)	Volume of 100 µg/mL Solution (mL)	Final Volume (mL)
0.03	0.030	100
0.50	0.100	100
1.0	0.200	100
1.5	0.500	100
2.0	1.000	100

Pipette 5 mL of each calibration standard into labeled Test 'N Tube vials.

Second Source Standard:

Use a 1000 □g/L P Inorgai
to make the calibration standards. Dilute this second-source stock solution to 100 g/mL P by adding 1.0 mL stock solution to 9.0 mL deionized water. Add 0.05 mL of this 100 g/mL P solution to 4.95 mL DI to make a 1.0 mg/L P quality control sample (QCS). Use Phosphate as P stock for PO4 as P analyses.

Quality Control:

- Continuing Calibration Blank (CCB):
  - Analyze the CCB at the beginning of each run, every 10 samples, and at the end.
- Low Level Calibration Verification (LCVL):
  - A solution containing the same concentration as the lowest calibration solution is measured at the beginning of the batch.
- Second Source Quality Control (QCS):
  - A mid-range solution made from a source that is different from that used to make the calibration is to be measured at least quarterly and every time that new calibration standards are prepared.
- Continuing Calibration Verification (CCV):
  - A mid-range CCV should be analyzed every ten samples or at least once per batch.
- Matrix Spikes:
  - A matrix spike and a matrix spike duplicate should be analyzed every ten samples or at least once per batch. If the matrix spikes do not meet the acceptance criteria, there may be matrix interference that warrants investigation.

Digestion Procedure:

All calibration standards and quality control samples are to be digested along with samples requiring total phosphorus. Samples requiring PO4-P are not to be digested.

1. Turn on the digestion block and allow it to warm up.
2. Neutralize at least 10 mL of sample to pH 6-8 using 5N NaOH.
3. Add 5 mL of the neutralized sample to a Test 'N Tube vial.
4. Add 1 packet of Potassium Persulfate reagent.
5. Cap and shake to mix and diffuse the heat generated and Vortex to thoroughly combine reagents and samples.
6. Samples may now be placed in the digestion block. Digest for 30 minutes at 150 °C.
7. Remove samples from the digestion block and allow them to cool to room temperature.
8. Add 2 mL of 1.54N NaOH

Instrument Warm-Up Procedure:

1. Turn on the spectrophotometer. Do not open the cover while the instrument is initializing.
2. Press enter to get to the main menu and select "PC Control" at the top right.
3. Open the software and select "Connect" at the bottom of the screen (or F9).
4. Open the Phosphorus method file and allow the instrument to warm up for at least 30 minutes.

5. Set up the Standard Table for the calibration curve. Be sure to include the concentration of each calibration standard.
6. Add each sample to be analyzed into the Sample Table.

Analysis Procedure:

1. For samples requiring PO4-P, add 2 mL of 1.54 N NaOH to the Test 'N Tube vial, neutralize at least 10 mL of sample to pH 6-8 using 5N NaOH, and then add 5 mL of the neutralized sample to the Test 'N Tube vial.
2. Starting with the calibration blank, add 1 PhosVer3 packet to the sample and mix. Not all of the reagent will dissolve.
3. Allow the color-developing reaction to occur for 3 minutes and then read the absorbance in the 1 cm cuvette. Press "Read Std" or "Read Unk" at the bottom of the screen (or F9) to record data.
4. Repeat steps 4-5 for each calibration standard and then each sample.

Shutdown Procedure:

1. Disconnect the software from the instrument by selecting "Disconnect" or pressing F10.
2. Save data before closing.
3. Power off the spectrophotometer.

References
Hach Method 8190 (DOC 316.53.01121)
Standard Methods 4500-P E; 21st Edition

Written By: Heather Craven Title: Chemist Date: 04/21/2014

Updated By: Sarah McGinnis Title: Chemist Date: 12/10/2014

Approved By: David Holland Title: Lab Director Date: 2/15/2015

PH METER

4500/H+B

Standard Methods 19th Edition

1. Check electrode for crystals. If crystals are present, empty solution, rinse with DI water until clear of crystals and refill with Orion Filling Solution #810007. Orion filling solution does not contain AgCl. The DI water and filling solution can be emptied from the electrode by pushing down on the top of the electrode.
2. A 3 point calibration (pH 4, pH 7, & pH 10) is performed daily following the Acumet 50 instructions. The slope and efficiency is checked. The efficiency must be above 98% and no greater than 102%. The calibration and efficiency is logged on the form. The pH is calibration is confirmed using a reference standard supplied by RICCA.
3. If the efficiency is below 98% clean the electrode and calibrate again. If a 98% efficiency can not be obtained the electrode should be replaced. Also the buffer solutions should be checked to make sure its expiration date has not been passed.
4. All remedial actions will be logged on the "corrective action" form in the pH section of the QA/QC notebook.
5. After the meter is calibrated, place the electrode in pH 7 buffer until ready for use. Rinse electrode with DI water before use.
6. Place electrode in sample and allow it to stabilize before taking pH reading. Afterwards rinse electrode in DI water and place in pH 7 buffer.

PORTABLE pH METER

1. Following instructions for removing crystals same as with Accumet electrode.
2. Calibrate pH as per the Orion instructions. Place in pH 7 buffer.
3. Calibrate daily or whenever the meter is used.
4. Rinse with DI water before placing in sample.
5. Allow pH to stabilize before taking reading.
6. Rinse with DI water after reading and replace in pH 7 buffer.

DISSOLVED OXYGEN

Hach Method 10360

City of Watsonville Environmental Laboratory

Updated 9-16-2015

Scope and Application

1. This method is for the measurement of dissolved oxygen (DO) in surface and ground water, and municipal and industrial wastewater.
2. The method may be used as a replacement for the modified Winkler and membrane electrode procedures for the measurement of DO in wastewater treatment processes such as aeration and biological nutrient basins, effluent outfalls, receiving water, and in Biochemical Oxygen Demand (BOD) determinations where it is desired to perform nondestructive DO measurements.
3. The method is for use in the United States Environmental Protection Agency's (EPA's) survey and monitoring programs for the measurement of DO and for the determination of BOD and cBOD under the Clean Water Act.
4. This method is capable of measuring DO in the range of 0.20 to 20 mg/L.
5. Calibration is by single-point water-saturated air (100% saturation).
6. The luminescence technology for measuring dissolved oxygen is a superior technique from that of Winkler titration and membrane potentiometric measurement and has no interferences associated with oxygen detection process (EPA Validation study, 2004). Therefore, do not adjust the luminescence measurement to that of Winker or membrane readings.

