Quality Assurance Project Plan (QAPP)

For

Phase II Small Municipal Separate Storm Sewer System General Permit Receiving Water Monitoring Program

Prepared for:

Mojave River Watershed Group

Town of Apply Valley
City of Victorville
City of Hesperia
County of San Bernardino

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Acronyms

%R	Percent Recovery
BMPs	Best Management Practices
CDFW	California Department of Fish and Wildlife
CEDEN	California Environmental Data Exchange Network
COC	Chain-of-custody
DBM	Database Manager
DHS	California or Oregon Department of Health Services
EDD	Electronic Data Deliverable
ELAP	California Environmental Laboratory Accreditation Program
HASP	Health and Safety Plan
LCS	Laboratory Control Standards
LCSD	Laboratory Control Standards Duplicate
LID	Low Impact Development
MDLs	Method Detection Limits
MQOs	Measurement Quality Objectives
MRWG	Mojave River Watershed Group
MS	Matrix Spike
MS4s	Municipal Separate Storm Sewer System
MSD	Matrix Spike Duplicates
NELAP	National Environmental Laboratory Accreditation Program
NPDES	National Pollutant Discharge Elimination System
PD	Project Director
PM	Project Manager
QA/QC	Quality Assurance/Quality Control
QA/QCM	Consultant QA/QC Manager
QAM	Quality Assurance Manual
QAPP	Quality Assurance Program Plan
Regional Board	Lahontan Regional Water Quality Control Board
RLs	Reporting Limits
RPD	Relative Percent Difference
RWMP	Receiving Water Monitoring Program
SA	Spike Added
SOP	Standard Operating Procedures
SR	Original Sample Result
SRM	Standard Reference Material
SSCs	Site Safety Coordinators
SSR	Spike Sample Result
SWAMP	Surface Water Ambient Monitoring Program
USGS	United States Geological Survey
WDID	Waste Discharge Identification Number
WDRs	Waste Discharge Requirements

1. Introduction

On July 1, 2013 the California State Water Resources Control Board renewed National Pollutant Discharge Elimination System (NPDES) General Permit No. CAS000004, by adopting Order No. 2013-0001-DWQ, *Waste Discharge Requirements* (WDRs) *for Storm Water Discharges from Small Municipal Separate Storm Sewer Systems* (MS4), otherwise known as the Phase II Small MS4 Permit (Permit). This statewide permit is enforced by the individual Regional Water Quality Control Boards (Regional Boards) and each Permittee is responsible for its implementation within their jurisdiction. The Lahontan Regional Board oversees Phase II Small MS4 Permittees within the Mojave River Watershed.

The Permit is intended to minimize, or eliminate, adverse surface water quality impacts by instituting controls on MS4 discharges that have the greatest potential to cause environmental degradation. Permit Section E.13.d.1 directs Permittees, with characteristics similar to the Mojave River Watershed Group (MRWG), to implement a Receiving Water Monitoring Program (RWMP).

2. Purpose

This interim Quality Assurance Program Plan (QAPP) is a guide to the MRWG, potential consultants or contractors, and analytical laboratories of anticipated sampling methods and laboratory performance criteria applicable to those implementing the proposed MRWG RWMP. Included in the QAPP are Standard Operating Procedures (SOPs) for safe comprehensive and representative water and sediment quality sampling, field and laboratory equipment testing and calibration, laboratory analytical methodologies, and data acceptance criteria. The purpose of this QAPP is to identify specific consistent Quality Assurance/Quality Control (QA/QC) objectives, procedures, and criteria for implementing the MRWG RWMP and reporting program activities. These QA/QC requirements are designed to assist in achieving the project measurement quality objectives (MQOs) and analytical MQOs for all sampling activities that will be performed in the field and laboratory. The QAPP serves as the controlling mechanism during monitoring and identifies the QA/QC techniques needed for sampling, sample handling, sample storage, Chain-of-Custody procedures, laboratory analytical protocols, data interpretation, reporting, and documentation requirements. The QAPP further provides a summary of the project, its organizational hierarchy, and objectives. QA/QC procedures will be in accordance with applicable professional technical standards, USEPA requirements, RWQCB requirements, specific project goals, and client requirements.

Prior to the initiation of monitoring, the QAPP should be modified to identify the staff accountable for identified responsibilities, including laboratory personnel. The laboratory may have its own QAPP, sections of which may be inserted into this interim document, or attached as an appendix and duly referenced in the revised document. All project personnel will be required to read the revised QAPP and a copy will be brought to the field during sampling events.

3. Project Background

The Permit states that traditional small MS4 Permittees listed in Attachment A that are not already conducting Area of Special Biological Significance (ASBS), Total Maximum Daily Load (TMDL), or 303 (d) monitoring efforts shall participate in Receiving Water Monitoring (E.13.d.1) or Special Studies (E.13.d.2). Pursuant to the requirements set forth in the Permit, the MRWG will implement the Receiving Water monitoring and reporting as outlined in the RWMP. Upstream receiving water quality samples will be collected at the Outlet of the Mojave Forks Reservoir, designated the MR-URI location, while the downstream samples will be collected at the Mojave River Narrows, near the northern border of the City of Victorville, designated the MR-UD location. Additional details are presented in Sections 2, 3, 4 and 5 of the RWMP.

3.1 Geographical Setting

Located within the Mojave River watershed, the MRWG consists of the Town of Apple Valley, Cities of Hesperia and Victorville, and County of San Bernardino. The Mojave River Watershed encompasses approximately 4,500 square miles and is located entirely within San Bernardino County. The total population in the Mojave River Watershed was approximately 390,000 people in 2010 with much of the existing population concentrated in Victor Valley, which is located north of the San Bernardino Mountains and borders the edge of the Mojave Desert. The Victor Valley includes the communities of Adelanto, Apple Valley, Hesperia, Lucerne Valley, Oak Hills, Phelan, Victorville, and Wrightwood. Additional urban growth is expected throughout the watershed.

4. Project Management

This section describes the overall project organization, schedule, quality objectives, and documentation.

4.1 Roles and Responsibilities

As the regulatory lead, the Lahontan Regional Board may assert external oversight of most aspects of monitoring, reporting, and will be informed of investigation findings and project activities through the Permit requirement for preparation of an annual monitoring report.

Project Director Marc Rodabaugh will serve as the MRWG Project Director (PD), primary point of contact for the Permittees, who will be responsible for coordination of the MRWG RWMP activities. Any proposed updates or revisions of the QAPP should first be proposed to identified with the then current MRWG PD.

Consultant The consultant, or contractor, will be appointed by MRWG to provide clear lines of authority and communication that will expedite and enhance the flow of information vital to effective technical controls, cost, and schedule performance. The functional roles of personnel within the establishment will also be clearly defined. Individuals are given the authority to accomplish their respective project assignments. Since the individuals to be listed may change from time to time, this QAPP uses "designee" to include an alternate to the proposed or normal project organization. The following paragraphs define functional titles, positions, and responsibilities.

Consultant Project Manager The Consultant Project Manager (PM) designee, will report directly to the PD. The Consultant PM is responsible for ensuring the availability of resources and overall quality of the activities completed under the RWMP. The Consultant PM will provide programmatic guidance to support staff and ensure that documents, procedures, and project activities meet respective standards and quality requirements. The Consultant PM will also be responsible for resolving project concerns related to specific technical matters.

The Consultant PM is the focal point for control of project activities, continuity, quality, accountability, and leadership responsibility throughout all phases of the project. The Consultant PM will be supported by QA personnel, who provide reviews, guidance, and technical advice on project execution and issues resolution. The project team, consisting of sampling manager, quality assurance/quality control manager, and technical personnel, will support the Consultant PM to ensure that the project meets professional standards, is safely executed, and in compliance with applicable laws, regulations, statutes, and industry codes. Individuals on the project team are responsible for fulfilling appropriate portions of the project QA program, in accordance with assignments made by the Consultant PM. The Consultant PM is responsible for satisfactory completion of the project QA program, may assign specific responsibilities to other members of the project staff, and will notify the PD of any significant changes in personnel.

Consultant Sampling Manager The Consultant Sampling Manager designee reports directly to the Consultant PM and will oversee all phases of technical work related to monitoring, reporting data and document generation. Additionally, the Consultant Sampling Manager is responsible for field activity preparations and execution of sampling activities. This includes overseeing sampling in accordance with approved procedures and methodologies, collection of QA/QC samples, completion of sampling forms, labels, chain-of-custody forms, applying custody seals, and packaging or shipping samples to the approved laboratory.

The Sampling Manager will also be responsible for approval of the Project Health and Safety Plan (HASP) to ensure that health and safety procedures are conducted in accordance with California Occupational Safety and Health Association regulations and guidelines. The designee will be responsible for updating the HASP as needed, ensuring proper health and safety procedures are followed, directing periodic field audits, and assigning Site Safety Coordinators (SSCs).

Consultant Quality Assurance/Quality Control Manager The Consultant QA/QC Manager (QA/QCM), designee, will be available to ensure that management activities are consistent with project objectives. The Consultant QA/QCM will be responsible for monitoring the project analytical QA/QC program. Additional responsibilities include laboratory coordination, project tracking, data validation, data quality assessment, data reporting procedures, calculations, and QC. The Consultant QA/QCM or designee will assume primary responsibility for maintaining and reviewing the QAPP.

Consultant Database Manager The Database Manager will report to the Consultant PM and be responsible for maintenance of the laboratory database. The DBM is responsible for providing routine data reporting deliverables as well as non-routine and special-circumstance data requests. All non-routine and special-circumstance data requests are routed through the Consultant PM to the Database Manager.

Consultant Field Scientist, Geologists, Engineers, and Technicians Consultant field scientist, geologists, engineers, and technicians report to the Consultant PM and are responsible for field activities including sampling and following the QA/QC elements of the QAPP.

Laboratory Project Manager Each Laboratory Project Manager will be the laboratory's primary project contact and will coordinate with the Consultant QA/QCM. The laboratory will be certified through the California Environmental Laboratory Accreditation Program (ELAP)[1] or National Environmental Laboratory Accreditation Program (NELAP) approved laboratory. Analytical services may be subcontracted with the prior approval of the Consultant Sampling Manager; however, the Laboratory PM holds primary responsibility for delivery of all subcontracted services. The lab is designated as the primary analytical subcontractor and will perform the analyses for the standard analytical methods. Key positions and quality related responsibilities for laboratory personnel are discussed in the Laboratory Quality Assurance Manual (QAM) or QAPP.

Laboratory Quality Assurance Manager The Laboratory Quality Assurance Manager, designated by each laboratory, is the QA Manager for the services and deliverables of by that laboratory. The Laboratory QA Manager will be responsible for implementing the laboratory's QA/QC programs, as described in the laboratory QAM and implementing any additional and project-specific QA/QC procedures included in this QAPP.

[1] http://www.waterboards.ca.gov/drinking_water/certlic/labs/index.shtml

4.2 Persons Responsible for QAPP Update and Maintenance

Following approval of the QAPP, changes and updates to this QAPP can be made by the Consultant PM with the concurrence of the Consultant QA/QCM. The Consultant PM will be responsible for making the changes, submitting drafts for review, preparing a final copy, and submitting the revised QAPP for signature.

4.3 Problem Statement

As a condition of Permit compliance, the MRWG RWMP was developed to assess whether development is affecting receiving water quality, and the effectiveness of stormwater low impact development (LID), best management practices (BMPs), and other programmatic water quality implementation efforts in protecting receiving water quality and beneficial uses. Permit Section E13.d.1 outlines the requirements for monitoring, reporting, and recordkeeping of receiving water quality data.

4.4 Project Schedule

As summarized in **Section** 3, the MRWG has elected to conduct Receiving Water Monitoring at, MR-URI and MR-UD. Section E.13.d.1.(ii) states, *within the second year of the effective date of the permit, the Permittee shall develop and implement a receiving water monitoring program.*

Monitoring of the MR-URI and MR-UD will commence in accordance to **Table 4-1**, which outlines the Receiving Water Monitoring parameter and sampling frequency. Samples will be collected in approved containers using sampling methods discussed in **Section 5.2** below. Collected samples and field data for all required parameters will be conducted in accordance to **Table 4-1**.

4.5 Project Constraints

The RWMP monitoring site MR-URI may require encroachment permits and coordination with the United States Army Corps of Engineers and the United States Department of Agriculture Forest Service. The PD and the Consultant PM will contact, coordinate, and complete the necessary documentation to obtain the necessary permits.

Table 4-1 Receiving Water Monitoring Parameters and Frequency				
Endpoint	**Frequency**			
	Spring	**Summer**	**Fall**	**Winter**
Field Measurement				
Dissolved oxygen (DO)	Continuous[1] Sampling - 1 Week	Continuous[1] Sampling - 1 Week	Continuous[1] Sampling - 1 Week	
Temperature	Continuous[1] Sampling - 2 Weeks	Continuous[1] Sampling - 2 Weeks	Continuous[1] Sampling - 2 Weeks	
Flow[2]	Continuous[1] Sampling - 2 Weeks	Continuous[1] Sampling - 2 Weeks	Continuous[1] Sampling - 2 Weeks	
PHAB assessment: - pH - Specific Conductance - Alkalinity	1			
Channel cross sections	1			
Photo documentation	1			
Nutrients (Algae): - Total Chlorophyll[3]	1	1	1	
Laboratory Analysis				
Pyrethroids[4]* (sediment)	1			
Bacteria			Early Fall. Once weekly for 4 weeks	
Nutrients (Algae)	1	1	1	
Benthic Macroinvertebrate (BMI) Bioassessment	1			

[1] Continuous sampling is the preferred approach, but physical limitations that might force the collection of grab samples. When stream depths are < 1 foot, grab samples will be collected.
[2] Flow data will be derived from the co-located USGS Stream Gauging Stations.
[3] Water column only
[4] *Pyrethroid monitoring is required at the urban/rural interface site only.*
** Currently, pyrethroids are the pesticide of greatest concern and abundance in urban/suburban waterways. However, new regulations enacted by the Dept. of Pesticide Regulation restrict how pyrethroids may be applied. Initial models by UC Davis researchers suggest that this could result in a runoff reduction of 80-90%, depending on the amount of impervious cover in the watershed. In the future, other pesticides may become more of a threat to aquatic life in urban waterways. One pesticide that is being used with greater frequency is fipronil, a phenylpyrazole insecticide, that is more water soluble than pyrethroids. In order to use the resources of the Permittees most efficiently, the State Water Resource Control Board reserves the right to modify the terms and conditions of the permit based on new information on pesticide use and toxicity. This could include substituting another pesticide for monitoring or eliminating this endpoint.*

4.6 Quality Objectives and Criteria for Measurement Data

Laboratory MQOs are quantitative and qualitative statements that specify the tolerable levels of potential errors in the data and ensure that the data generated meet the quantity and quality of data required to support the study objectives. These include

- Accuracy
- Precision
- Completeness

For this project the analysis specific MQOs are presented in **Table 4-2**, while additional SWAMP MQOs requirements are included in **Attachment 1**. The following subsections further characterize these QA/QC characterization and assessment tools.

Table 4-2 Measurement Quality Objectives

Parameter	Accuracy	Precision	Completeness
Field Measurements			
Water Velocity (for Flow calc.)[1]	2%	NA	90%
pH	+ 0.2 pH units	+ 0.5 pH units	90%
Temperature	+ 0.5° C	5%	90%
Dissolved Oxygen	+ 0.5 mg/L	+ 10%	90%
Conductivity	5%	5%	90%
Nutrient - Total Chlorophyll[2]	n/a	±10%	90%
Laboratory Analyses			
Conventional Pollutants and Solids	80 – 120%	0 – 25%	90%
Nutrients	80 – 120%	0 – 25%	90%
Pyrethroid	70 – 130%	0 – 25%	90%
Bioassessment	SAFIT Level 2	0 – 10%	≥99%

[1] Collected via USGS Gauging Stations
[2] Water column only

4.6.1 Accuracy

Accuracy, or measurement bias, is an assessment of the agreement between an experimental or observed value and the true value of the parameter being measured. A measurement is evaluated for accuracy by comparing a given observed value to a true value and against an established range specifying a lower limit and an upper limit of acceptability. Laboratory Control Standards (LCS), their duplicates (LCSD), and surrogate spikes will be used to evaluate the accuracy and bias for the project samples. Accuracy is expressed as percent recovery (%R), as determined from the formula:

$$\%\mathbf{R} = \frac{\mathbf{SSR} - \mathbf{SR}}{\mathbf{SS}} \times \mathbf{111}$$

Where:
%R = Percent recovery (percent)
SSR = Spike sample result (concentration units)
SR = Original sample result (concentration units)
SA = Spike added (concentration units)

Method-specific recovery criteria will be reported by the contracted laboratory. For data validation, the more stringent of either the laboratory-specific criteria or the method-specific criteria will be used.

4.6.2 Precision

Precision refers to the agreement or reproducibility of a set of duplicate or replicate results obtained from independent analyses completed under identical conditions. Both sampling and laboratory precision will be evaluated by the performance of field duplicates (if collected), laboratory duplicates, and Laboratory Control Samples/Laboratory Control Sample Duplicates (LCS/LCSDs).

Precision is expressed as the relative percent difference (RPD) in concentration between the original and duplicate analyses, as determined in the formula:

$$\mathbf{RRR} = \frac{|\mathbf{S} - \mathbf{R}|}{\frac{\mathbf{1}}{\mathbf{2}} \times (\mathbf{S} + \mathbf{R})} \times \mathbf{111}$$

Where:
RPD = Relative percent difference
S = Concentration of analyte in the original sample
D = Concentration of analyte in duplicate sample

4.6.3 Completeness

Completeness is an assessment of the adequacy of the available data resulting from the sampling and analysis program. It is evaluated for each method, matrix, and analyte combination in order to prevent misinterpretation of the data and to meet the needs of the sampling program. Another aspect of completeness involves the adequacy of the data package in documenting the associated QC data for the project samples. The validated data will provide a measure of completeness, but the usability of the validated data will be determined by the selected Consultants, the Project Director, and reviewed by the Regional Board. The completeness goal for this project is 90 percent; however, for critical samples, the completeness goal will be 95 percent. Percent completeness is expressed as (%PC), as determined from the formula:

$$\%\mathbf{RP} = \frac{\mathbf{N_S}}{\mathbf{N_1}} \times \mathbf{111}$$

N_A = actual number of valid analytical results obtained
N_1 = theoretical number of results obtainable under ideal conditions

4.7 Special Training Needs/Certification

The Consultant shall choose a laboratory that is certified through the ELAP or NELAP for the analytical methods proposed in the RWMP and QAPP. Other training requirements are summarized in the subsections below.

4.7.1 Training and Certification Documentation

Field personnel will be properly trained in the use of monitoring equipment and clean/dirty hand sample collection and handling techniques along with all appropriate health and safety protocols prior to conducting monitoring activities. The following elements will be included in the training of field personnel:

- Review of HASP
- Field training

Personnel will have had prior experience performing field sampling and laboratory analyses for the type of water quality monitoring required. SWAMP Standard Operating Procedures (SOP) for collection, records, handling, and analysis will be monitored by the Consultant QA/QCM and Laboratory QA Manager and can be found in **Attachment 2**.

4.7.2 Training Personnel

The Consultant QA/QCM and Sampling Manager will provide training to the field monitoring personnel. The Consultant Sampling Manager will train these personnel in sampling protocols and procedures in accordance with the RWMP and QAPP. The Consultant Sampling Manager or Consultant Project Manager will communicate any updates or revisions of these protocols in a timely manner.

The Consultants delegated analytical laboratory must provide training to all staff members to ensure they are adequately qualified and trained to perform assigned tasks. Details of the laboratories' training plans shall be described in Laboratories' Quality Assurance Manual.

4.7.3 Bioassessment and Physical Habitat Assessment Training

Bioassessment sampling and laboratory analysis require specialized training[2]. The Consultant QA/QCM is responsible for ensuring that training requirements are met by participating field crews and laboratories. Field sampling training can be provided during short courses offered by the California Department of Fish and Wildlife or similar agency. Laboratory analysis requires years of experience and mentoring by a qualified taxonomist.

All field crews must consist of at least two adults qualified to work in the State of California. However, it is strongly recommended that crews contain no fewer than four members as there are several indicators measured at each site (i.e., physical habitat, benthic macroinvertebrates, and benthic algae communities, water chemistry, toxicity, and California Rapid Assessment Methods (CRAM)). If smaller crews are used, conducting CRAM assessments on a separate day, independent of sampling other indicators, may be acceptable. Inadequate staffing of field crews is one of the most common sources of data errors, and may result in costly corrective actions or data deficiencies.

At least one member of each bioassessment crew must have received training in sampling procedures described in the Bioassessment SOP, **Attachment 2**. Training in basic first aid is also required. Crew chiefs are responsible for ensuring the safety of the crew and must use his or her discretion to end sampling if conditions become unsafe. At least one person per crew must have experience with the Bioassessment SOP at a minimum of 20 sites in California.

4.8 Documents and Records

All field observations will be recorded in standard Field Conditions Data Log sheets. The sheets will be reviewed for errors prior to leaving the sample site. Chain-of-custody (COC) forms will be completed for all field samples before the samples are delivered to the laboratory. Field sheets and COCs will be scanned and stored as an electronic PDF by the Project Director for a minimum of five years from the time the RWMP is completed. Example Field Conditions Data Log sheets and COCs are included in **Attachment 3**. Additionally, the documents and records to be saved should include the following information:

- Site identification and location
- Date and time that sampling or measurements were taken
- Individual(s) who performed the sampling or measurements
- Field Measurements
- Analytical methods used
- Results of analyses

[2] http://www.waterboards.ca.gov/water_issues/programs/swamp/reports.shtml#bmp_assess

The Laboratory Manager reviews the laboratory analytical results, verifies completeness, and logs the date of sample receipt, analysis, internal QA/QC and final reporting to the Consultant PM. The reports and data are then transferred to the Project Director and filed with all other original project documentation in order to maintain complete project records. The laboratory will provide analytical data in electronic format for maintenance and management in Microsoft® Excel or Access. **Table 4-3** summarizes the record retention, archival, and disposition guidelines for each type of document.

Table 4-3 Document and Record Retention, Archival, and Disposition Information

Records	Identify Type Needed	Retention	Archival	Disposition
Project Plan	RWMP	Paper/Electronic	Document	Minimum 5 years
	QAPP	Paper/Electronic	Document	Minimum 5 years
Field Data	Field Conditions Data Log Sheets	Paper/Electronic	Project File/PDFs	Minimum 5 years
	Photographs	Electronic	Project File	Minimum 5 years
Sample Collection Records	Chain-of-Custody	Paper/Electronic	Project File	Minimum 5 years
	Calibration and Maintenance	Paper	Project File	Minimum 3 years
	Original strip charts	Paper/Electronic	Project File	Minimum 3 years
Analytical Records	Lab Notebooks	Paper	Notebook	Minimum 5 years
	Lab Reports (include COCs)	Electronic	Notebook/Excel	Minimum 5 years
	Electronic Data File	Electronic	Database	Minimum 5 years
Assessment Records	QA/QC Assessment	Paper/Electronic	Document	Minimum 5 years
	Final Report	Paper/Electronic	Document	Minimum 5 years

5. Sampling Process and Procedures (Data Generation and Acquisition)

The purpose of the QAPP is to produce reliable data, assure and provide ongoing control of data quality, evaluate data quality through data quality indicators, and provide applicable, quantitative data for analysis, interpretation, and decision making. The subsections below present an overview of the monitoring program and the steps to be taken for receiving water monitoring.

5.1 Sampling Process Design

The plan schedule, rationale for the sampling design, and monitoring locations are outline in **Section** 3 and **4.4** of this QAPP and the RWMP. Samples will be collected in approved containers using sampling methods discussed in **Section 5.2** below. Collected samples and field data for all required parameters will be conducted in accordance to **Table 4-1**.

Upon receipt of the field samples (water, sediment, BMI and algae), the laboratory will take the appropriate actions to dispense the sample contents into containers that contain the required volume needed for analysis of the constituents. The laboratory will preserve the samples using the appropriate preservative, and will conduct the analysis within the maximum holding time limits.

5.2 Sampling Methods

All sampling procedures will adhere to the guidelines found in the Surface Water Ambient Monitoring Program (SWAMP) sampling (SOP), presented in **Attachment 2**, "Field measurement and field collection of water and bed sediment samples, water and bed sediment samples with associated field measurement and physical habitat, stream algae samples and associated physical habitat and chemical data, and benthic macroinvertebrates (BMI) samples and associated physical and chemical data." Should field crews feel that it is unsafe to collect samples for any reason, the field crews **SHOULD NOT COLLECT** a sample and note on the field log that the sample was not collected, why the sample was not collected, and provide photo documentation, when feasible.

A two-or three-person team will generally conduct the sampling event. The sampling team will have access to a cellular phone in order to alert rescue agencies should an accident occur. Sampling will be postponed if the sampling team determines that the conditions are unsafe. Failure to collect a sample due to safety concerns or technical issues will be promptly reported to the Consultant PM and Project Director, who will determine if any corrective action is needed and make arrangements to collect a replacement sample, if possible. The Consultant QA/QCM will document sampling failures and the effectiveness of corrective actions. A copy of the SOP can be found in **Attachment 2**.

Section 3.1.1, 3.1.2, and 3.1.3 of the RWMP presents the methods and procedures for the following:

- Direct Submersion: Grab Sample Technique (Water and Sediment Sampling);
- Physical Habitat (PHAB) and Bioassessment; and
- Nutrient (Algae)

Monitoring of these constituents should be conducted in accordance with the procedures outline in the RWMP and the SWAMP sampling SOPs located in **Attachment 2**.

5.3 Sampling Handling and Custody

The laboratory will provide appropriate sample containers according to **Table 5-1**. All samples will be pre-labeled with the project name, site ID, sample type, bottle number, sampler name, preservative, and analysis. All sample bottles will also be pre-labeled with a unique Sample ID to track the sample throughout its analyses. At the time of sample collection, the sample labels will be completed in the field with the date and time. The Sample IDs will also be entered directly onto the Field Conditions Data Log Sheets and the COC Forms. The COC forms will accompany the collection of all samples.

The following sample handling protocols will be followed when collecting samples to minimize the possibility of contamination:

- New unused sample bottles will be employed. Sample bottles and bottle caps will be protected from contact with solvents, dust, or other contaminants during storage and bottle handing.
- The sampler will attempt to prevent large gravel and floating debris from entering the sample containers. The sampler will also make an effort to not stir up sediments at the sample locations.
- The inside of the sampling container will not be touched to the maximum extent practicable during preparation and sampling activities.
- Vehicle engines will be turned off during sampling, to minimize sample exposure to fumes.
- All samples will be collected in accordance with the "clean sampling" techniques.
- Manual water grab samples will be collected by inserting the transfer container under or down current of the direction of flow, with the container opening facing upstream.
- Once sample containers are filled, they will be promptly placed on ice, in a clean cooler (target temperature 6 degrees Celsius), in the dark and transported to the laboratory for processing to meet holding times. All necessary pre-processing for analysis, such as filtration and acidification, will take place in the laboratory by certified personnel.
- After the field crew collects and delivers the samples to the laboratory, the laboratory will conduct the analysis within the holding times listed in **Table 5-1**. The field and laboratory activities will be coordinated to assure all samples are handled within the proper holding time.

After the laboratory receives the samples, the certified laboratory technicians will dispense the sample contents into containers that contain the required volume specified in **Table 5-1**. The laboratory will preserve the water samples using the appropriate preservative and the laboratory will conduct the analysis within the maximum holding time limits.

Table 5-1 Sampling Handling and Custody

Parameters	Container Type	Min Sample Volume	Preservation	Maximum Holding Time
Pyrethroids* (sediment)				
Allethrin	Solids in PSJ	8oz	Solids to be Frozen	14 days
Bifenthrin	Solids in PSJ	8oz	Solids to be Frozen	14 days
Cyfluthrin	Solids in PSJ	8oz	Solids to be Frozen	14 days
Lambda-Cyhalothrin	Solids in PSJ	8oz	Solids to be Frozen	14 days
Cypermethrin	Solids in PSJ	8oz	Solids to be Frozen	14 days
Deltamethrin:Tralomethrin	Solids in PSJ	8oz	Solids to be Frozen	14 days
Esfenvalerate:Fenvalerate	Solids in PSJ	8oz	Solids to be Frozen	14 days
Fenpropathrin	Solids in PSJ	8oz	Solids to be Frozen	14 days
Tau-Fluvalinate	Solids in PSJ	8oz	Solids to be Frozen	14 days
Permethrin	Solids in PSJ	8oz	Solids to be Frozen	14 days
Tetramethrin	Solids in PSJ	8oz	Solids to be Frozen	14 days
Bacteria				
E. coli	Sterile Plastic	100 mL	Sodium thiosulfate; Cool to 4°C; dark	6 hrs
Nutrients (Algae)				
Ammonia as N (NH_3-N)	Plastic	200 mL	H_2SO_4; Cool to ≤6°C	28 days
Nitrate as N (NO_3-N)	Plastic	100 mL	Cool to ≤6°C	48 hours
Nitrite as N (NO_2-N)	Plastic	100 mL	Cool to ≤6°C	48 hours
Nitrogen, Total (TN)	Plastic	200 mL	Cool to ≤6°C; H_2SO_4 to pH<2	28 days
Orthophosphate as P (dissolved; SRP)	Plastic	100 mL	Filter; Cool to ≤6°C	48 hrs once filtered
Phosphorous, Total (TPHOS)	Plastic	100 mL	Cool to ≤6°C; H_2SO_4 to pH<2	28 days
Dissolved Organic Carbon (DOC)	Glass	50 mL	Filter and preserve to pH<2 within 48 hours of collection; Cool to ≤6°C	28 days
Chloride (Cl)	Plastic	100 mL	None required	28 days
Chlorophyll *a* Pheophytin a	Amber Plastic	1000 mL	Cool to 4°C, dark	7 days
Bioassessment				
Benthic macroinvertebrate field samples	Plastic	500 mL	95% Ethanol for ≤ I month, Transfer to 70% Ethanol	5 years
Sorted specimens	Glass	Variable	70% Ethanol	5 years
Sorted subsample residue	Plastic	Variable	70% Ethanol	1 year from date of sorting
Unsorted sample	Plastic	Variable	70% Ethanol	2 years

PSJ – Poly Sleeve Jacket

5.3.1 Chain-of-Custody Procedures

Either the laboratory will supply the COC forms, or the exampler form in **Attachment 3** may be utilized by the Consultant field team. . COC procedures will be used for throughout the collection, transport, and analytical process to ensure accurate results. COCs will be pre-printed along with the bottle labels and will contain the same data as the labels. The COCs will be completed in the field with dates, times, and sample team names, and will be cross-checked with the bottles to make sure proper samples have been collected. Documentation of sample handling and custody will include the following:

- Sample identification;
- Type of sample;
- Sample collection date and time;
- Any special notations on sample characteristics or analysis;
- Analyses to be performed;
- Initials of the sampling team member that collected the sample; and
- Date the sample was delivered to/sent to the laboratory.

The COC forms will be transported with the samples to the analytical laboratory. Sampled water will be properly chilled and transferred to an analytical laboratory within holding times. When custody of the samples is transferred to the laboratory, the COC will be signed and dated, and a PDF copy will be sent from the laboratory. The COCs will be reviewed by personnel at the receiving laboratory to make sure no samples have been lost in transport. The laboratory will also verify that each sample has been received within holding times. COC records will be included in the final reports prepared by the analytical laboratory and are considered an integral part of the report.

5.3.2 Sample Disposal Procedures

After analysis, including QA/QC procedures, any excess sample will be disposed of by the analytical laboratory.

5.4 Analytical Methods Requirements

The analytical methods are described in the Standard Methods for the Examination of Water and Wastewater (APHA et al, 2005) and US EPA standard methods. The list of constituents, measurement techniques, method detection limits (MDLs), and reporting limits (RLs) are presented in **Table 5-2**. After the contract has been awarded, the designated laboratory shall revise **Table 5-2** to support their methods and procedures.

Table 5-2 Analytical Methods					
Parameters	**Matrix**	**Analytical Method**	**Units**	**MDL**	**RL**
Pyrethroids					
Allethrin	Sediment	GCMS-NCI-SIM/8270M	ug/kg (ng/g)	0.05	0.33
Bifenthrin	Sediment	GCMS-NCI-SIM/8270M	ug/kg (ng/g)	0.1	0.33
Cyfluthrin	Sediment	GCMS-NCI-SIM/8270M	ug/kg (ng/g)	0.11	0.33
Lambda-Cyhalothrin	Sediment	GCMS-NCI-SIM/8270M	ug/kg (ng/g)	0.06	0.33
Cypermethrin	Sediment	GCMS-NCI-SIM/8270M	ug/kg (ng/g)	0.1	0.33
Deltamethrin:Tralomethrin	Sediment	GCMS-NCI-SIM/8270M	ug/kg (ng/g)	0.12	0.33
Esfenvalerate:Fenvalerate	Sediment	GCMS-NCI-SIM/8270M	ug/kg (ng/g)	0.13	0.33
Fenpropathrin	Sediment	GCMS-NCI-SIM/8270M	ug/kg (ng/g)	0.07	0.33
Tau-Fluvalinate	Sediment	GCMS-NCI-SIM/8270M	ug/kg (ng/g)	0.04	0.33
Permethrin	Sediment	GCMS-NCI-SIM/8270M	ug/kg (ng/g)	0.11	0.33
Tetramethrin	Sediment	GCMS-NCI-SIM/8270M	ug/kg (ng/g)	0.06	0.33
Bacteria					
E. coli (Fecal Coliform)	Water	SM 9223B	MPN/100mL	2	2
Nutrients					
Ammonia as N (NH_3-N)	Water	EPA 300.0	mg/L	0.059	0.1
Nitrate as N (NO_3-N)	Water	EPA 300.0	mg/L	0.01	0.01
Nitrite as N (NO_2-N)	Water	EPA 300.0	mg/L	0.01	0.01
Nitrogen, Total (TN)	Water	EPA 300.0	mg/L	-	-
Orthophosphate as P (dissolved; SRP)	Water	EPA 365.1	mg/L	-	0.01
Phosphorous, Total (TPHOS)	Water	EPA 365.1	mg/L	0.014	0.05
Dissolved Organic Carbon (DOC)	Water	EPA 415.3	mg/L	-	0.6
Chloride (Cl)	Water	EPA 9212	mg/L	0.25	0.25
Chlorophyll *a* Pheophytin a	Water	EPA 445.0	mg/L	-	0.002
Bioassessment					
Benthic macroinvertebrate field samples	Water	SWAMP SOP for benthic macroinvertebrates and physical habitat (Ode et al. 2007, Appendix B)	-	-	-

5.5 Quality Assurance/Quality Control Requirements

This section addresses QA/QC requirements associated with both laboratory analyses and field sampling. Laboratory QA/QC samples provide information to assess potential laboratory contamination, analytical precision, and accuracy. The field QA/QC samples are used to evaluate potential contamination and sampling errors introduced prior to submittal of the samples to the analytical laboratory. If any QA/QC standards are not met, the appropriate corrective actions will be taken in accordance with **Section 5.5.2** and **5.5.4** of this document and the laboratory's QAM.

5.5.1 Laboratory quality control

Internal laboratory quality control checks will include the use of laboratory replicate/split, method blanks, matrix spike and matrix spike duplicates (MS/MSDs), laboratory control samples, and standard reference material (SRMs). These quality control samples are as follows:

1. **Laboratory Replicate/Split** – A sample is split by the laboratory into two portions and each sample is analyzed. Once the duplicate analyses have been analyzed, the results are evaluated by calculating the RPD between the two sets of results. This serves as a measure of the reproducibility, or precision, of the sample analysis. Typically, duplicate results should fall within an accepted RPD range, depending upon the analysis.

2. **Method Blanks** – A method blank is an analysis of a known clean sample matrix that has been subjected to the same complete analytical procedure as the field sample to determine if potential contamination has been introduced during processing. Blank analysis results are evaluated by checking against reporting limits for that analyte. Results obtained should be less than the reporting limits for each analysis.

3. **Matrix Spike and Matrix Spike Duplicates (MS/MSDs**) – Matrix spikes and matrix spike duplicates (MS/MSDs) involve adding a known amount of the chemical(s) of interest to one of the actual samples being analyzed. One sample is split into three separate portions. One portion is analyzed to determine the concentration of the analyte in question in an un-spiked state. The other two portions are spiked with a known concentration of the analytes of interest. The recovery of the spike, after accounting for the concentration of the analyte in the original sample, is a measure of the accuracy of the analysis. By determining spike duplicate recoveries, another measure of precision is accomplished. An additional precision measure is made by calculating the RPD of the duplicate spike recoveries. Both the RPD values and spike recoveries are compared against accepted and known method dependent acceptance limits. Analyses outside these limits are subject to corrective action.

4. **Laboratory Control Sample (LCS)** – The laboratory control sample procedure involves spiking known amounts of the analyte of interest into a known, clean, sample matrix to assess the possible matrix effects on spike recoveries. High or low recoveries of the analytes in the matrix spikes may be caused by interferences in the sample. Laboratory control samples assess these possible matrix effects since the LCS is known to be free from interferences.

5. **Standard Reference Material (SRM)** –SRMs may be used in lieu of laboratory control samples. An SRM is a sample containing a known and certified amount of the analyte of interest and is typically analyzed with the analyst not knowing the analyte concentration. SRMs are typically purchased from independent suppliers who prepare them and certify the analyte concentrations. Results are evaluated by comparing results obtained against the known quantity and the acceptable range of results supplied by the manufacturer.

Table 5-3 presents the frequency of analysis for each of the laboratory quality control samples.

Table 5-3 Laboratory Quality Control Sample Frequency

QA/QC Sample Type	Minimum Sampling Frequency	Acceptance Limits
Laboratory Replicate/Split	One per batch or per 20 samples (5%), per sampling event.	The relative percent difference between the primary sample result and duplicate sample result should meet the objective for precision.
Method Blank	One per batch or per 20 samples (5%).	Procedural blanks should be below 10x the MDL.
Matrix Spike/Matrix Spike Duplicate (MS/MSD's)	One per batch or per 20 samples (5%), per sampling event.	The percent recovery should be within the accuracy acceptance limits listed in Table 1-7.
Laboratory Control Spike (LCS)	One per batch or per 20 samples (5%).	The percent recovery should be within the accuracy acceptance limits listed in Table 1-7.
Standard Reference Material (SRM)	One per batch or per 20 samples (5%).	The percent recovery should be within the accuracy acceptance limits listed in Table 1-7.

5.5.2 Laboratory corrective action

Failures in laboratory measurement systems include, but are not limited to: instrument malfunction, calibration failure, sample container breakage, contamination, and QC sample failure. If the failure can be corrected, the analyst must document it and its associated corrective actions in the laboratory record and complete the analysis. If the failure is not resolved, it is conveyed to the respective supervisor who should determine if the analytical failure compromised associated results. The nature and disposition of the problem must be documented in the data report that is sent to the Consultant PM, who will also notify the Project Director.

5.5.3 Field quality control

The main types of field quality control samples that will be utilized for the RWMP are as follows:

1. **Field Blanks** – Field blanks verify that field conditions, field sampling activities, and air deposition are non-contaminating. Field blanks are submitted blind to the laboratory. Sample bottles are filled with reagent-grade, analyte-free deionized water in the field during an event.

2. **Equipment Blanks** – Equipment blanks verify that the sampling containers, sampling equipment, and tubing are contaminant free prior to sampling. A representative number of bottles or sections of tubing from each lot is submitted to the laboratory. The laboratory will use reagent-grade, analyte-free deionized water to fill the bottles or rinse through the tubing and then analyze the water. Blank analysis results are evaluated by checking against reporting limit for that analyte. Results obtained should be less than the reporting limit for each analyte. If results are above the reporting limits then the entire lot must be cleaned and re-analyzed.

3. **Field Duplicates** – Field duplicates evaluate sampling error introduced by both field sampling and laboratory analyses. Field duplicates are submitted blind to the laboratory. Procedures for collecting field duplicates should be the same as those used for collecting field samples. Duplicates of manual grab samples will be collected by filling two grab sample containers at the same time, or in rapid sequence.

The blank samples and duplicate samples need not all come from the same monitoring site during a particular sampling event. However, each of these QA/QC analyses will be provided along with the

standard analyses if enough sample volume has been collected. The field QA/QC samples for field blanks and field duplicates are submitted blind to the analytical laboratory. **Table 5-4** presents the frequency of analysis for each of the field quality control samples.

Table 5-4 Sampling (Field) QC

QA/QC Sample Type	Minimum Sampling Frequency	Constituent Class	Acceptance Limits
Field Blank	Every 20 samples collected at a given site, per sampling event.	All	Field blanks shall find no detectable amounts or less than 1/5 of sample amounts. Accuracy at 1 per culture medium or reagent lot.
Equipment Blank	Sample bottles should be blanked at 10% frequency, or per lot.	All	Equipment blank shall be less than the reporting limit for that analyte.
Field Duplicate	Every 10 samples collected at a given site, or per sampling event.	All	The relative percent difference between the primary sample result and the duplicate sample result should meet the objective for precision listed in table 1-7.

5.5.4 Field corrective action

The field team is responsible for responding to failures in their sampling and field measurement systems. If monitoring equipment fails, personnel are to record the problem according to their documentation protocols. Failing equipment must be replaced or repaired prior to subsequent sampling events. It is the combined responsibility of all members of the field organization to determine if the performance requirements of the specific sampling method have been met, and to collect additional samples if necessary.

5.6 Instrument and Equipment Maintenance

The Consultant Sampling and Laboratory Mangers are responsible for the maintenance of all equipment. All Mangers will also maintain all records of equipment maintenance. All field and laboratory devices will be maintained per the manufacturer's guidelines and are outlined in the sections below.

5.6.1 Sampling Equipment

Prior to each sampling event, field sampling equipment will be checked for proper operation. Field technicians will be responsible for preparing sampling kits that include field logs, COC forms, sample labels, sampling bottles, field equipment and tools. Equipment will be inspected for damage when first handed out and returned from use.

5.6.2 Analytical Instruments

The contract Consultants delegated laboratory will maintain analytical equipment in accordance with their QAM, which include those specified by the manufacturer and those specified by the method. If deficiencies occur, the laboratory will resolve and document the issue in accordance with their QA procedures. These SOPs will be reviewed by the Consultant QA/QCM and will need to be in compliance with criteria.

If failures or errors occur with analytical instrumentation, proper corrective action must be taken. The laboratory is responsible for taking the appropriate measures in accordance with their QA procedures and/or manufacturer's agreements. The Laboratory Manager is responsible for notifying the Consultant PM. Refer to **Section 5.5.2** for more details regarding corrective action procedures.

5.7 Instrument and Equipment Calibration

All laboratory equipment is calibrated based on manufacturer recommendations and accepted laboratory protocol. The laboratory maintains calibration practices as part of their method SOPs maintained in their laboratory by their Laboratory Manager/QA officer and can be provided upon request.

5.8 Inspection/Acceptance Requirement for Supplies and Consumables

All glassware, sample bottles, and collection equipment will be inspected prior to their use. Some sampling containers and caps will be obtained from the participating laboratory. The Consultant Sampling Manager will be in charge of ordering sampling containers. All ordered supplies will be examined for damage as they are received. The laboratory will maintain logbooks for all consumables that are checked against all materials received. Bottles and caps will be inspected for damage prior to sampling, and only sound bottles with intact threads will be used. The container caps will be tested for tightness prior to the transport of samples.

The Consultant Sampling Manager will make sure sufficient field supplies are on hand prior to the start of sampling for each period, shown in **Table 5-5**. Field supplies will be stored at the awarded consultant's facility and laboratory supplies will be stored at the delegated laboratory.

Table 5-5 Inspection/Acceptance Testing Requirements for Consumables and Supplies

Project Related Supplies/Consumables	Inspection/Testing Specifications	Acceptance Criteria	Frequency	Responsible Party
Pre-Cleaned Sample Bottles	Open bottle	Lids screwed on bottles	100%	Consultant
Laboratory Glassware	Dirty	Clean	100%	Delegated Laboratory
Lab Solvents and Acids	Leaks	No cracks or chips	Prior to use	Delegated Laboratory
19-Liter Glass	Laboratory blanked	Pass blanking analysis	New bottles each monitoring event	Delegated Laboratory/ Consultant
1-Gallon Glass	If not certified pre-cleaned then laboratory blanked	Pass blanking analysis	New bottles each monitoring event	Delegated Laboratory / Consultant
125-Milliliter Plastic	Laboratory sterilized	Lids screwed on containers	New bottles each monitoring event	Delegated Laboratory
125-Milliliter Glass Container	Laboratory cleaned and blanked	Lids screwed on containers	New bottles each monitoring event	Delegated Laboratory / Consultant
Grab Bags	Dirty, open	Sealed bags	New bottles each monitoring event	Delegated Laboratory
10-Liter HDPE Cubitainers	Laboratory cleaned and blanked	Lids screwed on containers	New bottles each monitoring event	Delegated Laboratory
Silicone Tubing	Laboratory cleaned and blanked	Pass blanking analysis	New tubing at start of program	Delegated Laboratory / Consultant
Teflon Tubing	Laboratory cleaned and blanked	Pass blanking analysis	New tubing at start of program	Delegated Laboratory / Consultant
Gloves	New box	New box	Monthly	Consultant

5.9 Non-Direct Measurements

The RWMP will not use any non-direct measurements.

5.10 Data Management

All data collected for the RWMP will be uploaded to SMARTS and conform to California Environmental Data Exchange Network (CEDEN) minimum data templates format. Data management will be initiated with the use of field and laboratory data sheets. The data are generated by the Consultant Field Sampling Team and the analytical laboratory. Each of the two categories of data management is summarized below.

5.10.1 Laboratory Data Management

The Consultant PM is responsible for leading laboratory data management. Overall management of the data will be consistent with established consultant procedures for receiving water monitoring projects. The Consultant Sampling Manager will be responsible for tracking the analytical process to assure that the laboratory is meeting the required turnaround times and providing a complete deliverable package.

The laboratory will conduct the quality control checks prior to data submittal, for more details regarding laboratory quality assurance and record keeping protocols refer to the QAM. The Consultant Sampling Manager receives the original hard copy from the laboratory, verifies completeness, and logs the date of receipt. Analysis results will be electronically sent to the Consultant Database Manager following the completion of quality control checks by the laboratory. Data will be screened for the following major items:

- A 100 percent check between electronic data provided by the laboratory and the hard copy reports;
- Conformity check between the COC forms and laboratory reports;
- A check for laboratory data report completeness;
- A check for typographical errors on the laboratory reports; and
- A check for suspect values.

The originals are then transferred to the Project Director and filed with all other original project documentation in order to maintain complete project records.

Following the initial screening, a more complete QA/QC review process will be performed, which will include an evaluation of holding times, method and equipment blank contamination, and analytical accuracy and precision.

The laboratory will be requested to provide data in both hard copy and electronic formats. The form of electronic submittals will conform to reporting protocols that are compatible with SWAMP. A relational database will be developed by the Consultant and used for all data. Laboratory data will be maintained and managed with Microsoft Excel and/or Microsoft Access by the Consultant Database Manager.

The Consultant Database Manager will control the access to the project's database. The laboratory EDDs will be maintained in a file separate to the cumulative database so the original is maintained and can be used as a reference. If data is reissued, the file name will include the date and the word 'revised'. To manage the revision and prevent duplicate entries, the erroneous dataset will be removed from the database prior to uploading the revised dataset.

The Laboratory Manager at the delegated laboratory will maintain their respective analytical laboratory records. The Laboratory Project Manager will oversee the actions of these persons and will arbitrate any issues relative to records retention and any decisions to discard records. All original laboratory notebooks and data summaries will be maintained in secure areas and electronic databases will be maintained and backed up.

5.10.2 Field Data Management

The Consultant Sampling Manager will be responsible for the proper management of field measurement and observation data. The Consultant Sampling Manager will review all Field Conditions Data Log Sheets for completeness and maintain the original hardcopies in the project file. The Field Conditions Data Log Sheet responses will also be manually entered into an electronic version of the Field Conditions Data Log Sheet and these fields will be saved in the Microsoft Access Database. The data will be manually entered by one individual and the entries will be checked against the hard copies for accuracy by a second individual. Photographs of the monitoring sites taken by field personnel will be uploaded into the project file within three days of taking the photograph. Field team members will name the photographs using the photograph naming convention developed specifically for this project.

6. Assessment and Oversight

6.1 Assessments and Response Actions

The Consultant PM will be responsible for the day-to-day oversight of the project. The Consultant QA/QCM will review progress of the monitoring program. The managers and coordinators of the project, along with the Consultant QA/QCM, will meet to discuss the site location, sampling, laboratory analyses, data management, and the overall status of the project. This information will be communicated monthly between the MRWG Project Director and the Consultant PM and Consultant Sampling Manager. The Consultant Sampling Manager will review laboratory data and field data. The Consultant QA/QCM has the power to halt all sampling and analytical work by the monitoring personnel and the delegated laboratory if the deviations noted are considered detrimental to data quality.

Three types of assessments will be performed as part of this project to ensure that the sampling and analysis activities are in accordance with the approved QAPP. They are as follows:

1. **Surveillance of Sample Collection Activities**. The Consultant Sampling Manager will be responsible for oversight of sampling activities and will review field datasheets to verify that the samples were collected in accordance with QAPP requirements. The Consultant QA/QCM will accompany the field crew at least once, toward the beginning of the data collection phase of the project, and again at some later point, if deemed necessary, to audit field activities. If the Consultant QA/QCM finds any of the field activities to be in violation of QAPP requirements, the Consultant QA/QCM has the authority to stop these activities until corrective actions are successfully implemented. These include additional training to improve field team performance and QAPP compliance, and appropriate re-sampling of sites, as needed. The Consultant QA/QCM will report all such actions to the Consultant PM and document it in the project file.

2. **Data Quality Assessment.** The Consultant Sampling Manager is responsible for reviewing laboratory reports to verify that the performance criteria of the QAPP were met. This will occur following receipt of each report from the contracted laboratory. If it is determined that the precision and accuracy objectives were not met the Consultant Sampling Manager will notify the Consultant QA/QCM and Consultant PM. Then the contract laboratory QA Officer will review laboratory techniques to minimize errors, and samples will be re-analyzed, if possible.

3. **Assessment of Data Entry.** Once the performance criteria are met, data analysis can be conducted. The Consultant Sampling Manager will review data files to ensure that errors are detected and corrected.

If an audit discovers any discrepancy, the Consultant QA/QCM will discuss the observed discrepancy with the appropriate personnel responsible for the activity. The discussion will determine whether the information collected is accurate, what caused the deviation, how the deviation impacts data quality, and what corrective actions are necessary as provided in **Sections 5.5.2** and **5.5.4**. Any corrective actions taken will be verified based on satisfactory collection of data in accordance with the QAPP, following these actions. The QAPP violation(s), corrective action(s), and verification of correction will be reported in a Corrective Action Plan by the Consultant QA/QCM to the Consultant PM and kept on record.

6.2 Reports to Management

The Consultant designated laboratory will complete an EDD following each sampling event. The EDDs will be submitted to MRWG with the Annual Monitoring Report. The EDD will contain the following information:

- Laboratory results
- Laboratory QA/QC results
- Field Forms

The laboratory results will be submitted in Microsoft Excel format. The field forms will include the completed Field Conditions Data Log Sheets in PDF format. Responses to the Field Conditions Data Log Sheets will also be provided in Microsoft Excel format. The Consultant will prepare a draft and final annual monitoring report to be attached to the Annual Report. The report will provide a review and analysis of the data provided in the EDD. The draft report will be submitted to MRWG for a two-week period for review and comment. The consultant will address MRWG's comments and incorporate into the final report. A summary of the deliverables and frequencies are presented in **Table 6-1**.

Table 6-1 QA Management Reports

Type of Report	Frequency	Person(s) Responsible for Report Preparation	Report Recipients
Electronic Data Deliverable	Once Annually	Consultant	MRWG
Draft Annual Monitoring Report	Once Annually	Consultant	MRWG
Final Annual Monitoring Report	Once Annually	Consultant	MRWG

7. Data Validation and Usability

7.1 Data Review, Verification, and Validation

All analytical data will be reviewed and compared to the MQO presented in **Table 4-2**. If results fail to meet any MQOs, the Consultant Sampling Manager and/or the Consultant QA/QCM will flag them for further review. Batch QA samples will be reviewed to determine the potential cause of failure to meet the MQO. If the cause cannot be readily ascertained, reserve samples will be reanalyzed, if within designated holding times. If subsequent analyses meet the MQO, the samples will be deemed acceptable.

If samples fail to meet the MQOs a second time or cause of the failure cannot be identified and rectified, the data will be excluded from inclusion in the results of the Annual Monitoring Report. All rejected data will be retained in the project database, and qualified as "rejected." The ultimate decision of whether to accept or reject a data point will be made by the Consultant PM in consultation with the Consultant QA/QCM.

If the analysis for more than ten percent of any given analyte fails to meet the MQOs, the Consultant PM and Consultant QA/QCM will meet to discuss the appropriateness of the MQOs and any potential modifications. All proposed modifications of MQOs will be reviewed by MRWG.

7.2 Verification and Validation Methods

Data verification is the process of evaluating the completeness, correctness, and conformance of the dataset against the method, procedural, or contractual requirements. Data quality indicators will be continuously monitored by the analyst producing the data (field and lab personnel), as well as the Consultant Sampling Manager, with assistance from the Consultant QA/QCM, throughout the project to make sure corrective actions are taken in a timely manner. Laboratory and field personnel responsible for conducting QA analysis will be responsible for documenting when data does not meet MQOs as determined by data quality indicators.

In coordination with the Consultant QA/QCM, the Consultant Sampling Manager will validate and verify field measurements and activities (sample collection and handling) and the Laboratory Quality Assurance Manager will validate and verify laboratory analysis (sample analysis and handling). Following sample delivery, the laboratory will maintain COCs and sample manifests. Laboratory validation and verification of the data generated is the responsibility of the laboratory. The Laboratory Project Manager maintains analytical reports in a database format as well as all QA/QC documentation for the laboratory. The Laboratory Quality Assurance Manager will perform checks of all of its records.

The Laboratory Project Manager and Consultant Sampling Managers are responsible for oversight of data collection and the analysis of the raw data obtained from the laboratory and the field. Reconciliation and correction of data that fails to meet the MQOs will be done by the responsible manager in consultation with the Consultant QA/QCM and the Consultant PM. Corrections require a unanimous agreement that the correction is appropriate.

Data verification and validation of field sample collection and handling consists of the following tasks:

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

Data verification and validation for the laboratory sample analysis and handling activities will include the following tasks:

- Verification that all samples recorded on COCs forms were received by the laboratory.
- Verification that the appropriate analytical methodology has been followed.
- Verification that QC samples meet performance criteria.
- Verification that analytical results and documentation are complete.

Verification and validation of data entry includes:

- Sorting data to identify missing or mistyped (too large or too small) values.
- Double-checking all typed values.

- Data is entered in the proper format for each database fields (i.e., text for text, integers for integers, number for numbers, dates for dates, times for times, etc.).

7.3 Reconciliation with User Requirements

The data quality will be evaluated according to this document with respect to the sampling design, sampling methods, field and laboratory analyses, quality control, and maintenance. By properly following the guidelines in this document and references, the data quality will be validated. If samples or procedures used in this study fail to meet the guidelines listed in this document, the data will be flagged and reported to the Consultant PM. The limitations and assumptions of the data will be provided to the end-user to allow the user to determine the data's usefulness.

The end-user will use this data to determine the compliance of the MS4 discharges within the management area. This data will help to characterize pollutant loads and the whether BMPs are effective at maintain water quality during new development of the Mojave Watershed area. The results will identify if this is the case and any pollutants found in excess of maximum levels. A summary of this will be presented in the Annual Monitoring Report, to be submitted with the Annual Report to the Regional Water Board.

8. References

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Attachment 1

SWAMP MQOs

Ancillary Parameters
in Freshwater Sediment and Marine Sediment

A list of parameters included in this category may be found in the associated QAPrPTableReference.

Terms appearing in the tables are defined in the *Surface Water Ambient Monitoring Program Quality Assurance Program Plan*, which contains a glossary (Appendix E), as well as a list of abbreviations and acronyms (Appendix F).

Table 1: Quality Control[1]: Ancillary Parameters in Freshwater Sediment and Marine Sediment

Laboratory Quality Control	Frequency of Analysis	Measurement Quality Objective
Laboratory Blank	One per analytical batch	Per method
Laboratory Duplicate	One per analytical batch	RPD<25%
Field Quality Control	**Frequency of Analysis**	**Measurement Quality Objective**
Field Duplicate	5% of total project sample count	RPD<25%
Field Blank, Travel Blank, Equipment Blank	Per method	<30% of lowest sample

[1] Unless method specifies more stringent requirements

Table 2: Sample Handling: Ancillary Parameters in Freshwater Sediment and Marine Sediment

Recommended Container[1]	Recommended Preservation	Required Holding Time[2]
G	Cool to ≤6 ˚C	7 days

[1] "G" is glass

[2] Each "Required Holding Time" is based on the assumption that the "Recommended Preservation" (or a method-mandated alternative) has been employed. If a "Required Holding Time" for filtration, preservation, preparation, or analysis is not met, the project manager and SWAMP Quality Assurance Officer must be notified. Regardless of preservation technique, data not meeting the "Required Holding Time" will be appropriately flagged in the SWAMP database.

Table 3: Recommended Corrective Action: Ancillary Parameters in Freshwater Sediment and Marine Sediment

Laboratory Quality Control	Recommended Corrective Action
Laboratory Blank	Reanalyze the blank to confirm the result. Investigate the source of contamination. If the source of the contamination is isolated to the sample preparation, the entire batch of samples, along with the new laboratory blanks and associated QC samples, should be prepared and/or re-extracted and analyzed. If the source of contamination is isolated to the analysis procedures, reanalyze the entire batch of samples. If reanalysis is not possible, the associated sample results must be flagged to indicate the potential presence of the contamination.
Laboratory Duplicate	Reanalyze the duplicate samples to confirm the results. Visually inspect the samples to determine if a high RPD between the results could be attributed to sample heterogeneity. For duplicate results due to matrix heterogeneity, or where ambient concentrations are below the reporting limit, qualify the results and document the heterogeneity.
Field Quality Control	**Recommended Corrective Action**
Field Duplicate	Visually inspect the samples to determine if a high RPD between results could be attributed to sample heterogeneity. For duplicate results due to matrix heterogeneity, or where ambient concentrations are below the reporting limit, qualify the results and document the heterogeneity. All failures should be communicated to the project coordinator, who in turn will follow the process detailed in the method.
Field Blank, Travel Blank, Equipment Blank	Investigate the source of contamination. Potential sources of contamination include sampling equipment, protocols, and handling. The laboratory should report evidence of field contamination as soon as possible so corrective actions can be implemented. Samples collected in the presence of field contamination should be flagged.

Conventional Parameters in Freshwater Sediment and Marine Sediment

A list of parameters included in this category may be found in the associated QAPrPTableReference.

Terms appearing in the tables are defined in the *Surface Water Ambient Monitoring Program Quality Assurance Program Plan*, which contains a glossary (Appendix E), as well as a list of abbreviations and acronyms (Appendix F).

Table 1: Quality Control[1]: Conventional Parameters in Freshwater Sediment and Marine Sediment

Laboratory Quality Control	Frequency of Analysis	Measurement Quality Objective
Calibration Standard	Per analytical method or manufacturer's specifications	Per analytical method or manufacturer's specifications
Laboratory Blank	Total organic carbon only: one per analytical batch (n/a for other parameters)	<RL or <30% of lowest sample
Reference Material	Total organic carbon only: one per 20 samples or per analytical batch, whichever is more frequent (n/a for other parameters)	80-120% recovery
Laboratory Duplicate	One per analytical batch	RPD<25% (n/a if native concentration of either sample<RL)
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 or <30% of lowest sample

[1] Unless method specifies more stringent requirements

Table 2: Sample Handling: Conventional Parameters in Freshwater Sediment and Marine Sediment

Parameter	Recommended Container[1,2]	Recommended Preservation	Required Holding Time[3]
Acid-Volatile Sulfides	G	Freeze to ≤-20 °C	1 year
Grain Size	G	Wet ice to ≤6 °C in the field, then refrigerate at ≤6 °C	1 year
Organic Carbon (Total)	G	Cool to ≤6 °C or freeze to ≤-20 °C	28 days at ≤6 °C; 1 year at ≤-20 °C
Phosphorus (Total)	G	Cool to ≤6 °C	14 days

[1] "G" is glass

[2] Samples for total organic carbon and grain size analysis can be combined in one 250-mL clear glass jar, and sub-sampled at the laboratory in order to utilize holding time differences for the two analyses. If this is done, the 250 mL combined sediment sample must be refrigerated only (not frozen) at ≤6 °C for up to 28 days, during which time the sub-samples must be aliquoted in order to comply with separate storage requirements.

[3] Each "Required Holding Time" is based on the assumption that the "Recommended Preservation" (or a method-mandated alternative) has been employed. If a "Required Holding Time" for filtration, preservation, preparation, or analysis is not met, the project manager and SWAMP Quality Assurance Officer must be notified. Regardless of preservation technique, data not meeting the "Required Holding Time" will be appropriately flagged in the SWAMP database.

Table 3: Recommended Corrective Action: Conventional Parameters in Freshwater Sediment and Marine Sediment

Laboratory Quality Control	Recommended Corrective Action
Calibration Standard	Recalibrate the instrument. Affected samples and associated quality control must be reanalyzed following successful instrument recalibration.
Laboratory Blank	Reanalyze the blank to confirm the result. Investigate the source of contamination. If the source of the contamination is isolated to the sample preparation, the entire batch of samples, along with the new laboratory blanks and associated QC samples, should be prepared and/or re-extracted and analyzed. If the source of contamination is isolated to the analysis procedures, reanalyze the entire batch of samples. If reanalysis is not possible, the associated sample results must be flagged to indicate the potential presence of the contamination.
Reference Material	Reanalyze the reference material to confirm the result. Compare this to the matrix spike/matrix spike duplicate recovery data. If adverse trends are noted, reprocess all of the samples associated with the batch.
Laboratory Duplicate	Reanalyze the duplicate samples to confirm the results. Visually inspect the samples to determine if a high RPD between the results could be attributed to sample heterogeneity. For duplicate results due to matrix heterogeneity, or where ambient concentrations are below the reporting limit, qualify the results and document the heterogeneity.
Field Quality Control	**Recommended Corrective Action**
Field Duplicate	Visually inspect the samples to determine if a high RPD between results could be attributed to sample heterogeneity. For duplicate results due to matrix heterogeneity, or where ambient concentrations are below the reporting limit, qualify the results and document the heterogeneity. All failures should be communicated to the project coordinator, who in turn will follow the process detailed in the method.
Field Blank, Travel Blank, Equipment Blank	Investigate the source of contamination. Potential sources of contamination include sampling equipment, protocols, and handling. The laboratory should report evidence of field contamination as soon as possible so corrective actions can be implemented. Samples collected in the presence of field contamination should be flagged.

Inorganic Analytes
in Freshwater Sediment and Marine Sediment

A list of analytes included in this category may be found in the associated QAPrPTableReference.

Terms appearing in the tables are defined in the *Surface Water Ambient Monitoring Program Quality Assurance Program Plan*, which contains a glossary (Appendix E), as well as a list of abbreviations and acronyms (Appendix F).

Table 1: Quality Control[1]: Inorganic Analytes in Freshwater Sediment and Marine Sediment

Laboratory Quality Control	Frequency of Analysis	Measurement Quality Objective
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	75-125% recovery (70-130% for methylmercury)
Matrix Spike	Per 20 samples or per analytical batch, whichever is more frequent	75-125% recovery (70-130% for methylmercury)
Matrix Spike Duplicate	Per 20 samples or per analytical batch, whichever is more frequent	75-125% recovery (70-130% for methylmercury); RPD<25%
Laboratory Duplicate	Per 20 samples or per analytical batch, whichever is more frequent	RPD<25% (n/a if native concentration of either sample<RL)
Internal Standard	Accompanying every analytical run when method appropriate	60-125% recovery
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), unless otherwise specified by method
Field Blank, Equipment Blank	Per method	Blanks<RL for target analyte

[1] Unless method specifies more stringent requirements

Table 2: Sample Handling: Inorganic Analytes in Freshwater Sediment and Marine Sediment

Analyte	Recommended Container[1]	Recommended Preservation	Required Holding Time[2]
Methylmercury	G	Freeze to ≤-20 °C immediately	1 year
Trace Metals[3]	G	Cool to ≤6 °C within 24 hours, then freeze to ≤-20 °C	1 year; samples must be analyzed within 14 days of collection or thawing

[1] "G" is glass

[2] Each "Required Holding Time" is based on the assumption that the "Recommended Preservation" (or a method-mandated alternative) has been employed. If a "Required Holding Time" for filtration, preservation, preparation, or analysis is not met, the project manager and SWAMP Quality Assurance Officer must be notified. Regardless of preservation technique, data not meeting the "Required Holding Time" will be appropriately flagged in the SWAMP database.

[3] With the exception of methylmercury

Table 3: Recommended Corrective Action: Inorganic Analytes in Freshwater Sediment and Marine Sediment

Laboratory Quality Control	Recommended Corrective Action
Calibration Standard	Recalibrate the instrument. Affected samples and associated quality control must be reanalyzed following successful instrument recalibration.
Calibration Verification	Reanalyze the calibration verification to confirm the result. If the problem continues, halt analysis and investigate the source of the instrument drift. The analyst should determine if the instrument must be recalibrated before the analysis can continue. All of the samples not bracketed by acceptable calibration verification must be reanalyzed.
Laboratory Blank	Reanalyze the blank to confirm the result. Investigate the source of contamination. If the source of the contamination is isolated to the sample preparation, the entire batch of samples, along with the new laboratory blanks and associated QC samples, should be prepared and/or re-extracted and analyzed. If the source of contamination is isolated to the analysis procedures, reanalyze the entire batch of samples. If reanalysis is not possible, the associated sample results must be flagged to indicate the potential presence of the contamination.
Reference Material	Reanalyze the reference material to confirm the result. Compare this to the matrix spike/matrix spike duplicate recovery data. If adverse trends are noted, reprocess all of the samples associated with the batch.
Matrix Spike	The spiking level should be near the midrange of the calibration curve or at a level that does not require sample dilution. Reanalyze the matrix spike to confirm the result. Review the recovery obtained for the matrix spike duplicate. Review the results of the other QC samples (such as reference materials) to determine if other analytical problems are a potential source of the poor spike recovery.
Matrix Spike Duplicate	The spiking level should be near the midrange of the calibration curve or at a level that does not require sample dilution. Reanalyze the matrix spike duplicate to confirm the result. Review the recovery obtained for the matrix spike. Review the results of the other QC samples (such as reference materials) to determine if other analytical problems are a potential source of the poor spike recovery.
Laboratory Duplicate	Reanalyze the duplicate samples to confirm the results. Visually inspect the samples to determine if a high RPD between the results could be attributed to sample heterogeneity. For duplicate results due to matrix heterogeneity, or where ambient concentrations are below the reporting limit, qualify the results and document the heterogeneity.
Internal Standard	Check the response of the internal standards. If the instrument continues to generate poor results, terminate the analytical run and investigate the cause of the instrument drift.
Field Quality Control	**Recommended Corrective Action**
Field Duplicate	Visually inspect the samples to determine if a high RPD between results could be attributed to sample heterogeneity. For duplicate results due to matrix heterogeneity, or where ambient concentrations are below the reporting limit, qualify the results and document the heterogeneity. All failures should be communicated to the project coordinator, who in turn will follow the process detailed in the method.
Field Blank, Equipment Blank	Investigate the source of contamination. Potential sources of contamination include sampling equipment, protocols, and handling. The laboratory should report evidence of field contamination as soon as possible so corrective actions can be implemented. Samples collected in the presence of field contamination should be flagged.

Conventional Parameters in Fresh and Marine Water

A list of parameters included in this category may be found in the associated QAPrPTableReference.

Terms appearing in the tables are defined in the *Surface Water Ambient Monitoring Program Quality Assurance Program Plan*, which contains a glossary (Appendix E), as well as a list of abbreviations and acronyms (Appendix F).

Table 1: Quality Control: Conventional Parameters in Fresh and Marine Water

Laboratory Quality Control	Frequency of Analysis	Measurement Quality Objective
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[2]	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

[1] Unless method specifies more stringent requirements

[2] Field duplicate relative percent differences are not calculated for chlorophyll a analyses for bioassessment

Table 2: Sample Handling: Conventional Parameters in Fresh and Marine Water

Analyte	Recommended Container[1]	Recommended Preservation[2,3]	Required Holding Time[4]
Alkalinity (as $CaCO_3$)[5]	P	Cool to ≤6 °C	14 days
Biochemical Oxygen Demand	P	Cool to ≤6 °C; add 1 g FAS crystals per liter if residual chlorine is present	48 hours
Chemical Oxygen Demand (Titrametric)	G	Cool to ≤6 °C; H_2SO_4 to pH<2	28 days; biologically active samples should be tested as soon as possible
Chloride	P	None required	28 days
Chlorophyll a Pheophytin a	Per method	Centrifuge or filter as soon as possible after collection; if processing must be delayed, keep samples on ice or at ≤6 °C; store in the dark	Samples must be frozen or analyzed within 4 hours of collection; filters can be stored frozen for 28 days
Cyanide (Total)	P	Cool to ≤6 °C; NaOH to pH>10; add 0.6 g $C_6H_8O_6$ if residual chlorine is present	14 days
Fluoride	P	None required	28 days
Hardness (as $CaCO_3$)	P	Cool to ≤6 °C; HNO_3 or H_2SO_4 to pH<2	6 months
Oil and Grease	G	Cool to ≤6 °C; HNO_3 or H_2SO_4 to pH<2	28 days
Organic Carbon (Dissolved)	G	Filter and preserve to pH<2 within 48 hours of collection; cool to ≤6 °C	28 days
Organic Carbon (Total)	G	Cool to ≤6 °C; acidify to pH<2 with HCl, H_3PO_4, or H_2SO_4 within 2 hrs	28 days
Perchlorate	P, G	Protect from temperature extremes	28 days
Phenols[6]	G	Cool to ≤6 °C; H_2SO_4 to pH<2	28 days
Silica	P	Cool to ≤6 °C; HNO_3 to pH<2	28 days; 6 months if acidified
Specific Conductance	P	Cool to ≤6 °C; if analysis is not completed within 24 hours of sample collection, sample should be filtered through a 0.45 micron filter and stored at ≤6 °C	28 days
Sulfate	P	Cool to ≤6 °C	28 days
Turbidity	P	Cool to ≤6 °C	48 hours

[1] "P" is polyethylene; "G" is glass

[2] Per the draft *National Coastal Assessment Quality Assurance Project Plan* (August 2009), marine waters in plastic containers may be ultra-frozen to ≤-50 °C for a maximum of six months.

[3] Per 40 CFR 136.3, aqueous samples must be preserved at ≤6 °C, and should not be frozen unless data demonstrating that sample freezing does not adversely impact sample integrity is maintained on file and accepted as valid by the regulatory authority. The preservation temperature does not apply to samples that are analyzed immediately (less than 15 minutes).

[4] Each "Required Holding Time" is based on the assumption that the "Recommended Preservation" (or a method-mandated alternative) has been employed. If a "Required Holding Time" for filtration, preservation, preparation, or analysis is not met, the project manager and SWAMP Quality Assurance Officer must be notified. Regardless of preservation technique, data not meeting the "Required Holding Time" will be appropriately flagged in the SWAMP database.

[5] Marine samples for alkalinity (as $CaCO_3$) may be cooled to ≤6 °C for a maximum of 24 hours.

[6] This table applies to phenols analysis using colorimetry. Guidelines for the chromatographic analysis of phenols are located in *Synthetic Organic Compounds in Water* Table 4*: Sample Handling.*

Table 3: Recommended Corrective Action: Conventional Parameters in Fresh and Marine Water

Laboratory Quality Control	Recommended Corrective Action
Calibration Standard	Recalibrate the instrument. Affected samples and associated quality control must be reanalyzed following successful instrument recalibration.
Calibration Verification	Reanalyze the calibration verification to confirm the result. If the problem continues, halt analysis and investigate the source of the instrument drift. The analyst should determine if the instrument must be recalibrated before the analysis can continue. All of the samples not bracketed by acceptable calibration verification must be reanalyzed.
Laboratory Blank	Reanalyze the blank to confirm the result. Investigate the source of contamination. If the source of the contamination is isolated to the sample preparation, the entire batch of samples, along with the new laboratory blanks and associated QC samples, should be prepared and/or re-extracted and analyzed. If the source of contamination is isolated to the analysis procedures, reanalyze the entire batch of samples. If reanalysis is not possible, the associated sample results must be flagged to indicate the potential presence of contamination.
Reference Material	Reanalyze the reference material to confirm the result. Compare this to the matrix spike/matrix spike duplicate recovery data. If adverse trends are noted, reprocess all of the samples associated with the batch.
Matrix Spike	The spiking level should be near the midrange of the calibration curve or at a level that does not require sample dilution. Reanalyze the matrix spike to confirm the result. Review the recovery obtained for the matrix spike duplicate. Review the results of the other QC samples (such as reference materials) to determine if other analytical problems are a potential source of the poor spike recovery.
Matrix Spike Duplicate	The spiking level should be near the midrange of the calibration curve or at a level that does not require sample dilution. Reanalyze the matrix spike duplicate to confirm the result. Review the recovery obtained for the matrix spike. Review the results of the other QC samples (such as reference materials) to determine if other analytical problems are a potential source of the poor spike recovery.
Laboratory Duplicate	Reanalyze the duplicate samples to confirm the results. Visually inspect the samples to determine if a high RPD between the results could be attributed to sample heterogeneity. For duplicate results due to matrix heterogeneity, or where ambient concentrations are below the reporting limit, qualify the results and document the heterogeneity.
Internal Standard	Check the response of the internal standards. If the instrument continues to generate poor results, terminate the analytical run and investigate the cause of the instrument drift.
Field Quality Control	**Recommended Corrective Action**
Field Duplicate	Visually inspect the samples to determine if a high RPD between results could be attributed to sample heterogeneity. For duplicate results due to matrix heterogeneity, or where ambient concentrations are below the reporting limit, qualify the results and document the heterogeneity. All failures should be communicated to the project coordinator, who in turn will follow the process detailed in the method.
Field Blank, Travel Blank, Equipment Blank	Investigate the source of contamination. Potential sources of contamination include sampling equipment, protocols, and handling. The laboratory should report evidence of field contamination as soon as possible so corrective actions can be implemented. Samples collected in the presence of field contamination should be flagged.

Field Measurements in Fresh and Marine Water

A list of parameters included in this category may be found in the associated QAPrPTableReference.

Terms appearing in the tables are defined in the *Surface Water Ambient Monitoring Program Quality Assurance Program Plan*, which contains a glossary (Appendix E), as well as a list of abbreviations and acronyms (Appendix F).

Table 1: Quality Control[1]: Field Measurements in Fresh and Marine Water

Water Quality Parameter	Recommended Device	Units	Resolution	Instrument Accuracy Specs	Points per Calibration	Pre-Sampling Calibration Check Frequency[2]	Post-Sampling Calibration Check Frequency[2]	Allowable Drift[3]
Dissolved Oxygen	Polarographic or luminescence quenching probe	mg/L	0.01	±0.2	1	Before every monitoring day on-site (re-calibrate if change of elevation is ≥500 m or barometric pressure > 2mmHg)	After every monitoring day (within 24 hours)	±0.5 or 10%
pH	Electrode	pH	0.01	±0.2	2	Per manufacturer	Per manufacturer	±0.2 units
Salinity	Refractometer or conductivity cell	ppt	0.01	±2%	Per manufacturer	Per manufacturer	Per manufacturer	Per manufacturer
Secchi Depth	Stadia rod (top setting)/staff gauge	m or decimal ft	0.01	n/a	2	n/a	n/a	n/a
Specific Conductance	Conductivity cell	µS/cm*	1	±0.5%	Per manufacturer	Per manufacturer	Per manufacturer	±10%
Temperature	Thermistor or bulb	°C	0.1	±0.15	Per manufacturer	Per manufacturer	Per manufacturer	±0.5
Total Chlorophyll[4]	Optical fluorescence chlorophyll probe	µg/L	0.1	n/a	2	Per manufacturer	Per manufacturer	±10%

Water Quality Parameter	Recommended Device	Units	Resolution	Instrument Accuracy Specs	Points per Calibration	Pre-Sampling Calibration Check Frequency[2]	Post-Sampling Calibration Check Frequency[2]	Allowable Drift[3]
Turbidity	Portable turbidimeter or optical probe	NTU	0.1	±1% up to 100 NTU; ±3% from 100-400 NTU; and ±5% from 400-3000 NTU	2	Per manufacturer	Per manufacturer	±0.2 or 10%
Velocity	Flow meter[5]	ft/s	0.1	Per manufacturer	Per manufacturer	Per manufacturer[6]	Per manufacturer[6]	Per manufacturer

[1] Unless manufacturer specifies more stringent requirements

[2] SWAMP requires daily pre- and post-sampling calibration checks when the manufacturer or documented procedure (e.g., standard operating procedure) do not provide calibration instructions

* mS/cm for marine water

[3] Unit or percentage, whichever is greater

[4] Water column only

[5] Electromagnetic meters should undergo periodic maintenance according to manufacturer instructions

[6] Price AA meter: spin test>2 minutes; pygmy meter: spin test>45 seconds; electromagnetic meter: zero check

Table 2: Corrective Action: Field Measurements in Fresh and Marine Water

Corrective Action
The instrument should be recalibrated following manufacturer cleaning and maintenance procedures. If measurements continue to fail measurement quality objectives, affected data should not be reported and the instrument should be returned to the manufacturer for maintenance. All troubleshooting and corrective actions should be recorded in calibration and field data logbooks.

Indicator Bacteria in Fresh Water

The following tables are not applicable to marine water samples.

A list of species included in this category may be found in the associated QAPrPTableReference.

Terms appearing in the tables are defined in the *Surface Water Ambient Monitoring Program Quality Assurance Program Plan*, which contains a glossary (Appendix E), as well as a list of abbreviations and acronyms (Appendix F).

Table 1: Quality Control[1]: Indicator Bacteria in Fresh Water

Laboratory Quality Control	Frequency of Analysis	Measurement Quality Objective
Sterility Checks[3]	Per new lot of dehydrated culture media as instructed in SM 9020B.4.i.5[2] and SM 9222D.1.a	No growth
	For non-sterile filters and pads per lot as instructed in SM 9020B.4.h.1.1	No growth
	Membrane Filter Media, filters, buffered dilution water, rinse water, and all equipment per series of samples as instructed in SM 9020B.8.a.5[2]	No growth
	Multiple Tube Media, dilution water, and glassware as instructed in SM 9020B.8.a.5[2]	No growth
Laboratory Positive Control	Per new lot of dehydrated culture media for the following methods: Colilert, Colilert -18, Colisure, Enterolert, or other chromogenic/fluorogenic methods. Per new lot of commercially-prepared culture media ampules for USEPA-approved fecal coliform and E. coli membrane filter methods (e.g. SM 9222, m-ColiBlue24, EPA 1603) Per batch for laboratory-prepared culture media for USEPA-approved fecal coliform and E. coli membrane filter methods (e.g., SM 9222)	Positive response
Laboratory Negative Control	Per new lot of dehydrated culture media for the following methods: Colilert, Colilert -18, Colisure, Enterolert, or other chromogenic/fluorogenic methods. Per new lot of commercially-prepared culture media ampules for USEPA-approved fecal coliform and E. coli membrane filter methods (e.g. SM 9222, m-ColiBlue24, EPA 1603) Per batch for laboratory-prepared culture media for USEPA-approved fecal coliform and E. coli membrane filter methods (e.g., SM 9222)	Negative response
Laboratory Duplicate	Per 10 samples or per analytical batch, whichever is more frequent	$R_{log} \leq 3.27 \times R^4$ Computation of R from duplicate laboratory sample analyses
Laboratory Blank[5]	Required only when samples are diluted; dilution water must be tested	No growth

Field Quality Control[6]	Frequency of Analysis	Measurement Quality Objective
Field Blank, Equipment Blank	Per method or SOP	Negative response

[1] Unless method specifies more stringent requirements

[2] Citations from *Standard Methods for the Examination of Water and Wastewater*, 20th edition

[3] Sterility Checks

The specific type and number of sterility checks are method-dependent. For example, membrane filter tests require the testing of filters for sterility, while multiple-tube or pour plate procedures do not.

[4] Method for Determining Precision

In order to determine precision for bacterial analysis, the following procedure (adapted from Standard Methods 9020 Section 8.b) will be used. Note: When determining the precision of bacterial analyses, it is important to distinguish between different matrices (drinking water, wastewater, ambient water). Duplicate results from different matrices must be kept separate when calculating precision.

In order to calculate the laboratory precision for bacterial analyses, the results from the preceding 15 positive samples of a specific type (matrix) are used to calculate a running mean. The results used to calculate the running mean must all correspond to the same quality control parameter, in this instance laboratory duplicates (as opposed to field duplicates). The results of different quality control parameters such as laboratory and field duplicates must not both be used to calculate a single running mean. Note: Field duplicates are not a current SWAMP requirement (see footnote 6).

