Ventura Countywide Stormwater Quality Management Program

2014-2015 Permit Year

Ventura Countywide Stormwater Quality Management Program Annual Report

Attachment E16 Ventura River and Tributaries Algae, Eutrophic Conditions, and Nutrients TMDL Comprehensive Monitoring Plan for Receiving Waters

Camarillo
County of Ventura
Fillmore
Moorpark
Ojai
Oxnard
Port Hueneme
Santa Paula
Simi Valley
Thousand Oaks
Ventura
Ventura County Watershed Protection District

December 14, 2015

VENTURA RIVER AND TRIBUTARIES
ALGAE, EUTROPHIC CONDITIONS, AND NUTRIENTS
TOTAL MAXIMUM DAILY LOAD

COMPREHENSIVE MONITORING PLAN FOR RECEIVING WATERS

Approved By
California Regional Water Quality Control Board
Los Angeles Region
October 20, 2014

On Behalf of the
Ojai Valley Sanitary District, Ventura County Watershed Protection District, County of Ventura, City of Ojai, City of San Buenaventura (Ventura), California Department of Transportation, and the Ventura County Agricultural Irrigated Lands Group (represented by the Farm Bureau of Ventura County)

Prepared by the
Ventura County Watershed Protection District

TABLE OF CONTENTS

TABLE OF FIGURES

APPENDICES

1 INTRODUCTION

1.1 Background and Purpose

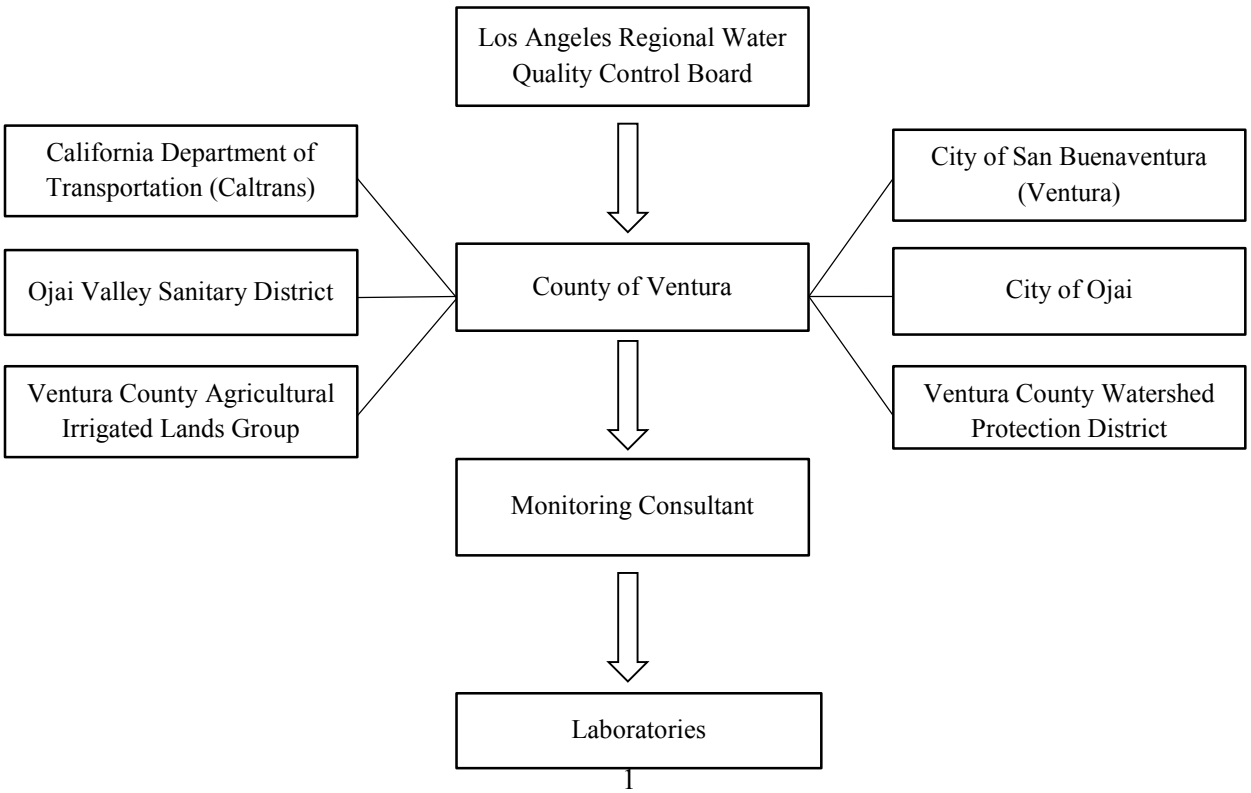
The Ventura River Estuary and the Ventura River (including its tributaries) are identified on the 1998, 2002, 2006, and 2010 Clean Water Act (CWA) Section 303(d) list of impaired waterbodies due to algae (Estuary and Reach 1+2), eutrophic conditions (Estuary), low dissolved oxygen (Cañada Larga), and nitrogen (San Antonio Creek). The CWA requires the development of Total Maximum Daily Loads (TMDLs) to restore impaired waterbodies to fully support their beneficial uses.

The Ventura River and Tributaries Algae, Eutrophic Conditions, and Nutrients TMDL (TMDL) was adopted by the Los Angeles Regional Water Quality Control Board (Regional Board) on December 6, 2012 and became effective on June 28, 2013. The TMDL requires the development and implementation of a comprehensive monitoring plan (CMP) for receiving water monitoring to assess numeric attainment and measure in-stream nutrient concentrations. This document is designed to meet that objective by identifying receiving water monitoring sites, specifying monitoring parameters, schedules, and protocols, and describing measurement quality objectives for data collection and analysis. The final draft of this CMP was submitted to the Regional Board on June 27, 2014 and was approved by the Regional Board on October 20, 2014.

1.2 Responsible Parties

The Responsible Parties for this CMP are the Ojai Valley Sanitary District, Ventura County Watershed Protection District, County of Ventura, City of Ojai, City of San Buenaventura (Ventura), California Department of Transportation, and the Ventura County Agricultural Irrigated Lands Group (represented by the Farm Bureau of Ventura County).

1.3 Organizational Chart



1.4 Numeric Targets

The numeric targets used to assess impairment for this TMDL are based on the numeric and narrative water quality objectives (WQO) listed in Chapter 3 of the Los Angeles Region Basin Plan. For dissolved oxygen (DO) and pH, the numeric target is equal to their numeric WQOs. The numeric targets for algal and phytoplankton biomass and percent cover are established as a numeric interpretation of the narrative water quality objective for biostimulatory substances. Numeric targets to interpret narrative water quality objectives are based on the California Nutrient Numeric Endpoints (NNE) approach, developed by USEPA Region 9 and the State and Regional Water Quality Control Boards and are listed in Table 1.

Table 1. TMDL Numeric Targets

Indicator	Numeric Target	Waterbody
Total Algal Biomass	150 mg/m^2 chlorophyll *a* as seasonal average	Ventura River and Tributaries
Macroalgal Cover (attached & unattached)	≤ 30 percent as seasonal average	Ventura River and Tributaries
Phytoplankton Biomass	20 µg/L chlorophyll *a* as seasonal average	Estuary (shallow subtidal area)
Macroalgal Cover	≤ 15 percent as seasonal average	Estuary (intertidal and shallow subtidal areas)
Dissolved Oxygen	≥ 7 mg/L as a daily minimum	Ventura River, Tributaries, and Estuary
pH	6.5 – 8.5 (instantaneous value)	Ventura River, Tributaries, and Estuary

1.5 Ventura River Watershed

The Ventura River Watershed is located in northwestern Ventura County with a small portion in southeastern Santa Barbara County. The watershed drains a fan-shaped area of about 220 square miles with an elevation from 6,000 feet to sea level. The Ventura River has several major tributaries, including Matilija Creek, North Fork Matilija Creek, San Antonio Creek, Coyote Creek and Cañada Larga.

The uppermost reach of the Ventura River Watershed (Reach 5) includes Matilija Creek and North Fork Matilija Creek from their headwaters in the Los Padres National Forest to approximately 0.3 miles below their confluence, at Camino Cielo Road. This reach is primarily open space and contains both wilderness and semi-primitive forest areas. Flow in this reach is both ephemeral and perennial, depending on location. Reach 5 is not identified in the TMDL and is not part of this CMP. The Matilija Dam and Reservoir are located on Matilija Creek, less than one mile above the confluence with North Fork Matilija Creek and the start of the Ventura River, from which point the River then flows for about 16 miles in a southerly direction to the estuary and the Pacific Ocean.

Reach 4 begins at Camino Cielo Road near the unincorporated community of Meiners Oaks and continues approximately 10 miles south to Casitas Vista Road, south of the unincorporated community of Casitas Springs. The River is predominantly dry during the summer in the upper 7-8 miles of this reach. San Antonio Creek and Coyote Creek both join the River within this Reach. San Antonio Creek and its tributaries drain the northeastern section of the watershed, including urban, residential, agriculture, and

open space land uses. This section of the watershed includes the Los Padres National Forest, the City of Ojai, and public and private golf courses and parks. Flow is ephemeral along much of the length of the Creek and its tributaries. Coyote Creek drains the western portion of the Ventura River Watershed, which is largely open space, but the flow is captured by the Lake Casitas Dam and Reservoir and is used for water supply. Consequently, flow from Coyote Creek only enters the River as a result of discharges from the Dam, which only occurs when needed to prevent over-topping during heavy storms.

Reach 3 starts at Casitas Vista Road and ends less than a mile downstream at Weldon Canyon, near the northern boundary of the Ojai Valley Sanitary Plant. Flow is typically perennial in this reach due to the surfacing of the River upstream of Casitas Vista Road near Foster Park. Land use is predominantly agriculture and open space.

Reach 2 starts at Weldon Canyon and stretches south for approximately 4.5 miles to the Main Street Bridge. Land use in this reach includes agriculture and open space, as well as residential, institutional and industrial land uses from the community of West Ventura. The River is primarily perennial in this reach, mostly as a result of the Ojai Valley Sanitary Plant discharge which occurs upstream of the confluence with Cañada Larga. Cañada Larga drains mostly open space used for ranching and is dry during most of the year.

Reach 1 covers about 0.25 mile between the Main Street Bridge and the Ventura River Estuary. The lower boundary of this reach changes depending on the status of the sand berm at the mouth of the Estuary. Land use in this reach includes agriculture, open space, residential, and institutional. The Estuary and Reaches 1+2 have a history of homeless encampments, although efforts in recent years have largely discontinued this practice.

The Ventura River Estuary extends from the ocean to approximately 150 meters upstream of the railroad bridge based on tidal influence and whether the sand berm at the mouth of the Estuary is open or closed. The Estuary includes an open water area that is separated from the ocean by a berm that forms during the dry season. The berm is breached during storm events and slowly rebuilds through the summer, sometimes not fully building until August or September. The Estuary is flushed by tides when the berm is open and is dominated by slightly brackish to freshwater when the berm is closed. The Ventura River has intermittent direct discharge to the ocean; long shore transport of sand can cause a sand bar to form at the mouth of the estuary in the late summer and early fall.

2 MONITORING PROGRAM DESIGN

2.1 Sampling Locations

The TMDL requires that sampling locations must be adequate to assess beneficial use condition and attainment of applicable WQOs. To meet this requirement, sampling locations were selected with the following guidelines: presence of historic/ongoing monitoring, presence of flow, safe physical access, land use contribution, data logger suitability[1], sensitive species presence/absence (so as not to interfere with known populations), canopy, substrate, cobble areas (suitable for SPWN), avoidance of unauthorized interference, position relative to confluences, and proximity to other CMP monitoring sites.

[1] Considerations for data logger locations include reliable and ongoing access and retrieval, vandalism avoidance, and the manufacturer's criteria and specifications for installation.

Many segments of the Ventura River and its tributaries are non-perennial (including Ventura River above Reach 3, Cañada Larga, and San Antonio Creek). The exact time of drying depends on the location and environmental conditions, such as the intensity, duration, and frequency of rainfall events leading up to the monitoring date and the presence, length, and quantity of upwelling. Sites have been selected to maximize the likelihood of sampleable flow; however sites that are dry will be noted as such and will not be monitored during that sampling visit.

The selected monitoring sites are described below and fact sheets are included in Appendix A.

2.1.1 Estuary (TMDL-Est)

Macroalgal transect locations within the Estuary should be selected using the protocols listed in Appendix D, SOP 2, when applicable. The SOP is largely meant to be used in tidal estuaries, however the Ventura River Estuary is non-tidal when the sand berm is intact at the mouth of the estuary, and tidal when the sand berm is breached (e.g. as a result of storm activity/higher flows). This can result in changes to estuary shape, profile, and boundaries which limits pre-site selection. The Estuary was non-tidal at the time of CMP development due to consecutive low rainfall years. The selection of transect locations should consider flow regime, spatial variability in biomass and/or percent cover, data logger suitability, physical access, unauthorized interference, sensitive species presence/absence, and ongoing access permission. Modifications to the protocols for non-tidal transect location are described in Section 4.7.

2.1.2 Reach 1 (TMDL-R1)

The upstream boundary of the monitoring reach will be the Main Street Bridge. The downstream boundary will be 150 meters downstream of the bridge, or less if salinity from the Estuary is determined to be influencing the reach[2]. If salinity in the lower section of the monitoring reach is above 2.0[3] parts per thousand (ppt), then the monitoring reach should be shortened and the lower boundary should be at the lowest point in the reach with salinity less than 2.0 ppt.

2.1.3 Reach 2 (TMDL-R2)

The lower boundary of the monitoring reach is located at the Ojai Valley Sanitary District's R5 sampling site, directly above the confluence with Cañada Larga.

2.1.4 Cañada Larga (TMDL-CL)

The monitoring reach is located approximately 0.3 miles east of Highway 33 where Cañada Larga crosses beneath Cañada Larga Road. The monitoring reach starts south of the bridge and continues 150 meters upstream.

2.1.5 Reach 3 (TMDL-R3)

The lower boundary of the monitoring reach is located above the Ojai Valley Sanitary District's R3 sampling site, directly above the northern end of the first non-wadeable pool.

[2] The boundary between the Estuary and Reach 1 fluctuates depending on flow regime. Shortening the monitoring reach to exclude areas with salinity above 2.0 ppt helps ensure that the monitoring reach represents Reach 1 and not the Estuary.

[3] The highest level of salinity measured in Ventura River Watershed freshwater surface flows by the Ventura County Watershed Protection District is 1.1 ppt.

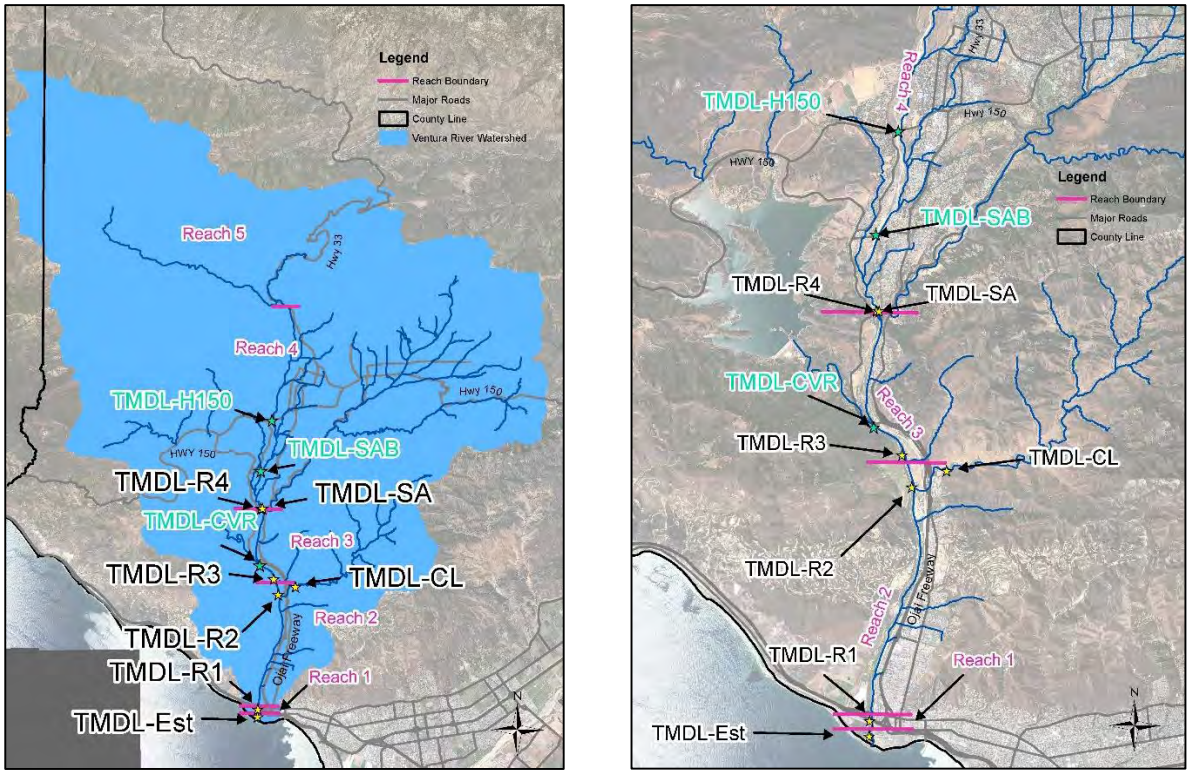
2.1.6 Reach 4 (TMDL-R4)

The lower boundary of the monitoring reach is located just above the confluence with San Antonio Creek.

2.1.7 San Antonio Creek (TMDL-SA)

The lower boundary of the monitoring reach is located just above the confluence with the Ventura River.

Figure 1. Sampling Sites and Flow Observation Locations



Note: Yellow site markers (black labels) are sampling locations. Blue site markers (blue labels) are flow observation locations.