Summary of Method

This luminescence-based sensor procedure measures the light emission characteristics from a luminescence-based reaction that takes place at the sensor-water interface. A light emitting diode (LED) provides incident light required to excite the luminophore substrate. In the presence of dissolved oxygen the reaction is suppressed. The resulting dynamic lifetime of the excited luminophore is evaluated and equated to DO concentration.

Interferences

1. There are no known interferences at normal wastewater concentrations that interfere with DO detection and quantification with this method.
2. As the temperature of water increases, the solubility of oxygen decreases. The HQ40d meter automatically accounts for temperature.
3. As salinity increases, the solubility of oxygen decreases. If measuring dissolved oxygen in marine or brackish waters, use a conductivity meter to determine the salinity of the water to be measured and use the meter's Salinity Correction function
4. As pressure decreases (altitude increases), the solubility of oxygen decreases. The HQ40d meter automatically accounts for barometric pressure.

Precaution

1. Read the user manual for the meter and probe and follow the instructions for maintaining, calibrating, and measuring with the instrument.
2. Use care not to scratch the luminophore substrate on the surface of the sensor cap. If the cap is scratched, it should be replaced. The probe shroud helps protect the cap and should be kept in place when possible.
3. Shield the sensor cap from direct sunlight when taking measurements. The probe shroud helps protect the cap from light interference. Indirect light should not affect dissolved oxygen measurements.

Equipment

1. Hach HQ40d meter
2. Hach LDO101 probe
3. Narrow-necked bottle and stopper (BOD bottle)
4. Oxygen demand free water

Calibration

1. Prepare a calibration chamber by adding about 1cm of demand-free water to a BOD bottle, stoppering, and shaking vigorously for several minutes. Alternatively, if a bottle is not available, a wet paper towel may be wrapped around the probe shroud to create a saturated-air calibration environment.
2. Press the BLUE/LEFT key under Calibrate. (If using the HQ40d meter with two probes, the display must be in single screen mode.)
3. Dry the probe and place it in the calibration chamber.
4. Press the GREEN/LEFT key under Read. (Be sure that no water is on the probe after placing it in the calibration chamber.)
5. When the reading is stable the standard value will be highlighted on the screen and the calibrated reading value will appear on the screen. Press the UP key under Done.
6. The Calibration Summary will appear. Press the GREEN/RIGHT key under Store to accept the calibration and return to the measurement mode. The calibration is recorded in the data log.
7. When the calibration is successful, the display will show OK in the upper left corner. A question mark will be displayed if the calibration has expired.
8. If the calibration slope does not meet the acceptance criteria, the display will show "Slope out of range". If this happens, allow the probe to stand in the water-saturated air for several minutes to reach equilibrium and re-press the GREEN/RIGHT key under Read.

Measuring Dissolved Oxygen

1. Whenever possible, the dissolved oxygen measurement should be taken *in situ* by directly placing the probe into the water to be tested. If a sample must be collected in a container prior to the measurement, the sample must be collected with minimal agitation and air entrainment in a zero head-space container and analyzed at the same temperature as soon as possible.
2. Place the probe into the sample
3. Press the GREEN/RIGHT key under Read.
4. The display will show "Stabilizing..." and a progress bar will fill from 0% to 100% as the probe stabilizes in the sample. When the result has stabilized, the lock icon will appear and the result will be stored automatically in the data log.
5. To make another measurement, repeat this procedure.

Quality Control

1. Calibrate the probe prior to running each batch of samples.
2. Perform a duplicate measurement every ten samples and at least once per batch. The relative percent difference (RPD) of the last 20 to 30 replicate samples is plotted on a control chart. If

the RPD of any set of duplicates falls beyond the control limits, or if the RPD of any successive sets of duplicates falls beyond the warning limits, the cause is investigated and corrected. Any corrective actions are logged into the QA/QC notebook. These actions may include recalibrating, replacing the sensor cap, recollecting or re-measuring the sample, or checking for other instrument problems according the troubleshooting section in the manual.

Solid Phase Adsorption Toxin Tracking (SPATT), to monitor microcystin levels in Pinto Lake and other water bodies

SPATT was first proposed for HAB monitoring as a means by which disadvantages associated with shellfish or other indicator organisms could be circumvented. These passive sampling systems had previously been utilized to detect a wide range of non-HAB environmental pollutants. SPATT deployment provides an inexpensive, simple, highly sensitive and precise means for environmental detection of microcystins, greatly facilitating efforts to link environmental triggers for toxin production with direct toxin measurements. SPATT bags are constructed from 100 micron Nitex bolting cloth sealed on three sides using a sealer to form a 55 mm wide open bag. The bag is filled with 3 g (dry weight) HP20 (Diaon) resin and the fourth side sealed to form a 55 mm^2 SPATT bag. For activation, SPATT bags are soaked in 100% MeOH for 48 hours, then rinsed thoroughly in Milli-Q and transferred into a fresh volume of Milli-Q for MeOH residue removal by sonication. The bags are stored in Milli-Q at 4-6 C prior to use. For field deployment, the bags are attached to plastic embroidery hoops which are used to fasten the bags in place. For microcystins, we achieve 100% recovery with simple extraction procedures (sequential 50% MeOH column extractions).