Step 1: Record the results from duplicate analyses (these results are here designated as D_1 and D_2).

Step 2: Calculate the logarithm (here designated as L_1 and L_2) of each duplicate result. Note: If either of the values D1 or D2 are less than 1, add 1 to both values before calculating the logarithms.

$$L_1 = \log D_1$$
$$L_2 = \log D_2$$

Step 3: Calculate the range of logarithms (R_{log}) for each pair of duplicates. R_{log} is equal to the absolute value of the difference between the two numbers.

$$R_{log} = | L_1 - L_2 |$$

Step 4: Calculate the mean of R_{log} ($\bar{R}$) for the duplicates analyzed

$$\bar{R} = \frac{\sum R_{log}}{n}$$

Where

$\sum R_{log}$ = the sum of the ranges of logarithms calculated for each pair of duplicates n

= the number of pairs of duplicates (in this case, n = 15)

Step 5: Assess the precision of the duplicate analyses. In order for the laboratory to demonstrate an acceptable level of precision, the range of logarithms for a particular duplicate must be less than the mean of the range of logarithms multiplied by 3.27.

$$R_{log} \leq 3.27 \times \bar{R}$$

[5] Laboratory Blanks

Analysis and reporting of laboratory blanks is required only when samples are diluted prior to analysis. If samples are not diluted in the sample batch, no laboratory blanks are required for that specific sample batch.

[6] Field Duplicates

While SWAMP recommends that field duplicates be collected and analyzed, they are not a current SWAMP requirement. Projects are encouraged to require field duplicates in their QA project plan (QAPP) if it supports their specific quality objectives.

Table 2: Sample Handling: Indicator Bacteria in Fresh Water

Recommended Container	Recommended Preservation	Required Holding Time[1,2,3]
Factory-sealed, pre-sterilized, disposable whirlpak bags or 125-mL sterile plastic (high density polyethylene, polystyrene, or polypropylene) or glass container	Cool to ≤10 °C; for samples containing chlorine, sodium thiosulfate is pre-added to the containers in the laboratory	24 hours (8 hours for regulatory data)

[1] Each "Required Holding Time" is based on the assumption that the "Recommended Preservation" (or a method-mandated alternative) has been employed. If a "Required Holding Time" for filtration, preservation, preparation, or analysis is not met, the project manager and SWAMP Quality Assurance Officer must be notified. Regardless of preservation technique, data not meeting the "Required Holding Time" will be appropriately flagged in the SWAMP database.

[2] Sample analysis should begin as soon as possible after receipt; sample incubation must be started no later than 8 hours from time of collection.

[3] For fecal coliform samples for sewage sludge (biosolids) only, the holding time is extended to 24 hours for the following sample types using either EPA Method 1680 (LTB–EC) or 1681 (A–1): Class A composted, Class B aerobically digested, and Class B anaerobically digested.

Table 3: Corrective Action: Indicator Bacteria in Fresh Water

Laboratory Quality Control	Corrective Action
Sterility Checks	Identify contamination source and take appropriate action; discard membrane filter/pad or prepared media lot; discard sample results if checks made during analysis
Laboratory Positive Control	Identify cause and take appropriate action; discard prepared media and remake from start or purchase new lot
Laboratory Negative Control	Identify cause and take appropriate action; discard prepared media and remake from start or purchase new lot
Laboratory Duplicate	Verify results; qualify data as appropriate
Laboratory Blank	Identify contamination source and take appropriate action; qualify data as needed
Field Quality Control	**Corrective Action**
Field Blank, Equipment Blank	Examine field log; identify potential contamination source; qualify data as needed

Inorganic Analytes in Fresh and Marine Water

A list of analytes included in this category may be found in the associated QAPrPTableReference.

Terms appearing in the tables are defined in the *Surface Water Ambient Monitoring Program Quality Assurance Program Plan*, which contains a glossary (Appendix E), as well as a list of abbreviations and acronyms (Appendix F).

Table 1: Quality Control[1]: Inorganic Analytes in Fresh and Marine Water

Laboratory Quality Control	Frequency of Analysis	Measurement Quality Objective
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[2]	Per 20 samples or per analytical batch, whichever is more frequent	75-125% recovery (70-130% for MMHg)
Matrix Spike	Per 20 samples or per analytical batch, whichever is more frequent	75-125% recovery (70-130% for MMHg)
Matrix Spike Duplicate	Per 20 samples or per analytical batch, whichever is more frequent	75-125% recovery (70-130% for MMHg); RPD<25%
Laboratory Duplicate	Per 20 samples or per analytical batch, whichever is more frequent	RPD<25% (n/a if native concentration of either sample<RL)
Internal Standard	Accompanying every analytical run when method appropriate	60-125% recovery
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), unless otherwise specified by method
Field Blank, Equipment Blank	Per method	Blanks<RL for target analyte

[1] Unless method specifies more stringent requirements

[2] Not applicable to selenium speciation

Table 2: Sample Handling: Inorganic Analytes in Fresh and Marine Water

Analyte	Recommended Container[1]	Recommended Preservation[2,3]	Required Holding Time[4]
Hexavalent Chromium (Filtered)	P, G	Cool to ≤6 °C, pH 9.3 – 9.7 within 24 hours	28 days at ≤6 °C[5]
Mercury (Dissolved)	G, PA	Filter and preserve with 0.5% v:v pre-tested 5% BrCl or 12N HCl within 48 hours	90 days at room temperature following acidiciaftion
Mercury (Total)	G, PA	Preserve with 0.5% v:v pre-tested 5% BrCl or 12N HCl within 48 hours	90 days at room temperature following acidification
Methylmercury (Dissolved)[6]	G, PA	Immediately after collection, cool to ≤6 °C in the dark; filter and acidify to 0.5% with pre-tested HCl within 48 hours; if salinity is >0.5 ppt, acidify with H_2SO_4	6 months at to ≤6 °C in the dark following acidification
Methylmercury (Total)[6]	G, PA	Immediately after collection, cool to ≤6 °C in the dark; acidify to 0.5% with pre-tested HCl within 48 hours; if salinity is >0.5 ppt, acidify with H_2SO_4	6 months at to ≤6 °C in the dark following acidification
Selenium Speciation[7]	P	Filter and preserve with 0.4% HCl within 15 minutes of collection; maintain collection temperature as best as possible	6 months
Trace Metals[8] (Dissolved)	P	Filter within 15 minutes of collection; HNO_3 to pH<2 within 48 hours and at least 24 hours prior to analysis	6 months at room temperature following acidification
Trace Metals[8] (Total)	P	HNO_3 to pH<2 within 48 hours and at least 24 hours prior to analysis	6 months at room temperature following acidification

[1] "P" is polyethylene; "G" is glass; "PA" is any plastic that is made of a sterilizable material (polypropylene or other autoclavable plastic)

[2] Per 40 CFR 136.3, aqueous samples must be preserved at ≤6 °C, and should not be frozen unless data demonstrating that sample freezing does not adversely impact sample integrity is maintained on file and accepted as valid by the regulatory authority. The preservation temperature does not apply to samples that are analyzed immediately (within 15 minutes).

[3] Per 40 CFR 136.3, an aqueous sample may be collected and shipped without acid preservation. However, acid must be added at least 24 hours before analysis to dissolve any metals that adsorb to the container walls. If the sample must be analyzed within 24 hours of collection, add the acid immediately.

[4] Each "Required Holding Time" is based on the assumption that the "Recommended Preservation" (or a method-mandated alternative) has been employed. If a "Required Holding Time" for filtration, preservation, preparation, or analysis is not met, the project manager and SWAMP Quality Assurance Officer must be notified. Regardless of preservation technique, data not meeting the "Required Holding Time" will be appropriately flagged in the SWAMP database.

[5] If the analytical method doesn't include preservation, analysis must occur within 24 hours.

[6] Methylmercury samples may be shipped to the laboratory unpreserved if they are collected in fluoropolymer bottles, filled to the top with no head space, capped tightly, and maintained at ≤6 °C from the time of collection until preservation. The samples must be acid-preserved within 48 hours of sampling.

[7] Including the species selenite, selenate, and selenocyanate

[8] With the exception of mercury, methylmercury, hexavalent chromium, and selenium speciation

Table 3: Recommended Corrective Action: Inorganic Analytes in Fresh and Marine Water

Laboratory Quality Control	Recommended Corrective Action
Calibration Standard	Recalibrate the instrument. Affected samples and associated quality control must be reanalyzed following successful instrument recalibration.
Calibration Verification	Reanalyze the calibration verification to confirm the result. If the problem continues, halt analysis and investigate the source of the instrument drift. The analyst should determine if the instrument must be recalibrated before the analysis can continue. All of the samples not bracketed by acceptable calibration verification must be reanalyzed.
Laboratory Blank	Reanalyze the blank to confirm the result. Investigate the source of contamination. If the source of the contamination is isolated to the sample preparation, the entire batch of samples, along with the new laboratory blanks and associated QC samples, should be prepared and/or re-extracted and analyzed. If the source of contamination is isolated to the analysis procedures, reanalyze the entire batch of samples. If reanalysis is not possible, the associated sample results must be flagged to indicate the potential presence of the contamination.
Reference Material	Reanalyze the reference material to confirm the result. Compare this to the matrix spike/matrix spike duplicate recovery data. If adverse trends are noted, reprocess all of the samples associated with the batch.
Matrix Spike	The spiking level should be near the midrange of the calibration curve or at a level that does not require sample dilution. Reanalyze the matrix spike to confirm the result. Review the recovery obtained for the matrix spike duplicate. Review the results of the other QC samples (such as reference materials) to determine if other analytical problems are a potential source of the poor spike recovery.
Matrix Spike Duplicate	The spiking level should be near the midrange of the calibration curve or at a level that does not require sample dilution. Reanalyze the matrix spike duplicate to confirm the result. Review the recovery obtained for the matrix spike. Review the results of the other QC samples (such as reference materials) to determine if other analytical problems are a potential source of the poor spike recovery.
Laboratory Duplicate	Reanalyze the duplicate samples to confirm the results. Visually inspect the samples to determine if a high RPD between the results could be attributed to sample heterogeneity. For duplicate results due to matrix heterogeneity, or where ambient concentrations are below the reporting limit, qualify the results and document the heterogeneity.
Internal Standard	Check the response of the internal standards. If the instrument continues to generate poor results, terminate the analytical run and investigate the cause of the instrument drift.
Field Quality Control	**Recommended Corrective Action**
Field Duplicate	Visually inspect the samples to determine if a high RPD between results could be attributed to sample heterogeneity. For duplicate results due to matrix heterogeneity, or where ambient concentrations are below the reporting limit, qualify the results and document the heterogeneity. All failures should be communicated to the project coordinator, who in turn will follow the process detailed in the method.
Field Blank, Equipment Blank	Investigate the source of contamination. Potential sources of contamination include sampling equipment, protocols, and handling. The laboratory should report evidence of field contamination as soon as possible so corrective actions can be implemented. Samples collected in the presence of field contamination should be flagged.

Nutrients in Fresh and Marine Water

A list of analytes included in this category may be found in the associated QAPrPTableReference.

Terms appearing in the tables are defined in the *Surface Water Ambient Monitoring Program Quality Assurance Program Plan*, which contains a glossary (Appendix E), as well as a list of abbreviations and acronyms (Appendix F).

Table 1: Quality Control[1]: Nutrients in Fresh and Marine Water

Laboratory Quality Control	Frequency of Analysis	Measurement Quality Objective
Calibration Standard	Per analytical method or manufacturer's specifications	Per analytical method or manufacturer's specifications
Calibration Verification	Per 10 analytical runs	90-110% 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	90-110% recovery
Matrix Spike	Per 20 samples or per analytical batch, whichever is more frequent	80-120% recovery
Matrix Spike Duplicate	Per 20 samples or per analytical batch, whichever is more frequent	80-120% recovery RPD<25% for duplicates
Laboratory Duplicate	Per 20 samples or per analytical batch, whichever is more frequent	RPD<25% (n/a if native concentration of either sample<RL)
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

[1] Unless method specifies more stringent requirements

Table 2: Sample Handling: Nutrients in Fresh and Marine Water

Analyte	Recommended Container[1]	Recommended Preservation[2]	Required Holding Time[3]
Ammonia (as N)	P	Cool to ≤6 °C; samples may be preserved with 2 mL of H_2SO_4 per L	48 hours; 28 days if acidified
Kjeldahl Nitrogen (Total)	P	Cool to ≤6 °C; H_2SO_4 to pH<2	7 days; 28 days if acidified
Nitrate (as N)	P	Cool to ≤6 °C	48 hours (unless calculated from nitrate + nitrite (as N) and nitrite (as N) analyses)
Nitrate + Nitrite (as N)	P	Cool to ≤6 °C; H_2SO_4 to pH<2	48 hours; 28 days if acidified
Nitrite (as N)	P	Cool to ≤6 °C	48 hours
Nitrogen (Total)	P	Cool to ≤6 °C; H_2SO_4 to pH <2	28 days
Orthophosphate (Dissolved, as P; ***Soluble Reactive Phosphorus***)	P	Filter within 15 minutes of collection[4]; cool to ≤6 °C	48 hours
Orthophosphate (Total, as P)	P	Cool to ≤6 °C	48 hours
Phosphorus (Dissolved, as P)	P	Filter within 15 minutes of collection; cool to ≤6 °C; H_2SO_4 to pH <2	28 days
Phosphorus (Elemental)	G	Cool to ≤6 °C	48 hours
Phosphorus (Total, as P)	P	Cool to ≤6 °C; H_2SO_4 to pH <2	28 days

[1] "P" is polyethylene; "G" is glass

[2] Per 40 CFR 136.3, aqueous samples must be preserved at ≤6 °C, and should not be frozen unless data demonstrating that sample freezing does not adversely impact sample integrity is maintained on file and accepted as valid by the regulatory authority. The preservation temperature does not apply to samples that are analyzed immediately (less than 15 minutes).

[3] Each "Required Holding Time" is based on the assumption that the "Recommended Preservation" (or a method-mandated alternative) has been employed. If a "Required Holding Time" for filtration, preservation, preparation, or analysis is not met, the project manager and SWAMP Quality Assurance Officer must be notified. Regardless of preservation technique, data not meeting the "Required Holding Time" will be appropriately flagged in the SWAMP database.

[4] Per 40 CFR 136.3, the immediate filtration requirement in orthophosphate measurement is to assess the dissolved or bio-available form of orthophosphorus (i.e., that which passes through a 0.45-micron filter), hence the requirement to filter the sample immediately upon collection (i.e., within 15 minutes of collection).

Table 3: Recommended Corrective Action: Nutrients in Fresh and Marine Water

Laboratory Quality Control	Recommended Corrective Action
Calibration Standard	Recalibrate the instrument. Affected samples and associated quality control must be reanalyzed following successful instrument recalibration.
Calibration Verification	Reanalyze the calibration verification to confirm the result. If the problem continues, halt analysis and investigate the source of the instrument drift. The analyst should determine if the instrument must be recalibrated before the analysis can continue. All of the samples not bracketed by acceptable calibration verification must be reanalyzed.
Laboratory Blank	Reanalyze the blank to confirm the result. Investigate the source of contamination. If the source of the contamination is isolated to the sample preparation, the entire batch of samples, along with the new laboratory blanks and associated QC samples, should be prepared and/or re-extracted and analyzed. If the source of contamination is isolated to the analysis procedures, reanalyze the entire batch of samples. If reanalysis is not possible, the associated sample results must be flagged to indicate the potential presence of the contamination.
Reference Material	Reanalyze the reference material to confirm the result. Compare this to the matrix spike/matrix spike duplicate recovery data. If adverse trends are noted, reprocess all of the samples associated with the batch.
Matrix Spike	The spiking level should be near the midrange of the calibration curve or at a level that does not require sample dilution. Reanalyze the matrix spike to confirm the result. Review the recovery obtained for the matrix spike duplicate. Review the results of the other QC samples (such as reference materials) to determine if other analytical problems are a potential source of the poor spike recovery.
Matrix Spike Duplicate	The spiking level should be near the midrange of the calibration curve or at a level that does not require sample dilution. Reanalyze the matrix spike duplicate to confirm the result. Review the recovery obtained for the matrix spike. Review the results of the other QC samples (such as reference materials) to determine if other analytical problems are a potential source of the poor spike recovery.
Laboratory Duplicate	Reanalyze the duplicate samples to confirm the results. Visually inspect the samples to determine if a high RPD between the results could be attributed to sample heterogeneity. For duplicate results due to matrix heterogeneity, or where ambient concentrations are below the reporting limit, qualify the results and document the heterogeneity.
Field Quality Control	**Recommended Corrective Action**
Field Duplicate	Visually inspect the samples to determine if a high RPD between results could be attributed to sample heterogeneity. For duplicate results due to matrix heterogeneity, or where ambient concentrations are below the reporting limit, qualify the results and document the heterogeneity. All failures should be communicated to the project coordinator, who in turn will follow the process detailed in the method.
Field Blank, Travel Blank, Equipment Blank	Investigate the source of contamination. Potential sources of contamination include sampling equipment, protocols, and handling. The laboratory should report evidence of field contamination as soon as possible so corrective actions can be implemented. Samples collected in the presence of field contamination should be flagged.

Solid Parameters in Fresh and Marine Water

A list of parameters included in this category may be found in the associated QAPrPTableReference.

Terms appearing in the tables are defined in the *Surface Water Ambient Monitoring Program Quality Assurance Program Plan*, which contains a glossary (Appendix E), as well as a list of abbreviations and acronyms (Appendix F).

Table 1: Quality Control[1]: Solid Parameters in Fresh and Marine Water

Laboratory Quality Control	Frequency of Analysis	Measurement Quality Objective
Laboratory Blank[2]	Per 20 samples or per analytical batch, whichever is more frequent	<RL for target analyte
Laboratory Duplicate[3]	Per 20 samples or per analytical batch, whichever is more frequent	RPD<25% (n/a if native concentration of either sample<RL)
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, Equipment Blank	Per method	<RL for target analyte

[1] Unless method specifies more stringent requirements

[2] Not applicable to volatile suspended solids

[3] Applicable only to total suspended solids, total dissolved solids, and ash-free dry mass

Table 2: Sample Handling: Solid Parameters in Fresh and Marine Water

Parameter	Recommended Container[1]	Recommended Preservation[2]	Required Holding Time[3]
Ash-Free Dry Mass	Pre-combusted glass-fiber filter	Field filter; cool to ≤6 °C (foil-wrapped); freeze to ≤-20 °C	28 days
Fixed & Volatile Dissolved Solids **Volatile Suspended Solids**	Per method	Cool to ≤6 °C	7 days
Suspended Sediment Concentration **Total Suspended Solids**	G, P	Cool to ≤6 °C	7 days
Total Dissolved Solids	P	Cool to ≤6 °C	7 days

[1] "P" is polyethylene; "G" is glass

[2] Per 40 CFR 136.3, aqueous samples must be preserved at ≤6 °C, and should not be frozen unless data demonstrating that sample freezing does not adversely impact sample integrity is maintained on file and accepted as valid by the regulatory authority. The preservation temperature does not apply to samples that are analyzed immediately (less than 15 minutes).

[3] Each "Required Holding Time" is based on the assumption that the "Recommended Preservation" (or a method-mandated alternative) has been employed. If a "Required Holding Time" for filtration, preservation, preparation, or analysis is not met, the project manager and SWAMP Quality Assurance Officer must be notified. Regardless of preservation technique, data not meeting the "Required Holding Time" will be appropriately flagged in the SWAMP database.

Table 3: Recommended Corrective Action: Solid Parameters in Fresh and Marine Water

Laboratory Quality Control	Recommended Corrective Action
Laboratory Blank	Reanalyze the blank to confirm the result. Investigate the source of contamination. If the source of the contamination is isolated to the sample preparation, the entire batch of samples, along with the new laboratory blanks and associated QC samples, should be prepared and/or re-extracted and analyzed. If the source of contamination is isolated to the analysis procedures, reanalyze the entire batch of samples. If reanalysis is not possible, the associated sample results must be flagged to indicate the potential presence of the contamination.
Laboratory Duplicate	Reanalyze the duplicate samples to confirm the results. Visually inspect the samples to determine if a high RPD between the results could be attributed to sample heterogeneity. For duplicate results due to matrix heterogeneity, or where ambient concentrations are below the reporting limit, qualify the results and document the heterogeneity.
Field Quality Control	**Recommended Corrective Action**
Field Duplicate	Visually inspect the samples to determine if a high RPD between results could be attributed to sample heterogeneity. For duplicate results due to matrix heterogeneity, or where ambient concentrations are below the reporting limit, qualify the results and document the heterogeneity. All failures should be communicated to the project coordinator, who in turn will follow the process detailed in the method.
Field Blank, Equipment Blank	Investigate the source of contamination. Potential sources of contamination include sampling equipment, protocols, and handling. The laboratory should report evidence of field contamination as soon as possible so corrective actions can be implemented. Samples collected in the presence of field contamination should be flagged.

Semi-Volatile Organic Compounds in Fresh and Marine Water

A list of compounds included in this category may be found in the associated QAPrPTableReference.

Terms appearing in the tables are defined in the *Surface Water Ambient Monitoring Program Quality Assurance Program Plan*, which contains a glossary (Appendix E), as well as a list of abbreviations and acronyms (Appendix F).

Table 1: Quality Control[1]: Semi-Volatile Organic Compounds in Fresh and Marine Water[2]

Laboratory Quality Control	Frequency of Analysis	Measurement Quality Objective
Tuning[3]	Per analytical method	Per analytical method
Calibration	Initial method setup or when the calibration verification fails	• Correlation coefficient (r^2 >0.990) for linear and non-linear curves • If RSD<15%, average RF may be used to quantitate; otherwise use equation of the curve • First- or second-order curves only (not forced through the origin) • Refer to SW-846 methods for SPCC and CCC criteria[3] • Minimum of 5 points per curve (one of them at or below the RL)
Calibration Verification	Per 12 hours	• Expected response or expected concentration ±20% • RF for SPCCs=initial calibration[3]
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	70-130% recovery if certified; otherwise, 50-150% recovery
Matrix Spike	Per 20 samples or per analytical batch, whichever is more frequent	50-150% or based on historical laboratory control limits (average±3SD)
Matrix Spike Duplicate	Per 20 samples or per analytical batch, whichever is more frequent	50-150% or based on historical laboratory control limits (average±3SD); RPD<25%
Surrogate	Included in all samples and all QC samples	Based on historical laboratory control limits (50-150% or better)
Internal Standard	Included in all samples and all QC samples (as available)	Per laboratory procedure

[1] Unless method specifies more stringent requirements

[2] All detected analytes must be confirmed with a second column, second technique, or mass spectrometry

[3] Mass spectrometry only

Table 1: Quality Control[1]: Semi-Volatile Organic Compounds in Fresh and Marine Water[2] (continued)

Field Quality Control	Frequency of Analysis	Measurement Quality Objective
Field Duplicate	5% of total project sample count	Per method
Field Blank, Travel Blank, Equipment Blank	Per method	<RL for target analyte

[1] Unless method specifies more stringent requirements

[2] All detected analytes must be confirmed with a second column, second technique, or mass spectrometry

[3] Mass spectrometry only

Table 2: Sample Handling: Semi-Volatile Organic Compounds in Fresh and Marine Water

Recommended Container[2]	Recommended Preservation[3]	Required Holding Time[1]
G	Cool to ≤6 °C	7 days until extraction, 40 days after extraction

[1] Each "Required Holding Time" is based on the assumption that the "Recommended Preservation" (or a method-mandated alternative) has been employed. If a "Required Holding Time" for filtration, preservation, preparation, or analysis is not met, the project manager and SWAMP Quality Assurance Officer must be notified. Regardless of preservation technique, data not meeting the "Required Holding Time" will be appropriately flagged in the SWAMP database.

[2] "G" is glass

[3] Per 40 CFR 136.3, aqueous samples must be preserved at ≤6 °C, and should not be frozen unless data demonstrating that sample freezing does not adversely impact sample integrity is maintained on file and accepted as valid by the regulatory authority. The preservation temperature does not apply to samples that are analyzed immediately (less than 15 minutes).

Table 3: Recommended Corrective Action: Semi-Volatile Organic Compounds in Fresh and Marine Water

Laboratory Quality Control	Recommended Corrective Action
Calibration	Recalibrate the instrument. Affected samples and associated quality control must be reanalyzed following successful instrument recalibration.
Calibration Verification	Reanalyze the calibration verification to confirm the result. If the problem continues, halt analysis and investigate the source of the instrument drift. The analyst should determine if the instrument must be recalibrated before the analysis can continue. All of the samples not bracketed by acceptable calibration verification must be reanalyzed.
Laboratory Blank	Reanalyze the blank to confirm the result. Investigate the source of contamination. If the source of the contamination is isolated to the sample preparation, the entire batch of samples, along with the new laboratory blanks and associated QC samples, should be prepared and/or re-extracted and analyzed. If the source of contamination is isolated to the analysis procedures, reanalyze the entire batch of samples. If reanalysis is not possible, the associated sample results must be flagged to indicate the potential presence of the contamination.
Reference Material	Reanalyze the reference material to confirm the result. Compare this to the matrix spike/matrix spike duplicate recovery data. If adverse trends are noted, reprocess all of the samples associated with the batch.
Matrix Spike	The spiking level should be near the midrange of the calibration curve or at a level that does not require sample dilution. Reanalyze the matrix spike to confirm the result. Review the recovery obtained for the matrix spike duplicate. Review the results of the other QC samples (such as reference materials) to determine if other analytical problems are a potential source of the poor spike recovery.
Matrix Spike Duplicate	The spiking level should be near the midrange of the calibration curve or at a level that does not require sample dilution. Reanalyze the matrix spike duplicate to confirm the result. Review the recovery obtained for the matrix spike. Review the results of the other QC samples (such as reference materials) to determine if other analytical problems are a potential source of the poor spike recovery.
Internal Standard	Check the response of the internal standards. If the instrument continues to generate poor results, terminate the analytical run and investigate the cause of the instrument drift.
Surrogate	Analyze as appropriate for the utilized method. Troubleshoot as needed. If no instrument problem is found, samples should be re-extracted and reanalyzed if possible.
Field Quality Control	**Recommended Corrective Action**
Field Duplicate	Visually inspect the samples to determine if a high RPD between results could be attributed to sample heterogeneity. For duplicate results due to matrix heterogeneity, or where ambient concentrations are below the reporting limit, qualify the results and document the heterogeneity. All failures should be communicated to the project coordinator, who in turn will follow the process detailed in the method.
Field Blank, Travel Blank, Equipment Blank	Investigate the source of contamination. Potential sources of contamination include sampling equipment, protocols, and handling. The laboratory should report evidence of field contamination as soon as possible so corrective actions can be implemented. Samples collected in the presence of field contamination should be flagged.

Synthetic Organic Compounds in Fresh and Marine Water

Groups associated with this category are defined in the following compound lists:

Carbamate Pesticides
Diesel Range Organics
Glyphosates
Organochlorine Pesticides
Organophosphate Pesticides
Organotins
Polynuclear Aromatic Hydrocarbons
Polybrominated Diphenyl Ethers
Polychlorinated Biphenyls
Phenols
Pyrethroid Pesticides
Surfactants
Triazine Pesticides
Wastewater Organochlorine Pesticides

Terms appearing in the tables are defined in the *Surface Water Ambient Monitoring Program Quality Assurance Program Plan*, which contains a glossary (Appendix E), as well as a list of abbreviations and acronyms (Appendix F).

Table 1: Quality Control[1, 2]: Synthetic Organic Compounds in Fresh and Marine Water[3]

Laboratory Quality Control	Frequency of Analysis	Measurement Quality Objective
Tuning[4]	Per analytical method	Per analytical method
Calibration	Initial method setup or when the calibration verification fails	• Correlation coefficient (r^2 >0.990) for linear and non-linear curves • If RSD<15%, average RF may be used to quantitate; otherwise use equation of the curve • First- or second-order curves only (not forced through the origin) • Refer to SW-846 methods for SPCC and CCC criteria[4] • Minimum of 5 points per curve (one of them at or below the RL)
Calibration Verification	Per 12 hours	• Expected response or expected concentration ±20% • RF for SPCCs=initial calibration[4]
Laboratory Blank	Per 20 samples or per analytical batch, whichever is more frequent	<RL for target analytes
Reference Material	Per 20 samples or per analytical batch (preferably blind)	70-130% recovery if certified; otherwise, 50-150% recovery
Matrix Spike	Per 20 samples or per analytical batch, whichever is more frequent	50-150% or based on historical laboratory control limits (average±3SD)
Matrix Spike Duplicate	Per 20 samples or per analytical batch, whichever is more frequent	50-150% or based on historical laboratory control limits (average±3SD); RPD<25%
Surrogate	Included in all samples and all QC samples	Based on historical laboratory control limits (50-150% or better)
Internal Standard	Included in all samples and all QC samples (as available)	Per laboratory procedure

Table 1: Quality Control[1, 2]: Synthetic Organic Compounds in Fresh and Marine Water[3] (continued)

Field Quality Control	Frequency of Analysis	Measurement Quality Objective
Field Duplicate	5% of total project sample count	Per method
Field Blank, Travel Blank, Equipment Blank	Per method	<RL for target analytes

[1] Unless method specifies more stringent requirements; ELISA results must be assessed against kit requirements.

[2] Pyrethroids quality control guidelines are presented in Table 2 immediately below.

[3] All detected analytes must be confirmed with a second column, second technique, or mass spectrometry.

[4] Mass spectrometry only

Table 2: Quality Control[1]: Synthetic Organic Compounds in Whole Water - Pyrethroids Only

Laboratory Quality Control	Frequency of Analysis	Measurement Quality Objective
Tuning[2]	Per analytical method	Per analytical method
Calibration	Daily, or just prior to analysis; five or more standards spanning the sample result range[3], with the lowest standard at or below the RL	r ≥0.995 (or r^2 ≥0.995, all curve types not forced through origin)
Calibration Verification	Per 10 analytical samples[4]	80-120%[5]
Laboratory Blank	Per 20 samples or per analytical batch, whichever is more frequent	<RL for target analytes
Laboratory Control Sample[6],	Per 20 samples or per analytical batch, whichever is more frequent	50-150%
Matrix Spike	Per 20 samples or per analytical batch, whichever is more frequent	50-150%
Matrix Spike Duplicate	Per 20 samples or per analytical batch, whichever is more frequent	50-150%; RPD≤35%
Surrogate[7]	Included in all samples and all QC samples	Based on historical laboratory control limits (50-150% or better)
Internal Standard	Included in all samples and all QC samples (as available)	Per laboratory procedure
Field Quality Control[8]	**Frequency of Analysis**	**Measurement Quality Objective**
Field Duplicate	5% of total project sample count	RPD ≤ 35%

[1] Unless project specifies more stringent requirements

[2] Mass spectrometry only

[3] Sample results above the highest standard are to be diluted and re-analyzed.

[4] Analytical samples include samples only and do not include clean-out or injection blanks.

[5] Limit applies to a mid-level standard; low-level calibration checks near the reporting limit may have a wider range that is project - specific

[6] Laboratory control samples must be matrix-specific. A clean sediment, roasted sand, or roasted sodium sulfate may be used for sediments.

[7] Laboratory historical limits for surrogate recovery must be submitted to the SWAMP database in the lab result comment section.

[8] A technical group consisting of regional, laboratory, and research representatives determined that field blanks do not provide technical value to a pyrethroids data set.

Table 3: Sample Handling: Synthetic Organic Compounds in Fresh and Marine Water[1]

Matrix	Recommended Container[2]	Recommended Preservation[4]	Required Holding Time[2]
Carbamate Pesticides **Organochlorine Pesticides** **Organophosphate Pesticides** **Wastewater Organochlorine Pesticides**	G	Cool to ≤6 °C; pH 5-9	7 days until extraction, 40 days after extraction
Diesel Range Organics **Triazine Pesticides**	G	Cool to ≤6 °C	7 days until extraction, 40 days after extraction
Glyphosate	G	Cool to ≤6 °C; store in the dark; 0.008% $Na_2S_2O_3$ if residual chlorine is present; freeze to ≤-20 °C	18 months (14 days if unfrozen)
Phenols[5]	G	Cool to ≤6 °C; 0.008% $Na_2S_2O_3$ if residual chlorine is present	7 days until extraction, 40 days after extraction
Polychlorinated Biphenyls (as Congeners/Aroclors)	G	Cool to ≤6 °C	1 year until extraction, 1 year after extraction
Polynuclear Aromatic Hydrocarbons	G	Cool to ≤6 °C; store in the dark; 0.008% $Na_2S_2O_3$ if residual chlorine is present	7 days until extraction, 40 days after extraction
Pyrethroids	G	Cool ≤ 6 °C in the dark; samples must be extracted or preserved according to laboratory procedures with suitable preservative or extraction solvent within 72 hours of collection	7 days until extraction, 40 days after extraction
Surfactants	G	Cool to ≤6 °C, store in the dark	7 days until extraction, 40 days after extraction

[1] Pyrethroids information applies to a whole water matrix.

[2] "G" is glass

[3] Per 40 CFR 136.3, aqueous samples must be preserved at ≤6 °C, and should not be frozen unless data demonstrating that sample freezing does not adversely impact sample integrity is maintained on file and accepted as valid by the regulatory authority. The preservation temperature does not apply to samples that are analyzed immediately (less than 15 minutes).

[4] Each "Required Holding Time" is based on the assumption that the "Recommended Preservation" (or a method-mandated alternative) has been employed. If a "Required Holding Time" for filtration, preservation, preparation, or analysis is not met, the project manager and SWAMP Quality Assurance Officer must be notified. Regardless of preservation technique, data not meeting the "Required Holding Time" will be appropriately flagged in the SWAMP database.

[5] This table applies to phenols analysis using gas chromatography. Guidelines for the colorimetric analysis of phenols are located in *Conventional Parameters in Water* Table 2*: Sample Handling.*

Table 4: Recommended Corrective Action: Synthetic Organic Compounds in Fresh and Marine Water[1]

Laboratory Quality Control	Recommended Corrective Action
Calibration	Recalibrate the instrument. Affected samples and associated quality control must be reanalyzed following successful instrument recalibration.
Calibration Verification	Reanalyze the calibration verification to confirm the result. If the problem continues, halt analysis and investigate the source of the instrument drift. The analyst should determine if the instrument must be recalibrated before the analysis can continue. All of the samples not bracketed by acceptable calibration verification must be reanalyzed.
Laboratory Blank	Reanalyze the blank to confirm the result. Investigate the source of contamination. If the source of the contamination is isolated to the sample preparation, the entire batch of samples, along with the new laboratory blanks and associated QC samples, should be prepared and/or re-extracted and analyzed. If the source of contamination is isolated to the analysis procedures, reanalyze the entire batch of samples. If reanalysis is not possible, the associated sample results must be flagged to indicate the potential presence of the contamination.
Reference Material	Reanalyze the reference material to confirm the result. Compare this to the matrix spike/matrix spike duplicate recovery data. If adverse trends are noted, reprocess all of the samples associated with the batch.
Matrix Spike	The spiking level should be near the midrange of the calibration curve or at a level that does not require sample dilution. Reanalyze the matrix spike to confirm the result. Review the recovery obtained for the matrix spike duplicate. Review the results of the other QC samples (such as reference materials) to determine if other analytical problems are a potential source of the poor spike recovery.
Matrix Spike Duplicate	The spiking level should be near the midrange of the calibration curve or at a level that does not require sample dilution. Reanalyze the matrix spike duplicate to confirm the result. Review the recovery obtained for the matrix spike. Review the results of the other QC samples (such as reference materials) to determine if other analytical problems are a potential source of the poor spike recovery.
Internal Standard	Check the response of the internal standards. If the instrument continues to generate poor results, terminate the analytical run and investigate the cause of the instrument drift.
Surrogate	Analyze as appropriate for the utilized method. Troubleshoot as needed. If no instrument problem is found, samples should be re-extracted and reanalyzed if possible.
Field Quality Control	**Recommended Corrective Action**
Field Duplicate	Visually inspect the samples to determine if a high RPD between results could be attributed to sample heterogeneity. For duplicate results due to matrix heterogeneity, or where ambient concentrations are below the reporting limit, qualify the results and document the heterogeneity. All failures should be communicated to the project coordinator, who in turn will follow the process detailed in the method.
Field Blank, Travel Blank, Equipment Blank	Investigate the source of contamination. Potential sources of contamination include sampling equipment, protocols, and handling. The laboratory should report evidence of field contamination as soon as possible so corrective actions can be implemented. Samples collected in the presence of field contamination should be flagged.

[1] Pyrethroids corrective actions are presented in Table 5 immediately below

Table 5: Recommended Corrective Action: Synthetic Organic Compounds in Whole Water – Pyrethroids Only

Laboratory Quality Control	Recommended Corrective Action
Calibration	Affected samples and associated quality control must be reanalyzed following successful instrument recalibration.
Calibration Verification	Initial calibration is analyzed immediately after calibration and should be from a source different than the calibration curve. Bracketing continuing calibration standards are used every ten sample runs for quantitation per method protocol. The analysis must be halted, the problem investigated, and the instrument recalibrated. All samples after the last acceptable continuing calibration verification must be reanalyzed.
Laboratory Blank	The sample analysis must be halted, the source of the contamination investigated, the samples along with a new laboratory blank prepared and/or re-extracted, and the sample batch and fresh laboratory blank reanalyzed. If reanalysis is not possible due to sample volume, flag associated samples.
Laboratory Control Sample	The LCS is analyzed in the same manner as an environmental sample and the spike recovery demonstrates the accuracy of the method. Affected samples and associated quality control must be reanalyzed following LCS troubleshooting and resolution. After troubleshooting, compare to matrix spike/matrix spike duplicate recovery data. If adverse trends are noted, reprocess all samples associated with the batch.
Matrix Spike	The spiking level should be near the midrange of the calibration curve or at a level that does not require sample dilution. Appropriately spiked results should be compared to the matrix spike duplicate to investigate matrix interference. If matrix interference is suspected, the matrix spike result must be flagged. Appropriately spiked results should be compared to the matrix spike duplicate to investigate matrix interference. If matrix interference is suspected and LCS recoveries are acceptable, the matrix spike and matrix spike duplicate results must be flagged.
Matrix Spike Duplicate	The spiking level should be should be near the midrange of the calibration curve or at a level that does not require sample dilution. Appropriately spiked results should be compared to the matrix spike to investigate matrix interference. If matrix interference is suspected and LCS recoveries are acceptable, the matrix spike duplicate result must be flagged.
Surrogate	Analyze as appropriate per method. Trouble shoot as appropriate, if no instrument problem is found samples should be re-extracted and re-analyzed if possible.
Internal Standard	Analyze as appropriate per method. Troubleshoot as appropriate. If, after trouble-shooting, the responses of the internal standards remain unacceptable, the analysis must be terminated and the cause of drift investigated.
Field Quality Control	**Recommended Corrective Action**
Field Duplicate	For duplicates with a heterogeneous matrix or ambient levels below the reporting limit, failed results may be flagged. All failures should be communicated to the project coordinator, who in turn will follow the process detailed in the method.

Volatile Organic Compounds in Fresh and Marine Water

A list of compounds included in this category may be found in the associated QAPrPTableReference.

Terms appearing in the tables are defined in the *Surface Water Ambient Monitoring Program Quality Assurance Program Plan*, which contains a glossary (Appendix E), as well as a list of abbreviations and acronyms (Appendix F).

Table 1: Quality Control[1]: Volatile Organic Compounds in Fresh and Marine Water[2]

Laboratory Quality Control	Frequency of Analysis	Measurement Quality Objective
Tuning[3]	Per analytical method	Per analytical method
Calibration	Initial method setup or when the calibration verification fails	• Correlation coefficient (r^2 >0.990) for linear and non-linear curves • If RSD<15%, average RF may be used to quantitate; otherwise use equation of the curve • First- or second-order curves only (not forced through the origin) • Refer to SW-846 methods for SPCC and CCC criteria[3] • Minimum of 5 points per curve (one of them at or below the RL)
Calibration Verification	Per 12 hours	• Expected response or expected concentration ±20% • RF for SPCCs=initial calibration[3]
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	70-130% recovery if certified; otherwise 50-150% recovery
Matrix Spike	Per 20 samples or per analytical batch, whichever is more frequent	50-150% or based on historical laboratory control limits (average±3SD)
Matrix Spike Duplicate	Per 20 samples or per analytical batch, whichever is more frequent	50-150% or based on historical laboratory control limits (average±3SD); RPD<25%
Surrogate	Included in all samples and all QC samples	Based on historical laboratory control limits (50-150% or better)
Internal Standard	Included in all samples and all QC samples (as available)	Per laboratory procedure
Field Quality Control	**Frequency of Analysis**	**Measurement Quality Objective**
Field Duplicate	5% of total project sample count	Per method
Field Blank, Travel Blank, Equipment Blank	Per method	<RL for target analyte

[1] Unless method specifies more stringent requirements

[2] All detected analytes must be confirmed with a second column, second technique, or mass spectrometry

[3] Mass spectrometry only

Table 2: Sample Handling: Volatile Organic Compounds in Fresh and Marine Water

Recommended Container[2]	Recommended Preservation[3]	Required Holding Time[1]
G	Cool to ≤6 °C; prior to sampling, all vials are pre-acidified (50% HCl or H_2SO_4) at the laboratory	7 days; 14 days if acidified

[1] Each "Required Holding Time" is based on the assumption that the "Recommended Preservation" (or a method-mandated alternative) has been employed. If a "Required Holding Time" for filtration, preservation, preparation, or analysis is not met, the project manager and SWAMP Quality Assurance Officer must be notified. Regardless of preservation technique, data not meeting the "Required Holding Time" will be appropriately flagged in the SWAMP database.

[2]"G" is glass

[3] Per 40 CFR 136.3, aqueous samples must be preserved at ≤6 °C, and should not be frozen unless data demonstrating that sample freezing does not adversely impact sample integrity is maintained on file and accepted as valid by the regulatory authority. The preservation temperature does not apply to samples that are analyzed immediately (less than 15 minutes).

Table 3: Recommended Corrective Action: Volatile Organic Compounds in Fresh and Marine Water

Laboratory Quality Control	Recommended Corrective Action
Calibration	Recalibrate the instrument. Affected samples and associated quality control must be reanalyzed following successful instrument recalibration.
Calibration Verification	Reanalyze the calibration verification to confirm the result. If the problem continues, halt analysis and investigate the source of the instrument drift. The analyst should determine if the instrument must be recalibrated before the analysis can continue. All of the samples not bracketed by acceptable calibration verification must be reanalyzed.
Laboratory Blank	Reanalyze the blank to confirm the result. Investigate the source of contamination. If the source of the contamination is isolated to the sample preparation, the entire batch of samples, along with the new laboratory blanks and associated QC samples, should be prepared and/or re-extracted and analyzed. If the source of contamination is isolated to the analysis procedures, reanalyze the entire batch of samples. If reanalysis is not possible, the associated sample results must be flagged to indicate the potential presence of the contamination.
Reference Material	Reanalyze the reference material to confirm the result. Compare this to the matrix spike/matrix spike duplicate recovery data. If adverse trends are noted, reprocess all of the samples associated with the batch.
Matrix Spike	The spiking level should be near the midrange of the calibration curve or at a level that does not require sample dilution. Reanalyze the matrix spike to confirm the result. Review the recovery obtained for the matrix spike duplicate. Review the results of the other QC samples (such as reference materials) to determine if other analytical problems are a potential source of the poor spike recovery.
Matrix Spike Duplicate	The spiking level should be near the midrange of the calibration curve or at a level that does not require sample dilution. Reanalyze the matrix spike duplicate to confirm the result. Review the recovery obtained for the matrix spike. Review the results of the other QC samples (such as reference materials) to determine if other analytical problems are a potential source of the poor spike recovery.
Internal Standard	Check the response of the internal standards. If the instrument continues to generate poor results, terminate the analytical run and investigate the cause of the instrument drift.
Surrogate	Analyze as appropriate for the utilized method. Troubleshoot as needed. If no instrument problem is found, samples should be re-extracted and reanalyzed if possible.
Field Quality Control	**Recommended Corrective Action**
Field Duplicate	Visually inspect the samples to determine if a high RPD between results could be attributed to sample heterogeneity. For duplicate results due to matrix heterogeneity, or where ambient concentrations are below the reporting limit, qualify the results and document the heterogeneity. All failures should be communicated to the project coordinator, who in turn will follow the process detailed in the method.
Field Blank, Travel Blank, Equipment Blank	Investigate the source of contamination. Potential sources of contamination include sampling equipment, protocols, and handling. The laboratory should report evidence of field contamination as soon as possible so corrective actions can be implemented. Samples collected in the presence of field contamination should be flagged.

Attachment 2

SWAMP SOPs

SWAMP Field Data Sheet (Water Chemistry & Discrete Probe) - EventType=WQ

Entered in d-base (initial/date) | Pg of Pgs

*StationID: _____ | *Date (mm/dd/yyyy): / / | *Group: | *Agency:

*Funding: ___ ___ ___ ___ ___ ___ ___ ___ | ArrivalTime: | DepartureTime: | *SampleTime (1st sample): | *Protocol:

*ProjectCode: | *Personnel: | *Purpose (circle applicable): WaterChem WaterTox Habitat FieldMeas | *PurposeFailure:

*Location: Bank Thalweg Midchannel OpenWater | *GPS/DGPS | Lat (dd.ddddd) | Long (ddd.ddddd) | OCCUPATION METHOD: Walk-in Bridge R/V _____ Other

GPS Device: | Target: | - | STARTING BANK (facing downstream): LB / RB / NA

Datum: NAD83 | Accuracy (ft / m): | *Actual: | - | Point of Sample (if Integrated, then -88 in dbase)

Habitat Observations (CollectionMethod = Habitat_generic)

WADEABILITY: Y / N / Unk | BEAUFORT SCALE (see attachment): | DISTANCE FROM BANK (m): | STREAM WIDTH (m): | WATER DEPTH (m):

SITE ODOR: None,Sulfides,Sewage,Petroleum,Smoke,Other_____

SKY CODE: Clear, Partly Cloudy, Overcast, Fog, Smoky, Hazy

WIND DIRECTION (from): 

HYDROMODIFICATION: None, Bridge, Pipes, ConcreteChannel, GradeControl, Culvert, AerialZipline, Other | LOCATION (to sample): US / DS / WI /

OTHER PRESENCE: Vascular,Nonvascular,OilySheen,Foam,Trash,Other_____

PHOTOS (RB & LB assigned when facing downstream; RENAME to StationCode_yyyy_mm_dd_uniquecode): 1: (RB / LB / BB / US / DS / ##)

DOMINANT SUBSTRATE: Bedrock, Concrete, Cobble, Boulder, Gravel, Sand, Mud, Unk, Other_____

WATERCLARITY: Clear (see bottom), Cloudy (>4" vis), Murky (<4" vis) | PRECIPITATION: None, Fog, Drizzle, Rain, Snow | 2: (RB / LB / BB / US / DS / ##)

WATERODOR: None, Sulfides, Sewage, Petroleum, Mixed, Other_____ | PRECIPITATION (last 24 hrs): Unknown, <1", >1", None

WATERCOLOR: Colorless, Green, Yellow, Brown | EVIDENCE OF FIRES: No, <1 year, <5 years | 3: (RB / LB / BB / US / DS / ##)

OVERLAND RUNOFF (Last 24 hrs): none, light, moderate / heavy, unknown

OBSERVED FLOW: NA, Dry Waterbody Bed, No Obs Flow, Isolated Pool, Trickle (<0.1cfs), 0.1-1cfs, 1-5cfs, 5-20cfs, 20-50cfs, 50-200cfs, >200cfs

Field Measurements (SampleType = FieldMeasure; Method = Field)

	DepthCollec (m)	Velocity (fps)	Air Temp (°C)	Water Temp (°C)	pH	O_2 (mg/L)	O_2 (%)	Specific Conductivity	Salinity (ppt)	Turbidity (ntu)			
SUBSURF/MID/ BOTTOM/REP													
SUBSURF/MID/ BOTTOM/REP													
SUBSURF/MID/ BOTTOM/REP													
Instrument:													
Calib. Date:													

Samples Taken (# of containers filled) - Method=Water_Grab

Field Dup YES / NO: (SampleType = Grab / Integrated; LABEL_ID = FieldQA; create collection record upon data entry

SAMPLE TYPE: Grab / Integrated | COLLECTION DEVICE: Indiv bottle (by hand, by pole, by bucket); Teflon tubing; Kemmer; Pole & Beaker; Other

	DepthCollec	Inorganics	Bacteria	Chl a	TSS / SSC	TOC / DOC	Total Hg	Dissolved	Total Metals	Dissolved	Organi	Toxicity	VOAs
Sub/Surface													
Sub/Surface													

COMMENTS:

SWAMP Field Data Sheet (Sediment Chemistry) - EventType=WQ

Entered in d-base (initial/date) | Pg of Pgs

*StationID: _____ | *Date (mm/dd/yyyy): / / | *Group: | *Agency:

*Funding: ___ ___ ___ ___ ___ ___ ___ ___ | ArrivalTime: | DepartureTime: | *SampleTime (1st sample): | *Protocol:

*ProjectCode: | *Personnel: | *Purpose (circle applicable): SedChem SedTox Habitat Benthic | *PurposeFailure:

*Location: Bank Thalweg Midchannel OpenWater

GPS Device:

Datum: NAD83 Accuracy (ft / m):

*GPS/DGPS	Lat (dd.ddddd)	Long (ddd.ddddd)
Target:		-
*Actual:		-

Same as Water/Probe Collection? YES NO

OCCUPATION METHOD: Walk-in Bridge R/V _____ Other

STARTING BANK (facing downstream): LB / RB / NA

Point of Sample (if Integrated, then -88 in dbase)

DISTANCE FROM BANK (m):

STREAM WIDTH (m):

WATER DEPTH (m):

HYDROMODIFICATION: None, Bridge, Pipes, ConcreteChannel, GradeControl, Culvert, AerialZipline, Other

LOCATION (to sample): US / DS / WI / NA

Habitat Observations (CollectionMethod = Habitat_generic) **Only complete Sed Observations (bolded) if WQ Observations are already recorded

WADEABILITY: Y / N / Unk

BEAUFORT SCALE see Attachtment

SITE ODOR: None,Sulfides,Sewage,Petroleum,Smoke,Other_____

SKY CODE: Clear, Partly Cloudy, Overcast, Fog, Smoky, Hazy

WIND DIRECTION (from): N W E S

OTHERPRESENCE: Vascular,Nonvascular,OilySheen,Foam,Trash,Other____

DOMINANTSUBSTRATE: Bedrock, Concrete, Cobble, Boulder, Gravel, Sand, Mud, Unk, Other_____

PHOTOS (RB & LB assigned when facing downstream; RENAME to StationCode_yyyy_mm_dd_uniquecode):

1: (RB / LB / BB / US / DS / ##)

2: (RB / LB / BB / US / DS / ##)

3: (RB / LB / BB / US / DS / ##)

SEDODOR: None, Sulfides, Sewage, Petroleum, Mixed, Other_____

SEDCOLOR: Colorless, Green, Yellow, Brown

SEDCOMPOSITION: Silt/Clay, FineSand, CoarseSand, Gravel, Cobble, Mixed, HardPanCla

PRECIPITATION: None, Fog, Drizzle, Rain, Snow

PRECIPITATION (last 24 hrs): Unknown, <1", >1", None

EVIDENCE OF FIRES: No, <1 years, <5 years

OBSERVED FLOW: NA, Dry Waterbody Bed, No Obs Flow, Isolated Pool, Trickle (<0.1cfs), 0.1-1cfs, 1-5cfs, 5-20cfs, 20-50cfs, 50-200cfs, >200cfs

Samples Taken (# of containers filled) - Method=Sed_Grab

Field Dup YES / NO: (SampleType = Grab / Integrated; LABEL_ID = FieldQA; create collection record upon data entry

COLLECTION DEVICE: Scoop (SS / PC / PE, Core (SS / PC / PE), Grab (Van Veen / Eckman / Petite Ponar)

COLLECTION DEVICE AREA (m2): _____

Sample Type:	DepthCollec (cm)	Equipment Used	Sediment Only (Y / N)	Grain Size/TOC	Organics	Metals/HgT	Selenium	Toxicity	SWI	Archive Chemistry	Benthic Infauna	Benthic Coll. Area (m^2)	Sieve Size (mm)
Integrated Grab													
Integrated Grab													
Integrated Grab													
Integrated Grab													

COMMENTS:

Modified 06/27/08

REACH DOCUMENTATION

Standard Reach Length (wetted width ≤ 10 m) = 150 m Distance between transects = 15 m
Alternate Reach Length (wetted width >10 m) = 250 m Distance between transects = 25 m

Project Name:	Date: / / 2014	Sample Collection Time:
Stream Name:	Site Name/ Description:	
Site Code:	Crew Members:	
Latitude (actual – decimal degrees): ºN	datum: NAD83	
Longitude (actual – decimal degrees): ºW	*other:*	GPS Device:

AMBIENT WATER QUALITY MEASUREMENTS

** Turbidity, silica, oxygen saturation, and air temp are optional; calibration date required on page 24*

Water Temp (Deg C)	pH	Alkalinity (mg/L)	Turbidity (ntu)*	Oxygen Sat. (%)*

Dissolved O^2 (mg/L)	Specific. Conduct (uS/cm)	Salinity (ppt)	Silica (mg/L)*	Air Temp (Deg C)*

Actual Reach Length (m) *(see reach length guidelines at top of form)*

Explanation:

DISCHARGE MEASUREMENTS

1st measurement = left bank (looking downstream)

check if discharge measurements not possible (explain in field notes section) ☐

VELOCITY AREA METHOD (preferred)

cal. date

Transect Width (m):

	Distance from Left Bank (cm)	Depth (cm)	Velocity (ft/sec)		Distance from Left Bank (cm)	Depth (cm)	Velocity (ft/sec)
1				11			
2				12			
3				13			
4				14			
5				15			
6				16			
7				17			
8				18			
9				19			
10				20			

BUOYANT OBJECT METHOD (use ONLY if velocity area method not possible)

	Float 1	Float 2	Float 3
Distance (m)			
Float Time (sec)			

Float Reach Cross Section

width (m) depth(cm)	Upper Section	Middle Section	Lower Section
Width			
Depth 1			
Depth 2			
Depth 3			
Depth 4			
Depth 5			

NOTABLE FIELD CONDITIONS (check one box per topic)

Evidence of recent rainfall (enough to increase surface runoff)	NO	☐	minimal	☐	>10% flow increase	☐
Evidence of fires in reach or immediately upstream (<500 m)	NO	☐	< 1 year	☐	< 5 years	☐
Dominant landuse/ landcover in area surrounding reach	Agriculture	☐	Forest	☐	Rangeland	☐
	Urban/ Industrial	☐	Suburb/Town	☐	Other	☐

ADDITIONAL COBBLE EMBEDDEDNESS MEASURES

(carry over from transect forms if needed to attain target count of 25; measure in %)

1	2	3	4	5	6	7	8	9	10	11	12	13
14	15	16	17	18	19	20	21	22	23	24	25	

Site Code:	Date: ____ / ____ / **2014**

SLOPE and BEARING FORM (transect based - for Full PHAB only)

AUTOLEVEL	
CLINOMETER	
HANDLEVEL	
OTHER	

Starting Transect	MAIN SEGMENT (record percent of inter-transect distance in each segment **if supplemental segments are used**)					SUPPLEMENTAL SEGMENT (record percent of inter-transect distance in each segment **if supplemental segments are used**)				
	Stadia rod measurements	Slope (%) or Elevation Difference cm☐ %☐	Segment Length (m)	Bearing (0°-359°)	Percent of Total Length (%)	Stadia rod measurements	Slope or Elevation Difference cm☐ %☐	Segment Length (m)	Bearing (0°-359°)	Percent of Total Length (%)
K										
J										
I										
H										
G										
F										
E										
D										
C										
B										
A										
additional calculation area										

ADDITIONAL HABITAT CHARACTERIZATION

High Gradient ☐ **Low Gradient** ☐

Parameter	Optimal	Suboptimal	Marginal	Poor
Epifaunal Substrate/ Cover	Greater than 70% of substrate favorable for epifaunal colonization and fish cover (50% for low-gradient streams); mix of submerged logs, undercut banks, cobble or other stable habitat	40-70% mix of stable habitat (30-50% for low-gradient streams); well-suited for full colonization potential	20-40% mix of stable habitat (10-30% in low-gradient streams); substrate frequently disturbed or removed	Less than 20% stable habitat (10% in low-gradient streams); lack of habitat is obvious; substrate unstable or lacking
Score:	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
Sediment Deposition	Little or no enlargement of islands or point bars and less than 5% of the bottom affected by sediment deposition (<20% in low-gradient streams)	Some new increase in bar formation, mostly from gravel, sand, or fine sediment; 5-30% of the bottom affected (20-50% in low-gradient streams)	Moderate deposition of new gravel, sand, or fine sediment on bars; 30-50% of the bottom affected (50 - 80% in low-gradient streams)	Heavy deposits of fine material, increased bar development; more than 50% of the bottom changing frequently (>80% in low-gradient streams)
Score:	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
Channel Alteration	Channelization or dredging absent or minimal; stream with normal pattern	Some channelization present, (e.g., bridge abutments); evidence of past channelization (> 20yrs) may be present but recent channelization not present	Channelization may be extensive: embankments or shoring structures present on both banks; 40 to 80% of stream reach disrupted	Banks shored with gabian or cement; Over 80% of the stream reach channelized and disrupted. Instream habitat greatly altered or removed entirely
Score:	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0

Site Code:	Site Name:		Date: _____ / _____ / 2014
Wetted Width (m):	Bankfull Width (m):	Bankfull Height (m):	**Transect A**

Transect Substrates

Position	Dist from LB (m)	Depth (cm)	mm/ size class	% Cobble Embed.	CPOM	Microalgae Thickness Code	Macroalgae Attached	Macroalgae Unattached	Macrophytes
Left Bank					P A D		P A D	P A D	P A D
Left Center					P A D		P A D	P A D	P A D
Center					P A D		P A D	P A D	P A D
Right Center					P A D		P A D	P A D	P A D
Right Bank					P A D		P A D	P A D	P A D
	Note: Substrate sizes can be recorded either as direct measures of the median axis of each particle or one of the size class categories listed on the supplemental page (direct measurements preferred)								

Microalgae Thickness Codes
0 = No microalgae present, Feels rough, not slimy;
1 = Present but not visible, Feels slimy;
2 = Present and visible but <1mm; Rubbing fingers on surface produces a brownish tint on them, scraping leaves visible trail.
3 = 1-5mm;
4 = 5-20mm;
5 = >20mm;
UD = Cannot determine if microalgae present, substrate too small or covered with silt (formerly Z code).
D = Dry, not assessed

Riparian Vegetation
(facing downstream, 5 m u/s, 5 m d/s, 10 m from wetted width)

0 = Absent (0%) 3 = Heavy (40-75%)
1 = Sparse (<10%) 4 = Very Heavy (>75%)
2 = Moderate (10-40%)

Vegetation Class	Left Bank	Right Bank
Upper Canopy (>5 m high)		
Trees and saplings >5 m high	0 1 2 3 4	0 1 2 3 4
Lower Canopy (0.5 m-5 m high)		
All vegetation 0.5 m to 5 m	0 1 2 3 4	0 1 2 3 4
Ground Cover (<0.5 m high)		
Woody shrubs & saplings <0.5 m	0 1 2 3 4	0 1 2 3 4
Herbs/ grasses	0 1 2 3 4	0 1 2 3 4
Barren, bare soil/ duff	0 1 2 3 4	0 1 2 3 4

Instream Habitat Complexity
(5 m u/s, 5 m d/s)

0 = Absent (0%)
1 = Sparse (<10%)
2 = Moderate (10-40%)
3 = Heavy (40-75%)
4 = Very Heavy (>75%)

Filamentous Algae	0 1 2 3 4
Aquatic Macrophytes/ Emergent Vegetation	0 1 2 3 4
Boulders	0 1 2 3 4
Woody Debris >0.3 m	0 1 2 3 4
Woody Debris <0.3 m	0 1 2 3 4
Undercut Banks	0 1 2 3 4
Overhang. Vegetation	0 1 2 3 4
Live Tree Roots	0 1 2 3 4
Artificial Structures	0 1 2 3 4

Densiometer Readings (0-17)
count covered dots

Center Left	
Center Upstream	
Center Right	
Center Downstream	
Optional	
Left Bank	
Right Bank	

Human Influence
(circle only the closest to wetted channel; assess 5 m u/s, 5 m d/s)

0 = Not Present;
B = On Bank;
C = Between Bank & 10m from Channel;
P = >10m+<50m from Channel;
Channel (record Yes or No; if Y for an analyte, do not assess banks)

	Left Bank	Channel	Right Bank
Walls/ Rip-rap/ Dams	P C B 0	Y N	0 B C P
Buildings	P C B 0	Y N	0 B C P
Pavement/ Cleared Lot	P C B 0		0 B C P
Road/ Railroad	P C B 0	Y N	0 B C P
Pipes (Inlet/ Outlet)	P C B 0	Y N	0 B C P
Landfill/ Trash	P C B 0	Y N	0 B C P
Park/ Lawn	P C B 0		0 B C P
Row Crop	P C B 0		0 B C P
Pasture/ Range	P C B 0		0 B C P
Logging Operations	P C B 0		0 B C P
Mining Activity	P C B 0	Y N	0 B C P
Vegetation Management	P C B 0		0 B C P
Bridges/ Abutments	P C B 0	Y N	0 B C P
Orchards/ Vineyards	P C B 0		0 B C P

Bank Stability
(score zone 5m upstream and 5m downstream of transect between bankfull - wetted width)

Left Bank	eroded	vulnerable	stable
Right Bank	eroded	vulnerable	stable

TAKE PHOTOGRAPHS
(check box if taken & record photo code)

Downstream (**optional**)	☐
Upstream (**required**)	☐

Inter-Transect: AB

Wetted Width (m):

Inter-Transect Substrates

Position	Dist from LB (m)	Depth (cm)	mm/ size class	% Cobble Embed.	CPOM	Microalgae Thickness Code	Macroalgae Attached	Macroalgae Unattached	Macrophytes
Left Bank					P A D		P A D	P A D	P A D
Left Center					P A D		P A D	P A D	P A D
Center					P A D		P A D	P A D	P A D
Right Center					P A D		P A D	P A D	P A D
Right Bank					P A D		P A D	P A D	P A D
	Note: Substrate sizes can be recorded either as direct measures of the median axis of each particle or one of the size class categories listed on the supplemental page (direct measurements preferred)								

Microalgae Thickness Codes

0 = No microalgae present, Feels rough, not slimy;
1 = Present but not visible, Feels slimy;
2 = Present and visible but <1mm; Rubbing fingers on surface produces a brownish tint on them, scraping leaves visible trail.
3 = 1-5mm;
4 = 5-20mm;
5 = >20mm;
UD = Cannot determine if microalgae present, substrate too small or covered with silt (formerly Z code).
D = Dry, not assessed

FLOW HABITATS
(% between transects, total=100%)

Channel Type	%
Cascade/ Falls	
Rapid	
Riffle	
Run	
Glide	
Pool	
Dry	

Site Code:	Site Name:		Date: _____ / _____ / 2014
Wetted Width (m):	Bankfull Width (m):	Bankfull Height (m):	**Transect B**

Transect Substrates

Position	Dist from LB (m)	Depth (cm)	mm/ size class	% Cobble Embed.	CPOM	Microalgae Thickness Code	Macroalgae Attached	Macroalgae Unattached	Macrophytes
Left Bank					P A D		P A D	P A D	P A D
Left Center					P A D		P A D	P A D	P A D
Center					P A D		P A D	P A D	P A D
Right Center					P A D		P A D	P A D	P A D
Right Bank					P A D		P A D	P A D	P A D
	Note: Substrate sizes can be recorded either as direct measures of the median axis of each particle or one of the size class categories listed on the supplemental page (direct measurements preferred)								

Microalgae Thickness Codes
0 = No microalgae present, Feels rough, not slimy;
1 = Present but not visible, Feels slimy;
2 = Present and visible but <1mm; Rubbing fingers on surface produces a brownish tint on them, scraping leaves visible trail.
3 = 1-5mm;
4 = 5-20mm;
5 = >20mm;
UD = Cannot determine if microalgae present, substrate too small or covered with silt (formerly Z code).
D = Dry, not assessed

Riparian Vegetation
(facing downstream, 5 m u/s, 5 m d/s, 10 m from wetted width)

0 = Absent (0%) 3 = Heavy (40-75%)
1 = Sparse (<10%) 4 = Very Heavy (>75%)
2 = Moderate (10-40%)

Vegetation Class	Left Bank	Right Bank
Upper Canopy (>5 m high)		
Trees and saplings >5 m high	0 1 2 3 4	0 1 2 3 4
Lower Canopy (0.5 m-5 m high)		
All vegetation 0.5 m to 5 m	0 1 2 3 4	0 1 2 3 4
Ground Cover (<0.5 m high)		
Woody shrubs & saplings <0.5 m	0 1 2 3 4	0 1 2 3 4
Herbs/ grasses	0 1 2 3 4	0 1 2 3 4
Barren, bare soil/ duff	0 1 2 3 4	0 1 2 3 4

Instream Habitat Complexity
(5 m u/s, 5 m d/s)

0 = Absent (0%)
1 = Sparse (<10%)
2 = Moderate (10-40%)
3 = Heavy (40-75%)
4 = Very Heavy (>75%)

Filamentous Algae	0 1 2 3 4
Aquatic Macrophytes/ Emergent Vegetation	0 1 2 3 4
Boulders	0 1 2 3 4
Woody Debris >0.3 m	0 1 2 3 4
Woody Debris <0.3 m	0 1 2 3 4
Undercut Banks	0 1 2 3 4
Overhang. Vegetation	0 1 2 3 4
Live Tree Roots	0 1 2 3 4
Artificial Structures	0 1 2 3 4

Densiometer Readings (0-17)
count covered dots

Center Left	
Center Upstream	
Center Right	
Center Downstream	
Optional	
Left Bank	
Right Bank	

Human Influence
(circle only the closest to wetted channel; assess 5 m u/s, 5 m d/s)

0 = Not Present;
B = On Bank;
C = Between Bank & 10m from Channel;
P = >10m+<50m from Channel;
Channel (record Yes or No; if Y for an analyte, do not assess banks)

	Left Bank	Channel	Right Bank
Walls/ Rip-rap/ Dams	P C B 0	Y N	0 B C P
Buildings	P C B 0	Y N	0 B C P
Pavement/ Cleared Lot	P C B 0		0 B C P
Road/ Railroad	P C B 0	Y N	0 B C P
Pipes (Inlet/ Outlet)	P C B 0	Y N	0 B C P
Landfill/ Trash	P C B 0	Y N	0 B C P
Park/ Lawn	P C B 0		0 B C P
Row Crop	P C B 0		0 B C P
Pasture/ Range	P C B 0		0 B C P
Logging Operations	P C B 0		0 B C P
Mining Activity	P C B 0	Y N	0 B C P
Vegetation Management	P C B 0		0 B C P
Bridges/ Abutments	P C B 0	Y N	0 B C P
Orchards/ Vineyards	P C B 0		0 B C P

Bank Stability
(score zone 5m upstream and 5m downstream of transect between bankfull - wetted width)

Left Bank	eroded	vulnerable	stable
Right Bank	eroded	vulnerable	stable

Inter-Transect: BC

Wetted Width (m):

Inter-Transect Substrates

Position	Dist from LB (m)	Depth (cm)	mm/ size class	% Cobble Embed.	CPOM	Microalgae Thickness Code	Macroalgae Attached	Macroalgae Unattached	Macrophytes
Left Bank					P A D		P A D	P A D	P A D
Left Center					P A D		P A D	P A D	P A D
Center					P A D		P A D	P A D	P A D
Right Center					P A D		P A D	P A D	P A D
Right Bank					P A D		P A D	P A D	P A D
	Note: Substrate sizes can be recorded either as direct measures of the median axis of each particle or one of the size class categories listed on the supplemental page (direct measurements preferred)								

Microalgae Thickness Codes

0 = No microalgae present, Feels rough, not slimy;
1 = Present but not visible, Feels slimy;
2 = Present and visible but <1mm; Rubbing fingers on surface produces a brownish tint on them, scraping leaves visible trail.
3 = 1-5mm;
4 = 5-20mm;
5 = >20mm;
UD = Cannot determine if microalgae present, substrate too small or covered with silt (formerly Z code).
D = Dry, not assessed

FLOW HABITATS
(% between transects, total=100%)

Channel Type	%
Cascade/ Falls	
Rapid	
Riffle	
Run	
Glide	
Pool	
Dry	

Site Code:	Site Name:		Date: _____ / _____ / 2014
Wetted Width (m):	Bankfull Width (m):	Bankfull Height (m):	**Transect C**

Transect Substrates

Position	Dist from LB (m)	Depth (cm)	mm/ size class	% Cobble Embed.	CPOM	Microalgae Thickness Code	Macroalgae Attached	Macroalgae Unattached	Macrophytes
Left Bank					P A D		P A D	P A D	P A D
Left Center					P A D		P A D	P A D	P A D
Center					P A D		P A D	P A D	P A D
Right Center					P A D		P A D	P A D	P A D
Right Bank					P A D		P A D	P A D	P A D
	Note: Substrate sizes can be recorded either as direct measures of the median axis of each particle or one of the size class categories listed on the supplemental page (direct measurements preferred)								

Microalgae Thickness Codes
0 = No microalgae present, Feels rough, not slimy;
1 = Present but not visible, Feels slimy;
2 = Present and visible but <1mm; Rubbing fingers on surface produces a brownish tint on them, scraping leaves visible trail.
3 = 1-5mm;
4 = 5-20mm;
5 = >20mm;
UD = Cannot determine if microalgae present, substrate too small or covered with silt (formerly Z code).
D = Dry, not assessed

Riparian Vegetation
(facing downstream, 5 m u/s, 5 m d/s, 10 m from wetted width)

0 = Absent (0%) 3 = Heavy (40-75%)
1 = Sparse (<10%) 4 = Very Heavy (>75%)
2 = Moderate (10-40%)

Vegetation Class	Left Bank	Right Bank
Upper Canopy (>5 m high)		
Trees and saplings >5 m high	0 1 2 3 4	0 1 2 3 4
Lower Canopy (0.5 m-5 m high)		
All vegetation 0.5 m to 5 m	0 1 2 3 4	0 1 2 3 4
Ground Cover (<0.5 m high)		
Woody shrubs & saplings <0.5 m	0 1 2 3 4	0 1 2 3 4
Herbs/ grasses	0 1 2 3 4	0 1 2 3 4
Barren, bare soil/ duff	0 1 2 3 4	0 1 2 3 4

Instream Habitat Complexity
(5 m u/s, 5 m d/s)

0 = Absent (0%)
1 = Sparse (<10%)
2 = Moderate (10-40%)
3 = Heavy (40-75%)
4 = Very Heavy (>75%)

Filamentous Algae	0 1 2 3 4
Aquatic Macrophytes/ Emergent Vegetation	0 1 2 3 4
Boulders	0 1 2 3 4
Woody Debris >0.3 m	0 1 2 3 4
Woody Debris <0.3 m	0 1 2 3 4
Undercut Banks	0 1 2 3 4
Overhang. Vegetation	0 1 2 3 4
Live Tree Roots	0 1 2 3 4
Artificial Structures	0 1 2 3 4

Densiometer Readings (0-17)
count covered dots

Center Left	
Center Upstream	
Center Right	
Center Downstream	
Optional	
Left Bank	
Right Bank	

Human Influence
(circle only the closest to wetted channel; assess 5 m u/s, 5 m d/s)

0 = Not Present;
B = On Bank;
C = Between Bank & 10m from Channel;
P = >10m+<50m from Channel;
Channel (record Yes or No; if Y for an analyte, do not assess banks)

	Left Bank	Channel	Right Bank
Walls/ Rip-rap/ Dams	P C B 0	Y N	0 B C P
Buildings	P C B 0	Y N	0 B C P
Pavement/ Cleared Lot	P C B 0		0 B C P
Road/ Railroad	P C B 0	Y N	0 B C P
Pipes (Inlet/ Outlet)	P C B 0	Y N	0 B C P
Landfill/ Trash	P C B 0	Y N	0 B C P
Park/ Lawn	P C B 0		0 B C P
Row Crop	P C B 0		0 B C P
Pasture/ Range	P C B 0		0 B C P
Logging Operations	P C B 0		0 B C P
Mining Activity	P C B 0	Y N	0 B C P
Vegetation Management	P C B 0		0 B C P
Bridges/ Abutments	P C B 0	Y N	0 B C P
Orchards/ Vineyards	P C B 0		0 B C P

Bank Stability
(score zone 5m upstream and 5m downstream of transect between bankfull - wetted width)

Left Bank	eroded	vulnerable	stable
Right Bank	eroded	vulnerable	stable

Inter-Transect: CD

Wetted Width (m):

Inter-Transect Substrates

Position	Dist from LB (m)	Depth (cm)	mm/ size class	% Cobble Embed.	CPOM	Microalgae Thickness Code	Macroalgae Attached	Macroalgae Unattached	Macrophytes
Left Bank					P A D		P A D	P A D	P A D
Left Center					P A D		P A D	P A D	P A D
Center					P A D		P A D	P A D	P A D
Right Center					P A D		P A D	P A D	P A D
Right Bank					P A D		P A D	P A D	P A D
	Note: Substrate sizes can be recorded either as direct measures of the median axis of each particle or one of the size class categories listed on the supplemental page (direct measurements preferred)								

Microalgae Thickness Codes

0 = No microalgae present, Feels rough, not slimy;
1 = Present but not visible, Feels slimy;
2 = Present and visible but <1mm; Rubbing fingers on surface produces a brownish tint on them, scraping leaves visible trail.
3 = 1-5mm;
4 = 5-20mm;
5 = >20mm;
UD = Cannot determine if microalgae present, substrate too small or covered with silt (formerly Z code).
D = Dry, not assessed

FLOW HABITATS
(% between transects, total=100%)

Channel Type	%
Cascade/ Falls	
Rapid	
Riffle	
Run	
Glide	
Pool	
Dry	

Site Code:	Site Name:		Date: _____ / _____ / 2014
Wetted Width (m):	Bankfull Width (m):	Bankfull Height (m):	**Transect D**

Transect Substrates

Position	Dist from LB (m)	Depth (cm)	mm/ size class	% Cobble Embed.	CPOM	Microalgae Thickness Code	Macroalgae Attached	Macroalgae Unattached	Macrophytes
Left Bank					P A D		P A D	P A D	P A D
Left Center					P A D		P A D	P A D	P A D
Center					P A D		P A D	P A D	P A D
Right Center					P A D		P A D	P A D	P A D
Right Bank					P A D		P A D	P A D	P A D
	Note: Substrate sizes can be recorded either as direct measures of the median axis of each particle or one of the size class categories listed on the supplemental page (direct measurements preferred)								

Microalgae Thickness Codes

0 = No microalgae present, Feels rough, not slimy;
1 = Present but not visible, Feels slimy;
2 = Present and visible but <1mm; Rubbing fingers on surface produces a brownish tint on them, scraping leaves visible trail.
3 = 1-5mm;
4 = 5-20mm;
5 = >20mm;
UD = Cannot determine if microalgae present, substrate too small or covered with silt (formerly Z code).
D = Dry, not assessed

Riparian Vegetation

(facing downstream, 5 m u/s, 5 m d/s, 10 m from wetted width)

0 = Absent (0%) 3 = Heavy (40-75%)
1 = Sparse (<10%) 4 = Very Heavy (>75%)
2 = Moderate (10-40%)

Vegetation Class	Left Bank	Right Bank
Upper Canopy (>5 m high)		
Trees and saplings >5 m high	0 1 2 3 4	0 1 2 3 4
Lower Canopy (0.5 m-5 m high)		
All vegetation 0.5 m to 5 m	0 1 2 3 4	0 1 2 3 4
Ground Cover (<0.5 m high)		
Woody shrubs & saplings <0.5 m	0 1 2 3 4	0 1 2 3 4
Herbs/ grasses	0 1 2 3 4	0 1 2 3 4
Barren, bare soil/ duff	0 1 2 3 4	0 1 2 3 4

Instream Habitat Complexity

(5 m u/s, 5 m d/s)

0 = Absent (0%)
1 = Sparse (<10%)
2 = Moderate (10-40%)
3 = Heavy (40-75%)
4 = Very Heavy (>75%)

Filamentous Algae	0 1 2 3 4
Aquatic Macrophytes/ Emergent Vegetation	0 1 2 3 4
Boulders	0 1 2 3 4
Woody Debris >0.3 m	0 1 2 3 4
Woody Debris <0.3 m	0 1 2 3 4
Undercut Banks	0 1 2 3 4
Overhang. Vegetation	0 1 2 3 4
Live Tree Roots	0 1 2 3 4
Artificial Structures	0 1 2 3 4

Densiometer Readings (0-17)

count covered dots

Center Left	
Center Upstream	
Center Right	
Center Downstream	
Optional	
Left Bank	
Right Bank	

Human Influence

(circle only the closest to wetted channel; assess 5 m u/s, 5 m d/s)

0 = Not Present;
B = On Bank;
C = Between Bank & 10m from Channel;
P = >10m+<50m from Channel;
Channel (record Yes or No; if Y for an analyte, do not assess banks)

	Left Bank	Channel	Right Bank
Walls/ Rip-rap/ Dams	P C B 0	Y N	0 B C P
Buildings	P C B 0	Y N	0 B C P
Pavement/ Cleared Lot	P C B 0		0 B C P
Road/ Railroad	P C B 0	Y N	0 B C P
Pipes (Inlet/ Outlet)	P C B 0	Y N	0 B C P
Landfill/ Trash	P C B 0	Y N	0 B C P
Park/ Lawn	P C B 0		0 B C P
Row Crop	P C B 0		0 B C P
Pasture/ Range	P C B 0		0 B C P
Logging Operations	P C B 0		0 B C P
Mining Activity	P C B 0	Y N	0 B C P
Vegetation Management	P C B 0		0 B C P
Bridges/ Abutments	P C B 0	Y N	0 B C P
Orchards/ Vineyards	P C B 0		0 B C P

Bank Stability

(score zone 5m upstream and 5m downstream of transect between bankfull - wetted width)

Left Bank	eroded	vulnerable	stable
Right Bank	eroded	vulnerable	stable

Inter-Transect: DE

Wetted Width (m):

Inter-Transect Substrates

Position	Dist from LB (m)	Depth (cm)	mm/ size class	% Cobble Embed.	CPOM	Microalgae Thickness Code	Macroalgae Attached	Macroalgae Unattached	Macrophytes
Left Bank					P A D		P A D	P A D	P A D
Left Center					P A D		P A D	P A D	P A D
Center					P A D		P A D	P A D	P A D
Right Center					P A D		P A D	P A D	P A D
Right Bank					P A D		P A D	P A D	P A D
	Note: Substrate sizes can be recorded either as direct measures of the median axis of each particle or one of the size class categories listed on the supplemental page (direct measurements preferred)								

Microalgae Thickness Codes

0 = No microalgae present, Feels rough, not slimy;
1 = Present but not visible, Feels slimy;
2 = Present and visible but <1mm; Rubbing fingers on surface produces a brownish tint on them, scraping leaves visible trail.
3 = 1-5mm;
4 = 5-20mm;
5 = >20mm;
UD = Cannot determine if microalgae present, substrate too small or covered with silt (formerly Z code).
D = Dry, not assessed

FLOW HABITATS
(% between transects, total=100%)

Channel Type	%
Cascade/ Falls	
Rapid	
Riffle	
Run	
Glide	
Pool	
Dry	

Site Code:	Site Name:		Date: _____ / _____ / 2014
Wetted Width (m):	Bankfull Width (m):	Bankfull Height (m):	**Transect E**

Transect Substrates

Position	Dist from LB (m)	Depth (cm)	mm/ size class	% Cobble Embed.	CPOM	Microalgae Thickness Code	Macroalgae Attached	Macroalgae Unattached	Macrophytes
Left Bank					P A D		P A D	P A D	P A D
Left Center					P A D		P A D	P A D	P A D
Center					P A D		P A D	P A D	P A D
Right Center					P A D		P A D	P A D	P A D
Right Bank					P A D		P A D	P A D	P A D
	Note: Substrate sizes can be recorded either as direct measures of the median axis of each particle or one of the size class categories listed on the supplemental page (direct measurements preferred)								

Microalgae Thickness Codes
0 = No microalgae present, Feels rough, not slimy;
1 = Present but not visible, Feels slimy;
2 = Present and visible but <1mm; Rubbing fingers on surface produces a brownish tint on them, scraping leaves visible trail.
3 = 1-5mm;
4 = 5-20mm;
5 = >20mm;
UD = Cannot determine if microalgae present, substrate too small or covered with silt (formerly Z code).
D = Dry, not assessed

Riparian Vegetation
(facing downstream, 5 m u/s, 5 m d/s, 10 m from wetted width)

0 = Absent (0%) 3 = Heavy (40-75%)
1 = Sparse (<10%) 4 = Very Heavy (>75%)
2 = Moderate (10-40%)