Table 2. Sampling Locations

Reach/Tributary	Site ID	Site Location	Latitude	Longitude
Ventura River Estuary	TMDL-Est	Estuary	34°16'35.80"N	119°18'30.06"W
Ventura River Reach 1	TMDL-R1	Below Main Street Bridge	34°16'49.46"N	119°18'29.79"W
Ventura River Reach 2	TMDL-R2	Directly above confluence with Cañada Larga	34°20'16.33"N	119°17'48.28"W
Ventura River Reach 3	TMDL-R3	Above non-wadeable pool above OVSD-R3 site	34°20'44.51"N	119°17'58.93"W
Ventura River Reach 4	TMDL-R4	Directly above confluence with San Antonio Creek	34°22'51.55"N	119°18'28.26"W
Cañada Larga	TMDL-CL	0.3 miles east of Hwy 33, upstream of bridge	34°20'31.16"N	119°17'11.32"W
San Antonio Creek	TMDL-SA	Between Hwy 33 and confluence with Ventura River	34°22'51.19"N	119°18'25.25"W

Reach/Tributary	Site ID	Site Location	Latitude	Longitude
Field Duplicate	TMDL-FD	Collected concurrently at one of the above sites	TBD	TBD

2.2 Flow Observation Locations

Visual observations are to be recorded during each sampling event, documenting the status of the sand berm at the estuary, i.e. whether the estuary is open or closed, and the status of the river (flowing, ponded, or dry) at the locations listed in Table 3. Any wet/dry delineations and locations of groundwater upwelling should also be noted.

Table 3. Flow Observation Locations

Reach/Tributary	Site ID	Site Location	Latitude	Longitude
Ventura River Reach 3 + 4	TMDL-CVR	Ventura River at Casitas Vista Rd Bridge	34°21'8.85"N	119°18'28.98"W
Ventura River Reach 4	TMDL-SAB	Ventura River at Santa Ana Blvd Bridge	34°23'58.47"N	119°18'29.75"W
Ventura River Reach 4	TMDL-H150	Ventura River at Highway 150 (Baldwin Rd) Bridge	34°25'30.10"N	119°18'8.87"W

2.3 Analytical Parameters

Analytical parameters that are to be measured at all sampling locations include flow, nutrients (total and dissolved nitrogen, total and dissolved phosphorus, and nitrate plus nitrite as nitrogen), *in situ* water quality parameters (pH, temperature, electrical conductivity, and dissolved oxygen (DO)), algal percent cover, and algal biomass (chlorophyll *a*). The collection method and sample matrix for chlorophyll *a* is different for the River and tributaries (total algal biomass from algae sample) and the Estuary (phytoplankton biomass from water column sample).

2.4 Cofactor Observations

Observations of cofactors should be recorded on the field data sheets, including percent cover (shade), weather conditions (sun/wind/temperature), wet/dry delineations, ponding, and locations of groundwater upwelling.

2.5 Analytical Methods

SWAMP does not mandate specific analytical methods for field or laboratory use but has adopted a performance-based approach to promote comparability, including the use of measurement quality objectives (Section 3) and the application of reporting limits (Table 10) that are universal to all program participants. A reporting limit (RL) is the lowest concentration at which an analyte can be detected in a sample and its concentration can be reported with a reasonable degree of accuracy and precision. A method detection limit (MDL) is the minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero. Sample results below the MDL are considered "Not Detected" or "ND". Sample results between the MDL and the RL are considered "Detected Not Quantified" or "DNQ". The RLs for this project are listed in Table 10 and reflect this project's objectives.

2.6 Sampling Schedule

In the TMDL, the Regional Board specified that the sampling frequency must be adequate to assess beneficial use condition and attainment of applicable WQOs.

In compliance with the TMDL, algal biomass and percent cover will be monitored once per month in the dry season (May 1st to September 30th). After two years, if a significant difference between monthly algal biomass measurements is not observed, algal biomass monitoring may be reduced to three times per dry season, during the months of May, July, and September.

DO and pH shall be measured continuously for two week periods on a quarterly basis, including the months of May[4] (2nd quarter) and September[2] (3rd quarter). February and November may be targeted for monitoring during the 1st and 4th quarters, respectively. All other parameters shall be monitored monthly.

The TMDL states that "nutrients are loaded from the watershed to the Ventura River and Estuary in both dry and wet weather, but the nutrients loaded in the dry season are predominately responsible for the algae, eutrophic conditions, and nutrient impairments in the Ventura River and Estuary." Therefore, dry weather is targeted for monitoring in this CMP and scheduled sampling events should be postponed for at least three days following significant (>0.1" in 24 hours) amounts of rainfall.

Table 4. Sampling Schedule

Type of Analysis	Sampling Period	Sampling Frequency
Algal biomass and percent cover	Dry Season (May 1 – Sep 30)	Monthly for first two years; after first two years, if criteria met, then May, Jul, Sep
DO and pH	February*, May, September, November*	Quarterly two week continuous monitoring
Nutrients (TP, TDP, TN, TDN, NO3+NO2-N)	Jan 1 – Dec 31	Monthly
In situ parameters (EC, Temp, DO)	Jan 1 – Dec 31	Monthly
Flow Measurements	Jan 1 – Dec 31	Monthly

* February and November may be targeted to complete the 1st and 2nd quarter sampling events

Sampling will begin no later than 90 days after the Regional Board staff approves the Final Comprehensive Monitoring Plan. Receiving water monitoring shall continue beyond the final implementation date of the TMDL unless the Regional Board Executive Officer approves a reduction or elimination of such monitoring.

2.7 Sampling Protocols

As required by the TMDL, monitoring procedures/methods, analysis, and quality assurance will be Surface Water Ambient Monitoring Program (SWAMP) comparable, where appropriate. River indicators shall be averaged over a monitoring reach as described in the SWAMP monitoring protocol -

[4] The TMDL requires that continuous monitoring of DO and pH shall occur during the months of May and September in the 2nd and 3rd quarters.

Bioassessment SOP 02 (SOP 02) in Appendix D (SOP 1). Estuary macroalgal cover is to be measured using three transects and evaluating percent cover at 10 random points along each transect as described in the Southern California Coastal Water Research Project (SCCWRP) Estuarine Eutrophication Assessment Field Operations Manual (Estuarine Manual) used for the Southern California Bight 2008 Regional Monitoring Program: Estuarine Eutrophication Assessment project. A customized version of the Estuarine Manual is included in Appendix D (SOP 2).

The complete SOP 02 and Estuarine Manual contain components/measurements outside of the scope of this CMP. Per SOP 02, these components may be omitted if not needed for a project. Since stream and estuary productivity in terms of algae is the primary concern of the assessment, the relevant river indicators incorporated into this CMP are biomass samples (chlorophyll *a* analysis), macroalgal cover (algal cover point-intercept data), shade (canopy cover), and water width and depth.

2.8 Changes to the Sampling Design and Schedule

Changes to sampling locations may be required due to circumstances that are beyond the control of the Responsible Parties (Section 1.2). Such circumstances include a change in access permission status, changes in stream or estuary position (e.g. following storms or water releases), and changes in flow regime. In the event that a site needs to be relocated, the Responsible Parties will notify Regional Board staff and propose a site replacement strategy.

If a monitoring site is completely dry during a monthly visit, then it will be noted on the field data form and photo documentation of the dry conditions will be collected at the lower, middle, and upper boundaries of the monitoring reach. Algae, water quality, and other monitoring data will not be collected. If water is present but ponded or present in only part of the reach, then it will be noted on the field data sheet and the monitoring reach will be evaluated as described in Section 4.3. If no more than three transects are dry, monitoring samples will be collected as scheduled.

The protocols for algae sampling (SOP 02 and the Estuarine Manual) state that (algae) 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. Since monitoring of algae under this CMP occurs in the dry season as defined by the TMDL (May 1st through September 30th), it is unlikely that high-velocity storm flows will occur during the monitoring period. However, should a storm occur with sufficient velocity to remove macroalgae and biofilms from the stream bottom, then sampling should continue as planned (to track the recolonization process) but the date and size of the storm should be recorded on the field data sheet.

2.9 Permits

Right of entry permits will be obtained by the Responsible Parties or their designee for sampling for each location. California Department of Fish and Wildlife scientific collection permits will be obtained for each field team and a U.S. Fish and Wildlife Service recovery permit will be obtained for the group, as needed.

2.10 Data Management

Completed COCs, field data sheets, and laboratory reports will be maintained by the monitoring consultant and provided to the Responsible Parties after the end of each monitoring season as shown in Table 5, or as decided by mutual agreement between the Responsible Parties and monitoring consultant.

Table 5. Monitoring Data Provisional Due Dates

Monitoring Season	Data Due Date
Dry Season (May – September)	December 31st (Annually)
Wet Season (October – April)	July 31st (Annually)

3 DATA QUALITY OBJECTIVES

Per the TMDL, the monitoring procedures/methods, analysis, and quality assurance shall be SWAMP comparable, where appropriate. The SWAMP 2013 Guidelines for quality control are provide in Table 6, Table 7, and Table 8 below.

3.1 Field Measurements DQO

Table 6. SWAMP 2013 Guidelines Quality Control[5]: 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[1]	Post-Sampling Calibration Check Frequency[1]	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 ≥500m or barometric pressure >2mmHg)	After every monitoring day (within 24 hours)	±0.5 or 10%
pH	Electrode	pH	0.01	±0.2	2	PM	PM	±0.2
Specific Conductance	Conductivity cell	µS/cm[2]	1	±0.5%	PM	PM	PM	±10%
Temperature	Thermistor or bulb	°C	0.1	±0.15	PM	PM	PM	±0.5
Velocity	Flow meter[5]	Ft/s	0.1	PM	PM	PM[6]	PM[6]	PM

PM Per Manufacturer

[1] 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.

[2] mS/cm for marine water

[3] Unit or percentage, whichever is greater

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

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

[5] Unless manufacturer specifies more stringent requirements.

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.

3.2 Laboratory Analysis DQO

Nutrients: Nitrate + Nitrite as nitrogen, Total and Dissolved Nitrogen, Total and Dissolved Phosphorus

Conventionals: Chlorophyll *a*

Table 7. SWAMP 2013 Guidelines Quality Control[6] : 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

Table 8. SWAMP 2013 Guidelines Quality Control[6]: Conventionals 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

[6] Unless method specifies more stringent requirements.

Laboratory Quality Control	Frequency of Analysis	Measurement Quality Objective
Reference Material	Per 20 samples or per analytical batch, whichever is more frequent	80-120% recovery
Laboratory Duplicate	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 Blank, Travel Blank, Equipment Blank	Per method	<RL for target analyte

If samples do not meet the applicable criteria listed in Table 7/Table 8, then the associated recommended corrective action (Table 9) should be implemented.

Table 9. SWAMP 2013 Quality Control Recommended Corrective Actions

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. 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.

Laboratory Quality Control	Recommended Corrective Action
	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.

3.3 Field and Laboratory Reporting Limits

Targeted reporting limits for this project are provided in Table 10 below. SWAMP recommended reporting limits are also provided for comparison.

Table 10. Reporting Limits

Water Quality Parameter	Project Reporting Limit	SWAMP Recommended Reporting Limit
Dissolved Oxygen	0.2 mg/L	Not listed
pH	Not Applicable	Not listed
Specific Conductance	10 µS/cm	2.5 µS/cm
Temperature	0 °C	Not listed
Flow	Not Applicable	Not listed
Chlorophyll *a*	0.002 mg/L	0.002 mg/L
Algal Percent Cover	Not Applicable	Not listed
Total and Dissolved Nitrogen	0.1 mg/L	Not listed
Total and Dissolved Phosphorus	0.01 mg/L	Not listed
Nitrate + Nitrite as Nitrogen	0.1 mg/L	0.1 mg/L

4 FIELD METHODS AND PROCEDURES

4.1 Health and Safety

The safety of field staff is of primary importance. Field staff must remain vigilant at each site to ensure that potential hazards are avoided or mitigated. Potential hazards include uneven and slippery surfaces, hot and cold weather, vehicular traffic, homeless individuals and encampments, chemicals (sample preservatives), plants and animals (e.g. poison oak, stinging nettle, venomous snakes, insects etc.), viruses and bacteria. Sampling crews should always consist of a minimum of two people. If for any reason sample collection appears to be unsafe, do not attempt to sample. Leave the area, notify the relevant authorities if necessary, and document the reason/incident. If possible, samples should be collected at a later time when conditions are safe. A throw rope should be easily accessible in each sampling vehicle and at the ready during grab sample collection if conditions warrant it. First aid manuals should be kept in each sampling vehicle. Prevention and treatment facts for some common field hazards are included in Appendix E (National Institute for Occupational Safety and Health (NIOSH) Fast Facts).

General: Wear a reflective safety vest in high-traffic areas. Use equipment properly. Wear gloves when handling samples and sampling equipment, and ensure sample preservatives do not come into contact with skin or clothing. Clean hands frequently using hand sanitizer or soap and water, especially after sampling and before eating, drinking, or smoking. Waterproof waders or boots should be worn during sample collection to minimize contact with water. Gloves should be worn when handling sample bottles, especially those containing preservatives. Field staff should be aware of current recommended guidelines for lifting and carrying items prior to going into the field (such as those in the *Ergonomic Guidelines for Manual Material Handling* produced by the California Department of Industrial Relations, http://www.cdc.gov/niosh/docs/2007-131/pdfs/2007-131.pdf).

Slips/Trips/Falls: Be aware that algae, mud, and water can be slippery to walk on. Sampling in the Ventura River Watershed frequently involves traversing rocky and uneven terrain. Be sure that the surface is stable and not slippery prior to placing weight on it.

Hot/Cold Weather (Appendix E): Dress in layers appropriate for the weather conditions. Brimmed hats, sunscreen, and long sleeves should be worn to protect skin from sun damage. Long sleeves also provide some protection from insect bites and poison oak. Stay hydrated. Be on the lookout for symptoms of heat or cold stress. If symptoms develop, apply first aid and seek professional assistance, if necessary.

Plants and animals (Appendix E): Do not come into contact with poison oak. It is known to be present in riparian and estuarine areas in the Ventura River Watershed. Oils from poison oak are present in the leaves and stems of the plant and can remain active for up to five years after the death of a plant. Take care when traveling through potential snake and insect habitats.

Homeless Persons and Encampments: Treat all individuals in a professional and courteous manner. Use discretion in all interactions and if at any time field staff feel uncomfortable or in danger, activities must immediately cease and all staff must return to a safe location and notify the appropriate authorities.

4.2 Preparation for Sampling

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 D (SOP 3) for additional information.

• Use the equipment checklist provided in Appendix C to make sure all necessary supplies are brought along.

• Check with contract lab on sampling containers, and shipping and storage of samples.

• Prepare, and double check, site dossiers to make sure they are complete with maps and directions to sites. Bring along county maps, atlases, or Thomas Guides to further aid location of sites. Also bring business cards, and any site access permits, passes, and/or keys, as needed (and be aware that some landowners may require notice prior to each site visit).

4.3 At the Site

Park the vehicle in a safe spot where there are no "No Parking" signs. Stick a business card with cell phone number in the driver's window. 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 monitoring 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 monitoring reach, strive for as few dry transects as possible (and preferably none). Sites should be safe to sample, wadeable, and legally accessible.

4.4 Sample Types (water + algae, estuary/tributary etc.)

The protocol for the collection of nutrient and *in situ* parameter data is the same for the river, tributaries, and estuary. Chlorophyll *a* is used in the TMDL to determine phytoplankton biomass in the estuary (Estuarine Manual protocols) and total algal biomass in the river and tributaries (SOP 02 protocols). Estuarine phytoplankton biomass is measured from a water column sample as described in the Estuarine Manual. River and tributary total algal biomass is measured from macroalgae samples as described in SOP 02. Site type, parameters, and sample matrix are shown in Table 11.

Table 11. Sample Type and Field Preparation

Site Type	Sample Type	Analysis	Sample Field Preparation
Estuary	Water – ambient	*in situ* parameters (DO, pH, EC, temp)	N/A
Estuary	Water – grab	Nutrients (total)	N/A
Estuary	Water – grab	Nutrients (dissolved)	Field Filtered
Estuary	Water – grab	Chlorophyll *a*	Field Filtered
River or Tributary	Water – ambient	*in situ* parameters (DO, pH, EC, temp)	N/A
River or Tributary	Water – grab	Nutrients (total)	N/A

Site Type	Sample Type	Analysis	Sample Field Preparation
River or Tributary	Water – grab	Nutrients (dissolved)	Field Filtered
River or Tributary	Algae – composite	Chlorophyll *a*	Field Filtered

4.5 Sampling Parameters

A list of sampling parameters is presented above in Section 2.3. The methods selected for the analysis of the samples are at the discretion of the responsible parties in consultation with the monitoring staff, but should aim to meet the measurement quality objectives listed in Section 3.

4.6 River and Tributary Algae and Water Quality Assessment

SOP 02 is a detailed SOP for conducting a complete physical habitat and algal assessment of a monitoring reach. This SOP is referenced in the TMDL for macroalgal cover and total algal biomass comparisons with TMDL numeric targets. Therefore, there are sections of SOP 02 that are not relevant for this CMP. Sampling should follow the protocols listed in SOP 02 Sections 3 – 8, with the following changes:

Reach Delineation (Section 3):

1. Customized field forms have been created for this CMP (Appendix B) and should be used in place of those included in SOP 02.
2. Section 3.3: Ignore dominant land use as a notable field condition.
3. Section 3.4: The water quality parameters listed in Section 2.2 of this CMP should be used instead of those listed in SOP 02.

Reachwide Benthos Sampling of Algae (Section 4):

1. Parameters: Only chlorophyll *a* and percent macroalgal cover are relevant for this CMP, all other parameters/measurements should be omitted.
2. Relevant sections: 4.1, 4.2 (omit BMI), and 4.3 (quantitative only[7]).

Algal Sample Processing (Section 5)

1. Chlorophyll *a* is the only parameter of interest in Section 5. If no macroalgal clumps are present prepare chlorophyll *a* samples as described in SOP 02 Section 5.1, 5.2 and 5.2.4; or if macroalgal clumps are present prepare chlorophyll *a* samples as described in SOP 02 Section 5.1, 5.3 and 5.3.5.
2. Section 5.5: Follow instructions relevant to chlorophyll *a* biomass QA/QC.
3. The rest of SOP 02 Section 5 does not apply to this CMP.

Physical Habitat Transect-based Measurements to Accompany Algal Bioassessment (Section 6)

1. Relevant parts of Section 6 are 6.1 (wetted width), 6.5 (depth), 6.9 (algal cover), 6.11 (dry substrates), and 6.14 (densiometer readings – canopy cover).

[7] The TMDL requires the collection of algae for determining chlorophyll *a* as a measure of biomass. Taxonomic identification of algae is not required to meet the intent of the TMDL and this CMP.