The preferred method of analysis after extraction is LCMS, following the protocols of Mekebri et al. (2008). Briefly, the assay (used for all cyanotoxin measurements) employs certified cyanoHAB standards (LR, RR, LF, LA, YR, LW, NOD-R and anatoxin-A) from various sources, along with HPLC-grade solvents (acetonitrile, methanol, water), glass fiber filters (Type A/E, 90mm, 1 µm), Gelman Acrodisc® CR PTFE syringe filters (13 mm, 0.45 µm), mobile phase additives, ACS grade formic acid (98%) and trifluoroacetic acid (99%). A combined intermediate working solution of microcystins will be made in methanol and a matrix spiking solution (20 ppb) used to fortify samples will be prepared and serial diluted to make a seven level calibration curve, ranging from 0.2 to 200 ppb. To determine total microcystin concentrations and congener types in water, the cell wall will be ruptured by repeated freeze-thawing and sonication. A 100ml aliquot will be filtered under vacuum through a glass fiber filter. Water and filters will be extracted separately and filters containing planktonic material will be extracted twice with 15 mL of methanol:acidified water (90:10, v/v) by homogenizing for 1-2 minutes using a Polytron, followed by 10 minute sonication in an ultrasonic bath. For SPATT, only dissolved toxins are measured, so the cell disruption steps are not needed.

LABORATORY PROCESSING, IDENTIFICATION, AND ENUMERATION OF STREAM ALGAE

Surface Water Ambient Water Monitoring Projram

Updated September 2015

Section 2: Laboratory Sample Receipt

Three separate stream algae samples are collected in the field and delivered to the lab as described by Ode et al. (2015).

SBA qualitative sample: Unpreserved sample consisting of a composite of all types of SBA macroalgae visible within the stream reach. This sample is collected in a 100 mL Whirl-Pak® bag and kept cool (4°C) and dark until it is received by the laboratory.

SBA quantitative sample: Sample preserved with 2% glutaraldehyde in 50 mL plastic centrifuge tube. If the samples arrive unpreserved, follow steps listed in Section 2.2.2.

Upon delivery, the laboratory technician receives, inspects, and documents the incoming samples. A unique laboratory sample identification code (lab sample ID) for internal tracking purposes may be assigned to each sample. The condition of each sample upon receipt is assessed against the SWAMP required sample handling criterion (see Section 5.3).**2.1 Sample Receipt**
Upon receipt, the laboratory must confirm that the sample labels match the chain of custody (COC) forms and all samples are accounted for. Sample site IDs should be written legibly on labels. Copies of the COCs must be retained as a record.

2.2 Sample Integrity Check

Following receipt, the laboratory must inspect each sample and confirm sample integrity has been maintained to the level indicated. Sample handling requirements and associated corrective actions are specified in Table 1 and Table 2 of Section 5.3.1.

2.2.1 SBA Qualitative Sample Integrity Check

- Confirm the sample is received in a 100 mL Whirl-Pak® bag.
- Confirm the sample is cool (4°C) upon receipt. Note if warm on the COC.
- Inspect the sample for evidence of freezing. Note evidence of freezing on the COC.
- Inspect the sample for evidence of leaking during shipping. Leaking can result in cross contamination of samples. Note evidence of leaking on the COC.
- Confirm the sample has been received within 2 weeks of collection.
- If the qualitative SBA sample is received more than 2 weeks from collection, or if the integrity of the sample upon receipt is in question, the taxonomist must inspect the sample to determine the extent of sample degradation and document these findings on the COC.

2.2.2 SBA Quantitative Sample Integrity Check

- SBA quantitative sample may arrive unpreserved.
- Confirm that the SBA quantitative sample has been preserved. If the sample is received unpreserved, it must be preserved as soon as possible within 4 days of collection with 2% glutaraldehyde final concentration. The volume of the unpreserved sample, amount of glutaraldehyde added, and date and time of preservation must be documented on the COC.
- Samples preserved in the field are preserved with 2% glutaraldehyde in 50 mL plastic centrifuge tube.
- The total volume of the field-preserved sample should be 50 mL (45 mL sample and 5 mL preservative). Vials received with less than 50 mL of preserved sample may indicate the sample was not preserved or had leaked during transport.

2.2.4 Receipt of Broken Sample Vials

If a vial is cracked or leaking it must be transferred to a new vial according to the following procedure:

- Transfer the affected sample to a new 50 mL plastic centrifuge tube with a label containing the sample information.
- Measure and record the remaining sample volume.
- Document the sample condition.
- Add additional preservative and note volume added on COC.

Note any action taken on the COC and notes section of the laboratory database sample log in.

Section 3: Sample Preparation

After algal samples are received, samples are prepared for taxonomic analysis. The sample preparation

process is different for the three different algae samples: (1) SBA qualitative sample; (2) SBA quantitative sample (macroalgae and microalgae fractions).

The purpose of analysis of qualitative SBA samples is to identify as many macroalgal taxa present in the sample as possible. Macroalgal species identification requires observation of enough unfixed material representing different life stages to determine vegetative features, reproductive mode, and characteristics of completely developed reproductive structures of each species. All macroalgal taxa are identified to lowest possible taxonomic level (usually to species). Quantitative SBA samples contain algae of different sizes requiring detailed observations of many cellular, vegetative and reproductive structures in order for the species to be identified. For proper identification and enumeration of SBA taxa, macroalgal and microalgal fractions of each sample are processed separately. The purpose of analysis of quantitative SBA samples is to identify as many SBA taxa present in the sample as possible, to provide an accurate algal taxa list and uniform biovolume estimate of each algal taxon in a sampled stream reach. This procedure is designed to produce a comprehensive list of all algal taxa identified to lowest possible taxonomic level (usually species) together with a precise estimate of their individual volumetric contribution per unit area sampled.

During the sample preparation and consequent taxonomic analysis, care should be given to avoid sample cross contamination by using disposable materials, or carefully washed and DI rinsed materials. Instrumentation should be used only for an individual sample and then immediately stored for decontamination. Dropper bottles with DI or Lugol's Iodine Solution, used multiple times, should not touch the algal material. Sample splashing should be avoided when multiple samples are processed.

3.1 SBA Qualitative and Quantitative Sample Preparation

This section describes initial preparation of SBA samples for taxonomic analysis. The processed samples are used for semi-permanent water mounts prepared by the taxonomist prior to algae identification and enumeration (Sections 3.1.4 and 4.1).