Vegetation Class	Left Bank	Right Bank
Upper Canopy (>5 m high)		
Trees and saplings >5 m high	0 1 2 3 4	0 1 2 3 4
Lower Canopy (0.5 m-5 m high)		
All vegetation 0.5 m to 5 m	0 1 2 3 4	0 1 2 3 4
Ground Cover (<0.5 m high)		
Woody shrubs & saplings <0.5 m	0 1 2 3 4	0 1 2 3 4
Herbs/ grasses	0 1 2 3 4	0 1 2 3 4
Barren, bare soil/ duff	0 1 2 3 4	0 1 2 3 4

Instream Habitat Complexity
(5 m u/s, 5 m d/s)

0 = Absent (0%)
1 = Sparse (<10%)
2 = Moderate (10-40%)
3 = Heavy (40-75%)
4 = Very Heavy (>75%)

Filamentous Algae	0 1 2 3 4
Aquatic Macrophytes/ Emergent Vegetation	0 1 2 3 4
Boulders	0 1 2 3 4
Woody Debris >0.3 m	0 1 2 3 4
Woody Debris <0.3 m	0 1 2 3 4
Undercut Banks	0 1 2 3 4
Overhang. Vegetation	0 1 2 3 4
Live Tree Roots	0 1 2 3 4
Artificial Structures	0 1 2 3 4

Densiometer Readings (0-17)
count covered dots

Center Left	
Center Upstream	
Center Right	
Center Downstream	
Optional	
Left Bank	
Right Bank	

Human Influence
(circle only the closest to wetted channel; assess 5 m u/s, 5 m d/s)

0 = Not Present;
B = On Bank;
C = Between Bank & 10m from Channel;
P = >10m+<50m from Channel;
Channel (record Yes or No; if Y for an analyte, do not assess banks)

	Left Bank	Channel	Right Bank
Walls/ Rip-rap/ Dams	P C B 0	Y N	0 B C P
Buildings	P C B 0	Y N	0 B C P
Pavement/ Cleared Lot	P C B 0		0 B C P
Road/ Railroad	P C B 0	Y N	0 B C P
Pipes (Inlet/ Outlet)	P C B 0	Y N	0 B C P
Landfill/ Trash	P C B 0	Y N	0 B C P
Park/ Lawn	P C B 0		0 B C P
Row Crop	P C B 0		0 B C P
Pasture/ Range	P C B 0		0 B C P
Logging Operations	P C B 0		0 B C P
Mining Activity	P C B 0	Y N	0 B C P
Vegetation Management	P C B 0		0 B C P
Bridges/ Abutments	P C B 0	Y N	0 B C P
Orchards/ Vineyards	P C B 0		0 B C P

Bank Stability
(score zone 5m upstream and 5m downstream of transect between bankfull - wetted width)

Left Bank	eroded	vulnerable	stable
Right Bank	eroded	vulnerable	stable

Inter-Transect: EF

Wetted Width (m):

Inter-Transect Substrates

Position	Dist from LB (m)	Depth (cm)	mm/ size class	% Cobble Embed.	CPOM	Microalgae Thickness Code	Macroalgae Attached	Macroalgae Unattached	Macrophytes
Left Bank					P A D		P A D	P A D	P A D
Left Center					P A D		P A D	P A D	P A D
Center					P A D		P A D	P A D	P A D
Right Center					P A D		P A D	P A D	P A D
Right Bank					P A D		P A D	P A D	P A D
	Note: Substrate sizes can be recorded either as direct measures of the median axis of each particle or one of the size class categories listed on the supplemental page (direct measurements preferred)								

Microalgae Thickness Codes

0 = No microalgae present, Feels rough, not slimy;
1 = Present but not visible, Feels slimy;
2 = Present and visible but <1mm; Rubbing fingers on surface produces a brownish tint on them, scraping leaves visible trail.
3 = 1-5mm;
4 = 5-20mm;
5 = >20mm;
UD = Cannot determine if microalgae present, substrate too small or covered with silt (formerly Z code).
D = Dry, not assessed

FLOW HABITATS
(% between transects, total=100%)

Channel Type	%
Cascade/ Falls	
Rapid	
Riffle	
Run	
Glide	
Pool	
Dry	

Site Code:	Site Name:		Date: _____ / _____ / 2014
Wetted Width (m):	Bankfull Width (m):	Bankfull Height (m):	**Transect F**

Transect Substrates

Position	Dist from LB (m)	Depth (cm)	mm/ size class	% Cobble Embed.	CPOM	Microalgae Thickness Code	Macroalgae Attached	Macroalgae Unattached	Macrophytes
Left Bank					P A D		P A D	P A D	P A D
Left Center					P A D		P A D	P A D	P A D
Center					P A D		P A D	P A D	P A D
Right Center					P A D		P A D	P A D	P A D
Right Bank					P A D		P A D	P A D	P A D
	Note: Substrate sizes can be recorded either as direct measures of the median axis of each particle or one of the size class categories listed on the supplemental page (direct measurements preferred)								

Microalgae Thickness Codes
0 = No microalgae present, Feels rough, not slimy;
1 = Present but not visible, Feels slimy;
2 = Present and visible but <1mm; Rubbing fingers on surface produces a brownish tint on them, scraping leaves visible trail.
3 = 1-5mm;
4 = 5-20mm;
5 = >20mm;
UD = Cannot determine if microalgae present, substrate too small or covered with silt (formerly Z code).
D = Dry, not assessed

Riparian Vegetation
(facing downstream, 5 m u/s, 5 m d/s, 10 m from wetted width)

0 = Absent (0%) 3 = Heavy (40-75%)
1 = Sparse (<10%) 4 = Very Heavy (>75%)
2 = Moderate (10-40%)

Vegetation Class	Left Bank	Right Bank
Upper Canopy (>5 m high)		
Trees and saplings >5 m high	0 1 2 3 4	0 1 2 3 4
Lower Canopy (0.5 m-5 m high)		
All vegetation 0.5 m to 5 m	0 1 2 3 4	0 1 2 3 4
Ground Cover (<0.5 m high)		
Woody shrubs & saplings <0.5 m	0 1 2 3 4	0 1 2 3 4
Herbs/ grasses	0 1 2 3 4	0 1 2 3 4
Barren, bare soil/ duff	0 1 2 3 4	0 1 2 3 4

Instream Habitat Complexity
(5 m u/s, 5 m d/s)

0 = Absent (0%)
1 = Sparse (<10%)
2 = Moderate (10-40%)
3 = Heavy (40-75%)
4 = Very Heavy (>75%)

Filamentous Algae	0 1 2 3 4
Aquatic Macrophytes/ Emergent Vegetation	0 1 2 3 4
Boulders	0 1 2 3 4
Woody Debris >0.3 m	0 1 2 3 4
Woody Debris <0.3 m	0 1 2 3 4
Undercut Banks	0 1 2 3 4
Overhang. Vegetation	0 1 2 3 4
Live Tree Roots	0 1 2 3 4
Artificial Structures	0 1 2 3 4

Densiometer Readings (0-17)
count covered dots

Center Left	
Center Upstream	
Center Right	
Center Downstream	
Optional	
Left Bank	
Right Bank	

Human Influence
(circle only the closest to wetted channel; assess 5 m u/s, 5 m d/s)

0 = Not Present;
B = On Bank;
C = Between Bank & 10m from Channel;
P = >10m+<50m from Channel;
Channel (record Yes or No; if Y for an analyte, do not assess banks)

	Left Bank	Channel	Right Bank
Walls/ Rip-rap/ Dams	P C B 0	Y N	0 B C P
Buildings	P C B 0	Y N	0 B C P
Pavement/ Cleared Lot	P C B 0		0 B C P
Road/ Railroad	P C B 0	Y N	0 B C P
Pipes (Inlet/ Outlet)	P C B 0	Y N	0 B C P
Landfill/ Trash	P C B 0	Y N	0 B C P
Park/ Lawn	P C B 0		0 B C P
Row Crop	P C B 0		0 B C P
Pasture/ Range	P C B 0		0 B C P
Logging Operations	P C B 0		0 B C P
Mining Activity	P C B 0	Y N	0 B C P
Vegetation Management	P C B 0		0 B C P
Bridges/ Abutments	P C B 0	Y N	0 B C P
Orchards/ Vineyards	P C B 0		0 B C P

Bank Stability
(score zone 5m upstream and 5m downstream of transect between bankfull - wetted width)

Left Bank	eroded	vulnerable	stable
Right Bank	eroded	vulnerable	stable

TAKE PHOTOGRAPHS
(check box if taken & record photo code)

Downstream (**required**)	☐
Upstream (**required**)	☐

Inter-Transect: FG

Wetted Width (m):

Inter-Transect Substrates

Position	Dist from LB (m)	Depth (cm)	mm/ size class	% Cobble Embed.	CPOM	Microalgae Thickness Code	Macroalgae Attached	Macroalgae Unattached	Macrophytes
Left Bank					P A D		P A D	P A D	P A D
Left Center					P A D		P A D	P A D	P A D
Center					P A D		P A D	P A D	P A D
Right Center					P A D		P A D	P A D	P A D
Right Bank					P A D		P A D	P A D	P A D
	Note: Substrate sizes can be recorded either as direct measures of the median axis of each particle or one of the size class categories listed on the supplemental page (direct measurements preferred)								

Microalgae Thickness Codes

0 = No microalgae present, Feels rough, not slimy;
1 = Present but not visible, Feels slimy;
2 = Present and visible but <1mm; Rubbing fingers on surface produces a brownish tint on them, scraping leaves visible trail.
3 = 1-5mm;
4 = 5-20mm;
5 = >20mm;
UD = Cannot determine if microalgae present, substrate too small or covered with silt (formerly Z code).
D = Dry, not assessed

FLOW HABITATS
(% between transects, total=100%)

Channel Type	%
Cascade/ Falls	
Rapid	
Riffle	
Run	
Glide	
Pool	
Dry	

Site Code:	Site Name:		Date: _____ / _____ / 2014
Wetted Width (m):	Bankfull Width (m):	Bankfull Height (m):	**Transect G**

Transect Substrates

Position	Dist from LB (m)	Depth (cm)	mm/ size class	% Cobble Embed.	CPOM	Microalgae Thickness Code	Macroalgae Attached	Macroalgae Unattached	Macrophytes
Left Bank					P A D		P A D	P A D	P A D
Left Center					P A D		P A D	P A D	P A D
Center					P A D		P A D	P A D	P A D
Right Center					P A D		P A D	P A D	P A D
Right Bank					P A D		P A D	P A D	P A D
	Note: Substrate sizes can be recorded either as direct measures of the median axis of each particle or one of the size class categories listed on the supplemental page (direct measurements preferred)								

Microalgae Thickness Codes
0 = No microalgae present, Feels rough, not slimy;
1 = Present but not visible, Feels slimy;
2 = Present and visible but <1mm; Rubbing fingers on surface produces a brownish tint on them, scraping leaves visible trail.
3 = 1-5mm;
4 = 5-20mm;
5 = >20mm;
UD = Cannot determine if microalgae present, substrate too small or covered with silt (formerly Z code).
D = Dry, not assessed

Riparian Vegetation
(facing downstream, 5 m u/s, 5 m d/s, 10 m from wetted width)

0 = Absent (0%) 3 = Heavy (40-75%)
1 = Sparse (<10%) 4 = Very Heavy (>75%)
2 = Moderate (10-40%)

Vegetation Class	Left Bank	Right Bank
Upper Canopy (>5 m high)		
Trees and saplings >5 m high	0 1 2 3 4	0 1 2 3 4
Lower Canopy (0.5 m-5 m high)		
All vegetation 0.5 m to 5 m	0 1 2 3 4	0 1 2 3 4
Ground Cover (<0.5 m high)		
Woody shrubs & saplings <0.5 m	0 1 2 3 4	0 1 2 3 4
Herbs/ grasses	0 1 2 3 4	0 1 2 3 4
Barren, bare soil/ duff	0 1 2 3 4	0 1 2 3 4

Instream Habitat Complexity
(5 m u/s, 5 m d/s)

0 = Absent (0%)
1 = Sparse (<10%)
2 = Moderate (10-40%)
3 = Heavy (40-75%)
4 = Very Heavy (>75%)

Filamentous Algae	0 1 2 3 4
Aquatic Macrophytes/ Emergent Vegetation	0 1 2 3 4
Boulders	0 1 2 3 4
Woody Debris >0.3 m	0 1 2 3 4
Woody Debris <0.3 m	0 1 2 3 4
Undercut Banks	0 1 2 3 4
Overhang. Vegetation	0 1 2 3 4
Live Tree Roots	0 1 2 3 4
Artificial Structures	0 1 2 3 4

Densiometer Readings (0-17)
count covered dots

Center Left	
Center Upstream	
Center Right	
Center Downstream	
Optional	
Left Bank	
Right Bank	

Human Influence
(circle only the closest to wetted channel; assess 5 m u/s, 5 m d/s)

0 = Not Present;
B = On Bank;
C = Between Bank & 10m from Channel;
P = >10m+<50m from Channel;
Channel (record Yes or No; if Y for an analyte, do not assess banks)

	Left Bank	Channel	Right Bank
Walls/ Rip-rap/ Dams	P C B 0	Y N	0 B C P
Buildings	P C B 0	Y N	0 B C P
Pavement/ Cleared Lot	P C B 0		0 B C P
Road/ Railroad	P C B 0	Y N	0 B C P
Pipes (Inlet/ Outlet)	P C B 0	Y N	0 B C P
Landfill/ Trash	P C B 0	Y N	0 B C P
Park/ Lawn	P C B 0		0 B C P
Row Crop	P C B 0		0 B C P
Pasture/ Range	P C B 0		0 B C P
Logging Operations	P C B 0		0 B C P
Mining Activity	P C B 0	Y N	0 B C P
Vegetation Management	P C B 0		0 B C P
Bridges/ Abutments	P C B 0	Y N	0 B C P
Orchards/ Vineyards	P C B 0		0 B C P

Bank Stability
(score zone 5m upstream and 5m downstream of transect between bankfull - wetted width)

Left Bank	eroded	vulnerable	stable
Right Bank	eroded	vulnerable	stable

Inter-Transect: GH

Wetted Width (m):

Inter-Transect Substrates

Position	Dist from LB (m)	Depth (cm)	mm/ size class	% Cobble Embed.	CPOM	Microalgae Thickness Code	Macroalgae Attached	Macroalgae Unattached	Macrophytes
Left Bank					P A D		P A D	P A D	P A D
Left Center					P A D		P A D	P A D	P A D
Center					P A D		P A D	P A D	P A D
Right Center					P A D		P A D	P A D	P A D
Right Bank					P A D		P A D	P A D	P A D
	Note: Substrate sizes can be recorded either as direct measures of the median axis of each particle or one of the size class categories listed on the supplemental page (direct measurements preferred)								

Microalgae Thickness Codes

0 = No microalgae present, Feels rough, not slimy;
1 = Present but not visible, Feels slimy;
2 = Present and visible but <1mm; Rubbing fingers on surface produces a brownish tint on them, scraping leaves visible trail.
3 = 1-5mm;
4 = 5-20mm;
5 = >20mm;
UD = Cannot determine if microalgae present, substrate too small or covered with silt (formerly Z code).
D = Dry, not assessed

FLOW HABITATS
(% between transects, total=100%)

Channel Type	%
Cascade/ Falls	
Rapid	
Riffle	
Run	
Glide	
Pool	
Dry	

Site Code:	Site Name:		Date: _____ / _____ / 2014
Wetted Width (m):	Bankfull Width (m):	Bankfull Height (m):	**Transect H**

Transect Substrates

Position	Dist from LB (m)	Depth (cm)	mm/ size class	% Cobble Embed.	CPOM	Microalgae Thickness Code	Macroalgae Attached	Macroalgae Unattached	Macrophytes
Left Bank					P A D		P A D	P A D	P A D
Left Center					P A D		P A D	P A D	P A D
Center					P A D		P A D	P A D	P A D
Right Center					P A D		P A D	P A D	P A D
Right Bank					P A D		P A D	P A D	P A D
	Note: Substrate sizes can be recorded either as direct measures of the median axis of each particle or one of the size class categories listed on the supplemental page (direct measurements preferred)								

Microalgae Thickness Codes
0 = No microalgae present, Feels rough, not slimy;
1 = Present but not visible, Feels slimy;
2 = Present and visible but <1mm; Rubbing fingers on surface produces a brownish tint on them, scraping leaves visible trail.
3 = 1-5mm;
4 = 5-20mm;
5 = >20mm;
UD = Cannot determine if microalgae present, substrate too small or covered with silt (formerly Z code).
D = Dry, not assessed

Riparian Vegetation
(facing downstream, 5 m u/s, 5 m d/s, 10 m from wetted width)

0 = Absent (0%) 3 = Heavy (40-75%)
1 = Sparse (<10%) 4 = Very Heavy (>75%)
2 = Moderate (10-40%)

Vegetation Class	Left Bank	Right Bank
Upper Canopy (>5 m high)		
Trees and saplings >5 m high	0 1 2 3 4	0 1 2 3 4
Lower Canopy (0.5 m-5 m high)		
All vegetation 0.5 m to 5 m	0 1 2 3 4	0 1 2 3 4
Ground Cover (<0.5 m high)		
Woody shrubs & saplings <0.5 m	0 1 2 3 4	0 1 2 3 4
Herbs/ grasses	0 1 2 3 4	0 1 2 3 4
Barren, bare soil/ duff	0 1 2 3 4	0 1 2 3 4

Instream Habitat Complexity
(5 m u/s, 5 m d/s)

0 = Absent (0%)
1 = Sparse (<10%)
2 = Moderate (10-40%)
3 = Heavy (40-75%)
4 = Very Heavy (>75%)

Filamentous Algae	0 1 2 3 4
Aquatic Macrophytes/ Emergent Vegetation	0 1 2 3 4
Boulders	0 1 2 3 4
Woody Debris >0.3 m	0 1 2 3 4
Woody Debris <0.3 m	0 1 2 3 4
Undercut Banks	0 1 2 3 4
Overhang. Vegetation	0 1 2 3 4
Live Tree Roots	0 1 2 3 4
Artificial Structures	0 1 2 3 4

Densiometer Readings (0-17)
count covered dots

Center Left	
Center Upstream	
Center Right	
Center Downstream	
Optional	
Left Bank	
Right Bank	

Human Influence
(circle only the closest to wetted channel; assess 5 m u/s, 5 m d/s)

0 = Not Present;
B = On Bank;
C = Between Bank & 10m from Channel;
P = >10m+<50m from Channel;
Channel (record Yes or No; if Y for an analyte, do not assess banks)

	Left Bank	Channel	Right Bank
Walls/ Rip-rap/ Dams	P C B 0	Y N	0 B C P
Buildings	P C B 0	Y N	0 B C P
Pavement/ Cleared Lot	P C B 0		0 B C P
Road/ Railroad	P C B 0	Y N	0 B C P
Pipes (Inlet/ Outlet)	P C B 0	Y N	0 B C P
Landfill/ Trash	P C B 0	Y N	0 B C P
Park/ Lawn	P C B 0		0 B C P
Row Crop	P C B 0		0 B C P
Pasture/ Range	P C B 0		0 B C P
Logging Operations	P C B 0		0 B C P
Mining Activity	P C B 0	Y N	0 B C P
Vegetation Management	P C B 0		0 B C P
Bridges/ Abutments	P C B 0	Y N	0 B C P
Orchards/ Vineyards	P C B 0		0 B C P

Bank Stability
(score zone 5m upstream and 5m downstream of transect between bankfull - wetted width)

Left Bank	eroded	vulnerable	stable
Right Bank	eroded	vulnerable	stable

Inter-Transect: HI

Wetted Width (m):

Inter-Transect Substrates

Position	Dist from LB (m)	Depth (cm)	mm/ size class	% Cobble Embed.	CPOM	Microalgae Thickness Code	Macroalgae Attached	Macroalgae Unattached	Macrophytes
Left Bank					P A D		P A D	P A D	P A D
Left Center					P A D		P A D	P A D	P A D
Center					P A D		P A D	P A D	P A D
Right Center					P A D		P A D	P A D	P A D
Right Bank					P A D		P A D	P A D	P A D
	Note: Substrate sizes can be recorded either as direct measures of the median axis of each particle or one of the size class categories listed on the supplemental page (direct measurements preferred)								

Microalgae Thickness Codes

0 = No microalgae present, Feels rough, not slimy;
1 = Present but not visible, Feels slimy;
2 = Present and visible but <1mm; Rubbing fingers on surface produces a brownish tint on them, scraping leaves visible trail.
3 = 1-5mm;
4 = 5-20mm;
5 = >20mm;
UD = Cannot determine if microalgae present, substrate too small or covered with silt (formerly Z code).
D = Dry, not assessed

FLOW HABITATS
(% between transects, total=100%)

Channel Type	%
Cascade/ Falls	
Rapid	
Riffle	
Run	
Glide	
Pool	
Dry	

Site Code:	Site Name:		Date: _____ / _____ / 2014
Wetted Width (m):	Bankfull Width (m):	Bankfull Height (m):	**Transect I**

Transect Substrates

Position	Dist from LB (m)	Depth (cm)	mm/ size class	% Cobble Embed.	CPOM	Microalgae Thickness Code	Macroalgae Attached	Macroalgae Unattached	Macrophytes
Left Bank					P A D		P A D	P A D	P A D
Left Center					P A D		P A D	P A D	P A D
Center					P A D		P A D	P A D	P A D
Right Center					P A D		P A D	P A D	P A D
Right Bank					P A D		P A D	P A D	P A D
	Note: Substrate sizes can be recorded either as direct measures of the median axis of each particle or one of the size class categories listed on the supplemental page (direct measurements preferred)								

Microalgae Thickness Codes
0 = No microalgae present, Feels rough, not slimy;
1 = Present but not visible, Feels slimy;
2 = Present and visible but <1mm; Rubbing fingers on surface produces a brownish tint on them, scraping leaves visible trail.
3 = 1-5mm;
4 = 5-20mm;
5 = >20mm;
UD = Cannot determine if microalgae present, substrate too small or covered with silt (formerly Z code).
D = Dry, not assessed

Riparian Vegetation
(facing downstream, 5 m u/s, 5 m d/s, 10 m from wetted width)

0 = Absent (0%) 3 = Heavy (40-75%)
1 = Sparse (<10%) 4 = Very Heavy (>75%)
2 = Moderate (10-40%)

Vegetation Class	Left Bank	Right Bank
Upper Canopy (>5 m high)		
Trees and saplings >5 m high	0 1 2 3 4	0 1 2 3 4
Lower Canopy (0.5 m-5 m high)		
All vegetation 0.5 m to 5 m	0 1 2 3 4	0 1 2 3 4
Ground Cover (<0.5 m high)		
Woody shrubs & saplings <0.5 m	0 1 2 3 4	0 1 2 3 4
Herbs/ grasses	0 1 2 3 4	0 1 2 3 4
Barren, bare soil/ duff	0 1 2 3 4	0 1 2 3 4

Instream Habitat Complexity
(5 m u/s, 5 m d/s)

0 = Absent (0%)
1 = Sparse (<10%)
2 = Moderate (10-40%)
3 = Heavy (40-75%)
4 = Very Heavy (>75%)

Filamentous Algae	0 1 2 3 4
Aquatic Macrophytes/ Emergent Vegetation	0 1 2 3 4
Boulders	0 1 2 3 4
Woody Debris >0.3 m	0 1 2 3 4
Woody Debris <0.3 m	0 1 2 3 4
Undercut Banks	0 1 2 3 4
Overhang. Vegetation	0 1 2 3 4
Live Tree Roots	0 1 2 3 4
Artificial Structures	0 1 2 3 4

Densiometer Readings (0-17)
count covered dots

Center Left	
Center Upstream	
Center Right	
Center Downstream	
Optional	
Left Bank	
Right Bank	

Human Influence
(circle only the closest to wetted channel; assess 5 m u/s, 5 m d/s)

0 = Not Present;
B = On Bank;
C = Between Bank & 10m from Channel;
P = >10m+<50m from Channel;
Channel (record Yes or No; if Y for an analyte, do not assess banks)

	Left Bank	Channel	Right Bank
Walls/ Rip-rap/ Dams	P C B 0	Y N	0 B C P
Buildings	P C B 0	Y N	0 B C P
Pavement/ Cleared Lot	P C B 0		0 B C P
Road/ Railroad	P C B 0	Y N	0 B C P
Pipes (Inlet/ Outlet)	P C B 0	Y N	0 B C P
Landfill/ Trash	P C B 0	Y N	0 B C P
Park/ Lawn	P C B 0		0 B C P
Row Crop	P C B 0		0 B C P
Pasture/ Range	P C B 0		0 B C P
Logging Operations	P C B 0		0 B C P
Mining Activity	P C B 0	Y N	0 B C P
Vegetation Management	P C B 0		0 B C P
Bridges/ Abutments	P C B 0	Y N	0 B C P
Orchards/ Vineyards	P C B 0		0 B C P

Bank Stability
(score zone 5m upstream and 5m downstream of transect between bankfull - wetted width)

Left Bank	eroded	vulnerable	stable
Right Bank	eroded	vulnerable	stable

Inter-Transect: IJ

Wetted Width (m):

Inter-Transect Substrates

Position	Dist from LB (m)	Depth (cm)	mm/ size class	% Cobble Embed.	CPOM	Microalgae Thickness Code	Macroalgae Attached	Macroalgae Unattached	Macrophytes
Left Bank					P A D		P A D	P A D	P A D
Left Center					P A D		P A D	P A D	P A D
Center					P A D		P A D	P A D	P A D
Right Center					P A D		P A D	P A D	P A D
Right Bank					P A D		P A D	P A D	P A D
	Note: Substrate sizes can be recorded either as direct measures of the median axis of each particle or one of the size class categories listed on the supplemental page (direct measurements preferred)								

Microalgae Thickness Codes

0 = No microalgae present, Feels rough, not slimy;
1 = Present but not visible, Feels slimy;
2 = Present and visible but <1mm; Rubbing fingers on surface produces a brownish tint on them, scraping leaves visible trail.
3 = 1-5mm;
4 = 5-20mm;
5 = >20mm;
UD = Cannot determine if microalgae present, substrate too small or covered with silt (formerly Z code).
D = Dry, not assessed

FLOW HABITATS
(% between transects, total=100%)

Channel Type	%
Cascade/ Falls	
Rapid	
Riffle	
Run	
Glide	
Pool	
Dry	

Site Code:	Site Name:		Date: _____ / _____ / 2014
Wetted Width (m):	Bankfull Width (m):	Bankfull Height (m):	**Transect J**

Transect Substrates

Position	Dist from LB (m)	Depth (cm)	mm/ size class	% Cobble Embed.	CPOM	Microalgae Thickness Code	Macroalgae Attached	Macroalgae Unattached	Macrophytes
Left Bank					P A D		P A D	P A D	P A D
Left Center					P A D		P A D	P A D	P A D
Center					P A D		P A D	P A D	P A D
Right Center					P A D		P A D	P A D	P A D
Right Bank					P A D		P A D	P A D	P A D
	Note: Substrate sizes can be recorded either as direct measures of the median axis of each particle or one of the size class categories listed on the supplemental page (direct measurements preferred)								

Microalgae Thickness Codes
0 = No microalgae present, Feels rough, not slimy;
1 = Present but not visible, Feels slimy;
2 = Present and visible but <1mm; Rubbing fingers on surface produces a brownish tint on them, scraping leaves visible trail.
3 = 1-5mm;
4 = 5-20mm;
5 = >20mm;
UD = Cannot determine if microalgae present, substrate too small or covered with silt (formerly Z code).
D = Dry, not assessed

Riparian Vegetation
(facing downstream, 5 m u/s, 5 m d/s, 10 m from wetted width)

0 = Absent (0%) 3 = Heavy (40-75%)
1 = Sparse (<10%) 4 = Very Heavy (>75%)
2 = Moderate (10-40%)

Vegetation Class	Left Bank	Right Bank
Upper Canopy (>5 m high)		
Trees and saplings >5 m high	0 1 2 3 4	0 1 2 3 4
Lower Canopy (0.5 m-5 m high)		
All vegetation 0.5 m to 5 m	0 1 2 3 4	0 1 2 3 4
Ground Cover (<0.5 m high)		
Woody shrubs & saplings <0.5 m	0 1 2 3 4	0 1 2 3 4
Herbs/ grasses	0 1 2 3 4	0 1 2 3 4
Barren, bare soil/ duff	0 1 2 3 4	0 1 2 3 4

Instream Habitat Complexity
(5 m u/s, 5 m d/s)

0 = Absent (0%)
1 = Sparse (<10%)
2 = Moderate (10-40%)
3 = Heavy (40-75%)
4 = Very Heavy (>75%)

Filamentous Algae	0 1 2 3 4
Aquatic Macrophytes/ Emergent Vegetation	0 1 2 3 4
Boulders	0 1 2 3 4
Woody Debris >0.3 m	0 1 2 3 4
Woody Debris <0.3 m	0 1 2 3 4
Undercut Banks	0 1 2 3 4
Overhang. Vegetation	0 1 2 3 4
Live Tree Roots	0 1 2 3 4
Artificial Structures	0 1 2 3 4

Densiometer Readings (0-17)
count covered dots

Center Left	
Center Upstream	
Center Right	
Center Downstream	
Optional	
Left Bank	
Right Bank	

Human Influence
(circle only the closest to wetted channel; assess 5 m u/s, 5 m d/s)

0 = Not Present;
B = On Bank;
C = Between Bank & 10m from Channel;
P = >10m+<50m from Channel;
Channel (record Yes or No; if Y for an analyte, do not assess banks)

	Left Bank	Channel	Right Bank
Walls/ Rip-rap/ Dams	P C B 0	Y N	0 B C P
Buildings	P C B 0	Y N	0 B C P
Pavement/ Cleared Lot	P C B 0		0 B C P
Road/ Railroad	P C B 0	Y N	0 B C P
Pipes (Inlet/ Outlet)	P C B 0	Y N	0 B C P
Landfill/ Trash	P C B 0	Y N	0 B C P
Park/ Lawn	P C B 0		0 B C P
Row Crop	P C B 0		0 B C P
Pasture/ Range	P C B 0		0 B C P
Logging Operations	P C B 0		0 B C P
Mining Activity	P C B 0	Y N	0 B C P
Vegetation Management	P C B 0		0 B C P
Bridges/ Abutments	P C B 0	Y N	0 B C P
Orchards/ Vineyards	P C B 0		0 B C P

Bank Stability
(score zone 5m upstream and 5m downstream of transect between bankfull - wetted width)

Left Bank	eroded	vulnerable	stable
Right Bank	eroded	vulnerable	stable

Inter-Transect: JK

Wetted Width (m):

Inter-Transect Substrates

Position	Dist from LB (m)	Depth (cm)	mm/ size class	% Cobble Embed.	CPOM	Microalgae Thickness Code	Macroalgae Attached	Macroalgae Unattached	Macrophytes
Left Bank					P A D		P A D	P A D	P A D
Left Center					P A D		P A D	P A D	P A D
Center					P A D		P A D	P A D	P A D
Right Center					P A D		P A D	P A D	P A D
Right Bank					P A D		P A D	P A D	P A D
	Note: Substrate sizes can be recorded either as direct measures of the median axis of each particle or one of the size class categories listed on the supplemental page (direct measurements preferred)								

Microalgae Thickness Codes

0 = No microalgae present, Feels rough, not slimy;
1 = Present but not visible, Feels slimy;
2 = Present and visible but <1mm; Rubbing fingers on surface produces a brownish tint on them, scraping leaves visible trail.
3 = 1-5mm;
4 = 5-20mm;
5 = >20mm;
UD = Cannot determine if microalgae present, substrate too small or covered with silt (formerly Z code).
D = Dry, not assessed

FLOW HABITATS
(% between transects, total=100%)

Channel Type	%
Cascade/ Falls	
Rapid	
Riffle	
Run	
Glide	
Pool	
Dry	

Site Code:	Site Name:		Date: _____ / _____ / 2014
Wetted Width (m):	Bankfull Width (m):	Bankfull Height (m):	**Transect K**

Transect Substrates

Position	Dist from LB (m)	Depth (cm)	mm/ size class	% Cobble Embed.	CPOM	Microalgae Thickness Code	Macroalgae Attached	Macroalgae Unattached	Macrophytes
Left Bank					P A D		P A D	P A D	P A D
Left Center					P A D		P A D	P A D	P A D
Center					P A D		P A D	P A D	P A D
Right Center					P A D		P A D	P A D	P A D
Right Bank					P A D		P A D	P A D	P A D
	Note: Substrate sizes can be recorded either as direct measures of the median axis of each particle or one of the size class categories listed on the supplemental page (direct measurements preferred)								

Microalgae Thickness Codes
0 = No microalgae present, Feels rough, not slimy;
1 = Present but not visible, Feels slimy;
2 = Present and visible but <1mm; Rubbing fingers on surface produces a brownish tint on them, scraping leaves visible trail.
3 = 1-5mm;
4 = 5-20mm;
5 = >20mm;
U = Cannot determine if microalgae present, substrate too small or covered with silt (formerly Z code).
D = Dry, not assessed

Riparian Vegetation
(facing downstream, 5 m u/s, 5 m d/s, 10 m from wetted width)

0 = Absent (0%) 3 = Heavy (40-75%)
1 = Sparse (<10%) 4 = Very Heavy (>75%)
2 = Moderate (10-40%)

Vegetation Class	Left Bank	Right Bank
Upper Canopy (>5 m high)		
Trees and saplings >5 m high	0 1 2 3 4	0 1 2 3 4
Lower Canopy (0.5 m-5 m high)		
All vegetation 0.5 m to 5 m	0 1 2 3 4	0 1 2 3 4
Ground Cover (<0.5 m high)		
Woody shrubs & saplings <0.5 m	0 1 2 3 4	0 1 2 3 4
Herbs/ grasses	0 1 2 3 4	0 1 2 3 4
Barren, bare soil/ duff	0 1 2 3 4	0 1 2 3 4

Instream Habitat Complexity
(5 m u/s, 5 m d/s)

0 = Absent (0%)
1 = Sparse (<10%)
2 = Moderate (10-40%)
3 = Heavy (40-75%)
4 = Very Heavy (>75%)

Filamentous Algae	0 1 2 3 4
Aquatic Macrophytes/ Emergent Vegetation	0 1 2 3 4
Boulders	0 1 2 3 4
Woody Debris >0.3 m	0 1 2 3 4
Woody Debris <0.3 m	0 1 2 3 4
Undercut Banks	0 1 2 3 4
Overhang. Vegetation	0 1 2 3 4
Live Tree Roots	0 1 2 3 4
Artificial Structures	0 1 2 3 4

Densiometer Readings (0-17)
count covered dots

Center Left	
Center Upstream	
Center Right	
Center Downstream	
Optional	
Left Bank	
Right Bank	

Human Influence
(circle only the closest to wetted channel; assess 5 m u/s, 5 m d/s)

0 = Not Present;
B = On Bank;
C = Between Bank & 10m from Channel;
P = >10m+<50m from Channel;
Channel (record Yes or No; if Y for an analyte, do not assess banks)

	Left Bank	Channel	Right Bank
Walls/ Rip-rap/ Dams	P C B 0	Y N	0 B C P
Buildings	P C B 0	Y N	0 B C P
Pavement/ Cleared Lot	P C B 0		0 B C P
Road/ Railroad	P C B 0	Y N	0 B C P
Pipes (Inlet/ Outlet)	P C B 0	Y N	0 B C P
Landfill/ Trash	P C B 0	Y N	0 B C P
Park/ Lawn	P C B 0		0 B C P
Row Crop	P C B 0		0 B C P
Pasture/ Range	P C B 0		0 B C P
Logging Operations	P C B 0		0 B C P
Mining Activity	P C B 0	Y N	0 B C P
Vegetation Management	P C B 0		0 B C P
Bridges/ Abutments	P C B 0	Y N	0 B C P
Orchards/ Vineyards	P C B 0		0 B C P

Bank Stability
(score zone 5m upstream and 5m downstream of transect between bankfull - wetted width)

Left Bank	eroded	vulnerable	stable
Right Bank	eroded	vulnerable	stable

TAKE PHOTOGRAPHS
(check box if taken & record photo code)

Downstream (**required**) ☐

Upstream (**optional**) ☐

Site Code:	Date: ____ / ____ / 2014

BENTHIC INVERTEBRATE SAMPLES

Collection Method (indicate standard or margin-center-margin)			Replicate	# Jars
RWB (standard)	**RWB** (MCM)	**TRC**	**1**	
RWB (standard)	**RWB** (MCM)	**TRC**	**2**	
RWB (standard)	**RWB** (MCM)	**TRC**		
RWB (standard)	**RWB** (MCM)	**TRC**		

Field Notes/ Comments:

Was macroalgae (e.g., filamentous algae) collected in the composite algae sample? Yes / No
If YES, how many of the 11 transect samples contained macroalgae? _____
If YES, what was the original size of the macroalgae cylinder roll before sectioning into ¼ and ¾ pieces? _____ mm length x _____ mm diameter

Analyte	Equipment & Calibration Date
pH	Cal date: / /
Wat temp	Cal date: / /
dissolved oxygen	Cal date: / /
oxygen sat	Cal date: / /
specific cond	Cal date: / /
Salinity	Cal date: / /
Alkalinity	Cal date: / /
Turbidity	Cal date: / /
Silica	Cal date: / /
Air temp	Cal date: / /
Velocity	Cal date: / /

ALGAE SAMPLES

Collection Method (circle one or write new method if applicable)	SWAMP EMAP	SWAMP EMAP	SWAMP EMAP	SWAMP EMAP
Collection Device (sum # of transects per device)	**Rep. 1**	**Rep. 2**	**Rep.**	**Rep.**
Rubber Delimiter (area=12.6cm^2)				
PVC Delimiter (area=12.6cm^2)				
Syringe Scrubber (area=5.3cm^2)				
Other area=				
Number of transects sampled (0-11)				
Composite Volume (mL)				
Assemblage ID volume **(diatoms)** (50 mL tube)				
Assemblage ID volume **(soft algae)** (50 mL tube)				
Check if Qualitative Algae sample was collected with soft algae/diatom sample (required even if macroalgae not visible)	☐	☐	☐	☐
Check if a water chem. **integrated** sample was collected (chl, AFDM)	☐	☐	☐	☐
Chlorophyll a volume use GF/F filter (25 mL **(preferred volume)**				
Ash Free Dry Mass use GF/F filter (AFDM) volume (25 mL **(preferred vol)**				

Water and Sediment Chemistry Samples

Check if a WATER chemistry **grab** sample was collected (nutrients, SSC, etc.)	☐
Check if a DUPLICATE WATER chemistry **grab** sample was collected	☐
Check if a SEDIMENT chemistry sample was collected	☐
Check if a DUPLICATE SED chemistry sample was collected	☐

Sed Coll Device: S OOP CORE GRAB

Material: Stainless Steel Polyethylene Polycarbonate Other

Sediment Collection Depth (cm): 2 or 5

Create Lab Collection records for each checked box for integrated and grab water chemistry samples

Description	Photo Code	Description	Photo Code

Flow Habitat Type	DESCRIPTION
Cascades	Short, high gradient drop in stream bed elevation often accompanied by boulders and considerable turbulence
Falls	High gradient drop in elevation of the stream bed associated with an abrupt change in the bedrock
Rapids	Sections of stream with swiftly flowing water and considerable surface turbulence. Rapids tend to have larger substrate sizes than riffles
Riffles	Shallow sections where the water flows over coarse stream bed particles that create mild to moderate surface turbulence; (< 0.5 m deep, > 0.3 m/s).
Runs	Long, relatively straight, low-gradient sections without flow obstructions. The stream bed is typically even and the water flows faster than it does in a pool; (> 0.5 m deep, > 0.3 m/s). A **step-run** is a series of runs separated by short riffles or flow obstructions that cause discontinuous breaks in slope
Glides	A section of stream with little or no turbulence, but faster velocity than pools; (< 0.5 m deep, < 0.3 m/s)
Pools	A reach of stream that is characterized by deep, low-velocity water and a smooth surface; (> 0.5 m deep, < 0.3 m/s)

Size Class Code	Size Class Range	Size Class Description	Common Size Reference
RS	> 4 m	bedrock, smooth	larger than a car
RR	> 4 m	bedrock, rough	larger than a car
XB	1 - 4 m	boulder, large	meter stick to car
SB	25 cm - 1.0 m	boulder, small	basketball to meter stick
CB	64 - 250 mm	cobble	tennis ball to basketball
GC	16 - 64 mm	gravel, coarse	marble to tennis ball
GF	2 – 16 mm	gravel, fine	ladybug to marble
SA	0.06 – 2 mm	sand	gritty to ladybug
FN	< 0.06 mm	fines	not gritty
HP	< 0.06 mm	hardpan (consolidated fines)	
WD	NA	wood	
RC	NA	concrete/ asphalt	
OT	NA	other	

BANK STABILITY

Although this measure of the degree of erosive potential is subjective, it can provide clues to the erosive potential of the banks within the reach. Assign the category whose description best fits the conditions in the area between the wetted channel and bankfull channel (see figure below)

Eroded	Banks show obvious signs of erosion from the current or previous water year; banks are usually bare or nearly bare
Vulnerable	Banks have some vegetative protection (usually annual growth), but not enough to prevent erosion during flooding
Stable	Bank vegetation has well-developed roots that protect banks from erosion; alternately, bedrock or artificial structures (e.g., concrete/ rip-rap) prevent bank erosion

CPOM/ COBBLE EMBEDDEDNESS

CPOM: Record presence (P) or absence (A) of coarse particulate organic matter (>1.0 mm particles) within 1 cm of each substrate particle; if point is dry, record Dry (D)

Cobble Embeddedness: Visually estimate % embedded by fine particles (record to nearest 5%)

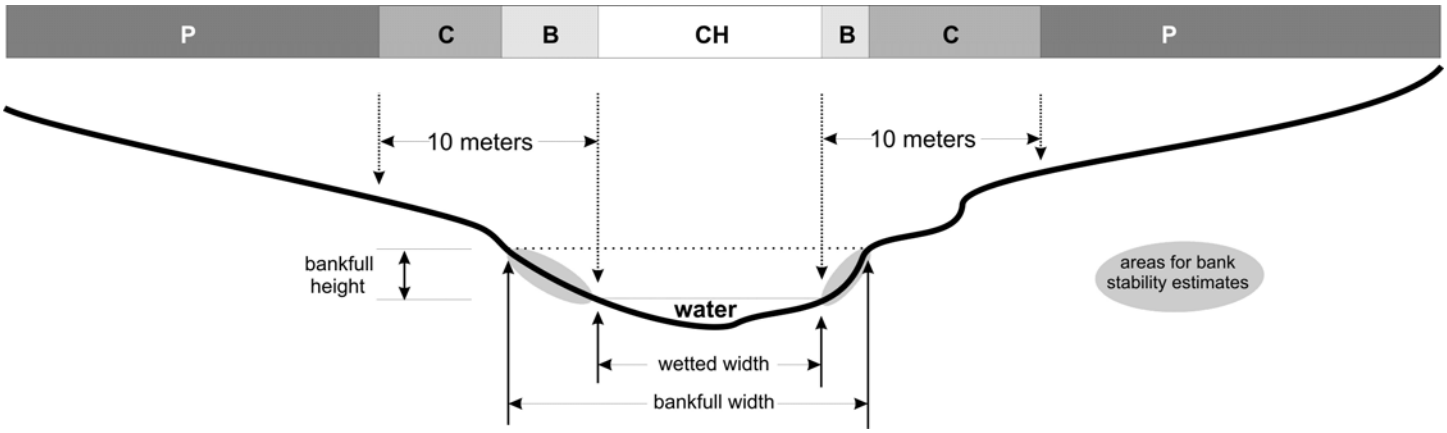
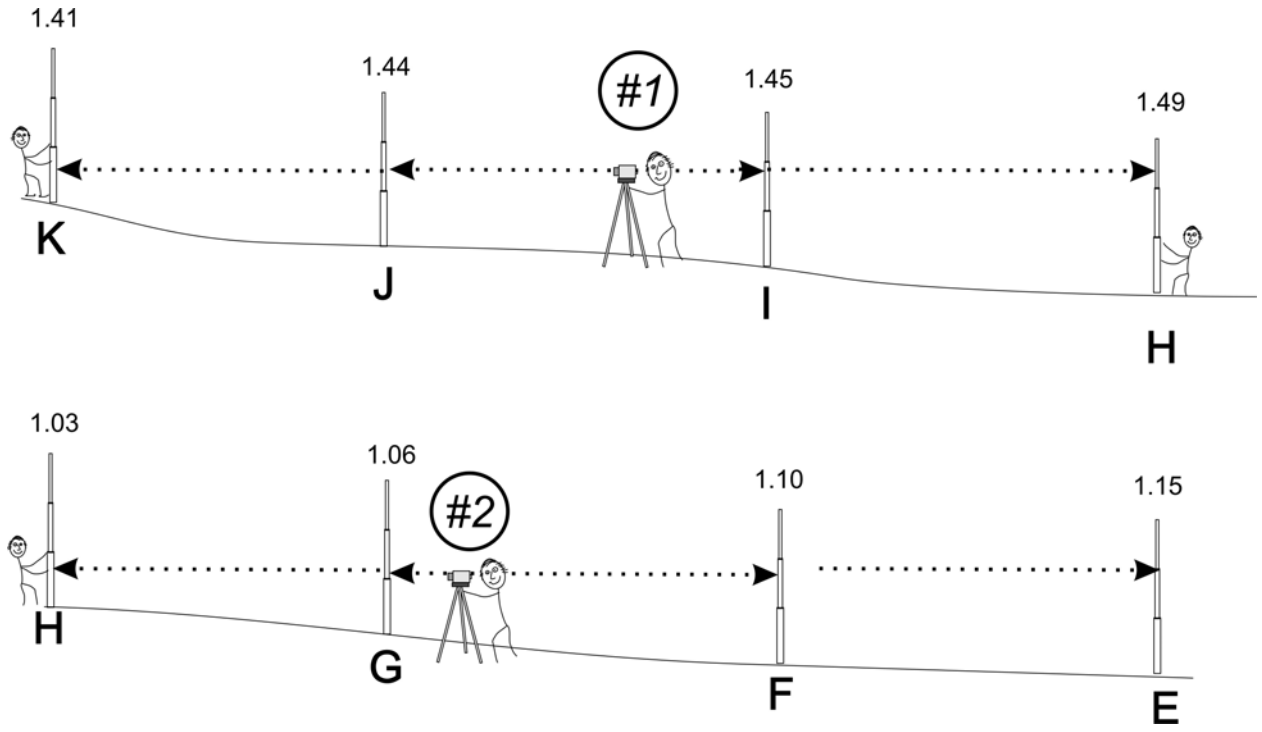


Figure 1. Cross-sectional diagram of stream transect indicating regions for assessing human influence measures:

- The measurement zone extends 5 meters upstream and 5 meters downstream of each transect
- Record one category for each bank and for the wetted channel (3 values possible)
- In reaches with wide banks, region "C" may be entirely overlapped by region "B"; in these cases, circle "B"
- Region "P" extends from 10 meters to the distance that can be seen from the channel, but not greater than 50 m

SLOPE and BEARING FORM	***EXAMPLE***	**AUTOLEVEL**	**X**
		CLINOMETER	
		HANDLEVEL	

Starting Transect	MAIN SEGMENT (record percent of inter-transect distance in each segment if supplemental segments are used)						SUPPLEMENTAL SEGMENT (record percent of inter-transect distance in each segment if supplemental segments are used)					
	Stadia rod measurements		Slope (%) or Elevation Difference cm ■ % ☐	Segment Length (m)	Bearing (0°-359°)	Percent of Total Length (%)	Stadia rod measurements		Slope or Elevation Difference cm ☐ % ☐	Segment Length (m)	Bearing (0°-359°)	Percent of Total Length (%)
K	1.41											
J	1.44		3	15	140	100						
I	1.45		1	15	145	100						
H	1.49	1.03	4	15	150	100						
G		1.06	3	15	143	100						
F		1.10	4	15	187	100						
E		1.15	5	15	195	100						



1. Level the autolevel at Position #1
2. Place base of stadia rod at water level every time
3. Sight to stadia rod at Transect K, then Transect J
4. Rotate scope and sight to Transects I and H.
5. Move level to Position #2 and re-level
6. Re-sight to stadia rod at Transect H, then Transect G
7. Rotate scope and sight to Transects F and E

Note: Sites will vary in the number of separate level positions needed to survey the reach.

Attachment 3

Example SWAMP Water Quality Field Conditions and Bioassessment Data Log Sheets, and Chain-of-Custody

Collections of Water and Bed Sediment Samples with Associated Field Measurements and Physical Habitat in California.Version 1.1 updated March-2014

The SOPs below are for reference and information purposes only, the documents are recommended, not required by the Surface Water Ambient Monitoring Program (SWAMP). Please see the SWAMP Quality Assurance Program Plan at: http://www.waterboards.ca.gov/water_issues/programs/swamp/tools.shtml#qa for more information regarding SWAMP QA/QC requirements.

Table of Contents

Acknowledgements:

This procedure has been modified from the Texas Natural Resources Conservation Commission's Procedure Manual for Surface Water Quality Monitoring, with major input from the United State's Geological Survey's (USGS's) National Water Quality Assessment (NAWQA) Protocol for Collection of Stream Water Samples, for which due credit is here with given.

The current version of these protocols was written by Sean Mundell (Moss Landing Marine Labs MPSL Field Sampling Team) with most of the credit to Max Puckett(CDFW) for originally writing this document for part of the original SWAMP QAMP, 2001. Significant contributions also came from Eric von der Geest and the (SWAMP) Quality Assurance (QA) Team, The SWAMP Data Management Team(DMT), Billy Jakl(MPSL), Mary Hamilton (RWQCB 3), and Bettina Sohst(former MPSL employee),

Field Measurements

Field Data Sheets

Field data sheets are used to record field observations, probe measurements, and water and sediment chemistry sampling. Field data sheets are provided through the Marine Pollution Studies Laboratory website at http://swamp.mpsl.mlml.calstate.edu/resources-and-downloads/database-management-systems/swamp-25-database/templates-25/field-data-sheets#WQFieldData

Click on the correct field data sheet for the most recent version. There are guidelines provided below to standardize what is recorded on all data sheets and that should be helpful in completing each form. The entries discussed below and on the field data sheets are recorded at each sampling site.

Notes to Standardize SWAMP Field Data Sheets (For in the field use)

KEY REMINDERS to IDENTIFY SAMPLES:

1. **SAMPLE TIME** is the SAME for all samples (Water, Sediment, & Probe) taken at the sampling event. Use time of FIRST sample; important for COC (is used for identification of sample).
2. **LEFT BANK/RIGHT BANK**
   Left bank is defined as the bank to the left of the observer when facing downstream, and the *right bank* is to the right of the observer when facing downstream
3. **GROUP;** many different ways to do a group, one suggestion is to create groups which assign trips to assess frequency of field QA

COLLECTION DETAILS:

1. **PERSONELL**: S. Mundell, G Ichikawa (first person listed is crew leader)
2. **LOCATION:** Bank, Thalweg, Mid-Channel, Open Water**.** Use "open water" in bay/estuary/harbor only if no distinguishable channel exists
3. **GRAB vs. INTEGRATED**: GRAB samples are when bottles are filled from a single depth; INTEGRATED sample are taken from MULTIPLE depths/grabs and combined.
   A. GRAB: use 0.1 for subsurface samples; if too shallow to submerge bottle; depth = 0
   B. INTEGRATED: -88 in depth sampled, record depths combined in sample comments
4. **TARGET LAT/LONG**: Refers to the existing station location that the sampling crew is trying to achieve; can be filled out prior to sampling
5. **ACTUAL LAT/ LONG**: is the location of the current sample event.
6. **HYDROMODIFICATION:** Describe existing hydro modifications such as a grade control, drainage pipes, bridge, culvert
7. **HYDROMOD LOC**: if there is an IMMEDIATE (with in range potentially effecting sample) hydro modification; Is the hydro modification upstream/downstream/within area of sample; if there is no hydro modification, NA is appropriate
8. **STREAM WIDTH and DEPTH**: describe in meters at point of sample.

FIELD OBSERVATIONS: (each one of these observations has a comment field in the database so use comment space on data sheet to add information about an observation if necessary)

1. **PICTURES:** use space to record picture numbers given by camera; be sure to rename accordingly back in the office. (StationCode_yyyy_mm_dd_unique code)
2. **WADEABILITY**: in general, is water body being sampled wadeable to the average person AT the POINT of SAMPLE
3. **DOMINANT SUBSTRATE**: if possible; describe DOMINANT substrate type; use UNK if you cannot see the dominant substrate type
4. **BEAUFORT SCALE**: use scale 0-12; refer to scales listed on page 28
5. **WIND DIRECTION**: records the direction from which the wind is blowing
6. **OTHER PRESENCE**: VASCULAR refers to terrestrial plants or submerged aquatic vegetation

(SAV) and NONVASCULAR refers to plankton, periphyton etc. These definitions apply to vegetation IN the water at the immediate sampling area.

7. **OBSERVED FLOW**: Visual estimates of flow range in cubic feet/second. Flow severity should be noted for each SWAMP visit to non-tidally influenced flowing streams and submitted on the SWAMP Field Data Sheet. It should be recorded even if flow is visible but not measurable on that sampling visit. This is an observational measurement that is highly dependent on the knowledge of monitoring personnel.
8. **WATER COLOR**: This is the color of the water from standing creek side
9. **WATER CLARITY**: this describes the clarity of the water while standing creek side; clear represents water that is clear to the bottom, cloudy may not be clear to bottom but greater than 4 inches can be seen through the water column.
10. **PRECIPITATION LAST24hrs:** refers to field crew's best categorization of rainfall in the last 24 hrs; may or may not effect Overland Runoff Last 24 hrs
11. **OVERLAND RUNOFF LAST 24 hrs**: Significant precipitation is defined as any amount that visibly influences water quality. Light Precipitation = fog, drizzle, and/or light rain with no overland runoff; Mod to Heavy Precipitation = rain such that site probably or definitely received at least some overland runoff.
12. **SEDIMENT COMP**: generally described sediments used for chemistry sample Note: these reminders do not give all details needed to maintain equivalent SWAMP sampling protocols, they are strictly for "infield" use to help insure comparability of field observations.
13. **WATER APPEARANCE:** Note general appearance (e.g., color, unusual amount of suspended matter, debris or foam)
14. **SEDIMENT APPEARANCE** Color, Odor and sediment composition should be noted.
15. **WEATHER:** Note recent meteorological events that may have impacted water quality; (e.g., heavy rains, cold front, very dry, very wet)
16. **BIOLOGICALACTIVITY:** Note excessive macrophyte, phytoplankton or periphyton growth. The observation of water color and excessive algal growth is very important in explaining high chlorophyll a values. Other observations such as presence of fish, birds and spawning fish are noted.
17. **WATERSHED or INSTREAM ACTIVITIES:** Note in stream or drainage basin activities or events that is impacting water quality (e.g., bridge construction, shoreline mowing, livestock watering upstream).
18. **RECORD of PERTINENT OBSERVATIONS RELATED to WATER QUALITY and STREAM USES:** If the water quality conditions are exceptionally poor, note that standards are not met in the observations, (e.g., dissolved oxygen is below minimum criteria). Note uses (e.g., swimming, wading, boating, fishing, irrigation pumps, navigation). Eventually, for setting water quality standards, the level of use will be based on comments related to the level of fishing and swimming activities observed at a station.
19. **SPECIFIC SAMPLE INFORMATION:** Note specific comments about the sample itself that may be useful in interpreting the results of the analysis (e.g., number of sediment grabs, or type and number of fish in a tissue sample). If the sample was collected for a complaint or fish kill, make a note of this in the observation section.
20. **MISSING PARAMETERS:** If a scheduled parameter or group of parameters is not collected, make some note of this in the comments.
21. **RECORD of DATA SUBMISSION:** Initials and date are recorded on the field data sheet showing a record that the data has been transcribed onto data forms and submitted to the SWAMP data management staff.

Record of Samples Collected for Purposes of Chemical Analysis

The general types of chemical samples to be collected are listed for each site, since this may vary from site-to-site (e.g., metals-in-water, pesticides-in-sediments, conventional water quality). Analyses authorization forms are recommended since different authorized laboratories perform different chemical analyses. The method of preservation for each chemical sample is recorded, as appropriate on the Chain of Custody Form (COC).

Field Data Measurements

While collecting water samples (see Field Collection Procedures for Water Samples page 29), record appropriate field measurements. When field measurements are made with a multi-parameter instrument, it is preferable to place the sonde in the body of water to be sampled and allow the dissolved oxygen (D.O.) to equilibrate. D.O. usually takes the longest to equilibrate out of the probe measurements (pH, Temperature, Conductivity and Turbidity) Field measurements are made at the centroid of flow, if the stream visually appears to be completely mixed from shore to shore. *Centroid* is defined as the midpoint of that portion of the stream width which contains 50% of the total flow. Probe measurements and water sampling are best to collect in the stream location that best represents the entire stream. For routine field measurements, the date, time and depth are reported as a grab. Quality control requirements for field measurements are listed in Quality Control and Sample Handling Tables for Field Measurements in Fresh and Marine Water.

Recommended Depths for Conducting Field Data Measurements

Water Depth Less than 5 ft (<1.5 m)

If the water depth is less than 5 ft (1.5 m), grab samples for water are taken at approximately 0.1 m (4 in.), and multi-probe measurements are taken at approximately 0.2 m (8 in.). This is because all sensors have to be submerged, so 0.1 m would not be deep enough. But taking a grab sample at 0.2 m is not always feasible, as it is difficult to submerge bottles to that depth, and in many cases the bottle will hit the stream bottom.

Water Depth Greater than 5 ft (>1.5 m)

If the water depth at the sampling point exceeds 5 ft (1.5 m) in depth, a vertical profile of dissolved oxygen, temperature, pH and specific conductance are made using the multi-parameter probe equipment. The depth of the sonde at the time of measurement is most accurately determined from the depth sensor on the multi-parameter sonde rather than depth labels on the cable.

Vertical Depth Profiles and Depth-Integrated Sample Collection

If depth integration sampling is being conducted, or if vertical profile measurements are requested, multi-probe measurements are made starting at a depth of 0.2 m, and are then conducted at 1.0, 2.0, 3.0, 4.0, and 5.0 m depths after that until 5.0 m depth is reached. Beginning at 5.0 m, measurements are made every 5.0 m through depth profile.

Field data for multi-parameter vertical depth profiles are recorded in final form on the SWAMP Field Data Sheets and submitted to the SWAMP data management staff. Go to http://www.waterboards.ca.gov/water_issues/programs/swamp/tools.shtml#qa for detailed information on data reporting.

Water Temperature (^{O}C)

Water temperature data are recorded for each site visit in final form on a Field Data Sheet and submitted to the SWAMP data management staff.

Temperature Sampling Procedures
Temperature is measured in-stream at the depth(s) specified above. Measuring temperature directly from the stream by immersing a multi-probe instrument or thermometer is preferred.

Hand Held Centigrade Thermometer
If an electronic meter is not available, the temperature is measured with a hand-held, centigrade thermometer (Rawson, 1982).

- In wadeable streams, stand so that a shadow is cast upon the site for temperature measurement.
- Hold the thermometer by its top and immerse it in the water. Position the thermometer so that the scale can be read.
- Allow the thermometer to stabilize for at least one minute, then without removing the thermometer from the water, read the temperature to the nearest 0.1^{o} C and record.
- Do not read temperature with the thermometer out of the water. Temperature readings made with modern digital instruments are accurate to within $\pm$ 0.1^{o} C.

Temperature Measurement from a Bucket
When temperature cannot be measured in-stream, it can be measured in a bucket-Nalgene or plastic container. Care must be taken to insure a measurement representative of in-stream conditions.

The following conditions must be met when measuring temperature from a bucket:

- The bucket must be large enough to allow full immersion of the probe or thermometer.
- The bucket must be brought to the same temperature as the water before it is filled.
- The probe must be placed in the bucket immediately, before the temperature changes.
- The bucket must be shaded from direct sunlight and strong breezes prior to and during temperature measurement.
- The probe is allowed to equilibrate for at least one minute before temperature is recorded.
- After these measurements are made, this water is discarded and another sample is drawn for water samples which are sent to the laboratory.

pH (standard units)

pH data is recorded for each SWAMP visit in final form on the Field Data Sheets and submitted to the SWAMP data management staff. Go to http://www.waterboards.ca.gov/water_issues/programs/swamp/tools.shtml#qa for detailed information on data reporting.

pH Sampling Equipment

The pH meter should be calibrated according to the recommended procedures for calibration and maintenance of SWAMP field equipment. Calibration directions are listed in the manufactures field equipment operations manual. The pH function is pre and post calibrated every 24 h of use for multi-parameter instruments.

pH Sampling Procedures

In-stream Method

Preferably, pH is measured directly in-stream at the depth(s) specified earlier in this document. Allow the pH probe to equilibrate for at least one minute before pH is recorded to the nearest 0.1 pH unit.

pH Measurement from a Bucket

When pH cannot be measured in-stream, it can be measured in a bucket-Nalgene or plastic container. The following precautions are outlined above; “Temperature Measurement from a Bucket”.

Potential Problems

- If the pH meter value does not stabilize in several minutes, out gassing of carbon dioxide or hydrogen sulfide, or the settling of charged clay particles may be occurring (Rawson, 1982).
- If out gassing is suspected as the cause of meter drift, collect a fresh sample, immerse the pH probe and read pH at one minute.
- If suspended clay particles are the suspected cause of meter drift, allow the sample to settle for 10 min, then read the pH in the upper layer of sample without agitating the sample.
- With care, pH measurements can be accurately measured to the nearest 0.1 pH unit.

Dissolved Oxygen (mg/L)

Dissolved oxygen (D.O.) data is recorded for each SWAMP visit in final form on a Field Data Sheet and submitted to the SWAMP data management staff.
See http://www.waterboards.ca.gov/water_issues/programs/swamp/tools.shtml#qa for detailed information on data reporting.

Dissolved Oxygen Sampling Equipment

The dissolved oxygen meter should be calibrated according to the recommended procedures for calibration and maintenance of SWAMP field equipment. Calibration directions are listed in the manufactures field equipment operations manual.

Multi-probe Instrument

Pre and post calibrate the D.O. sensor every 24 h and for elevations greater than 500 ft on the multi-probe instrument. Preferably, D.O. is measured directly in-stream at the depth(s) specified in the Field Measurements section above. The D.O. probe must equilibrate for at least 90 s before D.O. is recorded to the nearest 0.1 % saturation or mg/L. Care must be taken at profile stations to insure that the reading is stable for each depth. Since dissolved oxygen takes the longest to stabilize, record this parameter after temperature, conductivity and pH. If the D.O. probe has an operable, automatic stirrer attached, the D.O. probe does not have to be manually stirred. However, if the probe is not equipped with an automatic stirrer, manual stirring must be provided by raising and lowering the probe at a rate of 1 ft/s (0.3m/s) without agitating the water surface. If the stream velocity at the sampling point exceeds 1 ft/s, the probe membrane can be pointed upstream into the flow and manual stirring can be avoided (Rawson, 1982).

D.O. Measurement from a Bucket

When D.O. cannot be measured in-stream, it can be measured in a bucket-Nalgene or plastic container, following precautions outlined in the Temperature Measurement from a Bucket listed above. During equilibration and reading, water should be moved past the membrane surface at a velocity of 1 ft/s (0.3 m/sec), either by automatic stirrer or manual stirring. If stirred manually in a bucket, the water surface is not agitated (Rawson, 1982).

24-Hour Average D.O. Continuous Monitoring (if requested in special study)

Unattended 24-Hour D.O. Data Collection

Why Collect 24-Hour Data

Dissolved oxygen sampling for standards compliance is targeted to water bodies where low instantaneous D.O. levels indicate partial or nonsupport of designated aquatic life uses. Intensive monitoring is conducted with automated equipment that is preset to record and store field measurements hourly over one 24-h period. Four or more dissolved oxygen measurements may also be made manually at 4-6-h intervals over one 24-h period, as long as one is made near sunrise (0500-0900 h) to approximate the daily minimum. However, data collected with automated equipment is preferred.

When to Take Measurements

All 24-h D.O. monitoring events must be spaced over an index period representing warm-weather seasons of the year (approx March 15-October 15), with between one-half to two-thirds of the measurements occurring during the critical period (July 1-September 30). The ***critical period*** of the year is when minimum stream flows, maximum temperatures, and minimum dissolved oxygen concentrations typically occur in area streams. **A flow measurement must be taken at the time of deployment.** In a perennial stream, a 24-h data for standards compliance can not be used if the flow is less than the 7Q2. In perennial streams, the D.O. criterion to do not apply for flows under the 7Q2. A period of about one month must separate each 24-h sampling event. Additional samples may be collected outside the index period to further characterize a water body, but that information is generally not used for assessing standards compliance.

Frequency of Measurements

The measurement interval should be no more than once per 15 min and no less than once per hour.

Where to Take Measurements
For purposes of determining standards compliance with the 24-h average criteria, samples collected near the surface will be considered representative of the mixed surface layer. In deep streams, reservoirs, and tidally influenced water bodies, automated equipment is positioned between 1 foot (from the surface) to one-half the depth of the mixed surface layer. At least 10 24-h monitoring events (using the 24-h criteria and/or absolute minimum criteria) at each site within a 5-year period are recommended to provide adequate data for assessment.

When to Collect Other Routine Samples, if doing 24-hour D.O. measurements
Other routine field measurements and water samples should be collect at either the time of deployment, at the reference check, or when the multi-probe recording 24-h data is retrieved. When ever possible, flow must be measured at the 24-h site.

Priority for Scheduling 24-Hour Sampling Events

- < 303d listed waterbodies
- < Waterbodies with Concerns for DO problems (too few samples available for full use assessment).
- < Occurrence of low D.O. concentrations observed during the day
- < Waterbodies with trends indicating declining D.O. concentrations
- < Waterbodies which would contribute to an Eco-region data set

Data Reporting for 24-hour D.O. measurements
Dissolved oxygen values recorded over the 24-h period are summed and divided by the number of measurements to determine the average concentration, which is compared to the 24-h criterion. The lowest D.O. value from each 24-h set is compared to the minimum criterion. There will be occasions when a complete 24-h data set won't be possible. For example, if there are 18 measurements instead of 24, a time weighted diurnal average needs to be calculated. This can be easily done using GW Basic.

Support of assigned aquatic life use is based on 24-h D.O. average and minimum criteria for each monitoring event. Report the 24-h average D.O. value, number of measurements over a 24-h period, and the minimum, and maximum values. Report data as a time composite sample with a beginning and ending date and time, covering the 24-h period measured.

Specific Conductance (µS/cm)

Specific conductance should be recorded for each SWAMP visit in final form on a Field Data Sheet and submitted to the SWAMP data management staff.
See http://www.waterboards.ca.gov/water_issues/programs/swamp/tools.shtml#qa for detailed information on data reporting.
Specific Conductance Sampling Equipment

The conductivity meter should be calibrated according to the recommended procedures for calibration and maintenance of SWAMP field equipment. Calibration directions are listed in the manufactures field equipment operations manual.

Specific Conductance Sampling Procedure

Preferably, conductivity is measured directly in-stream at the depth(s) specified earlier in this document. Allow the conductivity probe to equilibrate for at least one minute before specific conductance is recorded to three significant figures (if the value exceeds 100). The primary physical problem in using a specific conductance meter is entrapment of air in the conductivity probe chambers. The presence of air in the probe is indicated by unstable specific conductance values fluctuating up to +/- 100 µS/cm. The entrainment of air can be minimized by slowly, carefully placing the probe into the water; and when the probe is completely submerged, quickly move it through the water to release any air bubbles.

If specific conductance cannot be measured in-stream, it should be measured in the container it can be measured in a bucket-Nalgene or plastic container. The following precautions are outlined above; “Temperature Measurement from a Bucket”.

Salinity (parts per thousand--ppt, or ‰)

The value for salinity is computed from chloride concentration or specific conductance. The calculation assumes a nearly constant ratio for major ions in an estuary when seawater is diluted by river water. This assumption does not hold for cases where salinity is less than about three parts per thousand. Salinity determinations at such low values are only approximate. In estuarine waters, salinity is a relevant and meaningful parameter. Often the salinity may be low, approaching that of freshwater. Nevertheless, this is useful information. Determine if a station is estuarine from historical records (i.e., experiences cases where salinity is >2.0 ppt) and always report salinity at this station, regardless of the salinity during periods of high flow.

Salinity is measured directly in-stream at the depth(s) specified earlier in this document. Salinity data should be recorded for each SWAMP visit in final form on a Field Data Sheet and submitted to the SWAMP data management staff. See http://www.waterboards.ca.gov/water_issues/programs/swamp/tools.shtml#qa for detailed information on data reporting.

Values between 2.0 ppt and 1.0 ppt should be reported as <2.0 ppt rather than the actual value and values <1.0 ppt should be reported as <1.0 ppt. The field instruments compute salinity from specific conductance and temperature, and display the value in parts per thousand. Report salinity values above 2.0 ppt to the nearest 0.1 ppt.

Secchi Disc Transparency (meters)--if requested in special study

Secchi disk transparency should be recorded for each SWAMP visit in final form on a Field Data Sheet and submitted to the SWAMP data management staff. See http://www.waterboards.ca.gov/water_issues/programs/swamp/tools.shtml#qa for detailed information on data reporting.

Secchi Disk Sampling Equipment

- < Secchi disk, 20 cm in diameter
- < Measuring tape

Secchi Disk Transparency Sampling Procedures

Preferably, Secchi disk transparency is measured directly in-stream wherever conditions allow. The Secchi disk should be clean, weighted and suspended with chain, wire, or Dacron line (the line used to suspend the Secchi disk should not be nylon or cotton; stretching may cause erroneous readings). Another option is to attach the Secchi disk to a metal rod calibrated in metric units.

Average Turbidity

The Secchi disk should be lowered vertically in a location shielded from direct sunlight. Glare from the water's surface will affect the accuracy of the measurement. Don't wear sunglasses.

Slowly lower the disk until it disappears from view. The person viewing the disk should maintain an eye level of less than two meters above the water's surface. Note the depth at which the disk disappears from view.

Slowly raise the disk until it becomes visible. Note the depth at which the disk reappears.

Compute the mathematical average of the two depths noted and record the average value to two significant figures on the field data sheet. The recorded average value is the Secchi disk transparency.

High Turbidity (Muddy Water)

In streams with very high turbidity, high velocity, and/or poor access, it may be necessary to measure Secchi disk transparency in a bucket. Fill the bucket from the centroid of flow being careful not to disturb the substrate.

Follow steps above for measuring the Secchi disk depth within 30 s after raising the filled bucket from the water's surface. Or, re-suspend the solids by stirring, then quickly make the measurement.

Record Secchi disk transparency to two significant figures.

Low Turbidity (Clear Water)

Some bodies of water will be so clear and shallow that it will not be possible to lower the Secchi disk until it disappears from view.

Measure and record the depth at the deepest point accessible. Report Secchi disk transparency as greater than the deepest depth measured.

Example (Low Turbidity): South Fork Rocky Creek is a small (<1 ft^3/s) clear stream. The stream in the vicinity of the sampling site was less than 1 m deep and the bottom was clearly visible everywhere. However, a pool was located in the stream next to a bridge. The maximum depth of the pool was 2.6 m at which depth the Secchi disk was still visible. Therefore, Secchi disk transparency for South Fork Rocky Creek was recorded as > 2.6 m.

Importance of Secchi Disk Data

Eutrophication, the natural aging process in reservoirs and lakes is accelerated by human activities which add nutrients to lakes, reservoirs, and the surrounding watersheds. Section 314 of the Clean Water Act (CWA) of 1987 requires all states to classify lakes and reservoirs according to trophic state. Although chlorophyll a is the most direct measure of algal biomass, other indices and programs utilize Secchi disk depth as the primary factor.

Turbidity Measurement with Turbidity Meter

Nephelometric Turbidity (turbidity standard unit is called Nephelometric Turbidity Units (NTU)) can be determined by measuring the amount of scatter when light is passed through a sample using a turbidity meter. The LaMotte 2020 Turbidity meter is a suitable instrument for example. There are also turbid-ometers attached to multi-probe instruments like YSI or Hydro-Lab.

Turbidity meters should be calibrated using a standard close to the expected sample value. Calibration standards should be used that are relative to the suspended sediment particles in the sampleable water column. Typical calibration standard values are 1, 10, 100, and 1000 NTU's.

For instructions on how to operate the instruments refer to the manufacturer's manual. Turbidity measurements can be executed together with water sampling. The turbidity sample has to be representative for the sampled water mass. Make sure that no gas bubbles are trapped in the vial for the reading and that the outside of the vial is wiped completely clean (i.e., meaning free of moisture, lint and fingerprints). Take several measurements to assure an accurate reading. Do not record values that vary greatly. If variations are small, record an average. If settling particles are present, record a reading before and one after settling. The meter might have to be recalibrated with a different standard, if the sample water readings are outside of the calibration standard limits.

Flow

Sampling crews should be notified on reconnaissance forms if it is known that there is an operational United States Geological Survey (USGS) gage located at or nearby a sampling site. If there is a USGS gage nearby, a gage height in feet is recorded and later converted to an instantaneous flow value and recorded on the field data sheet. The gage height is always to be reported to the USGS for conversion to flow. If a USGS gage is not available, a flow measurement should be taken, if requested. See Instantaneous Flow Measurement information starting on page 13 in this document. In addition, it is recommended that a flow severity value is recorded at each stream or river station that is not tidally influenced. See the Flow Severity section starting on page 13 of this document. Centroid velocity measurements may also be taken as a minimum acceptable rough characterization of the stream flow as requested, although this measurement is not to be recorded as a flow, since it is only a velocity measurement
Flow information for over 200 USGS sites is available on the Internet. The address is **http://water.usgs.gov/index.html**. This is useful information in determining flow conditions prior to sampling. This information may be included in general observations.

Flow Measurement Method (Reporting)

The method used to measure flow is noted by reporting which instrument or gage is used. Examples are, Flow Gage Station (USGS/IBWC), Electric Marsh-McBirney flo mate 4000, Mechanical (ex. Pigmy meter), Weir/Flume, Other (orange peel, etc.) Flow data transformers are used to enter flow data into the SWAMP database. Please contact the SWAMP data management team to obtain the flow data transformer.

Flow (ft^3/s)

If requested, flow data should be recorded for each monitoring visit to non-tidal, flowing streams. Flow data should be recorded in final form on a Field Data Sheet and submitted to the SWAMP data management staff. See http://www.waterboards.ca.gov/water_issues/programs/swamp/tools.shtml#qa for detailed information on data reporting. The following are two exceptions to the flow reporting requirement:

No Flow/ Pools — If there is no flow at a stream site and accessible, isolated pools remain in the stream bed, collect and report the required field data and laboratory samples from the pools and report instantaneous flow. Under these conditions, flow (ft^3/s) should be reported as zero. The reported flow severity value should be one. Pools may represent natural low-flow conditions in some streams and the chemistry of these pools will reveal natural background conditions.

Dry — If the stream bed holds no water, the sampling visit is finished. Report that the stream was "dry" in the observations and record a value of six (meaning "dry") for flow severity. No value is reported for flow since there is no water.

Flow Measurement

If a flow measurement is required at a site, measure and record flow after recording visual observations. The intent of measuring flow first is to delay collection of chemical and biological water samples with limited holding times. Care must be taken not to collect water samples in the area disturbed during flow measurement. There are several acceptable flow measurement methods that can be used.

U.S. Geological Survey (USGS) Gaging Station

Some SWAMP Stations are sampled at sites where the USGS maintains flow gaging equipment. On any type of sampling visit to a site that has a USGS flow gage, observe and record the gage height to the nearest hundredth of a foot in the field logbook. Upon return to the office, contact the USGS office responsible for maintaining the gage. USGS personnel can provide the flow value in cubic feet per second (ft^3/s) that corresponds to the gage height. Although SWAMP personnel may have a rating curve available to them, shifts associated with changes in the stream bed may occur over time. Always call the USGS to determine the shift. At some sites the shift changes frequently. At others, the relation between stream flow and gage height is almost unchanging. If a gage is no longer maintained by USGS, cross out the recorded gage height and be prepared to measure flow by another method on the return visit to that site.

Several factors may influence the accuracy of the USGS rating curves that are used to convert gage height to flow. If there is any doubt about the accuracy of a USGS gage height reading or flow rating curve, sampling personnel should measure the flow if possible.

Gage height may be indicated at a USGS gage by one of three methods:

Staff Gage

Staff gages are enameled steel plates (with the appearance of large measuring tapes) bolted to some stable structure. For example, staff gages may be bolted to concrete bridge abutments, pillars, or docks. The staff gage face is white with black lettering and gradations. The gradations shown are feet, tenths of a foot, and 0.02 of a foot. The point at which the water level crosses the staff gage should be recorded to the nearest hundredth of a foot.

Wire Weight Gage

Wire weight gages are locked, metal boxes with approximate dimensions of 15 in. long x 12 in. tall x 12 in. deep. Wire weight gages are usually affixed to bridge rails near mid-stream. They must be unlocked with a USGS key. The wire weight gages house a weight attached by wire cable to a graduated reel (gradations are tenths and hundredths of feet) with a counter at one end.

When the reel is released the weight can be gradually lowered until the bottom of the weight contacts the water surface. At the point of contact, the weight causes the water surface to ripple slightly. Maintaining the weight in that position, record the counter value to the nearest whole number and the point indicated by the stylus on the graduated reel to the nearest hundredth of a foot. Determine if the gage is the movable type that can be moved to multiple locations on the bridge. This type is common on braided streams. A correction value is stamped on the bridge near each point that the gage can be attached.

Record the corrected value as the gage height in feet.

Bubble Gage

Bubble gages are locked in metal sheds that are approximately 4 ft wide x 4 ft deep x 6.5 ft tall. The gage houses are most frequently located on the shore near a bridge but sometimes are attached to bridge pillars near mid-stream or established on the stream bank far from any bridge. The gage house must be unlocked with a USGS key. Bubble gages in gage houses usually indicate the gage height in two or three locations. A counter attached to the manometer system indicates gage height in feet. Some gage houses have stilling wells that can be entered. Often there is a staff gage on the inside wall.

Most bubble gages are also equipped with digital recorders. Digital recorders consist of two white, coded discs, approximately 4 in. in diameter with a punch tape overlapping a portion of each disc. The discs are marked with 100 gradations. As the front of the digital recorder is viewed, the stylus at the disc on the left indicates height in feet. The stylus at the disc on the right indicates gage height in hundredths of feet. The gage height from both discs should be added and the number recorded in the field logbook as gage height to the nearest hundredth of a foot.

Many USGS metal sheds also contain a surface level recorder. This devise can be opened to determine how stable stream flow has been prior to the sampling event. Record observations concerning the flow hydrograph.

Instantaneous Flow Measurement

Water quality monitoring visits to sites where there are no nearby USGS flow gauges will require water quality monitoring personnel to measure flow, when requested by Regional Water Quality Control Boards (Regional Boards).

Flow Measurement Equipment

Flow meter

One of the following or an equivalent:

- Marsh-McBirney Electronic meter
- Montedoro-Whitney Electronic meter
- Price Pigmy meter (with timer and beeper)
- Price meter, Type AA (with Columbus weight)

Additional Equipment

- Top-setting wading rod (preferably measured in tenths of feet)(see Figure 1).
- Tape measure (with gradations every tenth of a foot or every centimeter).

Flow Measurement Procedure (USGS, 1969)

Select a stream reach with the following characteristics:

- Straight reach with laminar flow (threads of velocity parallel to each other) and bank to bank. These conditions are typically found immediately upstream of riffle areas or places where the stream channel is constricted.

- The site should have an even streambed free of large rocks, weeds, and protruding obstructions that create turbulence. The site should not have dead water areas near the banks, and a minimum amount of turbulence or back eddies.

Flat Streambed Profile (cross section)

Stretch the measuring tape across the stream at right angles to the direction of flow. When using an electronic flow meter, the tape does not have to be exactly perpendicular to the bank (direction of flow). When using a propeller or pigmy type meter, however, corrections for deviation from perpendicular must be made.

If necessary and possible, modify the measuring cross section to provide acceptable conditions by building dikes to cut off dead water and shallow flows, remove rocks, weeds, and debris in the reach of stream one or two meters upstream from the measurement cross section. After modifying a streambed, allow the flow to stabilize before starting the flow measurement.

Record the following information on the flow measurement form (see example Flow Measurement Forms at end of this document):

- Station Location and Station ID
- Date
- Time measurement is initiated and ended
- Name of person(s) measuring flow
- Note if measurements are in feet or meters
- Total stream width and width of each measurement section
- For each cross section, record the mid-point, section depth and flow velocity

Measuring the Stream Width

Measure and record the stream width between the points where the tape is stretched (waters edge to waters edge).