2. Non-relevant sections are 6.2 (bankfull width), 6.3 (bankfull height), 6.4 (pebble count), 6.6 (particle size class), 6.7 (cobble embeddedness), 6.8 (CPOM), 6.10 (macrophytes), 6.12 (bank stability), and 6.13 (human influence).

Physical Habitat Inter-transect-based Measurements to Accompany Algal Bioassessment (Section 7)

1. Relevant parts of Section 7 are 7.1 (wetted width), 7.2 (percent algal cover only), and 7.4 (photographs – which should be in Section 6).
2. Non-relevant sections are 7.2 (substrates only) and 7.3 (flow habitats).

Reachwide Measurements (Section 8)

1. A gradient less than 1% can be assumed for all sampling sites listed in this CMP and does not need to be measured. The remainder of Section 8, stream discharge measurements (flow) should be taken as described.

4.7 Estuary Algae Assessment

The Estuarine Manual is referenced in the TMDL for macroalgal cover and phytoplankton biomass comparisons with TMDL numeric targets for the Estuary. It is designed to be used on intertidal channels and mudflats, and includes assessments and analysis that are outside of the scope of this CMP. Only the analysis for phytoplankton chlorophyll *a* and macroalgal transect evaluations are relevant for this CMP (Section VI).

Macroalgae percent cover and water column phytoplankton will be measured in three transects within the Estuary. In general, the location of the primary producer transects will be driven by the goal of capturing spatial variability in biomass and/or percent cover and be spaced throughout the system along spatial gradients (e.g. salinity/hydrology, grain size, and nutrient loading). It is possible for the observed gradients in these three variables to co-occur or be diametrically opposed. Optimally, the transects will be located in the same area of the estuary. Transect locations may need to be modified as Estuary size, depth, shape, and boundaries are dependent of flow regime (e.g. flow quantity, tide, and berm status). When the Estuary is non-tidal and the transects cannot be located according to the guidance in the Estuarine Manual, the procedures may need to be modified, e.g. locate transects at the waterline in areas of major algal accumulation. Site-specific considerations may cause a deviation from this general guidance and will be noted when this occurs.

When the berm is open, sampling should be scheduled around the low tide during daylight spring tides[8] (when the maximum amount of mudflat area is exposed). When the berm is closed and the estuary is non-tidal, sampling does not need to be scheduled around the tides.

Site measurements should follow the Estuarine Manual Section VI, part C.

4.8 QA/QC Field Samples

Field duplicates: Field duplicates shall be collected for 5% of the total number of project samples for water chemistry samples, or once per year and at 10 % of sites for chlorophyll *a*, as recommended in SOP 02. For the estuary, this equates to once every two years and should be scheduled in advance, e.g. June of odd years (e.g. 2015, 2017, 2019 etc.). For the river and tributaries, this translates to once per year and

[8] The exceptionally high and low tides that occur at the time of the new moon and the full moon.

should rotate between the sites in numerical order, e.g. TMDL-R1 in 2015, TMDL-R2 in 2016 etc., however if a site is dry, then the next site on the list should be substituted for that year.

4.9 Flow Observation

For each monitoring event (monthly), in addition to the visits to the monitoring sites, field staff will also visit the flow observation locations listed in Table 3 and document the presence/absence and quantity of flow (photograph site upstream and downstream and record observations/measurements on field data sheet).

4.10 Monitoring Stations

Per SOP 2, if the flow of water in the monitoring reach is split into two channels with an intervening (stable) island, then the transect should be placed across the dominant channel, and all samples should be collected from that channel. Stable islands are rarely, if ever inundated and so are typically vegetated, often with woody shrubs or trees, and have heights near or exceeding bankfull height. If the channels are not separated by an intervening island, then the transect should be placed to include both channels and samples collected accordingly.

4.11 Aquatic Invasive Species (AIS)

Known AIS in Ventura County include quagga mussels and New Zealand mud snails, although neither has been detected in the Ventura River Watershed at the time of writing this CMP. Care must be taken to prevent their introduction and spread. California Department of Fish and Wildlife protocols to prevent the spread of AIS are provided in Appendix D (SOP 3). Protocols should be updated as new information becomes available, or other invasive species are discovered in Ventura County.

4.12 Data Loggers

Data loggers will be installed at the seven monitoring sites for a two week period each quarter. Placement and timing of installation will consider reliable access, minimalize vandalism/theft potential, water depth (to keep the data logger wet), and changes in flow (e.g. due to stormwater runoff or drying out) in order to minimize the potential for interference and loss of data/data loggers. Use of existing support structures (e.g. bridge abutments, rail road trestles, etc.) will also be explored. Data loggers should be deployed at the bottom of the water column. The location of the continuous monitoring station should be recorded with a GPS unit and every effort should be made to install the data logger in the same location for each monitoring period. The same brand/model of data logger calibrated using the same calibration standards (if applicable) should be used at all sites to ensure that data is directly comparable. Data loggers should be programmed with the correct date and time prior to deployment, if applicable. Calibration of loggers shall be performed per manufacturer's specifications and records of calibration are to be kept in a log book.

4.13 Sample Bottles and Labels

The laboratory will supply appropriate sampling bottles to the monitoring crew, who will prepare the labels and apply them to the sample bottles, as needed. Each label/bottle will include the following information:

1. Project name: "VR Algae TMDL";

2. Sample ID: See Table 2;

3. Date and Time sample collected (to be completed by the monitoring crew in the field); and

4. Initials of individuals who collected the sample.

Each crew will be supplied with the bottles necessary to collect the samples at each site prior to the day of sampling. A waterproof marker should be used to make sure information on the label does not become distorted. Label information must also be entered on the chain of custody (COC), as described in Section 4.14.2. Table 12 details the types of containers, preservation, and holding time requirements for all analyses.

Table 12. Sample Containers, Preservatives, and Hold Times[9]

Parameter	Matrix/ Sample Type	Sample Volume	Sample Container	Preservation	Preferred/Maximum Holding Times
Dissolved Oxygen	Water *in situ*	NA	Sample directly with meter	None	Immediately
pH	Water *in situ*	NA	Sample directly with meter	None	Immediately
Specific Conductance	Water *in situ*	NA	Sample directly with meter	None	Immediately
Temperature	Water *in situ*	NA	Sample directly with meter	None	Immediately
Flow	Water *in situ*	NA	Sample directly with meter	None	Immediately
Algal Percent Cover	Algae *in situ*	NA	NA	NA	NA
Chlorophyll *a*	Algae grab	Varies	Glass fiber filter, foil wrapped (dark)	Freeze within 4 hours	Analyze within 28 days
Chlorophyll *a*	Water grab	2 x 1 L	Amber Poly → Glass fiber filter, foil wrapped (dark)	Freeze within 4 hours	Analyze within 28 days
Total Nitrogen	Water grab	500 ml	Polyethylene Bottle	Cool to ≤6 °C, H_2SO_4 to pH<2	28 days
Dissolved Nitrogen	Water grab	500 ml	Polyethylene Bottle	Filter within 15 minutes of collection; cool to ≤6 °C; H_2SO_4 to pH<2	28 days
Total Phosphorus	Water grab	500 ml	Polyethylene Bottle	Cool to ≤6 °C, H_2SO_4 to pH<2	28 days
Dissolved Phosphorus	Water grab	250 ml	Polyethylene Bottle	Filter within 15 minutes of collection; cool to ≤6 °C; H_2SO_4 to pH<2	28 days
Nitrate + Nitrite as Nitrogen	Water grab	250 ml	Polyethylene Bottle	Cool to ≤6 °C, H_2SO_4 to pH<2	48 hours/ 28 days if acidified

[9] Or as directed by laboratory.

4.14 Field Forms

An example Chain of Custody (COC) form and field data sheets are provided in Appendix B.

4.14.1 Monitoring Field Data Sheets

Field data sheets will be completed at each sample location for each event. Field data sheets ensure that the required data is collected for this project and that it is SWAMP comparable, where applicable. Digital photographs will also be taken at each site, showing the actual sample collection point, as well as conditions upstream and downstream of the sampling site. Each crew will complete the field data sheets and turn them in to the Field Team Leader.

4.14.2 Chain of Custody Forms

Chain of Custody forms (COC) will be supplied by the laboratory or created in-house. The COC will be filled out with the following information for each event:

1. Contact Person and Telephone Numbers;

2. Name of Study (Ventura River Algae TMDL);

3. Analyses to be performed;

4. Type of sample collected, e.g. matrix "Water" or “Algae”;

5. Number of bottles per sample and preservatives used (per bottles provided by the analytical laboratory);

6. Sample number;

7. Date and time sample collected (military time); and

8. Name of sampling staff and signature.

The COC should be kept in a dry location or in a re-sealable plastic bag to prevent smearing and water damage to the form. COC protocols will be followed as samples are transferred to the lab. This may require multiple signatures (i.e. field crew to field team leader to courier).

4.15 Sample Handling and Transport

All samples will be placed on wet ice or frozen ice packs immediately after collection. Identification information for each sample will be recorded on the field data sheets and COC forms.

Samples that are not processed immediately in the field will be labeled with the project name, sample ID, date and time of collection, sampler's name, and method used to preserve sample (if any). Samples will be handled, prepared, transported, and stored in a manner so as to minimize loss, misidentification, contamination, and/or degradation. Samples will be shipped on ice and in insulated containers (e.g., insulated cooler). All caps and lids will be checked for tightness prior to shipping. Efforts will be taken to minimize the leakage of any melted ice from the sample shipment container. It is assumed that samples in a sealed cooler are secure regardless of method of transportation to the selected analytical laboratory. Sample packaging will include the following steps:

1. Glass sample containers will be wrapped with plastic insulating material to prevent contact with other sample containers and the inner walls of the cooler;
2. Ice and/or reusable "blue ice" packs will be placed in the cooler with the samples to maintain the samples at 4° C during shipment;
3. The COC record will be enclosed in a waterproof plastic bag and taped to the underside of the cooler lid, or carried by the courier, if applicable;
4. Samples will be packaged in thermally insulated, rigid coolers.
5. If shipping a cooler by commercial carrier, such as Federal Express, all coolers should be shipped "Priority Overnight" and air bills will be completed and attached to the exterior lids of the containers. COC should be enclosed in a waterproof plastic bag and taped to the underside of the cooler lid. The cooler should be taped securely shut and efforts should be taken to prevent leakage of the cooler (e.g. wrap ice in plastic bags).

The collected samples are to be delivered to the laboratory for analyses as soon as practicable and within holding times of samples. Any delay in the receipt of the samples by the laboratory could necessitate a re-sampling and analysis effort.

At the end of the sampling activities, each crew will deliver the samples for chemical analyses with the respective COC forms to the laboratory, or coordinate with a reliable courier for sample pick up.

On receipt of the sample(s) at the laboratory, laboratory personnel will open the container and perform an initial inspection of the contents to check for evidence of breakage and/or leakage. The container will be inspected for COC documents and any other information or instructions. The sample custodian will verify that all information on the sample bottle labels is correct and in accordance with the COC documents and will sign for receipt. If discrepancies are noted between the COC and the sample labels, the project contact will be notified immediately. Contract laboratories will follow the sample custody procedures outlined in their QA plans. These QA plans are on file at the laboratory. All samples will be stored in a refrigerated, secure area. Samples will be removed from storage as needed by the analyst.

4.16 Site Issues (Inaccessibility, Dry etc.)

Site inaccessibility may be an issue for stream sites, under various circumstances described below. All instances should be documented on field data sheets.

1. If it would be dangerous to approach the stream during a sampling event due to swift water or other hazardous conditions, it is considered inaccessible. In this event, the sampling team will delay sampling until conditions are deemed safe. Length of delay will depend on site conditions. In the event that a site is considered inaccessible, the field team will take photographs as documentation of the site condition. Sampling events should be postponed for at least three days after significant (>0.1”) amounts of rainfall.

2. If sampling sites are temporarily or permanently blocked by a physical obstruction, such as downed trees or evidence of land-or rockslide, or ice or snow, the sampling team may move 25-50 ft. upstream or downstream from the site and conduct sampling there. If there still is no suitable access, the project team may discuss the possibility of sampling further away (up to 100 ft.) from the original station with the project manager, who will determine whether to approve the change.

3. If the sampling site comes under new ownership, such that previously granted access is denied, then permission will be requested from the new owner. If this is still denied, a permanent new location will be

<selected as near as possible to the original station, ideally within 1,000 feet, and not in a morphologically different stream reach.

Depending on flow status, tributary sampling may be more difficult to re-schedule at certain times of the year. A target of 90 percent completeness is reasonable as it accounts for the possibility of adverse weather conditions, safety concerns, equipment problems and access issues, however sites that go dry in the summer will be unlikely to meet this goal. In the event that a station is relocated or moved to facilitate sampling, field crews will record the GPS coordinates of the new location. This information will be recorded on the field form.

5 QUALITY ASSURANCE/QUALITY CONTROL

This monitoring plan describes procedures to collect representative water samples from the named reaches in the TMDL. The laboratory/laboratories must be Environmental Laboratory Accreditation Program (ELAP) certified for the relevant analyses and follow quality assurance and quality control programs in accordance with guidelines established by the State of California and the U.S. EPA.

Data will be compared to the data quality objectives in Section 3. Data outside of the data quality objectives will be flagged accordingly. Field duplicates will be collected according to the schedule provided in Section 4.8 and analyzed blind by the analytical laboratory. Sampling equipment which is reused should be decontaminated between samples.

Ventura River Estuary: TMDL-Est

Waterbody: Ventura River Estuary

Location: Varies depending on tides. The Ventura River Estuary upper boundary varies depending on whether the sand berm at the mouth of the estuary is open or closed. Site should be located as described in the relevant estuary protocol. General location of estuary is (34°16'35.80"N, 119°18'30.06"W)

Pros: N/A

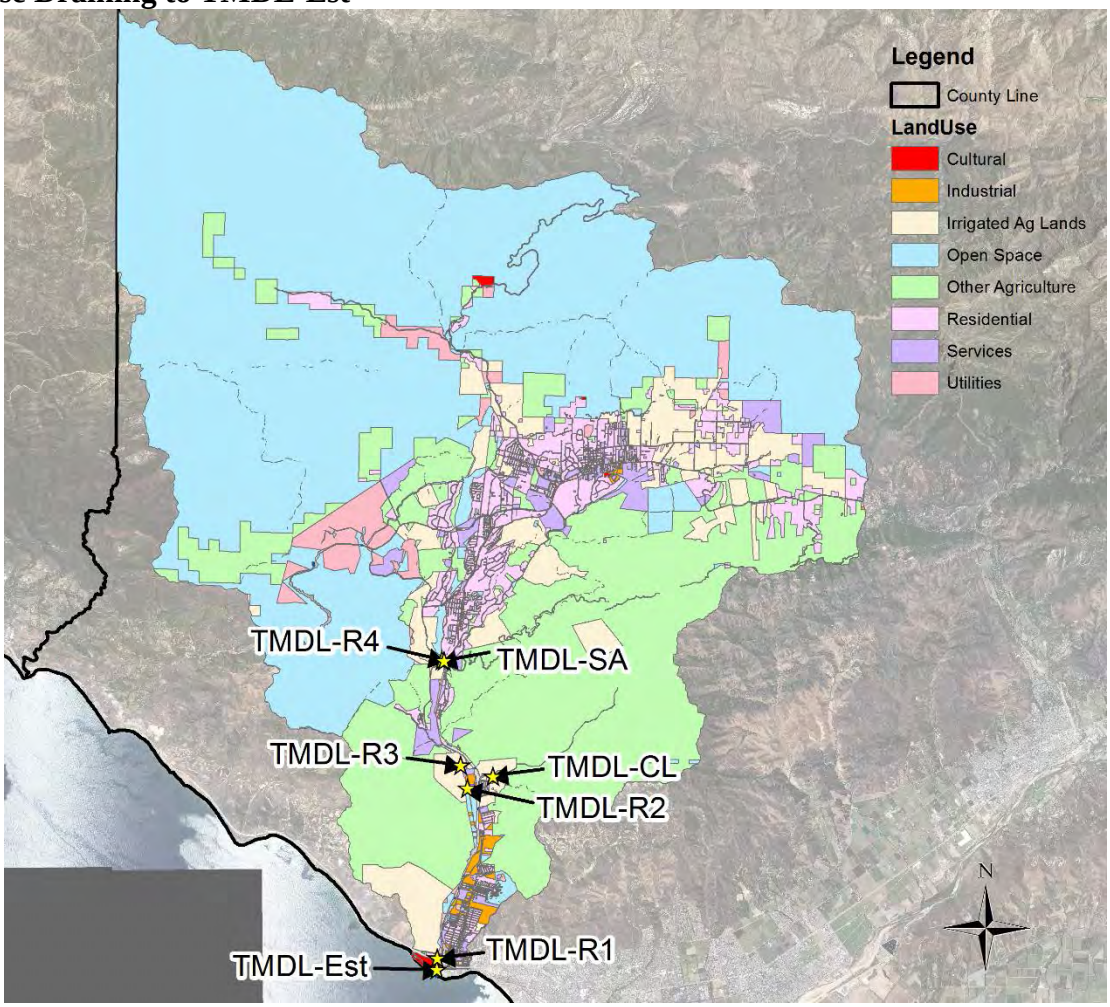
Cons: Site required by TMDL but has high potential for vandalism

Outstanding Site Selection Tasks: Landowner permission, transect location selection

Dry Season Flow Potential: Perennial

Ventura River Estuary: TMDL-Est

Land Use Draining to TMDL-Est



Land Use	Acres
Open Space	76280
Other Agriculture	37318
Irrigated Ag Land	9961
Residential	8346
Transportation	3165
Services	3050
Industrial	515
Cultural	153