3.1.1 SBA Qualitative Sample Preparation

Materials needed:

- Fresh sample in a 100 mL Whirl-Pak® bag
- Glass specimen dish
- Forceps (30 cm long) and jewelers forceps
- DI water
- Beakers (50 mL)
- Microscope slides
- Cover slip - 22 x 30 mm, No 1 thickness
- Dissecting and compound microscope, each with digital camera

Step 1: Very gently transfer the fresh macroalgae from the field plastic bag into a glass dish containing DI water.

Step 2: When the taxonomic work on the sample is completed (see Section 4.1.1) archive a portion of the fresh sample (see Section 4.3.1). Archiving of the SBA qualitative sample should be conducted as soon as possible following completion of the taxonomic analysis. Return the remaining material to the original plastic bag, loosely capped, adding DI water if needed. The fresh sample should be archived for two more weeks in the refrigerator at 4°C in case further examination is needed.

3.1.2 SBA Quantitative Sample Preparation – Macroalgal Fraction

Materials needed:

- Preserved composite sample in 50 mL plastic centrifuge tube
- Forceps (30 cm long) and jewelers forceps
- DI water

- 15 mL graduated centrifuge tube with graduations in 0.1 mL increments up to 1 mL, and 0.5 mL increments above
- 50 mL graduated centrifuge tube with graduations in 2.5 mL increments
- Grid bottom culture dish
- Microscope slides
- Cover slips - 22 x 30 mm, No 1 thickness
- Dissecting and compound microscope, each with digital camera

Step 1: Obtain the 50 mL centrifuge tube with preserved composite sample and visually inspect its content to estimate whether a 15 mL or 50 mL tube is needed for macroalgae fraction collection.

Step 2: Label a 15 mL or 50 mL graduated centrifuge tube with the following information:

- SWAMP sample ID
- Date of collection (MM/DD/YYYY)
- Note "macroalgae" on the label to distinguish from the microalgae fraction
- Macroalgae volume: xx mL

If the macroalgal fraction is very large, use a 50 mL graduated centrifuge tube with 2.5 mL increments.

Step 3: Place 10 mL of DI water into the labeled centrifuge tube.

Step 4: Using the forceps (30 cm long), very gently pinch the material at the bottom of the tube. Search for visible macroalgal clumps, and any solid particles in the sample, such as mosses, vascular plant tissues, roots, etc. Gently pull up the forceps and slowly move the macroalgae and all solid particles grasped between the forceps in the solution to remove extra clinging sediment and isolate any macroalgal filaments in the sample. Repeat this step at least three times before proceeding to the next step.

Step 5: If macroalgal clumps are present in the sample continue onto Step 6. If no macroalgal clumps are present, proceed with preparation of the microalgae fraction (Section 3.1.3). If no macroalgae and any solid particles are visible to the naked eye, inspect the sample tube under a dissecting microscope before proceeding with microalgae preparation.

Step 6: Using forceps, remove the macroalgae from sample very gently, squeeze it to remove as much liquid as possible and then place it into the tube with 10 mL DI water. Continue until no macroalgae remain.

Step 7: Determine the volume of macroalgal fraction by the increase (displacement) from the original 10 mL of water. When using 15 mL centrifuge tubes with graduated markings measuring 0.5 mL, estimate the water displacement to 0.1 mL (See Note 1 below). Record the volume of the macroalgal fraction (mL) in the ID Datasheet for SBA Sample- Heading: Qualitative sample – Heading: Macroalgal fraction-total volume: xx mL (Appendix C1) and on the label of the tube with the macroalgal fraction.

Note 1: The surface of water in a tube is not completely flat. Instead, the surface curves in a shallow U-shape meniscus. When measuring, read the line just at the bottom of the meniscus.

3.1.3 SBA Quantitative Sample Preparation – Microalgal Fraction

Materials needed:

- Preserved composite sample in 50 mL plastic centrifuge tube remaining after macroalgae removal
- 10 mL pipette
- 50 mL centrifuge tube with graduations in 2.5 mL increments
- 15 mL centrifuge tube with graduations in 0.1 mL increments up to 1 mL, and 0.5 mL increments above
- Dissecting needles

- Table-top centrifuge
- 146 mm borosilicate pipette
- Microscope slides
- Cover slip - 22 x 30 mm, No 1 thickness
- Compound microscope with digital camera

Step 1: Obtain the 50 mL centrifuge tube containing the SBA quantitative sample following removal of the macroalgae fraction. Homogenize the microalgal fraction of the SBA quantitative sample by gently but thoroughly inverting the centrifuge tube several times. The sample must be well homogenized prior to sub-sampling (Step 2).

Step 2: Pipette 5 mL of homogenized microalgae fraction into a 50 mL centrifuge tube labeled with the sample information.

Step 3: Fill the centrifuge tube with DI water to the 50 mL mark. Let the sample settle for a minimum of 12 hours.

Step 4: Once the sample has thoroughly settled, gently remove the supernatant layer down to a volume of 5 mL by using a pipette. Avoid disturbing the algal material on the bottom of the tube.

Step 5: Label a 15 mL graduated centrifuge tube with the following information:

- SWAMP sample ID
- Date of collection (MM/DD/YYYY)
- Note "microalgae" on the label to distinguish from the macroalgae fraction

Step 6: Transfer the 5 mL of sample material from the 50 mL centrifuge tube to the labeled 15 mL graduated centrifuge tube.

Step 7: Rinse down the sides of the 50 mL centrifuge tube several times with DI water to capture any remaining algae clinging to the sides. Transfer the rinse liquid to the labeled 15 mL graduated centrifuge tube.

Step 8: Fill the labeled 15 mL graduated centrifuge tube with DI water to the 15 mL mark.

Step 9: Centrifuge the sample for 5 min at 4000 RPM on a table-top centrifuge. Step 10: Remove the supernatant layer until 1 mL sample is left by using a pipette. Avoid disturbing the algal material on the bottom of the tube. This procedure concentrates the microalgal fraction 5 times while removing most of the glutaraldehyde before microscopic examination. From this 1 mL sample, a semi-permanent slide is prepared for analysis (Section 3.1.4 below).