Determining the Number of Flow Cross Sections

Determine the spacing and location of flow measurement sections. Some judgment is required depending on the shape of the stream bed. Measurements must be representative of the velocity within the cross-section. If the stream banks are straight and the depth is nearly constant and the bottom is free of large obstructions, fewer measurements are needed, because the flow is homogeneous over a large section. Flow measurement sections do not have to be equal width. However, they should be unless an obstacle or other obstruction prevents an accurate velocity measurement at that point. ***No flow measurement section should have greater than 10% of the total flow.***

If the *stream width is less than 5 ft*, use flow sections with a width of 0.5 ft (See example 1 on page 23 of this document). If the *stream width is greater than 5 ft*, the minimum number of flow measurements is 10. The preferred number of flow measurement cross sections is 20-30 (See Example 2 on page 24 on this document). The total stream width is 26 ft with 20 measurements, section widths will be 1.3 ft (26/20 = 1.3).

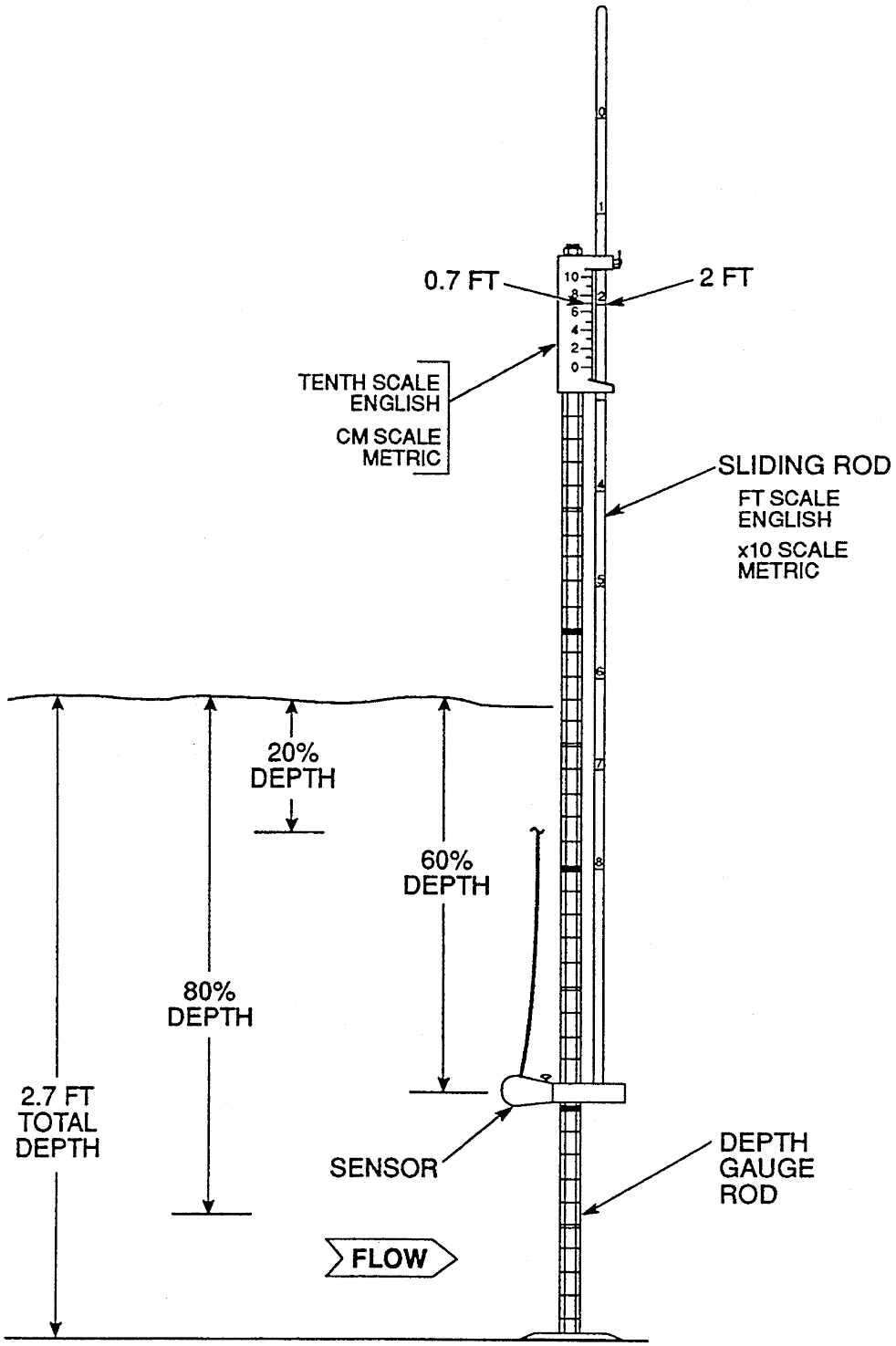
Determining the Mid-Point of the Cross Section

To find the mid-point of a cross section, divide the cross section width in half. Using Example 2 (see forms at end of document);

- The total stream width is 26 ft with 20 cross sections and each cross section width is equal to 1.3 ft.

- Divide 1.3 ft in half and the mid-point of the first section is 0.65 ft. In this example the tape at waters edge is set at zero (0) ft.
- By adding 0.65 to zero the mid-point of the first section is 0.65 ft.
- Each subsequent mid-point is found by adding the section width (1.3 ft) to the previous mid-point. For example; MIDPOINT #1 is 0.65 + 0.0 = 0.65; MIDPOINT #2 is 0.65 + 1.3= 1.95 ft; MIDPOINT #3 is 1.95 + 1.3 = 3.25 ft and .... MIDPOINT # 20 is 24.05 +1.3.
- Place the top setting wading rod at 0.65 ft for the first measurement.
- Using a top setting wading rod, measure the depth at the mid-point of the first flow measurement section and record to the nearest 0.01 ft.

Figure 1. Top-Setting Wading Rod (Marsh-McBirney)



Adjusting the Sensor Depth at a Cross Section

Adjust the position of the sensor to the correct depth at each mid-point. The purpose of the top setting wading rod is to allow the user to easily set the sensor at 20%, 60%, and 80% of the total depth. The total depth can be measured with the *depth gage rod*. Each single mark represents 0.10 foot, each double mark represents 0.50 foot, and each triple mark represents 1.00 foot (see Figure 2).

For Depths < 2.5 Ft	If the depth is less than 2.5 ft, only one measurement is required at each measurement section. To set the sensor at 60% of the depth, line up the foot scale on the *sliding rod* with the *tenth scale*, located on top of the depth gage rod. If, for example, the total depth is 2.7 ft (as shown on Figure 2), then line up the 2 on the foot scale with the 7 on the tenth scale (Marsh-McBirney 1990).
For Depths > 2.5 Ft	If the depth is greater than 2.5 ft, measurements should be taken at 20% and 80% of the total depth.

Measuring Velocity (this has typically been measured at 6/10 of the total depth, for velocity-only measurements)

- Position the meter at the correct depth and place at the mid-point of the flow measurement section. Measure and record the velocity and depth. The wading rod is kept vertical and the flow sensor kept perpendicular to the tape rather than perpendicular to the flow while measuring velocity with an electronic flow meter. When using a propeller or pigmy-type meter, however, the instrument should be perpendicular to the flow.
- Permit the meter to adjust to the current for a few seconds. Measure the velocity for a minimum of 20 s with the Marsh-McBirney and Montedoro-Whitney meters. Measure velocity for a minimum of 40 s (preferably 2 min with the Price and pigmy meters).
- When measuring the flow by wading, stand in the position that least affects the velocity of the water passing the current meter. The person wading stands a minimum of 1.5 ft downstream and off to the side of the flow sensor.
- A flow sensor, equipped with cable and weight may be used to measure flows where the water is too deep to wade. Follow the procedure involving meters attached to wading rods.
- Report flow values less than 10 ft^2/s to two significant figures. Report flow values greater than 10 ft^3/s to the nearest whole number, but no more than three significant figures.
- In cases where the flow is low and falling over an obstruction, it may be possible to measure the flow by timing how long it takes to fill a bucket of known volume.

Avoid measuring flow in areas with back eddies. The first choice would be to select a site with no back eddy development. However, this can not be avoided in certain situations. Measure the

negative flows in the areas with back eddies. These negative values will be included in the final flow calculation.

Calculating Flow

To calculate flow, multiply the width x depth (ft^2) to derive the area of the flow measurement section. The area of the section is then multiplied by the velocity (ft/s) to calculate the flow in cubic feet per second (cfs or ft^3/sec) for that flow measurement section. When flow is calculated for all of the measurement sections, they are added together for the total stream flow (see Figure 2). Flow data transformers are also provided by the SWAMP data management team. The transformer provides the calculations needed to obtain a final flow value in cubic feet per second.

Q=Total Flow (or discharge), W=Width, D=Depth, V=Velocity.

$$Q = (W_1 * D_1 * V_1) + (W_2 * D_2 * V_2) + ...(W_n * D_n * V_n)$$

What to Do with Negative Values

Do not treat cross sections with negative flow values as zero. Negative values obtained from areas with back eddies should be subtracted during the summation of the flow for a site.

Flow Estimate (ft^3/s)

Flow estimate data may be recorded for a non-tidally influenced stream when it is not possible to measure flows by one of the methods described above. Flow estimates are subjective measures based on field personnel's experience and ability to estimate distances, depths, and velocities. If flow can not be measured at a routine non-tidal station, a new site should be selected where flow can be measured.

Flow Estimate Procedure

- Observe the stream and choose a reach of the stream where it is possible to estimate the stream cross section and velocity.
- Estimate stream width (ft) at that reach and record.
- Estimate average stream depth (ft) at that reach and record. Estimate stream velocity (ft/s) at that reach and record. A good way to do this is to time the travel of a piece of floating debris. If doing this method from a bridge, measure the width of the bridge. Have one person drop a floating object (something that can be distinguished from other floating material) at the upstream side of the bridge and say start. The person on the downstream side of the bridge will stop the clock when the floating object reaches the downstream side of the bridge. Divide the bridge width by the number of seconds to calculate the velocity. The velocity can be measured at multiple locations along the bridge. These velocities are averaged. If this is done alone, watch for road traffic.
- Multiply stream width (ft) time's average stream depth (ft) to determine the cross sectional area (in ft^2) which when multiplied by the stream velocity (in ft/s) and a correction constant, gives an estimated flow (ft^3/s).

Example*:* A stream sampler conducted a sampling visit to a stream while the flow meter was being repaired. The sampler looked at the creek downstream from the bridge and saw a good place to estimate flow. The stream width was around 15 ft. It appeared the average depth on this reach was about 0.75 ft. The sampler timed a piece of floating debris as it moved a distance of 10 ft in 25 s downstream over the reach. An estimated flow with a smooth bottom was calculated using the following formula.

Width x Depth x Velocity x A (correction factor)= estimated flow
15 ft (width) x 0.75 ft (depth) x 2.5 ft/s (velocity) x A =25 ft^3/s (cfs)

A is a correction constant: 0.8 for rough bottom and 0.9 for smooth bottom

Estimated flow should be reported to one or two significant figures.

Experienced field personnel are able to estimate flow to within 20% of actual flow for total flows less than 50 ft^3/s. The best way to develop this skill is to practice estimating flow before making measurements at all monitoring visits to non-tidally influenced flowing streams and then compare estimated flows with those obtained from USGS gages or from instantaneous flow measurements

Figure 2. Stream Flow (Discharge) Measurement

Example 1.
Stream Flow (Discharge) Measurement
Small Stream < 5 Ft Wide and #2.5 Ft Deep

Stream: **OAK CREEK** Date: **5/29/91**
Station Description: **at US Hwy 90A**
Time Begin: **1545** Time End: **1630** Meter Type: **Marsh-McBirney**
Observers: **BK/MK** Stream Width*: **5 ft** Section Width: **0.5 ft**
Observations:

Section Midpoint (ft)	Section Depth (ft)	Observational Depth** Ft	Velocity		Area W x D (ft^2)	Discharge (Q) V x A (ft^3/s)
			At Point (ft/s)	Average (ft/s)		
0.25	0.55			0.05		0.01375
0.75	0.80			0.11		0.044
1.25	0.85			0.27		0.42635
1.75	0.90			0.49		0.2205
2.25	1.10			0.58		0.275
2.75	1.50			0.72		0.540
3.25	1.20			0.76		0.456
3.75	0.90			0.76		0.342
4.25	0.75			0.44		0.165
4.75	0.30			0.00		0.00

m^3/s x 35.3 = ft^3/s

Total Discharge (3Q) (ft^3/s)	2.4826

Example 2: Stream Discharge Measurement Example (Larger Stream > 5 Ft and #2.5 Ft Deep)

Stream:**RED RIVER** Date:**5/28/91**
Station Description:**Post Oak Creek 40 m Below Sherman WWTP Outfall**
Time Begin:**1542** Time End:**1601** Meter Type:**Marsh-McBirney**
Observers:**CM, EW, DO** Stream Width*:**26 ft** Section Width:**1.3 ft**
Observations:

Section Midpoint (ft)	Section Depth (ft)	Observational Depth** ft	Velocity		Area W x D (ft^2)	Discharge (Q) V x A (ft^3/s)
			At Point (ft/s)	Average (ft/s)		
0.65	0.55			2.03	0.715	1.451
1.95	0.40			2.04	0.520	1.061
3.25	0.42			2.02	0.546	1.103
4.55	0.38			1.77	0.494	0.874
5.25	0.40			1.75	0.520	0.910
7.15	0.42			1.93	0.546	1.054
8.45	0.40			1.99	0.52	1.035
9.75	0.37			1.92	0.481	0.924
11.05	0.37			1.56	0.481	0.750
12.35	0.43			1.32	0.559	0.738
13.65	0.40			1.36	0.520	0.707
14.95	0.42			1.33	0.546	0.726
16.25	0.40			1.35	0.520	0.702
17.55	0.45			1.64	0.585	0.959
18.85	0.48			1.70	0.624	1.061
20.15	0.48			2.00	0.624	1.248
21.45	0.50			1.95	0.650	1.268
22.75	0.40			2.18	0.520	1.134
24.05	0.48			1.71	0.624	1.067
25.35	0.50			0.60	0.650	0.390
				Total Discharge (3Q) (ft^3/s)		**19.162**

m^3/s x 35.3 = ft^3/s

Example 3: Stream Flow (Discharge) Measurement (Larger Stream > 5 Ft and >2.5 Ft Deep)

Stream:____ARROYO COLORADO_____Date:_**6/16/98**_____
Station Description:_____Downstream of Harlingen WWTP_____
Time Begin:_**1400**_____Time End:_**1445**_____Meter Type:___**Marsh-McBirney**_____
Observers:_____**JD, CK**_____Stream Width*:___**47.5 ft**___ Section Width:___**2.375 ft**_____
Observations: *Note that the starting point is at 4.7 ft on the measuring tape and not zero.

Section Midpoint (ft)	Section Depth (ft)	Observational Depth** ft	Velocity		Area W x D (ft^2)	Discharge (Q) V x A (ft^3/s)
			At Point (ft/sec)	Average (ft/sec)		
4.70	0.73			0.65	1.73	1.127
7.08	1.10			1.08	2.61	2.822
9.45	1.85			0.90	4.39	3.954
11.83	2.20			1.05	5.23	5.486
14.20	2.20			1.44	5.23	7.531
16.58	2.45			1.09	5.82	6.342
18.95	2.55	0.20	1.75	1.76	6.06	10.659
		0.80	1.76			
21.33	2.60	0.20	1.79	1.56	6.18	9.633
		0.80	1.32			
23.70	2.70	0.20	1.63	1.45	6.41	9.298
		0.80	1.26			
26.10	3.05	0.20	1.68	1.42	7.24	10.286
		0.80	1.15			
28.48	3.10	0.20	1.23	0.96	7.36	7.068
		0.80	0.69			
30.85	2.90	0.20	1.22	1.06	6.89	7.301
		0.80	0.89			
33.23	2.84	0.20	0.60	0.49	6.75	3.305
		0.80	0.37			
35.60	2.65	0.20	0.80	0.51	6.29	3.210
		0.80	0.21			
37.98	2.65	0.20	0.85	0.91	6.29	5.727
		0.80	0.96			
40.35	2.20			0.28	5.23	1.464
42.73	2.30			0.16	5.46	0.874
45.10	2.05			0.51	4.87	2.483
47.48	1.10			0.49	2.61	1.280
49.86	0.65			0.62	1.54	0.957

m^3/s x 35.3 = ft^3/s **Total Discharge (3Q) (ft^3/s)**

Stream Flow (Discharge) Measurement Form

Stream:______________________________ Date:__________

Station Description:______________________________

Time Begin:________ Time End:__________ Meter Type:__________________

Observers:______________ Stream Width*:__________ Section Width:________

Observations:

Section Midpoint (ft) (m)	Section Depth (ft) (m) (cm)	Observational Depth** ft-m-cm	Velocity		Area W x D (ft^2) (m^2)	Flow (Q) V x A (m^3/s) (ft^3/s)
			At Point (ft/s) (m/s)	Average (ft/s)(m/s)		

$m^3/s \times 35.3 = ft^3/s$

Total Flow (Discharge) (3Q) (ft^3/s)	

Make a minimum of 10 measurements when the total width is > 5.0 ft, 20 measurements preferred.

If the depth is less than 2.5 ft, only one measurement is required at each measurement section. To set the sensor at 60% of the depth, line up the foot scale on the *sliding rod* with the *tenth scale*, located on top of the depth gage rod. If, for example, the total depth is 2.7 ft (as shown on Figure 2), then line up the 2 on the foot scale with the 7 on the tenth scale (Marsh-McBirney 1990). If the depth is greater than 2.5 ft, measurements should be taken at 20% and 80% of the total depth.

Summary of Significant Figures for Reporting Field Parameters

Parameter	Field Data Reporting Requirements
Water Temperature (ºC)	Report temperature to the nearest tenth of a degree. Round insignificant figures 0 through 4 down and 5 thru 9 up.
pH (s.u.)	Report pH to the nearest tenth of a pH standard unit.
D.O. mg/L **D.O.** (% saturation)	Report dissolved oxygen to the nearest tenth of a mg/L. Report % saturation to the nearest tenth of a percent
Specific Conductance (micro siemens/cm)	Report specific conductance to only three significant figures if the value exceeds 100. Do not report ORP which is displayed by some multi-probes.
Salinity (ppt)	Report salinity values above 2.0 ppt to the nearest tenth of a part per thousand. In estuarine waters report the actual values displayed by the multi-probe above 2.0 ppt and values less than 2.0 as <2.0 or <1.0 only. Determine if a station is estuarine (i.e., experiences cases where salinity is >2.0 ppt) and always report salinity at this station, regardless of the salinity during periods of high flow.
Secchi Disk (meters)	Report Secchi depth transparency in meters to two significant figures.
Flow (ft^3/s)	Report instantaneous flow values less than 10 ft^3/s to two significant figures. Report flow values greater than 10 ft^3/s to the nearest whole number, but no more than three significant figures. When there is no flow (pools), report as 0.0. When there is no water, don't report a value, but report as "dry" in the observations.

BEAUFORT SCALE: Specifications and equivalent speeds for

use at sea

FORCE	EQUIVALEN SPEED 10 m above ground		DESCRIPTION	SPECIFICATIONS FOR USE AT SEA
	Miles/hour	knots		
0	0-1	0-1	Calm	Sea like a mirror
1	1-3	1-3	Light air	Ripples with the appearance of scales are formed, but without foam crests.
2	4-7	4-6	Light Breeze	Small wavelets, still short, but more pronounced. Crests have a glassy appearance and do not break.
3	8-12	7-10	Gentle Breeze	Large wavelets. Crests begin to break. Foam of glassy appearance. Perhaps scattered white horses.
4	13-18	11-16	Moderate Breeze	Small waves, becoming larger; fairly frequent white horses.
5	19-24	17-21	Fresh Breeze	Moderate waves, taking a more pronounced long form; many white horses are formed. Chance of some spray.
6	25-31	22-27	Strong Breeze	Large waves begin to form; the white foam crests are more extensive everywhere. Probably some spray.
7	32-38	28-33	Near Gale	Sea heaps up and white foam from breaking waves begins to be blown in streaks along the direction of the wind.
8	39-46	34-40	Gale	Moderately high waves of greater length; edges of crests begin to break into spindrift. The foam is blown in well-marked streaks along the direction of the wind.
9	47-54	41-47	Severe Gale	High waves. Dense streaks of foam along the direction of the wind. Crests of waves begin to topple, tumble, and roll over. Spray may affect visibility.
10	55-63	48-55	Storm	Very high waves with long over- hanging crests. he resulting foam, in great patches, is blown in dense white streaks along the direction of the wind. On he whole the surface of the sea takes on a white appearance. The 'tumbling' of the sea becomes heavy and shock-like. Visibility affected.

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Field Collection Procedures for Water Samples

Scope and Application

This protocol describes the techniques used to collect water samples in the field in a way that neither contaminates, loses, or changes the chemical form of the analytes of interest. The samples are collected in the field into previously cleaned and tested (if necessary) sample bottles of a material appropriate to the analysis to be conducted. Pre-cleaned sampling equipment is used for each site, whenever possible and/or when necessary. Appropriate sampling technique and measuring equipment may vary depending on the location, sample type, sampling objective, and weather. Trade names used in connection with equipment or supplies do not constitute an endorsement of the product. Safety equipment is always used while water sampling including gloves, waders and eye protection. Safety equipment helps to protect the sampler from potential contaminants and to prevent sample contamination.

Summary of Method

Appropriate sample containers and field measurement gear as well as sampling gear are transported to the site where samples are collected according to each sample's protocol. Water velocity, turbidity, temperature, pH, conductivity, dissolved oxygen as well as other field data are measured and recorded using the appropriate equipment. These field data measurement protocols are provided in this Field Measurement SOP. Samples are immediately put on ice and appropriately shipped to the authorized laboratories. This procedure has been modified from the Texas Natural Resources Conservation Commission's Procedure Manual for Surface Water Quality Monitoring, with major input from the United State's Geological Survey's (USGS's) National Water Quality Assessment (NAWQA) Protocol for Collection of Stream Water Samples.

WATER SAMPLE COLLECTION

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

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

Sample Collection Depth

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

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

Surface Grab Sample Samples are collected at the surface when water depth is <0.1 m. Since there is a difference in water chemistry on the surface, compared to subsurface, surface water should be noted on the field data sheet as 0 m.

Where to Collect Samples

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

In reservoirs, lakes, rivers, and coastal bays, samples are collected from boats at designated locations provided by Regional Water Quality Control Boards (Regional Boards). Samples from boats should be collected where the vessel does not interfere with the water being collected.

Sampling Order if Multiple Media are Requested to be Collected

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

Sample Container Labels

Label each container with the station ID, sample code, matrix type, analysis type, project ID, and date and time of collection (in most cases, containers will be pre-labeled). After sampling, secure the label by taping around the bottle with clear packaging tape.

Procedural Notes

For inorganic and organic water samples, bottles do not have to be rinsed if they are I-Chem 200 series or higher or ESS PC grade or higher. This means that the sample bottles are analyzed for contamination, and a certification of analysis is included with the bottles. Other sample containers are usually rinsed at least three times if the bottles do not meet these requirements. See filling instruction for each type of analyses if there is uncertainty. If applicable to the sample and analysis type, the sample container should be opened and re-capped under water.

Sample Short-term Storage and Preservation

Properly store and preserve samples as soon as possible. Usually this is done immediately after returning from the collection by placing the containers on bagged, crushed or cube ice in an ice chest. Sufficient ice will be needed to lower the sample temperature to at least 6 ° C within 45 min after time of collection. Sample temperature will be maintained at 6 ° C until delivered to the laboratory. Care is taken at all times during sample collection, handling and transport to prevent exposure of the sample to direct sunlight. Samples are preserved in the laboratory, if necessary, according to protocol for specific analysis (acidification in most cases).

Field Safety Issues

Proper gloves must be worn to prevent contamination of the sample and to protect the sampler from environmental hazards (disposable polyethylene, nitrile, or non-talc latex gloves are recommended, **however, metals and mercury sample containers can only be sampled and handled using clean polyethylene gloves as the outer layer**). Wear at least one layer of gloves, but two layers help protect against leaks. One layer of shoulder high gloves worn as a first (inside) layer is recommended to have the best protection for the sampler. Safety precautions are needed when collecting samples, especially samples that are suspected to contain hazardous substances, bacteria, or viruses.

Sample Handling and Shipping

Due to increased shipping restrictions, samples being sent via a freight carrier require additional packing. Although care is taken in sealing the ice chest, leaks can and do occur. Samples and ice should be bagged placed inside a large trash bag inside the ice chest for shipping. Ice should be double bagged to prevent melted ice water from leaking into the sample. The large trash bag can be sealed by simply twisting the bag closed (while removing excess air) and taping the tail down. Prior to shipping the drain plug of the ice chests have to be taped shut. Leaking ice chests can cause samples to be returned or arrive at the lab beyond the holding time.

Although glass containers are acceptable for sample collection, bubble wrap must be used when shipping glass.

Chain of Custody (COC) Forms

Every shipment must contain a complete Chain of Custody (COC) Form that lists all samples collected and the analyses to be performed on these samples.

Make sure a COC is included for every laboratory, every time you send a shipment of samples. Electronic COC's can also be emailed to the various laboratories but must be sent before the samples arrive at their destinations.
Include region and trip information as well as any special instructions to the laboratory on the COC.

The original COC sheet (not the copies) is included with the shipment (insert into ziplock bag) One copy goes to the sampling coordinator, and the sampling crew keeps one copy.

Samples collected should have the salinity (in parts per thousand) or specific conductivity (**µS/cm),** depth of collection, and date/time collected for each station on every COC.

Write a comment on this form, if you want to warn the laboratory personnel about possibly hazardous samples that contain high bacteria, chlorine or organic levels.

Field QC Samples for Water Analyses

Field duplicates are currently submitted at an annual rate of 5%. Field travel blanks are required for volatile organic compounds at a rate of one per cooler shipped. Field blanks are required for trace metals (including mercury and methyl mercury), DOC, and volatile organic compounds in water at a project rate of 5%. See the SWAMP Quality Control and

Sample Handling Guidelines for information regarding frequency and types of field QC samples.

SWAMP Field Data Sheets

Each visited field site requires a field observation completed SWAMP Field Data Sheet, even if no samples are collected (i.e. at a site which is found to be dry). If water and/or sediment samples are collected, all elements of the SWAMP Field Data Sheet must be completely filled out. Data sheets are provided from the SWAMP MPSL MLML website: http://swamp.mpsl.mlml.calstate.edu/resources-and-downloads/database-management-systems/swamp-25-database/templates-25/field-data-sheets#WQFieldData

General Pre-Sampling Procedures

Instruments. All instruments must be in proper working condition. Make sure all calibrations are current. Multi-probe sondes should be pre-calibrated every morning prior to sampling and post-calibrated within 24 h of the original calibration. Conductivity should also be calibrated between stations if there is a significant change in salinity. Dissolved oxygen sensors should be re-calibrated if there is a 500 ft change in elevation.

Calibration Standards. Pack all needed calibration standards.

Sample Storage Preparations. A sufficient amount of cube ice, blue ice and dry ice as well as enough coolers of the appropriate type/size must be brought into the field, or sources for purchasing these supplies identified in advance.

Sample Container Preparation. After arriving at the sample station, pack all needed sample containers for carriage to the actual collection site, and label them with a pre-printed label containing Station ID, Sample Code, Matrix info, Analysis Type info, Project ID and blank fields for date and time (if not already pre-labeled).

Safety Gear. Pack all necessary safety gear like waders, protective gloves and safety vests.

Walk to the site. For longer hikes to reach a sample collection site, large hiking backpacks are recommended for transport of gear, instruments and containers. Tote bins can be used, if the sampling site can be accessed reasonably close to the vehicle.

GPS. At the sampling site, compare/record reconnaissance GPS reading with current site reading and note differences. GPS coordinates should be in Decimal Degrees (e.g. 38.12345 -117.12345).

COLLECTION OF WATER SAMPLES FOR ANALYSIS OF CONVENTIONAL CONSTITUENTS

In most streams, sub-surface (0.1 m below surface) water is representative of the water mass. A water sample for analysis of conventional constituents is collected by the grab method in most cases, immersing the container beneath the water surface with the cap on to a depth of 0.1 m. Remove cap and fill container replacing the cap before removing the container from the water. Sites accessed by bridge can be sampled with a sample container-suspending device. Extreme care must be taken to avoid contaminating the sample with debris from the rope and bridge. Care must also be taken to rinse the device between stations. If the centroid of the stream cannot be sampled by wading, sampling devices can be attached to an extendable sampling pole. It should be noted on the field data sheet if using a bucket sampler that surface water is entering the sample bottle.

In some cases, depth-integrated sampling is required, as requested by Regional Boards. This is useful when lakes or rivers are stratified and a sample is wanted that represents the entire water column. Depth-integrated sample collection is explained later in this document.

Conventional Water Constituents, Routinely Requested in SWAMP

Chloride, sulfate, nitrite, nitrate (or nitrate+nitrate), ortho-phosphate, fluoride, total phosphorus, ammonia, TKN, alkalinity, chlorophyll a.

Conventional Water Constituents, Occasionally Requested in SWAMP

Total Suspended Solids (TSS) or Suspended Sediment Concentration (SSC), Total Dissolved Solids (TDS--especially if total metals requested), Total Organic Carbon (TOC), Dissolved Organic Carbon (DOC), hardness (if trace metals analysis is requested).

Conventional Water Constituents Sample Volume

Due to the potential for vastly different arrays of requested analyses for conventional constituents, please refer to table at the end of this document, as well as the Quality Control and

Sample Handling Guidelines for Conventional Parameters, for information on the proper volume to collect for the various types of analyses.

Conventional Water Constituents Sample Container Type

Due to the potential for vastly different arrays of requested analyses for conventional constituents, please refer to table at the end of this document, as well as the Quality Control and Sample Handling Guidelines for Conventional Parameters, for information on the proper type of sample containers.

Chlorophyll a Syringe Sample Method

Chlorophyll a syringe method: Chlorophyll a is sampled by forcing water with a 60-mL syringe through a filter holder containing a 25-mm glass microfiber filter. The 60-mL syringe and an in-line filter holder are rinsed three times with the ambient water before filtration. The syringe is then filled with 60 mL of ambient water. The filter holder is then removed and a 25-mm glass microfiber filter is placed inside. The filter holder is then screwed onto the syringe and the ambient water is then flushed through the filter. The filter holder is removed every time more water needs to be drawn into the syringe. The process is then repeated until the desired amount of Chlorophyll a is present (usually 60 to 360 mL depending on the water clarity). When filtering is complete the filter holder is opened and the filter is removed with tweezers without touching the Chlorophyll a. The filter is then folded in half, then again, in half with the Chlorophyll a inside the folds. The folded filter is then wrapped in aluminum foil and placed in an envelope labeled with the site information and the volume filtered. The envelope is then immediately placed on dry ice until transferred to the lab.

Collection of Water Samples for Analysis of Trace Metals (Including Mercury)

When deciding to measure total and dissolved metals in water the purpose of the sampling must be considered. Water quality standards for the protection of aquatic life are determined for the dissolved form of heavy metals in most cases, although this, too, can vary within different Basin Plans for different regions. The exception to routinely conducting dissolved metals analyses is usually mercury (and often selenium). Water quality standards usually apply to the total form of mercury (and often selenium), and not the dissolved form of these elements. Several regions are interested in conducting total metals analyses, in order to address specific issues. In order to budget inputs, transport, and accumulation of metals, it is necessary to know the concentration of total metals in the water column, sediments, effluent, etc. Sample collection for trace metals and mercury in water requires "Clean Hands/Dirty Hands" methodology.

Metals-in-water: General Information

Unless otherwise requested to collect for total metals analysis, dissolved metals are collected for all elements with the exception of mercury. Metals-in-water samples should **not** be collected during periods of abnormally high turbidity if at all possible. Samples with high turbidity are unstable in terms of soluble metals, and it is difficult to collect a representative grab sample. Special study sampling, however, may be an exception. For example, wet weather sampling is likely to include some samples with high turbidity.

Metals-in-water: Sample Collection Depth

Collect a metals sample from a depth of 0.1 m using a sub - surface grab method, or at discrete depths using a depth-integrated sampling method with a peristaltic pump (described further down). In most streams, sub-surface water is representative of the water mass. For the purpose of determining compliance with numerical toxic substance standards, a sample taken at the surface is adequate.

Metals-in-water: Sample Volume

Refer to table at end of this document for specific information on the proper volume to collect for trace metals analyses. Generally, for procedures most commonly used for analysis of metals in water (total or dissolved metals); one 60-mL polyethylene container is filled with the salinity recorded on the field data sheet and COC. Generally, for the procedures most commonly used for analysis of mercury in water (whether total or dissolved), one 250-mL glass or teflon container is filled, regardless of the salinity. All containers are pre-cleaned in the lab using HNO_3.

Metals-in-water:

Sampling Equipment

The method of choice for the collection of water samples for trace metals analysis in small, wadeable streams is the grab method, where the sampler submerges the sample bottle or syringe beneath the surface of the water until filled. The procedure for filtration of water samples for trace metals analysis must be performed within 15 minutes of collection to meet the required filtration holding time. For Mercury(Hg) samples, preservation may take place in the field or at the laboratory within 48 hours of collection. Extreme care must be taken to avoid contamination of the water sample. Considering these factors, it is best to use a **field** filtration system, such as a set-up with peristaltic pump with in-line filter, or a set-up with a syringe filter, if filtered water is required. Samples are pumped and/or filtered directly into the sample container. This minimizes contamination by using no intermediate sampling device. Samples can also be filtered in lab if need be Un-powdered (no-talc) polyethylene gloves are always worn during sampling for metals-in-water.

Depth-integrated sampling is useful when lakes or rivers are stratified and a representative sample is wanted which represents the entire water column. The method involves a peristaltic pump system with enough Teflon tubing to pump at the desired depth with an inline filter. Filter equipment blanks are analyzed for five percent of all cleaned equipment.

Equipment Preparation

It is best if the metals-in-water sampling materials are prepared by a laboratory that can guarantee contamination-free sampling supplies. If a laboratory assembles a Metals-in-Water Sample Collection Kit, it should contain the following items packaged together **for each sample**:

- Tubing with an in-line filter (disposable, 0.45 µm) attached for dissolved metals-in-water sampling. This same tubing is used for total metals-in-water samples without filter. If an in-line pumping system is not used, an acid cleaned syringe and filter are packed.
- Sample containers- polyethylene for total and dissolved samples and blanks; Glass or Teflon for total and dissolved mercury.
- Acid preservation is performed in the laboratory.
- Metals-free DI water (for blanks).
- Powder-free polyethylene gloves

If a laboratory is not assembling collection kits, individuals should take care to keep containers in the original packaging. When removed from the box, sample containers are placed in clean plastic bags (zipper closure bags). Although filters come individually wrapped, they should also be stored in new zipper closure bags to avoid possible contamination.

The filtering equipment is pre-cleaned according to laboratory protocol. Clean tubing is put into clean containers, such as large zipper closure bags. Metals-free filter cartridges with the capacity to filter several liters are commercially available. Equipment blanks are run at the laboratory on batches of metals-in-water sampling equipment prior to their distribution to field staff. One to two liter containers with metals-free deionized water are taken into the field for travel blanks. Metals-free deionized water is supplied by the laboratory performing metals analysis. The deionized water containers are kept clean and dust-free on the outside by wrapping in two plastic bags.

Dissolved and Total Metals-in-Water: Detailed Collection Techniques

- ***Sub-Surface Grab Method***
- ***Syringe Filtration Method (for sub- surface collection)***
- ***Peristaltic Pumping Method (Using Tubing/In-line Cartridge Filters)for sub- surface collection or for depth-integrated collection***

Metals-in-water Sample Collection:

Sub-Surface Grab Method

Clean Hands/Dirty Hands Technique

<u>Unfiltered Samples (for total metals analysis, if requested, and for mercury almost always, unless otherwise requested):</u> Some samples can be sampled directly from the ambient water either by wading into the stream and dipping bottles under the surface of the water until filled, or by sampling from a boat and dipping the bottle under the surface of the water until it is filled. The bottles are cleaned according to laboratory protocol. It is very critical that all the acid is rinsed out of the bottles before the samples are collected. Personnel involved in field sample collection/processing wear polyethylene gloves. The laboratory pre-cleaned glass or Teflon™ 250 mL (for mercury) or polyethylene 60 mL (for metals) sample bottles are taken from the double-wrapped

zipper closure plastic bags using "Clean Hands/Dirty Hands" techniques. The dirty hands collector opens the first outer bag, and the clean hands collector opens the inner bag around the bottle. The clean hands collector then removes the bottle from the inner bag. Clean hands collector then places the inner bag back inside the outer bag while sampling occurs. The clean hands collector dips the bottle into the ambient water, with the cap on, to approximately 0.1 m (avoiding disturbing surface scums), placing the cap back on the bottle before being removed from the water, rinses the bottle five times with ambient water, making sure the threads of the bottle get rinsed as well, and fills the bottle to the top. The lid is secured under the water surface and the bottle is put back into the inner clean bag and sealed by the clean hand collector. The sealed clean bag is then placed back inside the outer bag by the clean hands collector. The dirty hands collector then seals the outer bag.

Metals-in-water Sample Collection:

Syringe Filtration Method (for sub-surface collection)

Filtered Samples (for dissolved metals analyses): Sub-surface water samples are filtered for dissolved trace metals analysis (not for mercury, however, in almost all cases) using the following syringe filtration method.

The syringe (60 cc size, pre-cleaned in the laboratory) and in-line filter are pre-packed in two zipper closure bags. The syringe and filter are taken out of the bags using "Clean Hands/Dirty Hands" technique, as previously described. The sub-surface water sample is collected by 1) wading out into the centroid portion of the stream, or by leaning over the edge of the boat, and aspirating water into the syringe, filling and rinsing the syringe five times with ambient water; 2) attaching the filter onto the syringe and filling the syringe body; 3) rinsing the filter with a few milliliters of the sample; 4) rinsing the sample bottle five times with the filtered ambient water; and 5) extruding the sample through the syringe filter and completely filling each bottle. The bottles are taken out of and put back into their bags using "Clean Hands/Dirty Hands".

Metals-in-water Sample Collection--

Peristaltic Pump

The basic "Clean Hands/Dirty Hands" technique is also applied in the use of a peristaltic pump with an in-line filter cartridge for metals-in-water sample collection. Dirty Hands removes the plastic cover from the end of the pump tubing and inserts the tubing into the sampling container. Dirty Hands holds the tubing in place. The in-line cartridge filter is attached to the outlet end of the tubing.

Clean Hands takes the plastic cover off the other end of the tubing. Dirty Hands turns on the pump and flushes lL of ambient water through the tubing to purge it for dissolved metals.

Clean Hands removes the cap from the sample bottle and uses the pump to fill it with ambient water. Clean Hands puts the cap back on the bottle and places it in the plastic bag.

Metals-in-water Sample Collection:

Depth-Integrated Sampling, using In-line Cartridge Filter and Peristaltic Pump

Preparation for Depth-integrated sample collection:
Depth-integrated sampling is useful when lakes or rivers are stratified, and a representative sample is wanted that represents the entire water column to the extent possible. The method utilized to date for SWAMP involves a peristaltic pump system with enough Teflon tubing to pump from the desired depth. Regional Boards must request depth-integrated sampling.

The tubing set consists of a small length of CFLEX tubing that fits in the peristaltic pump, with an appropriate length of Teflon tubing on the suction side of the pump and a 3-ft section of Teflon tubing on the discharge side of the pump.

The tubing set is pre-cleaned in 10% reagent grade HCL at the laboratory, and to date in SWAMP, a new pre-cleaned tubing set is used for each site. However, the same peristaltic tubing set can be used at multiple sites, as long as it has been cleaned in the field between stations, according to protocol as outlined below. If this is to be done, however, and Dissolved or Total Organic Carbon samples are collected, equipment blanks should be collected at each site until it is determined that the blanks are acceptably low.

The field cleaning procedure for tubing that is to be re-used is:

- Pump phosphate free detergent through tubing.
- Pump 10% HCL through tubing.
- Pump methanol through tubing.
- Pump 1 l of blank water (Milli-Q) through.

All reagents must be collected in appropriate hazardous waste containers (separated by chemical), and transport, as well as disposal, must follow appropriate local, state, and federal

regulations.

If a field blank is needed, collect it after the 1 L of blank water is pumped through. Pump the amount of ambient water equivalent to 3 times the volume of the tubing before sampling the next site.

Filtered and Unfiltered Samples, Depth-integrated:
It is recommended to attach the tubing to a line with depth measurement markers (preferably in meters). At the end of this line should be a trace metal-safe weight, which hangs about one meter below the tubing end, avoiding any sediment intake from the bottom of the water column with the pump tubing.

At the site, Dirty Hands sets up the pump, while Clean Hands takes a bottle from the plastic bag and places it in a container holder or on a clean surface. A container holder can be anything trace metal clean that supports the bottle, freeing up the collector's hands. Clean Hands takes the outlet-end of the tubing (with the in-line filter cartridge attached) out of the bag, and places it in the peristaltic pump head. The outlet end is long enough to allow easy bottle filling; the other end is long enough to easily reach beneath the water surface and to the desired depth. Dirty Hands closes the pump head, locking the tubing in place.

Make sure that all bottles are filled with a depth-integrated water sample. This can be accomplished by dividing the total vertical length of the water column into 2 to 10 equal intervals, and sampling each interval equally, filling the bottles at each depth proportional to the number of intervals sampled. For example, if 10 intervals are sampled, every bottle is filled $1/10^{th}$ full at each depth sampled. A very common method of dividing the water column is by first determining the depth of the thermo-cline. Samples are taken at the midpoint between the surface and the thermo-cline, at the midpoint between the top of the thermo-cline and the bottom of thermo-cline, and at the midpoint between the bottom of the thermo-cline and just above the bottom of the water column. For these methods, all containers have to be filled at the same time. Note the number of intervals sampled on the data sheet.

When filling bottles, Clean Hands immerses the intake tube directly into the water at the appropriate depth, and Dirty Hands operates the pump to flush the tubing with a minimum of 1L of ambient water through the tubing and filter.

Clean Hands removes the cap from the sample bottle, holds the tubing outlet with the in-line filter cartridge over the container opening (without touching the container), and allows the container to fill. The container is filled and rinsed five times with ambient water, and is then filled to the top for the actual sample. Clean Hands puts the cap back on the bottle, and places the bottle back it in the zipper closure plastic bag. Whenever Clean Hands touches the boat or equipment, which may be contaminated, gloves should be changed immediately.

(Note for Unfiltered samples: *If an unfiltered sample is required for total metals, total mercury, conventional constituents, toxicity, or synthetic organics, the same procedure is used as described above, except the filter is detached from the end of the tubing before filling the bottles.)*

When sampling is finished, the tubing is brought to the surface, clean water (Milli-Q or deionized) is pumped through system, and the tubing is stored in a polyethylene bag.

The tubing set can be used at multiple sites, as long as it has been cleaned in the field between stations (see field cleaning procedure above). However, if Dissolved or Total Organic Carbon samples (in water) are collected, equipment blanks should be collected at enough sites until it is determined the blanks are appropriate.

Metals-in-water Sample Collection:

Collecting the Sample:

Composite Bottle

The sample collection methodologies are identical to those described above except the sample is collected first into a composite bottle(s). The sample is collected in an amber glass 4-L bottle for mercury and methyl mercury, and a 4-L polyethylene bottle for other trace metals. The compositing bottle is cleaned according to SWAMP SOP.SC.G.1. It is very critical that all the acid is rinsed out of the bottle and that the bottle is rinsed with sample water (five times) before the sample is taken. The sample is collected by the grab or pumping method after being rinsed five times with ambient

water and is brought inside the water quality vehicle or sampling box for processing. Personnel involved in sample processing don polyethylene gloves. During sampling the dirty hands person opens the bag holding the composite bottle and opens the outer plastic bag. The clean hands person opens the inner plastic bag, removes the bottle and holds the bottle while the Dirty Hands sampler controls the flow of water through the pump into the bottle.

Preparing sample aliquots from a composite bottle into smaller sample bottles using an inline pump and filter:

The dirty hands person opens the first bag, and the clean hands person opens the inner bag around the composite bottle. The clean hands person then removes the bottle from the inner bag and places the bags and the bottle in a designated clean place.

This process is repeated until all sample bottles are lined up on the clean bench with their tops still on.

The top of the bottles are loosened so that they fit very loosely on top of the bottles so the clean hands person can remove the caps and pour or pump water into the bottles easier.

The clean hands person shakes the 4-L sample in a steady and slow up and down motion for two full minutes.

Samples that are not to be filtered (including TSS/SSC) are sub-sampled out of the bottle by pouring out of the large compositing bottle into the sample bottles. The compositing bottle is shaken for 15 s between these subsamples.

Each sample bottle is rinsed five times with ambient water before filling.

For the clean pumping system setup procedure, see above.

(The equipment or field blank is processed exactly like a sample following the same steps.)

The clean end of the tubing used for suction is placed into 1 L bottle. Approximately 750 mL of Milli-Q are then pumped through the system to purge any residual contamination.

The 250-mL sample bottles are then filled to the neck and capped as soon as possible.

Note: if volatile organics are to be collected they should be pumped directly into the sample containers before the compositing procedure.

Metals-in-water: Short-term Sample Preservation

After collecting the sample, the double-bagged container is placed in another plastic bag for shipping, and placed on ice in the ice chest, cooled to 6 °C. This is to prevent possible contamination from other samples in the ice chest. Metals-in-water samples are acid-preserved in the lab.

Metals-in-water: Sample Container Label

Label each outer sample-bag with the station ID, sample code, matrix type, analysis type, project ID, and date and time of collection.

Metals-in-water: Field Equipment Blank

Pumping Method. If required, field blanks are collected at the last site of a sampling trip, with the same tube and filter used to collect the last dissolved metals-in-water sample of the day (before the ambient sample is collected); and with the tube used for the last total metals-in-water sample of the day. If each sample is taken using a new set of tubing, a separate tubing-set should be used for the blank.

The same Clean Hands/Dirty Hands collection techniques are followed for the field blank as the samples, pumping trace metal-free water from a clean container supplied by the laboratory.

Syringe Method. If required, field blanks are collected in much the same way as in the pumping method. "Clean Hands/ Dirty Hands" techniques are used. The syringe is taken out of the double bags, deionized water is aspirated into the syringe, syringe is rinsed five times with ambient water, the filter is attached, and the blank water is extruded into a sample bottle. A minimum of one blank per trip is taken, if required.

Grab Method. Bottles full of deionized water or Milli-Q are opened at the site for the same length of time the sample bottles are open.

COMPANION SAMPLES FOR METALS-IN-WATER

A hardness analysis should be requested by the Regional Water Control Board whenever metals-in-water are to be analyzed from an inland (freshwater) site. Estuarine/marine sites do not require hardness analysis.
If a total metals sample is collected, it is recommended to submit a sample for total suspended solids/suspended sediment concentration (TSS/SSC) in a companion sample for "conventionals in water".

Hexavalent Chromium

Very rarely, a request may be made for conducting hexavalent chromium analysis in water samples. Acidification alters the hexavalent form of chromium. A separate (un-acidified) sample must be submitted if hexavalent chromium is to be analyzed. Filter and submit a minimum of 500 mL water. The sample is collected in a DI-water-rinsed polyethylene or glass container, placed on ice, and shipped to the lab in time for analysis to begin within 24 h of collection. The lab must be notified when a hexavalent chromium sample will arrive. Hexavalent chromium is not usually analyzed on unfiltered samples.

FIELD QC SAMPLE COLLECTION REQUIREMENTS FOR METALS-IN-WATER

In order to assess contamination, "blanks" are submitted for analysis. Special projects may have other requirements for blanks. The same group of metals requested for the ambient samples are requested for the blank(s). Run a blank for each type of metal sample collected. Blanks results are evaluated (as soon as available) along with the ambient sample results to determine if there was contamination or not. See the Quality Control and Sample Handling Guidelines for Inorganic Analytes for information regarding frequency and types of field QC samples.

Field Equipment Blank (Ambient Blank)	Submit an equal volume (equal to the ambient sample) of metals-free deionized water that has been treated exactly as the sample at the same location and during the same time period. Use the same methods as described above (Grab sample, pumping method, syringe method). At least one ambient blank per field trip is required each for trace metal and Mercury samples in water. *If contamination is detected in field equipment blanks, blanks are required for every metals-in-water sample until the problem is resolved.*
Laboratory Equipment	Laboratory Equipment Blanks for pumping and sampling equipment (Metals-in-Water Sample Collection Kits and

Blank

Syringe Filtration Kits) are run by the laboratory that cleans and distributes the collection materials. It documents that the materials provided by the laboratory are free of contamination. When each batch of tubes, filters, bottles, acid and deionized water are prepared for a sampling trip, about five percent of the Mercury sampling materials are chosen for QC checks. Trace metal equipment needs to be subjected to an initial blank testing series. If these blanks are acceptable only occasional re-testing is required for TM equipment. The QC checks are accomplished by analyzing metals-free water which has been pumped through the filter and tube; collected in a sample container; and preserved.

Field Duplicates

Five percent Field Duplicates are submitted every year. (If fewer than 20 samples are collected during an event, submit one set of duplicates per event.)

Collection of Water Samples for Analysis of Synthetic Organic Compounds

Collect organic samples at a depth of 0.1 m by submerging the sample container by hand. If depth-integrated sampling is required, use the in-line peristaltic pump methodology described previously. Since organic compounds tend to concentrate on the surface of the sampling device or container, the sampling device and sample container are ***not*** to be rinsed with ambient water before being filled.

Sample Containers and Collection

Also refer the Quality Control and Sample Handling Guidelines for Synthetic Organic Compounds in Fresh and Marine Water for a list of recommended container types.

Pesticides/ Herbicides	The sample container for pesticides and herbicides is a new, clean, unused amber glass jar with a Teflon-liner inside the cap. Collect one liter of water for each of the three sample types (Organophosphorus Pesticides, Organochlorine Pesticides and Chlorinated Herbicides). **EACH ANALYSIS TYPE REQUIRES A SEPARATE JAR**. Minimize the air space in the top of the jar. Preserve immediately after collection by placing on ice out of the sunlight.
Semi-volatile Organics	The sample container for semi-volatile organics must also be new, clean, unused amber glass bottles with a Teflon-liner inside the cap, and pre-rinsed with pesticide-grade hexane, acetone, or methylene chloride. Fill jars to the top and place on ice in the dark. In addition to other sample information, label the jar Semi-volatiles.
Volatile Organics: **Volatile Organic Carbon (VOC), Methyl-Tert Butyl Ether (MTBE) and (BTEX)**	The sample containers for volatiles are VOA vials. Fill the 40-mL VOA vials to the top and cap without trapping any air bubbles. If possible, collect directly from the water, keeping the vial under water during the entire collection process. To keep the vial full while reducing the chance for air bubbles, cap the vials under the water surface. Fill one vial at a time and preserve on ice. The vials are submitted as a set. If the vial has been pre-acidified for preservation, fill the vial quickly, without shaking using a separate clean glass jar. Fill the vial till the surface tension builds a meniscus, which extends over the top end of the vial, then cap tightly and check for bubbles by turning the vial on its head. Ensure that the pH is less than 2. If the water may be alkaline or have a significant buffering capacity, or if there is concern that pre-acidified samples may have the acid wash out, take a few

	practice vials to test with pH paper. It may take more than two drops, and it will then be known how to preserve the other samples that are being submitted to the lab. If an alternative method has proven successful, continue with that method. **Note**: If vigorous foaming is observed following acidification, discard that sample and collect another set. Do not acidify the second set. Mark the sample clearly "not acidified" and the lab will run them immediately. Holding time is 14 days with acid, 7 days without acid. Collect three VOA vials, if VOC, MTBE and BTEX are required, two vials, if only VOC is required and two vials, if only MTBE and BTEX are require. The vials may be taped together to keep them together.
Perchlorate	Surface water samples for perchlorate should be collected in a new unused polyethylene or glass container. Perchlorate samples should be placed immediately on ice to maintain temperature at 6 ºC. The sample holding time is 28 days, under refrigeration.
Sample Treatment in Presence of Chlorine	If in stream chlorine residual is suspected, measure the chlorine residual using a separate water subsample. Free chlorine will oxidize organic compounds in the water sample even after it is collected. If chlorine residual is above a detectable level, (i.e., the pink color is observed upon adding the reagents) immediately add 100 mg of sodium thiosulfate to the pesticides, herbicides, semi-volatiles and VOA samples; invert until sodium thiosulfate is dissolved. Record the chlorine residual concentration in field logbook. If chlorine residual is below detectable levels, no further sample treatment necessary.
VOA Trip Blank	Submit one Trip Blank for VOA samples (2- 40 mL VOA vials) for each sampling event. Trip Blanks are prepared in advance just before the sampling trip and transported to the field. Ask the laboratory for DI water and specify that it is for a VOA trip blank. VOA blanks require special purged water. Trip blanks demonstrate that the containers and sample handling did not introduce contamination. The trip blank vials are never opened during the trip.
Field QC Samples	If required, field Duplicates and field blanks are submitted at a rate subject to the discretion of the project manager. Refer to the SWAMP Quality Control and Sample Handling Guidelines for details on required blanks and duplicates.

BACTERIA AND PATHOGENS IN WATER SAMPLES

Summary of Collection Procedure (Based on EPA water quality monitoring procedures)

Make sure the containers are sterilized; either factory-sealed or labeled.

Whirl-pak® bags

- Label the bottle as previously described for SWAMP.
- Tear off the top of the bag along the perforation above the wire tab just prior to sampling. Avoid touching the inside of the bag. If you accidentally touch the inside of the bag, use another one.
- If wading into the stream, try to disturb as little bottom sediment as possible. Be careful not to collect water that has sediment from bottom disturbance. Stand facing upstream. Collect the water sample on your upstream side, in front of you.
- If taking sample from a boat, carefully reach over the side and collect the water sample on the upstream side of the boat.
- Hold the two white pull-tabs in each hand and lower the bag into the water on your upstream side with the opening facing upstream. Open the bag midway between the surface and the bottom by pulling the white pull-tabs. The bag should begin to fill with water. You may need to "scoop" water into the bag by drawing it through the water upstream and away from you. Fill the bag no more than 3/4 full.
- Lift the bag out of the water. Pour out excess water. Pull on the wire tabs to close the bag. Continue holding the wire tabs and flip the bag over at least 4-5 times quickly to seal the bag. Don't try to squeeze the air out of the top of the bag. Fold the ends of the wire tabs together at the top of the bag, being careful not to puncture the bag. Twist them together, forming a loop.
- If the samples are to be analyzed in the lab, place them in a cooler with ice or cold packs for transport to the lab.

Screw cap containers

- Label the bottle as previously described for SWAMP.
- Remove the plastic seal from the bottle’s cap just before sampling. Avoid touching the inside of the bottle or cap. If you accidentally touch the inside, use another bottle.
- If wading into the stream, try to disturb as little bottom

sediment as possible. Be careful not to collect water that has sediment from bottom disturbance. Stand facing upstream. Collect the water sample on your upstream side, in front of you.

- If taking sample from a boat, carefully reach over the side and collect the water sample on the upstream side of the boat.
- Hold the bottle near its base with polyethylene gloves and submerge the bottle in the water with the cap on. Open the bottle collecting the water sample 0.1m beneath the surface. When the bottle is filled to the desired level recap the bottle and remove from water. You can only use this method if the sample bottles do not contain sodium thiosulfate.
- Turn the bottle underwater into the current and away from you. In slow moving stream reaches, push the bottle underneath the surface and away from you in an upstream direction.
- Alternative sampling method: In case the sample bottle contains preservatives/chlorine removers (i.e. Sodium-Thiosulfate), it cannot be plunged opening down. In this case hold the bottle upright under the surface while it is still capped. Open the lid carefully just a little to let water run in. Fill the bottle to the fill mark and screw the lid tight while the bottle is still underneath the surface.
- Leave a 1-in. air space so that the sample can be shaken just before analysis. Recap the bottle carefully, remembering not to touch the inside.
- If the samples are to be analyzed in the lab, place them in a cooler with ice or cold packs for transport to the lab. Samples should be placed immediately on ice to maintain temperature at 6 ºC

Pouring from another clean bottle

- Due to different sampling conditions (high turbidity, rough water etc.) it is sometimes easy to pour water from another clean bottle into the bacteria bottle. This helps to make sure that the sample water is only being filled to the desired line and no overfilling occurs.

TOXICITY IN WATER

Sample Collection

Using the standard grab sample collection method described previously for water samples, fill (for typical suite of water toxicity tests conducted) the required amount of 2.25-L amber glass bottles with sub surface water. Since the size of the 2.25-L amber bottle is bigger than your average sample bottle, find a spot in the centroid of the stream to completely submerge the toxicity bottle if possible. A clean water organics(1-L glass amber) bottle can be used if there is no sampling point deep enough to submerge a large toxicity bottle. If the stream is not deep enough to submerge any bottle, then comments should be made on the field data sheets that surface water was collected. Depth should also equal 0 for the sampling depth. All toxicity samples should be. put on ice, and cooled to 4 °C. Label the containers as described above and notify the laboratory of the impending sample delivery, since there is a 48-hr maximum sample hold time. Sample collection must be coordinated with the laboratory to guarantee appropriate scheduling.

Summary of Sample Container, Volume, Initial Preservation, and Holding Time Recommendations for Water Samples

Parameters for Analysis in WATER Samples	Recommended Containers (all containers pre-cleaned)	Typical Sample Volume (mL)	Initial Field Preservation	Maximum Holding Time (analysis must start by end of max)
Conventional Constituents in Water				
Alkalinity	Polyethylene bottles (see **NOTE(1)** below)	100 mL	Cool to ≤ 6 °C, dark	14 days at ≤ 6 °C, dark
Chloride (Cl), Sulfate (SO_4) and Fluoride (F)	Polyethylene bottles (see **NOTE(1)** below)	300 mL	Cool to ≤ 6 °C, dark	28 days at ≤ 6 °C, dark
Ortho-phosphate (OPO_4)	Polyethylene bottles (see **NOTE(1)** below)	150 mL	Filter within 15 minutes; Cool to ≤ 6 °C, dark	48 h at ≤ 6 °C, dark
Nitrate + Nitrite (00630) ($NO_3 + NO_2$)	Polyethylene bottles (see **NOTE(1)** below)	150 mL	Cool to ≤ 6 °C, dark	48 h at ≤ 6 °C, dark
Total Keldjahl Nitrogen (TKN)	Polyethylene bottles (see **NOTE(1)** below)	600 mL	Cool to ≤ 6 °C, dark; H_2SO_4 to pH<2	Unacidified: 7 days Acidified: 28 days Either one at ≤ 6 °C, dark
Total Dissolved Solids (TDS)	Polyethylene bottles (see **NOTE(1)** below)	1000 mL	Cool to ≤ 6 °C, dark Cool to 4°C, dark	7 days at ≤ 6 °C, dark
Ammonia (NH_3)	Polyethylene bottles (see **NOTE(1)** below)	500 mL	Cool to ≤6 °C; samples may be preserved with 2 mL of H_2SO_4 per L	Unacidified: 48 h Acidified: 28 days Either one at ≤ 6 °C, dark
Total Phosphorus (TPO_4)	Polyethylene bottles (see **NOTE(1)** below)	300 mL	Cool to ≤ 6 °C, dark	28 days at ≤ 6 °C, dark
(1)NOTE: **The volume of water necessary to collect in order to analyze for the above constituents is typically combined in four 1-L polyethylene bottles, which also allows enough volume for possible re-analysis and for conducting lab spike duplicates. This is possible since the same laboratory is conducting all of the above analyses; otherwise, individual volumes apply.**				
Total Organic Carbon (TOC),	125 mL amber glass vial	125 mL for TOC only	Cool to ≤6 °C; acidify to pH<2 with HCl, H_3PO_4, or H_2SO_4 within 2 hrs	28 days
Dissolved Organic Carbon (DOC)	250 ml amber for TOC/DOC	250 mL for TOC/DOC	Filter and preserve to pH<2 within 48 hours of collection; cool to ≤6 °C	28 days
Total Suspended Solids (TSS)	250 mL plastic bottle	250 mL	Cool to ≤6 °C, dark	7 days at ≤6 °C, dark
Suspended Sediment Concentration (SSC)	125 mL polyethylene bottle	Up to 125ml depending on	Cool to ≤6 °C, dark	7 days at ≤6 °C, dark

Parameters for Analysis in WATER Samples	Recommended Containers (all containers pre-cleaned)	Typical Sample Volume (mL)	Initial Field Preservation	Maximum Holding Time (analysis must start by end of max)
		turbidity of water		
Chlorophyll *a* **Pheophytin *a*** **Chlorophyll a** **Pheophytin *a***	1-L amber polyethylene bottle Aluminum Foil, GFC Filters	1000 mL (one bottle) 20-420 mL	Centrifuge or filter as soon as possible after collection; if processing must be delayed, keep samples on ice or at ≤6 °C; store in the dark	Samples must be frozen or analyzed within 4 hours of collection; filters can be stored frozen for 28 days
Non-Routine Compounds in Water Samples				
OIL AND GREASE	1-L glass jar with Teflon lid-liner, rinsed with hexane or methylene chloride	1000 mL (one jar)	Cool to ≤6 °C; HNO_3 or H_2SO_4 to pH<2	28 days at ≤6 °C, dark
PHENOLS	1-L glass jar with Teflon lid-liner	1000 mL (one jar)	Cool to ≤6 °C; H_2SO_4 to pH<2	28 days at ≤6 °C, dark
CYANIDE	1-L cubitainer	1000 mL (one cubitainer)	Cool to ≤6 °C; NaOH to pH>10; add 0.6 g $C_6H_8O_6$ if residual chlorine is present	14 days at ≤6 °C, dark
BIOCHEMICAL OXYGEN DEMAND (BOD)	4-L cubitainer	4000 mL (one cubitainer)	Cool to ≤6 °C; add 1 g FAS crystals per liter if residual chlorine is present	48 h at ≤6 °C, dark
CHEMICAL OXYGEN DEMAND (COD)	1-L cubitainer	110 mL (one cubitainer)	Cool to ≤6 °C; H_2SO_4 to pH<2	28 days at ≤6 °C, dark; biologically active samples should be tested as soon as possible
Trace Metals in Water Samples				
DISSOLVED METALS (except Dissolved Mercury)	60 mL polyethylene bottle, pre-cleaned in lab using HNO_3	60 mL (one bottle)	Filter at sample site using 0.45 micron in-line filter, or syringe filter (within 15 minutes of collection). Cool to 6°C, dark. Acidify in lab, within 48 hrs, using pre-acidified container (ultra-pure HNO_3) for pH<2.	Once sample is filtered and acidified, can store up to 6 months at room temperature

Parameters for Analysis in WATER Samples	Recommended Containers (all containers pre-cleaned)	Typical Sample Volume (mL)	Initial Field Preservation	Maximum Holding Time (analysis must start by end of max)
DISSOLVED MERCURY	250 mL glass or Teflon bottle, pre-cleaned in lab using HNO_3	250 mL (one bottle)	Filter within 15 minutes of collection. Cool to 6°C, dark. Acidify in lab within 48 hrs, with pre-tested HCL to 0.5%.	Once sample is filtered and acidified, can store up to 90 days at room temperature
TOTAL METALS (except Total Mercury)	60 mL polyethylene bottle, pre-cleaned in lab using HNO_3	60 mL (one bottle)	Cool to ≤6 °C, dark. Acidify in lab within 48 hrs, with pre-acidified container (ultra-pure HNO_3), for pH<2.	Once sample is acidified, can store up to 6 months at room temperature
TOTAL MERCURY	250 mL glass or Teflon bottle, pre-cleaned in lab using HNO_3	250 mL (one bottle)	Cool to ≤6 °C, dark. Acidify in lab within 48 hrs, with pre-tested HCL to 0.5%.	Once sample is acidified, can store up to 90 days at room temperature.
HEXAVALENT CHROMIUM (filtered)	600 mL plastic or glass bottle	600 mL (one bottle)	Cool to ≤6 °C, dark No acid	Keep at ≤6 °C, dark for up to 24 h; must notify lab in advance.
HARDNESS	200 mL polyethylene bottle	200 mL (one bottle)	Cool to 6°C, dark OR Cool to ≤6 °C; HNO_3 or H_2SO_4 to pH<2	48 h at 6°C, dark 6 months at ≤6 °C, dark
Synthetic Organic Compounds in Water Samples				
VOLATILE ORGANIC ANALYTES (VOA's) including VOC, MTBE and BTEX	40 mL VOA vials	120 mL (three VOA vials)	All vials are pre-acidified (50% HCl or H_2SO_4) at lab before sampling. Cool to 6°C, dark	unacidified: 7 days acidified: 14 days Both at 6°C, dark
PESTICIDES & HERBICIDES* Organophosphate Pesticides Organochlorine Pesticides Chlorinated Herbicides **SEMI-VOLATILE ORGANICS*** **POLYCHLORINATED* BIPHEYNYL AND AROCHLOR COMPOUNDS** **TPH, PAH, PCP/TCP***	1-L I-Chem 200-series amber glass bottle, with Teflon lid-liner (per each sample type)	1000 mL (one container) ***Each sample type requires 1000 mL in a separate container**	Cool to 6°C, dark If chlorine is present, add 0.1g sodium thiosulfate	Keep at 6°C, dark, up to 7 days. Extraction must be performed within the 7 days; analysis must be conducted within 40 days.

Parameters for Analysis in WATER Samples	Recommended Containers (all containers pre-cleaned)	Typical Sample Volume (mL)	Initial Field Preservation	Maximum Holding Time (analysis must start by end of max)
Toxicity Testing Water Samples				
TOXICITY IN WATER	Four 2.25 L amber glass bottles	9000 mL	Cool to 4°C, dark	48 hrs at 4°C, dark
Bacteria and Pathogens in Water Samples				
E. Coli	Factory-sealed, pre-sterilized, disposable Whirl-pak® bags or 125 mL sterile plastic (high density polyethylene or polypropylene) container	100 mL volume sufficient for both E. coli and Enterococcus analyses	Sodium thiosulfate is pre-added to the containers in the laboratory (chlorine elimination). Cool to ≤ 10°C; dark.	STAT: 8 hrs at ≤ 10°C, dark if data for regulatory purposes; otherwise, 24 hrs at ≤ 10°C, dark if non-regulatory purpose.
Enterococcus	Factory-sealed, pre-sterilized, disposable Whirl-pak® bags or 125 mL sterile plastic (high density polyethylene or polypropylene) container	100 mL volume sufficient for both E. coli and Enterococcus analyses	Sodium thiosulfate is pre-added to the containers in the laboratory (chlorine elimination). Cool to ≤ 10°C ; dark.	STAT: 8 hrs at ≤ 10°C, dark if data for regulatory purposes; otherwise, 24 hrs at ≤ 10°C, dark if non-regulatory purpose.
FECAL COLIFORM	Factory-sealed, pre-sterilized, disposable Whirl-pak® bags or 125 mL sterile plastic (high density polyethylene or polypropylene) container	100 mL volume sufficient for both fecal and total coliform analyses	Sodium thiosulfate is pre-added to the containers in the laboratory (chlorine elimination). Cool to ≤ 10°C; dark.	STAT: 8 hrs at ≤ 10°C, dark if data for regulatory purposes; otherwise, 24 hrs at ≤ 10°C, dark if non-regulatory purpose.
TOTAL COLIFORM	Factory-sealed, pre-sterilized, disposable Whirl-pak® bags or 125 mL sterile plastic (high density polyethylene or polypropylene) container	100 mL volume sufficient for both fecal and total coliform analyses	Sodium thiosulfate is pre-added to the containers in the laboratory (chlorine elimination). Cool to ≤ 10°C; dark.	STAT: 8 hrs at ≤ 10°C, dark if data for regulatory purposes; otherwise, 24 hrs at ≤ 10°C, dark if non-regulatory purpose.

Field Collection Procedures for Bed Sediment Samples

Bed sediment (hereafter termed "sediment") samples are collected after any water samples are collected where water and sediment are taken in the same reach. Care must be taken not to sample sediments that have been walked on or disturbed in any manner by field personnel collecting water samples. Sediment samples are collected into a composite jar, where they are thoroughly homogenized in the field, and then aliquoted into separate jars for chemical or toxicological analysis. Sediment samples for metals and organics are submitted to the respective analytical laboratories in separate glass jars, which have been pre-cleaned according to laboratory protocol.

Sediment chemistry samples give information regarding both trends in contaminant loading and the potential for adverse effects on sediment and aquatic biota. In order to compare samples over time and from site to site, they must be collected in a consistent manner. Recently deposited fine grain sediments (see attached table) are the target for sediment collection. If a suitable site for collecting sediments cannot be found at a station (it only contains larger grain material), sampling personnel should not collect the sediment sample, and should instead attempt to reschedule the sample collection or move to a different area that has more recently deposited fine sediment. If this is not possible, make a note so that the missing sample is accounted for in the reconciliation of monitoring events during preparation of sample collection "cruise reports". Sites that are routinely difficult to collect should be considered for elimination or relocation from the sample schedule, if appropriate.

Characteristics of Ideal Sediment Material to be Collected

Many of the chemical constituents of concern are adsorbed onto fine particles. One of the major objectives in selecting a sample site, and in actually collecting the sample while on site, is to obtain recently deposited fine sediment, to the extent possible. Avoid hard clay, bank deposits, gravel, disturbed and/or filled areas. Any sediment that resists being scooped by a dredge is probably not recently deposited fine sediment material. In following this guidance, the collection of sediment is purposefully being biased for fine materials, which must be discussed thoroughly in any subsequent interpretive reporting of the data, in regards to representation of the collected sample to the environment from which it was collected.

Characteristics of an Ideal Site

Quiescent areas are conducive to the settling of finer materials (EPA/USACOE, 1981).
Choose a sampling site with lower hydrologic energy, such as the inner (depositional) side of bends or eddies where the water movement may be slower. Reservoirs and estuaries are generally depositional environments, also.

Selecting the

Sediment will vary from site to site and can vary between sample

Appropriate Sediment Type for Analysis

events at a particular site.

Streams and Rivers: Sediment collection in flowing streams is often a challenge. In areas of frequent scouring there may not be sufficient sediment for collection during or following periods of high flow. Sediment collection during these times may prove unsuccessful and may have to be rescheduled or cancelled.

When the suspended load in rivers and streams precipitates due to reduction of velocity, most of the resulting sediment will be fine-grained. More often than not, a dredge or mechanical grab device does not function well for collection of sediment in smaller streams. In many cases, sediment will have to be collected using a pre-cleaned polyethylene scoop. Collect the top 2 cm for analysis. Five or more (depending on the volume of sediment needed for conducting analyses) fine-sediment sub-sites within a 100-m reach are sampled into the composite jar.

Reservoirs and Estuaries: Collect the top 2 cm for analysis. Grabs are composited for the sediment sample, depending on the volume of sediment needed for conducting analyses.

GENERAL PROCEDURE FOR COLLECTION OF BED SEDIMENT

After choosing an appropriate site, and identifying appropriate fine-grained sediment areas within the general reach, collect the sample using one or more of the following procedures, depending on the setting:

A. Sediment Scoop Method—Primary Method for Wadeable, Shallow Streams

- The goal is to collect the top 2 cm of recently-deposited fine sediment only.
- Wear gloves and protective gear, in areas of potential exposure hazards, per appropriate protocol (make sure gloves are long enough to prevent water from overflowing gloves while submerging scoop).
- Survey the sampling area for appropriate fine-sediment depositional areas before stepping into the stream, to avoid disturbing possible sediment collection sub-sites.
- Carefully enter the stream and start sampling at the closest appropriate reach, then continue sampling UPSTREAM. Never advance downstream, as this could lead to sampling disturbed sediment.
- Stir, do not shake, collected sediment with a polyethylene scoop for at least 5 min making sure all sediment is completely homogenized.
- Quickly scoop sediment out of the homogenizing jar into desired sampling jars making sure

to stir the sediment in the homogenizing jar in between each aliquot.

- Inspect each individual sediment jar making sure of consistent grain size throughout the entire sample collection.
- Single bag all sediment containers to prevent cross contamination.
- Make sure all containers are capped tightly and stored in a cooler on cube ice at 6 °C.

B. Hand Core Method-Alternate method for wadeable shallow streams with fine sediment

- A hand core is used in wadeable streams where there is very fine sediment.
- The hand core sampler consists of a 3-in. diameter polycarbonate core that is 8 inches long. Samplers push the core into the sediment to the desired depth, pull the core out of the sediment, and cap the bottom with a polyethylene core cap or by placing their hand underneath the cap to hold the sediment in place.
- Hand cores are usually measured and marked at 2 cm length so the sampler knows how far to deploy the core into the sediment.
- Sediment is then emptied into a homogenizing jug and aliquoted accordingly.

C. Sediment Grab Method—Primarily for Lake, River, Bridge, and Estuarine Settings (or deeper streams)

Description of sediment grab equipment:

- A mechanical sediment grab is used for the SWAMP bed sediment collection field effort for lake, river, bridge, and estuarine/coastal settings (or deeper, non-wadeable streams).
- The mechanical grab is a stainless steel “Young-modified Van Veen Grab", and is 0.5 m^2 in size.
- The mechanical grab is deployed primarily from a boat, and is used in deeper, non-wadeable waters, such as lakes, rivers, estuaries, and coastal areas.
- It is also deployed by field personnel from land in settings which allow its use: primarily from bridges; from smaller vessels in streams or drainage channels too deep or steep to wade into, but too shallow for a larger boat.

Deploying and retrieving the grab:

- Slowly lower the grab to the bottom with a minimum of substrate disturbance.
- Retrieve the closed dredge at a moderate speed (e.g., less than two feet per second).
- Upon retrieval, open the lids of the sediment grab, examine the sample to ensure that the sediment surface is undisturbed and that the grab sample should not be rejected.

Rejection Criteria—reject the sample if the following are not met:

- Mud surface must not be pressing out of the top of the sampler. If it is, lower the grab more slowly.
- Overlying water must not be leaking out along the sides of the sediment in the grab. This

ensures the surficial sediment is not washed out.

- Sediment surface is flat and level in the sampler. If it is not level, the grab has tilted over before closing.

Processing the sediment sample from the grab equipment:

- The water overlying the sediment in the grab is very gently decanted by slightly tipping the grab with the lid closed until the water runs out the top.
- The decanting process should remove all of the overlying water but not remove the surficial sediments. The laboratory reports percent water for the sample, so overlying water is not included in the sample container.
- The sediment is examined for depth of penetration, color and thickness of top aerobic zone, and texture. These observations are recorded on the field data sheet.
- Collect the top 2 cm from at least five sub samples, and otherwise, exclude the bottom-most layer and composite.
- In streams or other settings with excessive bottom debris (e.g., rocks, sticks, leaves) where the use of a grab is determined to be ineffective (e.g., dredge does not close, causing loss of sediment), samples may be collected by hand using a clean plastic scoop, or by a variety of coring methods, if appropriate for the situation.
- Sediment is handled as described below in the metals and organic sections.

Cleaning the Grab Equipment and Protection from Potential Contaminating Sources:

- The sediment sampler will be cleaned prior to sampling EACH site by: rinsing all surfaces with ambient water, scrubbing all sediment sample contact surfaces with Micro™ or equivalent detergent, rinsing all surfaces with ambient water, rinsing sediment sample contact surfaces with 5% HCl, and rinsing all sediment sample contact surfaces with methanol.
- The sediment grab will be scrubbed with ambient water between successive deployments at ONE site, in order to remove adhering sediments from contact surfaces possibly originating below the sampled layer, thus preventing contamination from areas beyond target sampling area.
- Sampling procedures will attempt to avoid exhaust from any engine aboard any vessel involved in sample collection. An engine will be turned off when possible during portions of the sampling process where contamination from engine exhaust may occur. It is critical that sample contamination be avoided during sample collection. All sampling equipment (e.g., siphon hoses, scoops, containers) will be made of non-contaminating material and will be appropriately cleaned before use. Samples will not be touched with un-gloved fingers. In addition, potential airborne contamination (e.g., from engine exhaust, cigarette smoke) will be avoided.

D. Core Method--alternative for fast-moving, wadeable streams

The core method is used in soft sediments when it is difficult to use the other methodologies. The cores can be used in depths of water from 0 to 10 ft by using a pole deployment device or in deeper water using SCUBA divers. The pole deployment device consists of a pole that attaches to the top

of the core. The top of the core is fitted with a one-way valve, which allows the core to be filled with sediment, but when pulled from the sediment catches the sediment within the core. The core is then brought to the surface and the sediments within the core are extruded out the top of the core so that 2 cm of sediment is above the top of the plastic core. The 2 cm of sediment is then sliced off and placed in the homogenizing jar. A new core, homogenizing jar, and device used to slice off the top two cm. are used at each station unless the equipment is cleaned using laboratory protocols.

E. Sediment Grab Method – Primarily used from bridges or for streams with restricted bank access.

Description and sampling procedure for the Eckman sediment grab

- The Eckman grab is 0.2 m^2 in size with a lead "messenger" that triggers the spring loaded doors.
- The primary use is for sampling from bridges or from small vessels in streams or drainage channels too deep or steep to wade into, but too shallow for a larger boat.
- The grab must be cleaned with a Micro™ and tap water rinse before sampling and in-between sample stations.
- To deploy the grab, pull the spring loaded doors open and hook the cables on the actuator plate.
- With a rope, lower the grab to the desired sample reach making sure that the grab has penetrated the sediment. Clip the "messenger" on the rope and release it while maintaining tension on the rope. Pull up the grab once the "messenger" has activated the doors.
- While wearing clean poly gloves, open the top hatch and remove the top 2 cm of sediment with a clean polyethylene scoop. Place the sediment into the homogenizing jug and repeat the sampling process until there is enough desired sediment. See general procedures for processing of bed sediment samples, once they are collected for sediment homogenization and aliquoting into sample jars.

GENERAL PROCEDURE FOR PROCESSING OF BED SEDIMENT SAMPLES, ONCE THEY ARE COLLECTED

Sediment Homogenization, Aliquoting and Transport

For the collection of bed sediment samples, the top 2 cm is removed from the scoop, or the grab, or the core, and placed in the 4-L glass compositing/homogenizing container. The composited sediment in the container is homogenized and aliquoted on-site in the field. The sample is stirred with a polyethylene scoop until sediment/mud appears homogeneous. All sample identification information (station numbers, etc.) will be recorded prior to homogenizing and aliquoting. Sediment samples will immediately then be cooled to 6 ° C and separated for preservation according to the: Summary of Sample Container, Volume, Preservation, and Storage Requirements for SWAMP Bed Sediment, Biota, and Tissue Samples (for contaminant analysis).Each container will be sealed in one large plastic bag to prevent contact with other samples or ice or water.

Metals and Semi-volatile Organics in Sediment
For trace metals and semi-volatile organics, a minimum of three grabs is distributed to the composite bottle and/or sample containers. Mixing is generally done with a polyethylene scoop. Make sure the sample volume is adequate, but the containers do not need to be filled to the top. Seal the jars with the Teflon liner in the lids.

Sediment Conventionals
Sediment conventionals are sometimes requested when sediment organics, sediment metals, and/or sediment toxicity tests are requested for analysis of samples. The collection method is the same as that for metals, semi-volatile organics, and pesticides. Sediment conventionals include: grain size analysis and total organic carbon. These are used in the interpretation of metals and organics in sediment data.

Sample Containers
See "Sediment Sample Handling Requirements" table at end of this document.

Sediment Sample Size
Must collect sufficient volume of sediment to allow for proper analysis, including possible repeats, as well as any requested archiving of samples for possible later analysis. See "Sediment Sample Handling Requirements" Table at end of this document.

Labeling
Label the jars with the station ID, sample code, matrix type, project ID, time, and date of collection, as well as the type of analysis requested (e.g., metals, conventionals, organics, or archives).

Short-term Field Preservation
Immediately place the labeled jar on ice, cool to 6 ° C, and keep in the dark at 4 ° C until delivery to the laboratory.

Field Notes
Fill out the SWAMP Sediment Data Sheet. Make sure to record any field notes that are not listed on the provided data sheets. This information can be reported as comments with the sediment analytical results.