Ventura River Reach 1: TMDL-R1

Waterbody: Ventura River

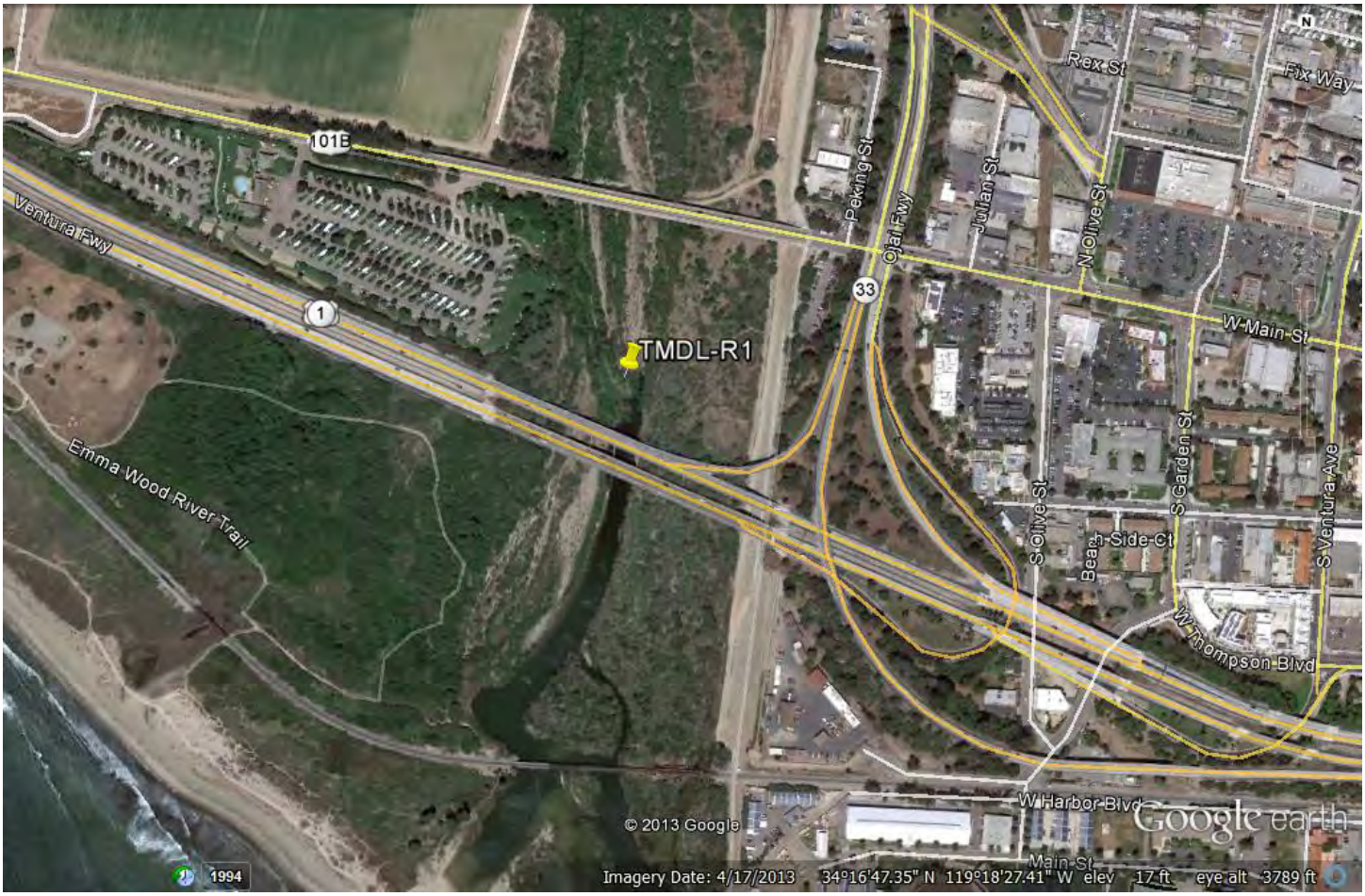
Location: Directly south of Main Street and above the Ventura River Estuary (34°16'49.46"N, 119°18'29.79"W)

Pros: Perennial, least likely location within reach to be influenced by estuary (i.e. salinity) during both tidal and non-tidal states

Cons: High potential for vandalism

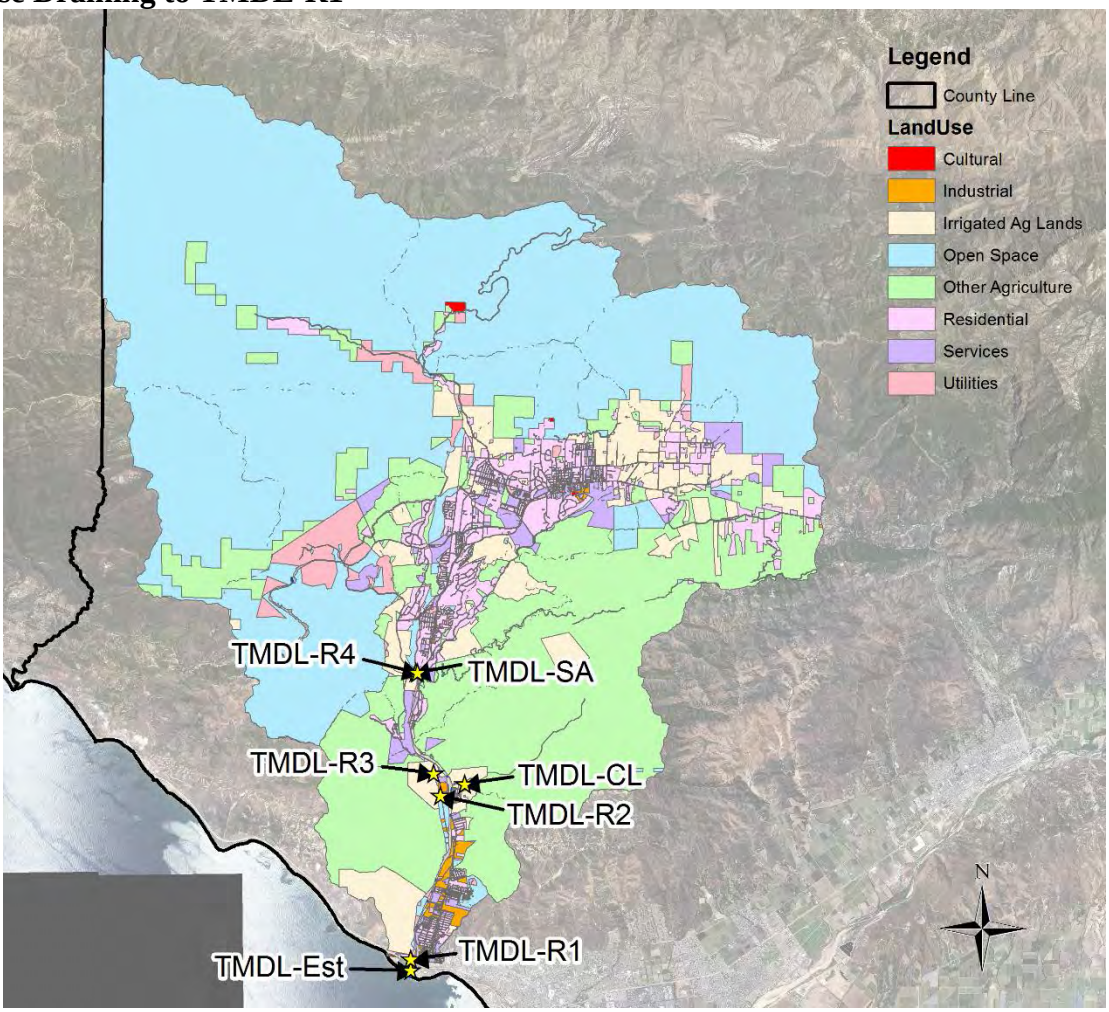
Outstanding Site Selection Tasks: Landowner permission

Dry Season Flow Potential: Perennial



Ventura River Reach 1: TMDL-R1

Land Use Draining to TMDL-R1



Land Use	Acres
Open Space	76271
Other Agriculture	37315
Irrigated Ag Land	9961
Residential	8336
Transportation	3146
Services	3040
Industrial	512
Cultural	78

Ventura River Reach 2: TMDL-R2

Waterbody: Ventura River

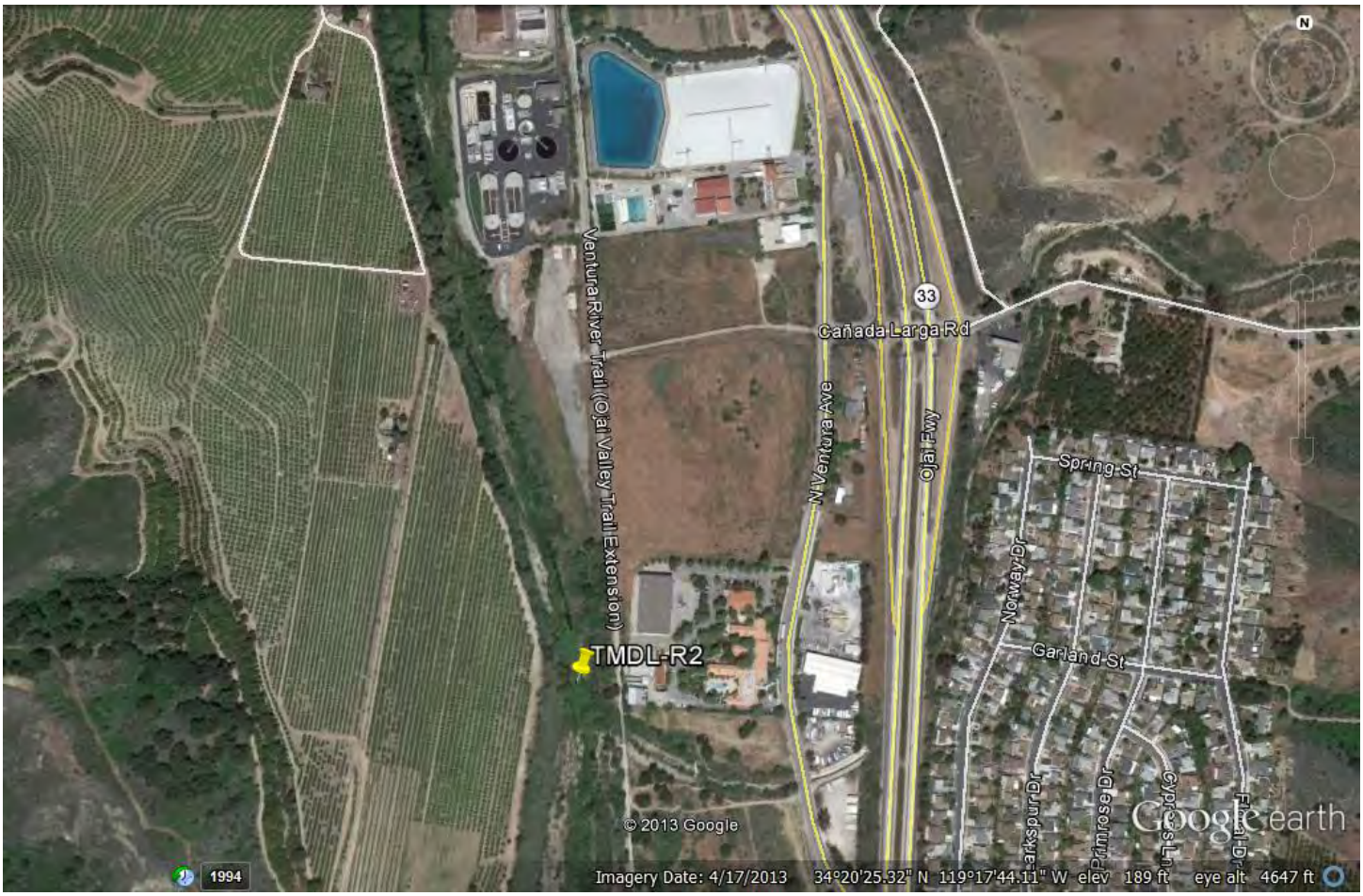
Location: Directly above the confluence with Cañada Larga (34°20'16.33"N, 119°17'48.28"W).

Pros: Perennial, co-located with Ojai Valley Sanitary District "R5" monitoring site

Cons:

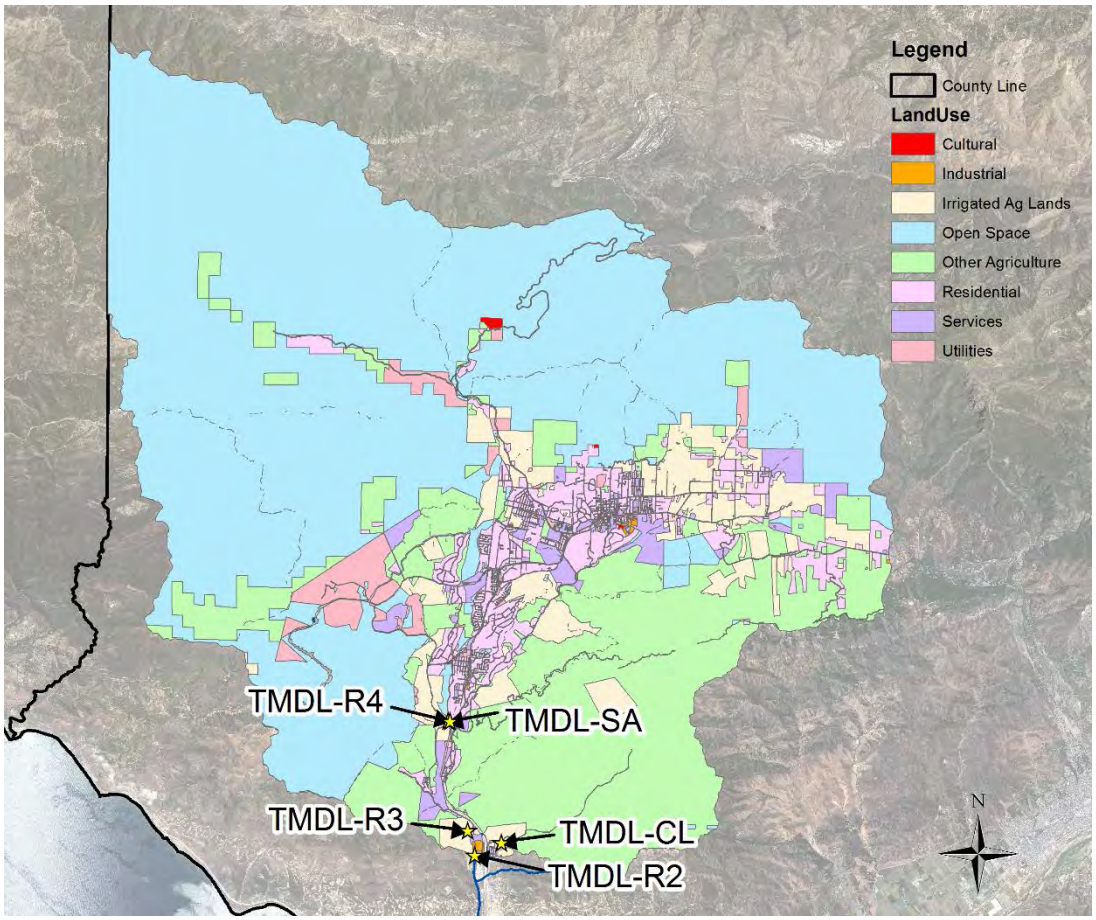
Outstanding Site Selection Tasks: Landowner permission

Dry Season Flow Potential: Perennial



Ventura River Reach 2: TMDL-R2

Land Use Draining to TMDL-R2



Land Use	Acres
Open Space	75864
Other Agriculture	31049
Irrigated Ag Land	8372
Residential	7806
Transportation	3022
Services	2806
Industrial	82
Cultural	72

Ventura River Reach 2 (Cañada Larga): TMDL-CL

Waterbody: Cañada Larga

Location: Approximately 0.3 miles east of Highway 33, on the upstream side of the second crossing of Cañada Larga by Cañada Larga Road (34°20'31.16"N, 119°17'11.32"W)

Pros: Low potential for groundwater influence, low in watershed, as likely to have flow as the rest of the watershed

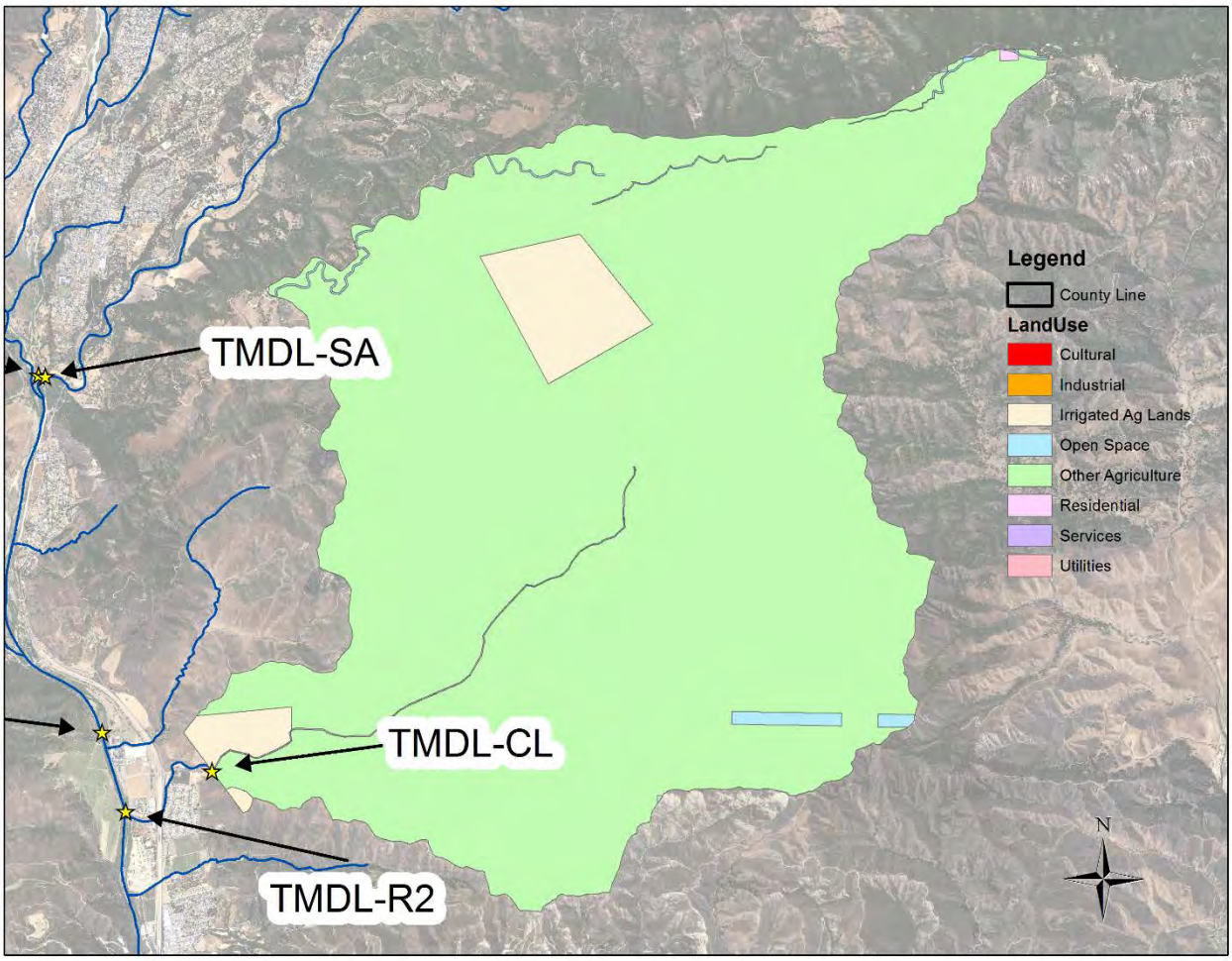
Cons: Moderate potential for vandalism, often dry (as is the rest of the watershed)