3.1.4 SBA Semi-permanent Slide Preparation of Quantitative Microalgal Fraction

Materials needed:

- 15 mL centrifuge tube with microalgal fraction of 1 mL
- DI water
- Dissecting needles
- 146 mm borosilicate pipette
- Microscope slides
- Cover slip - 22 x 30 mm, No 1 thickness
- Nail polish
- Compound microscope with digital camera

Step 1: Obtain the 15 mL centrifuge tube containing 1 mL concentrated microalgal fraction.

Step 2: Visually inspect the sample and evaluate the amount of material (sediment and algae) settled on

the bottom of the tube. If the sample contains more than 0.5 mL of material (sediment and algae) settled on the bottom, dilute with DI water to a final volume of 2, 3, 4 or 5 mL. If a small amount of material (sediment and algae) is present, centrifuge for 5 min at 4000 RPM on a table-top centrifuge and concentrate the sample to 0.5 mL. Record any additional dilution or concentration performed on the sample and the final sample volume used for slide preparation in the ID Datasheet for SBA Sample-Heading: Quantitative sample-Microalgal fraction-Sample volume after additional dilution/ concentration: xx mL (Appendix C1). This information is required for SBA biovolume calculations (see Section 4.1.4.2, Appendix F).

Step 3: Vortex or pipet-mix the sample and subsample with pipette from the center of the well-mixed material. Place 1 drop (0.05 mL) of sample on a standard microscope slide and cover with a 22 x 30 mm cover slip. Proper preparation of the slides is vital to performing identifications. The following should be noted while preparing slides:

- Ensure that the volume of the drop is not so large that it creates the formation of bubbles or causes the cover slip to float.
- Avoid having too much or too little material on the slide. Too much material results in layers of cells, specimen overlap and a non-flat cover slip which interferes with accurate identification. Too little material increases the amount of time required to complete analysis and may not be adequate for proper identification and enumeration.
- Small thick clumps of spreading filaments intermixed with colonial algae can sometimes occur in the microalgal fraction. Clumping of material not only interferes with accurate identification and enumeration, but can circumvent the assumption of random distribution of specimens on the sides. These clumps usually contain several different species, so they should to be dispersed before proceeding with analysis. Gentle tapping on the cover slip or spreading the clump apart with a pair of dissecting needles will reduce clumping.

Step 4: Inspect the semi-permanent microalgal slide at lower magnification (200x) using a compound microscope to confirm that microalgae are evenly distributed. Gently adjust the cover slip if algal clumps are present. Cover slip may be sealed with nail polish to prevent evaporation. This semi-permanent microalgal mount is good for analysis for at least two hours.

4.1.1 SBA Qualitative Sample Analysis

Step 1: Using a research-grade dissecting microscope carefully examine the macroalgal fresh sample placed into glass dish and determine the number of macroalgal genera in the sample appearing different in morphology or color.

Thoroughly examine all the material and identify key macroalgal features needed to separate the algae by genus. These may include:

- Colonial shape, size and color in cyanobacteria (such as Nostoc, Dichothrix, Rivularia);
- Different life stages, heterocyst position and akinete development in cyanobacteria (such as Anabaena, Cylindrospermum, Gloeotrichia);
- Male and female specimens with developed reproductive structures in red and green algae (such as Batrachospermum, Sirodotia, Oedogonium);
- Different life stages and completely matured reproductive structures in zygnematalean algae and tribophytes (such as Spirogyra, Zygnema, Mougeotia, Vaucheria).

Step 2: Place each macroalgal genus identified aside. When small rocks are collected, carefully examine the surface of the rocks for attached algae using dissecting microscope. If algae are present, scrape them out and place them on a microscope slide.

Step 3: Prepare microscope slides for each macroalgal genus which may be presented with more than one species in the sample. The number of slides prepared depends on the need to obtain sufficient information to successfully perform species identification. When reproducing filaments of zygnematalean

algae are observed, but completely matured zygospores/aplanospores are not available, further incubation under nutrient stress facilitates completion of sexual or asexual reproduction. The resulting mature zygospores (or akinetes, aplanospores) can provide the taxonomist with the additional information needed to identify the species. To incubate the algae under nutrient stress:

- Slect the conjugating filaments of Spirogyra, Zygnema or Mougeotia and place them in a 50 mL glass beaker filled with DI water.
- Keep the samples out of direct sunlight (in a north-facing window) at room temperature until reproductive structures completely develop. Check for reproductive filaments under the microscope every three days and document the different stages of conjugation and development of zygospores (or akinetes, aplanospores).

Step 4: Examine prepared slides under the compound microscope and identify SBA macroalgae to species level. If large colonial diatoms are observed in the sample, record them following the recommendations in Section 4.1.2 Step 5.

Step 5: Take sufficient photomicrographs of all newly recorded species to support harmonization of results. Take photomicrographs of previously reported species to demonstrate the key aspects of vegetative morphology and reproduction used in identification (see Section 1.5 and Appendix H).

Step 6: Record all macroalgal taxa identified in the SBA qualitative sample in the ID Datasheet for SBA Sample under the heading Qualitative sample – list of taxa (Appendix C1).

Step 7: Submit the remaining SBA qualitative sample and the algal material from the slides for archiving (see Section 4.3.1).

4.1.2 SBA Quantitative Sample Analysis – Macroalgal Fraction

Macroalgal fractions extracted from different quantitative SBA samples vary considerably in total volume and content. Typically this fraction consists of soft-bodied macroalgae sometimes mixed with large colonial diatoms. However, due to the field sampling protocol (Ode et al., 2015), the macroalgal fraction may contain non-algal matter, such as mosses, vascular plant tissues, roots or debris, macroinvertebrates, etc., which should be subtracted from the macroalgal sample total volume. Many SBA and diatom macroalgae, as well as vascular plants, support development of epiphytic SBA algae, which are identified and enumerated also. The approach for species identification of the macroalgae from the quantitative and qualitative samples (Section 4.1.1) is similar except for the process of algae incubation. Microscope slides for macroalgae and SBA epiphytic species identification need to be prepared during the identification process following the observations under the dissecting microscope.