Summary of Sample Container, Volume, Preservation, and Storage Requirements for SWAMP Bed Sediment, Biota, and Tissue Samples (for contaminant analysis)

Parameters for Analysis	Recommended Containers	Typical Sample Volume (mL)	Initial Field Preservation	Maximum Holding Time

Parameters for Analysis	Recommended Containers	Typical Sample Volume (mL)	Initial Field Preservation	Maximum Holding Time
Bed Sediment Samples				
Trace Metals, including Hg and As (except for Se--see below)	60-mL I-Chem 300- series clear glass jar with Teflon lid-liner; Pre-cleaned	60 mL (one jar)	Cool to ≤6 °C within 24 hours, then freeze to ≤-20 °C	12 months(1) (-20 °C)
Methylmercury	60-mL I-Chem 300- series clear glass jar with Teflon lid-liner; Pre-cleaned	60 mL (one jar)	Freeze to ≤-20 °C immediately	12 months(1) (-20 °C)
Selenium (separate container required)	60-mL I-Chem 300- series clear glass jar with Teflon lid-liner; Pre-cleaned	60 mL (one jar)	Cool to ≤6 °C within 24 hours, then freeze to ≤-20 °C	12 months(1) (-20 °C)
Synthetic Organic Compounds	250-mL I-Chem 300-series amber glass jar with Teflon lid-liner; Pre-cleaned	500 mL (two jars)	Cool to ≤6 °C within 24 hours, then freeze to ≤-20 °C	12 months(1) (-20 °C)
Sediment TOC	250-mL(3) clear glass jar; Pre-cleaned	125 mL (one jar)	Cool to ≤6 ˚C or freeze to ≤-20 ˚C	28 days at ≤6 ˚C; 1 year at ≤-20 ˚C (2)
Sediment Grain Size	250-mL(3) clear glass jar; Pre-cleaned	125 mL (one jar)	Wet ice to ≤6 ˚C in the field, then refrigerate at ≤6 ˚C	1 year (≤6 ˚C) ***Do not freeze***
Sediment Toxicity Testing	1-L I-Chem wide-mouth polyethylene jar with Teflon lid-liner; Pre-cleaned	2 (two jars filled completely)	Cool to 4 °C, dark, up to 14 days	14 days (4 °C) ***Do not freeze***

(1) Sediment samples for parameters noted with one asterisk (*) may be refrigerated at 6 °C for up to 14 days maximum, but analysis must start within the 14-day period of collection or thawing, or the sediment sample must be stored frozen at minus (-) 20 °C for up to 12 months.

(2) Sediment samples for sediment TOC analysis can be held at 4°C for up to 28 days, and should be analyzed within this 28-day period, but can be frozen at any time during the initial 28 days, for up to 12 months at minus (-) 20 °C.

(3) Sediment samples for TOC AND grain size analysis can be combined in one 250 mL clear glass jar, and sub-sampled at the laboratory in order to utilize holding time differences for the two analyses. If this is done, the 250 mL combined sediment sample must be refrigerated only (not frozen) at 4 °C for up to 28 days, during which time the sub-samples must be aliquoted in order to comply with separate storage requirements (as shown above).

SWAMP
Surface Water
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Program

SWAMP Bioassessment Procedures 2007

Standard Operating Procedures for Collecting Benthic Macroinvertebrate Samples and Associated Physical and Chemical Data for Ambient Bioassessments in California

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Standard Operating Procedures for Collecting Benthic Macroinvertebrate Samples and Associated Physical and Chemical Data for Ambient Bioassessments in California

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Preparation Date: January 23rd, 2007

Approved by: Beverly van Buuren, SWAMP QA Officer

Approval Date: 01/17/07

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ACKNOWLEDGEMENTS

The protocols described here represent the contributions of a wide range of researchers and field crews. Most of the physical habitat methods are close modifications of those used in the U.S. Environmental Protection Agency's (EPA's) Environmental Monitoring and Assessment Program (EMAP) and developed by EPA's Office of Research and Development (ORD, Peck et al. 2004). The benthic macroinvertebrate collection methods are based on EMAP methods (EPA's targeted riffle methods were derived in turn from methods developed at Utah State University; Hawkins et al. 2003).

The current version of these protocols was established by Peter Ode (Department of Fish and Game's (DFG's) Aquatic Bioassessment Laboratory (ABL)) and David Herbst (UC Santa Barbara's Sierra Nevada Aquatic Research Laboratory) with significant contributions from staff at the ABL (Jim Harrington, Shawn McBride, Doug Post, Andy Rehn, and Jennifer York), the Surface Water Ambient Monitoring Program (SWAMP) Quality Assurance (QA) Team, Thomas Suk and other members of the SWAMP bioassessment committee (Mary Adams, Lilian Busse, Matt Cover, Robert Holmes, Sean Mundell, and Jay Rowan) and three external reviewers: Chuck Hawkins, Dave Peck, and Phil Kaufmann.

Ode, P.R.. 2007. Standard operating procedures for collecting macroinvertebrate samples and associated physical and chemical data for ambient bioassessments in California. California State Water Resources Control Board Surface Water Ambient Monitoring Program (SWAMP) Bioassessment SOP 001.

SWAMP GUIDANCE SG

SWAMP GUIDANCE FOR MACROINVERTEBRATE FIELD PROTOCOLS FOR WADEABLE STREAMS

Background: The SWAMP Bioassessment Committee met in December, 2004, and agreed that the SWAMP Quality Assurance Management Plan (QAMP) should be amended to provide greater consistency in bioassessment sampling protocols for wadeable streams. The Committee's recommendations were reviewed and accepted by the full SWAMP Roundtable[1] in February, 2005 (some of the key considerations are contained in Appendix A).

The current guidance for macroinvertebrate sampling under the SWAMP program is as follows:

1. For ambient bioassessment monitoring of wadeable streams in California, two methods are to be used at sites with riffle habitats (i.e., one "multihabitat" sample, and one sample that targets the "richest" habitat):
   - **For sites with sufficient riffle habitat,** the two samples shall be: (1) the reachwide benthos (RWB) method (also known as "multihabitat" sampling.); and (2) the targeted-riffle composite (TRC) method.
   - **For low-gradient sites that do not have sufficient riffle habitat,** the RWB method is the standard method, but we also recommend the option of collecting a sample with (2) the "Margin-Center-Margin" (MCM) method until ongoing methods comparisons are completed (see Appendix A).
   - **Notes:** (1) The protocols for each method are provided in this document; (2) Other appropriate method(s) will be allowed if the specific monitoring objectives require use of alternative method(s). (See Item #2, below.); (3) The protocol recommendations specified above will be reevaluated as results become available from ongoing methods comparison studies. (See Appendix A for more information.)

2. The SWAMP QAMP allows flexibility in sampling methods so that the most appropriate method(s) may be used to address hypothesis tests and project-specific objectives that differ from program objectives. Such situations may include, but are not necessarily limited to, special studies (e.g., evaluation of point source discharges, above/below comparisons where statistical replication is needed), stressor identification investigations, and long-term monitoring projects where consistent data comparability is desired and an alternative method is needed to achieve that comparability. In addition, in some rare cases where funding limitations would make it cost-prohibitive to complete a project in compliance with the protocols listed in #1, above, the project proponent may request to complete laboratory analysis of only one sample, and "archive" one of the macroinvertebrate samples (i.e., the RWB sample in streams with riffles) to reduce lab costs. Deviations from the protocols specified in #1 above may be granted by the SWAMP Bioassessment Coordinator or the full SWAMP Roundtable.

1. The SWAMP Roundtable is the coordinating entity for the program. Participants include staff from the State and Regional Water Boards, USEPA, the Department of Fish and Game, the Marine Pollution Studies Laboratory, Moss Landing Marine Laboratories, contractors, and other interested entities.

SECTION 1 INTRODUCTION

This document describes two standard procedures (TRC and RWB) for sampling benthic macroinvertebrate (BMI) assemblages for ambient bioassessments. This document also contains procedures for measuring instream and riparian habitats and ambient water chemistry associated with BMI samples. These sampling methods replace previous bioassessment protocols referred to as the California Stream Bioassessment Procedure (CSBP, Harrington 1995, 1999, 2002).

These procedures can produce quantitative and repeatable measures of a stream's physical/habitat condition and benthic invertebrate assemblages, but they require field training and implementation of QA measures throughout the field season.

The sampling layout described here provides a framework for systematically collecting a variety of physical, chemical, and biological data. The biological sampling methods are designed to nest within the overall framework for assessing the biotic, physical, and chemical condition of a reach. The layout used in these procedures and most of the physical habitat methods are close modifications of those used in EPA's EMAP and developed by EPA's ORD (Peck et al. 2004). Data collected using this methodology are generally directly comparable to equivalent EMAP data, except for the difference in reach length. Other exceptions are noted in the text.

The following steps are presented in an order suggested for efficient data collection. The specific order of collection for the physical parameters may be modified according to preferences of field crews, with the caveat that care must always be taken to not disturb the substrates within the streambed before BMI samples are collected.

PHYSICAL HABITAT METHODS

The physical habitat scoring methods described here can be used as a stand-alone evaluation or used in conjunction with a bioassessment sampling event. However, measurements of instream and riparian habitat and ambient water chemistry are essential to interpretation of bioassessment data and should always accompany bioassessment samples. This information can be used to classify stream reaches, associate physical and chemical condition with biotic condition, and explain patterns in the biological data.

Because bioassessment samples can be collected to answer a variety of questions, this document describes the component measures of instream and riparian habitat as independent modules. Although individual modules can be added or subtracted from the procedure to reflect specific project objectives, a standard set of modules will normally accompany bioassessment samples. This document describes two standard groupings of modules that represent two different levels of intensity for characterizing the chemical and physical habitat data (Table 1). The BASIC physical habitat characterization represents a minimum amount of physical and chemical data that should be taken along with any ambient BMI sample, the FULL physical habitat characterization represents the suite of data that should be collected with most professional level bioassessment samples (e.g., SWAMP regional monitoring programs). In addition to these data, we also briefly introduce additional data modules (e.g., excess sediment, periphyton) that can be collected as supplements to the full set (OPTIONAL). Table 1 lists the physical and chemical variables that should be measured under the different levels.

Note: *SWAMP intends to develop guidance for selecting appropriate physical habitat modules to the intended uses of data. Until this guidance is available, users of these protocols should consult with representatives of the Regional Water Quality Control Boards (Regional Boards) or the SWAMP Bioassessment Coordinator when selecting modules.*

FIELD CREW SIZE AND TIME ESTIMATES

These methods are designed to be completed by either two or three (or more) person field crews. A very experienced field crew can expect to complete the full suite of physical habitat measurements and the two BMI sampling protocols in approximately two hours. Less experienced crews will probably take closer to three or four hours to complete the work depending on the complexity of the reach. Note that this estimate includes only time at the site, not travel time between sites.

Equipment and Supplies

Recommended equipment and supplies are listed in Table 2.

Table 1. Summary of physical habitat and water chemistry and proposal for basic, full, and optional levels of effort.

Survey Task	Parameter(s)	Basic	Full	Option	Comments
REACH DELINEATION and WATER QUALITY [Conducted before entering stream to sample BMIs or conduct any habitat surveys]	Layout reach and mark transects, record GPS coordinates	X	X		Use 150-m reach length if wetted width ≤10 m; Use 250-m reach length if wetted width > 10 m
	Temperature, pH, specific conductance, DO, alkalinity	X	X		Multi-meter (e.g., YSI, Hydrolab, VWR Symphony)
	Turbidity, Silica			X	Use test kit or meter
	Notable field conditions	X	X		Recent rainfall, fire events, dominant local landuse
CROSS-SECTIONAL TRANSECTS BASIC Measurements at main 11 transects only FULL Measurements at 11 main transects (A, B, C, D, E, F, G, H, I, J, K) or 21 transects (11 main plus 10 inter-transects) for substrate size classes only	Wetted width	X	X		Stadia rod is useful here
	Flow habitat delineation	X	X		Record proportion of habitat classes in each inter-transect zone
	Depth and Pebble Count + CPOM		X		5 -point substrate size, depth and CPOM records at all 21 transects
	Cobble embeddedness		X		All cobble-sized particles in pebble count. Supplement with "random walk" if needed for 25
	Slope (%)	See reach scale	X		Average slope calculated from 10 transect to transect slope measurements. Use autolevel for slopes ≤ 1%; clinometer is OK for steeper gradients
	Sinuosity		X		Record compass readings between transect centers
	Canopy cover	X	X		Four densiometer readings at center of channel (facing L bank R bank, Upstream +Downstream)
	Riparian Vegetation		X		Record % or categories
	Instream Habitat		X		
	Human Influence		X		
	Bank Stability	X	X		Eroding / Vulnerable / Stable
	Bankfull Dimensions		X		
	Excess Sediment Transect Measures (optional)				
	Bankfull width and height, bank angles			X	
	Large woody debris counts			X	Tallies of woody debris in several size classes
	Thalweg profile			X	100 equidistant points along thalweg

Survey Task	Parameter(s)	Basic	Full	Option	Comments
DISCHARGE TRANSECT	Discharge measurements		X		Velocity-Area Method or Neutrally Buoyant Object Method
REACH SCALE MEASUREMENTS:	EPA-RBP visual scoring of habitat features	*		X	*Used for citizen monitoring and comparison with legacy data
	Selected RBP visuals:		X		Channel alteration, sediment deposition, epifaunal substrate (redundant if doing EPA-RBP scoring)
	Slope (%, not degrees)	X	See transect scale		Single measurement for entire reach only for BASIC. Use autolevel for slopes ≤ 1%, clinometer is OK for higher gradients
	Photo documentation	X	X		Upstream (A, F, K) Downstream (F)
OTHER OPTIONAL COMPONENTS					
FOOD RESOURCE QUANTIFICATION	Periphyton (3 replicates)			X	Qualitative characterization of diatom growth and filamentous algal growth, quantification of biomass (AFDM, chl-a)
	CPOM & FPOM (3 replicates)			X	CPOM field measure of wet mass >1 mm particles, FPOM as 0.25 – 1 mm fraction (AFDM in lab)

Table 2. Field equipment and supplies

Physical Habitat	BMI Collection	General/ Ambient Chemistry
• GPS receiver • topographic maps • measuring tape (150-m) • small metric ruler or gravelometer for substrate measurements • digital watch, random number table or ten-sided die • stadia rod • clinometer • autolevel (for slopes < 1%) • handlevel (optional) • current velocity meter • stopwatch for velocity measurements • convex spherical densitometer • flags/ flagging tape • rangefinder	• D-frame kick net (fitted with 500-µ mesh bag) • standard # 35 sieve (500-µ mesh) • wide-mouth 500-mL or 1000 mL plastic jars • white sorting pan (enamel or plastic) • 95% EtOH • fine tipped forceps or soft forceps • waterproof paper and tape for attaching labels • 10-20-L plastic bucket for sample elutriation • preprinted waterproof labels (e.g., Rite-in-the-Rain™) • disposable gloves/ elbow length insulated gloves	• sampling SOP (this document) • hip or chest waders, or wading boots/shoes • field forms printed on waterproof paper (e.g., Rite-in-the-Rain™) • clip board and pencils • digital camera • centigrade thermometer • pH meter • DO meter • conductivity meter • field alkalinity meter • water chemistry containers • calibration standards • spare batteries for meters • first aid kit

SECTION 2
REACH DELINEATION AND WATER QUALITY

REACH LAYOUT AND GENERAL DOCUMENTATION

The systematic positioning of transects is essential to collecting representative samples and to the objective quantification of physical habitat measures. The standard sampling layout consists of a 150-m reach (length measured along the bank) divided into 11 equidistant transects that are arranged perpendicular to the direction of flow (Figure 1, Figure 2). Ten additional transects (designated "inter-transects") located between the main transects give a total of 21 transects per reach. Main transects are designated A through K while inter-transects are designated by their nearest upstream and downstream transects (e.g., AB, BC, etc.). In extreme circumstances, reach length can be shorter than 150 m (e.g., if upstream and downstream barriers preclude a 150-m reach), but this should be avoided whenever possible. If the actual reach length is other than 150 m or 250 m this should be noted and explained on the field forms.

Note 1: *The standard reach length differs from that used in the EMAP design, in which reach length was defined as 40x stream width, with a minimum reach length of 150 m. The EMAP reach length approach is used to ensure that enough habitat is sampled to support accurate fish assemblage estimates and relatively precise characterization of channel characteristics (e.g., residual pool volumes and woody debris estimates, which that are critical for relative bed stability estimates). Programs wishing to sample fish assemblages or produce relative bed stability estimates should strongly consider adopting the EMAP guidance for setting reach length.*

Note 2: *Streams > 10 m wetted width should use a reach length of 250 m. Some very large streams (i.e., > 20-m wetted width) may not be adequately represented even by a 250-m reach. In these cases, field crews should define a reach length that is representative of the larger stream segment being studied (i.e., attempt to include two to three meander cycles, or four to six riffle-pool sequences when possible).*

Note 3: *When the exact reach location is not restricted by the sampling design, attempt to position reaches upstream of bridges to avoid this influence.*

Step 1. Upon arrival at the sampling site, fill out the reach documentation section of the field forms (site and project identification, stream and watershed name, crew members, and date/time). If known at the time of sampling, record the Site Code following SWAMP site code formats. Determine the geographic coordinates of the downstream end of the reach (preferably in decimal degrees to at least four decimal places) with a GPS receiver and record the datum setting of the unit (preferably NAD83/ WGS84).

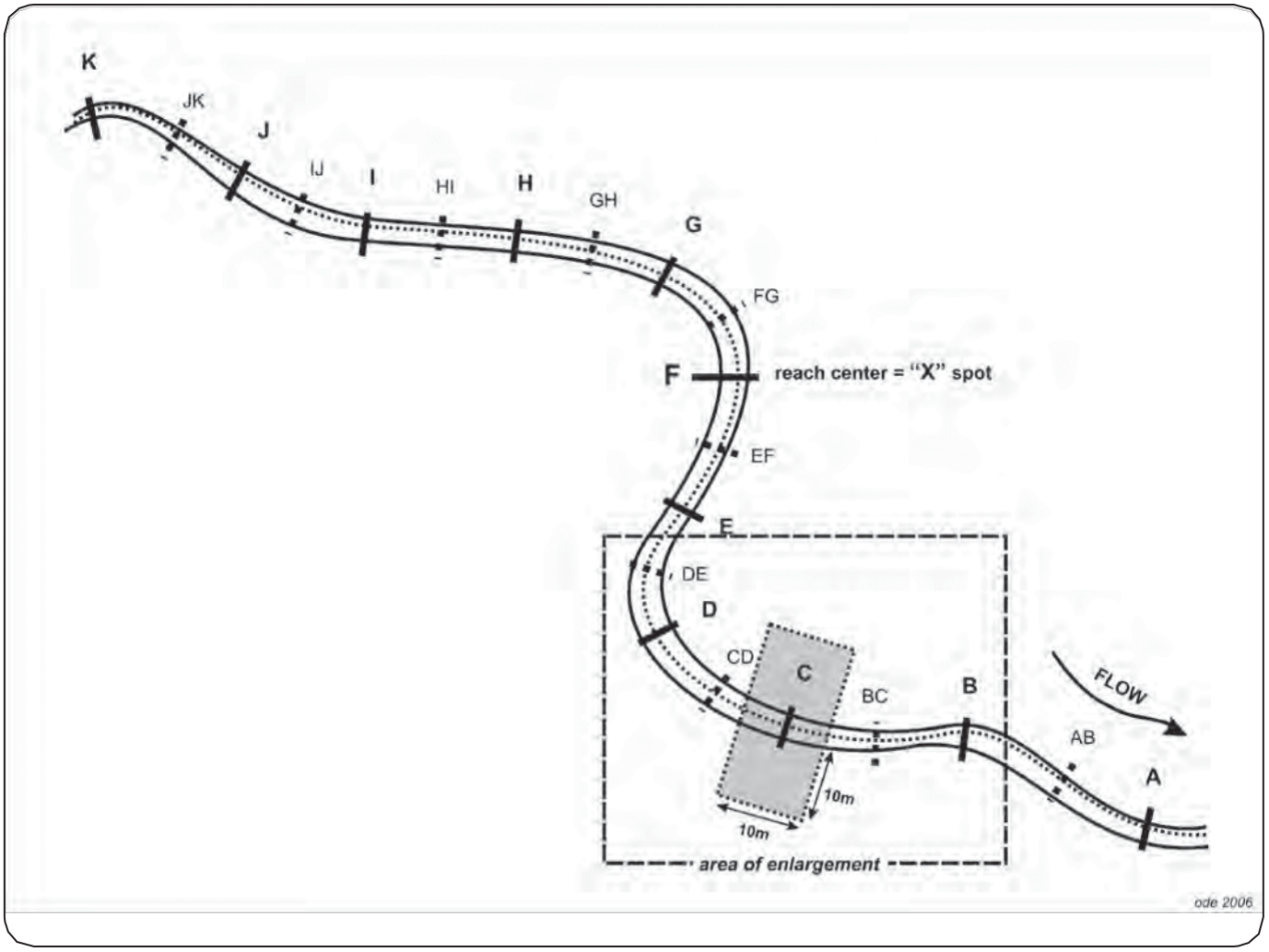

Figure 1. Reach layout geometry for physical habitat and biological sampling showing positions of 11 main transects (A – K) and the 10 supplemental inter-transects (AB- JK). The area highlighted in the figure is expanded in Figure 2. Note: reach length = 150 m for streams ≤ 10-m average wetted width, and reach length = 250 m for streams > 10-m average wetted width.

Step 2. Once a site has been identified, make an initial survey of the reach from the stream banks (being sure to not disturb the instream habitat). If TRC samples will be collected, identify all riffle habitats suitable for sampling (see Section IIIa for suitable habitat types) and note their positions so that a subset can be identified for sampling.

Step 3. Determine if the average wetted width is greater or less than 10 m. If the average wetted width ≤ 10 m, use a 150-m reach length. If the average wetted width > 10 m, use a 250-m reach length.

Step 4. Starting at one end of the reach, establish the position of the 11 main transects (labeled A-K from downstream to upstream) by measuring 15 m (25 m for streams > 10 m wetted width) along the bank from the previous transect. The 10 inter-transects should be established equidistant from the adjacent main transects (i.e., 7.5 m from main transects for 150-m reaches, 12.5 m for 250-m reaches). Since the data collection will start at the downstream end, is often easiest to establish transects starting from the upstream end. For easy setup and breakdown, mark the main transects with easily removable markers (e.g., large washers tied with strips of flagging, surveyor's flags).

Note 1: *While it is usually easiest to establish transect positions from the banks (this also reduces disturbance to the stream channel), this can result in uneven spacing of transects in complex stream reaches. To avoid this, estimate transect positions by projecting from the mid-channel to the banks.*

Note 2: *Flagging of a single bank is recommended to reduce mistakes caused by missed markers.*

Step 5. Measure and record common ambient water chemistry measurements (pH, DO, specific conductance, alkalinity, water temperature) at the downstream end of the reach (near same location as the GPS coordinates were taken). These are typically taken with a handheld water quality meter (e.g., YSI, Hydrolab), but field test kits (e.g., Hach) can provide acceptable information if they are properly calibrated. For appropriate calibration methods and calibration frequency, consult the current SWAMP QAMP (Appendix F), or follow manufacturer's guidelines.

Note 1: *If characteristics of the site prohibit downstream entry, measurements may be taken at other points in the reach. In all cases, ambient chemistry measurements should be taken at the beginning of the reach survey.*

Note 2: *Alkalinity test kits may not perform well in low ionic strength waters. Programs should consider collecting lab samples for these sites (see SWAMP QAMP for guidance on collecting water chemistry samples).*

Step 6. Take a minimum of four (4) photographs of the reach at the following locations: a) Transect A facing upstream, b) Transect F facing upstream, c) Transect F facing downstream, and d) Transect K facing downstream. It may also be desirable to take a photograph at Transect A facing downstream and Transect K facing upstream to document conditions immediately adjacent to the reach. Digital photographs should be used when possible. Record the image numbers on the front page of the field form.

Note 1: *When possible, photograph names should follow SWAMP coding conventions ("StationCode_yyyy_mm_dd_uniquecode"). The unique code should include one of the following codes to indicate direction: RB (right bank), LB (left bank), BB (both banks), US (upstream), DS (downstream). SWAMP suggests using unique codes created by the camera to facilitate file organization. Example: 603WQLB02_2004_03_20_RBDS1253.*

Step 7. Record the dominant land use and land cover in the area surrounding the reach (evaluate land cover within 50 m of either side of the stream reach).

Step 8. At the bottom of the form, record evidence of recent flooding, fire, or other disturbances that might influence bioassessment samples. Especially note if flow conditions have been affected by recent rainfall, which can cause significant under-sampling of BMI diversity (see note in the following section). If you are unaware of recent fire or rainfall events, select the "no" option on the forms.

SECTION 3
COLLECT BENTHIC MACROINVERTEBRATES

MULTIPLE HABITAT AND TARGETED RIFFLE PROTOCOLS

Note 1: *BMI samples intended for ambient bioassessments are generally collected when streams are at or near base flow (i.e., not influenced by surface runoff) as sudden flow increases can dramatically alter local community composition.*

Note 2: *Guidance for choosing among TRC sampling, RWB sampling or both will be provided in a separate document (see Appendix A for current guidance for sampling under SWAMP).*

Once the reach transects have been laid out, the biological samples (BMIs and algae if included) should be collected before any other physical habitat measures so that substrates are not disturbed prior to sampling. Both TRC and RWB methods use 500-µ mesh D-frame nets (see list of BMI sampling equipment in Table 2). The two samples can be collected at the same time by carrying two D-nets and compositing the material from the two samples in their respective nets. If a two person field crew is responsible for both the physical habitat data and benthic invertebrate samples, it is generally best to collect the benthos at each transect, then immediately record the physical habitat data before moving to the next transect. Obviously, this requires especially careful handling of the D-nets during the course of sampling to avoid loss or contamination of the samples. It can be helpful to clearly label the two D nets as RWB and TRC. Larger field crews may choose to split the sampling between biological team and a physical habitat team and have the biological team go through the reach first. The positions of the TRC and RWB subsampling locations are illustrated in Figure 2.

SECTION III A. TARGETED RIFFLE COMPOSITE PROCEDURE

The TRC method is designed for sampling BMIs in wadeable streams that contain fast-water (riffle/run) habitats and is not appropriate for waterbodies without fastwater habitats. The RWB protocol should be used in these situations. Riffles are often used for collecting biological samples (e.g., the old CSBP methods) because they often have the highest BMI diversity in wadeable streams. This method expands the definition to include other fast water habitats, however care should be taken when attempting to apply this method in low gradient streams.

Note: *Since all streams (even low gradient streams) have variation in flow habitats within the channel, this guidance should not be interpreted as including areas within low gradient streams that are only marginally faster than the surrounding habitats. The RWB protocol should be applied in these situations.*

The TRC was developed by the Western Center for Monitoring and Assessment of Freshwater Ecosystems (www.cnr.usu.edu/wmc) in Logan, Utah (Hawkins et al. 2003) and slightly modified by the EPA program (Peck et al. 2004). The TRC has been widely used in California (US Forest Service (USFS), the EMAP Western Pilot, and the California Monitoring and Assessment Program (CMAP)), and in the interest of methodological consistency between state and federal water resource agencies, has been adopted as the standard riffle protocol for bioassessment in California. The version described here is the EMAP modification, which distributes the sampling effort throughout the reach.

Sampling Locations - Acceptable Habitat Types

Riffles are the preferred habitat for TRC sampling, but other fast water habitats are acceptable for sampling if riffles are sparse. Common flow-defined habitat types are listed in Table 3 in decreasing order of energy. Most streams contain some or all of the following fast water habitat types: 1) cascades/falls, 2) rapids, 3) riffles, 4) runs. All of these are acceptable for TRC sampling if riffles are not available.

Note: *Because the common habitat types are arranged on a continuum between high to low energy environments, the categories grade into each other continuously and are not discrete. Thus, determination of habitat types requires somewhat subjective decision-making.*

Table 3. Common habitat types in stream channels, arranged in decreasing order of energy

Flow Habitat Type	Description
Cascades	Short, high gradient drop in stream bed elevation often accompanied by boulders and considerable turbulence
Falls	High gradient drop in elevation of the stream bed associated with an abrupt change in the bedrock
Rapids	Sections of stream with swiftly flowing water and considerable surface turbulence. Rapids tend to have larger substrate sizes than riffles
Riffles	Shallow sections where the water flows over coarse stream bed particles that create mild to moderate surface turbulence; (< 0.5 m deep, > 0.3 m/s)
Step-Runs	A series of runs that are separated by short riffles or flow obstructions that cause discontinuous breaks in slope
Runs	Long, relatively straight, low-gradient sections without flow obstructions. The stream bed is typically even and the water flows faster than it does in a pool; (> 0.5 m deep, > 0.3 m/s)
Glides	A section of stream with little or no turbulence, but faster velocity than pools; (< 0.5 m deep, < 0.3 m/s)
Pools	A reach of stream that is characterized by deep, low-velocity water and a smooth surface ; (> 0.5 m deep, < 0.3 m/s)

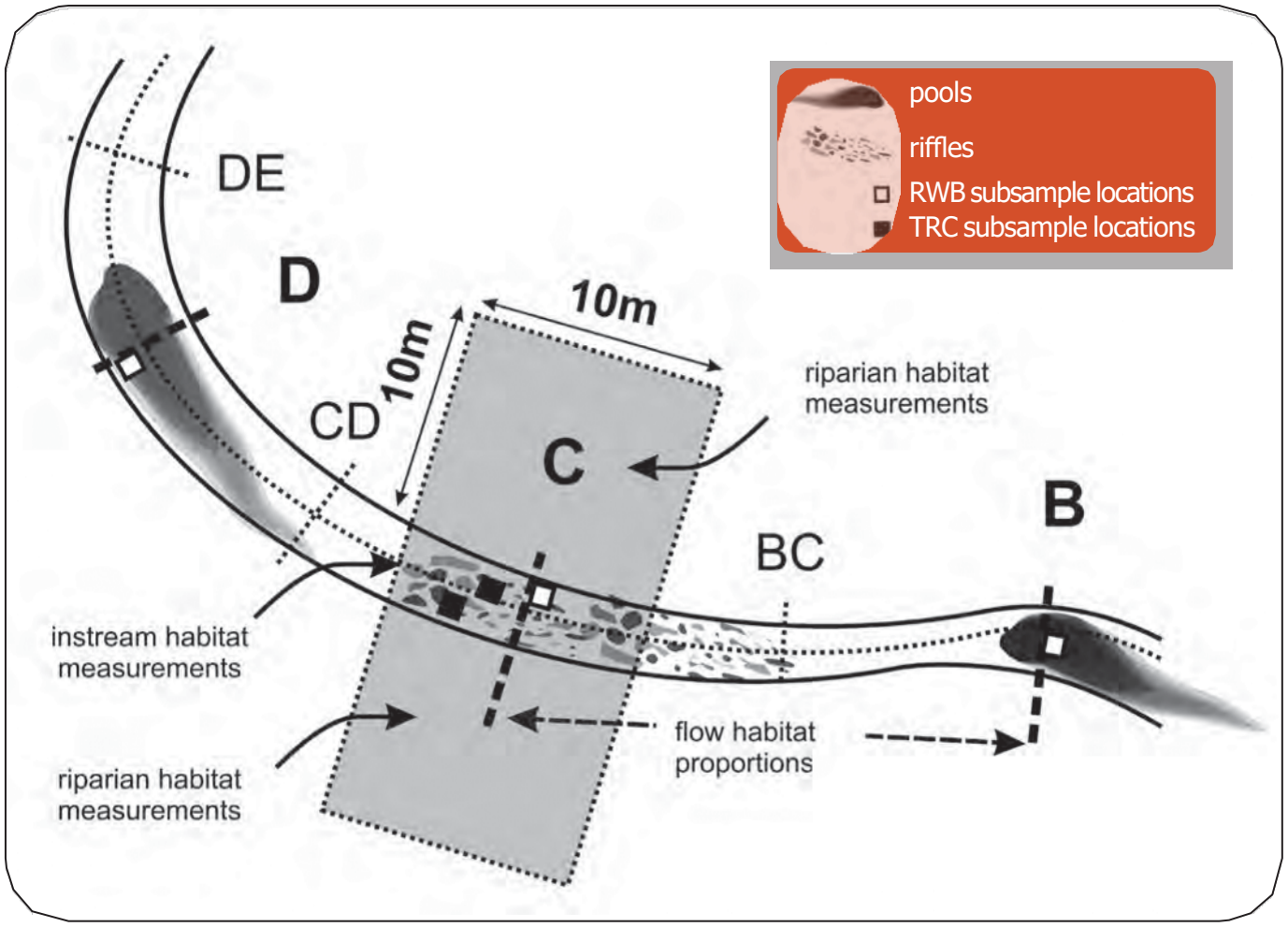

Figure 2. Section of the standard reach expanded from Figure 1 showing the appropriate positions for collecting benthic macroinvertebrate samples, instream and riparian habitat measurements and flow habitat proportion measurements.

Sampling Locations - Selecting Habitat Units

A TRC sample is a composite of eight individual kick samples of 1 ft^2 (0.09 m^2) of substrate each. During your initial layout of the reach, take a mental note of the number and position of the main riffles in a reach (and other fast water habitats if needed). Randomly distribute the eight sub-samples among the fast water habitats in the reach, giving preference to riffles where possible. Unless you are sampling in small streams, try to avoid very small riffle units (i.e., <5 ft^2). If fewer than eight riffles are present in a reach, more than one sample may be taken from a single riffle, especially if the riffles are large.

Sampling Procedure

Begin sampling at the downstream end of the reach at the first randomly selected riffle and work your way upstream.

TRC-Step 1. Determine net placement within each habitat unit by generating a pair of random numbers between 0 and 9. Examples of convenient random number generators include the hundredths place on the stopwatch feature of a digital watch, a 10 sided die and a random number chart. The first number in each pair (multiplied by 10) represents the percent upstream along the habitat unit's length. The second number in each pair represents the percent of the riffle width from right bank. For example, if the two generated random numbers are 4 and 7, you will walk upstream 40% of the distance of the riffle and then go 70% of the distance across the riffle (see Figure 3). This position is the center of the 1 ft^2 (0.09 m^2) sampling quadrat for that riffle. If you are unable to sample this location because it is too deep or it is occupied by a large boulder, select a new pair of random numbers and pick a new spot.

TRC-Step 2. Position a 500-µ D-net (with the net opening perpendicular to the flow and facing upstream) quickly and securely on the stream bottom to eliminate gaps under the frame. Avoid, and if necessary remove, large rocks that prevent the sampler from seating properly on the stream bottom.

TRC-Step 3. Holding the net in position on the substrate, visually define a square quadrat that is one net width wide and one net width long upstream of the net opening. Since D-nets are 12 inches wide, the area within this quadrat is 1ft^2 (0.09 m^2). Restrict your sampling to within that area. If desired, a wire frame of the correct dimensions can be placed in front of the net to help delineate the quadrat to be sampled, but it is often sufficient to use the net dimensions to keep the sampling area consistent.

TRC-Step 4. Working backward from the upstream edge of the sampling plot, check the quadrat for heavy organisms such as mussels, snails, and stone-cased caddisflies. Remove these organisms from the substrate by hand and place them into the net. Carefully pick up and rub stones directly in front of the net to remove attached animals. Remove and clean all of the rocks larger than a golf ball (~3 cm) within your sampling quadrat such that all the organisms attached to them are washed downstream into your net. Set these rocks outside your sampling quadrat after you have cleaned them. If the substrate is consolidated or comprised of large, heavy rocks, use your feet to kick and dislodge the substrate to displace BMIs into the net. If you cannot remove a rock from the stream bottom, rub it (concentrating on cracks or indentations) thereby loosening any attached insects. As you are disturbing the plot, let the water current carry all loosened material into the net.

Note 1: *Brushes are sometimes used in other bioassessment protocols to help loosen organisms, but in the interest of standardizing collections, do not use a brush when following this protocol.*

Note 2: *In sandy-bottomed streams, kicking within run habitats can quickly fill the sampling net with sand. In these situations, follow the standard procedures but use care to disturb the substrate gently and avoid kicking.*

TRC-Step 5. Once the coarser substrates have been removed from the quadrat, dig your fingers through the remaining underlying material to a depth of about 10 cm (this material is often comprised of gravels and finer particles). Thoroughly manipulate the substrates in the quadrat.

Note: *The sampler may spend as much time as necessary to inspect and clean larger substrates, but should take a standard time of 30 seconds to perform Step 5.*

TRC-Step 6. Let the water run clear of any insects or organic material before carefully lifting the net. Immerse the net in the stream several times to remove fine sediments and to concentrate organisms at the end of the net, but be careful to avoid having any water or foreign material enter the mouth of the net during this operation.

TRC-Step 7. Move upstream to the next randomly selected habitat unit and repeat steps one through six, taking care to keep the net wet but uncontaminated by foreign material when moving the net from riffle to riffle. Sometimes, the net will become so full of material from the streambed that it is no longer effective at capturing BMIs. In these cases, the net should be emptied into sample jars as frequently as necessary, following guidelines described below in the "Preparation of BMI Sample Jars" section. Continue until you have sampled eight $1ft^2$ ($0.09\ m^2$) of benthos.

TRC-Step 8. PROCEED to Section IIIc. Filling and Labeling BMI Sample Jars.

Figure 3. Example showing the method for selecting a subsampling position within a selected riffle under the TRC method. In this example, the random numbers 4 and 7 were selected

SECTION III B. REACHWIDE BENTHOS (MULTIHABITAT) PROCEDURE

The RWB procedure employs an objective method for selecting subsampling locations that is built upon the 11 transects used for physical habitat measurements. The RWB procedure can be used to sample any wadeable stream reach since it does not target specific habitats. Because sampling locations are defined by the transect layout, the position of individual sub-samples may fall in a variety of erosional or depositional habitats.

Note: *Sampling locations should be displaced one meter downstream of the transects to avoid disturbing substrates for subsequent physical habitat assessments.*

RWB -Step 1. The sampling position within each transect is alternated between the left, center and right positions along a transect (25%, 50% and 75% of wetted width, respectively) as you move upstream from transect to transect. Starting with the downstream transect (Transect

A), identify a point that is 25% of the stream width from the right bank (note that the right bank will be on your left as you face upstream). If you cannot collect a sample at the designated point because of deep water obstacles or unsafe conditions, relocate the point as close as possible to the designated position.

Note: *A modification to this procedure is currently being investigated by SWAMP. This "margin-center-margin" (MCM) modification replaces the samples at 25% and 75% of wetted width with samples of the marginal habitats (including emergent and submergent vegetation).*

RWB -Step 2. Place a 500-µ D-net in the water so the mouth of the net is perpendicular to and facing into the flow of the water. If there is sufficient current in the area at the sampling point to fully extend the net, use the normal D-net collection technique to collect the sub-sample (TRC-Step 3 through TRC-Step 6 above). If flow volume and velocity is not sufficient to use the normal collection technique, use the sampling procedure for "slack water" habitats (RWB-Step 3 through RWB-Step 7 below).

RWB -Step 3. Visually define a 1 ft^2 (0.09 m^2) quadrat that is one net-width wide and one net-width long at the sampling point.

RWB -Step 4. Working backward from the upstream edge of the sampling plot, check the quadrat for heavy organisms such as mussels and snails. Remove these organisms from the substrate by hand and place them into the net. Carefully pick up and rub stones directly in front of the net to remove attached animals. Remove and clean all of the rocks larger than a golf ball within your sampling quadrat such that all the organisms attached to them are washed downstream into your net. Set these rocks outside your sampling quadrat after you have cleaned them. Large rocks that are less than halfway into the sampling area should be pushed aside. If the substrate is consolidated or comprised of large, heavy rocks, use your feet to kick and dislodge the substrate to displace BMIs into the net. If you cannot remove a rock from the stream bottom, rub it (concentrating on cracks or indentations) thereby loosening any attached insects.

RWB -Step 5. Vigorously kick the remaining finer substrate within the quadrat with your feet while dragging the net repeatedly through the disturbed area just above the bottom. Keep moving the net all the time so that the organisms trapped in the net will not escape. Continue kicking the substrate and moving the net for 30 seconds. For vegetation-choked sampling points, sweep the net through the vegetation within a 1ft^2 (0.09 m^2) quadrat for 30 seconds.

Note: *If flow volume is insufficient to use a D- net, spend 30 seconds hand picking a sample from 1ft^2 of substrate at the sampling point, then stir up the substrate with your gloved hands and use a sieve with 500-µ mesh size to collect the organisms from the water in the same way the net is used in larger pools.*

RWB -Step 6. After 30 seconds, remove the net from the water with a quick upstream motion to wash the organisms to the bottom of the net.

RWB -Step 7. PROCEED to Section IIIc: Filling and Labeling BMI Sample Jars

SECTION III C. FILLING AND LABELING BENTHIC MACROINVERTEBRATE SAMPLE JARS

Step 1. Once all sub-samples (eight for TRC, 11 for RWB) have been collected, transfer benthos to a 500-mL or 1000-mL wide-mouth plastic sample jar using one of the following methods.

Note: *Field elutriation should only be used by well-trained field crews who are proficient at removing all benthic organisms from the discarded inorganic material. Training in the recognition of aquatic invertebrates is highly recommended.*

Step 1a. Complete Transfer of all Sampled Material – Invert the contents of the kick net into the sample jar. Perform this operation over a white enameled tray to avoid loss of any sampled material and make recovery of spilled organisms easier. If possible, remove the larger twigs and rocks by hand after carefully inspecting for clinging organisms, but be sure not to lose any organisms. Use forceps to remove any organisms clinging to the net and place these in the sample jar.

Step 1b. Field Elutriation of Samples – Empty the contents of the net into a large plastic bucket (10-20 L is sufficient). Use forceps to remove any organisms clinging to the net and place these in the bucket. Add stream water to the bucket and gently swirl the contents of the bucket in order to suspend the organic material (being certain to not introduce entrained organisms from the source water). Pour the organic matter from the bucket through a 500-µ sieve (or use the 500-µ net). Repeat this process until no additional material can be elutriated (i.e., only inorganic material is left in the bucket). If possible, remove the larger twigs and rocks by hand after carefully inspecting for clinging organisms, but be sure not to lose any organisms. Transfer all of the material in the sieve (invertebrates and organic matter) into the sample jar. Carefully inspect the gravel and debris remaining in the bottom of the bucket for any cased caddisflies, clams, snails, or other dense animals that might remain. Remove any remaining animals by hand and place them in the sample jar.

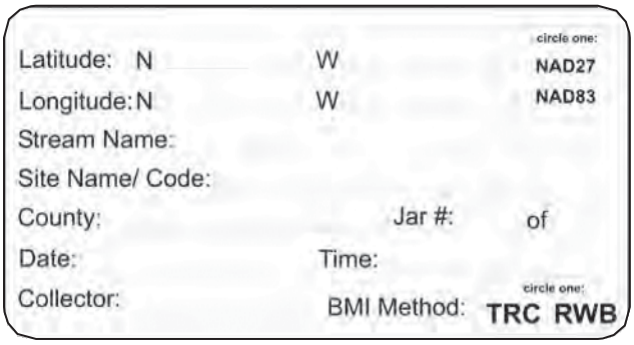

Figure 4. Example date - locality label for all BMI samples.

Step 2. Place a completed date/locality label (see Figure 4) on the inside of the jar (use pencil only as most "permanent" inks dissolve in ethanol) and completely fill with 95% ethanol. Place a second label on the outside of the jar. Note that the target concentration of ethanol is 70%, but 95% ethanol is used in the field to account for dilution from water in the sample. If organic and inorganic material does not accumulate in the net quickly, it may be possible to transfer all the material in the net into one jar. Otherwise, divide the material evenly among several jars

(being careful to clearly label them as part of a set). To ensure proper preservation of benthic macroinvertebrates it is critical that the ethanol is in contact with the BMIs in the sample jar. Never fill a jar more than 2/3 full with sampled material, and gently rotate jars that contain mostly mud or sand to ensure that the ethanol is well distributed. If jars will be stored for longer than a month prior to processing, jars should not contain more than 50% sample material.

SECTION 4
MAIN CROSS-SECTIONAL TRANSECT MEASURES

SECTION IVA. PHYSICAL MEASURES

The majority of physical habitat measurements in this protocol are made relative to the main cross-sectional transects (Figure 5). All the measures taken relative to each transect are recorded on forms specific to that transect. Start with the downstream transect (Transect A) and repeat steps 6-15 for all 11 main transects.

Module A. Transect Dimensions: Wetted Width and Bankfull Dimensions

Wetted Width – The wetted channel is the zone that is inundated with water and the wetted width is the distance between the sides of the channel at the point where substrates are no longer surrounded by surface water. Measure the wetted stream width and record this in the box at the top of the transect form.

Bankfull Width and Depth – The bankfull channel is the zone of maximum water inundation in a normal flow year (one to two year flood events). Since most channel formation processes are believed to act when flows are within this zone (Mount 1995), bankfull dimensions provide a valuable indication of relative size of the waterbody.

Note: *Bankfull dimensions are notoriously difficult to assess, even by experienced field crews (see Heil and Johnson 1995). It is often useful to discuss the interpretation of bankfull locations among the field crew members to reach a consensus. The USFS Stream Team provides a good set of instructional videos for improving consistency in accurate bankfull measurements (http://www.stream.fs.fed.us/publications/videos.html).*

Step 1. Scout along the stream margins to identify the location of the bankfull margins on either bank by looking for evidence of annual or semi-annual flood events. Examples of useful evidence includes topographic, vegetative, or geologic cues (changes in bank slope, changes from annual to perennial vegetation, changes in the size distribution of surface sediments). While the position of drift material caught in vegetation may be a helpful aid, this can lead to very misleading measurements.

Note: *The exact nature of this evidence varies widely across a range of stream types and geomorphic characteristics. It is helpful to investigate the entire reach when attempting to interpret this evidence because the true bankfull margin may be obscured at various points along the reach. Often the bankfull position is easier to interpret from one bank than the other; in these cases, it is easiest to infer the opposite bank position by projecting across the channel. Additionally, height can be verified by measuring the height from both edges of the wetted channel to the bankfull height (these heights should be equal).*

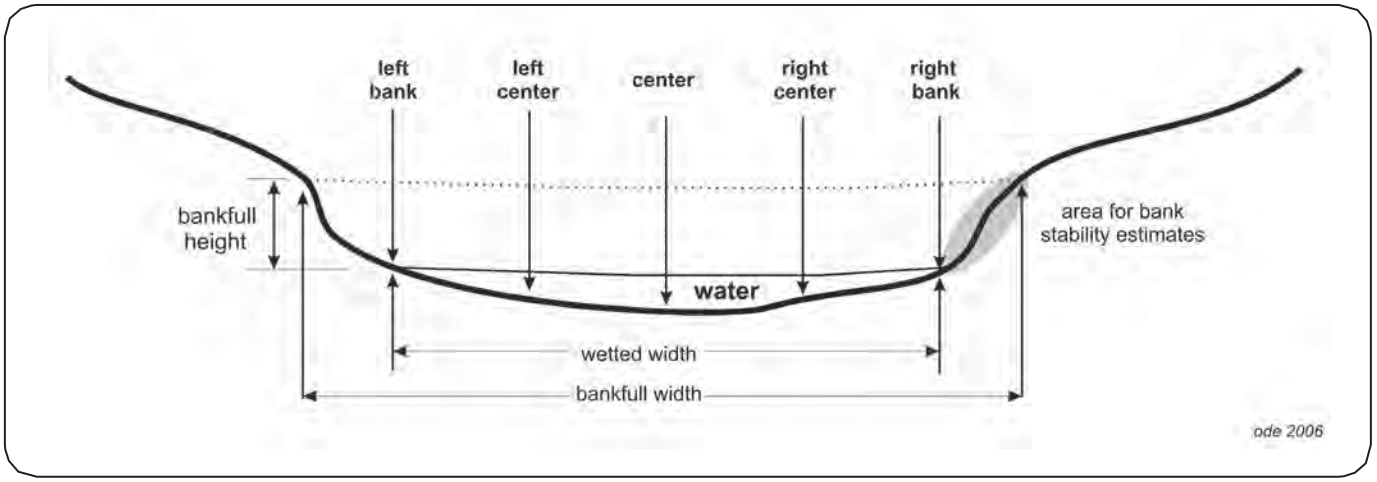

Figure 5. Cross sectional diagram of a typical stream channel showing locations of substrate measurements, wetted and bankfull width measurements, and bank stability visual estimates.

Step 2. Stretch a tape from bank to bank at the bankfull position. Measure the width of the bankfull channel from bank to bank at bankfull height and perpendicular to the direction of stream flow.

Step 3. Measure bankfull height (the vertical distance between the water height of the water and the height of the bank, Figure 5) and record.

Module B. Transect Substrate Measurements

Particle size frequency distributions often provide valuable information about instream habitat conditions that affect BMI distributions. The Wolman pebble count technique (Wolman 1954) is a widely used and cost-effective method for estimating the particle size distribution and produces data that correlates with costly, but more quantitative bulk sediment samples. The method described here follows the EMAP protocol, which records sizes of 105 particles in a reach (five particles from each of 11 main transects and 10 inter-transects).

Note: *The size cutoff for the finest particle sizes in the EMAP protocol (<0.06 mm) differs from that used by the Sierra Nevada Aquatic Research Laboratory (SNARL) program (0.25 mm), although the narrative description for this cutoff is the same (the point at which fine particles rubbed between one's fingers no longer feel gritty).*

Coarse particulate organic matter (CPOM, particles of decaying organic material such as leaves that are greater than 1.0 mm in diameter) is a general indicator of the amount of allochthonous organic matter available at a site, and its measurement can provide valuable information about the basis of the food web in a stream reach. The presence of CPOM associated with each particle is quantified at the same time that particles are measured for the pebble counts.

Step 1. Transect substrate measurements are taken at five equidistant points along each transect (Figure 5). Divide the wetted stream width by four to get the distance between the five points (Left Bank, Left Center, Center, Right Center and Right Bank) and use a measuring device to locate the positions of these points (a stadia rod is especially helpful here). Once the positions are identified, lower a graduated rod (e.g., a marked ski pole) though the water column perpendicular to both the flow and the transect to objectively select the particle located at the tip of the rod.

Step 2. Measure the depth from the water surface to the top of the particle with the graduated rod and record to the nearest cm.

Step 3. Record the presence or absence of CPOM >1mm within 1 cm of the particle.

Step 4. If the particle is cobble-sized (64-250 mm), record the percent of the cobble that is embedded by fine particles (<2 mm) to the nearest 5% (see cobble embeddedness text below).

Step 5. Remove the particle from the streambed, then measure and record the length of its intermediate axis to the nearest mm (see Figure 6). Alternatively, assign the particle to one of the size classes listed in the bottom of the transect form. Particle sizes classes can be estimated visually or with a quantitative measuring device (e.g., pass/ no-pass template, "gravelometer"). Regardless of the method, all particles less than 0.06 mm should be recorded as fines, all particles between 0.06mm and 2.0 mm recorded as sand. Field crews may want to carry vials containing sediment particles with these size ranges until they are familiar with these particles.

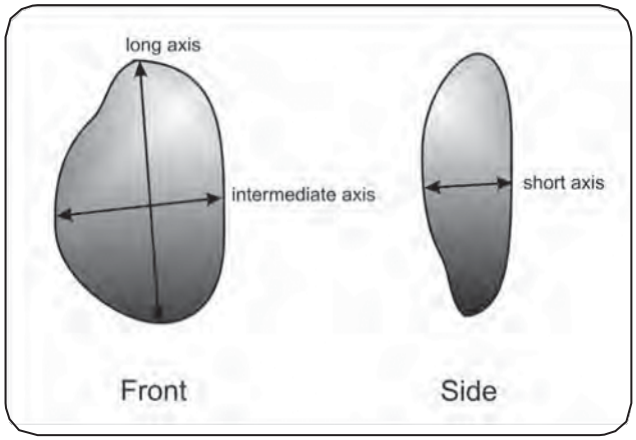

Figure 6. Diagram of three major perpendicular axes of substrate particles. The intermediate axis is recorded for pebble counts.

Module C. Cobble Embeddedness

The quantification of substrate embeddedness has long been a challenge to stream geomorphologists and ecologists (Klamt 1976, Kelley and Dettman 1980). It is generally agreed that the degree to which fine particles fill interstitial spaces has a significant impact on the ecology of benthic organisms and fish, but techniques for measuring this impact vary greatly (this is summarized well by Sylte and Fischenich 2002, http://stream.fs.fed.us/news/streamnt/pdf/StreamOCT4.pdf). Here we define embeddedness as the volume of cobble-sized particles (64-250 mm) that is buried by fine particles (<2.0 mm diameter).

Note: *This method differs from the EMAP method for measuring embeddedness, which measures embeddedness of all particles larger than 2 mm.*

Table 4. Size class codes and definitions for particle size measurements

Size Class Code	Size Class Description	Common Size Reference	Size Class Range
RS	bedrock, smooth	larger than a car	> 4 m
RR	bedrock, rough	larger than a car	> 4 m
XB	boulder, large	meter stick to car	1 - 4 m
SB	boulder, small	basketball to meter stick	25 cm - 1.0 m
CB	cobble	tennis ball to basketball	64 - 250 mm
GC	gravel, coarse	marble to tennis ball	16 - 64 mm
GF	gravel, fine	ladybug to marble	2 – 16 mm
SA	sand	gritty to ladybug	0.06 – 2 mm
FN	fines	not gritty	< 0.06 mm
HP	hardpan (consolidated fines)		< 0.06 mm
WD	wood		
RC	concrete/ asphalt		
OT	other		

Step 1. Every time a cobble-sized particle is encountered during the pebble count, remove the cobble from the stream bed and visually estimate the percentage of the cobble's volume that has been buried by fine particles. Since visual estimates of volume and surface area are subject to large amounts of observer error, field crews should routinely calibrate their estimates with each other and with other field crews.

Step 2. In the spaces to the right of the pebble count data, record the embeddedness of all cobble-sized particles encountered during the pebble count.

Note: *The cobble embeddedness scores do not correspond with the specific particles in the pebble count cells to the left, but are merely a convenient place to record the data.*

Step 3. If 25 cobbles are not encountered during the pebble count, supplement the cobbles by conducting a "random walk" through the reach. Starting at a random point in the reach, follow a transect from one bank to the other at a randomly chosen angle. Once at the other bank reverse the process with a new randomly chosen angle. Record embeddedness of cobble-sized particles in the cobble embeddedness boxes on the transect forms until you reach 25 cobbles. If 25 cobble-sized particles are not present in the entire reach, then record the values for cobbles that are present.

Module D. Canopy Cover

This method uses the Strickler (1959) modification of a convex spherical densiometer to correct for over-estimation of canopy density that occurs with unmodified readings. Read the densiometer by counting the number of line intersections that are obscured by overhanging vegetation (see Figure 7). Taping off the lower left and right portions of the mirror emphasizes overhead vegetation over foreground vegetation (the main source of bias in canopy density measurements). All densiometer readings should be taken with the bubble leveled and 0.3 m (1 ft) above the water surface.

Step 1. Using a modified convex spherical densitometer, take and record four 17-point readings all taken from the center of each transect: a) facing upstream, b) facing downstream, c) facing the left bank, d) facing the right bank.

Note: *This method deviates slightly from that of EMAP (in which two additional readings are taken at the left and right wetted edges to increase representation of bank vegetation).*

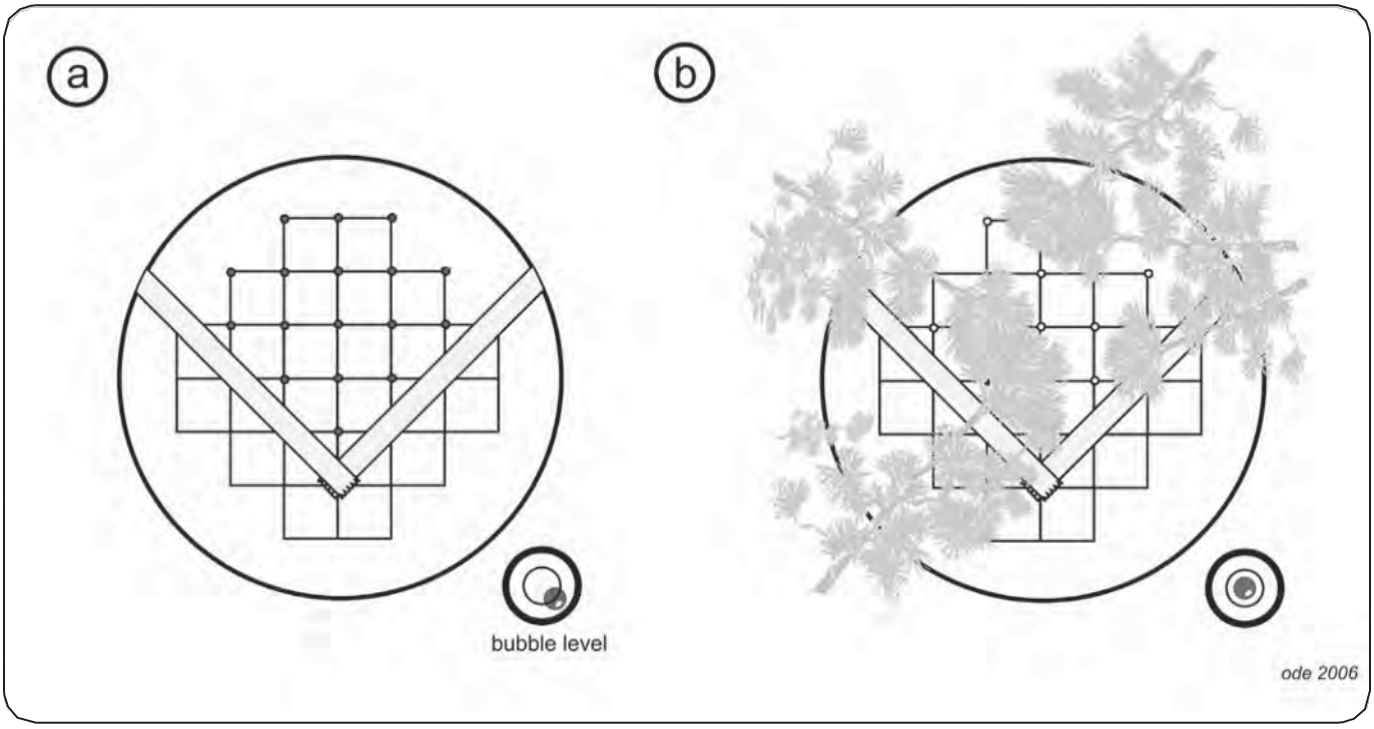

Figure 7. Representation of the mirrored surface of a convex spherical densiometer showing the position for taping the mirror and the intersection points used for the densiometer reading. The score for the hypothetical condition in (b) is 10 covered intersection points out of 17 possible. Note the position of the bubble level in (b) when the densiometer is leveled.

Module E. Gradient and Sinuosity

The gradient of a stream reach is one of the major stream classification variables, giving an indication of potential water velocities and stream power, which are in turn important controls on aquatic habitat and sediment transport within the reach. The gradient (slope) of a stream reach is often strongly correlated with many BMI metrics and other physical habitat measures and is therefore very useful when interpreting BMI data.

The "full" physical habitat method uses 10 transect to transect measurements to calculate the average slope through a reach. Although this is a little more time intensive than the reach-scale transect measures used in the "basic" protocol, it results in more precise slope determination and the ability to quantify slope variability within a reach. Sinuosity (calculated as the ratio of the length of the flow path between the ends of the reach and the straight line distance between the ends of the reach, Kaufmann et al. 1999) is measured at the same time as slope. These two measurements work best with two people, one taking the readings at the upstream transect ("backsighting") and the other holding a stadia rod at the downstream transect. If you cannot see the mid point of the next transect from the starting point, use the supplemental sections (indicating the proportion of the total length represented by each section). Otherwise, leave these blank.

Note 1: *An auto level should be used for reaches with a percent slope of less than or equal to 1%. All methods (clinometer, hand level, or auto level) may be used for reaches with a percent slope of greater than 1%. The following description is for clinometer-based slope measurements, but the same principles apply to use of an auto or hand level.*

Note 2: *In reaches that are close to 1%, you will not know whether you are above or below the 1% slope cutoff before taking readings. In these cases, default to use of an autolevel.*

Step 1. Beginning with the upper transect (Transect K), one person (the measurer) should stand at the water margin with a clinometer held at eye level. A second person should stand at the margin of the next downstream transect (Transect J) with a stadia rod flagged at the eye level of the person taking the clinometer readings. Be sure you mark your eye level while standing on level ground! Adjust for water depth by measuring from the same height above the water surface at both transects. This is most easily accomplished by holding the base of the pole at water level.

Note: *An alternative technique is to use two stadia rods pre-flagged at the eye-height of the person taking the readings.*

Step 2. Use a clinometer to measure the percent slope of the water surface (not the streambed) between the upstream transect and the downstream transect by sighting to the flagged position on the stadia rod. The clinometer reads both percent slope and degree of the slope. Be careful to read and record percent slope rather than degrees slope (these measurements differ by a factor of ~2.2). Percent slope is the scale on the right hand side as you look through most clinometers (e.g., Suunto models).

Note: *If an auto level or hand level is used, record the elevation difference (rise) between transects and the segment length (run) instead of the percent slope.*

Step 3. If the stream reach geometry makes it difficult to sight a line between transects, divide the distance into two or three sections and record the slope and the proportion of the total segment length between transects for each of these sections in the appropriate boxes on the slope form (supplemental segments).

Note: *Never measure slope across dry land (e.g., across a meander bend).*

Step 4. Take a compass reading from the center of each main transect to the center of the next main transect downstream and record this bearing to the nearest degree on the slope and bearing section of the form. Bearing measurements should always be taken from the upstream to downstream transect.

Step 5. Proceed downstream to the next transect pair (I-J) and continue to record slope and bearing between each pair of transects until measurements have been recorded for all transects.

SECTION IVB. VISUAL ESTIMATES OF HUMAN INFLUENCE, INSTREAM HABITAT, AND RIPARIAN VEGETATION

The transect-based approach used here permits semi-quantitative calculations from visual estimates even though most are categorical data (i.e., either presence/ absence or size classes) because we can calculate the percentage of transects that fall into different categories. These modules are adapted directly from EMAP protocols with some modifications as noted.

Module F. Human Influence

The influence of human activities on stream biota is of critical concern in bioassessment analyses. Quantification of human activities for these analyses is often performed with GIS techniques, which are very useful but are not capable of accounting for human activities occurring at the reach scale. Reach scale observations are often critical for explaining results that might seem anomalous on the basis of only remote mapping tools.

Step 1. For the left and right banks, estimate a 10 x 10 m riparian area centered on the edges of the transect (see Figure 2). Record the presence of 11 human influence categories in three spatial zones relative to this 10 x 10 m square (between the wetted edge and bankfull margin, between the bankfull margin and 10 m from the stream, and between 10 m and 50 m beyond the stream margins): 1) walls/rip-rap/dams, 2) buildings, 3) pavement/cleared lots, 4) roads/railroads, 5) pipes (inlets or outlets), 6) landfills or trash, 7) parks or lawns (e.g., golf courses), 8) row crops, 9) pasture/ rangelands, 10) logging/ timber harvest activities, 11) mining activities, 12) vegetative management (herbicides, brush removal, mowing), 13) bridges/ abutments, 14) orchards or vineyards. Circle all combinations of impacts and locations that apply, but be careful to not double-count any human influence observations.

Step 2. Record the presence of any of the 11 human influence categories in the stream channel within a zone 5 m upstream and 5 m downstream of the transect.

Module G. Riparian Vegetation

Riparian vegetation (vegetation in the region beyond the bankfull margins) has a strong influence on the composition of stream communities through its direct and indirect roles in controlling the food base, moderating sediment inputs and acting as a buffer between the stream channel and the surrounding environment. These methods provide a cursory survey of the condition of the riparian corridor. Observations are made in the same 10 x 10 m riparian area used for assessing human influence (see Figure 2).

Note: *Riparian vegetation measurements should only include living or recently dead vegetation.*

The riparian vegetation categories used here were condensed from the EMAP version, which further breaks the canopy classes into different components. However, because we have consolidated EMAP categories into fewer categories rather than creating new categories, existing EMAP data can be easily converted to this format simply by combining the appropriate categories.

Step 1. Divide the riparian zone into three elevation zones: 1) ground cover (<0.5 m), 2) lower canopy (0.5 m - 5 m), and 3) upper canopy (>5 m). Record the density of the following riparian classes: 1) Upper Canopy–Trees and Saplings, 2) Lower Canopy–Woody Shrubs and Saplings, 3) Woody Ground Cover–Shrubs, Saplings, 4) Herbaceous Ground Cover–Herbs and Grasses, and 5) Ground Cover–Barren, Bare Soil and Duff. Artificial banks (e.g., rip-rap, concrete, asphalt) should be recorded as barren.

Step 2. Indicate the areal cover (i.e., shading) by each riparian vegetative class as either: 1) absent, 2) sparse (<10%), 3) moderate (10-40%), 4) heavy (40-75%), or 5) very heavy (>75%).

Module H. Instream Habitat Complexity

Instream habitat complexity was developed by the EMAP program to quantify fish concealment features in the stream channel, but it also provides good information about the general condition and complexity of the stream channel. Estimates should include features within the banks and outside the wetted margins of the stream.

Step 1. Record the amount of nine different channel features within a zone 5m upstream and 5m downstream of the transect (see Figure 2): 1) filamentous algae (long-stranded algal forms that are large enough to see with the naked eye), 2) aquatic macrophytes (include mosses and vascular plants), 3) boulders (>25 cm), 4 and 5) woody debris (break into two classes- larger and smaller than 30 cm diameter), 6) undercut banks, 7) overhanging vegetation, 8) live tree roots and 9) artificial structures (includes any anthropogenic objects including large trash objects like tires and shopping carts). Indicate the areal cover of each feature as either: 1) absent, 2) sparse (<10%), 3) moderate (10-40%), 4) heavy (40-75%), or 5) very heavy (>75%).

SECTION 5
INTER-TRANSECT MEASURES

While most measures are taken at or relative to the main transects, a few measures are recorded at transects located at the midpoint between main transects. These are called "inter-transects".

Module B (Part 2) Pebble Counts (same as for transects, but no cobble embeddedness measures)

Step 1. Divide the wetted stream width by four to get the distance between the five points (Left Bank, Left Center, Center, Right Center and Right Bank) and use a measuring device to locate the positions of these points (a stadia rod is especially helpful here, see Figure 5). Once the positions are identified, lower a graduated rod through the water column perpendicular to both the flow and the transect to objectively select the particle located at its tip.

Step 2. With the graduated rod, measure the depth from the water surface to the top of the particle and record to the nearest cm.

Step 3. Remove the particle from the streambed, then measure and record the length of its intermediate axis to the nearest mm (see Figure 6). Alternatively, assign the particle to one of the size classes listed in the bottom of the transect form (see Table 3 for a list of size classes). Particle size classes may be estimated visually or with a quantitative measuring device (e.g., pass/ no-pass template, gravelometer). Regardless of the method, all particles less than 0.06 mm should be recorded as fines, while all particles between 0.06 mm and 2.0 mm should be recorded as sand. Field crews may want to carry vials containing sediment particles with these size ranges until they are familiar with these particle size classes.

Step 4. Record the presence (P) or absence (A) of any CPOM within 1 cm of each particle.

Module J. Flow Habitats

Because many benthic macroinvertebrates prefer specific flow and substrate microhabitats, the proportional representation of these habitats in a reach is often of interest in bioassessments. There are many different ways to quantify the proportions of different flow habitats (for example, see text on EMAP's "thalweg profile" below). Like the riparian and instream measures listed above, this procedure produces a semi-quantitative measure consisting of 10 transect-based visual estimates.

Note: *The categories used here are based on those used in the EMAP protocol, with pools combined into one class and cascades and falls combined into another class.*

Step 1. At each inter-transect, identify the proportion of six different habitat types in the region between the upstream transect and downstream transect: 1) cascades/falls, 2) rapids, 3) riffles, 4) runs, 5) glides, 6) pools, 7) dry areas. Record percentages to the nearest 5% — the total percentage of surface area for each section must total 100%.

SECTION 6 DISCHARGE

Stream discharge is the volume of water that moves past a point in a given amount of time and is generally reported as either cubic meters per second (cms) or cubic feet per second (cfs). Because discharge is directly related to water volume, discharge affects the concentration of nutrients, fine sediments and pollutants; and discharge measurements are critical for understanding impacts of disturbances such as impoundments, water withdrawals and water augmentation. Discharge is also closely related to many habitat characteristics including temperature regimes, physical habitat diversity, and habitat connectivity. As a direct result of these relationships, stream discharge is often also a strong predictor of biotic community composition. Since stream volume can vary significantly on many different temporal scales (diurnal, seasonal, inter-annually), it can also be very useful for understanding variation in stream condition.

This procedure (modified from the EMAP protocol) provides for two different methods for calculating discharge. It is preferable to take discharge measurements in sections where flow velocities are greater than 0.15 m/s and most depths are greater than 15 cm, but slower velocities and shallower depths can be used. If flow volume is sufficient for a transect-based "velocity-area" discharge calculation, this is by far the preferred method. If flow volume is too low to permit this procedure or if your flow meter fails, use the "neutrally buoyant object/ timed flow" method.

Note: *Programs that sample fixed sites repeatedly may want to consider installing permanent discharge estimation structures (e.g., stage gauges, wiers).*

Module K. Discharge: Velocity Area Method

The layout for discharge measurements under the velocity-area (VA) method is illustrated in Figure 8. Flow velocity should be measured with either a Swoffer Instruments propeller-type flow meter or a Marsh-McBirney inductive probe flow meter. Refer to the manufacturers' instrument manuals for calibration procedures.

VA-Step 1. Select the best location in the reach for measuring discharge. To maximize the repeatability of the discharge measurement, choose a transect with the most uniform flow (select hydraulically smooth flow whenever possible) and simplest cross-sectional geometry. It is acceptable to move substrates or other obstacles to create a more uniform cross-section before beginning the discharge measurements.

VA-Step 2. Measure the wetted width of the discharge transect and divide this into 10 to 20 equal segments. The use of more segments gives a better discharge calculation, but is impractical in small channels. A minimum of 10 intervals should be used when stream width permits, but interval width should not be less than 15 cm.

VA-Step 3. Record the distance from the bank to the end of the first interval. Using the top-setting rod that comes with the flow velocity meter, measure the median depth of the first interval.

VA-Step 4. Standing downstream of the transect to avoid interfering with the flow, use the top-setting rod to set the probe of the flow meter (either the propeller or the electromagnetic probe) at the midpoint of each interval, at 0.6 of the interval depth (this position generally approximates average velocity in the water column), and at right angles to the transect (facing upstream). See Figure 8 for positioning detail.

VA-Step 5. Allow the flow velocity meter to equilibrate for 10-20 seconds then record velocity to the nearest m/s. If the option is available, use the flow averaging setting on the flow meter.

Note: *Under very low flow conditions, flow velocity meters may register readings of zero even when there is noticeable flow. In these situations, record a velocity of 0.5x the minimum flow detection capabilities of the instrument.*

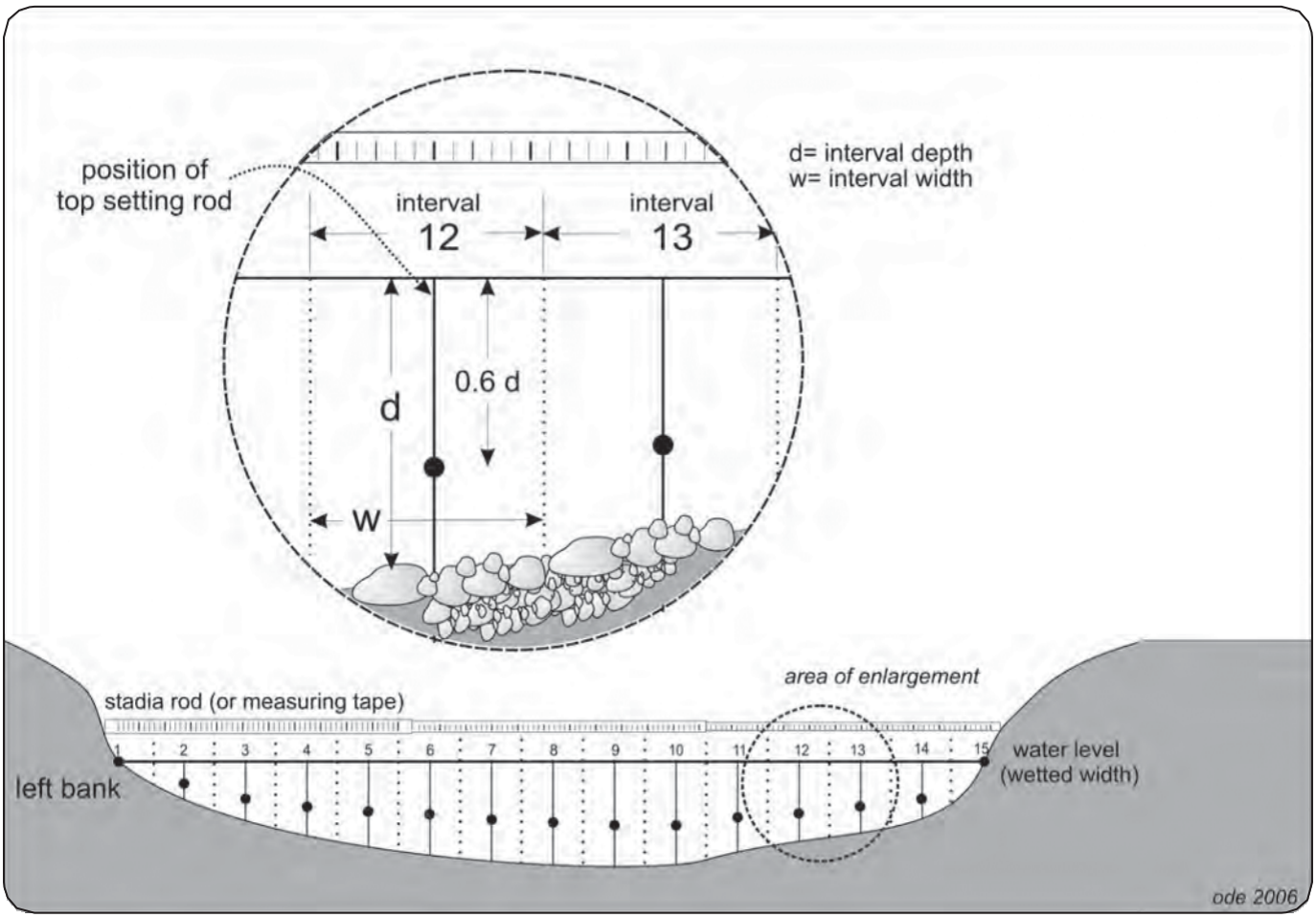

Figure 8. Diagram of layout for discharge measurements under the velocity-area method showing proper positions for velocity probe (black dots).

VA-Step 6. Complete Steps 3 through 5 on the remaining intervals.

Note: *The first and last intervals usually have depths and velocities of zero.*

Module L. Discharge: Neutrally Buoyant Object Method

If streams are too shallow to use a flow velocity meter, the neutrally buoyant object (NBO) method should be used to measure flow velocity. However, since this method is less precise than the flow velocity meter it should only be used if absolutely necessary. A neutrally buoyant object (one whose density allows it to just balance between sinking and floating) will act as if it were nearly weightless, thus it's movement will approximate that of the water it floats in better than a light object. To estimate the flow velocity through a reach, three transects are used to measure the cross-sectional areas within the test section sub-reach and three flow velocity estimates are used to measure average velocity through the test reach. To improve precision in velocity measurements, the reach segment should be long enough for the float time to last at least 10-15 seconds.

NBO-Step 1. The position of the discharge sub-reach is not as critical as it is for the velocity-area method, but the same criteria for selection of a discharge reach apply to the neutrally buoyant object method. Identify a section that has relatively uniform flow and a uniform cross sectional shape.

NBO-Step 2. The cross sectional area is estimated in a manner that is similar but less precise than that used in the velocity area method. Measure the cross sectional area in one to three places in the section designated for the discharge measurement (three evenly-spaced cross sections are preferred, but one may be used if the cross section through the reach is very uniform). Record the width once for each cross section and measure depth at five equally-spaced positions along each transect.

NBO-Step 3. Record the length of the discharge reach.

NBO-Step 4. Place a neutrally buoyant object (e.g., orange, rubber ball, heavy piece of wood, etc.) in the water upstream of the discharge reach and record the length of time in seconds that it takes for the object to pass between the upstream and downstream boundaries of the reach. Repeat this timed float three times.

SECTION 7
POST-SAMPLING OBSERVATIONS

Module M. Rapid Bioassessment Procedures Visual Assessment Scores (for Basic Physical Habitat, or optional supplement)

EPA's Rapid Bioassessment Procedures (RBPs, Barbour et al. 1999) include a set of 10 visual criteria for assessing instream and riparian habitat. The RBP has been used in the CSBP since its first edition (1995) and thus, this information is often valuable for comparison to legacy datasets. The criteria also have a useful didactic role since they help force the user to quantify key features of the physical environment where bioassessment samples are collected.

Module N. Additional Habitat Characterization (Full Physical Habitat only)

The RBP stream habitat visual estimates described in Step 1 are not included in the Full Physical Habitat version because they are generally replaced by more quantitative measurements of similar variables. However, we have found that three of the RBP measures are reasonably repeatable and include them in the reachwide assessment portion of the Full Physical Habitat version.

Note: *This is the only case in which a measurement included in the basic procedure is not included in the full.*

Module O. Reach Slope (for Basic Physical Habitat only)

Reach slope should be recorded as percent slope as opposed to degrees slope to avoid confusion. Slope measurements work best with two people, one taking the readings at the upstream transect and the other holding a stadia rod at the downstream transect. If you cannot see the mid point of the next transect from the starting point, use the supplemental sections (indicating the proportion of the total length represented by each section).

An auto level (with a tripod) should be used for reaches with a percent slope of less than or equal to 1%. All methods (clinometer, hand level, or auto level) may be used for reaches with a percent slope of greater than 1%. In reaches that are close to 1%, you will not know whether you are above or below the 1% slope cutoff. In these cases, default to use of an autolevel.

Step 1. Divide the reach into multiple segments such that stadia rod markings can be easily read with the measuring device to be employed (this is especially a factor for clinometer and hand level readings).

Step 2. Use a clinometer, hand level, or auto level to measure the percent slope of the water surface (not the streambed) between the top and bottom of each segment. Be sure to adjust for water depth by measuring from the same height above the water surface at both transects. Also be sure to record percent slope, not degrees slope. Record the segment length for each of these sections in the appropriate boxes on the BASIC slope form.

SECTION 8
OPTIONAL EXCESS SEDIMENT MEASURES

Future editions of these protocols will include supplemental modules, including a full discussion of the measurements used for calculating the excess sediment index (sometimes referred to as log relative bed stability, LRBS). However, since several of the measurements in EMAP's physical habitat protocols are interwoven into the layout of this protocol, a brief overview of the additional measurements collected for the LRBS calculations is included here for information purposes only. For detailed explanations of these measurements, consult Peck et al. 2004.

Woody Debris Tallies

Large woody debris (logs, snags, branches, etc.) that is capable of obstructing flow when the channel is at bankfull condition (just short of flood stage) contributes to the "roughness" of a channel. The effect of this variable is to reduce water velocity and thereby reduce the stream's competence to move substrate particles. The EMAP protocol tallies all woody debris with a diameter greater than 10 cm (~4") into one of 12 size classes based on the length and width of each object. Tallies are conducted in the zone between the main transects.

Thalweg Measurements

A stream's thalweg is a longitudinal profile that connects the deepest points of successive cross-sections of the stream. The thalweg defines the primary path of water flow through the reach. Thalweg measurements perform many functions in the EMAP protocols, producing measurements for the excess sediment calculations (residual pool volume, stream size, channel complexity) and flow habitat variability.

SECTION 9
OPTIONAL PERIPHYTON QUANTIFICATION

Periphyton Quantification

Characterization of periphyton has a dual role in bioassessments, as periphyton is both a food and habitat resource for benthic macroinvertebrates and fish and an effective bioindicator on its own. Quantification of periphytic resources will be covered under a separate SWAMP bioassessment protocol, but will include procedures for qualitative characterization of diatom assemblages, documentation of filamentous algal growth, and biomass quantification (e.g., ash-free dry mass and chlorophyll a).

SECTION 10
QUALITY ASSURANCE & CONTROL PROCEDURES

The SWAMP bioassessment group is currently developing guidelines for quality assurance and quality control for bioassessment procedures. Future revisions to this document will include guidance covering personnel qualifications, training and field audit procedures, procedures for field calibration, procedures for chain of custody documentation, requirements for measurement precision, health and safety warnings, cautions (actions that would result in instrument damage or compromised samples), and interferences (consequences of not following the standard operating procedure, SOP).