Outstanding Site Selection Tasks: Landowner permission

Dry Season Flow Potential: As likely to be wet as elsewhere in watershed

Ventura River Reach 2 (Cañada Larga): TMDL-CL

Land Use Draining to TMDL-CL



Land Use	Acres
Other Agriculture	11500
Irrigated Ag Land	559
Open Space	90
Residential	5

Ventura River Reach 3: TMDL-R3

Waterbody: Ventura River

Location: Above the deep pool upstream of the Ojai Valley Sanitary District R3 sampling location (34°20'44.51"N, 119°17'58.93"W)

Pros: Perennial, wadeable section of reach, accessible but with low potential for vandalism

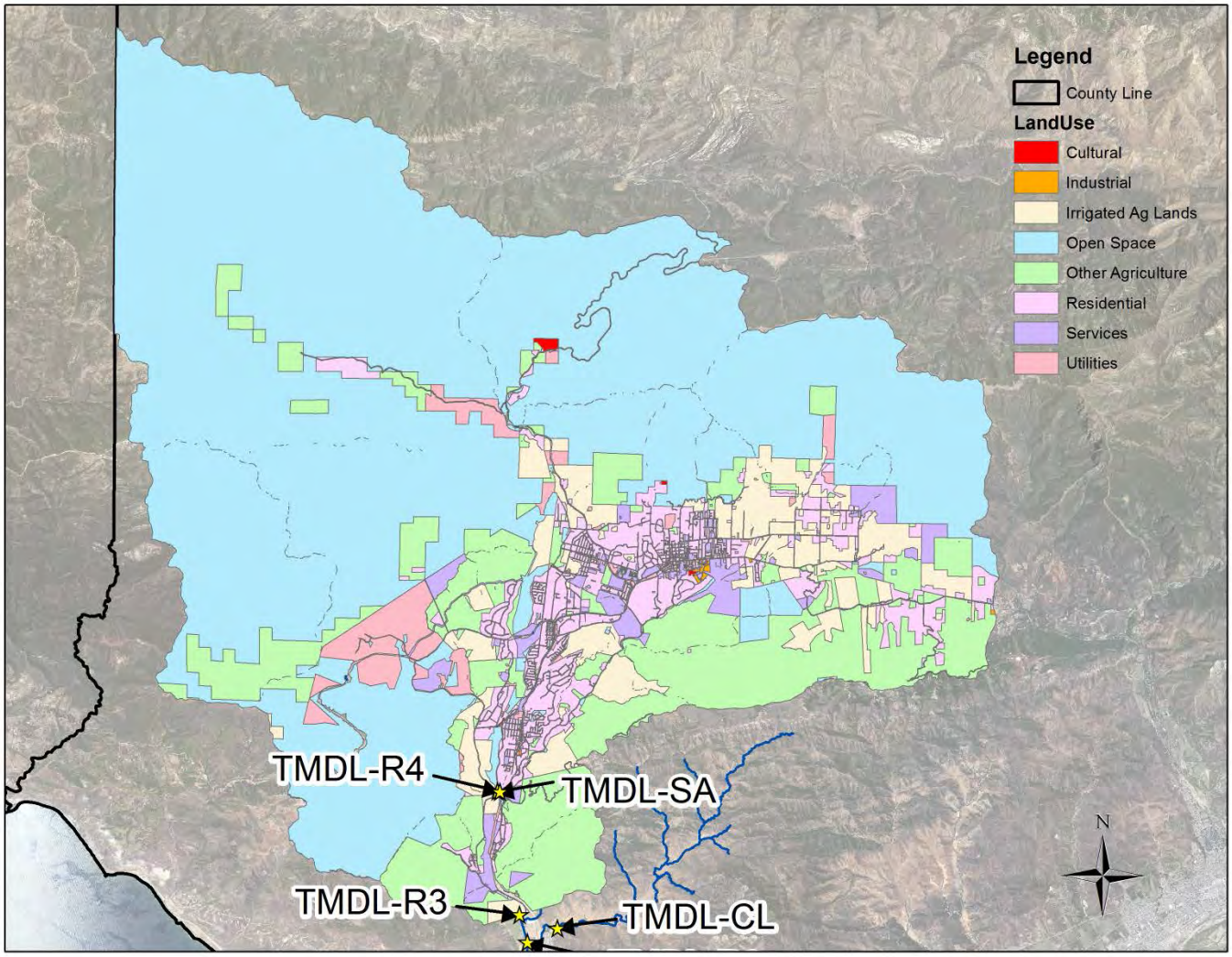
Cons:

Outstanding Site Selection Tasks: Landowner permission

Dry Season Flow Potential: Perennial

Ventura River Reach 3: TMDL-R3

Land Use Draining to TMDL-R3



Land Use	Acres
Open Space	75789
Other Agriculture	19516
Residential	7758
Irrigated Ag Land	7572
Transportation	3015
Services	2767
Cultural	72
Industrial	47

Ventura River Reach 4: TMDL-R4

Waterbody: Ventura River

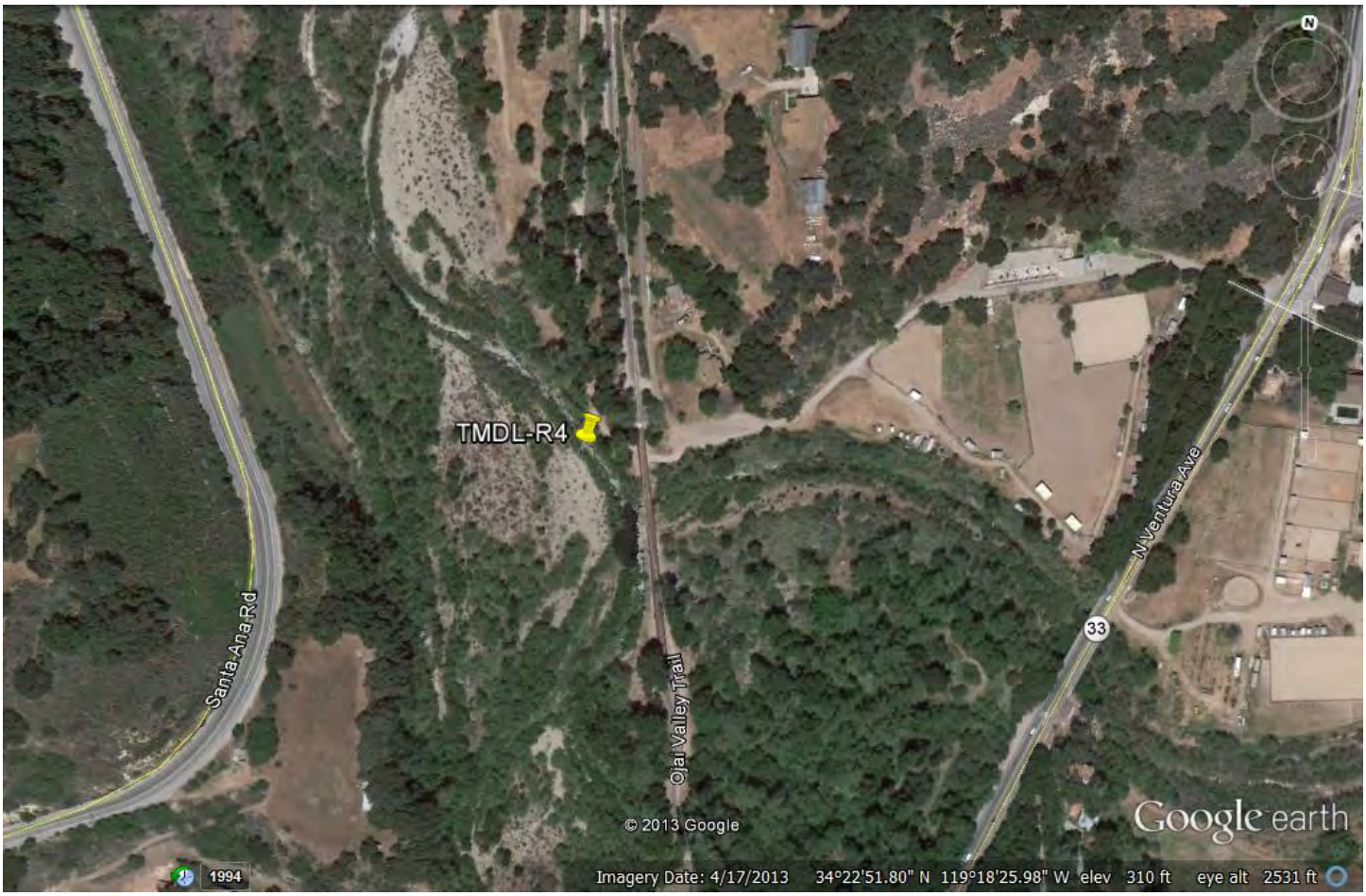
Location: Directly above the confluence with San Antonio Creek (34°22'51.55"N, 119°18'28.26"W)

Pros: Likelihood of flow, captures most of Reach 4 runoff, directly above known sensitive species habitat, above San Antonio Creek confluence, low potential for groundwater influence

Cons: Moderate potential for vandalism, non-perennial in dry years

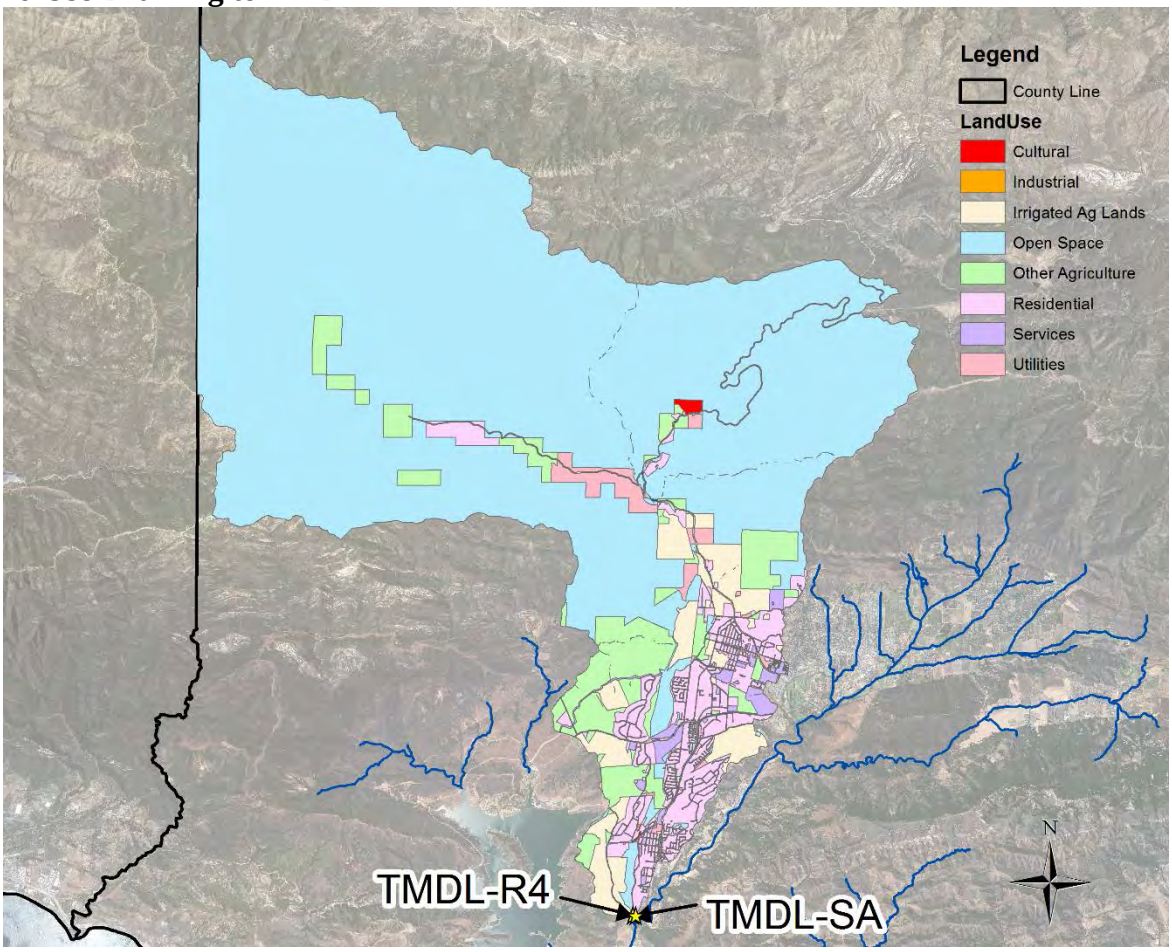
Outstanding Site Selection Tasks: Landowner permission

Dry Season Flow Potential: As likely to be wet as upstream locations



Ventura River Reach 4: TMDL-R4

Land Use Draining to TMDL-R4



Land Use	Acres
Open Space	43267
Other Agriculture	3828
Residential	3425
Irrigated Ag Land	2352
Transportation	763
Services	562
Cultural	60
Industrial	1

Ventura River Reach 4 (San Antonio Creek): TMDL-SA

Waterbody: San Antonio Creek

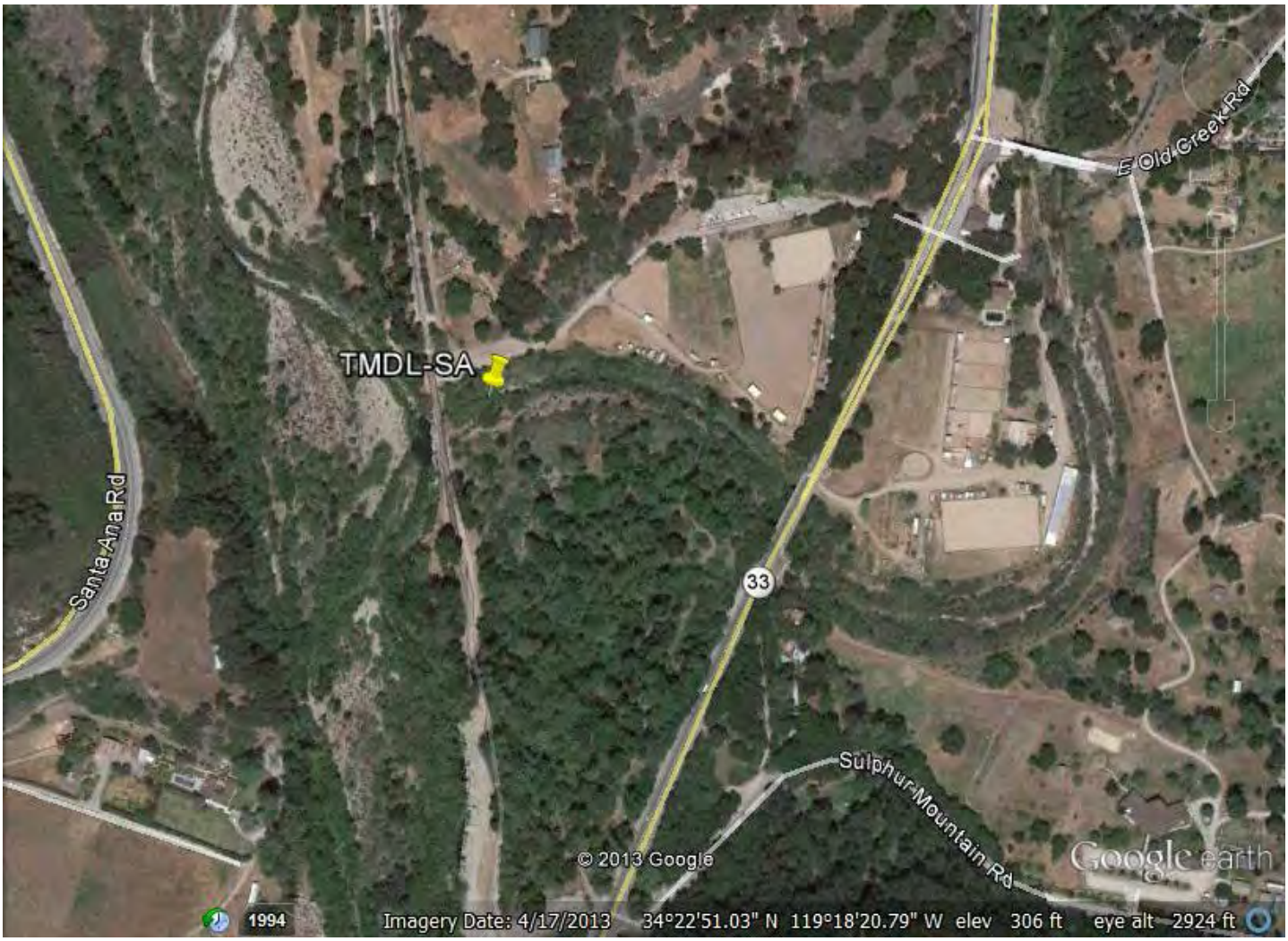
Location: Directly above the confluence with Ventura River (34°22'51.19"N, 119°18'25.25"W)

Pros: Likelihood of flow, captures all of San Antonio Creek runoff, directly above known sensitive species habitat, low potential for groundwater influence