Step 1: Start with the 15 mL or 50 mL centrifuge tube containing the macroalgal fraction extracted from the SBA quantitative sample.

Step 2: Transfer the contents of the centrifuge tube to a gridded Petri dish.

Step 3: Using forceps gently spread the material in the sample evenly throughout the Petri dish.

Step 4: Carefully examine the material under the dissecting microscope. If the sample contains non-algal matter such as mosses, vascular plant tissues, roots or debris, separate this material from the macroalgae and determine its volume. If it is possible to remove the non-algal matter, place it in centrifuge tube and measure the volume using water displacement. If the non-algal matter is not possible to remove, visually estimate its proportion. Calculate the fraction of the total sample volume represented by non-algal matter. Record the fraction represented by non-algal matter in the ID Datasheet for SBA Sample-Heading: non-algal matter xx % (Appendix C1).

Step 5: After the non-algal matter has been separated, gently distribute the soft-bodied macroalgae evenly using forceps. When working with the macroalgal fraction, care should be given to avoid breaking up large filaments or colonies and to preserve key features needed for species identification. Since large

reproductive structures are easily damaged, the clumps should be spread very carefully. If the sample contains macroalgal colonial diatoms (such as Melosira, Pleurosira, Terpsinoe, Cymbella, Gomphonema, Didymosphenia, Bacillaria, etc.) separate this material from the soft-bodied macroalgae and determine the representative proportion of the diatoms as a percent of total macroalgal fraction volume. If possible, identify diatoms to genus level, if not – use the general category "diatoms". This information can help recording invasive or bloom-forming macroalgal diatom taxa, which may be omitted in diatom analysis due to numerical dominance of smaller diatom species.

Step 6: Using a dissecting microscope, thoroughly examine the sample and identify key macroalgal features needed to segregate the SBA by genus. Determine the number of distinct soft-bodied macroalgal genera present in the sample.

Step 7: Prepare microscope slides for each soft-bodied macroalgal genus. The number of slides depends on the need to obtain sufficient information to successfully perform identification and support the STE.

Step 8: Examine prepared slides under the compound microscope. Identify soft-bodied macroalgae to species level.

Step 9: Estimate the representative proportion of each soft-bodied macroalgal species as a percent of total macroalgal fraction volume in order to determine their biovolume (see Section 4.1.4.1).

Step 10: Record the name and the representative proportion of each soft-bodied macroalgal taxon identified in the ID Datasheet for SBA Sample-Heading: Macroalgae taxon ID; Proportion of each taxon (%) (Appendix C1).

Step 11: When the total volume of macroalgal fraction is so low that it is not possible to be measured by water displacement, prepare a microscope slide containing all macroalgal material. Under compound microscope identify all macroalgal taxa and determine directly their biovolume by individual measuring of each specimen using geometric shapes (such as cylinder for filamentous algae, or sphere for young colonies of Nostoc, for details see Section 4.1.3 Step 4). Calculate the total biovolume of each macroalgal species by summing the biovolumes of all specimens measured. In this case the biovolume of each macroalgal species is directly measured in μm^3.

Step 12: Identify to species level and enumerate 100 NCEs of epiphytic SBA attached to the surface of the soft-bodied macroalgae and other aquatic substrates. If fewer than 100 NCEs of epiphytic SBA are observed, enumerate as many as there are in the entire macroalgal fraction.

Step 13: Record each SBA epiphytic taxon identified and the corresponding number of NCEs enumerated in the ID Datasheet for SBA Sample-Heading: Epiphyte taxon ID; #NCE (Appendix C1).

Step 14: Take sufficient photomicrographs of all newly recorded species to support harmonization of results. Take photomicrographs of previously reported species to demonstrate the key aspects of vegetative morphology and reproduction used in identification (see Section 1.5 Photographic Documentation of Algae and Appendix H).

Step 15: Return the entire content of the macroalgal fraction, including the material from the slides, and the non-algal matter into the tube with macroalgal fraction of the SBA quantitative sample and submit it for archiving (see Section 4.3.1).

4.1.3 SBA Quantitative Sample Analysis – Microalgal Fraction

The microalgal SBA fraction is examined on a semi-permanent water mount for best observation of algal cellular morphology. Water mounts allow adjusting of the cover slip to change the position of the cells and spreading out of multilayered cell clumps for observation of critical taxonomic features. Furthermore, specific techniques, such as staining (e. g. starch with Lugol's iodine solution) are possible to apply on water mount.

Step 1: Using a research quality compound microscope, scan the semi-permanent slide with microalgae at magnification 200x to assess the taxonomic composition of the sample.

Step 2: Switch to a magnification of 400x (e.g., 40x objective with 10x eyepieces). At a magnification of 400x, the cover slip is composed of many horizontal optical transects.

Step 3: Identify and enumerate 300 SBA NCEs across a known number of horizontal optical transects. Count only intact cells with complete cell contents. If 300 SBA NCEs have been counted before reaching the end of the last transect, continue enumeration and complete the transect. This ensures that a known fraction of the sample is analyzed. Record the number of horizontal transects traversed, each SBA microalgal taxon identified and the corresponding number of NCEs enumerated in ID Datasheet for SBA SampleHeading: Microalgal fraction-number of transects counted: xx, Microalgae taxon ID; #NCE (Appendix C1).

The taxonomist should adjust the total of NCEs enumerated under the following circumstances:

- If the sample contains numerous small single cells or new taxa appeared after counting 300 SBA NCEs, continue enumeration to 400 or 500 NCEs. If the morphological information obtained during the enumeration of 300 NCEs is not sufficient for species identification of certain taxon, continue observations of more specimens outside the counted area until species level identification is achieved.
- Stop counting after enumeration of the first complete slide if fewer than 20 SBA NCEs are recorded. Enumeration is stopped regardless of whether the sample received additional dilution or concentration prior to slide preparation.
- Prepare additional slides if more than 20 SBA NCEs are recorded in the first slide. Continue enumeration for 4 hours or until 150 SBA NCEs have been enumerated (whichever comes first). Exclude the time spent identifying and documenting new species from the total enumeration time.