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DEFINITIONS OF TERMS USED IN SOP D

Terms & Definitions	
TERM	**DEFINITION**
ABL	California Department of Fish and Game's Aquatic Bioassessment Laboratory
Allocthonous	Derived from a source external to the stream channel (e.g., riparian vegetation) as opposed to autochthonous, which indicates a source inside the stream channel (e.g., periphyton).
Ambient Bioassessment	Biological monitoring that is intended to describe general biotic condition as opposed to a diagnosis of sources of impairment
Bankfull	The bankfull channel is the zone of maximum water inundation in a normal flow year (one to two year flood events)
BMI	Benthic macroinvertebrates: bottom-dwelling invertebrates large enough to be seen with the unaided eye
Cobble Embeddedness	The volume of cobble-sized particles (64-250 mm) that is buried by fine particles (<2.0 mm diameter)
CPOM	Coarse particulate organic matter (CPOM, particles of decaying organic material such as leaves that are greater than 1.0 mm in diameter)
CSBP	California State Bioassessment Procedures
DFG	California Department of Fish and Game
EMAP	The U.S. Environmental Protection Agency's Environmental Monitoring and Assessment Program
EPA	The U.S. Environmental Protection Agency
Fines	Substrate particles less than 0.06 mm diameter (not gritty to touch)
Inter-transects	Transects established at points equidistant between the main transects
MCM	Margin-Center-Margin alternative procedure for sampling low gradient habitats
ORD	EPA's Office of Research and Development
QAMP	Quality assurance management plan
RBP	EPA's Rapid Bioassessment Procedures
Reach	A segment of the stream channel
Riparian	An area of land and vegetation adjacent to a stream that has a direct effect on the stream.
RWB	Reach-wide benthos composite sampling method for benthic macroinvertebrates, also referred to as multi-habitat method
SCCWRP	Southern Coastal California Water Research Project
SNARL	Sierra Nevada Aquatic Research Laboratory
Substrate	The composition of a streambed, including both inorganic and organic particles
SWAMP	The State Water Resources Control Board's Surface Water Ambient Monitoring Program
Thalweg	A longitudinal profile that connects the deepest points at successive cross-sections of the stream. The thalweg defines the primary path of water flow through the reach

TERM	DEFINITION
Transects	Lines drawn perpendicular to the path of flow used for standardizing sampling locations
TRC	Targeted riffle composite sampling method for benthic macroinvertebrates
USFS	The United States Forest Service
Wadeable Streams	Streams that can be sampled by field crews wearing chest waders (generally less that 0.5 m - 1.0 meters deep)

APPENDIX A

FACTORS TO CONSIDER WHEN RECOMMENDING/ CHANGING BIOASSESSMENT METHODS

Beyond the primary considerations of precision and accuracy, there are at least five other key issues that SWAMP has considered and should consider in the future, when recommending or changing its official methods for bioassessment. These issues include:

1. Costs of Collecting Samples via Multiple Protocols – Collecting, processing, and interpreting samples using more than one method for each indicator (e.g., algae, macroinvertebrates, fish) per site adds significant costs to bioassessment monitoring programs. SWAMP should strive to identify the minimum set of protocols necessary for each indicator. However, this should not come at the expense of sound monitoring. If more than one method is needed to interpret the biological response, then this decision should be based on a cost-benefit assessment.

2. Costs of Maintaining Multiple SWAMP Protocols – While multiple methods for monitoring a given indicator may provide additional accuracy in specific habitats, there are significant costs to maintaining multiple protocols:

a. Need to maintain method-specific infrastructure (e.g., separate reference samples, separate indices of biotic integrity (IBIs), separate O/E models, etc.).
b. May lose or impair ability to compare across sites if different methods are used (see Issue 5 below).
c. Guidance on when to use methods becomes more complex. For example, we need to define very specifically which methods to use at each water body type; and thus, which tools can be used to interpret them.

Recommendation: *SWAMP should maintain as few protocols as necessary. If we elect to add new or modified protocols it should be because we have determined that the added value is worth all of the costs listed above.*

3. Separating Physical Impairment from Water Quality Impairment – One of the original reasons for adding a multihabitat component to SWAMP bioassessment programs was the potential for distinguishing physical and water quality impairment sources (see recommendations in Barbour and Hill 2002). In regards to macroinvertebrate indicators, the conventional wisdom has been that reachwide (RW, sometimes referred to as multihabitat or MH) samples should be relatively more responsive to physical habitat alteration (i.e., fine sediment inputs) than targeted-riffle (TR) samples because it is believed that erosional habitats take longer

to respond to sediment stresses, and because pockets of riffle habitat are thought to act as refugia from habitat loss. To the extent that this is true, RW and TR samples may offer complementary information that allows us to separate these sources of impairment.

While very few studies have addressed this conventional wisdom directly, recent studies suggest that this may not be as much a factor as previously believed. In a recent comparison of TR and RW samples at nearly 200 sites statewide, the ABL found at most weak evidence to support this notion (Rehn et al. 2007). Gerth and Herlihy (2006) came to the same conclusion in their analysis of ~500 sites in the eastern and western United States. However, this issue is far from resolved and SWAMP scientists currently are not in agreement regarding this issue. Since the majority of bioassessment programs in California have emphasized targeted riffle sampling, SWAMP will undoubtedly want to evaluate this question further before making any policy decision to discontinue TR sampling.

Recommendation: *Until this issue can be evaluated further and resolved to SWAMP's satisfaction, ambient macroinvertebrate sampling should include collection of both RW samples and richest targeted habitat (TR or MCM) samples at every site. (The TR method should be used where sufficient riffles are present, and the MCM method should be used at low-gradient sites where sufficient riffle habitat is not available.)*

4. Compatibility with Previous Data – To address this issue, at least three sets of macroinvertebrate sampling method comparisons have been conducted in California.

a. **Targeted Riffle Methods –** Comparisons are complete. Samples collected under the current TR protocols are considered interchangeable with both CSBP and SNARL samples (Ode et al. 2005, Herbst and Silldorff 2006).
b. **Low Gradient Sand-Dominated Streams –** Collaborative studies are currently underway between Water Board Regions 3 and 5, the Southern California Coastal Water Research Project (SCCWRP), and ABL to compare the performance of: (1) the "low-gradient" CSBP; (2) RW samples; and (3) a modification of the RW method designed to emphasize habitats along stream margins (MCM). The results of these low-gradient methods comparisons are not yet available.
c. **Targeted Riffle vs. Reachwide Methods –** A recent comparison of RW and TR samples collected from nearly 200 EMAP/ CMAP sites is in peer review press (Rehn et al. 2007). Results demonstrate remarkably similar performance of the methods across a wide range of habitats. Gerth and Herlihy (2006) recently published a similar analysis with the same conclusions. However, the bioassessment committee has yet to carefully review and discuss these analyses and their implications for SWAMP biomonitoring.

5. Comparability Among Sites – The ability to compare biological condition across sites is a common requirement of most ambient bioassessment programs. This type of analysis is confounded if different methods are used at these sites. One of the big advantages of reachwide (i.e., multihabitat) methods is that they can be applied anywhere because they don't require a specific habitat for sampling. Statewide

bioassessments and most regional programs will require the ability to compare their bioassessment results among multiple sites (e.g., within a watershed, within a region, statewide).

INTERIM RECOMMENDATIONS FOR MACROINVERTEBRATE SAMPLING (UPDATED DECEMBER 2006):

1. Until we can reach consensus on the outstanding issues (i.e., whether a single method for macroinvertebrate sampling will meet our needs, and the outcome of RW vs. MCM comparison studies for low-gradient wadeable streams/rivers), SWAMP recommends collecting both a reachwide (i.e., multihabitat) and a targeted habitat sample at each site. In high gradient streams, this means using both the RW and TR methods. In low-gradient streams, we recommend collecting both RW and MCM samples until the results are available from the low-gradient ("non-riffle") comparison. In rare cases where monitoring objectives cannot be met following these recommendations, the SWAMP Bioassessment Coordinator may authorize deviations. For example, where project-specific objectives differ from ambient monitoring, the SWAMP Bioassessment Coordinator may authorize alternate methods. In rare cases where funding is extremely limited and the cost of following the above recommendations would be prohibitive, the SWAMP Bioassessment Coordinator may authorize cost-saving options such as collecting both samples, but archiving one of the samples for later lab analysis.

2. SWAMP should develop guidance specifying when and where different methods should be used. For example, at "low gradient" sites, what is the slope cut-off (or other channel feature criteria to use) when deciding whether to apply TR or MCM? In addition, while SWAMP may eventually choose to adopt a single method (such as RW) at most sites, some regions may determine that the value of targeted habitat sampling merits continued sampling with supplemental protocols. In the latter case, or if SWAMP determines that distinct methods are needed for different habitat types, the guidance should specify the types of waterbodies or classes of waterbodies that require different methods.

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SWAMP

Surface Water
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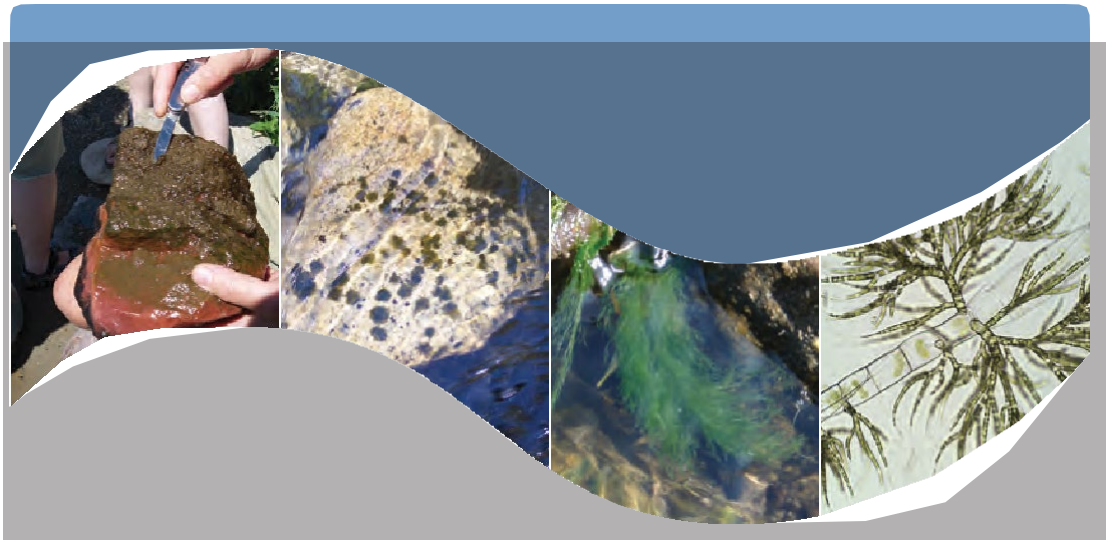

SWAMP Surface Water Ambient Monitoring Program

SWAMP Bioassessment Procedures 2010

Standard Operating Procedures for Collecting Stream Algae Samples and Associated Physical Habitat and Chemical Data for Ambient Bioassessments in California

June 2009, updated May 2010

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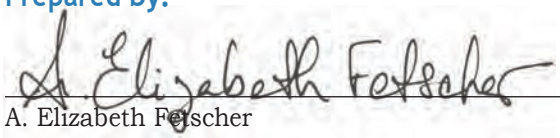

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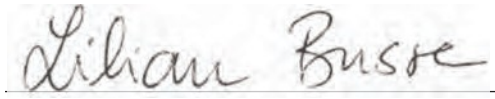

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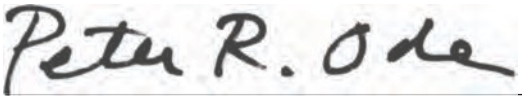

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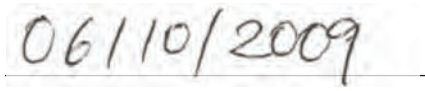
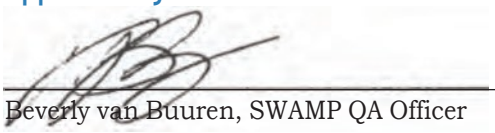


June 10, 2009

Approved by:



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Approval Date:

June 10, 2009

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ACKNOWLEDGEMENTS

This Standard Operating Procedures (SOP) manual represents the contributions of a wide range of researchers and field crews. The algal specimen collection methodology presented represents a modification of the U.S. Environmental Protection Agency's (EPA's) Environmental Monitoring and Assessment Program (EMAP) multihabitat sampling protocol (Peck et al. 2006). Point-intercept estimation of macroalgal cover has been adapted from the U.S. Geological Survey's (USGS's) National Water Quality Assessment (NAWQA) pilot procedures (J. Berkman, pers comm.), and assessment of microalgal thickness has been adapted from Stevenson and Rollins (2006). The physical habitat (PHab) methods are identical to those presented in the SWAMP Bioassessment protocol of Ode (2007), with the exception of the point-intercept method for determining algal cover, which is an add-on to the PHab pebble count procedure. The PHab procedures are, in turn, minor modifications of those used in EMAP and developed by EPA's Office of Research and Development (ORD, Peck et al. 2006).

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LIST OF ACRONYMS & ABBREVIATIONS

Acronyms & Abbreviations	Definitions
AFDM	Ash-Free Dry Mass
BMI	Benthic Macroinvertebrate
chl a	Chlorophyll *a*
CPOM	Coarse Particulate Organic Matter
DO	Dissolved Oxygen
EMAP	Environmental Monitoring and Assessment Program (of the U.S. EPA)
GPS	Global Positioning System
IBI	Index of Biotic Integrity
MCM	Margin-Center-Margin
NAD	North American Datum
NAWQA	National Water Quality Assessment (of the U.S. Geological Survey)
NBO	Neutrally Buoyant Object
NNE	Nutrient Numeric Endpoints
OSPR	Office of Spill Prevention and Response (of the California Department of Fish and Game)
PHab	Physical Habitat
QAPrP	Quality Assurance Program Plan (of SWAMP)
ORD	Office of Research and Development (of the U.S. EPA)
RBP	Rapid Bioassessment Procedures
RWB	Reachwide Benthos
SOP	Standard Operating Procedures
SWAMP	Surface Water Ambient Monitoring Program
TRC	Targeted Riffle Composite

SECTION 1 INTRODUCTION

This document is the Standard Operating Procedure (SOP) for collecting and field-processing stream algae for the California State Water Resources Control Board's Surface Water Ambient Monitoring Program (SWAMP). Instructions are provided for the following:

- collection of samples for taxonomic identification of diatoms and soft-bodied algae
- collection of samples for determination of biomass based on chlorophyll *a* and ash-free dry mass (AFDM)
- estimation of percent algal cover

The document is designed to serve as a stand-alone SOP if algae are the only bioindicators being assessed at a given site. However, it can also serve as an add-on module to the existing SWAMP SOP for bioassessment using benthic macroinvertebrates (BMIs). Much of the procedure for collecting physical habitat (PHab) data is identical for these two assemblages. However, some PHab elements assessed in conjunction with BMI bioassessment are not included for algal bioassessment, because they are more specific to BMI habitat needs than to algae. Conversely, one PHab element for algal bioassessment (i.e., point-intercept estimation of algal cover) is not part of the BMI SOP. It should also be noted that, while the standard PHab protocol associated with BMI sampling includes both a "Full" and a "Basic" (simplified) version, a distinction between basic and full protocols for algae has not been established.

This SOP requires the reachwide benthos (RWB) sampling method to be used whenever algae bioassessment is conducted under the SWAMP program. Other appropriate sampling methods will be allowed if specific monitoring objectives require the use of alternative methods or if consistent data comparability in long-term monitoring projects is desired. For SWAMP funded projects, the project proponent must have the approval of the SWAMP bioassessment coordinator and the SWAMP Quality Assurance Officer before the use of alternative methods. For other projects and/or programs working towards SWAMP comparability, deviations should be approved by their project manager and project Quality Assurance (QA) officer.

For quick reference, Table 1 provides a list of elements common and distinct to the two SWAMP bioassessment assemblages. In general, if both BMIs and algae are being collected at a given site, the PHab procedure as described in Ode (2007) should be followed, with the exception of the pebble count, which should be conducted according to this SOP, because it incorporates instructions for algal cover point-intercept data collection. More specifically, if bioassessment involving the Full BMI protocol plus algae is to be implemented at a given site, practitioners should follow the Full protocol of Ode (2007), and add only Section 3.4 (re: water chemistry), Sections 4 and 5, and Sections 6.9-6.11 from this SOP.

Table 1
Sample and data collection elements included in algal and BMI bioassessment (Ode 2007).
X indicates elements included in algal bioassessment. F indicates elements that are part of the "Full" protocol for conducting BMI bioassessment, B corresponds to elements of the "Basic" BMI protocol, and O indicates elements that are "Optional".

Element	Algae[1]	BMIs
Layout of reach, marking transects, recording GPS coordinates	X	B, F
Notable field conditions	X	B, F
Temperature, pH, specific conductance, DO, alkalinity	X	B, F
Turbidity, Silica	O	O
Water chemistry for lab analysis (see list in Section 3.4)	X	
Algal Sampling for Taxonomic IDs	X	
Algal Sampling for Biomass Assessment	X	O
BMI Sampling for Taxonomic IDs		B, F
Wetted Width	X	B, F
Bankfull Dimensions	X	F
Depth and Pebble Count + CPOM	X	F
Percent Algal Cover (point-intercept with Pebble Count)	X	
Cobble Embeddedness	X	F
Canopy Cover	X	B, F
Gradient	X	B[2], F
Sinuosity		F
Human Influence	X	F
Riparian Vegetation		F
Instream Habitat		F
Bank Stability	X	B, F
Flow Habitat Delineation	X	B, F
Discharge	X	F
Photo documentation	X	B,F
Selected Rapid Bioassessment Procedure (RBP) visuals		F

1. A distinction between Basic and Full protocols for algae has not been established.
2. For BMIs, a single, reachwide measurement of gradient is required for Basic, but gradient is measured between all adjacent transect pairs for Full.

Depending upon the requirements of the monitoring effort, different components of this SOP might be incorporated or omitted. For instance, if stream productivity in terms of algae is the primary concern of the assessment, one may wish to collect only biomass samples and algal cover point-intercept data. Alternatively, one will need to collect algal assemblages (for quantification of diatom and/or soft-bodied algal taxa) in order to make more refined inferences about water quality and stream condition (e.g., by applying an algal Index of Biotic Integrity (IBI)).

This SOP is organized in such a way as to facilitate the inclusion or omission of certain elements based on the goals of the monitoring effort. A list of field supplies is provided in Appendix A. It is organized according to the materials needed for each type of sampling and data collection. In order to facilitate decisions about algal indicators to assess for program-specific needs, the introduction to Section 4 discusses what algal indicators serve which monitoring purposes.

SECTION 2
GETTING STARTED

Several considerations come into play when planning an algae-sampling effort. For instance, time of year can be an important determinant of stream algae abundance as well as the type of community likely to be encountered. Likewise, a minimum amount of surface water is a prerequisite to conducting bioassessment. The following section provides guidelines to help practitioners determine when sampling is appropriate for a given reach and also some pointers to help prepare for field work.

2.1 WHEn TO SAMPLE

It is recommended that sampling for stream algae be carried out during the same period as BMI sampling, generally from May through September, depending on the region. This time frame may eventually be modified (e.g., expanded) based on the results of ongoing index period studies.

It should be noted that high-velocity storm flows can remove macroalgae and biofilms from the stream bottom. Sampling must be done at least a month after any storm event that has generated enough stream power to mobilize cobbles and sand/silt capable of scouring stream substrates, in order to allow ample time for recolonization of scoured surfaces (Round 1991; Kelly et al. 1998; Stevenson and Bahls in Barbour et al. 1999).

2.2 BEFORE SETTInG OuT FOR THE FIELD

- Proper precautions should be taken at all times in order to avoid transferring invasive organisms and pathogens between sites. This includes the implementation of effective equipment decontamination procedures. Refer to Appendix B for additional information.
- Use the equipment checklist provided in Appendix A to make sure all necessary supplies are brought along.
- Check with contract lab on sampling containers, and shipping and storage of samples.
- Have in mind at least three sites to visit per day (target two, but plan for at least one additional site as a back up if one of the first two sites is not useable.)
- Prepare, and double check, site dossiers to make sure they are complete with maps/directions to sites and scaled aerial photo(s). Bring along county maps, atlases, and Thomas Guides to further aid location of sites. Also bring along any site access permits, passes, and/or keys, as needed (and be aware that some landowners require notice prior to each site visit).

2.3 BEFORE LEAvInG vEHICLE FOR SITE

Make sure the vehicle is parked in a safe spot and there are no "No Parking" signs. Stick a business card with cell phone number in the driver's window. Be sure to display the brown administrative pass placard if you are on National Forest land (or a letter of permission, if applicable).

2.4 DETERMInInG WHETHER SITE IS APPROPRIATE FOR SAMPLInG

Make an initial survey of the potential monitoring reach from the stream banks (being sure to not disturb the instream habitat). Ensure that there is sufficient water in the stream reach to facilitate collection of algae and water samples. In order for a reach to be in appropriate condition for sampling, at least half of the reach should have a wetted width of at least 1m, and there should be no more than 3 transects that are completely dry. If there is some flexibility in terms of where to place the sampling reach, strive for as few dry transects as possible (and preferably none).

Sites should be safe to sample and legally accessible. The time required to access the sampling sites should also be a consideration in planning which sites to visit, in order to ensure that sample holding times can be met (see Table 2 on page 11 for holding-time information).

3 REACH DELINEATION AND WATER CHEMISTRY SAMPLING

Before sample and data collection can begin, the monitoring reach must be identified and delineated. This requires setting up sampling transects along the stream reach of interest. Once the reach is delineated, information about reach location and condition will need to be documented. Water chemistry parameters must also be recorded, and certain samples collected.

A set of field forms for recording information about monitoring sites, algal samples, and associated water chemistry and PHab data is provided on the SWAMP website (see below). The field forms are also available in electronic version on an portable computer. It is imperative that you confirm throughout the data collection effort at each site that all necessary data have been recorded on the field forms correctly, by double-checking values, and confirming spoken values with your field partner(s). As a general practice, you should conduct a final check across all datasheets to confirm that there are no missing values before you leave the site, and rectify any blanks. *Note: Field forms may be updated periodically. It is imperative that field crews ensure that they are always using the most current field forms. Updated forms can be accessed from the SWAMP website at: http://swamp.mpsl.mlml.calstate.edu/resources-and-downloads*

3.1 DELINEATING AND DOCUMENTING THE MONITORING REACH

To delineate the monitoring reach, you will need to scout it in its entirety in order to make sure that it is of adequate length for sampling algae. During this process, try to stay out of the channel as much as possible, to avoid disturbing the stream bottom, which could compromise the samples and data that will be collected.

SWAMP's standard algae (and BMI) sampling layout consists of a 150 m reach or a 250 m reach, depending upon the average wetted width of the channel. In some circumstances (see below), reach length can be < 150 m, but this should be avoided whenever possible. If the actual reach length is other than 150 m or 250 m, this should be noted and explained on the field forms. Under these circumstances, you will need to determine the useable length of the reach, and how to space your transects so that you can fit them into the reach at equal distances from one to the next.

The wetted channel is the zone that is inundated with water and the wetted width is the distance between the sides of the channel at the point where substrates are no longer surrounded by surface water. Estimate the average wetted width of the reach. If this value is ≤ 10 m, you will end up using 150 m for your monitoring reach length. If the average wetted width is > 10 m, you will use a 250 m long reach.

To set up the monitoring reach, begin a little outside of what you anticipate will be the outer boundary (based on aerials and maps) and count 150 large steps, or 250 large steps (for most adults, a large step is roughly equal to a meter), by walking along the bank. This will give a rough idea about the location of the ends of the study reach. However, keep in mind that once this is determined, the actual distances between transects and intertransects (and consequently, the reach length) will need to be more accurately measured.

As you go, identify where hydrologic inputs that could potentially modify the water chemistry environment occur along the length of the reach. If possible, there should be no tributaries or "end-of-pipe" outfalls feeding into the channel within the monitoring reach. Other features that should not be present within a monitoring reach are: bridge crossings (which shade the stream bottom and can artificially reduce or prevent algal growth), changes between natural and man-made (i.e., concrete) channel bottoms, waterfalls, and impoundments (dams and weirs). If any of such features occur within the reach, and there is not enough room to accommodate a 150-m reach or 250-m reach entirely upstream or downstream of such a feature, then the reach can be somewhat < 150 m. Whatever the reach length turns out to be (150 m, 250 m, or other), record it on the datasheet under "Reach Length".

3.2 MARkInG THE TRAnSECTS

The monitoring reach will be divided into 11 equidistant main transects that are arranged perpendicularly to the direction of flow. There will also be 10 additional transects (designated "inter-transects"), one between each pair of adjacent main transects, to give a total of 21 transects per monitoring reach. Main transects are designated "A" through "K", while inter-transects are designated by their nearest upstream and downstream main transects ("AB", "BC", etc.).

Once you have identified the upper and lower limits of the monitoring reach, determine the coordinates of the downstream end using a Global Positioning System (GPS) set to the North American Datum 1983 (NAD 83)[3], and record this information in decimal degrees (to five decimal places) on the datasheet under "Reach Documentation". Install a colored flag at water's edge on one of the banks at this location to indicate the first "main transect", or "A". Establish the positions of the remaining transects and inter-transects by heading along the entire length of the monitoring reach (again, staying out of the water/channel as much as possible) and using the transect tape or a segment of rope of appropriate length to measure off successive segments of 7.5 m (for streams of wetted width ≤ 10 m), or 12.5 m (for streams > 10 m wetted width). For monitoring reaches of non-standard length, you will divide the total, targeted length of the reach by 20 to derive the distance between the adjacent main, and inter-, transects. As you measure off the distances, always follow the virtual, mid-channel line, and not the water's edge (which may be irregular, and not reflective of the true stream curvilinear distance).

3. Be aware that some GPS units re-set themselves to factory default settings when the batteries are changed. This can include the datum. Therefore, anytime you remove batteries from your unit, double check that the unit is still set to the NAD83 datum after the batteries have been replaced.

At the end of each measured segment as you head along the stream, mark the transect location on the bank with a flag. We recommend alternating between two different flag colors (e.g., orange could correspond to main transects, and yellow to inter-transects). Determine transect orientations, and where on the banks to place the flags, by visually projecting perpendicularly from the mid-channel to the banks. Refer to Figure 1 for a visual representation of proper transect alignment relative to the stream's direction of flow. When you have finished, the downstream-most flag will correspond to main transect "A", and the upstream-most flag (the 21st in the entire series of main and inter- transects) will correspond to main transect "K".

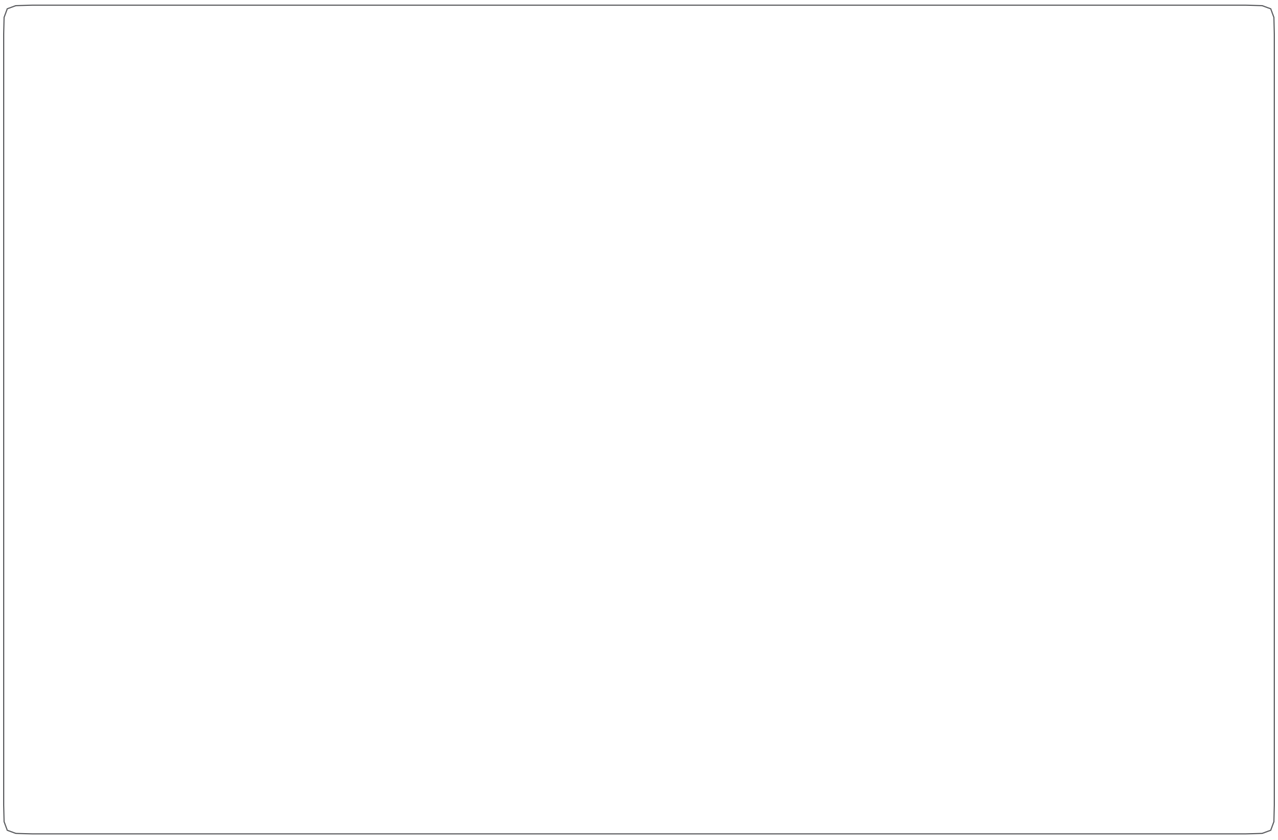

Figure 1. Reach layout geometry for physical habitat (PHab) and biological sampling showing positions of 11 main transects (A-K) and the 10 supplemental inter-transects (AB-JK). The area highlighted in the figure is expanded in Figure 11. Note: reach length = 150 m for streams ≤ 10 m average wetted width, and reach length = 250 m for streams > 10 m average wetted width (reprinted from Ode 2007).

3.3 nOTABLE FIELD COnDITIOnS

Record under "Notable Field Conditions" any evidence of recent flooding, fire, or other disturbances that might influence algae samples. Especially note if flow conditions have been affected by recent rainfall, which can cause significant under-sampling of algal biomass and diversity. If you are unaware of recent fire or rainfall events, select the "no" option on the forms. Record the dominant land use and land cover in the area surrounding the reach by evaluating land cover within 50 m of either side of the stream reach. You can

use a scaled aerial photograph of the site and vicinity to guide you. *Note: Before heading out to the field, it is convenient to add a 150 m (or 250 m) line adjacent the stream to be sampled in order to get an idea about the anticipated approximate upstream and downstream boundaries of the monitoring reach.*

3.4 WATER CHEMISTRy

Measure and record common ambient water chemistry measurements (pH, dissolved oxygen (DO), specific conductance, alkalinity, and water temperature) just outside of the reach, at the downstream end, near the same location that the GPS coordinates were taken. This should be done in such a way that it does not interfere with biotic sampling and PHab data collection, but also in such a way that water samples are not compromised by other sampling activities upstream (e.g., by suspension of matter from the stream bottom into the water column, and consequently the introduction of this matter into the water chemistry samples).

Water chemistry measurements are typically taken with a handheld, water-quality meter (e.g., YSI, Hydrolab), but field test kits (e.g., Hach) can provide acceptable information if they are properly calibrated. For appropriate calibration methods, calibration frequency, and accuracy checks, consult the current SWAMP Quality Assurance Program Plan (QAPrP)[4], or follow manufacturer's guidelines. *Note 1: If characteristics of the site prohibit downstream entry, measurements may be taken at other points in the reach. In all cases, ambient chemistry measurements should be taken at the start of the survey (i.e., before algae sampling and PHab data collection). Note 2: A digital titrator (e.g., Hach) using low-concentration acid (such as 0.16N H_2SO_4) as the titrant is recommended for determining alkalinity in low-alkalinity streams (i.e., approximately 100 mg/L $CaCO_3$ or less).*

A suite of analytes must also be evaluated to aid in interpretation of the algal data. These are listed below. Consult the SWAMP QAPrP for specific instructions on the proper techniques for collecting, preserving, and storing these water samples until analysis.

- Nitrate as N (NO_3)
- Nitrite as N (NO_2)
- Ammonia as N (NH_3)
- Nitrogen, Total (TN)[5]
- Orthophosphate as P (dissolved; SRP)
- Phosphorous, Total (TPHOS)
- Dissolved Organic Carbon (DOC)
- Chloride (Cl)

- Silica as SiO_2, dissolved (Note: this analyte is recommended for research purposes, but is not part of the standard algae protocol)

4. This document is available online from the SWAMP website: http://www.swrcb.ca.gov/water_issues/programs/swamp/docs/qapp/swamp_qapp_master090108a.pdf
5. Total Nitrogen can be calculated from Total Kjeldahl Nitrogen (TKN), Nitrate (NO_3) and Nitrite (NO_2)

SECTION 4
REACHWIDE BENTHOS SAMPLING OF ALGAE

The following is a short introduction of several types of algal indicators that can be monitored as part of a bioassessment effort. For a more detailed discussion, see Fetscher and McLaughlin (2008). The most appropriate indicators to include in a given program will ultimately depend upon that program's goals, because the various indicators provide information at varying levels of resolution and applicability to different uses. Likewise, the various indicators require different levels of investment in terms of field work and lab work. Percent algal cover, for instance, is a rapid means of estimating algal primary productivity that can be carried out entirely in the field and is conducted in tandem with the PHab pebble count. Therefore, percent algal cover is an appropriate, fast, and inexpensive parameter for citizen monitoring groups if they are concerned about increased algal biomass. Other estimators of algal biomass include chlorophyll *a* and AFDM, which involve quantitative collection of algae, preservation, and subsequent laboratory analysis. Algal biomass is a key component of the California Nutrient Numeric Endpoints (NNE) framework. Higher resolution information about algal assemblages can be used in algal IBIs, and offers more in-depth insight into water quality. For this type of data, algal specimens must be collected quantitatively (and qualitatively, in the case of soft-bodied algae). The quantitative samples are then fixed/preserved carefully and subjected to taxonomic analysis.

While the percent algal cover data are recorded in conjunction with standard PHab procedures, and do not require the collection of samples, all the other types of data described in this protocol require reachwide benthos (RWB) sampling of algal specimens in a manner analogous to that which is carried out for BMIs.

All four of the algal samples described in this SOP: chlorophyll *a*, AFDM, diatom assemblage, and soft-bodied algal assemblage, can be obtained from a single composite sample generated by the RWB method. Which combination of these samples to prepare and submit for laboratory processing will depend on the needs of the monitoring program. To aid in the selection of algal indicators, Table 2 provides a summary of their attributes.

Table 2
Types of algal indicators and considerations for their assessment.

	Algal indicator for	Collection method	Collection vessel	Preservation/fixation methods and holding times	Qualitative live sample required?
Percent algal cover	Stream productivity measured as algal abundance	Point-intercept add-on to the PHab pebble count	N/A	N/A	N/A
Chlorophyll a[6]	Stream productivity measured as algal biomass; key indicator for the Nutrient Numeric Endpoints (NNE) framework	RWB sample collection	Glass-fiber filter	Wet ice, dark (foil-wrapped); freezing within 4h, and analysis within 28d	N/A
AFDM	Stream productivity measured as biomass of organic matter (including algae); indicator for the NNE framework	RWB sample collection	Glass-fiber filter (pre-combusted[7])	Wet ice, dark (foil-wrapped); freezing within 4h, and analysis within 28d	N/A
Diatoms	Used in IBIs. Indicative of factors such as trophic status; organic enrichment; low DO; siltation; pH; metals	RWB sample collection	50 mL centrifuge tube	Add 10% buffered formalin for a 2% final concentration immediately after collection; keep dark and away from heat	Optional
Soft-bodied algae[8]	Used in IBIs. Indicative of factors such as nitrogen limitation/ trophic status; siltation; pH; temperature, light availability, nuisance/ toxic algal blooms	RWB sample collection	50 mL centrifuge tube	Keep unfixed samples in dark on wet (NOT DRY) ice; add glutaraldehyde (to a 2.5% final concentration) as soon as possible, but no later than 4 days after sampling; after fixing, keep dark and away from heat	Required

6. It is valuable to assess both chlorophyll *a* and phaeophytin *a* (the degradation product of the former) content of algal samples, as this may provide a more robust assessment of algal biomass.
7. Precombustion is recommended in order to remove any possible residual organic matter from the filter.
8. For the purposes of this SOP, the soft-bodied assemblage includes cyanobacteria (an explanation of the rationale for this is provided in Fetscher and McLaughlin 2008)

4.1 GEnERAL COnSIDERATIOnS FOR SAMPLInG ALGAE

This SOP describes the RWB method for collecting stream algae. It employs an objective approach for selecting sub-sampling locations that is built upon the 11 main transects described in the previous section. This approach is analogous to the SWAMP procedure for BMI sampling (Ode 2007), and is ultimately based on EPA's Environmental Monitoring and Assessment Program (EMAP; Peck et al. 2006). After collection, the 11 sub-samples are composited into a single sample per site (sampling reach).

The RWB method can be used to sample any wadeable stream reach since it does not target specific habitats. Because sampling locations are defined by the transect layout, the position of individual sub-sampling spots may fall within a variety of "erosional[9]" or "depositional[10]" habitats, each of which has implications for the type of substrate likely to be encountered and therefore the type of sampling device to use.

For the RWB method, the sub-sampling position alternates between left, center, and right portions of the transects, as one proceeds upstream from one transect to the next. These sampling locations are defined as the points at 25% ("left[11]"), 50% ("center") and 75% ("right") of the wetted width in high-gradient systems, and at "margin-center-margin" (MCM) positions in low-gradient systems. The RWB-MCM method should be only used in low-gradient streams where channel substrates are nearly uniform, resulting in low diversity within the channel. The interim cut-off between "low" and "high" gradient is 1%. Best professional judgment can be used to estimate whether the stream reach should be treated as low- or high-gradient. However, if there is uncertainty about the gradient, it should be measured prior to collecting the biotic assemblage samples. See Section 4.2 for specific instructions about where algae sampling locations should be positioned at the margins of low-gradient sampling reaches.

Algae should be sampled prior to PHab data collection (described in Sections 6-8), so as not to disturb the algae by trampling the transects, as occurs during the PHab process. Furthermore, to avoid disturbing the transects for eventual collection of PHab data, as with BMIs, algae should be collected at a location that is systematically offset from each transect (see Section 4.2).

4.2 COLLECTIOn OF ALGAE In COnJunCTIOn WITH BEnTHIC MACROInvERTEBRATES

If only algae (or only BMIs) are being collected for bioassessment, then the specimens should be collected 1m downstream of the transects. If both assemblages are being sampled, then the algae should be collected upstream of the spot where the BMIs are collected, according to the schematic in Figure 2. BMIs must be collected BEFORE algae at each of the transects, in order to minimize the chances disturbing BMIs during algal collection. After the BMIs are collected at each spot, the algae sample should be taken ¼ m upstream

9. Erosional - habitats in the stream that are dominated by fast-moving water, such as riffles, where stream power is more likely to facilitate erosion (suspension) of loose benthic material than deposition; examples of "erosional" substrates include cobbles and boulders.
10. Depositional - habitats in the stream that are dominated by slow-moving water, such as pools, where deposition of materials from the water column is more likely to occur than erosion (or (re)suspension) of bed materials; examples of "depositional" substrates include silt and sand.
11. For our purposes, "left" is defined as the left bank when facing downstream.

from the center of the upper edge of the "scar" in the stream bottom left from the BMI sampling. It is important to make sure that the surface from which algae will be collected has not been disturbed (by the BMI sampling, or otherwise) prior to sampling the algae.

Note: If only algae (and not BMIs) are being collected in a low-gradient reach, the collection location should be 1 m downstream of the transect and, for each of the "margin" positions, at a distance of 15 cm from the wetted margin of the bank. Fifteen centimeters is chosen because it is approximately ½ the width of a D-frame net.

If duplicates are to be sampled (of either or both assemblages), locations for sampling them should be arranged as depicted in Figure 2 (the duplicates are shown in light grey). *Note: For convenience, only Transects A through C are shown, but the same pattern of placement should be rotated across all 11 transects.*

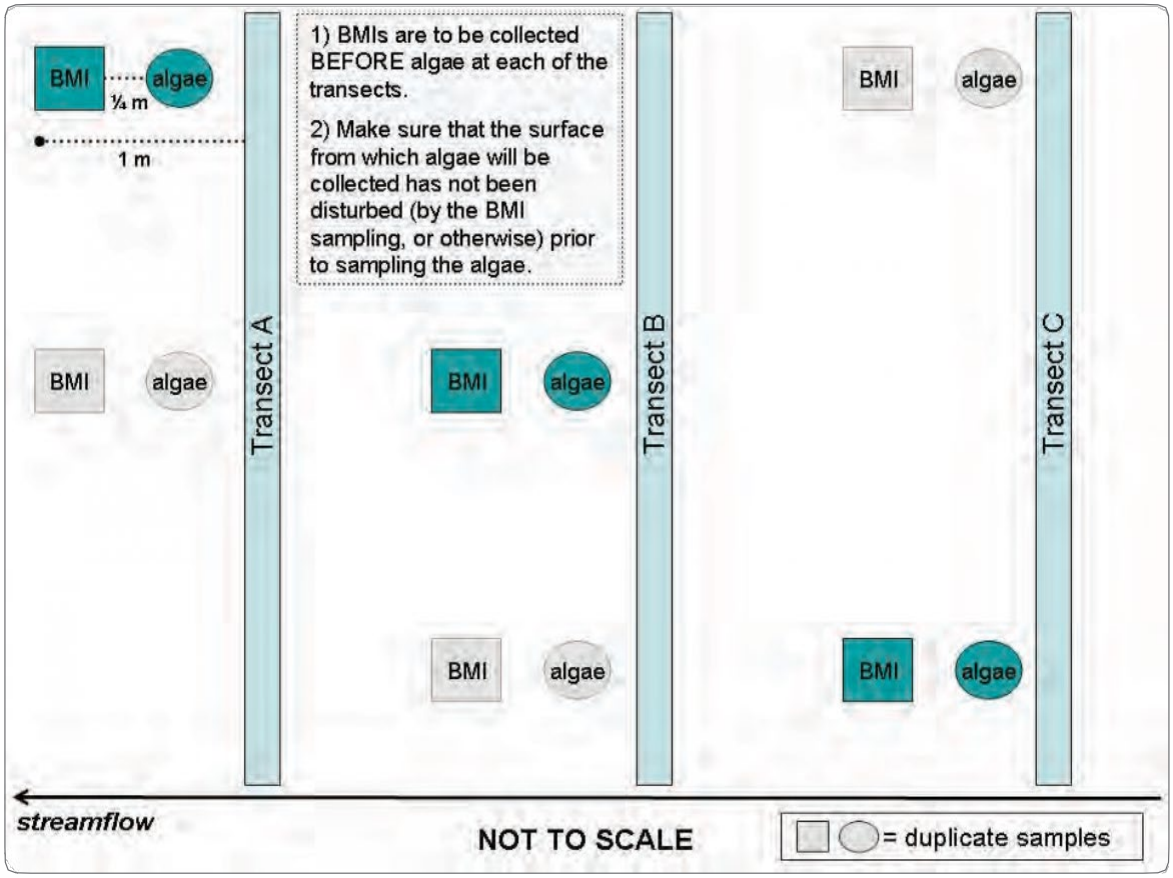

Figure 2. Sampling array for collection of algae, BMIs, and duplicates of each assemblage. For convenience, only Transects A through C are shown, but the same pattern of placement should be rotated across all 11 transects.

4.3 PROCEDuRE FOR COLLECTIOn OF QuAnTITATIvE ALGAL SAMPLES

During all phases of algae sampling and processing, in order to preserve specimen integrity, every attempt should be made to keep the sample material out of the sun, and in general, to protect the algae from heat and desiccation, as much as possible. This is necessary in order to reduce the risk of chlorophyll *a* degradation, limit cell division post-collection, and curb senescence/decay of live soft-bodied algae (especially for the qualitative samples; see Section 5.4). The need to maintain the integrity of the algal samples during collection and processing should always be borne in mind when planning the sampling scheme for a given site.

In addition, before sampling at any given site, the dish tub that will contain sample material must be scrubbed with a stiff-bristled brush or scouring pad and thoroughly rinsed with stream water, so that no algal material is carried over from the previous site to contaminate the current sample. The same applies to all other algae sampling apparati (toothbrushes for scrubbing, graduated cylinders, turkey basters, PVC and rubber delimiters, spatulas, syringe scrubbers, etc.).

4.3.1 Identifying the Sampling Locations

As with BMIs, algae sample collection should begin at Transect A and proceed upstream to Transect K. Except in circumstances in which the substrate to be sampled cannot be removed from the stream, a single sample of substrate material that corresponds to the objectively determined sampling point is gathered at each transect and placed in the plastic dish tub. The sample should always be collected from the substrate that is "uppermost" within the stream, and therefore has the highest probability of exposure to sun. For example, if at a given sampling point there is a thick layer of macroalgae above the stream bottom, the substrate collected at that point would be macroalgae itself, not the cobble, sand, or whatever other substrate lies beneath it. Proceeding from transect to transect with the dish tub, the sample collector rotates through the three collection positions in the following order: left at the first transect ("A"), center at the next transect ("B"), right at the next transect ("C"), then back to the left side ("D"), and so on through Transect K.

As substrates are gathered, a tally is taken of the number of samples that correspond to each of the classes of sampling device based on the surface area they sample: 1) 12.6 cm^2 for the PVC or rubber delimiters, and 2) 5.3 cm^2 for the syringe scrubber. The tallies are recorded in the Algae Samples field form under Collection Device. This information will ultimately be used to determine total stream surface area sampled at each site, which in turn will be used to calculate the soft-bodied algal biovolume and the biomass values. It may be helpful to use a tally meter in order to avoid having to carry a datasheet during substrate collection.

4.3.2 Collecting Erosional Substrates

If the substrate type that falls under the sampling spot is in erosional habitat and can be removed from the stream (e.g., a cobble, a piece of wood, or a piece of coarse gravel with an exposed surface area of at least 12.6 cm^2), carefully lift the substrate, moving slowly in an effort to disturb its top surface as minimally as

possible, and remove it from the stream. Then wipe any excess sand, silt, or BMIs, if present, off the bottom of the piece of substrate, and place it in the dish tub. It is helpful to place the substrate in such a way that makes it obvious what surface was facing upward when it was removed from the stream. Eventually, when you isolate a sample of algae from this substrate, you will want to obtain your sample from the portion of the substrate that had been exposed to the surface of the stream (and not buried) during the period leading up to the sampling event. For pieces of substrate with an exposed surface area that is < 12.6 cm^2, the PVC delimiter should be used (Section 4.3.3).

Be sure to place the substrate (e.g., cobble) in the dish tub in such a way that surfaces covered with non-target algae are not rubbing against anything, which could cause non-target algae to slough off into the tub, thus artificially inflating the amount of algae collected. To avoid this problem, and especially if a large number of cobbles are likely to be sampled across a given stream reach, one may choose to isolate the algal specimen from each cobble as it is selected, rather than collecting all the cobbles into the dish tub and then isolating the algal specimens from them after all transects have been sampled. See Section 4.3.9 for further elaboration on this alternative approach.

Figure 3. PVC Delimiter

4.3.3 Collecting Depositional Substrates

If the substrate type that falls under the sampling spot is removable and is in depositional habitat (e.g., silt, sand, fine gravel), and/or has an exposed surface area per particle that is < 12.6 cm^2, you will use a PVC delimiter. This is a plastic coring device with an internal diameter of 4 cm (Figure 3). Instructions for making a PVC delimiter are provided in Appendix C.

Isolate a specific quantity of sand/silt/gravel, centered on the sampling spot, by pressing into the top 1 cm of sediment with a PVC delimiter. Gently slide a masonry or kitchen spatula beneath the delimiter, being careful to keep the collected sediment contained within. Pull the PVC delimiter out of the water (with the spatula still in place) and remove any extra sediment from the spatula around the outside of the delimiter. Transfer the contents held in the delimiter by the spatula to the dish tub. Be sure not to pour the sediment sample on top of any cobbles that may be in the dish tub, as this could result in the sloughing of non-target algae from the cobbles into the dish tub, thus artificially inflating the amount of algae collected.

4.3.4 Collecting Sections of Macroalgae

If the substrate you hit on a given transect is a mass of macroalgae (e.g., a mass of attached filamentous algae underwater, or an unattached, floating mat that is believed to be native to the reach being sampled, and not imported from upstream), position the spatula directly under the macroalgae and press the PVC delimiter into the algae to define a 12.6 cm^2 area. Use a sharp razor blade or knife to cut away and discard any extra material from around the edges of the delimiter (do not simply pull it away, as this will distort the specimen and remove biomass from the targeted material). Add the macroalgal specimen that was isolated by the PVC delimiter to the dish tub.

When collecting a mass/mat of macroalgae, it is important to capture the full thickness of the macroalgae within the delimiter. To do this, from the side of the sampling area, slide your hand under the mat to feel where the bottom is, slide the spatula down to that spot, and then press the PVC delimiter downward slowly to "sandwich" the targeted section of macroalgae between the delimiter and the spatula. It is important to try not to bunch the macroalgae up nor stretch it out unnaturally, as the goal is to collect a representative sample of the algae as it occurs in the stream.

4.3.5 Collecting Sections of Macrophytes

If the substrate to be sampled is part of an immersed macrophyte, or old, dead leaves settled at the bottom of a pool, use the PVC delimiter/spatula combination to isolate a 12.6 cm^2 section of substrate that has been exposed to the surface of the stream. As with the macroalgae (Section 4.3.4), cut away and discard the extra material that falls outside the delimiter using a razor blade.

Figure 4. Syringe Scrubber

4.3.6 Collecting from Concrete, Bedrock, and Boulders

If the substrate falling under a sampling spot cannot be removed from the water (as in the case of bedrock, a boulder, or a concrete channel bottom), use a "syringe scrubber" device (Davies and Gee 1993; Figure 4) to collect an algae sample underwater. Instructions for making a syringe scrubber are provided in Appendix C.

To use this device, affix a fresh, white scrubbing pad circle onto the bottom of the syringe plunger using the Velcro® hooks on the end of the plunger. Press the plunger down so that the bottom of the scrubbing pad is flush with the bottom of the barrel. Then submerge the instrument, press the syringe firmly against the substrate, and rotate the syringe scrubber 3 times in order to collect the biofilm from the substrate surface onto the scrubbing pad. If the surface of the substrate where your sampling point fell is not flat enough to allow for a tight seal with the syringe barrel, objectively choose whatever sufficiently flat area on the exposed face of the substrate is closest to where the original point fell, and sample there.

After sampling, and before removing the syringe scrubber from the substrate, gently retract the plunger just slightly, so it is not up against the substrate anymore, but not so much that it pulls a lot of water into the barrel. Carefully slide the spatula under syringe barrel (which should be pulled just slightly away from the substrate on one side to allow the spatula to slide under), trying not to allow too much water to rush into the barrel. Then pull the instrument back up out of the water with the spatula still firmly sealed against the syringe-barrel bottom.

Hold the syringe scrubber over the dish tub and then remove the spatula, allowing any water to fall into the tub. Carefully detach the pad from the plunger and hold the pad over the tub. Using rinse water sparingly, remove as much algal material from the pad as possible by rinsing it off with the wash bottle, or a turkey baster, filled with stream water (from the current site—never carried over from a previous site), and

wringing it into the dish tub before discarding the used pad. Start this process by rinsing from the backside of the pad (the side that had been affixed to the plunger) to "push" the collected algae forward out of the front surface of the pad.

It is recommended that a fresh (new) pad be used each time a sample is collected, even within the same stream reach. Under no circumstances should the same pad be used at more than one site.

4.3.7 Collecting from Other Substrate Types

If other substrate types are encountered, they can be sampled from as long as there is good reason to believe that they were not recently introduced into the stream (e.g., by flowing from the upstream regions, or by recently falling into the stream), as they would then not be representative of the local instream environment. Use the collection instrument you deem to be most appropriate to sample the substrate and, as with any substrate, be sure to account for the surface area sampled (in this case, using the "Other" box on the Collection Device portion of the field forms).

4.3.8 Removal of Algae from Collected Substrates

After having sub-sampled substrates across the monitoring reach, there should be 11 transects' worth of material in the dish tub. Depending on the types of habitats in the stream and substrates encountered, the tub may contain cobbles, and/or sand, and/or gravel, and/or small pieces of wood, macroalgae, or macrophyte. Now a measured quantity of the algae clinging to these substrates must be removed and suspended in water to form a "composite sample" according to the instructions in the following sections.

For erosional substrate types that were removed from the stream (e.g., cobbles and small pieces of wood), use a rubber delimiter to isolate a 12.6 cm^2 area from which algae will be removed. A rubber delimiter can be made from a mountain bike tube with a hole cut out and reinforced with an appropriately sized rubber washer (Figure 5). Appendix C describes the procedure for making a rubber delimiter.

Figure 5. Rubber Delimiter

Wrap the rubber delimiter around the substrate to expose the desired sampling surface through the hole. Take care to ensure that the surface that will be scrubbed is truly the upper (generally at least somewhat "slimy") surface of the substrate as it had been oriented in the stream. Dislodge attached algae from this area by brushing it with a firm-bristled toothbrush (remember that this toothbrush must first have been thoroughly rinsed since the previous site to avoid contamination with algal specimens from other streams). If there is a thick mat of algae, or the algae is firmly encrusted on the surface of the substrate, use forceps or a razor blade first to dislodge the larger matter and put this in the dish tub. Then scrub the area with the brush.

Make sure that the entire surface within the delimiter has been scrubbed well in order to remove all the algae in that area. Fill a wash bottle or turkey baster with stream water from the current site (never carried over from a previous stream). Using as minimal a volume of water as possible, rinse the scrubbed algae from the sample area into the dish tub. Take care to squirt water only on the surface that is showing through the hole in the delimiter, and not anywhere else on the substrate's surface. It is helpful to invert the rock when rinsing so that the target surface is facing down toward the dish tub, and the rinsate drips off the sampling spot directly into the tub rather than flowing along the (non-target) sides of the substrate. Use water sparingly for each piece of substrate, because you should attempt to use no more than 400-500 mL total for the full suite of 11 samples collected along the transects (this includes any water used for rinsing algae off of sampling devices into the dish tub). After scrubbing is complete, rinse the delimiter and the brush into the dish tub, also. The scrubbed part of the substrate should feel relatively rough when you have finished, meaning that essentially all of the algae have been removed. After the sampling area on the piece of substrate has been thoroughly scrubbed and rinsed, the piece of substrate can be returned to the stream.

For depositional samples (e.g., silt, sand, or gravel), there is no need to isolate a specific area of the substrate within the dish tub, because the sample area was pre-isolated by using the PVC delimiter during collection. Simply massage all the sand and/or silt in the dish tub thoroughly between the fingers to dislodge any clinging algae. For pieces of gravel, use a toothbrush to remove algal material from surfaces.

Rinse the sediment thoroughly (but as sparingly as possible) with stream water so as to create a suspension of the dislodged microalgae (i.e., the sample). The final volume of the sample liquid in the dish tub will be measured before the algal taxonomic and biomass samples are prepared (described below). To do this, the liquid in the tub will be separated from the rinsed sediment such that the volume measured does not include sediment. After the liquid sample has been retrieved and measured, the rinsed sediment will be discarded back into the stream.

Other types of substrate, like pieces of macrophyte or dead leaves that had been collected with the PVC delimiter, should also be massaged between the fingers and rinsed into the tub in order to remove the algae coating them.

For macroalgal clumps there is a special step required for processing the samples. This procedure is described in detail in Section 5.3.

4.3.9 Alternative Approach: Processing Samples at Each Transect

It is also acceptable to isolate the algal specimens from each "piece" of substrate collected before moving on to the next transect. This approach has the disadvantage of requiring that all algae sampling/scraping tools be carried along with the collector as s/he proceeds up the stream, and that s/he pause to isolate the algae several times across the stream reach rather than one time at the end of all the transects. However, it limits the amount of substrate material that needs to be carried in the dish tub, thus making it lighter. This could be particularly important if a large number of cobbles are encountered across sampling points, such that it

could be difficult or impossible to carry them all to Transect K, or to carry them in such a way that non-target algae can easily be prevented from sloughing off into the tub via abrasion. For convenience, one may elect to wear a fisherman's vest to facilitate carrying all the algae sampling/scraping tools that will need to be brought along on the substrate sampling trip if employing this alternative approach.

SECTION 5
ALGAL SAMPLE PROCESSING

Four different types of laboratory samples may be prepared from the composite sample:

- Identification/Enumeration Samples
  1. Diatoms
  2. Soft-bodied algae
- Biomass Samples
  3. Chlorophyll *a* ("chl *a*")
  4. Ash-free dry mass ("AFDM")

5.1 GEnERAL COnSIDERATIOnS FOR PROCESSInG ALGAL SAMPLES

The general process for sample preparation is as follows. The Identification/enumeration samples are each aliquoted into 50-mL centrifuge tubes and chemically fixed (preserved). Diatom samples are fixed in the field with formalin immediately following collection, and soft-bodied algae samples are fixed in a laboratory with glutaraldehyde within four days of collection. The chlorophyll *a* and AFDM samples are collected on filters in the field and stored on wet ice, and then frozen as soon as possible after returning from the field (and within four hours of collection). The filters are kept frozen until analysis, which should occur within 28 days of collection. If the field crew is spending the night in a hotel, it is necessary to buy dry ice to freeze the biomass filters upon finishing the day's fieldwork, and to keep them on dry ice until the samples can be transferred to the freezer back at the lab.

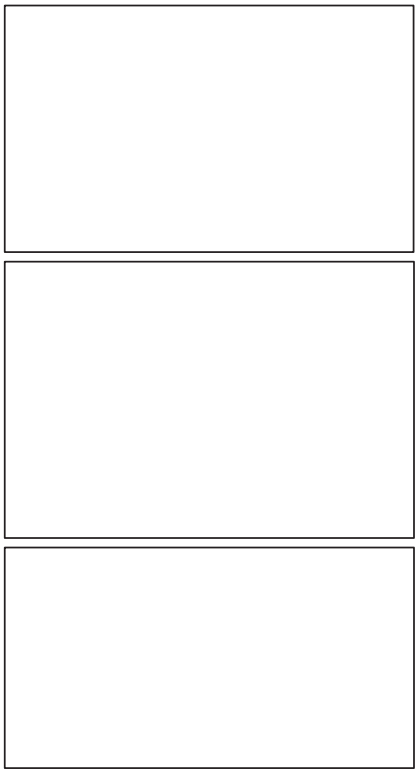

Figure 6. Labels for biomass and taxonomic identification samples.

Algae sample labels are shown in Figure 6. Recorded on each sample label are the volume of the composite sample (described in Sections 5.2.1 and 5.3.2), and the TOTAL area of stream bottom sampled (based on which sampling devices were used; described in Sections 4.3.2 - 4.3.7), as well as the volume of sample aliquoted (for the taxonomic ID samples) or filtered (for the chlorophyll *a* and ADFM samples). All of these values should be recorded on the field forms, as well, under the Algae Samples section. On the sample labels, the sample type: "chl *a*", "AFDM", "diatoms", or "soft" is circled, and all the remaining information on each label, like Site Code, Date, and site coordinates is filled out.

Before preparing the algae samples it is necessary to determine two things:

- Are there any clumps of macroalgae in the composite sample (as opposed to just microalgae suspended in liquid)?

AND

- Is a soft-bodied algal taxonomic sample going to be prepared?

The answers to these questions will determine the course of action for preparing the algae samples for a given site:

- If there is no macroalgal clump, liquid composite sample will simply be added to each taxonomic ID sample tube (40 mL for diatoms and 45 mL for soft-bodied algae). Biomass samples will also be prepared using the liquid composite sample, as is.
- If there is a macroalgal clump present, but no soft-bodied sample will be prepared, the entire clump will be chopped into fine bits (resulting in strands that are eyelash-length or shorter) and incorporated directly into the liquid portion of the composite sample, and the mixture will be shaken to homogenize it before preparing the diatom and/or biomass samples.
- If there is a macroalgal clump AND a soft-bodied algal taxonomic ID sample is to be prepared, then a more complex procedure must be employed in order to properly process the macroalgae before preparing the various samples.

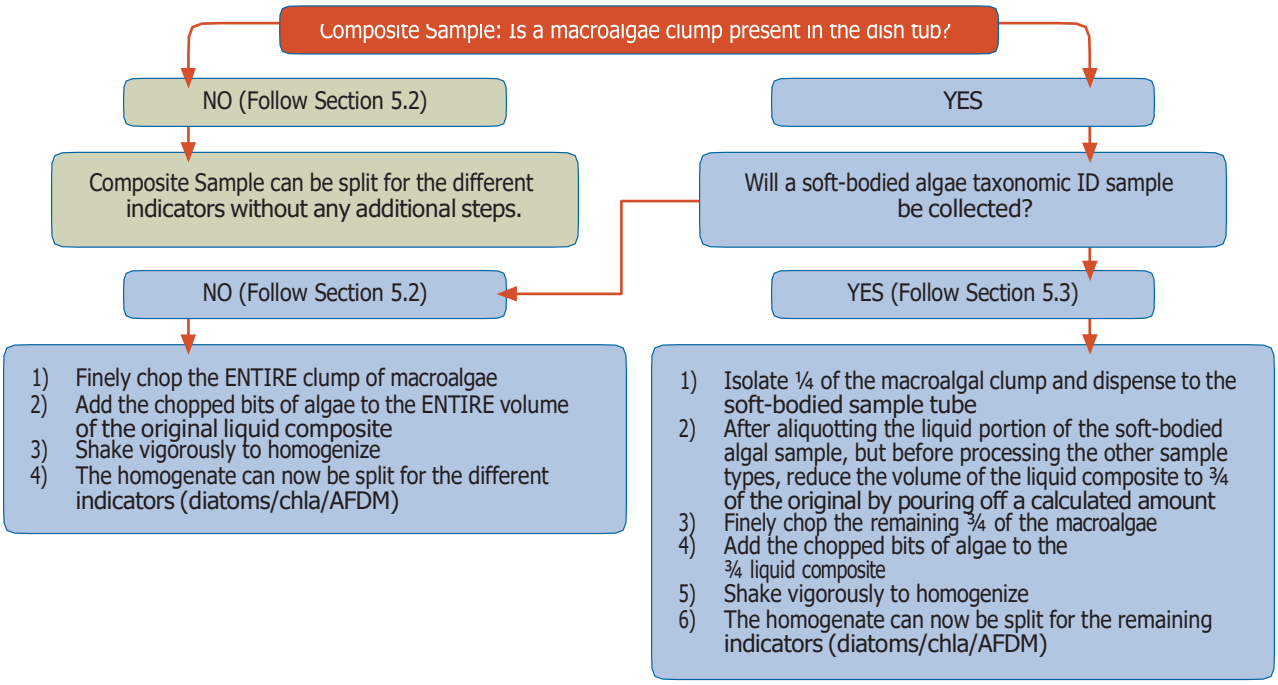

Figure 7. Summary of major sample-processing decision points based on presence of macroalgal clump(s) and need to prepare soft-bodied algal samples.

Figure 7 provides a summary of the various sample-processing steps that are involved, and the following sections describe the procedures in detail. Use Section 5.2. if there is NO macroalgal clump present in the dish tub OR a soft-bodied algal sample will be NOT be prepared. Use Section 5.3 if there IS a macroalgal clump present AND a soft-bodied algal sample will be prepared.

5.2 SAMPLE PROCESSInG WHEn THERE IS nO MACROALGAL CLuMP OR WHEn nO SOFT-BODIED SAMPLE IS BEInG PREPARED

This section describes the sample-processing procedure for the situation in which there was either 1) no macroalgal clump in the dish tub containing the composite sample material, or 2) no soft-bodied algal sample will be prepared. If there was no macroalgal clump but both soft bodied algae AND other sample types are to be prepared, follow all the instruction in this section with the exception of the final portion of Section 5.2.1 that is italicized and in orange font, and discusses how to process macroalgae when preparing only diatom and/or biomass samples.

If there is a macroalgal clump, but no soft-bodied algae sample is to be collected, follow the instructions in this section, including the final, italicized portion of Section 5.2.1, and skip Section 5.2.2, which deals with soft-bodied algal sample processing.

5.2.1 Measuring the Composite Liquid volume

Once algal specimens have been removed from all the substrates (e.g., sand, gravel, cobble, wood, leaves) in the dish tub, according to the procedure described in Section 4, thoroughly agitate the liquid to get as much as possible of the microalgae into suspension, and then immediately pour the liquid into a CLEAN graduated cylinder to measure its volume. Try to leave all substrate material (e.g., silt, sand) behind. Transfer the measured liquid into a CLEAN 1L plastic bottle. Using a minimal amount of stream water, rinse the substrate once or twice with stream water until it appears that little to no additional suspended material (microalgae) is coming off. Add this rinsate to the graduated cylinder to measure it also. If necessary, repeat this process (regularly agitating the dish tub) until all the liquid has been measured and transferred to the sample bottle. Use water sparingly, because the total sample volume (plus rinsate) should be no more than about 400-500 mL.

Because you are leaving as much as possible of the silt, sand, and any large substrate material behind, the final volume should reflect only the liquid component of the sample plus rinse water. Record the total volume of all the liquid that had been in the dish tub, including any that was used for rinsing the substrates and sampling devices, on the field sheet under the Algae Samples section. This is the COMPOSITE VOLUME. This value will also be recorded on all algae sample labels (i.e., for the diatom and soft-bodied algae taxonomic ID samples, the chlorophyll *a*, and the AFDM).

Note: If no soft-bodied algae sample is to be prepared, but there is a macroalgal clump in the sample, separate the clump from the liquid portion of the sample, measure and record the composite volume of the liquid (as described above), then cut the macroalgal clump into very fine pieces (resulting in strands that are eyelash-length or shorter) with CLEAN scissors and add these pieces to the composite liquid. The pieces should be chopped small enough so that they practically "blend" into the liquid (i.e., distinct fragments of macroalgae are not easily discernible), because the goal is to "homogenize" the macroalgae into the liquid as much as possible. Shake vigorously to homogenize the macroalgal fragments into the liquid. Then proceed to Section 5.2.3 and beyond to prepare the diatom and/or biomass samples.

5.2.2 Preparing the Soft-Bodied Algae Taxonomic ID Sample

Pour freshly-agitated liquid composite sample into the soft-bodied algae sample tube to the 45 mL mark. Midway through pouring, the composite sample should be swirled some more (first clockwise, then counter-clockwise) to ensure that the microalgae are still fully suspended. Cap the tube tightly. Completely fill out a sample label and affix it to the sample tube. Cover the label completely with clear plastic tape to prevent the writing on the label from smearing. Place the tube in the wet ice chest to keep it in the dark and as cold as possible, but make sure it is never allowed to freeze.

Note: As soon as possible, and no longer than four days after collection of the sample, glutaraldehyde must be added to the tube (to a final concentration of 2.5%, by adding 5 mL of 25% glutaraldehyde to 45 mL of sample) and distributed throughout the sample by agitation and turning the tube upside down repeatedly. Glutaraldehyde is necessary for fixing soft-bodied algal samples in order to preserve fine morphological features and the color of pigments, as both can be crucial characters for taxonomic determination. Glutaraldehyde is a hazardous substance that poses a number of safety risks. As such, it should be handled in a fume hood by trained personnel wearing appropriate gear. Refer to Appendix D for an SOP for the use of glutaraldehyde.

Members of the field crew can either have the glutaraldehyde added to the samples back at their own lab, or arrange for the glutaraldehyde to be added to the samples by the taxonomy lab. In either case, the unfixed samples must be kept in the dark and on wet ice (but not allowed to freeze), and must be fixed within four days of collection (and preferably sooner). If the taxonomy lab will be adding the fixative, it is imperative to plan ahead to arrange for this to be done, and also to clearly mark which tubes will need to have fixative added to them. Once the samples are fixed, it is no longer imperative to store them on wet ice. Following fixation, they can simply be stored in a cool, dark place.

5.2.3 Preparing the Diatom Taxonomic ID Sample

Diatom samples should be fixed as soon as possible after collection to reduce the possibility of cell division post-sampling. A 10% solution of buffered formalin is used to fix diatoms, and instructions for preparing this solution are provided in Appendix C.

To prepare the diatom sample, aliquot 40 mL of freshly-agitated composite liquid into the diatom ID sample tube, swirling the composite sample bottle again midway through pouring to keep the microalgae suspended. Add 10 mL of the 10% buffered formalin to the sample. This can be done using a small syringe or bulb pipette. Alternatively, if preferred, the centrifuge tubes for the diatom samples can be pre-loaded with 10 mL of the 10% buffered formalin and 40 mL of sample can be added carefully to the fixative, to avoid having to dispense the fixative in the field.

Notes: Fixatives such as formalin must be used with great care. Be sure to wear formalin-safe gloves and safety goggles when using the fixative, as it should never be touched with bare hands or allowed to splash onto skin or into eyes. Also make sure it is used only in a very well-ventilated place and avoid breathing in any fumes. Minimize the amount of time that vessels containing formalin are open. Fixative added to the sample must not be allowed to ooze outside the vessel that contains it, including the sample tubes. Refer to Appendix E for an SOP for the use of formalin.

Cap the tube tightly and shake it to mix the formalin into the sample. Fill out a sample label and affix it to the sample tube. Cover the label completely with clear plastic tape to prevent the writing on the label from smearing. Keep the fixed diatom samples in the dark and away from extreme heat.

5.2.4 Preparing the Biomass Samples

The remaining composite sample liquid can be used to prepare the chlorophyll *a* and AFDM filters as described below.

Chlorophyll *a* samples:

The procedure to filter chlorophyll *a* samples should be carried out quickly, and in the shade as much as possible, to minimize exposure of the sample to light, and minimize chlorophyll *a* degradation thereby. For the chlorophyll *a* samples, use CLEAN filter forceps (rinsed with DI water three times) to center a glass fiber filter (47 mm, 0.7 µm pore size) onto the mesh platform of a CLEAN filtering tower apparatus (rinsed with DI water three times), and rinse the filter a little with DI water to seat it well into the mesh before attaching the filter reservoir on top. Never touch the filters with hands or anything other than clean forceps.

Agitate the composite sample to resuspend all the microalgal material. Carefully measure 25 mL using a small, CLEAN graduated cylinder (rinsed with DI water three times). Midway through pouring the 25 mL, swirl the composite sample again to ensure that the material is still fully suspended. Pour the remainder of the 25 mL, and then pour the measured sample into the filter reservoir. Once empty, rinse the graduated cylinder with a few mL of DI water, and add this to the reservoir.

To filter the sample, create a gentle vacuum with the hand pump. Be sure to proceed very slowly, and pump only one stroke at a time until all of the liquid in the sample is passed through the filter. Pressure on the sample should never exceed 7 psi, as this could cause cells to burst and release contents, including chlorophyll *a*, into the filtrate and be lost. If it becomes impossible to filter a whole 25 mL of the sample and

remove the water efficiently, discard the filter and try again with a smaller volume (e.g., 10 mL). It is not necessary to collect on multiple filters to try to achieve a total volume of 25 mL. Simply filter as much as possible on a single filter, up to 25 mL, and then use that filter as the sample. Be sure to record the volume of the composite sample that was actually filtered, both on the datasheet, and on the sample label.

Rinse the sides of the filter reservoir with a few mL of DI water, and continue filtering until the water is drawn down. The filter should not be sucked dry, but rather left slightly moist, in order to avoid applying excessive pressure to the sample, which could cause algal cells to burst. After the liquid has passed through, check the filter to see if there are any bits of non-algal plant matter (like tiny seedlings or bits of leaves). If so, remove them with clean forceps, being careful not to remove any algae in the process. If possible, rinse the removed items with DI water onto the filter before discarding them. Remove the filter from the filtering device. *Note: Always thoroughly rinse the sides of the filter reservoir and the interface between the mesh filter seating and the screw-on part of the reservoir with DI water between samples.*

Being careful not to remove any of the collected material from the filter, fold the filter in half (with the sample material on the inside) using the forceps, and place it inside a clean, snap-top Petri dish[13]. Envelop the Petri dish completely within a small sheet of aluminum foil in order to prevent any light from reaching the filter. Place the covered Petri dish and its corresponding, completely filled-out sample label (face outward) into a 100 mL Whirl-pak bag[14], purge as much of the air out of the bag as possible, "whirl" it shut, and seal it tightly with its wire tabs, so that water in the cooler will not be able to enter the bag. *Note 1: If the Whirl-pak bags contain a lot of air, they will float on top of the ice water in the cooler, and they then run the risk of not being kept cold enough. Shove the sample packet down into the ice in the cooler. Note 2: A clean, clear plastic centrifuge tube is also an acceptable container in which to store the filter. It must also be properly labeled, wrapped in aluminum foil, and kept submerged in wet ice.*

Keep chlorophyll *a* filters as cold as possible and place in the freezer or dry ice as soon as possible (and within four hours of collection); the holding time for the chlorophyll *a* filters is 28 days from collection, when kept frozen.

Ash-free dry mass (AFDM) samples:

For the AFDM samples, you should use glass-fiber filters (47 mm, 0.7 µm pore size) that have been precombusted. Never touch the filters with hands or anything other than a CLEAN forceps (rinsed with DI water three times). The filters to use should be labeled "for AFDM", and stored in aluminum sleeves. Follow the same process as that used for chlorophyll *a* sample filtering. After all the liquid has passed through, check the filter to see if there are any pieces of non-algal plant matter (such bits of leaves or wood). If so, remove them with a clean forceps (rinsed with DI water three times), being careful not to remove any algae in the process. The goal with AFDM, for the purposes of this SOP, is to target the ALGAL portion of the organic matter in the sample, and therefore field crews should do their best to remove non-algal contributors

13. It may be beneficial to write the Site Code or sample ID code on the Petri dish itself, in addition to filling out the full sample label.
14. Other bag types are acceptable only if they are water-tight (note that Ziploc bags often leak when submerged).

of organic matter from the sample. Remove the filter from the filtering device. *Note: Always thoroughly rinse the sides of the filter reservoir and the interface between the mesh filter seating and the screw-on part of the reservoir with DI water between samples.*

Use the forceps to fold the AFDM filter in half (with the sample material on the inside) and wrap it loosely in a small sleeve of clean aluminum foil. Be careful not to squeeze the filter, which could cause the sample to ooze from the filter onto the aluminum sleeve. Store the filter in a sealed 100 mL Whirl-pak bag containing a completely filled-out sample label, including the volume that was filtered (i.e., 25 mL or otherwise). As with the chlorophyll *a*, purge as much of the air out of each bag as possible, "whirl" it shut, and seal tightly with the wire tabs. Shove the sample packet down into the ice in the cooler. *Note: A clean, clear plastic centrifuge tube is also an acceptable container in which to store the filter. It must also be properly labeled and kept submerged in wet ice.*

Keep AFDM filters as cold as possible until the samples can be frozen back at the lab that evening, or place on dry ice until they can be stored in the lab freezer. The holding time for the AFDM samples is 28 days from collection, when kept frozen.

5.3 PROCESSInG SOFT-BODIED AnD OTHER SAMPLE TyPES WHEn A MACROALGAL CLuMP IS PRESEnT

The following is a description of how to proceed when a soft-bodied algal taxonomic ID sample is to be prepared AND macroalgal clump(s) are present in the sample in the dish tub. A flowchart of this procedure is provided in Appendix F. It is recommended that this flowchart be printed out in color, laminated (if possible) or printed out on water-proof paper, and brought along to the field for quick reference on handling macroalgal clumps in the composite sample. The reason for the extra step in the processing of the macroalgae for the purposes of the soft-bodied algae sample is that it maintains larger, more intact macroalgal specimens for examination in the laboratory, rather than chopping up all of the macroalgal specimens before sending them to the lab. This is important, because availability of intact specimens greatly improves the chances that the taxonomist will be able to identify the soft-bodied algae to low taxonomic levels.

5.3.1 Isolating and Dividing the Macroalgal Clump

For this procedure, the macroalgal clump is first removed from the dish tub, wrung out gently, and rolled into a cylinder shape that is relatively even in thickness along its length. If there is more than one type of macroalgae in the sample, the various types should be layered on top of one another lengthwise so that they are represented in roughly constant proportions across the length of the "cylinder". The cylinder is measured with a ruler and a quarter of its length is cut off with scissors and put into the (still empty) soft-bodied algae ID centrifuge tube[15]. The clump is pushed down into the tube, and the top is flattened, so that the volume

15. It is unlikely that the ¼ macroalgal clump will occupy all the space in the sample tube, but if it does, a second tube will be needed in order to accommodate all the sample material plus liquid. If such an action is taken, it should be noted in the Comments section of the field sheets and the tubes should be clearly identified as belonging to the same sample, for record keeping purposes.

of the clump can be estimated using the graduations on the tube. The estimated volume of this clump will be used in a calculation (see Equation 1 and Figure 8). The remaining three-quarters length of cylinder is set aside in the shade/cool. It is recommended that this section be placed in a Ziploc bag, sealed, and put in the wet ice cooler.

5.3.2 Measuring the Composite Liquid volume

Once algal specimens have been removed from all the substrates (e.g., sand, gravel, cobble, wood, leaves) in the dish tub, according to the procedure described in Section 4, gently agitate the dish tub to suspend the microalgae in the liquid, and then start pouring this suspension into a CLEAN graduated cylinder to measure the volume of the liquid. Try to leave all substrate material (e.g., silt, sand) behind. Transfer the measured liquid into a CLEAN 1L plastic bottle. Using a minimal amount of stream water, rinse the substrate once or twice with stream water until it appears that little to no additional suspended material (microalgae) is coming off. Add this rinsate to the graduated cylinder to measure it also. If necessary, repeat this process (regularly agitating the dish tub) until all the liquid has been measured and transferred to the sample bottle. Use water sparingly, because the total sample volume plus rinsate should be no more than about 400-500 mL.

Because you are leaving as much of the silt, sand, and any large substrate material behind as possible, the final volume should reflect only the liquid component of the sample plus rinse water. Record the total volume of all the liquid that had been in the dish tub, including any that was used for rinsing the substrates and sampling devices, on the field sheet under the Algae Samples section. This is the COMPOSITE VOLUME. This value will also be recorded on all algae sample labels (i.e., for the diatom and soft-bodied algae taxonomic ID samples, the chlorophyll *a*, and the AFDM).

5.3.3 Preparing the Soft-Bodied Algae Taxonomic ID Sample

Pour freshly-agitated liquid composite sample from the 1-L bottle into the soft-bodied algae sample tube (on top of the clump of macroalgae) up to the 45 mL mark. Midway through pouring, the composite sample should be swirled some more (first clockwise, then counter-clockwise) to ensure that the microalgae are still fully suspended. Cap the tube tightly. Completely fill out a sample label and affix it to the sample tube. Cover the label completely with clear plastic tape to prevent the writing on the label from smearing. Place the tube in the wet ice chest to keep it in the dark and as cold as possible, but make sure it is never allowed to freeze.

Note: As soon as possible, and no longer than four days after collection of the sample, glutaraldehyde must be added to the tube (to a final concentration of 2.5%, by adding 5 mL of 25% glutaraldehyde to 45 mL of sample) and distributed throughout the sample by agitation and turning the tube upside down repeatedly. Glutaraldehyde is necessary for soft-bodied algal samples in order to preserve fine morphological features and the color of pigments, as both can be crucial characters for taxonomic determination. Glutaraldehyde is a hazardous substance that poses a number of safety risks. As such, it should be handled in a fume hood by trained personnel wearing appropriate gear. Refer to Appendix D for an SOP for the use of glutaraldehyde.

Members of the field crew can either have the glutaraldehyde added to the samples back at their own lab, or arrange for the glutaraldehyde to be added to the samples by the taxonomy lab. In either case, the unfixed samples must be kept in the dark and on wet ice (but not allowed to freeze), and must be fixed within four days of collection (and preferably sooner). If the taxonomy lab will be adding the fixative, it is imperative to plan ahead to arrange for this to be done, and also to clearly mark which tubes will need to have fixative added to them. Once the samples are fixed, it is no longer imperative to store them on wet ice. Following fixation, they can simply be stored in a cool, dark place.

5.3.4 Preparing the Diatom Taxonomic ID Sample

After the soft-bodied algal sample has been prepared, and before preparing the diatom sample (and biomass samples, which will be discussed in the next section), the volume of the remaining composite liquid must be reduced to equal ¾ of the original volume[16]. This is necessary because ¼ of the macroalgae clump was taken out of the composite sample but a full ¼ was not removed from the water portion. As such, the original ratio between water and macroalgae must be restored before further sample preparation.

The following procedure is used to reduce the volume of liquid composite to ¾ of the original. For convenience, you can use this formula (Figure 8) to calculate how many mL to pour off and discard from the composite:

Equation 1. Adjusting the volume of composite sample

volume (mL) of composite to pour off = (0.25 * C) - 45 + A

where "C" is the original composite volume and "A" is the approximate volume of the clump of macroalgae that was placed in the soft-bodied algae sample tube (tamped down and flattened). You may wish to fill out a copy of the Ratio Restoration worksheet shown in Figure 8 to calculate the amount of composite to pour off.

Liquid Portion of Composite Sample: _____ mL = C

volume of 1/4 macroalgal chunk: _____ mL = A

volume of Liquid Composite to Pour Off: (0.25 * _____ C) – 45 + _____ A = _____ mL

Figure 8. Ratio restoration worksheet.

16. For example, if the original composite volume was 480mL, you will be discarding enough composite liquid to get down to 360 mL.

As always, be sure to agitate the composite liquid adequately in order to resuspend any settled microalgae before pouring off the calculated volume.

Once the required amount of composite liquid has been discarded, the remaining ¾ of the macroalgal clump ("cylinder") is cut into very fine pieces with a scissors (resulting in strands that are eyelash-length or shorter), and these are added to the reduced-volume composite liquid. The pieces should be chopped small enough so that they practically "blend" into the liquid (i.e., distinct fragments of macroalgae are not easily discernible), because the goal is to "homogenize" the macroalgae into the liquid as much as possible. Now the ratio of macroalgae to liquid from the original sample in the dish tub is restored. Cap the composite bottle and shake vigorously to homogenize the bits into the liquid as much as possible, while not agitating so hard as to risk busting cells and releasing chlorophyll.