Cons: Moderate potential for vandalism, non-perennial in dry years

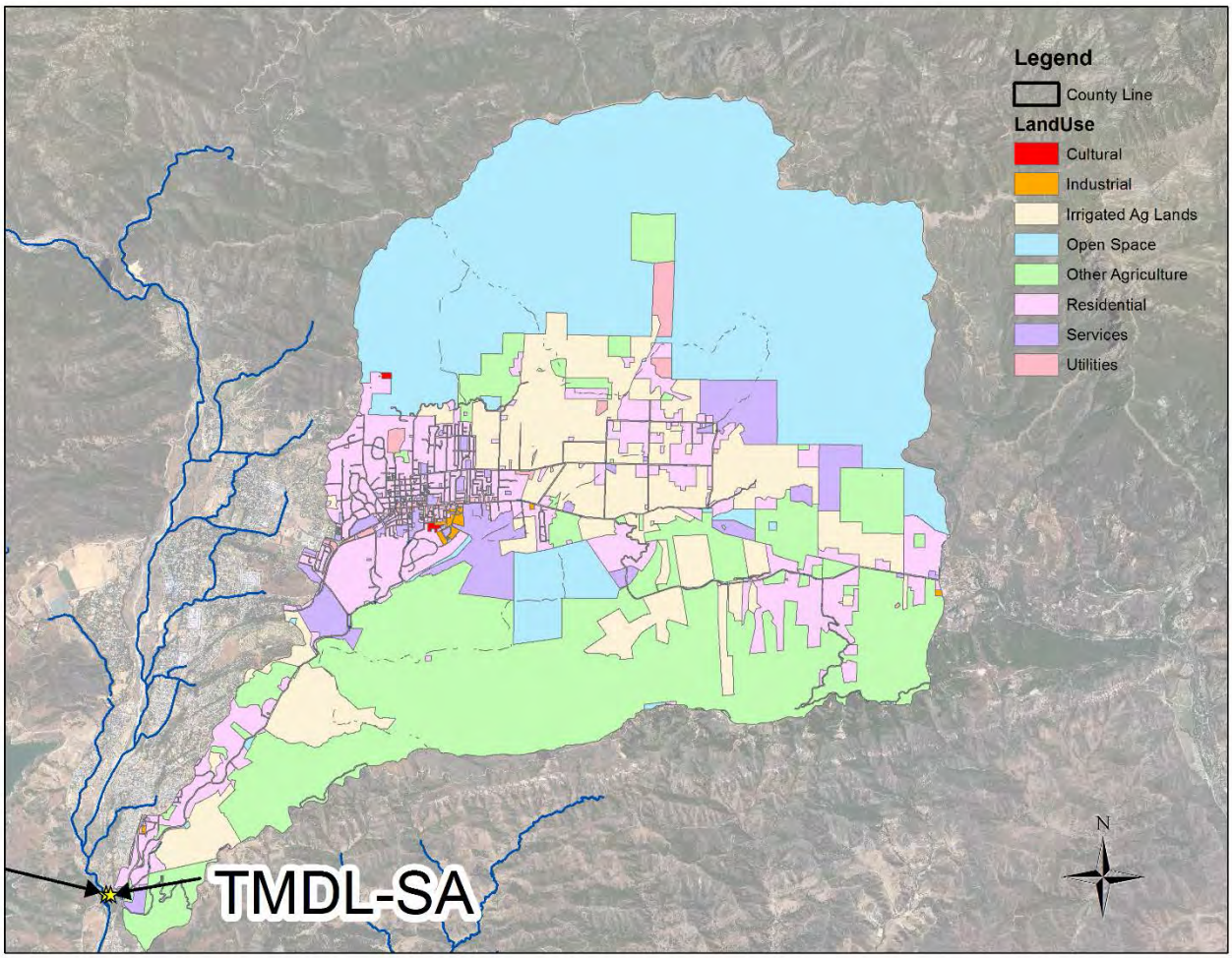
Outstanding Site Selection Tasks: Landowner permission

Dry Season Flow Potential: Likely in average water years



Ventura River Reach 4 (San Antonio Creek): TMDL-SA

Land Use Draining to TMDL-SA



Land Use	Acres
Open Space	12287
Other Agriculture	9200
Irrigated Ag Land	4842
Residential	4088
Services	1471
Transportation	289
Industrial	46
Cultural	12

Ventura River and Tributaries Algae, Eutrophic Conditions, and Nutrients TMDL Monitoring Program

CHAIN-OF-CUSTODY RECORD _____ OF _____

CLIENT: County of Ventura, Cities of Ojai and Ventura, Ojai Valley Sanitary District, VCAILG, Caltrans

SAMPLING EVENT: (E.g. May 2015 - Dry Season)

SAMPLING DATE:

SAMPLERS:

GRAB SAMPLES

SAMPLE ID		DATE/TIME	Total Nitrogen, Total Phosphorus	Dissolved Nitrogen, Dissolved Phosphorus	Nitrate + Nitrite as Nitrogen	Chlorophyll a			NOTES
	TMDL-Est		X	X	X	X			
	TMDL-R1		X	X	X	X			
	TMDL-R2		X	X	X	X			
	TMDL-R3		X	X	X	X			
	TMDL-R4		X	X	X	X			
	TMDL-CL		X	X	X	X			
	TMDL-SA		X	X	X	X			
	TMDL-FD		X	X	X	X			(Note which site)

Signature	Relinquished By:	Date/Time
Printed Name		
Affiliation		

Signature	Received By:	Date/Time
Printed Name		
Affiliation		

Miscellaneous Notes (Hazardous Materials, Quick turn-around time, etc.): _____

Event ID (Month Year): _____

Date: _____

Crew Members: _____

Weather (circle): Clear / Partly Cloudy / Overcast / Showers / Rain / Other _____

Event Type (check): □ Dry (<0.1" rain per day for the preceding three days)

□ Wet (days with ≥0.1" rain and the three days following)

Notes : _____

_____

_____

_____

OBSERVATIONS (BERM STATUS and RIVER FLOW)

Estuary

Berm Status (Open/Closed): _____ Photos Taken: Estuary and Mouth

Description of mudflats and subtidal area: _____

_____

_____

_____

_____

Notes: _____

_____

_____

Ventura River at Casitas Vista Road

Flow Status (circle one): Flowing / Ponded / Dry Photos Taken: Upstream / Downstream

Estimated Flow: _____ cfs.

Notes: _____

_____

_____

Ventura River at Santa Ana Blvd

Flow Status (circle one): Flowing / Ponded / Dry Photos Taken: Upstream / Downstream

Estimated Flow: _____ cfs

Notes: _____

_____

_____

Ventura River at Highway 150 (Baldwin Road)

Flow Status (circle one): Flowing / Ponded / Dry Photos Taken: Upstream / Downstream

Estimated Flow: _____ cfs

Notes: _____

_____

_____

Additional Observation Site: _____

Flow Status (circle one): Flowing / Ponded / Dry Photos Taken: Upstream / Downstream

Estimated Flow: _____ cfs

Notes: _____

_____

_____

Ventura River Algae TMDL Field Data Sheet (Estuary) - Page 1 of 2

Ventura River Algae TMDL—Estuary Details

Site ID: TMDL-Est

Event ID (Month Year):_____ **Date:** _____

Crew Members: _____

Weather (circle one): Clear / Partly Cloudy / Overcast / Rainy / Foggy **Ocean Inlet (circle one):** Open / Restricted / Closed

Direction of Tide: Ebb / Flood / Slack / N/A **Time of Low Tide:** _____ **Time of High Tide:** _____

Wind Strength: Calm / Slight Breeze / Moderate Breeze / Strong Breeze / Windy / Strong Wind **Wind Direction:** Blowing From / To _____

Notes (e.g. homeless, wildlife, dogs, swimming/recreation): _____

_____

TRANSECT 1

***In Situ* Measurements (Measure at Floating Macroalgae Quadrat 1, Transect 1)**

Monthly (Jan—Dec):

pH: _____ pH units EC: _____ µS/cm Water Temp: _____ °C

DO: _____ mg/L SC: _____ µS/cm

DO: _____ % Salinity: _____ ppt

Water Samples Collected (check box)

[Collect at Floating Macroalgae Quadrat 1, Transect 1]

Monthly Water (Jan—Dec):

Nitrogen, total and dissolved: □

Phosphorus, total and dissolved: □

Nitrate + Nitrite as Nitrogen: □

Dry Season Algae (May—Sep):

Chlorophyll a (phytoplankton): □

Volume filtered per sample: _____

Photos: □ Oceanward □ Landward	Start Time:	End Time:
Start Latitude:	Start Longitude:	
End Latitude:	End Longitude:	
PVC Latitude:	PVC Longitude:	

	MACROALGAE—LAND BASED										FLOATING MACROALGAE			
Quadrat	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**1**	**2**	**3**	**4**
Distance (m)														
Water Depth (must be ≤ 0.3 m)														
Condition [Frsh=Fresh, Int=Intermediate, Des=Dessicated, Dd=Dead]	Frsh Int Des Dd	Frsh Int Des Dd	Frsh Int Des Dd	Frsh Int Des Dd	Frsh Int Des Dd	Frsh Int Des Dd	Frsh Int Des Dd	Frsh Int Des Dd	Frsh Int Des Dd	Frsh Int Des Dd	Frsh Int Des Dd	Frsh Int Des Dd	Frsh Int Des Dd	Frsh Int Des Dd
No. Crosshairs with Macroalgae Present														
No. Crosshairs with Macroalgae Absent														
Crosshair Total (must equal 49)														

Ventura River Algae TMDL Field Data Sheet (Estuary) - Page 2 of 2

Ventura River Algae TMDL— Estuary Transect Measurements Date: _____ Crew: _____

TRANSECT 2

Photos: □ Oceanward □ Landward	Start Time:	End Time:
Start Latitude:	Start Longitude:	
End Latitude:	End Longitude:	
PVC Latitude:	PVC Longitude:	

	MACROALGAE—LAND BASED										FLOATING MACROALGAE			
Quadrat	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**1**	**2**	**3**	**4**
Distance (m)														
Water Depth (must be ≤ 0.3 m)														
Condition [Frsh=Fresh, Int=Intermediate, Des=Dessicated, Dd=Dead]	Frsh Int Des Dd	Frsh Int Des Dd	Frsh Int Des Dd	Frsh Int Des Dd	Frsh Int Des Dd	Frsh Int Des Dd	Frsh Int Des Dd	Frsh Int Des Dd	Frsh Int Des Dd	Frsh Int Des Dd	Frsh Int Des Dd	Frsh Int Des Dd	Frsh Int Des Dd	Frsh Int Des Dd
No. Crosshairs with Macroalgae Present														
No. Crosshairs with Macroalgae Absent														
Crosshair Total (must equal 49)														

TRANSECT 3

Photos: □ Oceanward □ Landward	Start Time:	End Time:
Start Latitude:	Start Longitude:	
End Latitude:	End Longitude:	
PVC Latitude:	PVC Longitude:	

	MACROALGAE—LAND BASED										FLOATING MACROALGAE			
Quadrat	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**1**	**2**	**3**	**4**
Distance (m)														
Water Depth (must be ≤ 0.3 m)														
Condition [Frsh=Fresh, Int=Intermediate, Des=Dessicated, Dd=Dead]	Frsh Int Des Dd	Frsh Int Des Dd	Frsh Int Des Dd	Frsh Int Des Dd	Frsh Int Des Dd	Frsh Int Des Dd	Frsh Int Des Dd	Frsh Int Des Dd	Frsh Int Des Dd	Frsh Int Des Dd	Frsh Int Des Dd	Frsh Int Des Dd	Frsh Int Des Dd	Frsh Int Des Dd
No. Crosshairs with Macroalgae Present														
No. Crosshairs with Macroalgae Absent														
Crosshair Total (must equal 49)														

Ventura River Algae TMDL Field Data Sheet (Reaches 1—4) - Page 1 of 2

Ventura River Algae TMDL—River or Tributary Site Details

Event ID (Month Year):_____

Site ID: _____

Date/Time: _____

Crew Members: _____

_____

Latitude/Longitude: _____

Reach Length (150 m if wetted width ≤ 10 m; 250 m if wetted width > 10 m): _____

Flow (circle one): Flowing / Ponded / Dry

Wind Strength: Calm / Light-Moderate Breeze / Strong Breeze / Windy

Wind Direction: Blowing (circle one) From / To _____

Photos (check): □ Upstream □ Downstream

Notes (e.g. homeless, wildlife, horses, swimming/recreation):

_____

_____

***In Situ* Measurements**

Monthly (Jan—Dec):

pH: _____ pH units EC: _____ µS/cm

DO: _____ mg/L SC: _____ µS/cm

DO: _____ % Salinity: _____ ppt

Water Temp: _____°C

Flow: _____ cfs

Samples Collected (check box)

Monthly Water (Jan—Dec):

Nitrogen, total and dissolved: □

Phosphorus, total and dissolved: □

Nitrate + Nitrite as Nitrogen: □

Dry Season Algae (May—Sep):

Chlorophyll *a* (algae): □

Discharge Measurement

1st Measurement = left bank (looking downstream)

Buoyant Object Method
(Use only if velocity area method not possible)

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

Float Reach Cross Section (ft)

	Upper Section	Middle Section	Lower Section
Width			
Depth 1			
Depth 2			
Depth 3			
Depth 4			
Depth 5			

Discharge measurement not possible: ☐

Reason: _____

Velocity Area Method (preferred)

No.	Distance from Left Bank (ft)	Depth (ft)	Velocity (ft/sec)
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
17			
18			
19			
20			

Algae Collection for Chlorophyll *a* (May—September)

Collection Device (sum # transects per Device)	Quantity
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)	
Chlorophyll *a* Volume (use GF/F filter, 25 mL preferred volume)	

Ventura River Algae TMDL Field Data Sheet (Reaches 1—4) - Page 2 of 2

Ventura River Algae TMDL Transect Measurements (for % cover, May—September)

Site: _____ Date: _____ Crew: _____

	Wetted Width (ft)	Macroalgae Presence/Absence (P/A)					Densiometer (0-17) Count covered dots				Photo (✓ when Taken)
Transect		Left Bank	Left Center	Center	Right Center	Right Bank	Center Left	Center Up	Center Right	Center Down	Upstream/ Downstream
A											
AB											
B											
BC											
C											
CD											
D											
DE											
E											
EF											
F											
FG											
G											
GH											
H											
HI											
I											
IJ											
J											
JK											
K											

TMDL-Est – Event Information

Event ID (Month Year):	Date:	
Crew members:		
Current weather: Clear / Partly Cloudy / Overcast / Rainy / Foggy (circle one)		
Wind strength: Calm / Slight Breeze / Moderate Breeze / Strong Breeze / Windy / Strong Wind		
Wind direction: Blowing From / To:		
Days since last rainfall (prior to sample date):	Amount of rainfall event:	
Ocean inlet condition: Open / Restricted / Closed	Direction of tide: Ebb / Flood / Slack / NA	
Water clarity:	Time low tide:	Time high tide:
Recent human activity in/near site: Water activity / intertidal activity / activity near site		
Notes (e.g. homeless, wildlife, dogs, swimming/recreation etc.):		

Transect 1

Start Time:	End Time:	Photos: Oceanward □ Landward □
Oceanward Latitude:		Landward Latitude:
Oceanward Longitude:		Landward Longitude:

Water (floating macroalgae quadrat 1, transect 1 only)

Water Samples Collected (check box)	***In Situ* Measurements**
Monthly Water (Jan—Dec):	**Monthly (Jan—Dec):**
Nitrogen, total and dissolved: □	DO: _____ mg/L pH: _____ pH units
Phosphorus, total and dissolved: □	DO: _____ % Water Temp: _____°C
Nitrate + Nitrite as Nitrogen: □	EC: _____ µS/cm
Dry Season Algae (May—Sep):	SC: _____ µS/cm
Chlorophyll a (phytoplankton): □	Salinity: _____ ppt
Volume filtered per sample: _____(ml)	

Floating Macroalgae

Quadrat	Oceanward – Facing Ocean	Oceanward – Facing Land	Landward – Facing Ocean	Landward – Facing Land
Mat Thickness (mm)	Est: Y/N	Est: Y/N	Est: Y/N	Est: Y/N
Condition	Frsh / Int / Des/ Dd	Frsh / Int / Des/ Dd	Frsh / Int / Des/ Dd	Frsh / Int / Des/ Dd
Absent				
Ulva intestinalis				
Ulva lactuca				
Macrocystis spp.				
Duck weed				
Filamentous algae				
Other 1:				
Other 2:				
Total:				
Biomass (Y/N)				

Vertical zonation of macroalgae? Y/N Describe:

Comments on location of floating algae:

Transect 1 continued

Macroalgae Transect - Distance from PVC (at oceanward end):										
Quadrat	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Distance (m)										
Mat Thick (mm)										
Estimated?	Y / N	Y / N	Y / N	Y / N	Y / N	Y / N	Y / N	Y / N	Y / N	Y / N
Condition	Frsh/Int/ Des/Dd	Frsh/Int/ Des/Dd	Frsh/Int/ Des/Dd	Frsh/Int/ Des/Dd	Frsh/Int/ Des/Dd	Frsh/Int/ Des/Dd	Frsh/Int/ Des/Dd	Frsh/Int/ Des/Dd	Frsh/Int/ Des/Dd	Frsh/Int/ Des/Dd
Bare										
Ulva intestinalis (string-like)										
Ulva lactuca (sheet-like)										
Ceramium										
Gracilaria										
Filamentous algae										
Ruppia (spp.)										
Macrocystis Wrack: Y / N										
Phyllospadix Wrack: Y / N										
Decayed and Unidentifiable										
Other 1:										
Other 2:										
Total:										

Comments:

Transect 2

Start Time:	End Time:	Photos: Oceanward □ Landward □
Oceanward Latitude:		Landward Latitude:
Oceanward Longitude:		Landward Longitude:

Floating Macroalgae

Quadrat	Oceanward – Facing Ocean	Oceanward – Facing Land	Landward – Facing Ocean	Landward – Facing Land
Mat Thickness (mm)	Est: Y/N	Est: Y/N	Est: Y/N	Est: Y/N
Condition	Frsh / Int / Des/ Dd	Frsh / Int / Des/ Dd	Frsh / Int / Des/ Dd	Frsh / Int / Des/ Dd
Absent				
Ulva intestinalis				
Ulva lactuca				
Macrocystis spp.				
Duck weed				
Filamentous algae				
Other 1:				
Other 2:				
Total:				
Biomass (Y/N)				

Vertical zonation of macroalgae? Y/N Describe:

Comments on location of floating algae:

Macroalgae Transect - Distance from PVC (at oceanward end):