Step 4: Using the techniques described by Hillebrand et al. (1999), determine the appropriate geometric model for each microalgal species identified. The list of geometric shapes corresponding to the SBA genera reported for SWAMP are detailed in Appendix E. Perform microscopic measurements of the cell dimensions for each algal entity according to the closest geometric shape Record measurements in the ID Datasheet for SBA SampleHeading: Cell diameter (µm); Cell/Filament length (µm); Cell depth (µm); Total number of cells; Total filament length (µm) (Appendix C1).

The following should be noted while estimating biovolumes:

- Measurements for each single SBA NCE are made concurrent with identification and enumeration. Most microalgal NCE can be viewed as cylinders, spheres, spheroids, or cones in order to estimate biovolume. Proper biovolume estimate requires measurements of two or three dimensions, such as cell width, length and depth. Depth measurements are made on specimens from a side view. This can be achieved by lightly tapping the coverslip to turn the specimen. If the specimen cannot be measured from the side, estimate the depth and include a note in the data record. SBA size measurements should be made using an ocular micrometer or by image analysis software when photomicrographs are available.
- A multicellular coccoid colony is considered to be a single NCE. However, the number of cells in the colony varies among the species and in many taxa increases with the age (for instance, cyanobacterial species belonging to Aphanocapsa and Aphanothece). When the colony consists of a small number of cells, measure the dimensions of all individual cells. If the colony contains a large number of cells average the cell dimensions (diameter and length) obtained by measurement of 20 cells and record the total cell number.
- Often the colonies break into fragments with different sizes, each one of which is considered a NCE. The biovolume of colonial NCE is calculated by multiplying averaged cell biovolume by number of cells per colony.

- For a filament, which is considered a single NCE, measure the width and the whole length of the filament. When some filamentous taxa are abundant in the sample (such as, species belonging to Leptolyngbya and Heteroleibleinia) average the width and the length of the filaments based on the measurements of 20 NCE.
- However, size averaging should be applied only for an individual sample, and not extrapolated to other samples, because filaments and colonies break apart into fragments with different lengths under variable conditions.

Step 5: Take sufficient photomicrographs of all newly recorded species to support harmonization of results. Take photomicrographs of previously reported species to demonstrate the key aspects of vegetative morphology and reproduction used in identification (see Section 1.5 and Appendix H).

Step 6: Submit remaining microalgal fraction of the SBA quantitative sample for archiving (see Section 4.3.2).

4.1.4 Biovolume Calculations for SBA

The biovolume for each identified SBA taxon is measured during the identification and enumeration. Precision of the SBA biovolume estimates is achieved by separate processing of macroalgal and microalgal fractions and by cell size measurements made for each single microalgal NCE (see Stancheva et al., 2012a for details).

4.1.4.1 Biovolume Calculations: SBA Quantitative Sample – Macroalgal Fraction

Step 1: Determine the representative proportion of each macroalgal species as a percentage of the total volume of macroalgal fraction, from which the non-algal matter has been subtracted, to calculate the biovolume of each species.

Determine the biovolume of each species in mL and convert it to µm 3 when multiplied by 1012. Note that in some cases the biovolume of macroalgal species is directly estimated in µm 3 (see Section 4.1.2 Step 11).

This is a total biovolume of i-species (Va) in macroalgal fraction of sample in µm3 .

Step 2: The value (Va) is multiplied by 4 in order to estimate the biovolume of each macroalgal species in the original field composite sample (Va'), from which only 1/4 of the macroalgae were transferred into the 50 mL tube received in the laboratory for SBA analysis (Ode et al., 2015, p. F-1).

Va' = 4 Va (µm3)

Use the following formula to calculate the biovolume of each macroalgal species in µm 3 per cm2 stream bottom area sampled. The result unit is µm 3 cm-2 .

Vi=Va' A-1 (µm 3 cm-2)

where:

Vi = biovolume of i-species (µm 3) per 1 cm2 stream bottom area sampled

Va' = biovolume of i-species (µm 3) in the original composite sample

A = stream bottom area of substratum sampled. It is the total area of substratum surface from which benthic algae were collected (Ode et al., 2015). This information is provided for each sample in cm2 .

4.1.4.2 Biovolume Calculations: SBA Quantitative Sample – Microalgal Fraction

Step 1: Calculate the biovolume of each microalgal NCE using the measured dimensions and formulae for geometric shapes closest to the cell's shape, proposed by Hillebrand et al. (1999) and specified for the

SBA genera recorded by SWAMP (see Appendix E). For each microalgal taxon, sum the biovolumes of all NCE recorded in the known number of analyzed optical transects.

This is a total biovolume of i-species (Va) in the analyzed optical transects in µm3 .

Step 2: This value (Va) needs to be corrected for the sample dilution, caused by addition of 5 mL of glutaraldehyde to the composite sample. The correction factor (Vcr) is calculated as follows:

Vcr= (Vt-Vm) (Vt-Vm-5)-1

where:

Vcr = a correction factor for sample dilution with fixative (assuming 5 mL of fixative was added to the sample)

Vt = total initial sample volume in the sample vial (generally~50 mL)

Vm = volume of macroalgal fraction in the sample (will be 0 if no macroalgae detected)

Then to correct biovolume of i-species (Va) for fixative dilution use the following formula:

Va'=Va Vcr (µm3)

Step 3: Use the following formula to calculate the biovolume of each microalgal species (µm 3) per 1 cm2 stream bottom area sampled. The result unit is µm 3 cm-2 .