Diatom samples should be fixed as soon as possible after collection to reduce the possibility of cell division post-sampling. A 10% solution of buffered formalin is used to fix diatoms, and instructions for preparing this solution are provided in Appendix C.

To prepare the diatom sample, aliquot 40 mL of freshly-agitated sample homogenate into the diatom ID sample tube, swirling the composite sample bottle again midway through pouring to keep the algal material suspended. Add 10mL of the 10% buffered formalin to the sample. This can be done using a small syringe or bulb pipette. Alternatively, if preferred, the centrifuge tubes for the diatom samples can be pre-loaded with 10 mL of the 10% buffered formalin and 40 mL of sample can be added carefully to the fixative, to avoid having to dispense the fixative in the field.

Note: Fixatives such as formalin must be used with great care. Be sure to wear formalin-safe gloves and safety goggles when using the fixative, as it should never be touched with bare hands or allowed to splash onto skin or into eyes. Also make sure it is used only in a very well-ventilated place and avoid breathing in any fumes. Minimize the amount of time that vessels containing formalin are open. Fixative added to the sample must not be allowed to ooze outside the vessel that contains it, including the sample tubes. Refer to Appendix E for an SOP for the use of formalin.

Cap the tube tightly and shake it to mix the formalin into the sample. Fill out a sample label and affix it to the sample tube. Cover the label completely with clear plastic tape to prevent the writing on the label from smearing. Keep the fixed diatom samples in the dark and away from extreme heat.

5.3.5 Preparing the Biomass Samples

The remaining composite sample homogenate can be used to prepare the chlorophyll *a* and AFDM filters according to the following procedure.

Chlorophyll *a* samples:

The procedure to filter chlorophyll *a* samples should be carried out quickly, and in the shade as much as possible, to minimize exposure of the sample to light, and minimize chlorophyll *a* degradation thereby. For the chlorophyll *a* samples, use CLEAN filter forceps (rinsed with DI water three times) to center a glass fiber filter (47 mm, 0.7 µm pore size) onto the mesh platform of CLEAN filtering tower apparatus (rinsed with DI water three times), and rinse the filter a little with DI water to seat it well into the mesh before attaching the filter reservoir on top. Never touch the filters with hands or anything other than clean forceps.

Agitate the composite sample homogenate to resuspend all the macroalgal fragments and microalgal material. Carefully measure 25 mL using a small, CLEAN graduated cylinder (rinsed with DI water three times). Midway through pouring the 25 mL, swirl the homogenate again to ensure that the material is still fully suspended. Pour the remainder of the 25 mL, and then pour the measured homogenate into the filter reservoir. Once empty, rinse the graduated cylinder with a few mL of DI water, and add this to the reservoir.

To filter the sample, create a gentle vacuum with the hand pump. Be sure to proceed very slowly, and pump only one stroke at a time until all of the liquid in the sample is passed through the filter. Pressure on the sample should never exceed 7 psi, as this could cause cells to burst and release contents, including chlorophyll *a*, into the filtrate and be lost. If it becomes impossible to filter a whole 25 mL of the sample and remove the water efficiently, discard the filter and try again with a smaller volume (e.g., 10 mL). It is not necessary to collect on multiple filters to try to achieve a total volume of 25 mL. Simply filter as much as possible on a single filter, up to 25 mL, and then use that filter as the sample. Be sure to record the volume of the composite sample that was actually filtered, both on the datasheet, and on the sample label.

Rinse the sides of the filter reservoir with a few mL of DI water, and continue filtering until the water is drawn down. The filter should not be sucked dry, but rather left slightly moist, in order to avoid applying excessive pressure to the sample, which could cause algal cells to burst. After all the liquid has passed through, check the filter to see if there are any bits of non-algal plant matter (like tiny seedlings or bits of leaves). If so, remove them with clean forceps, being careful not to remove any algae in the process. Remove the filter from the filtering device. *Note: Always thoroughly rinse the sides of the filter reservoir and the interface between the mesh filter seating and the screw-on part of the reservoir with DI water between samples.*

Fold the filter in half (with the sample material on the inside) using the forceps, and place it inside a clean, snap-top Petri dish. Envelope the Petri dish completely within a small sheet of aluminum foil in order to prevent any light from reaching the filter. Place the covered Petri dish and its corresponding, completely filled-out sample label (face outward) into a 100 mL Whirl-pak bag, purge as much of the air out of the bag as possible, "whirl" it shut, and seal it tightly with its wire tabs, so that water in the cooler will not be able to enter the bag. *Note: If the Whirl-pak bags contain a lot of air, they will float on top of the ice water in the cooler, and they then run the risk of not being kept cold enough. Shove the sample packet down into the ice in the cooler.*

Keep chlorophyll *a* filters as cold as possible and place in the freezer or dry ice as soon as possible (and within four hours of collection); the holding time for the chlorophyll *a* filters is 28 days from collection, when kept frozen.

Ash-free dry mass (AFDM) samples:

For the AFDM samples, you should use glass-fiber filters (47 mm, 0.7 µm pore size) that have been precombusted. Never touch the filters with hands or anything other than a clean forceps (rinsed with DI water three times). The filters to use should be labeled "for AFDM", and stored in aluminum sleeves. Follow the same process as that used for chlorophyll *a* sample filtering. After all the liquid has passed through, check the filter to see if there are any bits of non-algal plant matter (such as bits of leaves or wood). If so, remove them with clean forceps (rinsed with DI water three times), being careful not to remove any algae in the process. Remove the filter from the filtering device. *Note: Always thoroughly rinse the sides of the filter reservoir and the interface between the mesh filter seating and the screw-on part of the reservoir with DI water between samples.*

Use the forceps to fold the AFDM filter in half (with the sample material on the inside) and wrap it loosely in a small sleeve of clean aluminum foil. Be careful not to squeeze the filter, which could cause the sample to ooze from the filter onto the aluminum sleeve. Store the filter in a sealed 100 mL Whirl-pak bag containing a completely filled-out sample label, including the volume that was filtered (i.e., 25 mL or otherwise). As with the chlorophyll *a*, purge as much of the air out of each bag as possible, "whirl" it shut, and seal tightly with the wire tabs. Shove the sample packet down into the ice in the cooler.

Keep AFDM filters as cold as possible until the samples can be frozen back at the lab that evening, or place on dry ice until they can be stored in the lab freezer. The holding time for the AFDM samples is 28 days from collection, when kept frozen.

5.4 PROCEDuRE FOR COLLECTInG QuALITATIvE ALGAL SAMPLES

If your program calls for the collection of soft-bodied taxonomic ID samples, then you will also need to collect a "qualitative" sample at every monitoring reach. The qualitative samples consist of a composite of all types of soft-bodied algae visible within the reach. This is of value because it can provide a fairly exhaustive list of soft-bodied algal taxa present at the site and can also aid identification of taxa captured in the RWB sampling, since it allows larger, more intact specimens to be collected than those that may end up in the more heavily processed quantitative sample (described above). In addition, if the qualitative sample is kept cool and in the dark, and is delivered to the lab in a timely manner (i.e., as quickly as possible, and within two weeks of collection), the live specimens can be cultured, which can also aid in identification. For example, some taxa in the Zygnematales cannot be identified to species level unless they are in a sexual phase during examination. If asexual at the time of collection (which is the typical situation), live specimens could be induced to a sexual phase in the lab. Collection of a qualitative diatom sample is optional, and is typically not needed for general bioassessment purposes.

For qualitative soft-bodied algal samples, collect specimens of all obviously different types of macroalgal filaments and mats, as well as microalgae (in the forms of scrapings using a razor blade or knife), and depositional samples (suctioned from along the surface of sediments using a clean turkey baster). Note that some algae (e.g., species of *Chara*, *Paralemanea*, and *Vaucheria*) have morphologies similar to submerged macrophytes or mosses. In addition, algae are not always green, and may instead be dark-brownish, golden, reddish, or bluish-green. Some cyanobacteria (which should also be collected for the qualitative sample), such as members of the genus *Nostoc*, look like gelatinous globules, or sacs, ranging in size anywhere from smaller than a pea to larger than a lime. Online image searches of these taxa and others will help the collector develop an eye for the variety of types of algae that may be encountered in streams. If you suspect something may be algae, but are not sure, it is always preferable to collect some of it for the qualitative sample. The laboratory will determine whether it qualifies for inclusion in the species list. Collect from as many distinct locations as possible throughout the reach so as to capture as much of the apparent diversity in the reach as you can. Also, when possible, try to grab part of the holdfast structures that attached the macroalgae to the substrate, as these structures can be useful for taxonomic identification.

Since these samples are merely qualitative, and not quantitative, you need not worry about collecting them in a manner that is representative of their relative abundances within the reach. *Note: If there is only a small amount of macroalgae in the stream, it should be allocated preferentially to the soft-bodied algae laboratory sample, as opposed to the diatoms (if a diatom qualitative sample is being collected), because it is primarily needed for the soft-bodied algal identification work (although diatoms can live as epiphytes on macroalgae, so macroalgal samples are also of values for the diatom work).*

Using a thick, waterproof marker, label a Whirl-pak bag with the Site Code, Date, Sample ID, and "soft" (or "diatom", if also collecting a diatom sample). Fill the bag with a total volume of up to 100 mL of qualitative algae sample + water. Purge any extra air from the bag, seal with the wire tabs by twisting them together (not just folding them, as this can result in leakage), tuck the ends of the wire tabs inward so that they cannot poke other bags, and store in the cooler on wet ice in the field. Be careful not to place the bags right up against ice or frozen blue-ice bags, because this could cause the algae to freeze and thus destroy the sample. Unlike with the quantitative samples, **do not add glutaraldehyde or formalin** (or any other fixative) to these qualitative samples. Keep the qualitative samples on wet ice and refrigerate immediately upon return to the lab. Because they are not preserved, these samples should be examined by a taxonomist as soon as possible (and within two weeks, at most), as they can decompose fairly rapidly. Decomposition is of particular concern for the soft-bodied algae sample.

If it is impossible to get the soft-bodied qualitative samples to a taxonomist within two weeks of sample collection, then split the qualitative samples in half, transfer one half to a 50 mL centrifuge tube and preserve it with glutaraldehyde (to a 2.5% final concentration) and leave the other half un-fixed (but continue to store in the cold/dark until examination by a taxonomist). This should be done in order to preserve part of the sample for morphological identification, but still maintain some possibility of keeping some specimens alive, in case culturing is necessary. *Note: Glutaraldehyde is a hazardous substance that can*

pose health and safety risks. Add glutaraldehyde in a fume hood, wearing safety goggles and glutaraldehyde-safe gloves. Refer to Appendix D for more detailed instructions on the safe handling of glutaraldehyde.

5.5 ALGAL SAMPLInG QuALITy ASSuRAnCE / QuALITy COnTROL

The SWAMP bioassessment group is currently developing guidelines for quality assurance and quality control for bioassessment procedures. Future revisions to this document will include more specific information covering personnel qualifications, training and field audit procedures, procedures for field calibration, procedures for chain of custody documentation, requirements for measurement precision, health and safety warnings, cautions (actions that would result in instrument damage or compromised samples), and interferences (consequences of not following the SOP).

It is recommended that duplicate sampling of algae occur at 10% of study sites. The recommended method for collecting duplicates is at adjacent positions along the sampling transect according to the scheme depicted in Figure 2. Both samples should be collected at each transect before moving on to the next transect. When duplicate samples are collected at a site, the full suite of information about the algae samples (composite volume, numbers of each sampling device used, amount filtered, etc.) will need to be recorded for each replicate. This information can be recorded on a duplicate copy of the "Algae Samples" field sheet. Alternatively, the data cells on this sheet can be divided in half to accommodate information for each replicate. If the latter, it is important to keep track of which values go with which replicate.

In addition to including composite volume, area sampled (total, for all sampling devices used), and amount filtered (for the biomass samples) on the sample labels and field sheets, this information should also be included on the chain-of-custody sheets that are submitted to the algae analytical and taxonomy laboratories. This will facilitate efficient calculation of several types of data output, because this information is needed both for the biomass results and for the soft-bodied algae biovolume results.

6 PHYSICAL HABITAT TRANSECT-BASED MEASUREMENTS TO ACCOMPANY ALGAL BIOASSESSMENT

Once all algae samples have been collected at a given transect, PHab data collection can begin there. PHab data are designed to assess the physical habitat conditions of the stream reach being sampled. Knowledge about the PHab parameters can aid interpretation of the biotic assemblage data collected. Data for the following PHab parameters will be entered on transect-specific datasheets (corresponding to each of the 11 main transects along the monitoring reach). These datasheets are provided on the SWAMP website.

It should be noted that the data collection procedures for the parameters below reflect those that are described in the SWAMP BMI Bioassessment SOP (Ode 2007). With respect to PHab assessment, the only deviation between this SOP and that of Ode (2007) is in terms of omission of certain parameters. However, where there is overlap in parameters between the two SOPs, they are assessed in exactly the same manner. The one exception to this is the addition, in this SOP, of percent algal cover determination to the pebble count as described in Ode (2007). Also, note that because the datasheets are multi-purpose datasheets, developed for both BMIs and algae, they include some PHab parameters that are not a part of this SOP. Specifically, the following PHab data that appear on the datasheets are not collected when only algae are being sampled: 1) Riparian vegetation, and 2) Instream habitat complexity. As such, these sections are not filled out on the datasheets when only algae samples are being collected.

6.1 WETTED WIDTH

The wetted channel is the zone that is inundated with water and the wetted width is the distance between the sides of the channel at the point where substrates are no longer surrounded by surface water. Measure the wetted stream width and record this in the box at the top of the Transect data form.

6.2 BAnkFuLL WIDTH

The bankfull channel is the zone of maximum water inundation in a normal flow year (one-to-two year flood events). Since most channel formation processes are believed to act when flows are within this zone, bankfull dimensions provide a valuable indication of relative size of the waterbody.

Scout along the stream margins to identify the location of the bankfull margins on either bank by looking for evidence of annual or semi-annual flood events. Examples of useful evidence include topographic, vegetative, or geologic cues (changes in bank slope, changes from annual to perennial vegetation, changes in the size distribution of surface sediments). While the position of drift material caught in vegetation may be

a helpful aid, this can lead to very misleading measurements. *Note: The exact nature of this evidence varies widely across a range of stream types and geomorphic characteristics. It is helpful to investigate the entire reach when attempting to interpret this evidence because the true bankfull margin may be obscured at various points along the reach. Often the bankfull position is easier to interpret from one bank than the other; in these cases, it is easiest to infer the opposite bank position by projecting across the channel. Additionally, height can be verified by measuring the height from both edges of the wetted channel to the bankfull height (these heights should be equal).*

Stretch a tape from bank to bank at the bankfull position. Measure the width of the bankfull channel from bank to bank at bankfull height and perpendicular to the direction of stream flow.

6.3 BAnkFuLL HEIGHT

Measure bankfull height (the vertical distance between the **water surface** and the height of the bank, Figure 9) and record in the boxes at the top of the Transect data form under "Bankfull Width" and "Bankfull Height".

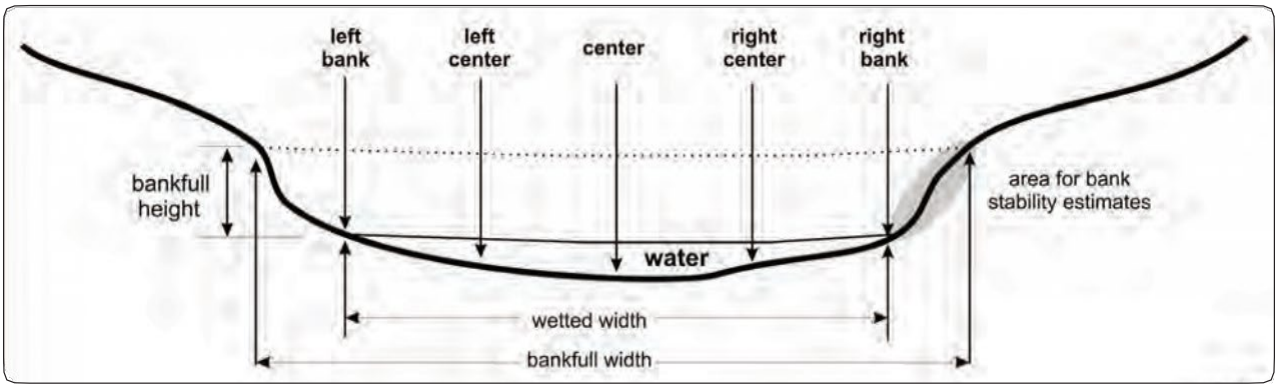

Figure 9. Cross sectional diagram of a typical stream channel showing locations of substrate measurements, wetted and bankfull width measurements, and bank stability visual estimates (reprinted from Ode 2007).

6.4 "PEBBLE COunT": TRAnSECT SuBSTRATES

Particle size frequency distributions often provide valuable information about instream habitat conditions that affect benthic communities. The Wolman pebble count technique is a widely used and cost-effective method for estimating the particle size distribution and produces data that correlate with costly, but more quantitative bulk sediment samples. Coarse particulate organic matter (CPOM, particles of organic material such as leaves that are greater than 1.0 mm in diameter) is a general indicator of the amount of

allochthonous organic matter available at a site, and its measurement can provide valuable information about the basis of the food web in a stream reach. The presence of CPOM associated with each particle is quantified at the same time that particles are measured for the pebble counts.

Transect substrate measurements are taken at five equidistant points along each transect (Figure 9). Divide the wetted stream width by four to get the distance between the five points (Left Bank, Left Center, Center, Right Center and Right Bank) and use a measuring device to locate the positions of these points (e.g., a stadia rod or measuring tape). Once the positions are identified, lower a folding meter stick through the water column perpendicular to both the flow and the transect to identify the particle located at the tip of the meter stick. *Note: It is important that you are not subjective about selecting a particle, as this will result in failing to generate an accurate assessment of the size class distribution of particles present in that stream reach.*

6.5 DEPTH

With the folding meter stick, measure the depth from the water surface to the top of the particle to the nearest cm and record on the datasheet.

Table 3
Particle size class codes, descriptions, and measurements (adapted from Ode 2007)

Size Class	Code Size Class Description	Common Size Reference	Size Class Range
RS	bedrock, smooth	larger than a car	> 4 m
RR	bedrock, rough	larger than a car	> 4 m
XB	boulder, large	meter stick to car	1 - 4 m
SB	boulder, small	basketball to meter stick	25 cm - 1.0 m
CB	cobble	tennis ball to basketball	64 - 250 mm
GC	gravel, coarse	marble to tennis ball	16 - 64 mm
GF	gravel, fine	ladybug to marble	2 – 16 mm
SA	sand	gritty to ladybug	0.06 – 2 mm
FN	fines	not gritty	< 0.06 mm
HP	hardpan (consolidated fines)		< 0.06 mm
WD	wood		
RC	concrete/ asphalt		
OT	other		

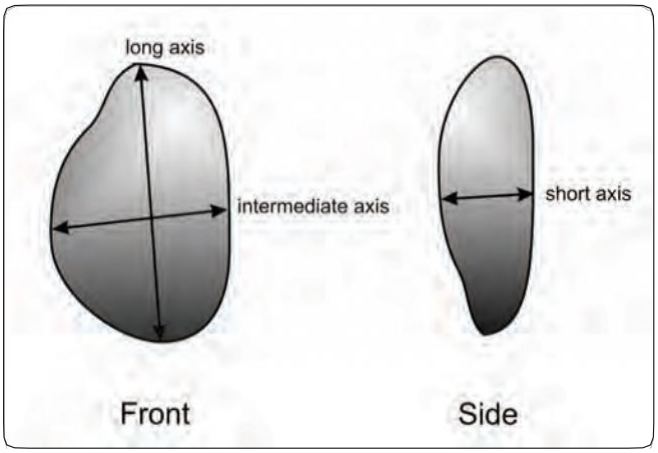

Figure 10. Diagram of three major perpendicular axes of substrate particles. The intermediate axis is recorded for pebble counts (reprinted from Ode 2007).

6.6 PARTICLE SIzE CLASS

Remove the particle from the streambed. Assign the particle to one of the size classes listed in Table 3 (these are also provided in a box on the transect form), based on its intermediate axis length (Figure 10). Record this information under Substrate size class.

Be sure to use measurements or the established codes for particle size class. If the latter, confirm the 2-letter codes for the particles as you call them out to your partner recording the data to ensure you are using the correct codes.

6.7 COBBLE EMBEDDEDnESS

It is generally agreed that the degree to which fine particles fill interstitial spaces has a significant impact on the ecology of benthic organisms and fish, but techniques for measuring this impact vary greatly. Here we define embeddedness as the volume of cobble-sized particles (64-250 mm) that is buried by fine and sand particles (<2.0 mm diameter).

When a cobble-sized particle is encountered during the pebble count, visually estimate the percentage of the cobble's volume that has been buried by fine/sand particles (this will likely require removing the cobble from the streambed). Record, to the nearest 5%, the embeddedness of up to 25 cobble-sized particles within the sampling reach in the corresponding "% Cobble Embed" field for each cobble.

If 25 cobbles are not encountered during the pebble count, supplement the cobbles by conducting a "random walk" through the reach. Starting at a random point in the reach, follow a line from one bank to the other at a randomly chosen angle. Once at the other bank reverse the process with a new randomly chosen angle. Enter any of these additional embeddedness values at the bottom of the first page of the set of field forms, under "ADDITIONAL COBBLE EMBEDDEDNESS MEASURES".

If 25 cobble sized particles are not present in the entire reach, then record the values for however many cobbles are present.

6.8 CPOM

Record the presence or absence of Coarse Particulate Organic Matter (CPOM) that is > 1 mm diameter, and within 1 cm of the particle.

6.9 ALGAL COvER

Algal cover refers to the amount of algae in the stream reach, both in terms of 1) microalgal coatings ("slimy-ness") on stream substrates and 2) macroalgae (e.g., filaments, mats, globules). Algal cover is estimated by a point-intercept approach that entails collecting information about the presence/absence of both types of algae (as well as thickness, for the microalgae) at each of the points along the transects associated with the pebble count. If the imaginary point corresponding to each pebble in the pebble count intercepts algae, then algae is recorded as "present" at that point. The percentage of the points across the sampling reach that have algae present yields an estimate of the percent algal cover.

For each point along the pebble count, record information about algae as follows. For any film-like coating of algae (referred to as "Microalgae" on the datasheet) present on the surface of the substrate at that point, estimate the presence/thickness category according to the scheme in Table 4. For thicker microalgal layers, a small metal or plastic rod with demarcations at 1, 5, and 20 mm can be used for measurement. For layers too thin to measure, use the diagnostic criteria listed in the last column of Table 4. Note that these thickness codes refer only to microalgal coatings/films on substrate. They do not refer to thickness of macroalgal filaments/mats; macroalgal thickness is not assessed in this protocol. Be sure to collect microalgal thickness data from whatever substrate is topmost within the stream and therefore is most likely to be exposed to sunlight. Sometimes this substrate is not the actual pebble used in the pebble count, but rather a substrate type that occurs above the pebble, such as a thick mat of macroalgae that is above (and covering) the stream bottom. Microalgal species (which can include diatoms and unicellular soft-bodied algae) can grow as "epiphytes" upon macroalgal filaments and mats, coating them with a slimy, tinted film.

Table 4
Microalgal thickness codes and descriptions (adapted from Stev enson and Rollins 2006).

Code	Thickness	Diagnostics
0	No microalgae present	The surface of the substrate feels rough, not slimy.
1	Present, but not visible	The surface of the substrate feels slimy, but the microalgal layer is too thin to be visible.
2	<1mm	Rubbing fingers on the substrate surface produces a brownish tint on them, and scraping the substrate leaves a visible trail, but the microalgal layer is too thin to measure.
3	1-5mm	
4	5-20mm	
5	> 20mm	
UD	Cannot determine if a microalgal layer is present	
D	Dry point	

Note: Sometimes, due to the nature of the substrate, it can be difficult to discern whether a microalgal layer is present (particularly if it is very thin). For example, in the case of very fine sediments, the dark color of the silt can obscure the diagnostic color of a microalgal layer, and the inherent "sliminess" of very fine silt may make tactile determination of microalgae impossible. Therefore, when silt is the substrate, only relatively thick layers of microalgae might be easily discernible. If presence/absence of a microalgal layer cannot be determined with certainty, score microalgal thickness as "UD".

In addition to recording the presence and thickness of microalgae on the surfaces of substrates, record the presence/absence of attached macroalgae in the water column, as well as unattached, floating macroalgal mats on the water's surface, corresponding to each pebble count sampling point. Do this by envisioning an imaginary "line" extending from the water's surface down to the stream bottom where the target "pebble" lies (particularly in turbulent water, it may be helpful to use a viewing bucket (Appendix C) in order to see below the water's surface; the use of the viewing bucket is optional). If this line intercepts macroalgae, either floating on the water's surface, or somewhere within the water column, the appropriate algal class(es) should be recorded as "present". Attached macroalgal filaments have an obvious physical connection to something (like a cobble, boulder, or a gravel bed) lying on the bottom of the stream, whereas for unattached macroalgae, there is no obvious physical connection with the streambed, and the algae is just freely floating at or near the water's surface. For each class of macroalgae (Attached and Unattached), mark "P" (for "present") if intercepted by the sampling point and "A" (for "absent") if not intercepted.

Bear in mind that, because pebble counts span the "wetted width" of each transect, the expectation is that even the pebbles at the bank positions will generally be at least moist, and sometimes even submerged. As such, it is important to realize that algal cover can occur at the bank positions of the pebble count as well as intermediate positions across the stream. An exception to this is when the pebble surface is completely dry. Section 6.11 provides instructions for data collection in this situation.

6.10 MACROPHyTES

If a vascular plant (i.e., a macrophyte) is intercepted by the imaginary line associated with the pebble count point, mark "P" for "present" under Macrophytes. Otherwise, mark "A" for absent. Include only herbaceous plants that are rooted underwater. Examples of macrophytes include cattails, tules, rushes, sedges, monkeyflowers, speedwells, knotweeds, and watercress.

6.11 DRy SuBSTRATES

To determine how to collect data at dry sampling points, it is necessary to first establish whether the dry area in question lies within the stream's active channel (i.e., therefore regularly inundated during storms), or whether the point is on a stable island (i.e., therefore rarely, if ever, inundated). Stable islands are typically

vegetated, often with woody shrubs or trees, and have heights near or exceeding bankfull height. Pebble counts should not be conducted on stable islands. If the transect spans a portion of the study reach in which the channel is bifurcated such that there are two channels with an intervening island, the entire transect should be placed across the dominant channel, and all five pebble count points should be located on that side.

If the point falls on a dry surface that is within the usual active channel (i.e., subject to regular disturbance by flows), then pebble count/algal cover data from the dry point should be recorded as follows:

- score Depth as 0
- score particle Size Class and Embeddedness as described above for wet particles
- score all the algae variables (Microalgae, Macroalgae Attached, and Macroalgae Unattached) as "D" for "dry"
- leave CPOM and Macrophytes "blank" (i.e., do not circle anything). These parameters will register as NR (Not Recorded) in the database.

Ordinarily, the sampling transect would span the wetted width of the channel, but when no water is present at a given transect, evidence of the typical wetted extent of the active channel will need to be used to infer appropriate transect boundaries. Such indicators can include the transition from vegetated to unvegetated area (i.e., moving from banks to active channel), as well as the presence of dried algae, water stains, microtopographic transitions, changes in substrate composition, and others.

6.12 BAnk STABILITy

The vulnerability of stream banks to erosion is often of interest in bioassessments because of its direct relationship with sedimentation.

For each transect, record a visual assessment of bank vulnerability in the region between the wetted width and bankfull width of the stream margins and between the upstream and downstream inter-transects. Choose one of three vulnerability states: eroded (evidence of mass wasting), vulnerable (obvious signs of bank erosion or unprotected banks), or stable.

6.13 HuMAn InFLuEnCE

For the left and right banks, estimate a 10 x 10 m riparian area centered on the edges of the transect (see Figure 11). In the "Human Influence" section of the Transect data sheet, record the presence of 14 human influence categories in three spatial zones relative to this 10 x 10 m square (between the wetted edge and bankfull margin, between the bankfull margin and 10 m from the stream, and between 10 m and 50 m beyond the stream margins): 1) walls/rip-rap/dams, 2) buildings, 3) pavement/cleared lots, 4) roads/

railroads, 5) pipes (inlets or outlets), 6) landfills or trash, 7) parks or lawns (e.g., golf courses), 8) row crops, 9) pasture/ rangelands, 10) logging/ timber harvest activities, 11) mining activities, 12) vegetative management (herbicides, brush removal, mowing), 13) bridges/ abutments, 14) orchards or vineyards. Circle all combinations of impacts and locations that apply, but be careful to not double-count any human influence observations.

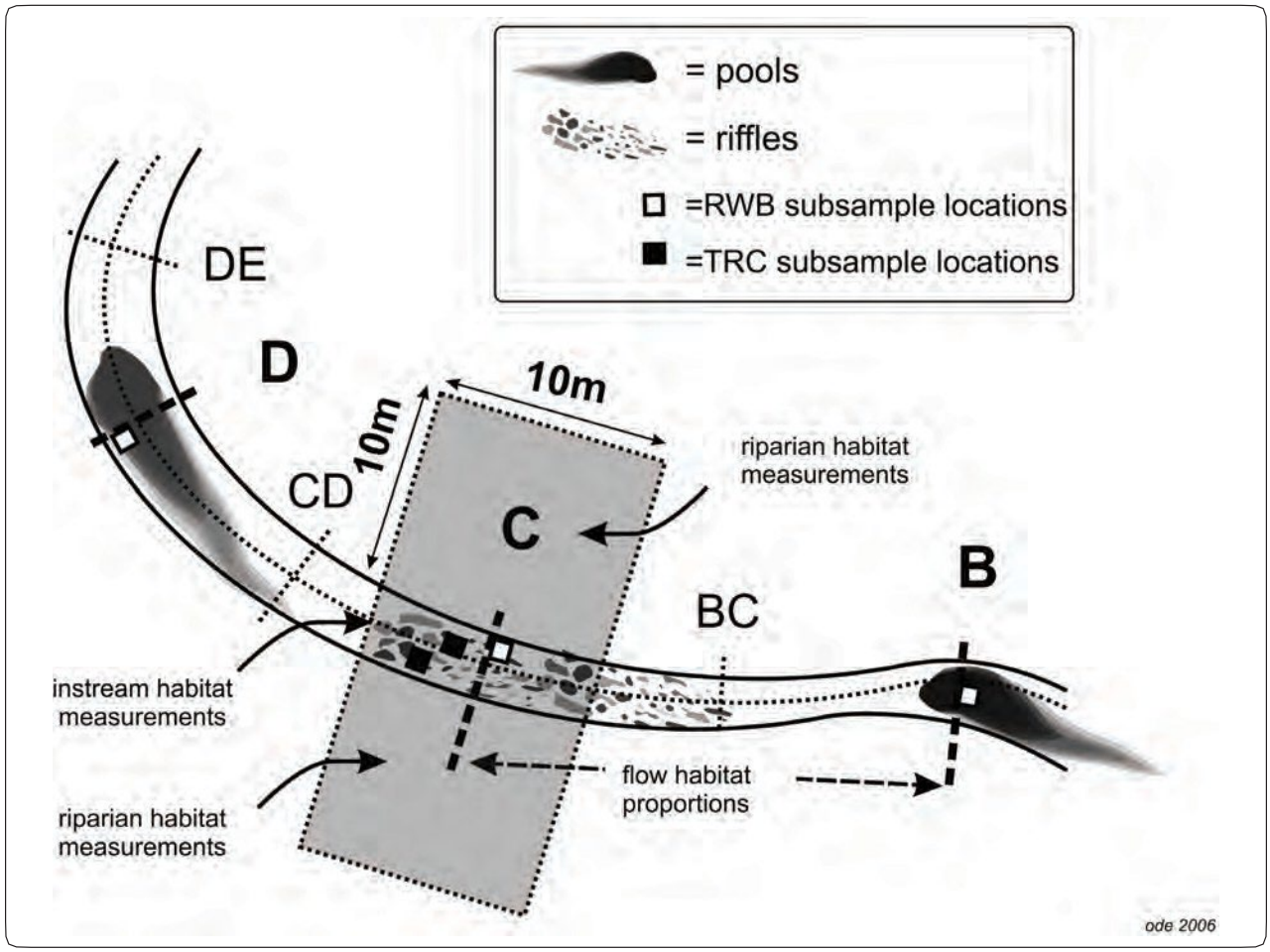

Figure 11. Section of the standard reach expanded from Figure 1 showing the appropriate positions for collecting algae samples (the white square, labeled "RWB" in the legend box) and flow habitat proportion measurements (reprinted from Ode 2007).

Record the presence of any of the 14 human influence categories in the stream channel within a zone 5 m upstream and 5 m downstream of the transect.

6.14 DEnSIOMETER READInGS (CAnOPy COvER)

The densiometer is read by counting the number of line intersections that are obscured by overhanging vegetation. Before using, the densiometer should be modified by taping off the lower left and right portions of the mirror in order to emphasize overhead vegetation over foreground vegetation (the main source of bias in canopy density measurements; see Figure 12.)

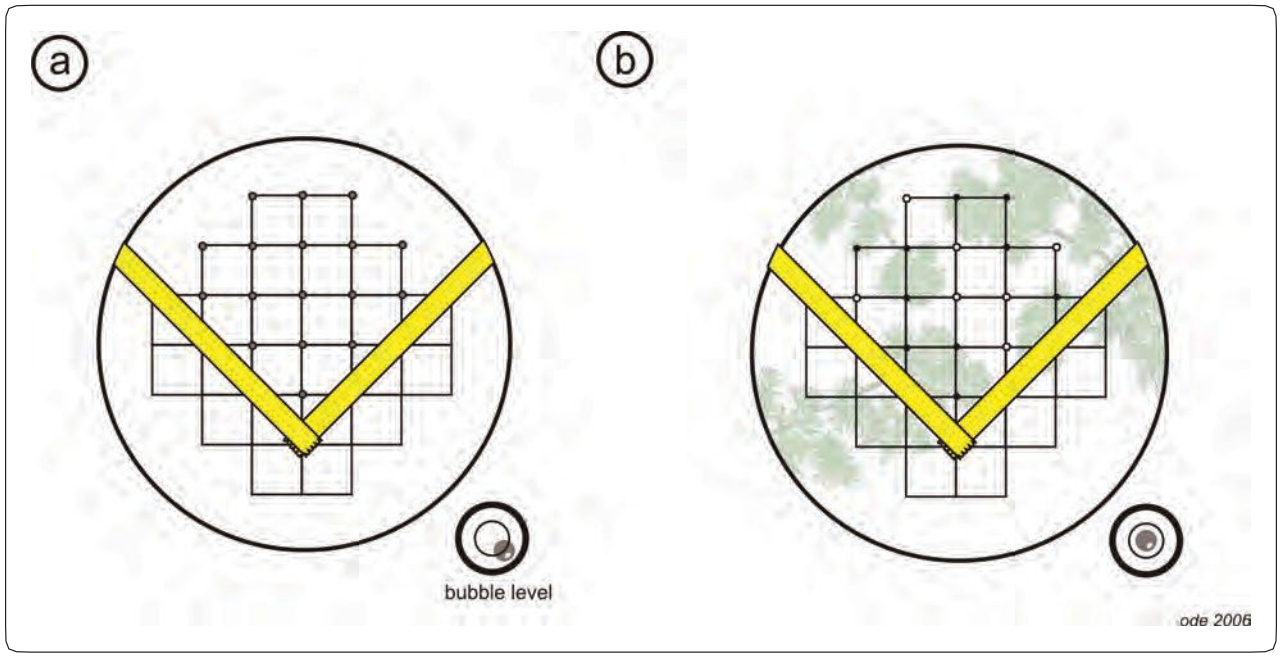

Figure 12. Representation of the mirrored surface of a convex spherical densiometer showing the position for taping the mirror and the intersection points used for the densiometer reading. The score for the hypothetical condition in (b) is 10 covered intersection points out of 17 possible. Note the position of the bubble level in (b) when the densiometer is leveled (reprinted from Ode 2007).

All densiometer readings should be taken with the bubble leveled, and 0.3 m (1 ft) above the water surface. The densiometer should be held just far enough from the squatting observer's body so that his/her forehead is just barely obscured by the intersection of the two pieces of tape.

Take and record four 17-point readings from the center of each transect: a) facing upstream, b) facing downstream, c) facing the left bank, d) facing the right bank. Optional readings can also be taken at the left and right banks (facing away from the stream, for these positions).

7 PHYSICAL HABITAT INTER-TRANSECT-BASED MEASUREMENTS

While most measures are taken at or relative to the main transects, a few measures are recorded at transects located at the midpoint between main transects. These are called "Inter-transects". The following measurements are taken relative to the Inter-transects: 1) Wetted Width, 2) Flow Habitats, and 3) "Pebble Count": Transect Substrates (including algal cover, as for the main transects).

7.1 InTER-TRAnSECT WETTED WIDTH

Measure the same way that Transect wetted width was measured.

7.2 InTER-TRAnSECT SuBSTRATES AnD PERCEnT ALGAL COvER

Collect these data the same way that Transect substrates and percent algal cover data were collected.

7.3 FLOW HABITATS

Because many benthic organisms prefer specific flow and substrate microhabitats, the proportional representation of these habitats in a reach is often of interest in bioassessments. There are many different ways to quantify the proportions of different flow habitats. This procedure produces a semi-quantitative measure consisting of 10 transect-based visual estimates.

At each Inter-transect, identify the percentage of six different habitat types in the region between the upstream Transect and downstream Transect: 1) cascades, 2) falls, 3) rapids, 4) riffles, 5) runs, 6) glides, 7) pools, and 8) dry areas. Record percentages to the nearest 5% — the total percentage of surface area for each section must equal 100%.

A description of each of these flow habitat types is provided below:

- cascades: short, high-gradient drops in stream bed elevation often accompanied by boulders and considerable turbulence
- falls: high-gradient drops in elevation of the stream bed associated with an abrupt change in the bedrock
- rapids: sections of stream with swiftly flowing water and considerable surface turbulence (rapids tend to have larger substrate sizes than riffles)
- riffles: "shallow/fast"; riffles are shallow sections where the water flows over coarse stream bed particles that create mild to moderate surface turbulence (< 0.5 m deep, > 0.3 m/s)
- runs: "deep/fast"; long, relatively straight, low-gradient sections without flow obstructions. The stream bed is typically even and the water flows faster than it does in a pool (> 0.5 m deep, > 0.3 m/s)
- glides: "shallow/slow"; sections of stream with little or no turbulence, but faster velocity than pools (< 0.5 m deep, < 0.3 m/s)
- pools: "deep/slow"; a reach of stream that is characterized by deep, low-velocity water and a smooth surface (> 0.5 m deep, < 0.3 m/s)
- dry: any surface area within the channel's wetted width that is above water

After you have collected all the above Transect-, and Inter-transect-, based measurements, collect data on Gradient. Also, if you have not already done so, take photographs at specific Transects, as indicated below. After you have collected Gradient data at each Transect, and have taken photographs where indicated, remove the corresponding flag from the stream bank.

7.4 PHOTOGRAPHS

Take a minimum of four (4) photographs of the reach at the following locations: a) Transect A facing upstream, b) Transect F facing upstream, c) Transect F facing downstream, and d) Transect K facing downstream. It is also desirable to take a photograph at Transect A facing downstream and Transect K facing upstream to document conditions immediately adjacent to the reach. Digital photographs should be used. Record the image numbers on the front page of the field form under "Photographs". *Note: An easy way to keep track of which site each series of photographs belongs to is to take a close-up of the front data sheet (containing legible site code and date) for that site prior to taking the series of photos.*

SECTION 8
REACHWIDE MEASUREMENTS

This last section describes PHab measurements of attributes specific to the stream reach as a whole. These include gradient of the reach and stream discharge.

8.1 GRADIEnT

The gradient of a stream reach is one of the major stream classification variables, giving an indication of potential water velocities and stream power, which are in turn important controls on aquatic habitat and sediment transport within the reach. The data collected for gradient are recorded on the "Slope and Bearing" form.

Note: An autolevel should be used for reaches with a percent slope of less than or equal to 1%. Either a clinometer or an autolevel may be used for reaches with a percent slope of greater than 1%, and sometimes a clinometer is preferable in really steep areas that are also heavily vegetated. The following description is for clinometer-based slope measurements. In reaches that are close to 1%, you will not know whether you are above or below the 1% slope cutoff. In these cases, default to use of an autolevel, which is described further below.

Clinometer method:

Transect to transect measurements taken with a clinometer are used to calculate the average slope through a reach. This measurement works best with two people, one taking the readings at the upstream transect ("backsighting") and the other holding a stadia rod at the downstream transect. If you cannot see the mid point of the next transect from the starting point, use the supplemental sections (indicating the proportion of the total length represented by each section). Otherwise, leave these blank.

Beginning with the upper transect (Transect K), one person (the measurer) should stand at the water margin with a clinometer held at eye level. A second person should stand at the margin of the next downstream transect (Transect J) with a stadia rod flagged at the eye level of the person taking the clinometer readings. Be sure you mark your eye level while standing on level ground! Adjust for water depth by measuring from the same height above the water surface at both transects. This is most easily accomplished by holding the base of the stadia rod at water level. *Note: An alternative technique is to use two stadia rods pre-flagged at the eye-height of the person taking the readings.*

Use a clinometer to measure the percent slope of the water surface (not the streambed) between the upstream transect and the downstream transect by sighting to the flagged position on the stadia rod. The clinometer reads both percent slope and degree of the slope. Be careful to read and record percent slope

rather than degrees slope (the measurements differ by a factor of ~2.2). Percent slope is the scale on the right hand side as you look through most clinometers. *Note: If an autolevel or hand level is used, record the elevation difference (rise) between transects and the segment length (run) instead of the percent slope (autolevel instructions are provided in the following section).*

If the stream reach geometry makes it difficult to sight a line between transects, divide the distance into two sections and record the slope and the proportion of the total segment length between transects for each of these sections in the appropriate boxes on the slope form (supplemental segments). Do not measure slope across dry land (e.g., across a meander bend).

Proceed downstream to the next transect pair (I-J) and continue to record slope between each pair of transects until measurements have been recorded for all transects. If you have finished all the other transect and inter-transect based measurements for PHab, you may remove the transect flags as you go.

Autolevel method (preferred):

To measure gradient using an autolevel, identify a good spot to set up the autolevel, preferably somewhere around the center of the reach (if there is good visibility from this location to both the upstream and downstream ends of the reach.) Set up the autolevel on very stable, and preferably fairly flat, ground. Set the height of the autolevel to comfortable eye level for the operator. Level the plane of view of the autolevel by balancing it using the bubble. Start by adjusting the legs, and then fine-tune the adjustment using the knobs. Once balanced, begin "shooting" the change in the height of the water level of the stream from transect to transect. Try to start with one of the outer transects (like A). Have a field partner at Transect A hold the Stadia rod at water's edge and perpendicular to the ground. Viewing through the autolevel (and focusing as necessary), look at the Stadia rod and note to the smallest demarcation on the stadia rod the height at which the autolevel line of view (i.e., the middle line in the viewfinder) hits. Record this information, and then have the Stadia rod holder proceed to the next transect (e.g., Transect B), again holding the base of the Stadia rod at water's edge. Very carefully, rotate the head of the autolevel so that it points to the new Stadia rod location. *Note: Take care not to bump the autolevel out of its position, because if this happens, you will not be able to take a height measurement of Transect B's water surface relative to that of Transect A, to determine the slope between the two transects.*

If the autolevel is bumped out of position before all the measurements are done, or if there is a point along the reach at which there is no longer a clear line of sight from the autolevel to the Stadia rod positioned at the transect, at water's edge, a new location must be set up for the autolevel. In order to maintain a relationship between water heights of the various transects already measured, it will be necessary to "re-shoot" the height of the water at the last transect for which a valid measurement was attained. From there, assuming there is no more disturbance to the position of the autolevel, you can continue cycling through the remaining transects from the new position. On the Slope and Bearing Form corresponding to autolevel use, indicate when the autolevel's position has been changed. If it is necessary to move the autolevel at some point, the transect that was measured from the original and the new position will be listed twice on

the datasheet: once for the original position, and once for the new. Also indicate the distance between main transects (i.e., 15 m, 25 m or other). These pieces of information will later be used to determine the slopes between transects and for the reach as a whole.

8.2 STREAM DISCHARGE

Stream discharge is the volume of water that moves past a point in a given amount of time and is generally reported as cubic feet per second (cfs) or cubic meters per second (cms). Because discharge is directly related to water volume, discharge affects the concentration of nutrients, fine sediments and pollutants; and discharge measurements are critical for understanding impacts of disturbances such as impoundments, water withdrawals and water augmentation. Discharge is also closely related to many habitat characteristics including temperature regimes, physical habitat diversity, and habitat connectivity. As a direct result of these relationships, stream discharge is often also a strong predictor of biotic community composition. Since stream volume can vary significantly on many different temporal scales (diurnal, seasonal, inter-annually), it can also be very useful for understanding variation in stream condition.

It is preferable to take discharge measurements in sections where flow velocities are greater than 0.5 ft/s and most depths are greater than 15 cm, but slower velocities and shallower depths can be used. If flow volume is sufficient for a transect-based "velocity-area" discharge calculation, this is by far the preferred method. If flow volume is too low to permit this procedure or if your flow meter fails, use the "neutrally buoyant object/ timed flow" method.

8.2.1 Discharge: velocity Area Method

The layout for discharge measurements under the velocity-area (VA) method is illustrated in Figure 13. Flow velocity should be measured with either a Swoffer Instruments propeller-type flow meter or a Marsh-McBirney inductive probe flow meter.

Select the best location in the reach for measuring discharge. To maximize the repeatability of the discharge measurement, choose a transect with the most uniform flow (select hydraulically smooth flow whenever possible) and simplest cross-sectional geometry. It is acceptable to move substrates or other obstacles to create a more uniform cross-section before beginning the discharge measurements.

Data for this parameter will be entered in the "Discharge Measurements" section of the datasheet with the basic site information at the top ("Reach Documentation"). Measure the wetted width of the discharge transect and divide this into 10 to 20 equal segments. The use of more segments gives a better discharge calculation, but is impractical in small channels. A minimum of 10 intervals should be used when stream width permits, but interval width should not be less than 15 cm.

At each interval, record the distance from the bank to the end of the interval. Using the top-setting rod that comes with the flow velocity meter, measure the median depth of the interval. Standing downstream of the

transect to avoid interfering with the flow, use the top-setting rod to set the probe of the flow meter at the midpoint of the interval, at 0.6 of the interval depth (this position generally approximates average velocity in the water column), and at right angles to the transect (facing upstream). See Figure 13 for positioning detail.

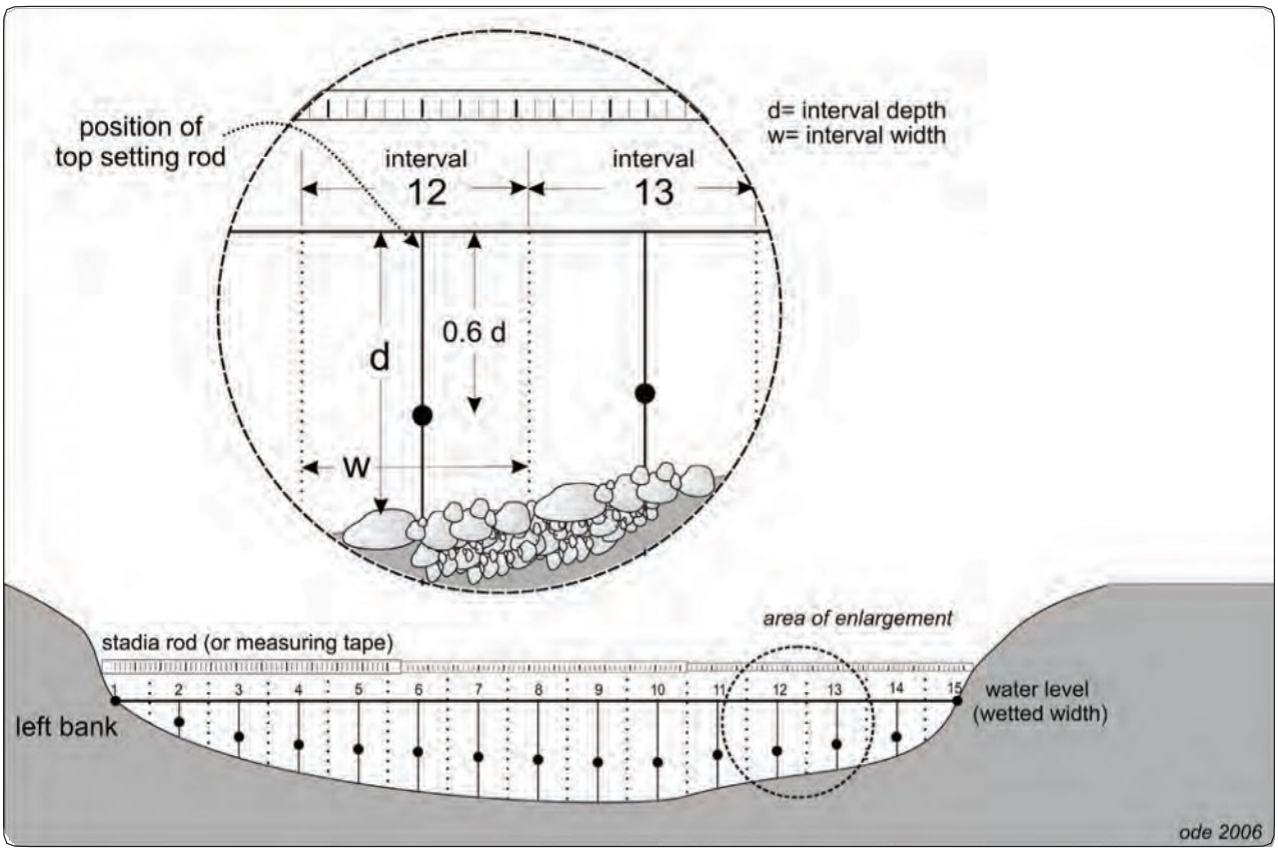

Figure 13. Diagram of layout for discharge measurements under the velocity-area method showing proper positions for velocity probe (black dots; reprinted from Ode 2007).

Allow the flow velocity meter to equilibrate for 10-20 seconds, then record velocity to the nearest ft/s. If the option is available, use the flow averaging setting on the flow meter. *Note 1: Under very low flow conditions, flow velocity meters may register readings of zero even when there is noticeable flow. In these situations, record a velocity of 0.5x the minimum flow detection capabilities of the instrument. Complete these steps on each of the intervals across the stream. Note 2: The first and last intervals usually have depths and velocities of zero.*

8.2.2 Discharge: neutrally Buoyant Object Method

If streams are too shallow to use a flow velocity meter, the neutrally buoyant object (NBO) method should be used to measure flow velocity. However, since this method is less precise than the flow velocity meter

it should only be used if absolutely necessary. A neutrally buoyant object (one whose density allows it to just balance between sinking and floating) will act as if it were nearly weightless, thus its movement will approximate that of the water it floats in better than a light object. A piece of orange peel works well. To estimate the flow velocity through a reach, three transects are used to measure the cross-sectional areas within the test section sub-reach and three flow velocity estimates are used to measure average velocity through the test reach. To improve precision in velocity measurements, the reach segment should be long enough for the float time to last at least 10-15 seconds.

The position of the discharge sub-reach is not as critical as it is for the velocity-area method, but the same criteria for selection of a discharge reach apply to the neutrally buoyant object method. Identify a section that has relatively uniform flow and a uniform cross sectional shape.

The cross sectional area is estimated in a manner that is similar to, but less precise than, that used in the velocity area method. Measure the cross sectional area in one to three places in the section designated for the discharge measurement (three evenly-spaced cross sections are preferred, but one may be used if the cross section through the reach is very uniform). Record the width once for each cross section and measure depth at five equally-spaced positions along each transect.

Record the length of the discharge reach.

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GLOSSARY OF TERMS USED IN SOP G

Terms & Definitions	
TERM	**DEFInITIOn**
Aliquot	a measured portion of a sample, or subsample, or to measure a portion of a sample or subsample
Ash-free dry mass (AFDM)	the portion, by mass, of a dried sample that is represented by organic matter; the concentration of AFDM per stream surface area sampled can be used as a surrogate for algal biomass
Benthic algae	algae that are anchored to, or have at one point been anchored to, the stream bottom, in contrast to planktonic algae which are free-floating in the water column
Biofilm	a matrix/film adhering to stream substrata and consisting of microorganisms (e.g., algae, fungi, bacteria, protozoans) and detritus
Chlorophyll *a*	primary light receptor/photosynthetic pigment in algae and higher plants; the concentration of this pigment per stream surface area sampled provides an estimate of algal biomass
Composite sample	volume of all the liquid material amassed during sampling, including water used for rinsing substrate and sampling devices. Final composite volume should not exceed 400-500 ml.
Cyanobacteria	historically referred to as "blue-green" algae, but actually bacteria that are capable of photosynthesis and co-occur with true benthic algae in streams; useful as a bioindicator, and field-sampled and laboratory-processed concurrently with soft-bodied algae
Depositional	habitats in the stream that are dominated by slow-moving water, such as pools, where deposition of materials from the water column is more likely to occur than erosion (or (re)suspension) of loose bed materials; examples of "depositional" substrates include silt and sand
Diatom	a unicellular alga that possesses a rigid, silicified (silica-based) cell wall in the form of a "pill box"
Erosional	habitats in the stream that are dominated by fast-moving water, such as riffles, where stream power is more likely to facilitate erosion (suspension) of loose benthic material than deposition; examples of "erosional" substrates include cobbles and boulders
Homogenate	mixture of liquid composite sample and finely chopped fragments of macroalgae
Index of Biotic Integrity (IBI)	a quantitative assessment tool that uses information about the composition of one or more assemblages of organisms to make inferences about condition the environment they occupy (e.g., the assemblage of interest could be diatoms or benthic macroinvertebrates living in a stream)
Macroalgae	soft bodied algae that form macroscopically discernible filaments, mats, or globose structures
Microalgae	diatoms and microscopic soft-bodied algae, including unicellular forms; can co-occur with other microorganisms in a biofilm
Reachwide benthos (RWB)	method for biotic assemblage sample collection that does not target a specific substrate type, but rather objectively selects sampling locations across the reach, allowing for any of a number of substrate types to be represented in the resulting composite sample
Soft-bodied algae	non-diatom algal taxa; for the purposes of this SOP, cyanobacteria are subsumed under this assemblage
Wetted width	the distance between the sides of the channel at the point where substrates are no longer surrounded by surface water

List of Supplies for Stream Algae Sampling and Associated Data Collection A

Table 5.
General Supplies and Ambient Water Chemistry Collection

Item	Quantity / Site	Specifications
Sampling SOP (this document)	1/person	
Equipment decontamination supplies		See Appendix B
Hip or chest waders, or wading boots/shoes (not felt-soled)	at least 1 pair/ person	
Digital camera	1	
Full set of datasheets printed on waterproof paper (e.g., Rite-in-the-Rain™)	1 full set (and spare set recommended)	
Fine-tipped and thick-tipped waterproof/alcohol-proof pens and markers; pencils	2-3 each	
Clipboard	2-3	
Site dossier containing site maps, aerials, etc.	1	Add a 150 m scale line to aerials adjacent to stream
Thomas Guide and regional maps	as needed	
Centigrade thermometer	1	
pH meter	1	
DO meter and spare membrane	1	
Conductivity meter	1	
Turbidimeter and vial(s) (optional)	1	
Field alkalinity meter or test kit (e.g., Hach)	1	
Water chemistry containers	as needed	
Calibration standards	1	
Spare batteries for meters	as needed	
First aid kit	1	

Table 6.
Algal Taxonomic and Biomass Sample Collection

needed for[1]:	Item	Quantity / Site	Specifications
D, S, C, A	White dish tub, rectangular, plastic, 11.5 qt	1	Use white, not colored
D, S, C, A	Scrubbing brush or scouring pad to clean dish tub, etc.	1	
D, S, C, A	Composite sample receiving bottle with cap, 1 L, plastic	1	Fisher 02-912-038
D, S, C, A	Graduated cylinder, 500 mL and 25 mL, plastic	1 each	Fisher 03-007-42 & 03-007-39
D, S, C, A	Bottle brush to clean graduated cylinders, etc.	1 sm, 1 lg	
D, S, C, A	PVC delimiter, 12.6 cm^2 area	1	See Appendix C
D, S, C, A	Spatula (> 12.6 cm^2 surface area)	1	
D, S, C, A	Rubber delimiter, 12.6 cm^2 area	1	See Appendix C
D, S, C, A	Toothbrush, firm-bristled	1	
D, S, C, A	Syringe scrubber, 60 mL syringe, 5.3 cm^2 area	1	See Appendix C
D, S, C, A	White (non-pigmented) scrubbing-pad circles	11 per replicate	See Appendix C
D, S, C, A	Tally meter (optional)	1	Ben Meadows 9JB-102385
D, S, C, A	Scissors	1	
D, S, C, A	Wash bottles	2	Label bottles with "stream water", and "DI water"
D, S, C, A	Razor blades or Swiss army knife	1	
D, S, C, A	Sample labels (printed on waterproof paper)	4 per replicate	See Figure 6
D, S, C, A	Clear plastic tape, 5 cm wide	Length of ~20cm per replicate	
D, S, C, A	Ice chest with wet ice	1 (2 preferred if multiple sites to be sampled)	
D, S, C, A	Fisherman's vest (optional)	1	
D, S	Centrifuge tubes, 50 mL, plastic	2 per replicate	Cole Parmer 06344-27
D, S	Rack for 50 mL centrifuge tubes	1	
D	10% formalin solution buffered with borax	10 mL per replicate	See Appendix C
D	Formalin-resistant gloves	1 pair	
D	Safety goggles or face shield	1	
D	Small syringe or bulb pipette	1	

1. "D" = diatom sample, "S" = soft-bodied algal sample, "C" = chlorophyll a sample, "A" = ash-free dry mass sample

needed for[1]:	Item	Quantity / Site	Specifications
D	Vermiculite packing material	as needed	
S	Turkey baster	1	
S (see note)	25% glutaraldehyde solution (to be dispensed in a laboratory fume hood, wearing appropriate safety gear)	5 mL per replicate	*Note: could be added by taxonomy lab, with prior notification*
S	Calculator	1	
S	Small metric ruler (waterproof)	1	
S	Small Ziploc bag	1	
S, C, A	Whirl-pak bag, 100 mL	3 per replicate	Cole Parmer 06498-00
C, A	Filter forceps	1	Fisher 0975350
C, A	Filtering chamber/tower, 47 mm, plastic	1	Hach 2254400
C, A	Hand vacuum pump	1	Fisher 13-874-612B
C, A	Aluminum foil	~100 cm^2 per replicate	
C, A	Deionized water	500 mL	
C, A	Dry ice (if not returning to lab immediately following the day's fieldwork)	10 lbs	
C	Glass fiber filter, 47 mm, 0.7 µm pore size	1 per replicate	Fisher 09804142H
C	Snapping Petri dish, 47 µm	1 per replicate	Fisher 08-757-105
A	Glass fiber filter, 47 mm, 0.7 µm pore size; foil-wrapped and pre-combusted for ash-free dry mass (AFDM)	1 per replicate	

1. "D" = diatom sample, "S" = soft-bodied algal sample, "C" = chlorophyll a sample, "A" = ash-free dry mass sample

Table 7.
Physical Habitat Data Collection

Item	Quantity / Site	Specifications
GPS receiver	1	
Transect tape; 150 m	1	
Lengths of rope (7.5 m and 12.5 m)	1 each	
Small metric folding ruler (waterproof)	1	
Digital watch	1	
Stadia rod	1	
Clinometer	1	
Autolevel and tripod	1	
Current velocity meter and top-setting rod	1	
Convex spherical densiometer	1	Taped to expose only 17 intersections of the grid (see Figure 12)
Transect flags	21 total	Two colors; label with main transect (11 ct.) and inter-transect (10 ct.) names
Algae viewing bucket (optional)	1	See Appendix C
Small/slender rod with 1, 5, and 20 mm marks	1	For measuring microalgal thickness
Rangefinder (optional)	1	
Fresh orange peel	1	

Information Resources for Avoiding Introduction of Invasive Species and Pathogens into Streams

B

The following is an adaptation of an excerpt taken from an EMAP-based Quality Assurance Project Plan developed by the California Department of Fish and Game Aquatic Bioassessment Laboratory (2008).

Organisms of concern in the U.S. include, but may not be limited to, Eurasian watermilfoil (*Myriophyllum spicatum*), New Zealand mud snail (*Potamopyrgus antipodarum*), zebra mussel (*Dreissena polymorpha*), *Myxobolus cerebralis* (the sporozoan parasite that causes salmonid whirling disease), and *Batrachochytrium dendrobatidis* (a chytrid fungus that threatens amphibian populations).

Field crews must be aware of regional species of concern, and take appropriate precautions to avoid transfer of these species. Crews should make every attempt to be apprised of the most up-to-date information regarding the emergence of new species of concern, as well as new advances in approaches to hygiene and decontamination to prevent the spread of all such organisms (e.g., Hosea and Finlayson, 2005; Schisler et al., 2008).

There are several online sources of information regarding invasive species, including information on cleaning and disinfecting gear:

Whirling Disease Foundation
www.whirling-disease.org

uSDA Forest Service - Preventing Accidental Introductions of Freshwater Invasive Species
www.fs.fed.us/invasivespecies/documents/Aquatic_is_prevention.pdf

California Department of Fish and Game
www.dfg.ca.gov

u.S. Geological Survey nonindigenous Aquatic Species: general information about freshwater invasive species
http://nas.er.usgs.gov

Protect your Waters - Co-sponsored by the u.S. Fish and Wildlife Service
www.protectyourwaters.net/hitchhikers

The California State Water Resources Control Board Aquatic Invasive Species website

www.swrcb.ca.gov/water_issues/programs/swamp/ais

REFEREnCES

Hosea, R.C. and B. Finlayson. 2005. Controlling the spread of New Zealand mudsnails of wading gear. California Department of Fish and Game, Office of Spill Prevention and Response, Administrative Report 2005-02, Sacramento.

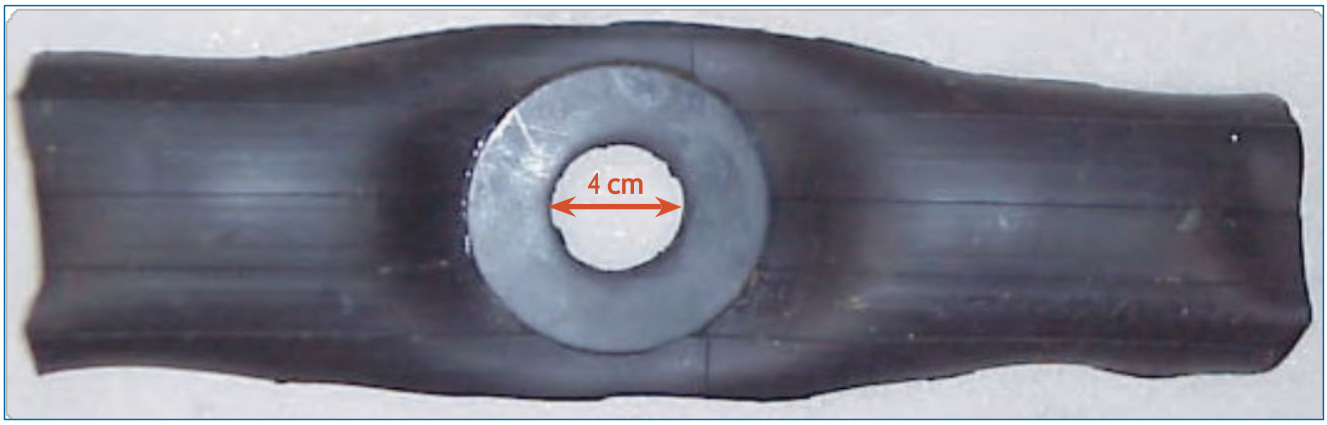
Schisler, G.J., N.K.M. Vieira, and P.G. Walker. 2008. Application of Household Disinfectants to Control New Zealand Mudsnails. North American Journal of Fisheries Management 28:1171-1176.

CONSTRUCTION OF ALGAE SAMPLING TOOLS C

This appendix provides step-by-step instructions for constructing the devices used for sampling algae. It also provides a recipe for the formalin fixative for diatoms.

1. RuBBER DELIMITER

The rubber delimiter for use on "erosional"/hard substrates like cobbles and wood is made from a sliced-open mountain bike inner tube that has a 4-cm diameter hole cut in the middle. The hole should be reinforced with a rubber gasket affixed to the tube with rubber cement.

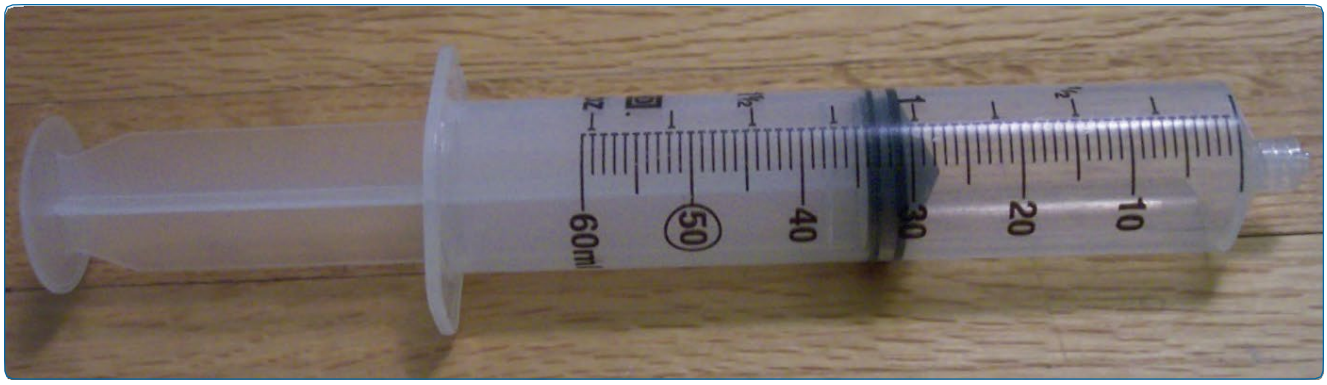


2. PvC DELIMITER

The PVC delimiter for "depositional"/soft substrates like sand, small gravel, and silt is made from a 1½" sewer cleanout, which can be found at a home-improvement or plumbing supply store. The hole in the bottom of the cleanout is 4 cm in diameter. The bottom edge of the cleanout is filed to make it sharp, to ease insertion into silt/sand. To facilitate consistent sampling, it is useful to paint a bright line indicating a depth of 1 cm around the outer surface of the bottom of the sampling device. This indicates the depth to which to insert the delimiter when sampling.

3. SyRInGE SCRuBBER

The syringe scrubber is for use on hard substrates that cannot be picked up out of the stream, like submerged bedrock and concrete channel bottoms. It is made from a 60 mL syringe barrel with the end cut off and its plunger fitted with Velcro-type material. Disposable, white (non-pigmented) scrubbing pads circles are then affixed to the end of the plunger and used to scrub the algae from the substrate.



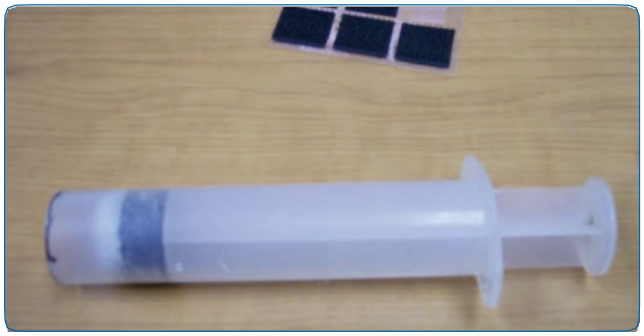
You will need a 60-mL plastic syringe for each sampler you want to make. Remove the plunger and saw the conical end off the plastic syringe, then sand the bottom so it is flat all the way around and fits tightly against a flat surface.

Firmly affix the rubber end to the plastic plunger by removing the rubber tip, applying glue to the "naked" end of the plunger, and replacing the rubber cap. Allow glue to cure. Then cut the conical part off the plunger tip so that only a flat surface of rubber remains.

Cut a circle of Velcro®-style hook material to fit the size of the plunger. Use a waterproof adhesive to affix the "Velcro®" circle to the end of the plunger.

Obtain some white scrubbing pad material (make sure it is not pigmented so it will not end up interfering with eventual chlorophyll *a* analysis of the samples collected.) Cut a supply of circles to fit the size of the plunger.

Before each sampling event, attach a fresh circular scrubbing pad to the end of the plunger. This is a head-on view of the plunger, with the scrubbing pad circle attached.



This is what the syringe sampler looks like when it is ready to be used.

4. vIEWInG BuCkET (OPTIOnAL)

A viewing bucket can be useful for visualizing submerged algae, particularly in instances of a turbulent stream surface that obscures the stream bottom. A viewing bucket can be constructed from a narrow cylinder of clear Plexiglas (approximately 8 inches in diameter) whose bottom is fitted with a circle of thick glass, and secured in place with a silicone seal. If desired, one or two handles can also be fashioned out of Plexiglas and attached to the side(s) of the cylinder. The use of the viewing bucket is optional.

5. PREPARInG A 1-L SOLuTIOn OF 10-PERCEnT BuFFERED FORMALIn (MOuLTOn ET AL. 2002)

1. Add 100 mL of formaldehyde (37-40%) to 900 mL of water in a chemically resistant, non-breakable bottle.
2. Add about 3 g of borax to 10 mL of water and mix.
3. Add dissolved borax solute, to buffer formalin solution.
4. Tightly seal the bottle and mix by carefully inverting the bottle several times.
5. Label the outside of the bottle with "10-percent buffered formalin," the date of preparation, and related hazardous chemical stickers.

REFEREnCE

Moulton II, S.R., J.G. Kennen, R.M. Goldstein, and J.A. Hambrook. 2002. Revised protocols for sampling algal, invertebrate, and fish communities as part of the National Water-Quality Assessment Program, Open-File Report 02-150.

STANDARD OPERATING PROCEDURES (SOP) FOR USING GLUTARALDEHYDE FOR THE PRESERVATION OF SOFT ALGAE D

(adapted from the Aquatic Bioassessment Laboratory, California Department of Fish and Game)

Note: Glutaraldehyde must only be handled by trained individuals who understand the safe handling and use of this chemical

1. SCOPE AnD APPLICATIOn

Glutaraldehyde is a colorless liquid with a pungent odor used as a preservative and sterilant. This SOP covers the use of Glutaraldehyde by Department of Fish and Game OSPR laboratories as a preservative for soft bodied algae.

2. PHySICAL HAzARDS

The physical hazards associated with the use of Glutaraldehyde include:

- Incompatibility with strong oxidizing substances and bases
- Corrosive to metals
- Production of Carbon Monoxide and Carbon Dioxide during decomposition
- Discolors on exposure to air

3. HEALTH HAzARDS

The health hazards associated with the use of Glutaraldehyde include;

Inhalation

- Regulatory limit of 0.05 ppm as a ceiling level
- Chemical burns to the respiratory tract
- Asthma and shortness of breath
- Headache, dizziness, and nausea

Skin

- Sensitization or allergic reactions, hives
- Irritations and burns
- Staining of the hands (brownish or tan)

Eyes

- Irritation and burns. Eye contact causes moderate to severe irritation, experienced as discomfort or pain, excessive blinking and tear production
- May cause permanent visual impairment
- Conjunctivitis and corneal damage

Ingestion

- Gastrointestinal tract burns; Central nervous system depression, excitement
- Nausea, vomiting
- Unconsciousness, coma, respiratory failure, death

*Note: Oral toxicity of Glutaraldehyde **increases** with dilution*

4. EnGInEERInG COnTROLS

Strict engineering controls will be followed when using Glutaraldehyde. This chemical and processes using this chemical will only be used under a laboratory fume hood meeting the requirements of Title 8, CCR Section 5154.1. At no time will containers of Glutaraldehyde be opened outside of an operating fume hood. Personnel using Glutaraldehyde will designate an area of the lab for its use. The area where it is used will be noticed with a sign reading:

CAuTIOn GLuTARALDEHyDE In uSE

Only trained personnel will be allowed to enter the designated area when using Glutaraldehyde.

5. PERSOnAL PROTECTIvE EQuIPMEnT

Personal Protective Equipment (PPE) is required to be worn at all times when working with Glutaraldehyde. This includes:

Eye Protection

- Chemical splash goggles; or
- Safety glasses with face shield

Hand Protection

- Nitrile or Polyvinyl Chloride (vinyl) gloves

Body Protection

- Lab coat with polypropylene splash apron that cover the arms

Any PPE with noticeable contamination will be immediately removed and the affected area washed with water. Gloves and apron will be removed before leaving the designated area. Disposable PPE (gloves and aprons) will not be re-worn. Disposable PPE will be disposed of in a sealed waste receptacle approved for hazardous waste. Any non-disposable PPE (lab coats, chemical goggles) with noticeable contamination will be rinsed or cleaned as soon as practical, and secured in a manner that does not allow contamination of laboratory personnel. Respiratory protection will not be required as long as strict engineering controls are followed.

6. SAFETy SHOWER AnD EyEWASH

All employees using Glutaraldehyde must be aware of the location and use of the laboratory safety shower and eyewash, and must be able to reach it within 10 seconds from the time of contamination. At no time will processes using Glutaraldehyde be allowed that do not provide access to a safety shower and eyewash. Employees who have skin or eye contact with Glutaraldehyde will immediately stop all processes and proceed to the safety shower and eyewash station. The employee will rinse the affected area for a minimum of 15 minutes. If eye contact has occurred, the upper and lower eyelids must be lifted to allow adequate flushing of the eyes.

7. SPECIAL HAnDLInG PROCEDuRES AnD STORAGE REQuIREMEnTS

Procedures will be followed that reduce exposure to Glutaraldehyde vapor to the lowest reasonable level. This includes:

- Ensure Glutaraldehyde is only used under a fume hood
- Use only enough Glutaraldehyde to perform the required procedure
- Every effort must be made to minimize splashing, spilling, and personnel exposure
- Once specimens are preserved, they will be capped or secured in a way that does not allow Glutaraldehyde vapor to escape into the lab
- At no time will open containers be removed from the fume hood
- All containers of Glutaraldehyde or solutions containing Glutaraldehyde will be appropriately marked with the chemical name, and hazard warning label at the end of the work day or whenever there is a personnel change
- Glutaraldehyde will be stored in tightly closed containers in a cool, secure, and properly marked location

8. WASTE DISPOSAL

Excess Glutaraldehyde and all waste material containing Glutaraldehyde must be placed in an unbreakable secondary container labeled with the following “HAZARDOUS WASTE GLUTARALDEHYDE.” Wastes will be disposed of through the laboratory hazardous waste contract.

9. SPILL AnD ACCIDEnT PROCEDuRES

Drips and splashes will be wiped up immediately with a sponge, towel, or mop. Any material used to clean spills will be disposed of as hazardous waste. Large spills (Greater than 300 CC) require response by a local Hazmat team. The Hazmat team will be called by the laboratory supervisor. In the event of a large spill personnel will immediately leave the laboratory, and not re-enter until cleared by the laboratory supervisor.

10. TRAInInG

All personnel engaged in the use of Glutaraldehyde will be trained on the hazards associated with this chemical, before use. The training will include;

- OSPR's Hazard Communication Program and information contained in the chemical's Material Safety Data Sheet (MSDS)
- Health hazards and routes of exposure
- Specific procedures and techniques for use and handling
- Use of PPE and engineering controls
- The contents and requirements of this Standard Operating Procedure.

STANDARD OPERATING PROCEDURES (SOP) FOR USING FORMALIN FOR THE PRESERVATION OF DIATOMS

E

(adapted from the US EPA EMAP program; Peck et al. 2006)

Note: Formalin must only be handled by trained individuals who understand the safe handling and use of this chemical. All personnel engaged in the use of formalin will be trained on the hazards associated with this chemical before use. The training will include the information contained in the chemical's Material Safety Data Sheet (MSDS).

Formaldehyde (or formalin) is highly allergenic, toxic, and dangerous to human health (potentially carcinogenic) if utilized improperly. Formalin vapors and solution are extremely caustic and may cause severe irritation on contact with skin, eyes, or mucous membranes. Formaldehyde is a potential carcinogen, and contact with it should be avoided. Wear gloves and safety glasses and always work in a well-ventilated area. In case of contact with skin or eyes, rinse immediate with large quantities of water. Store stock solution in sealed containers in a safety cabinet or cooler lined with vermiculite or other absorbent material. If possible, transport outside the passenger compartment of a vehicle.

During the course of field activities, a team may observe or be involved with an accidental spill or release of hazardous materials. In such cases, take the proper action and do not become exposed to something harmful. The following guidelines should be applied:

- First and foremost during any environmental incident, it is extremely important to protect the health and safety of all personnel. Take any necessary steps to avoid injury or exposure to hazardous materials. You should always err on the side of personal safety for yourself and your fellow field crew members.
- Never disturb, or even worse, retrieve improperly disposed hazardous materials from the field and bring them back to a facility for disposal. To do so may worsen the impact to the area of the incident, incur personal or organizational liability, cause personal injury, or cause unbudgeted expenditures of time and money for proper treatment and disposal of material. However, it is important not to ignore environmental incidents. You are required to notify the proper authorities of any incident of this type so they can take the necessary actions to respond properly to the incident.

Follow Department of Transportation (DOT) and the Occupational Safety and Health Administration (OSHA) regulations for handling, transporting, and shipping hazardous material such as formalin and ethanol. Regulations pertaining to formalin are in the Code of Federal Regulations (CFR, specifically 29 CFR 1910.1048).

These requirements should be summarized for all hazardous materials being used for the project and provided to field personnel. Transport formalin and ethanol in appropriate containers with absorbent material. Dispose of all wastes in accordance with approved procedures (e.g., National Institute for Occupational Safety and Health 1981, US EPA 1986).

To dispense formalin in the field, wear formalin-safe gloves and safety goggles. Use a small syringe or bulb pipette to add 10 mL of 10% buffered formalin solution to 40 mL of the diatom sample in a 50 mL centrifuge tube. Alternatively, in order to avoid dispensing formalin solution in the field, clean 50 mL centrifuge tubes that will hold the diatom samples can also be pre-loaded with 10 mL of 10% buffered formalin in a laboratory fume hood prior to going into the field.

The preparation of the 10% buffered formalin stock solution should always be done by trained personnel under a laboratory fume hood while wearing protective gloves, clothing, and goggles.

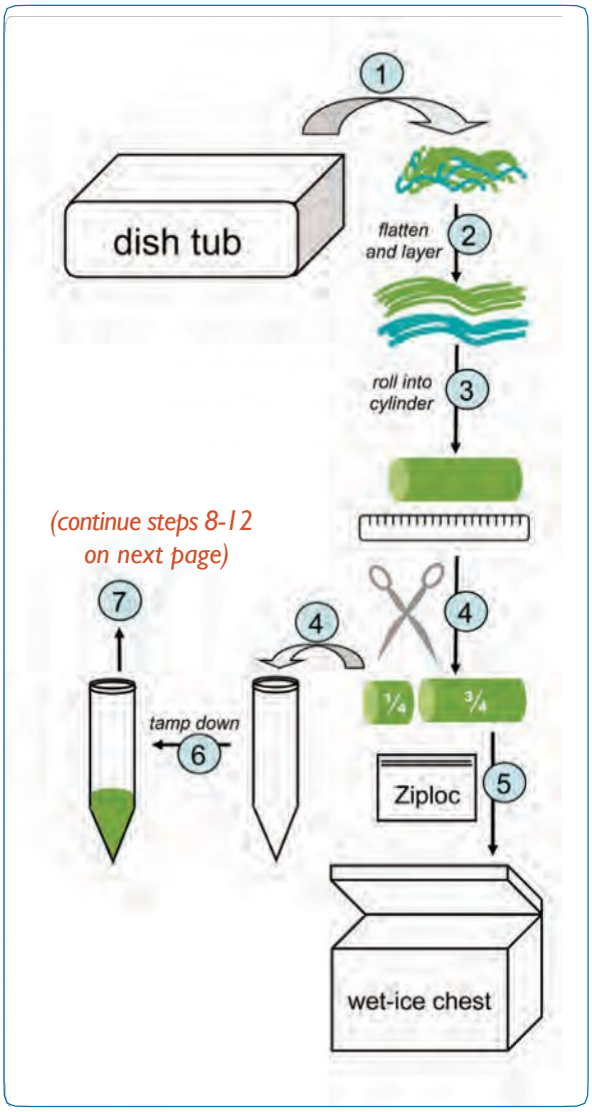
REFEREnCE

Peck, D. V., A. T. Herlihy, B. H. Hill, R. M. Hughes, P. R. Kaufmann, D. Klemm, J. M. Lazorchak, F. H. McCormick, S. A. Peterson, P. L. Ringold, T. Magee, and M. Cappaert. 2006. Environmental Monitoring and Assessment Program-Surface Waters Western Pilot Study: Field operations manual for wadeable streams. U.S. Environmental Protection Agency, Washington, D.C. EPA/620/R-06/003.