Quadrat	1	2	3	4	5	6	7	8	9	10
Distance (m)										
Mat Thick (mm)										
Estimated?	Y / N	Y / N	Y / N	Y / N	Y / N	Y / N	Y / N	Y / N	Y / N	Y / N
Condition	Frsh/Int/ Des/Dd	Frsh/Int/ Des/Dd	Frsh/Int/ Des/Dd	Frsh/Int/ Des/Dd	Frsh/Int/ Des/Dd	Frsh/Int/ Des/Dd	Frsh/Int/ Des/Dd	Frsh/Int/ Des/Dd	Frsh/Int/ Des/Dd	Frsh/Int/ Des/Dd
Bare										
Ulva intestinalis (string-like)										
Ulva lactuca (sheet-like)										
Ceramium										
Gracilaria										
Filamentous algae										
Ruppia (spp.)										
Macrocystis Wrack: Y / N										
Phyllospadix Wrack: Y / N										
Decayed and Unidentifiable										
Other 1:										
Other 2:										
Total:										

Transect 3

Start Time:	End Time:	Photos: Oceanward □ Landward □
Oceanward Latitude:		Landward Latitude:
Oceanward Longitude:		Landward Longitude:

Floating Macroalgae

Quadrat	Oceanward – Facing Ocean	Oceanward – Facing Land	Landward – Facing Ocean	Landward – Facing Land
Mat Thickness (mm)	Est: Y/N	Est: Y/N	Est: Y/N	Est: Y/N
Condition	Frsh / Int / Des/ Dd	Frsh / Int / Des/ Dd	Frsh / Int / Des/ Dd	Frsh / Int / Des/ Dd
Absent				
Ulva intestinalis				
Ulva lactuca				
Macrocystis spp.				
Duck weed				
Filamentous algae				
Other 1:				
Other 2:				
Total:				
Biomass (Y/N)				

Vertical zonation of macroalgae? Y/N Describe:

Comments on location of floating algae:

Macroalgae Transect - Distance from PVC (at oceanward end):

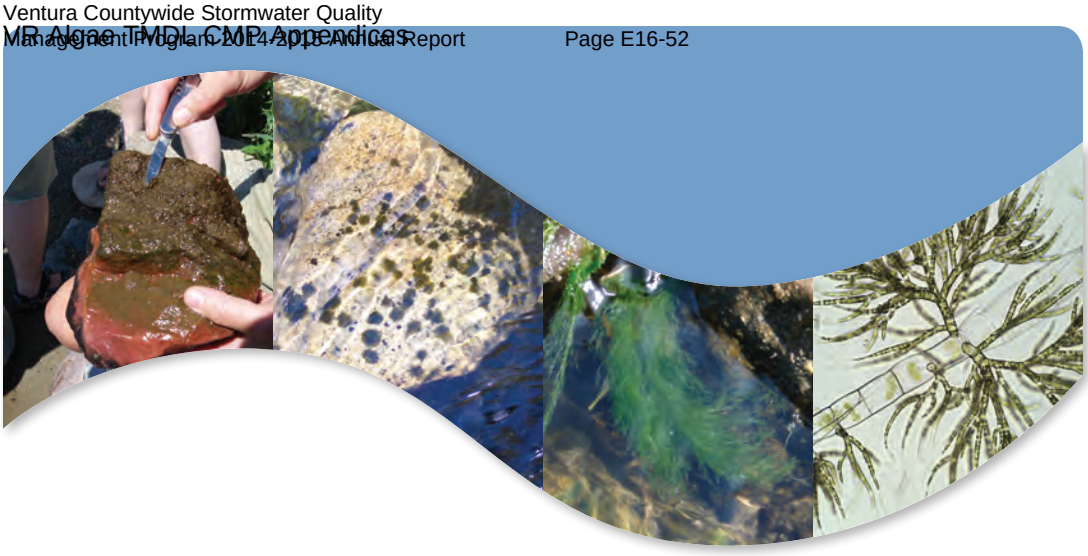
Quadrat	1	2	3	4	5	6	7	8	9	10
Distance (m)										
Mat Thick (mm)										
Estimated?	Y / N	Y / N	Y / N	Y / N	Y / N	Y / N	Y / N	Y / N	Y / N	Y / N
Condition	Frsh/Int/ Des/Dd	Frsh/Int/ Des/Dd	Frsh/Int/ Des/Dd	Frsh/Int/ Des/Dd	Frsh/Int/ Des/Dd	Frsh/Int/ Des/Dd	Frsh/Int/ Des/Dd	Frsh/Int/ Des/Dd	Frsh/Int/ Des/Dd	Frsh/Int/ Des/Dd
Bare										
Ulva intestinalis (string-like)										
Ulva lactuca (sheet-like)										
Ceramium										
Gracilaria										
Filamentous algae										
Ruppia (spp.)										
Macrocystis Wrack: Y / N										
Phyllospadix Wrack: Y / N										
Decayed and Unidentifiable										
Other 1:										
Other 2:										
Total:										

Appendix C: Equipment List

Visit Type	Item	Quantity / Site	Specifications/Examples
All	Sampling SOP (this document)	1/person	
All	Equipment decontamination supplies		See Appendix D SOP 4
All	Hip or chest waders, or wading boots/shoes (not felt-soled)	at least 1 pair/ person	
All	Digital camera	1	
All	Chain of Custodies	1	
All	Full set of datasheets printed on waterproof paper (e.g., Rite-in-the-Rain™)	1 full set (and spare set recommended)	
All	Fine-tipped and thick-tipped waterproof/alcohol-proof pens and markers; pencils	2-3 each	
All	Clipboard	2-3	
All	Thomas Guide and regional maps	as needed	
All	Centigrade thermometer	1	
All	pH meter	1	
All	DO meter and spare membrane	1	
All	Conductivity/Salinity meter	1	
All	Spare batteries for meters	as needed	
All	Calibration standards	1	
	Nitrile gloves	as needed	
All	Water chemistry containers	as needed	
All	Ice chest with wet ice	as needed	
All	First aid kit	1	
All	GPS unit	1	
All	Current velocity meter and top-setting rod	1	
All	Digital watch with stopwatch	1	
All	Fresh orange peel	1	
All	Luer-lok 60 mL syringes	1 per site	
All	0.45MCE filters for dissolved nutrients/TDN/TDP	as needed	
Algae	Quadrat (0.5mx0.5m PVC pipe strung with two orthogonal sets of 7 evenly spaced taut strings	1	
Algae	Whatman GF/F filters (47 mm)	as needed	
Algae	Transect tape - 30 m	1	
Algae	Transect tape; 150 m	1	
Algae	Lengths of rope (7.5 m and 12.5 m)	1 each	
Algae	Small metric folding ruler (waterproof)	1	

Appendix C: Equipment List

Visit Type	Item	Quantity / Site	Specifications/Examples
Algae	Stadia rod	1	
Algae	Convex spherical densiometer	1	Taped to expose only 17 intersections of the grid (see Figure 12)
Algae	Transect flags	21 total	Two colors; label with main transect (11 ct.) and inter- transect (10 ct.) names
Algae	Algae viewing bucket (optional)	1	See Appendix C
Algae	Rangefinder (optional)	1	
Algae	Glass fiber filter, 47 mm, 0.7 µm pore size	1 per replicate	Fisher 09804142H
Algae	Snapping Petri dish, 47 µm	1 per replicate	Fisher 08-757-105
Algae	Filter forceps	1	Fisher 0975350
Algae	Filtering chamber/tower, 47 mm, plastic	1	Hach 2254400
Algae	Hand vacuum pump	1	Fisher 13-874-612B
Algae	Aluminum foil	~100 cm2 per replicate	
Algae	Deionized water	500 mL	
Algae	Dry ice (if not returning to lab immediately following the day's fieldwork)	10 lbs	
Algae	White dish tub, rectangular, plastic, 11.5 qt	1	Use white, not colored
Algae	Scrubbing brush or scouring pad to clean dish tub, etc.	1	
Algae	Composite sample receiving bottle with cap, 1 L, plastic	1	Fisher 02-912-038
Algae	Graduated cylinder, 500 mL and 25 mL, plastic	1 each	Fisher 03-007-42 & 03-007-39
Algae	Bottle brush to clean graduated cylinders, etc.	1 sm, 1 lg	
Algae	PVC delimiter, 12.6 cm2 area	1	See Appendix C
Algae	Spatula (> 12.6 cm2 surface area)	1	
Algae	Rubber delimiter, 12.6 cm2 area	1	See Appendix C
Algae	Toothbrush, firm-bristled	1	
Algae	Syringe scrubber, 60 mL syringe, 5.3 cm2 area	1	See Appendix C
Algae	White (non-pigmented) scrubbing-pad circles	11 per replicate	See Appendix C
Algae	Tally meter (optional)	1	Ben Meadows 9JB-102385
Algae	Scissors	1	
Algae	Wash bottles	2	Label bottles with "stream water", and "DI water"
Algae	Razor blades or Swiss army knife	1	
Algae	Sample labels (printed on waterproof paper)	4 per replicate	See Figure 6 in SOP 02
Algae	Clear plastic tape, 5 cm wide	Length of ~20cm per replicate	
Algae	Fisherman's vest (optional)	1	
Algae	Whirl-pak bag, 100 mL	3 per replicate	Cole Parmer 06498-00



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

A. Elizabeth Fetscher
Southern California Coastal Water Research Project
3535 Harbor Blvd., Suite 110
Costa Mesa, CA 92626

Lilian Busse
San Diego Regional Water Quality Control Board
State Water Resources Control Board
9174 Sky Park Court
San Diego, CA 92123

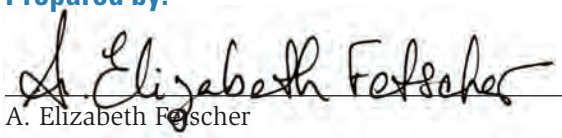
Peter R. Ode
Aquatic Bioassessment Laboratory/Water Pollution Control Laboratory
California Department of Fish and Game
2005 Nimbus Road
Rancho Cordova, CA 95670

CALIFORNIA Water Boards

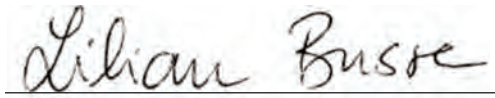
http://www.waterboards.ca.gov/water_issues/programs/swamp

Standard Operating Procedures for Collecting Stream Algae Samples and Associated Physical Habitat and Chemical Data for Ambient Bioassessments in California

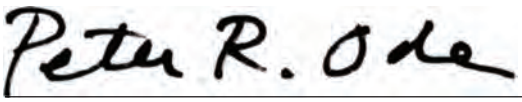
Prepared by:



A. Elizabeth Fetscher
Senior Scientist at the Southern California Coastal Water Research Project

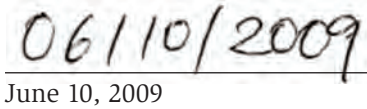


Lilian Busse
Environmental Scientist at the San Diego Regional Board, State Water Resources Control Board



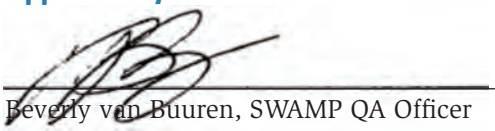
Peter R. Ode
SWAMP Bioassessment Coordinator, California Department of Fish and Game, Aquatic Bioassessment Laboratory

Preparation Date:



June 10, 2009

Approved by:



Beverly van Buuren, SWAMP QA Officer
SWAMP QA Officer

Approval Date:

June 10, 2009

TABLE OF CONTENTS TOC

TABLE OF CONTENTS TOC

TABLE OF CONTENTS TOC

Figures

TABLE OF CONTENTS TOC

Tables

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).

The current version of the protocol was established with contributions from the following people:

Karissa Anderson (San Francisco Regional Water Quality Control Board)
Dean Blinn (Northern Arizona University)
Erick Burres (State Water Resources Control Board, Clean Water Team)
Will Hagan (SWAMP QA Team)
Julie Hambrook Berkman (US Geological Survey)
Jim Harrington (Aquatic Bioassessment Laboratory/Department of Fish and Game)
David Herbst (Sierra Nevada Aquatic Research Laboratory, University California Santa Barbara)
Gary Ichikawa (Moss Landing Marine Laboratories)
Bill Isham (Weston Solutions)
Scott Johnson (Aquatic Bioassay & Consulting Laboratories)
Revital Katznelson (Consultant for SWAMP)
Patrick Kociolek (University of Colorado)
Berengere Laslandes (University of Colorado)
Carrieann Lopez (San Francisco Regional Water Quality Control Board)
Marc Los Huertos (California State University Monterey Bay)
Raphael Mazor (Southern California Coastal Water Research Project)
Shawn McBride (Aquatic Bioassessment Laboratory/Department of Fish and Game)
Sean Mundell (Marine Pollution Studies Laboratory, California Department of Fish and Game)
Yangdong Pan (Portland State University)
Craig Pernot (Aquatic Bioassay & Consulting Laboratories)
Doug Post (Aquatic Bioassessment Laboratory/Department of Fish and Game)

Scott Rollins (Spokane Falls Community College)
Robert Sheath (California State University San Marcos)
Marco Sigala (SWAMP data management team)
Rosalina Stancheva Hristova (California State University San Marcos)
Martha Sutula (Southern California Coastal Water Research Project)
Karen Taberski (San Francisco Regional Water Quality Control Board)
Evan Thomas (University of Colorado)
Melinda Woodard (SWAMP QA Team)

CITATION FOR THIS DOCUMENT:

Fetscher, A.E., L. Busse, and P. R. Ode. 2009. Standard Operating Procedures for Collecting Stream Algae Samples and Associated Physical Habitat and Chemical Data for Ambient Bioassessments in California. California State Water Resources Control Board Surface Water Ambient Monitoring Program (SWAMP) Bioassessment SOP 002. (updated May 2010)

LIST OF ACRONYMS & ABBREVIATIONS L

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".

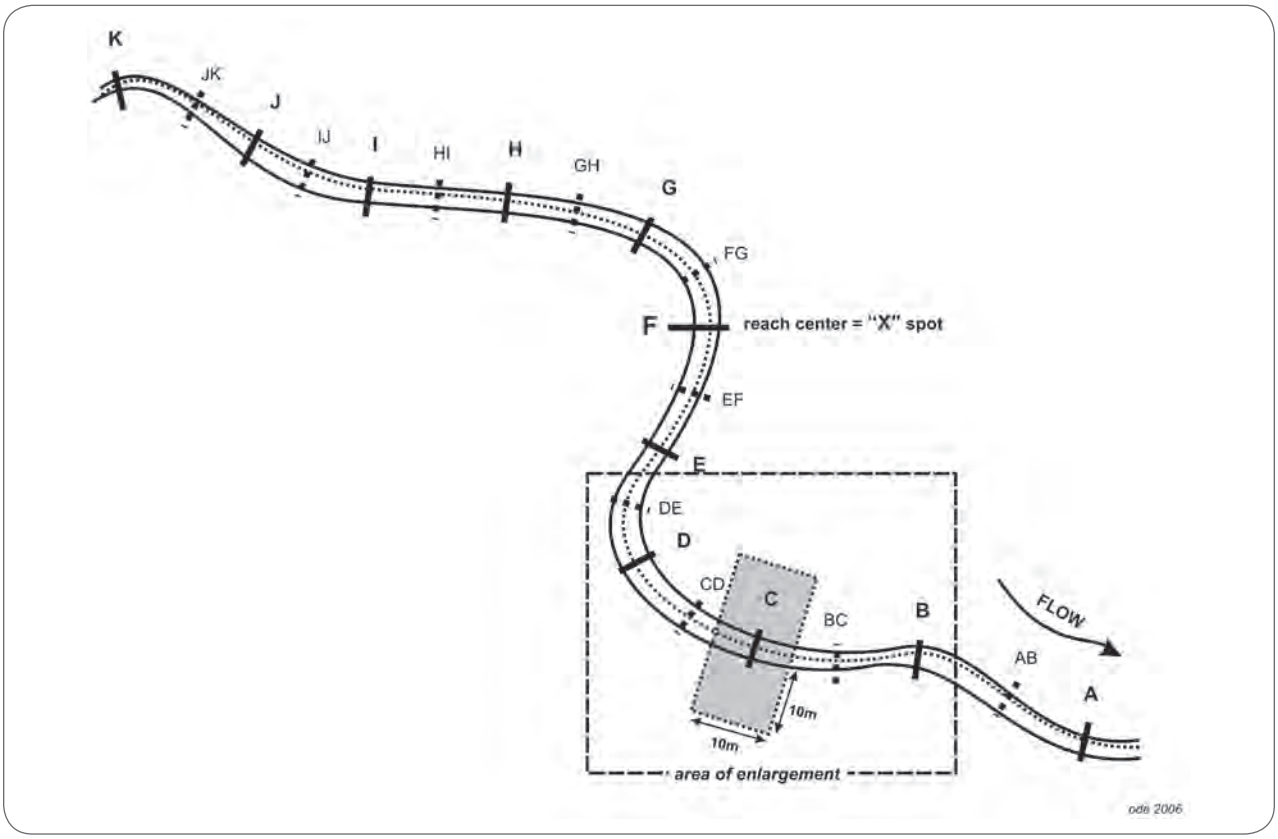


Figure 1. Reach layout geometry for physical habitat (PHab) and biological sampling showing positions of 11 main transects (A-K) and the 10 supplemental inter-transects (AB-JK). The area highlighted in the figure is expanded in Figure 11. Note: reach length = 150 m for streams ≤ 10 m average wetted width, 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.*

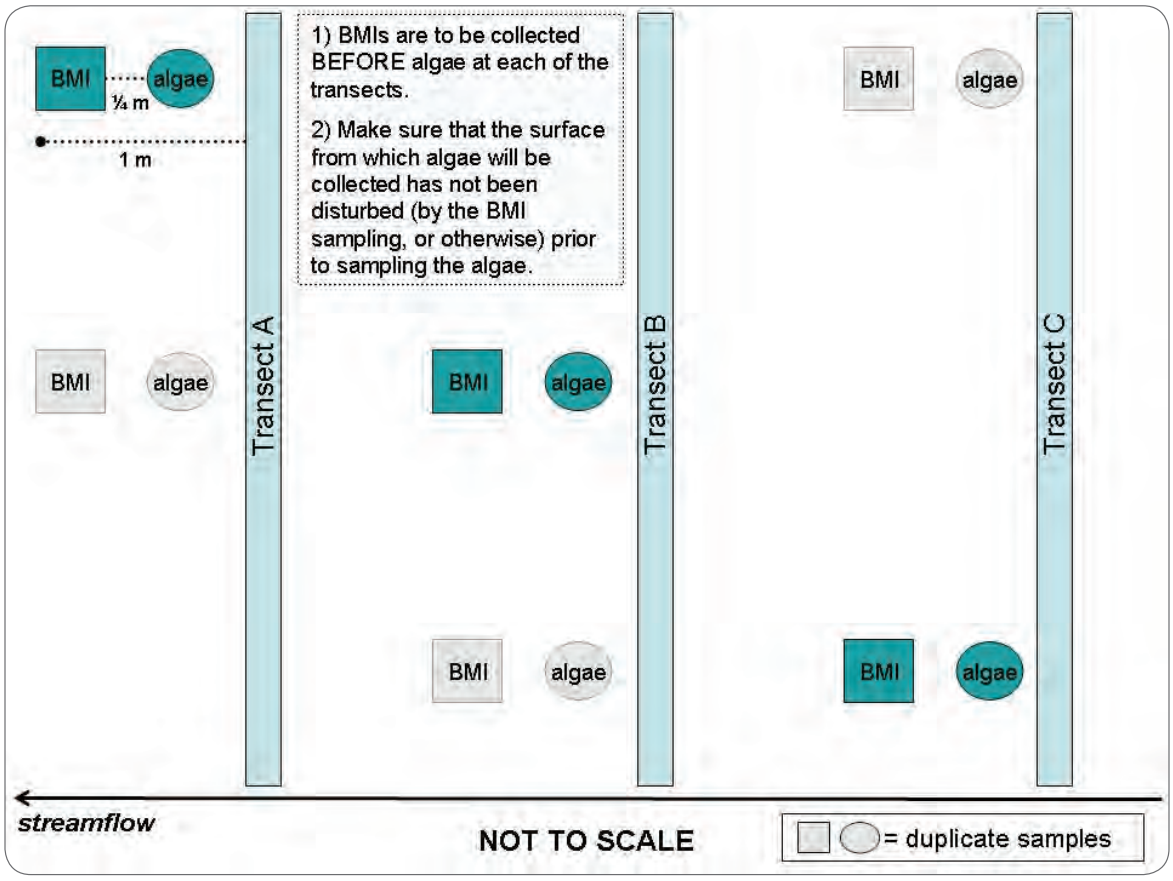


Figure 2. Sampling array for collection of algae, BMIs, and duplicates of each assemblage. For convenience, only Transects A through C are shown, but the same pattern of placement should be rotated across all 11 transects.