Vi=Va' Vs Vc-1 A -1 (µm 3 cm-2)

where:

Vi = biovolume of i-species (µm 3) per 1 cm2 stream bottom area sampled

Va' = biovolume of i-species (µm 3) per sample counted (known number of optical transects in which the enumeration has been done) corrected for the dilution with fixative (Vcr)

Vs = composite sample volume (mL). It is the volume of all the liquid material amassed during sampling, including water used for rinsing substrate and sampling devices. Final composite volume typically does not exceed 400-500 mL (Ode et al., 2015). This information is provided for each sample in mL.

Vc = volume of sample counted (mL) [this is the number of transects counted multiplied by the sample volume per transect and by dilution factor (see Note 5 below)];

A = stream bottom area of substratum sampled. It is the total area of substratum surface from which benthic algae were collected (Ode et al., 2015). This information is provided for each sample in cm2 .

Note 5: The sample volume contained in one horizontal transect is determined as follows: a transect is a rectangular area of the slide in which the width is equal to the field of view and the length is equal to the length of the cover slip. With our microscope condition, at a 40x objective, the 0.55 mm width of the transect results in a cover slip (22 x 30 mm) consisting of 40 optical horizontal transects. For microscopes where the 40x field of view differs from 0.55 mm, calculate the transect width required. Sample volume held by one horizontal optical transect is calculated as follows: on the counting slide, 0.05 mL of subsample is placed. This subsample has been concentrated 5 times the original sample, thus 0.25 mL from the original sample is analyzed. Therefore the original sample volume held by one horizontal optical transect is 0.00625 mL (=0.25 mL/40 horizontal transects). When additional dilutions or concentrations are applied to the initial microalgal subsample of 1 mL (see Section 4.1.3 Step 2), the sample volume per transect must be corrected by multiplying with the dilution factor (DF). Most often, the subsample of 1 mL is counted without dilutions/concentrations (DF 1), but sometimes is concentrated to 0.5 mL (DF 2), or diluted to 2 mL (DF 1/2), to 3 mL (DF 1/3), to 4 mL (DF 1/4), to 5 mL (DF 1/5).

4.3 Sample Labeling and Archiving

All SBA and diatom samples and slides must be retained as voucher specimens until harmonization and reporting of data is complete. All samples will be archived and stored as reference collections. Archives of samples and slides should be retained by the laboratory for two years. The following types of samples will be archived:

SBA

- Vials with qualitative SBA sample fixed with 2% glutaraldehyde
- 15 mL or 50 mL graduated centrifuge tube with minimally disturbed SBA macroalgal fraction fixed with 2% glutaraldehyde
- 15 mL graduated centrifuge tube with remaining SBA microalgal fraction fixed with 2% glutaraldehyde
- 50 mL graduated centrifuge tube with remaining content of original SBA sample fixed with 2% glutaraldehyde
- Sealed semi-permanent slides with analyzed quantitative SBA microalgal fraction

4.3.1 Archiving of SBA – Qualitative Samples
Materials needed:

- Plastic scintillation vials (20-40 mL)
- 2% glutaraldehyde • Parafilm

Step 1: Select a representative subsample that contains all identified macroalgal taxa and the algal material from the slides. Place the material in plastic scintillation vial, fix it with 2% glutaraldehyde final concentration and parafilm the cap.

Step 2: Labeled the vial by SWAMP sample ID, sampling date (MM/DD/YYYY), and note "qualitative sample" or "Q".

4.3.2 Archiving of SBA – Quantitative Samples
Materials needed:

- Nail polish
- Slide box
- Parafilm

Macroalgal fraction

Step 1: Place the entire content of the macroalgal fraction which has been analyzed back in the 15 mL or 50 mL tube, including the macroalgae investigated on microscope slide under compound microscope. This should be done very careful preserving the entirety of the sample.

Step 2: Refix the sample with glutaraldehyde to 2% final concentration and parafilm the cap. Label the tube by SWAMP sample ID, collection date (MM/DD/YYYY), and note "macroalgae".

Microalgal fraction

Step 1: When finished with taxonomic work and enumeration, seal the cover slip with nail polish, label the microscopic slide by SWAMP sample ID, collection date (MM/DD/YYYY), and note "microalgae", and keep it in a slide box.

Step 2: Refix the subsample with 2% glutaraldehyde final concentration, parafilm the cap, and keep it separately from original sample for reference purposes. Label it by SWAMP sample ID, collection date (MM/DD/YYYY), and note "microalgae".

TOTAL SUSPENDED SOLIDS

2540 d. Standard methods 19th edition

City of Watsonville Environmental Laboratory

Updated 1-26-2012

NOTE: filters for this test must be prepared in advance. Place a 934AH grade filter in the filtering funnel and apply vacuum. Wash the filter with three successive rinses of distilled water (about 20 mls each) dry in muffle furnace for at least 1/2 hour. Place in desiccator until ready to use.

Effluent suspended solids are run on a 250 mls aliquot of composite sample, 100 mls of roughing filter sample, 100 mls for primary and 50 mls for influent twice a week.

Weigh a prepared filter and record weight on composite work sheet.

Place filter paper in the filtering funnel and apply vacuum. Wash down the filter with distilled water completely and press filter onto the sides and bottom of the funnel so that it is well-sealed. (Use tweezers to do this)

Mix the composite sample well and pour into graduated cylinder. Pour sample precisely to the desired volume, making sure the bottom of the meniscus is on the volume mark.

Pour sample slowly into the center of the filter paper. Avoid allowing the sample to creep up the sides. Rinse the cylinder at least three times with DI water and pour rinsing onto the filter.

Turn off the vacuum, break the vacuum seal by lifting the funnel out of the vacuum flask, and remove the filter with tweezers. Carefully place the filter on a wire mess and place in the drying oven. Allow to dry for at least 1 hour at 103° - 105° C

When dry carefully remove from drying oven and place in desiccator until cool. Weigh and record weight on work sheet

Belt press filtrate: follow procedure above using 50-100 ml sample size. Rinse well to get the particles clinging to sides.

CALCULATION: MG TOTAL SUSPENDED SOLIDS /L = $\frac{(A-B) \times 1000}{\text{SAMPLE VOLUME, ML}}$

A = WEIGHT OF FILTER + DRIED RESIDUE (MG)

B = WEIGHT OR FILTER