PROCESSING SOFT-BODIED ALGAL AND DIATOM SAMPLES WHEN MACROALGAL CLUMPS ARE IN THE SAMPLE

F

The first step involves delivering a known quantity of macroalgae to the soft-bodied algae sample tube.



1) Gently wring excess water out of macroalgae and remove from dish tub.

2) Flatten each distinct taxon of macroalgae into even "sheets" and lay atop one another to distribute the volume of each as equally as possible.

3) Once the various layers of macroalgae are evenly spread upon one another, gently roll the stack into a cylinder shape that is roughly straight and even in thickness along its length.

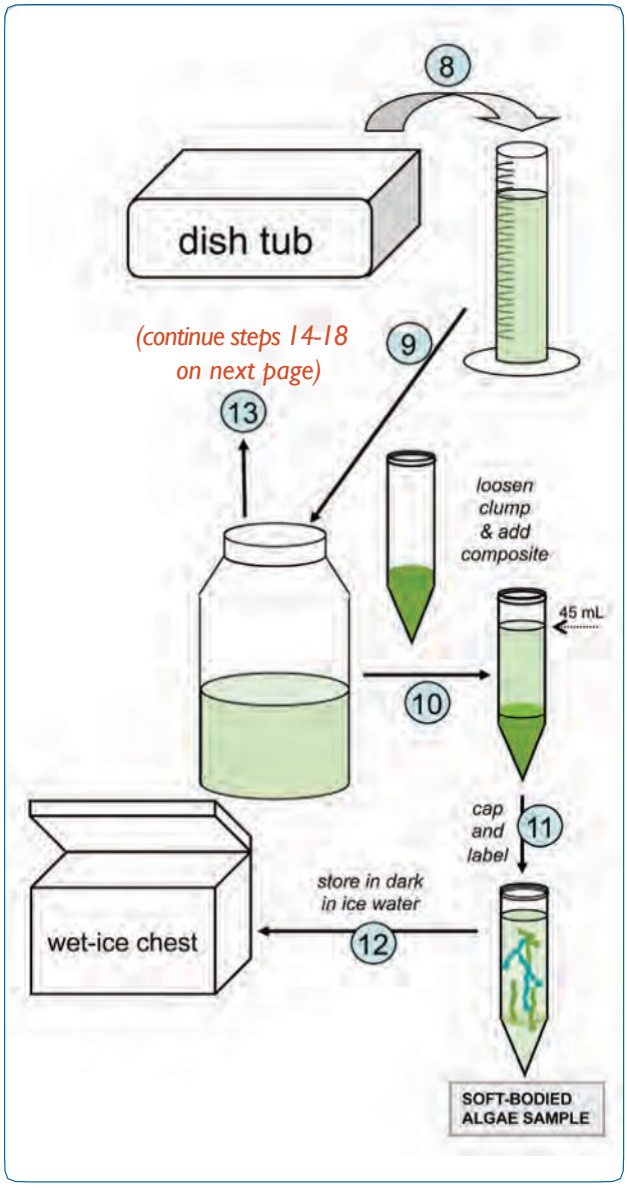
4) Measure and cut off ¼ of the cylinder and place that piece into the empty soft-bodied algae taxonomic ID sample tube.

5) Seal the remaining ¾ of the macroalgae in a clean plastic bag and place inside a cool, dark place such as the wet-ice chest.

6) Using a clean, blunt-ended object, tamp down the ¼ clump of macroalgae in the tube to make it dense and flatten the top surface. Estimate the volume of the macroalgal clump using the graduations on the tube and record this value on the Ratio Restoration worksheet (Figure 8).

7) Add composite sample solution to the tube according to directions on next page.

Some of the liquid composite sample is now added to the tube containing the macroalgae, but first the volume of the entire liquid composite collected must be measured.



8) Agitate the composite sample in the dish tub in order to suspend and mix the microalgae. Wait a few seconds to let the sand/silt settle. Quickly pour only the liquid (leaving silt/sand behind) into a graduated cylinder to measure its volume. Rinse substrate as necessary. Record TOTAL volume of the composite liquid (+ rinsate) on the datasheet, sample labels, and Ratio Restoration worksheet.

9) Pour the liquid composite sample into a clean, 1 L sample bottle

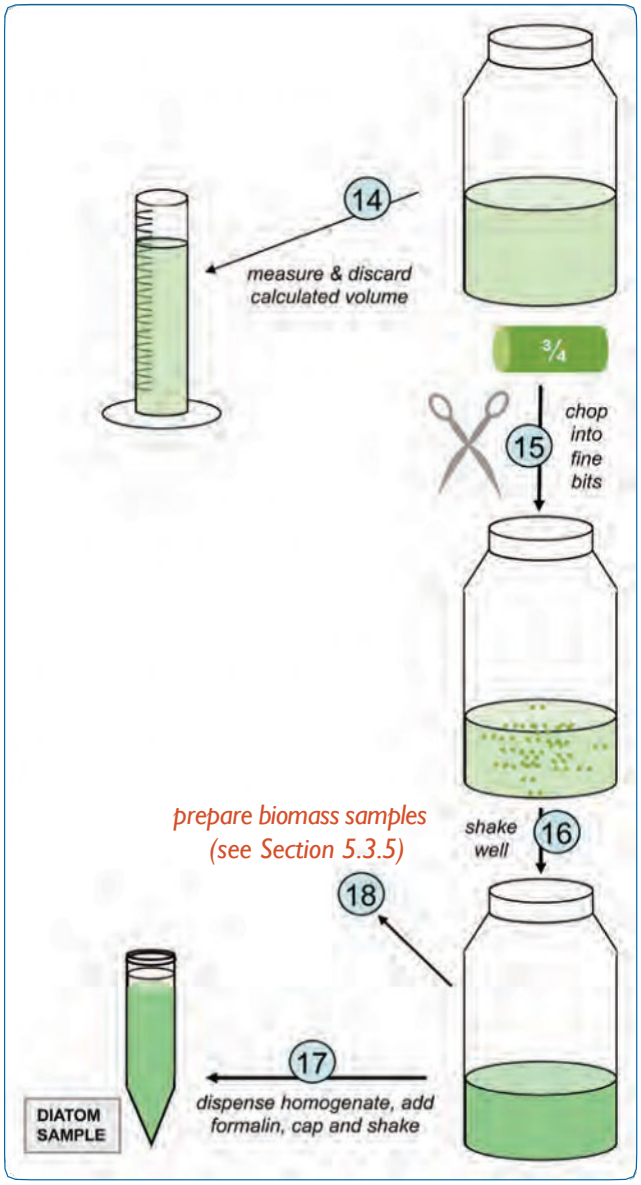
10) Loosen the macroalgae in the sample tube a little so it is no longer a dense clump lodged in the bottom and then pour freshly-agitated composite liquid into sample tube up to the 45 mL mark.

11) Cap the sample tube tightly. Affix a filled-out label to sample tube and cover with clear tape.

12) Place the tube in the dark in a wet-ice chest (not dry ice). Do not allow the algae to freeze. **Glutaraldehyde will need to be added to the tube within 4 days of sample collection, and preferably as soon as possible.**

13) According to directions on the next page, restore the original ratio of macroalgae to liquid composite in order to prepare the remainder of the samples.

The remainder of the macroalgae is now cut into tiny bits, which are added back to the liquid composite. **But the original ratio of macroalgae:liquid must first be restored.** The diatom and biomass samples are then prepared.



14) Use your Ratio Restoration worksheet to determine how much of the liquid composite to pour off. First shake the bottle vigorously, then measure and discard the appropriate volume.

15) Remove the macroalgal clump from the wet-ice chest. Chop the algae into **very fine** (eyelash length or smaller) pieces and add these to the liquid composite.

16) Cap and shake the bottle vigorously in order to homogenize the chopped algae into the liquid as thoroughly as possible.

17) Pour 40 mL of the freshly-agitated homogenate into the diatom sample tube. Add 10 mL 10% buffered formalin solution, observing all formalin safety precautions. Cap the tube, shake, and affix a sample label.

18) After both taxonomic ID samples have been prepared, the remainder of the homogenate is used for the biomass samples (chlorophyll *a* and ash-free dry mass). 25 mL of freshly shaken homogenate is filtered for each biomass sample. See Section 5.3.5.

SWAMP
Surface Water
Ambient Monitoring
Program

For more information, please contact:

Betty Fetscher
Southern California Coastal Water Research Project
3535 Harbor Blvd., Suite 110
Costa Mesa, California 92626
bettyf@sccwrp.org

Water Boards

www.waterboards.ca.gov/water_issues/programs/swamp/

Recent Advances in the Analysis of Pyrethroid Insecticides in Surface Water and Sediments

Abdou Mekebri, Ph.D.

November 2, 2011

CALIFORNIA DEPT OF FISH AND GAME
OFFICE OF SPILL PREVENTION AND RESPONSE
FISH AND WILDLIFE WATER POLLUTION CONTROL LABORATORY

SWAMP
Surface Water Ambient Monitoring Program

Outline

- Aquatic Toxicity of Pyrethroids
- GC/QQQ with Chemical Ionization and Backflushing
- Results
- Conclusions

SWAMP
Surface Water Ambient Monitoring Program

Why go to Lower Detection Limits?

- There is a direct (toxic) effect to aquatic and terrestrial organisms from pyrethroid exposure and an indirect effect because of the threat to their food supply.

- Synthetic pyrethroids are known endocrine disruptors and many may be carcinogenic (USEPA).

- Synthetic pyrethroids are extremely toxic to aquatic organisms with LC_{50} values < 1 ppb.

SWAMP
Surface Water Ambient Monitoring Program

Toxicity in Water and Sediment

Pesticide	Water		Sediment	
	LC_{50} (ng/L)	Organism	LC_{50} (μg/kg)	Organism
Bifenthrin	70	*Hyalella azteca*[1]	4-10	*Hyalella azteca*[2]
Cyfluthrin	140	*Ceriodaphnia dubia*[1]	4-10	*Hyalella azteca*[2]
Cypermethrin	1.5	*Hyalella azteca*[3]	3-6	*Hyalella azteca*[3]
Esfenvalerate	70	*Ceriodaphnia lacustris*[1]	4-10	*Hyalella azteca*[2]
λ-Cyhalothrin	4	*Hyalella azteca*[4]	4-10	*Hyalella azteca*[2]
Permethrin	30	*Hyalella azteca*[1]	4-10	*Hyalella azteca*[2]

1 From previous slide

2 Amwag et al., 2005, *Environ. Toxicol. Chem.,* 24, 966-972.
3 Maund et al., 2002, *Environ. Toxicol. Chem.,* 21, 9-15.
4 Maund et al, 1998, *Pestic. Sci.*, 54, 408-417.

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Detection Limits Needed

Optimal MDL use $LC_{50}/10$

- Water: ~ **0.2** ng/L
- Sediment: ~ **0.3** ng/g

MDL equal to LC_{50}

- Water: ~ **2** ng/L
- Sediment: ~ **3** ng/g

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Improving MDLs

- Larger sample and/or injection volumes
- Use of MS/MS – lowers background noise
- Negative Chemical Ionization (nCI) – effective for pyrethroids with Cl, F, or Br

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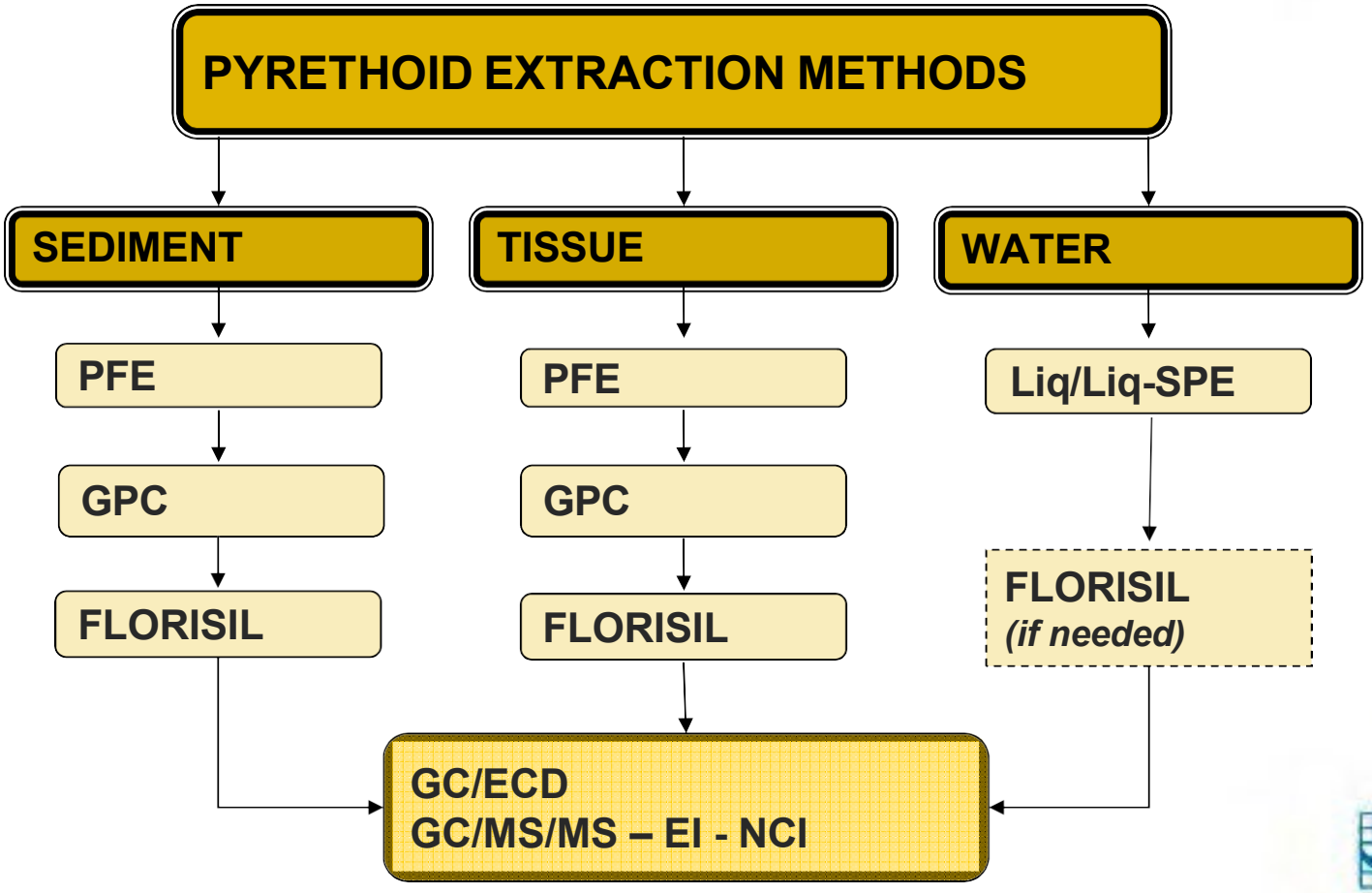
DFG Sample Preparation/Analysis



PFE: Pressurized Fluid Extraction
GPC: Gel Permeation Chromatography

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Analytical Instrument Comparison for Pyrethroids

Instrument: GC-ECD

Advantage	Disadvantage
very sensitive (1-5 pg on column)	dual column required for confirmation, therefore dual data processing
specific for halogenated compounds	rely on retention times for confirmation
excellent screening tool	requires extensive clean-up (sediment and tissue)
affordable	extensive matrix interference
easy to operate	cannot distinguish between deltamethrin and tralomethrin

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Instrument: GC-MS Ion trap in MS-MS mode

Advantage	Disadvantage
sensitivity comparable with ECD (2-10 pg)	narrow linearity range
MS spectra confirmation	trap clean-up needed for tissue
no matrix interference	requires experienced MS chemist
less data handling	cannot distinguish between deltamethrin and tralomethrin
short run time	

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Instrument: LC-MS-MS

Advantage	Disadvantage
sensitivity comparable with GC-MS-ion trap	not as sensitive as GC-MS-QqQ due to polarity of compounds
best result will be obtained by APCI	requires specially trained MS chemist
distinguishes between deltamethrin and tralomethrin	very expensive
excellent for thermo labile compounds	
minimum sample clean-up required	
excellent linearity	
very promising for chiral separation	
green chemistry	

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Instrument: GC-MS Triple-quadrupole (QqQ)

Advantage	Disadvantage
extremely sensitive (0.1-2.0 pg on column)	maintenance needed frequently
excellent for trace level in sediment and tissue	very experienced MS chemist needed
detects halogenated and non-halogenated pyrethroids	expensive
best analytical tool for pyrethroids	cannot distinguish between deltamethrin and tralomethrin

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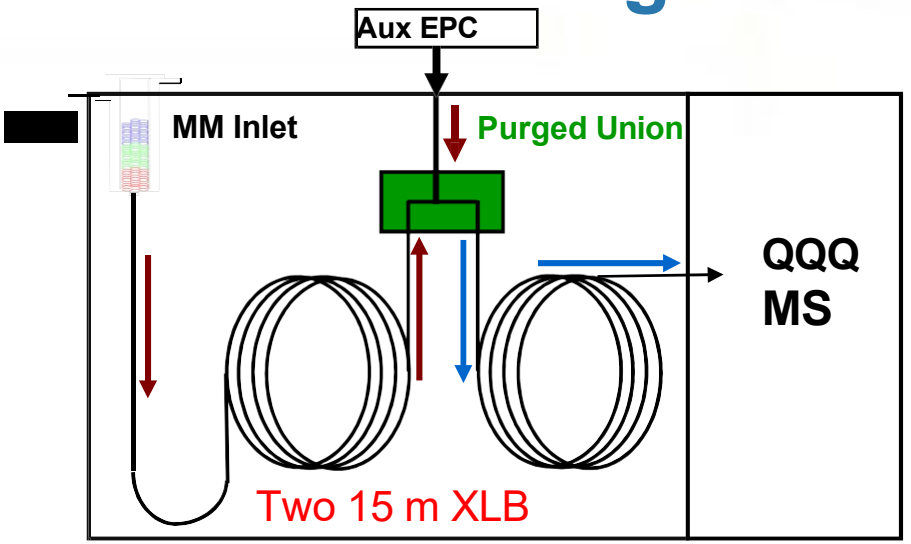
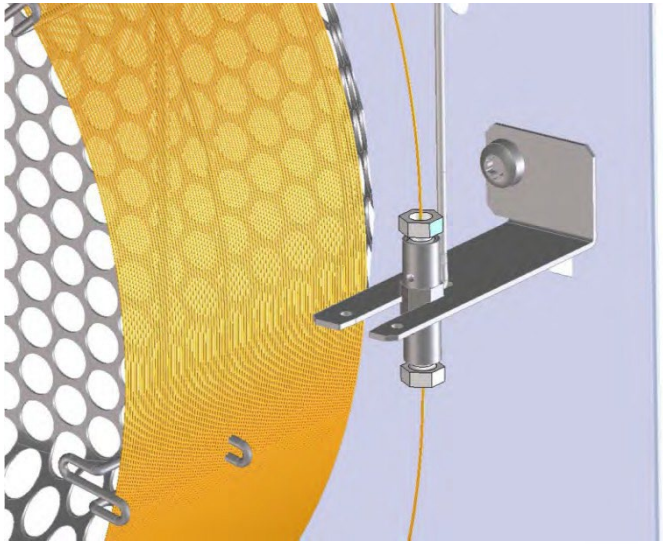
Instrumentation & Backflush Configuration

Agilent 7890A/7000B GC/QQQ
With Chemical Ionization





Purged Ultimate Union

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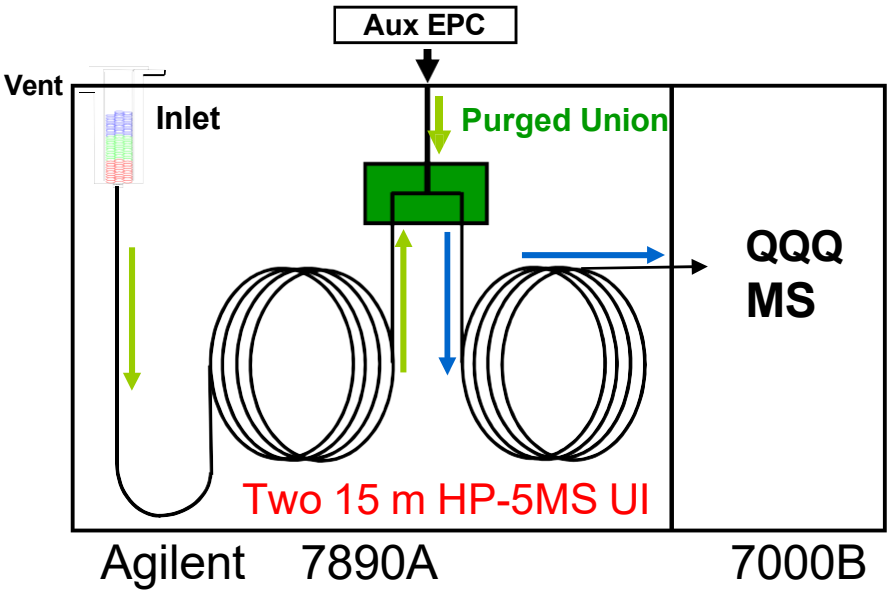
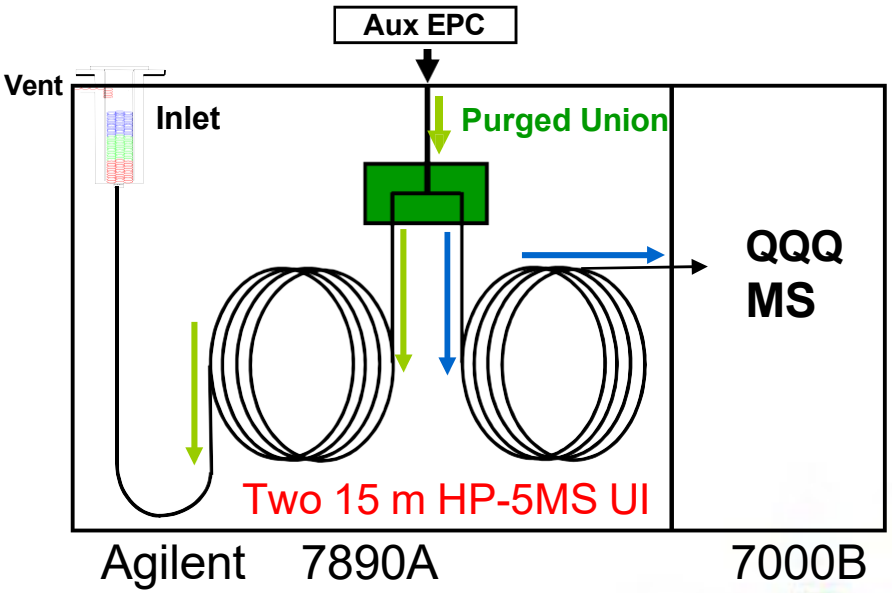
Mid-column Backflush

Injection & analysis



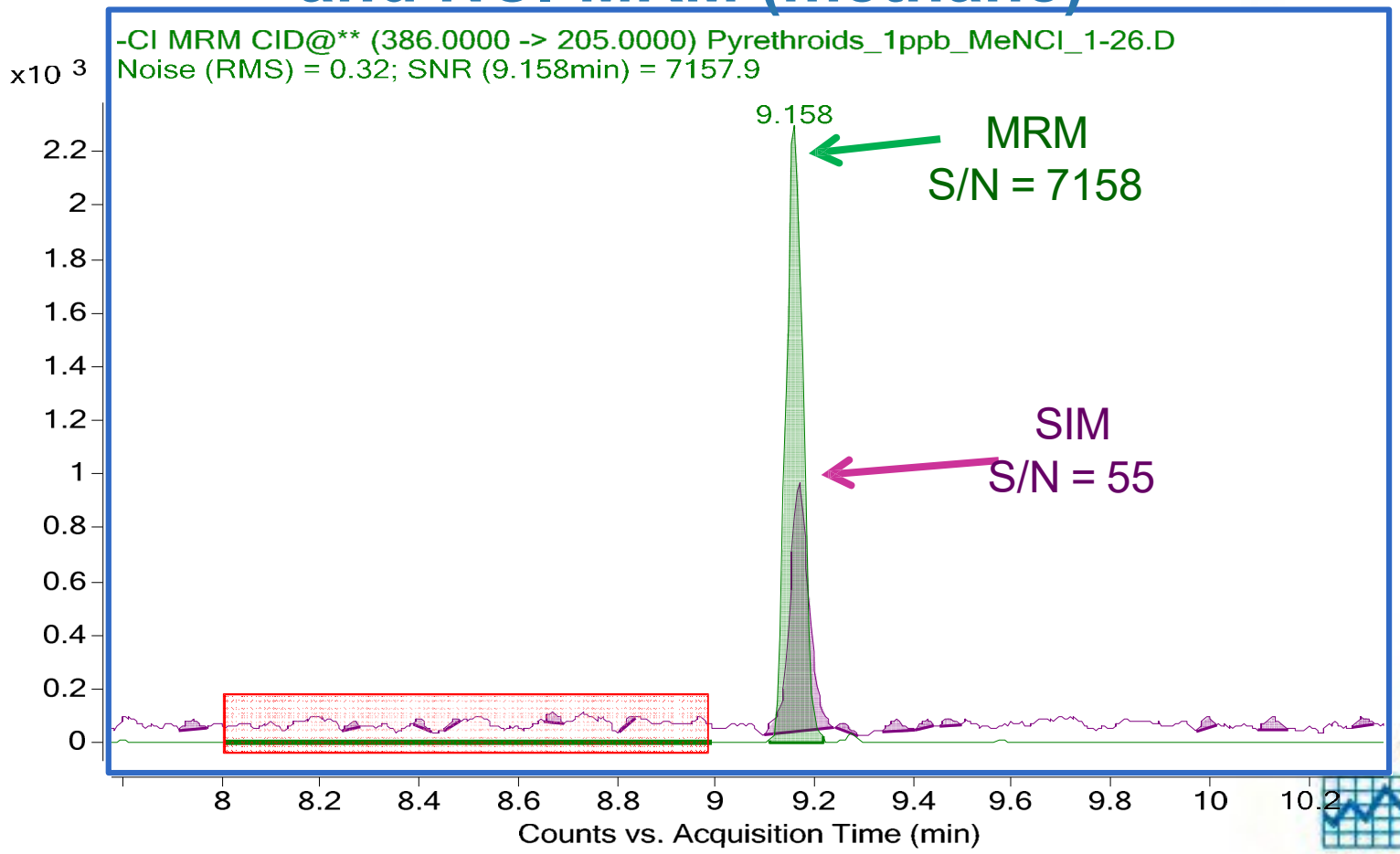
During Backflush



Begin backflushing after last analyte passes purged union or during post run

Ambient Monitoring Program

Bifenthrin, 1 ppb Standard: NCI-SIM and NCI-MRM (methane)



MRM transition: 386→205 (green)
SIM ion: 386 (purple)

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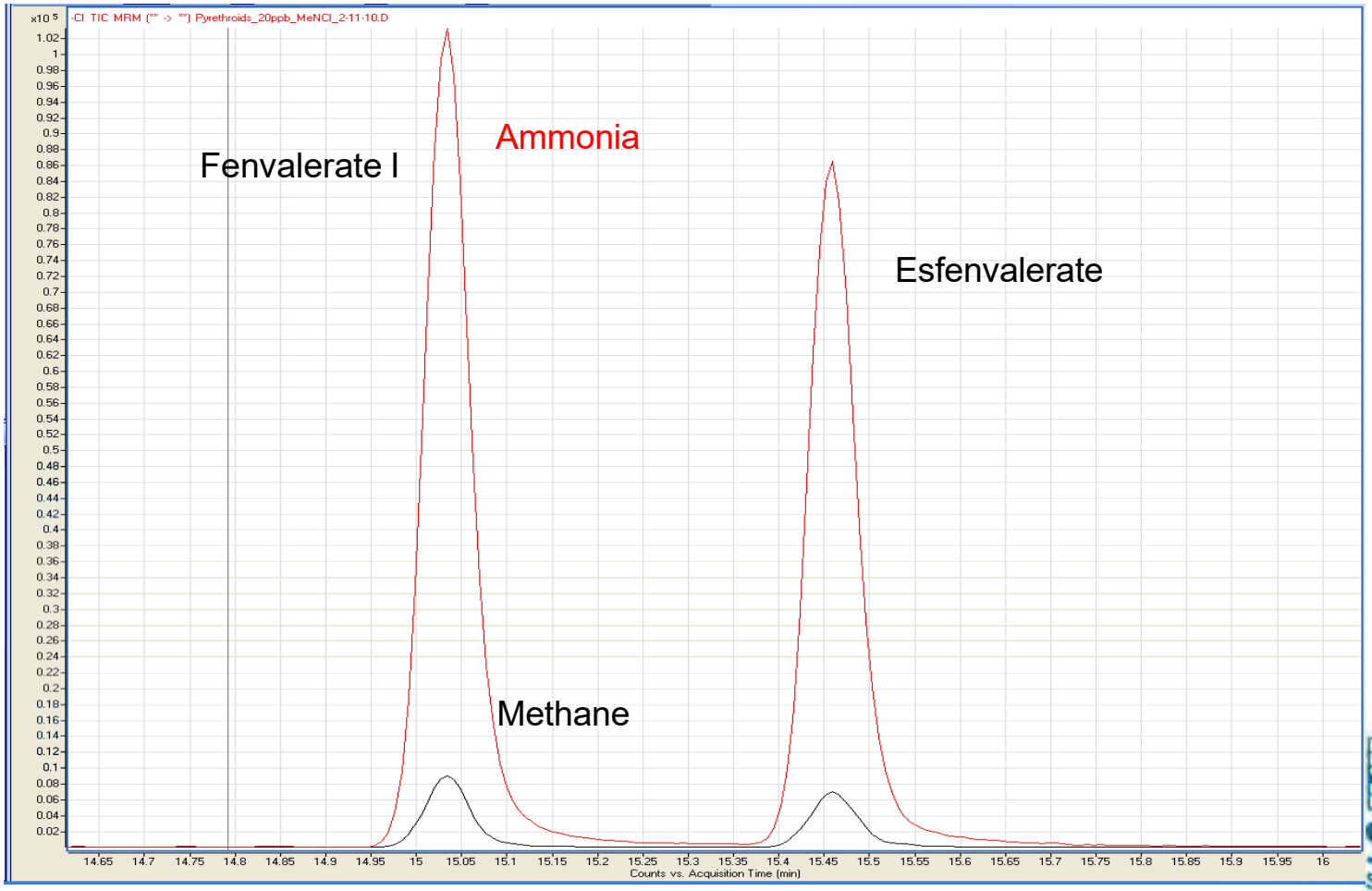
Comparing Methane and Ammonia Reagent Gases for NCI GC/MS/MS Analysis of Pyrethroids



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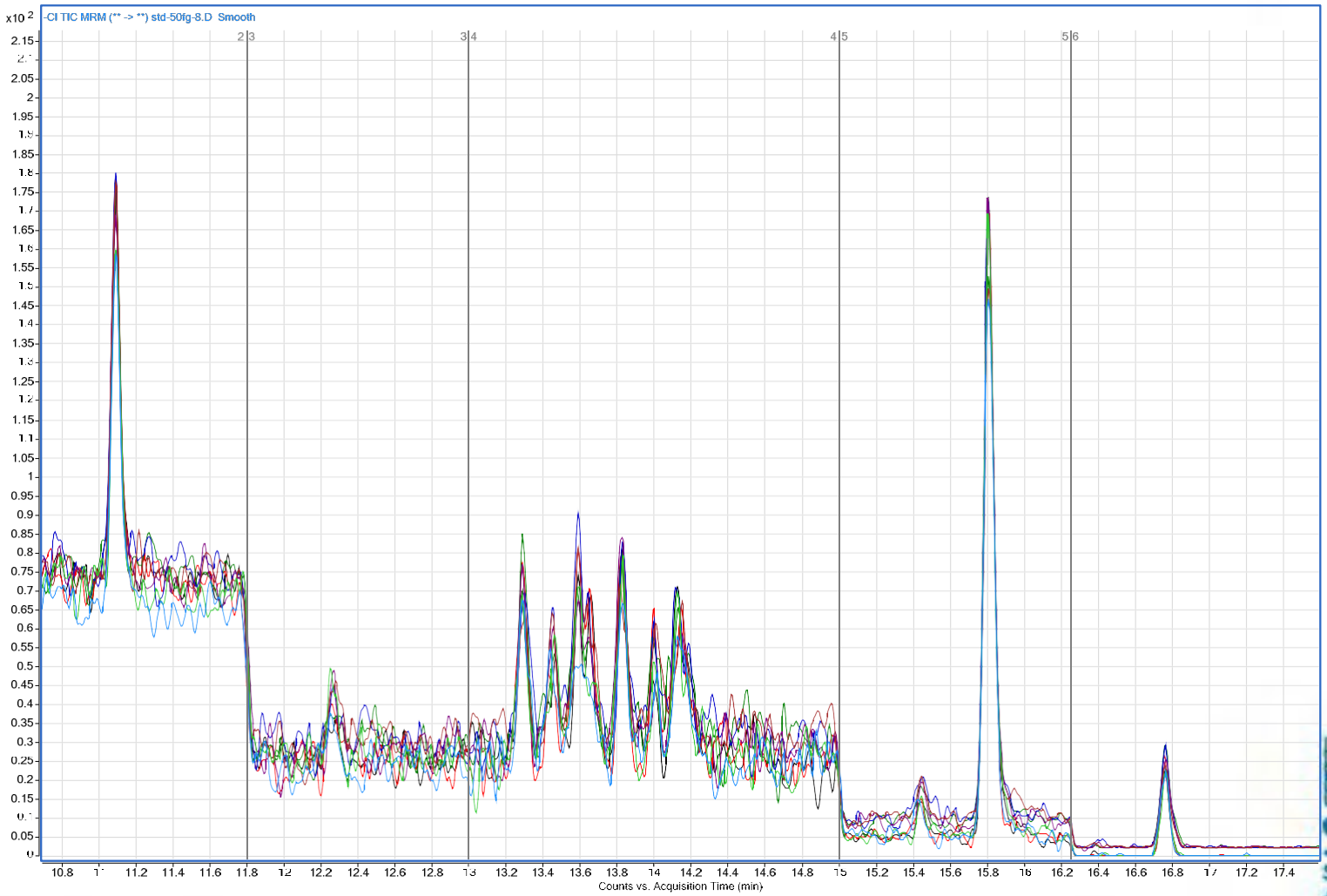
Pyrethroids-50fg (n=8)-NCI



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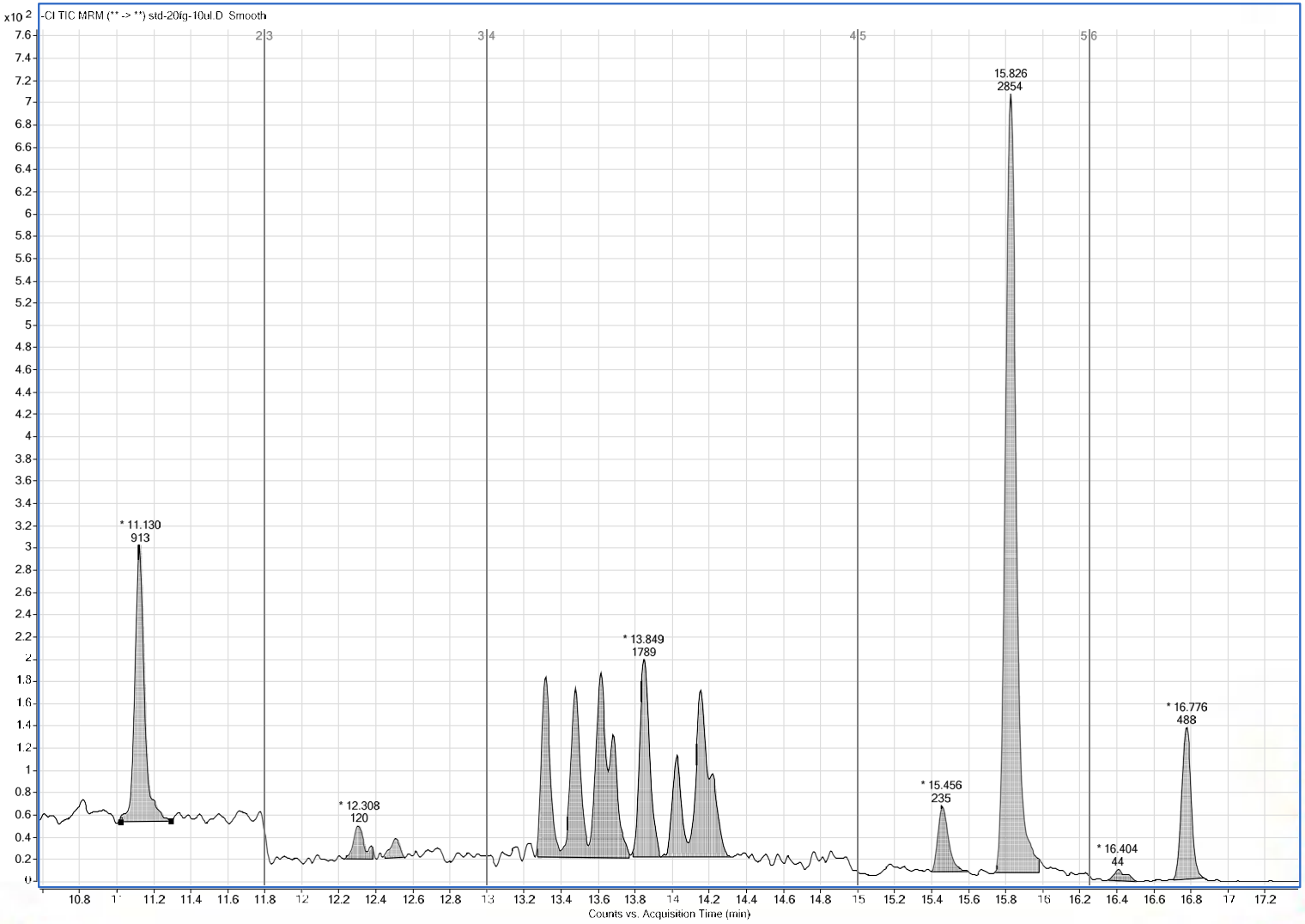
Pyrethroids-20fg-NCI-large volume injection



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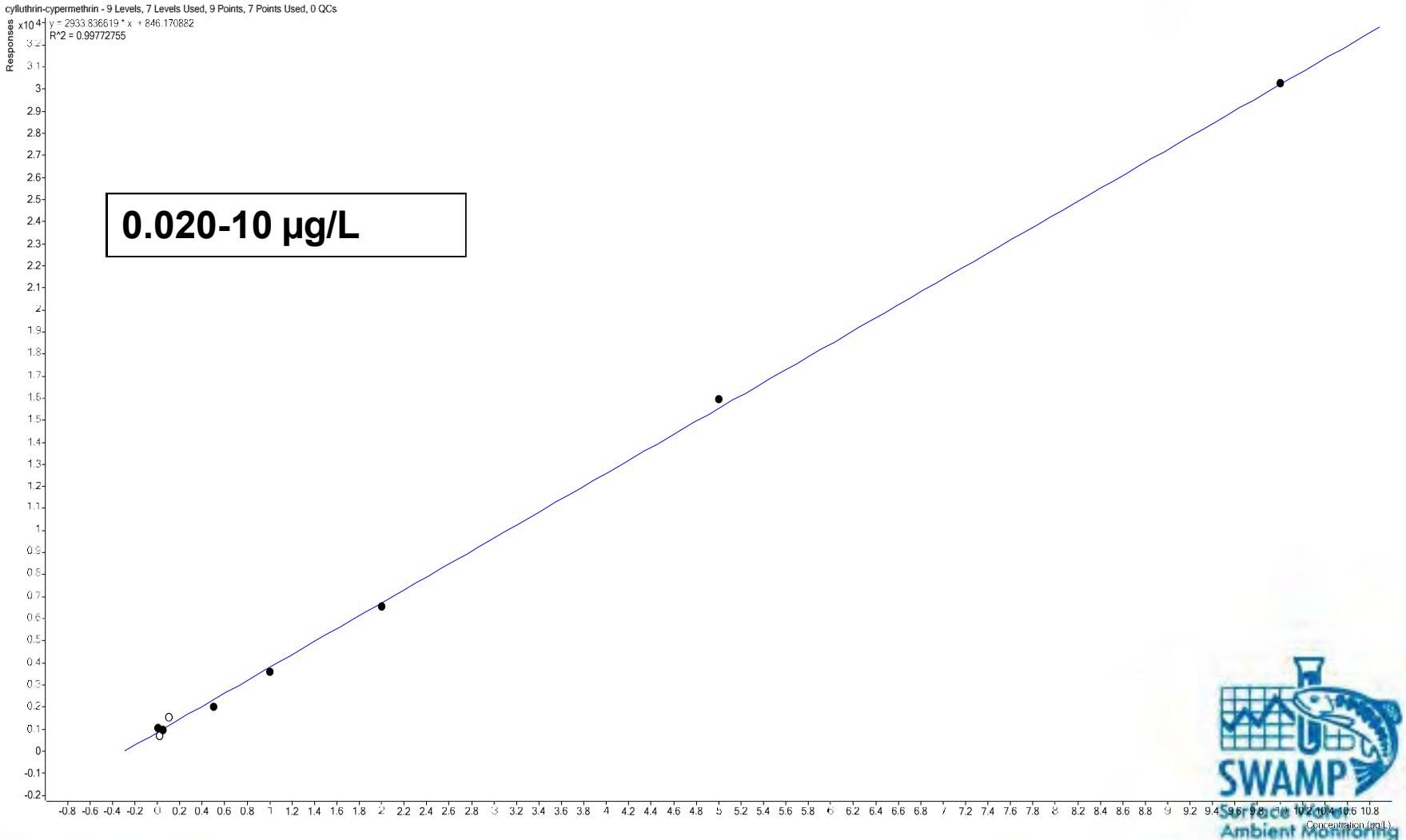
Cypermethrin MRM by NCI Ammonia



0.020-10 µg/L

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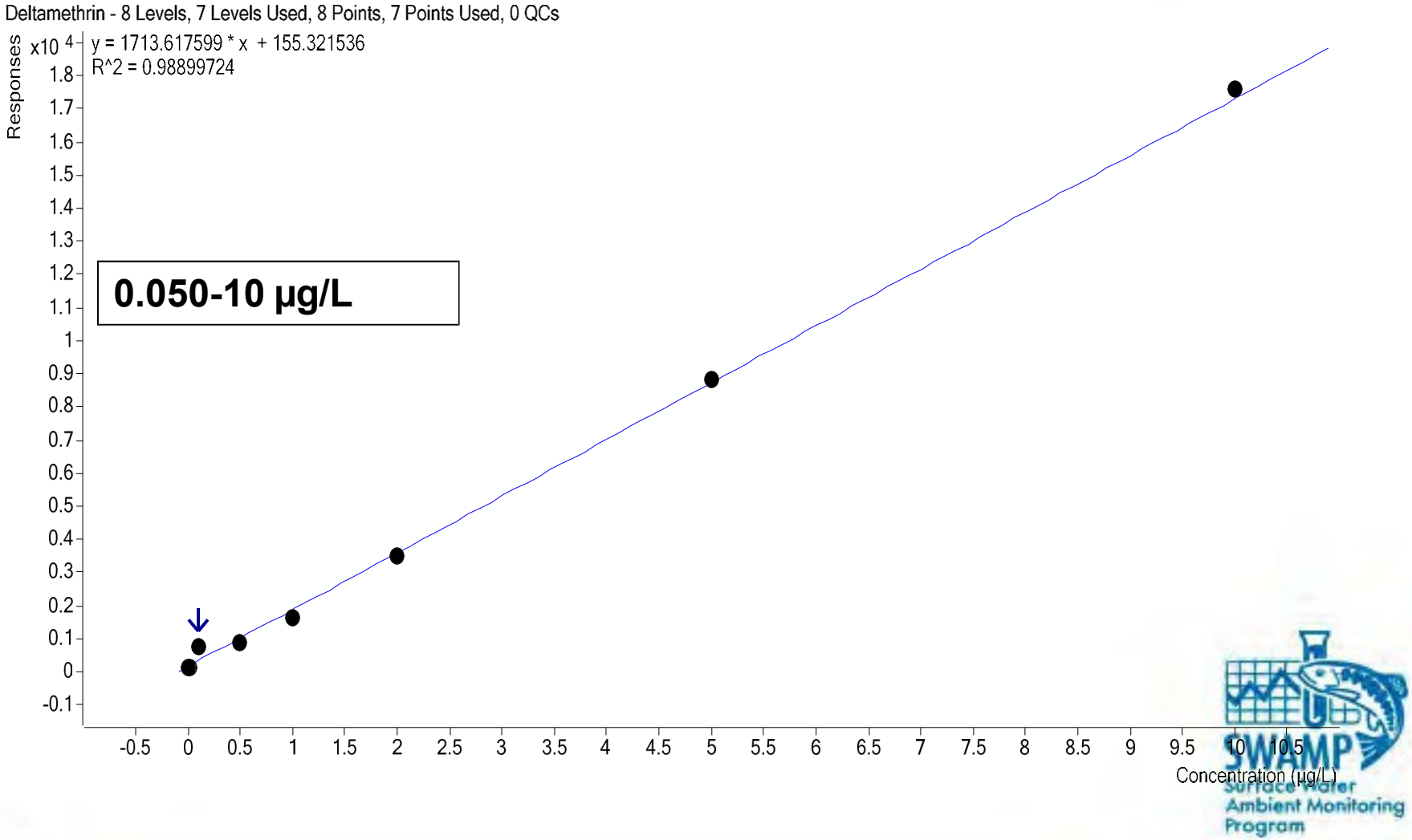
Deltamethrin MRM by NCI Methane



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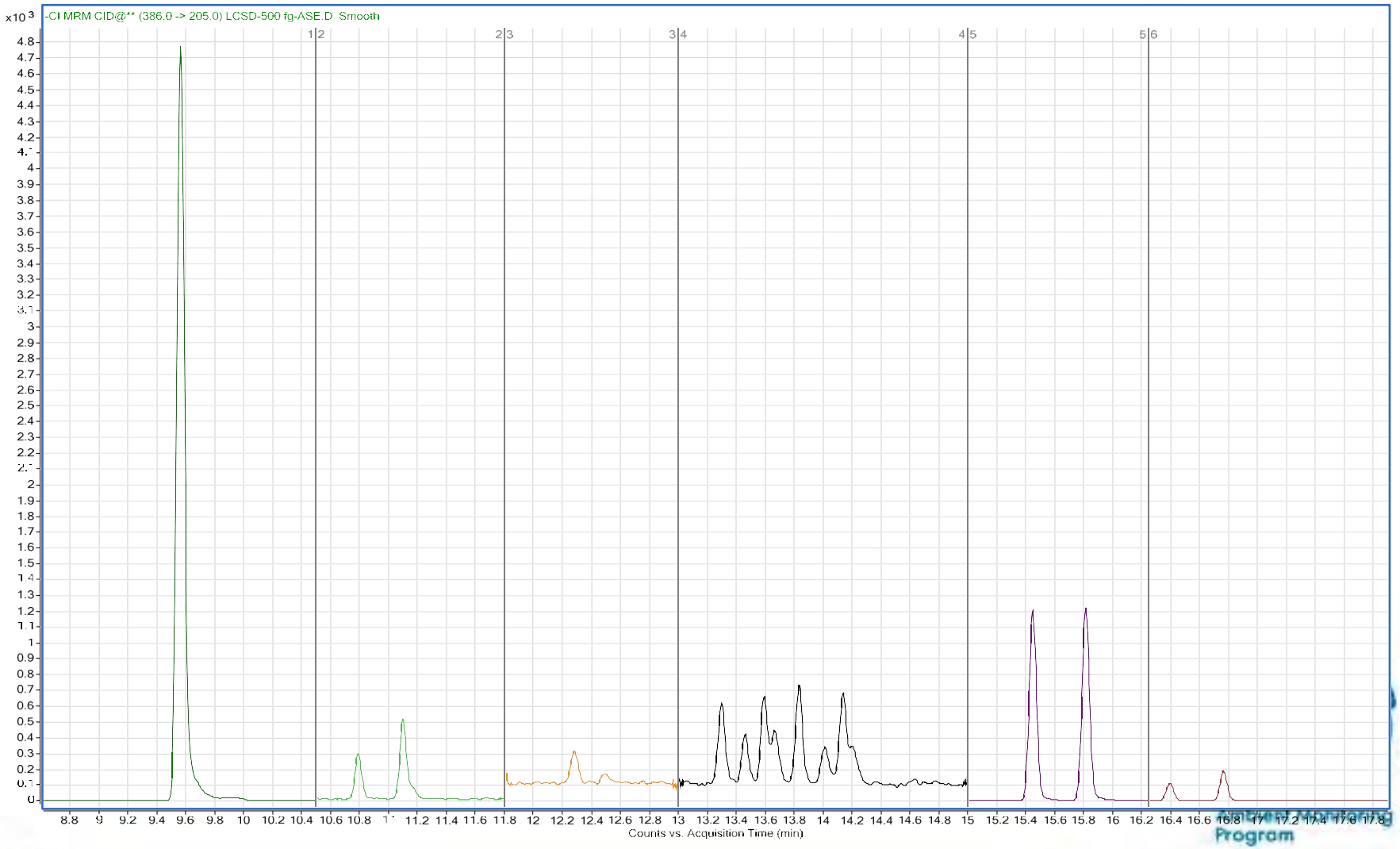
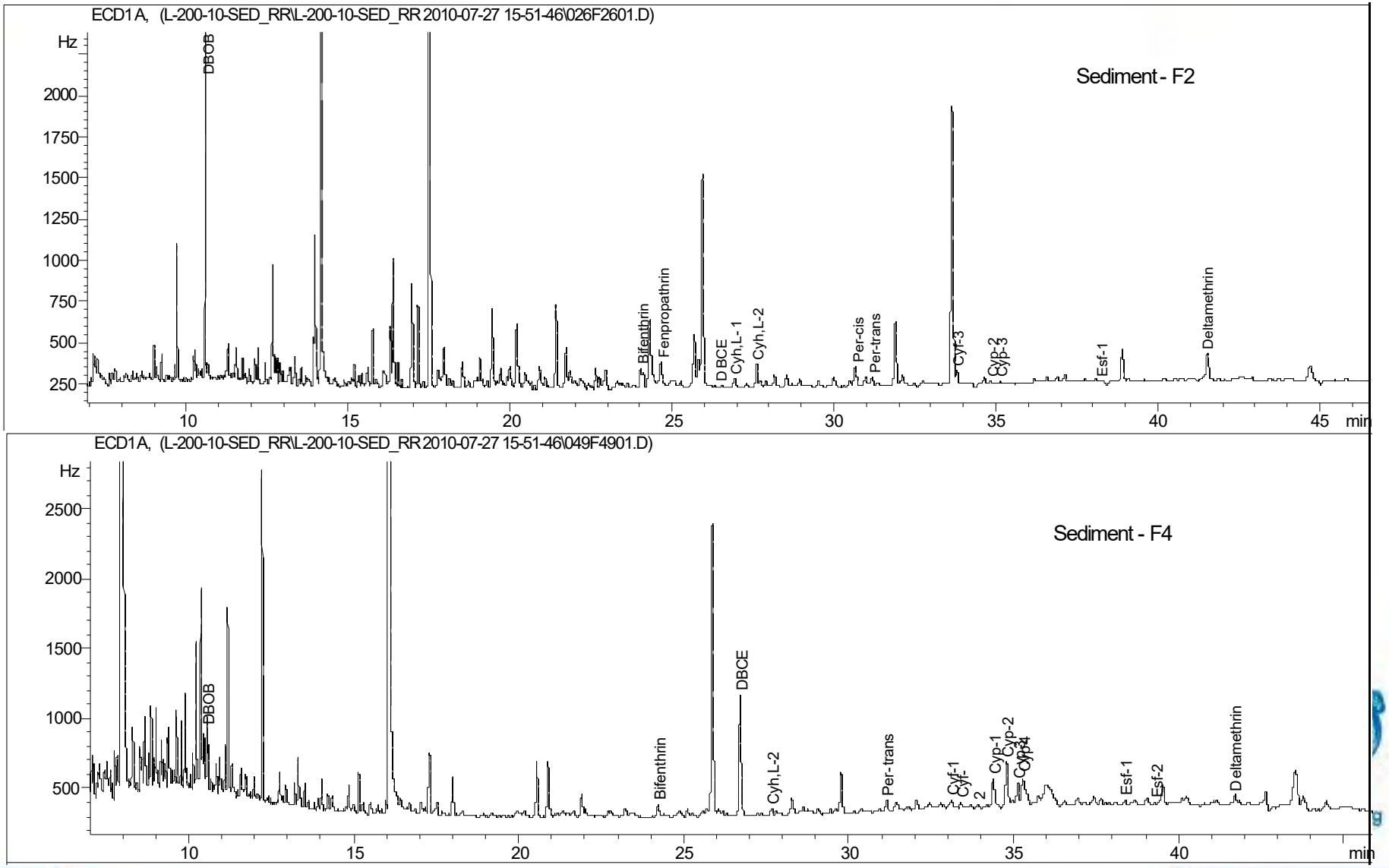
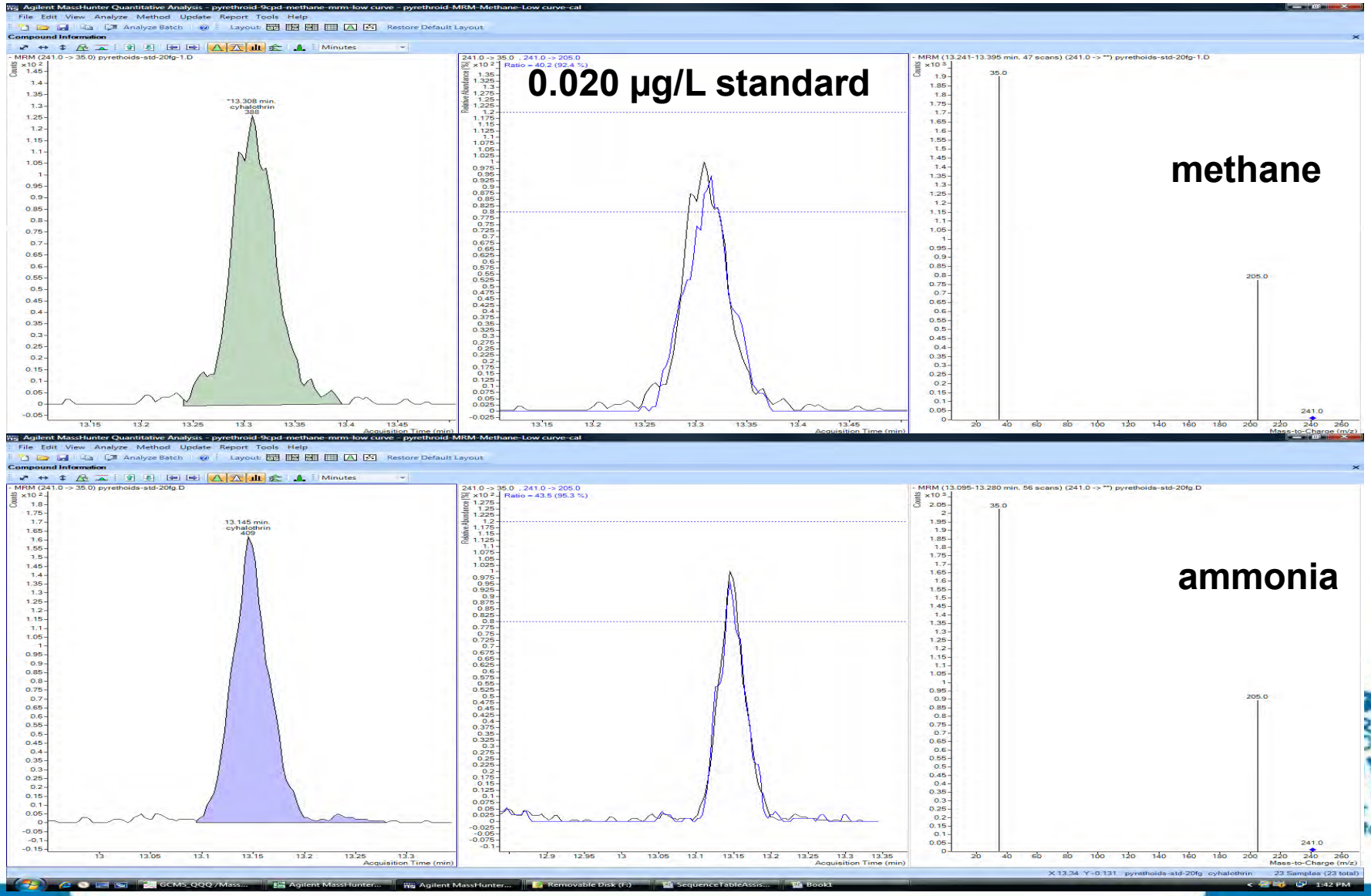
Sediment-100fg spike



GC/ECD DB5 - Sediment Extracts

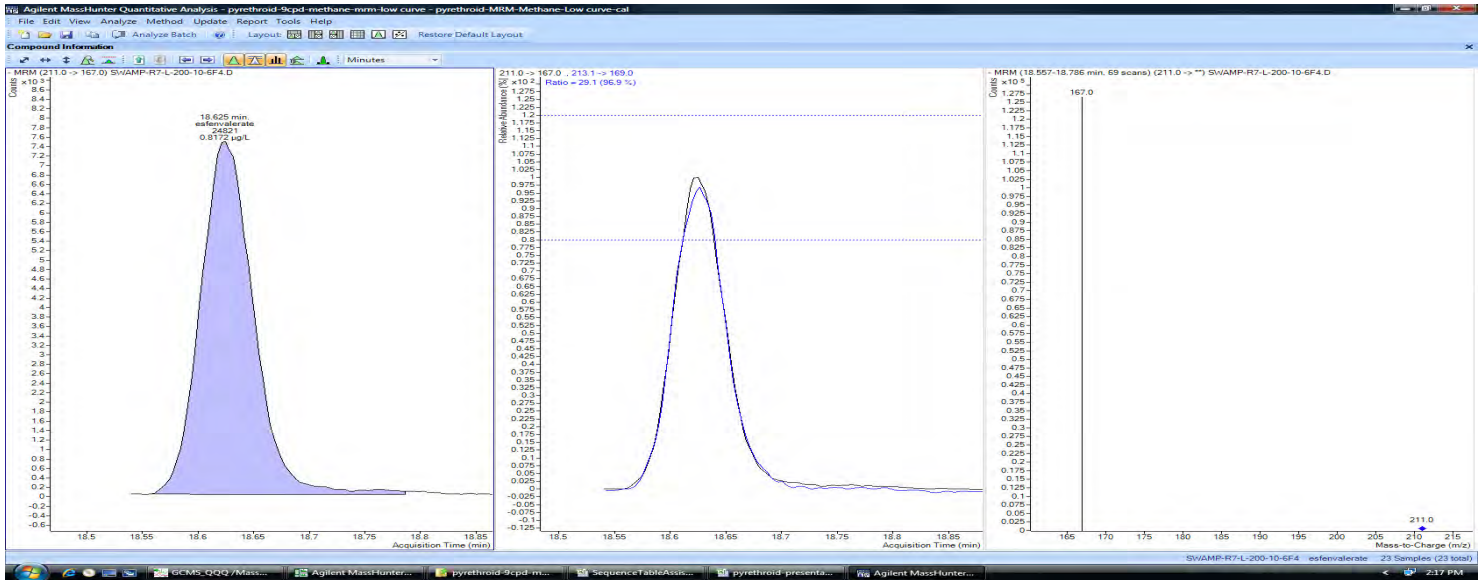


Lambda-cyhalothrin MRM-NCI-MSMS

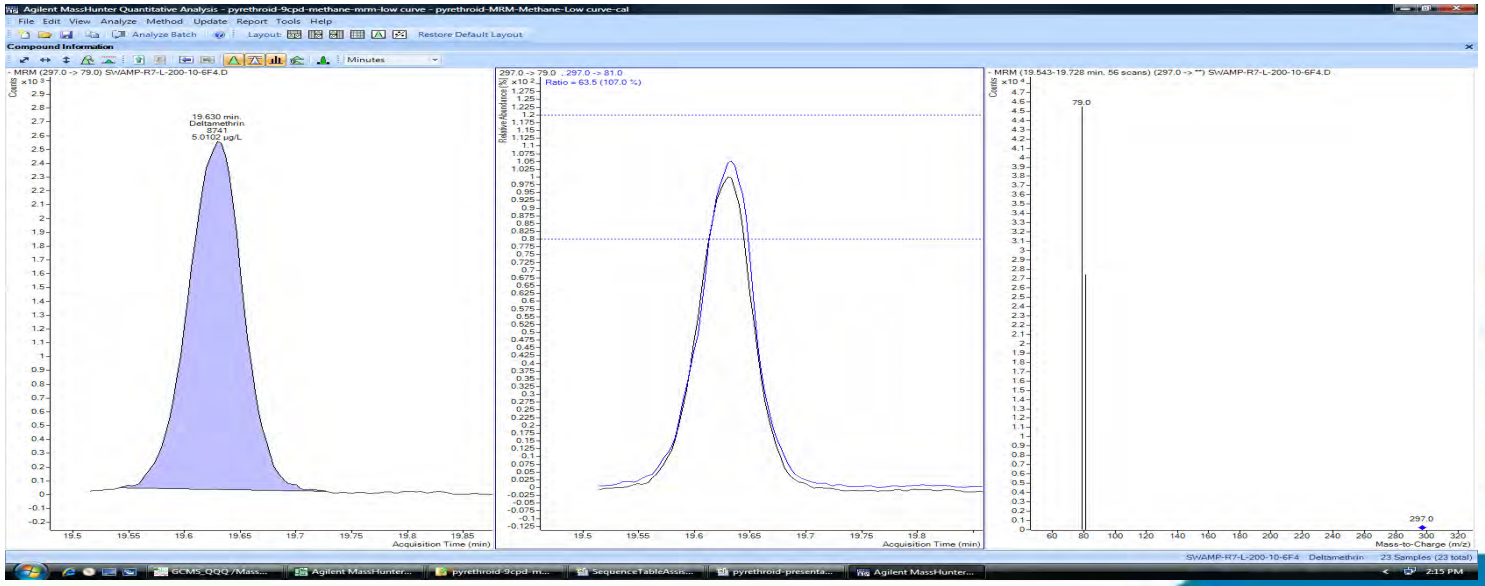


Sediment Sample Extract by NCI - methane

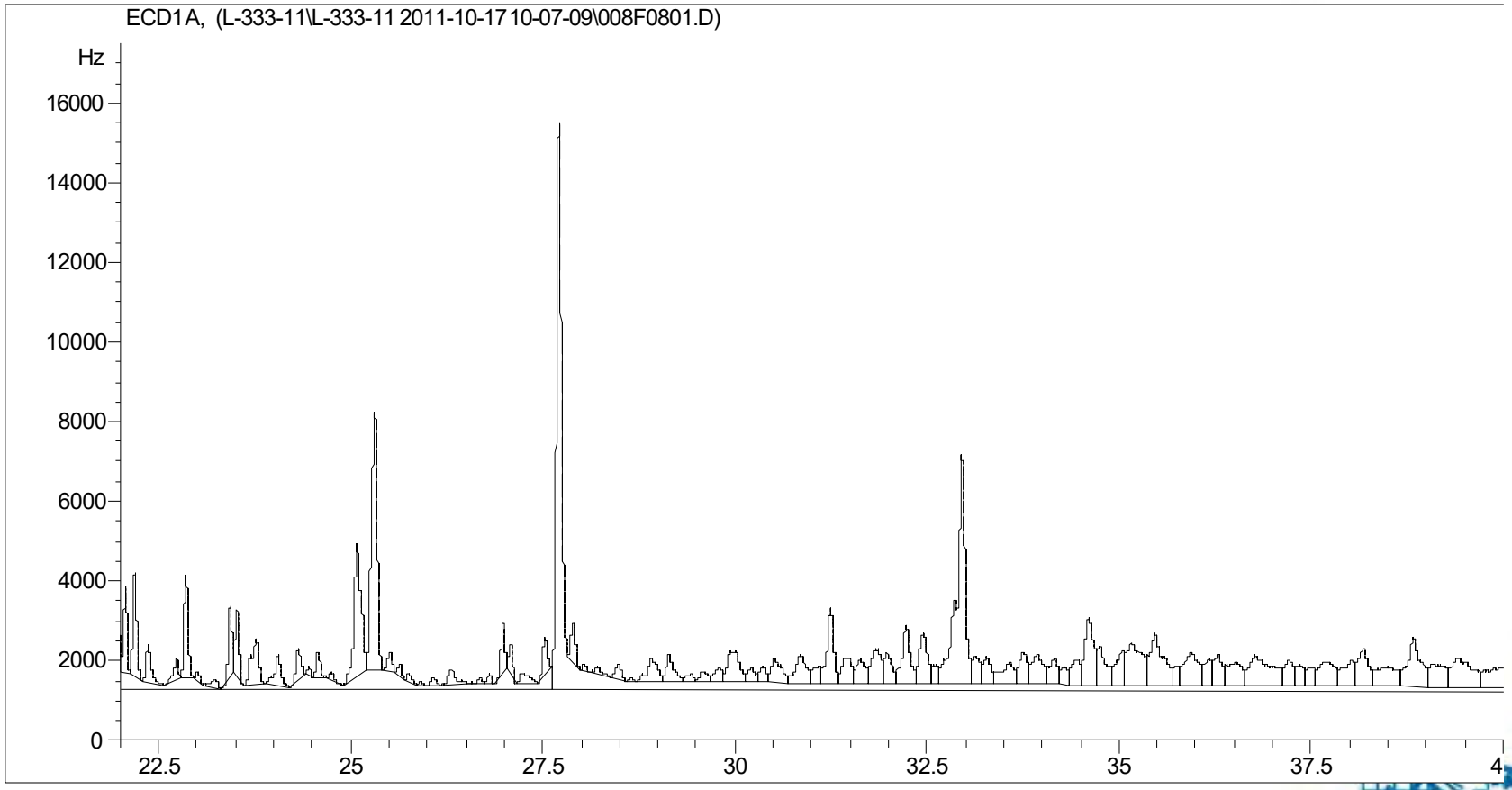
es-fenvalerate
0.81 ng/g



deltamethrin
5.01 ng/g



ECD - Round Robin Sediment Extract – 2011



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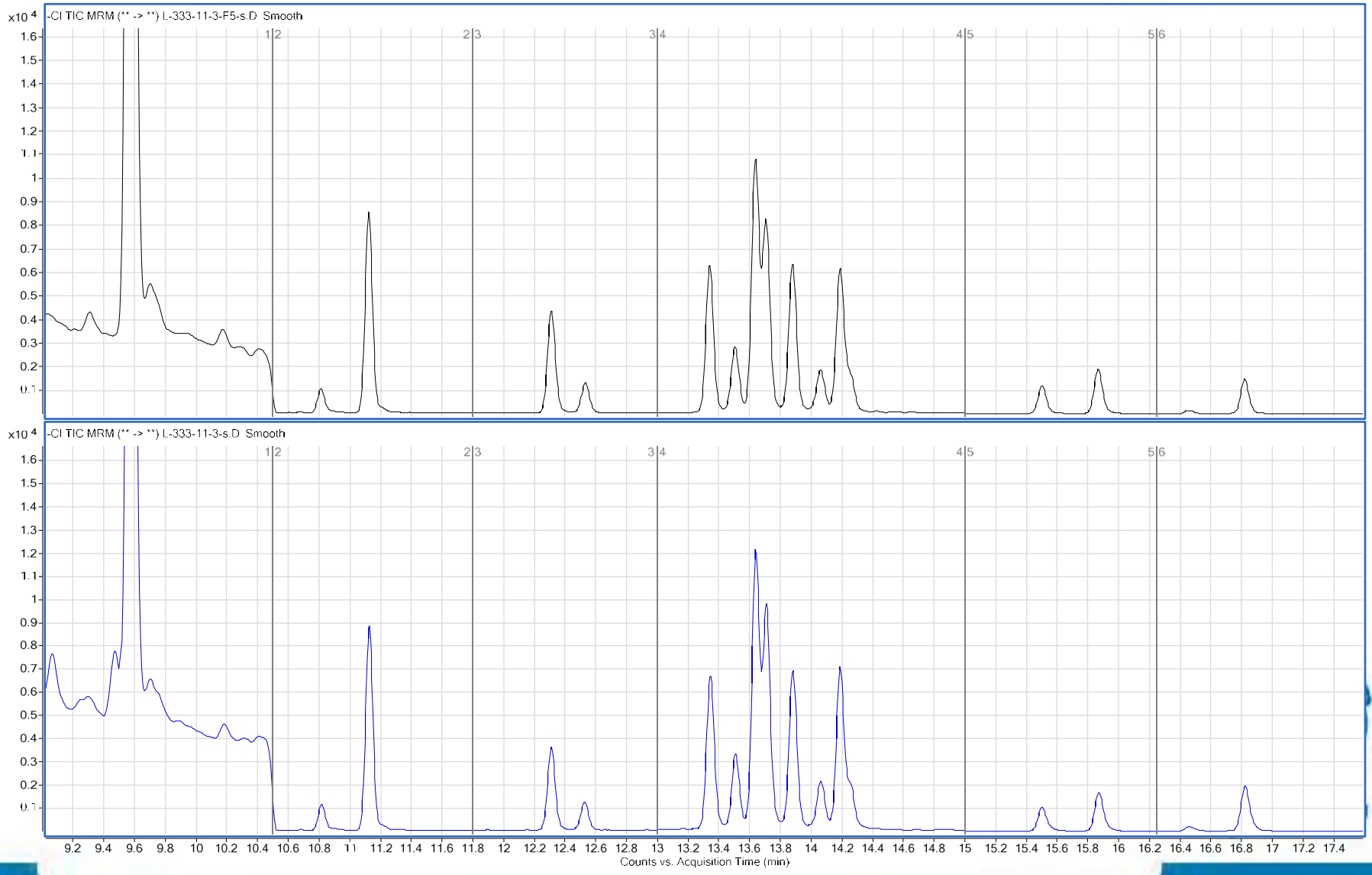
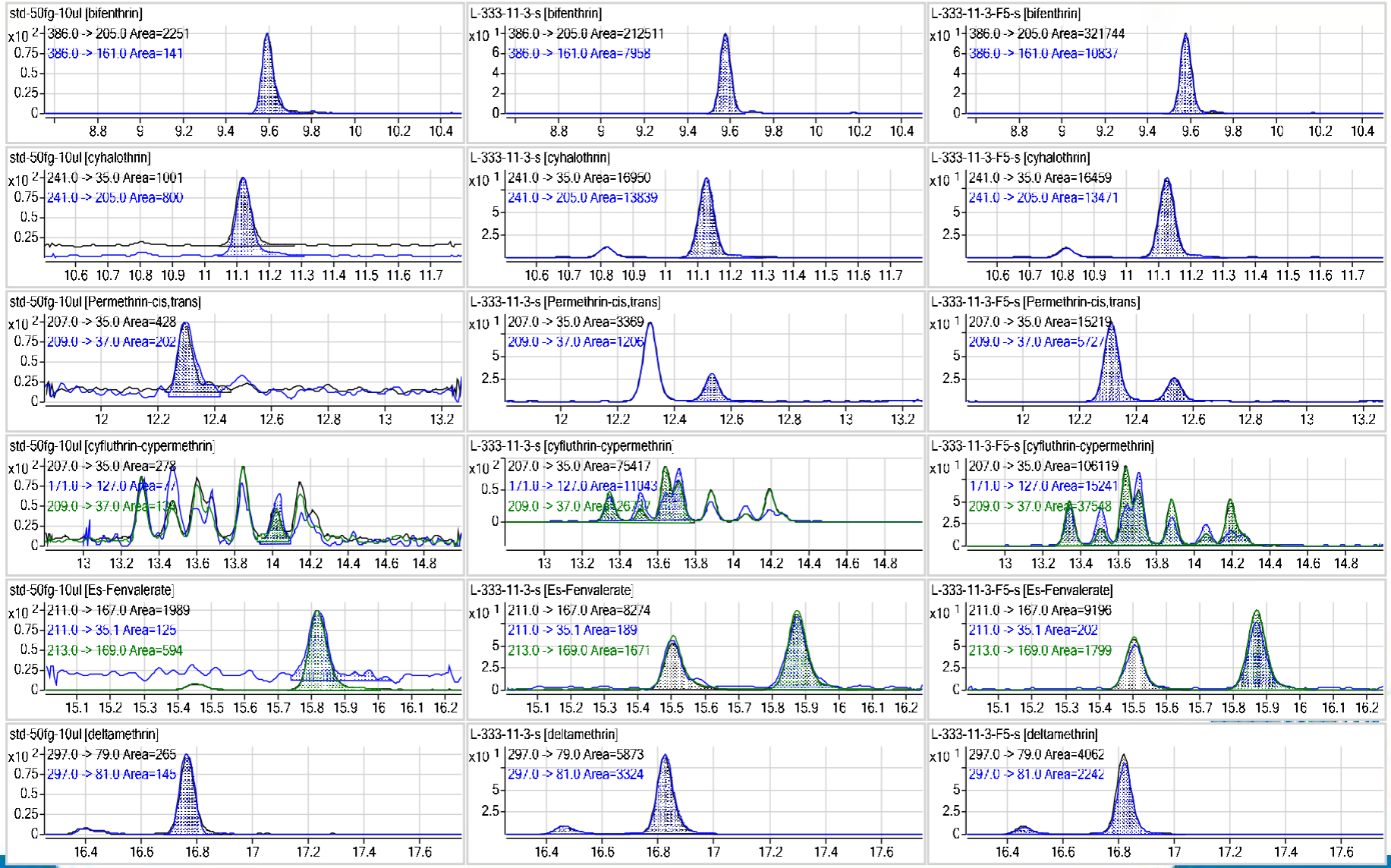
NCI - Round Robin Sediment Extract – 2011



NCI - Round Robin Sediment Extract – 2011



MS/MS-EI-Signal to Noise and ELOD

Synthetic pyrethroid	std conc µg/L	MS/MS-EI-S/N	Estimated Limit of Detection (ELOD) Sediment ng/g
Allethrin	0.500	629	1.50
Parallethrin	0.500	325	0.80
Resmethrin	0.500	501	0.80
Bifenthrin	0.500	1408	0.30
Fenpropathrin	0.500	165	1.20
Tetramethrin	0.500	262	0.50
Phenothrin	0.500	686	0.50
Cyhalothrin	0.500	197	0.35
Permethrin cis	0.500	423	0.75
Permethrin trans	0.500	362	0.95
Cyfluthrin	0.500	24.5	0.35
Cypermethrin	0.500	40.0	0.45
Es-fenvalerate	0.500	57.4	0.25
Deltamethrin	0.500	30.9	0.50

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MS/MS-NCI-Signal to Noise and ELOD

Synthetic pyrethroid	std conc µg/L	MS/MS-NCI-Signal to Noise S/N		Estimated Limit of Detection (ELOD)	
		Methane	Ammonia	Water ng/L	Sediment ng/g
Bifenthrin	0.050	973	2345	0.10	0.08
Cyhalothrin	0.050	683	1890	0.20	0.1
Permethrin	0.050	25	150	1.00	1.5
Cyfluthrin	0.050	277	1217	0.20	0.2
Cypermethrin	0.050	336	1410	0.20	0.18
Es-fenvalerate	0.050	2757	6450	0.05	0.05
Deltamethrin	0.050	190	463	0.15	0.3

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Pyrethroid Method Detection and Reporting Limits for Water, Sediment and Tissue - ECD

Sample Matrix	Water		Sediment		Tissue	
Pyrethroid Pesticides	MDL ppb (ug/L)	RL ppb (ug/L)	MDL Dry wt ppb (ng/g)	RL Dry wt ppb (ng/g)	MDL Fresh wt ppb (ng/g)	RL Fresh wt ppb (ng/g)
Bifenthrin	0.001	0.002	0.50	1.00	0.65	2.00
Cyfluthrin	0.002	0.004	2.00	4.00	2.70	6.00
Cypermethrin	0.004	0.004	2.00	4.00	1.70	4.00
Deltamethrin	0.002	0.004	2.00	4.00	0.60	2.00
Es/Fenvalerate	0.001	0.002	1.00	2.00	1.70	4.00
Fenpropathrin	0.002	0.004	2.00	4.00	1.35	4.00
Lambda-cyhalothrin	0.001	0.002	1.00	2.00	1.50	4.00
Permethrin	0.003	0.005	4.00	8.00	2.20	6.00

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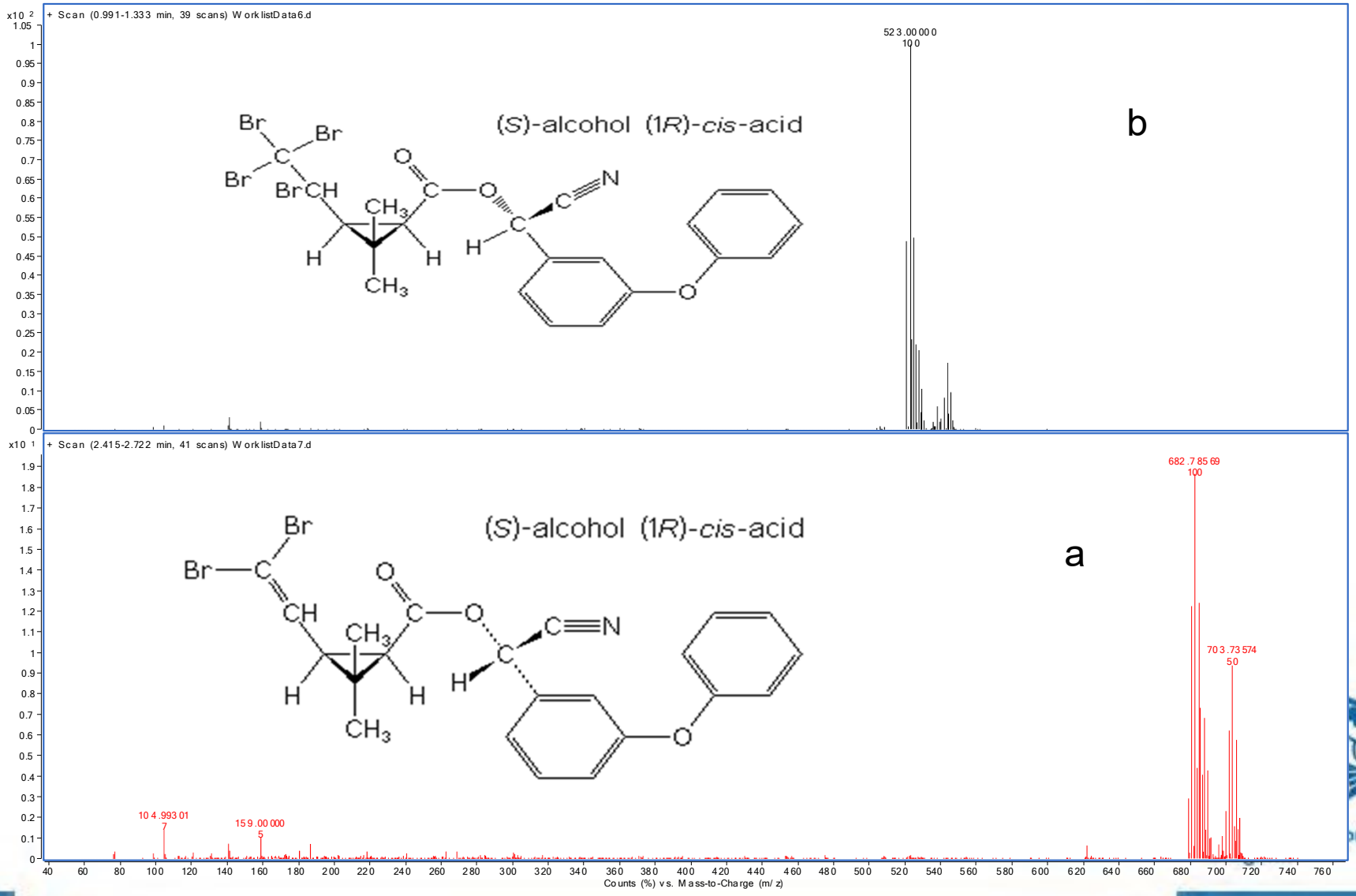
Analysis of Deltamethrin(a) and Tralomethrin(b) using LC/MS



Conclusions

- Analysis of pyrethroids by GC-MSMS-NCI yields low detection limits that are necessary for toxicity of these analytes
- GC-NCI-MSMS is at least 5 times more sensitive than GC-EI-MSMS but is not suitable for all pyrethroid pesticides
- Ammonia NCI is 5-10 times more sensitive than methane
- LC-MS is necessary to distinguish between deltamethrin and tralomethrin

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Thank You

Any questions?

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