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.

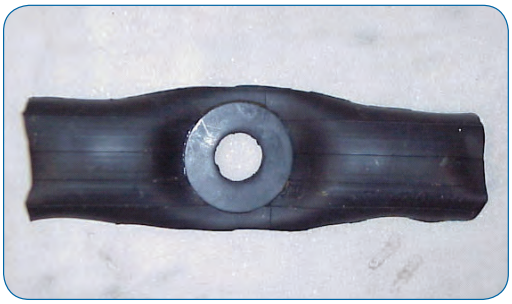


Figure 5. Rubber Delimiter

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.

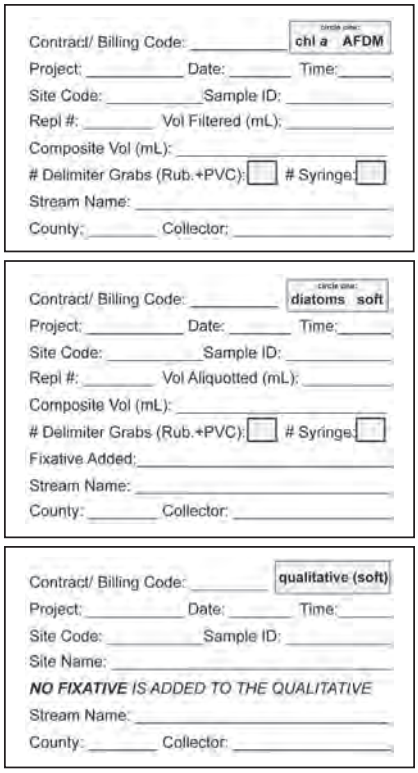
SECTION 5
ALGAL SAMPLE PROCESSING

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.


Contract/ Billing Code: _____ circle one: chl a AFDM
Project: _____ Date: _____ Time: _____
Site Code: _____ Sample ID: _____
Repl #: _____ Vol Filtered (mL): _____
Composite Vol (mL): _____
Delimiter Grabs (Rub.+PVC): ☐ # Syringe: ☐
Stream Name: _____
County: _____ Collector: _____

Contract/ Billing Code: _____ circle one: diatoms soft
Project: _____ Date: _____ Time: _____
Site Code: _____ Sample ID: _____
Repl #: _____ Vol Aliquotted (mL): _____
Composite Vol (mL): _____
Delimiter Grabs (Rub.+PVC): ☐ # Syringe: ☐
Fixative Added: _____
Stream Name: _____
County: _____ Collector: _____

Contract/ Billing Code: _____ qualitative (soft)
Project: _____ Date: _____ Time: _____
Site Code: _____ Sample ID: _____
Site Name: _____
***NO FIXATIVE** IS ADDED TO THE QUALITATIVE*
Stream Name: _____
County: _____ Collector: _____

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.

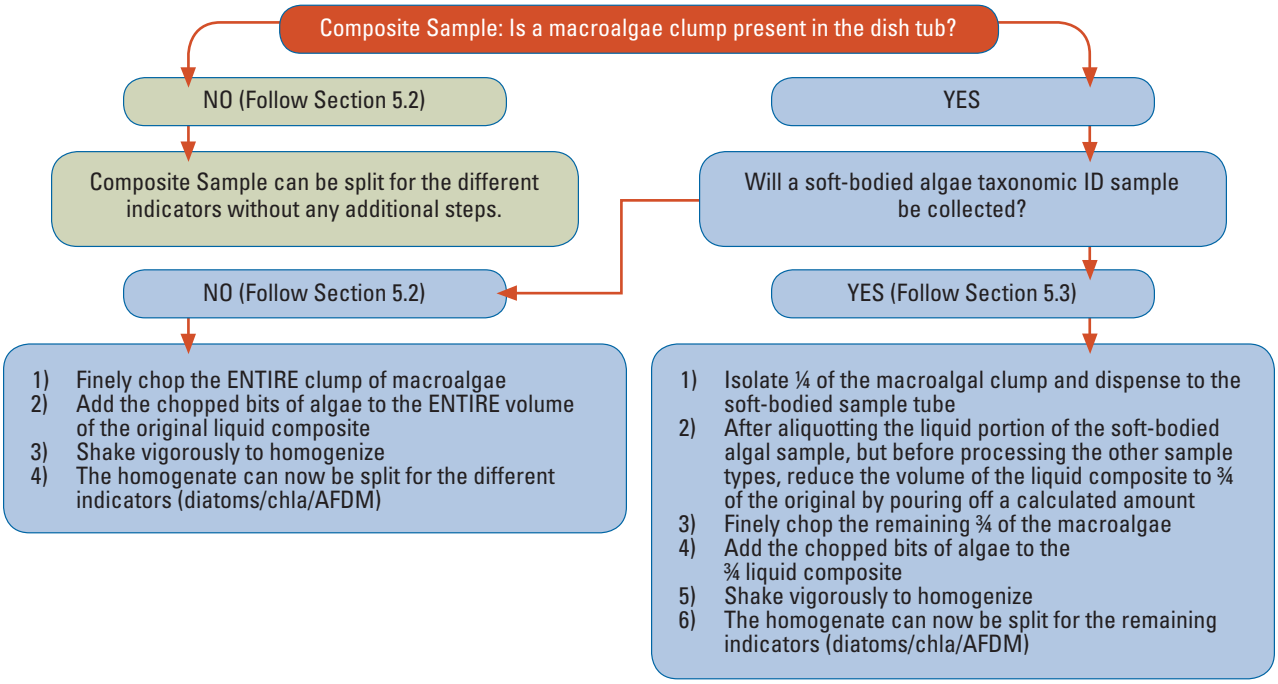


Figure 7. Summary of major sample-processing decision points based on presence of macroalgal clump(s) and need to prepare soft-bodied algal samples.

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.

PHYSICAL HABITAT TRANSECT-BASED MEASUREMENTS TO ACCOMPANY ALGAL BIOASSESSMENT 6

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".

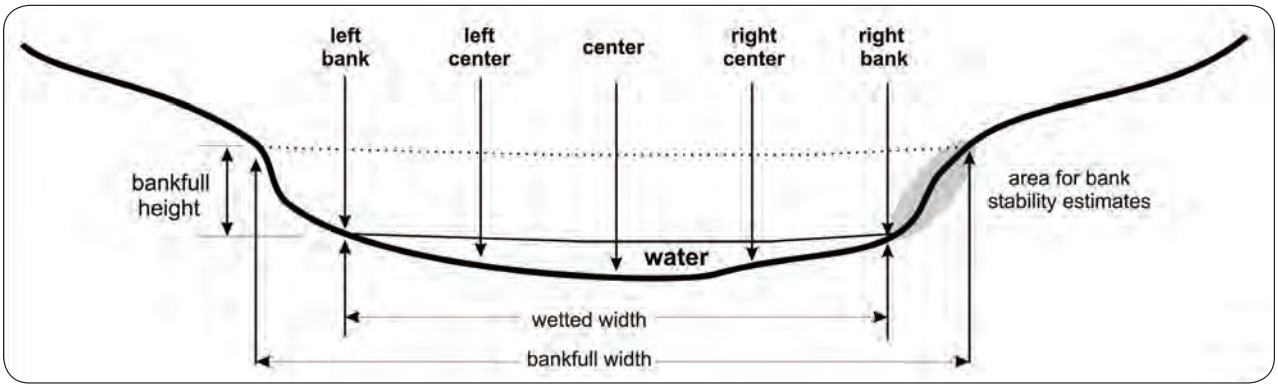


Figure 9. Cross sectional diagram of a typical stream channel showing locations of substrate measurements, wetted and bankfull width measurements, and bank stability visual estimates (reprinted from Ode 2007).

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		

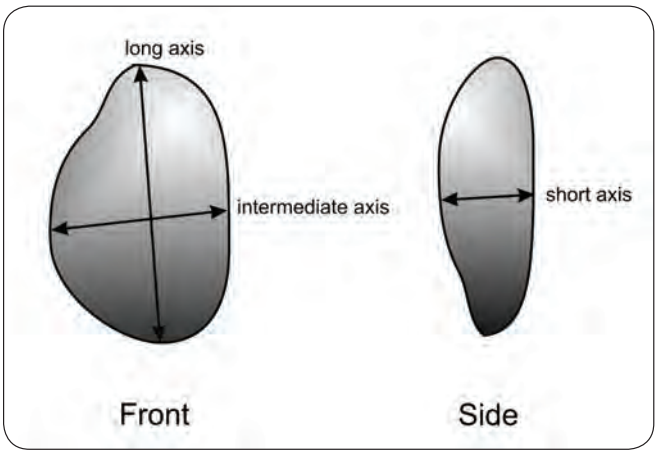


Figure 10. Diagram of three major perpendicular axes of substrate particles. The intermediate axis is recorded for pebble counts (reprinted from Ode 2007).

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 Stevenson 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.

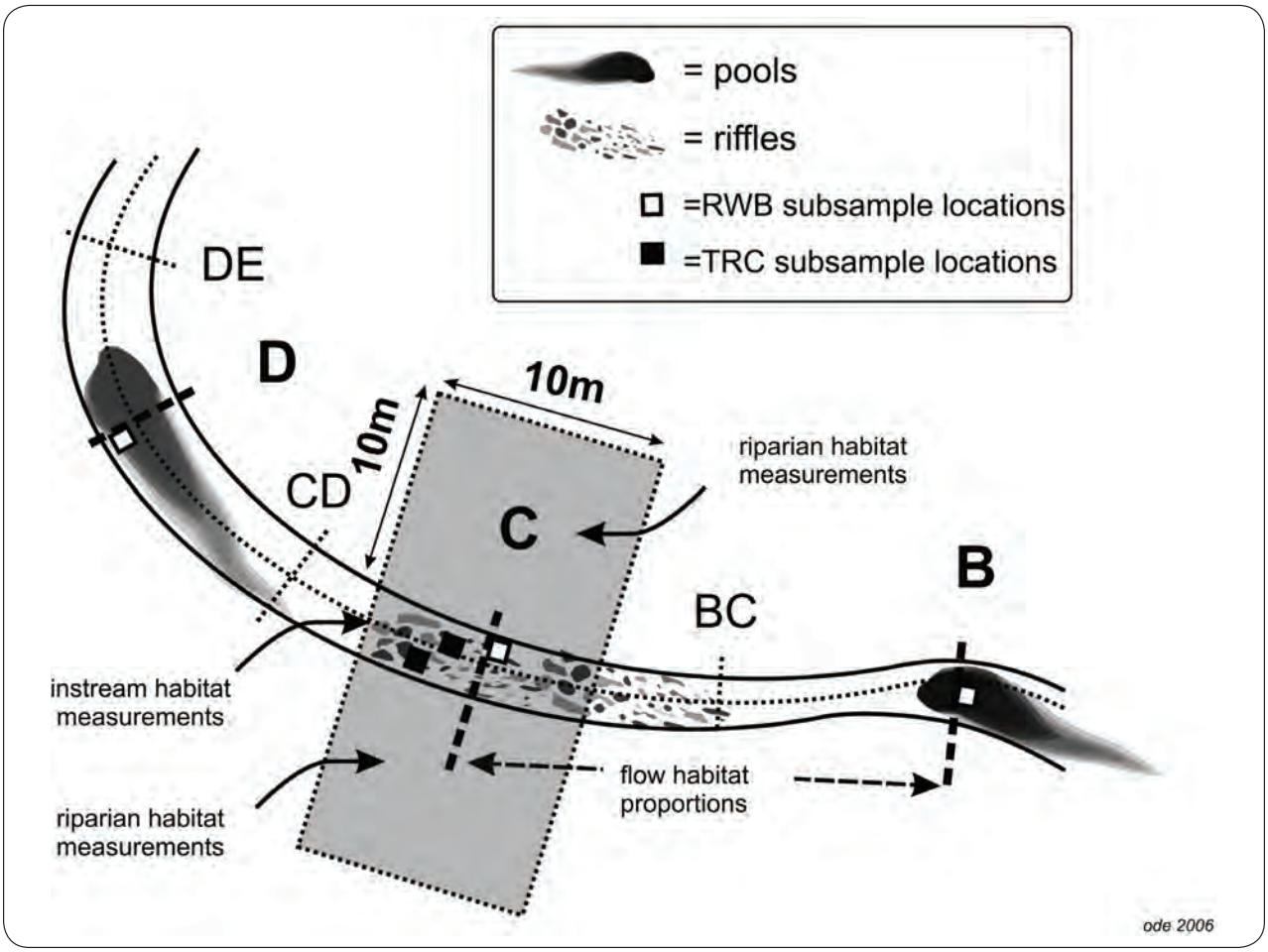


Figure 11. Section of the standard reach expanded from Figure 1 showing the appropriate positions for collecting algae samples (the white square, labeled "RWB" in the legend box) and flow habitat proportion measurements (reprinted from Ode 2007).

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.)

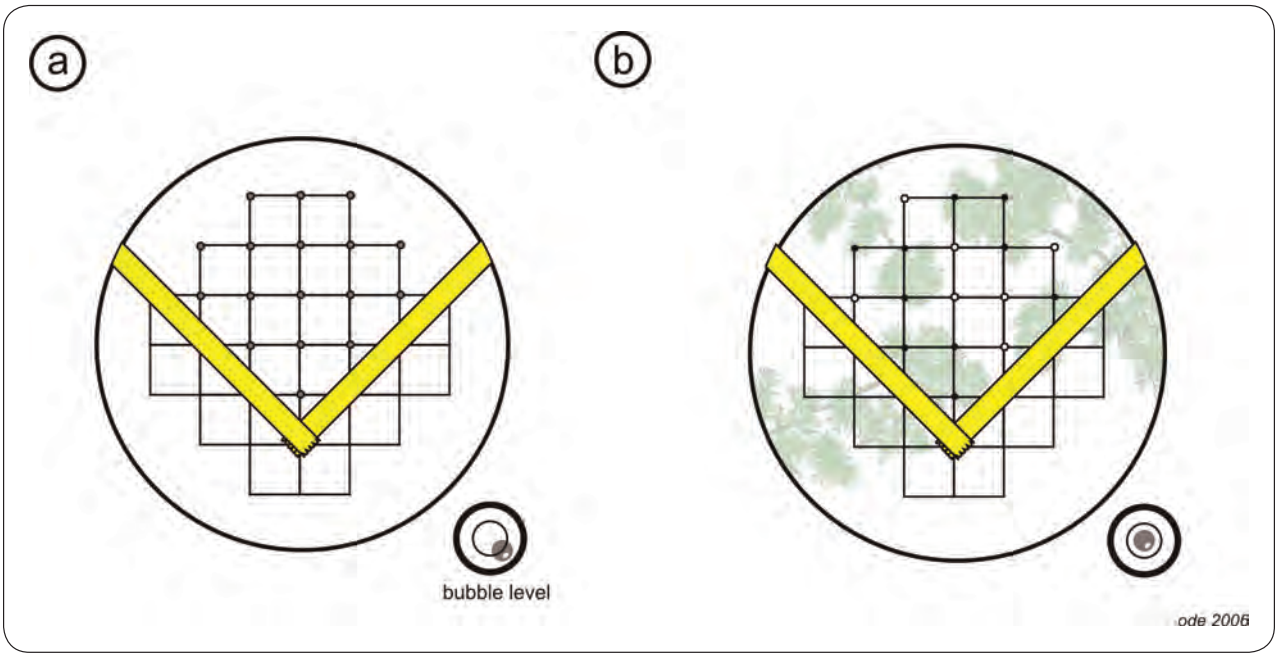


Figure 12. Representation of the mirrored surface of a convex spherical densiometer showing the position for taping the mirror and the intersection points used for the densiometer reading. The score for the hypothetical condition in (b) is 10 covered intersection points out of 17 possible. Note the position 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).

PHYSICAL HABITAT INTER-TRANSECT-BASED MEASUREMENTS 7

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.

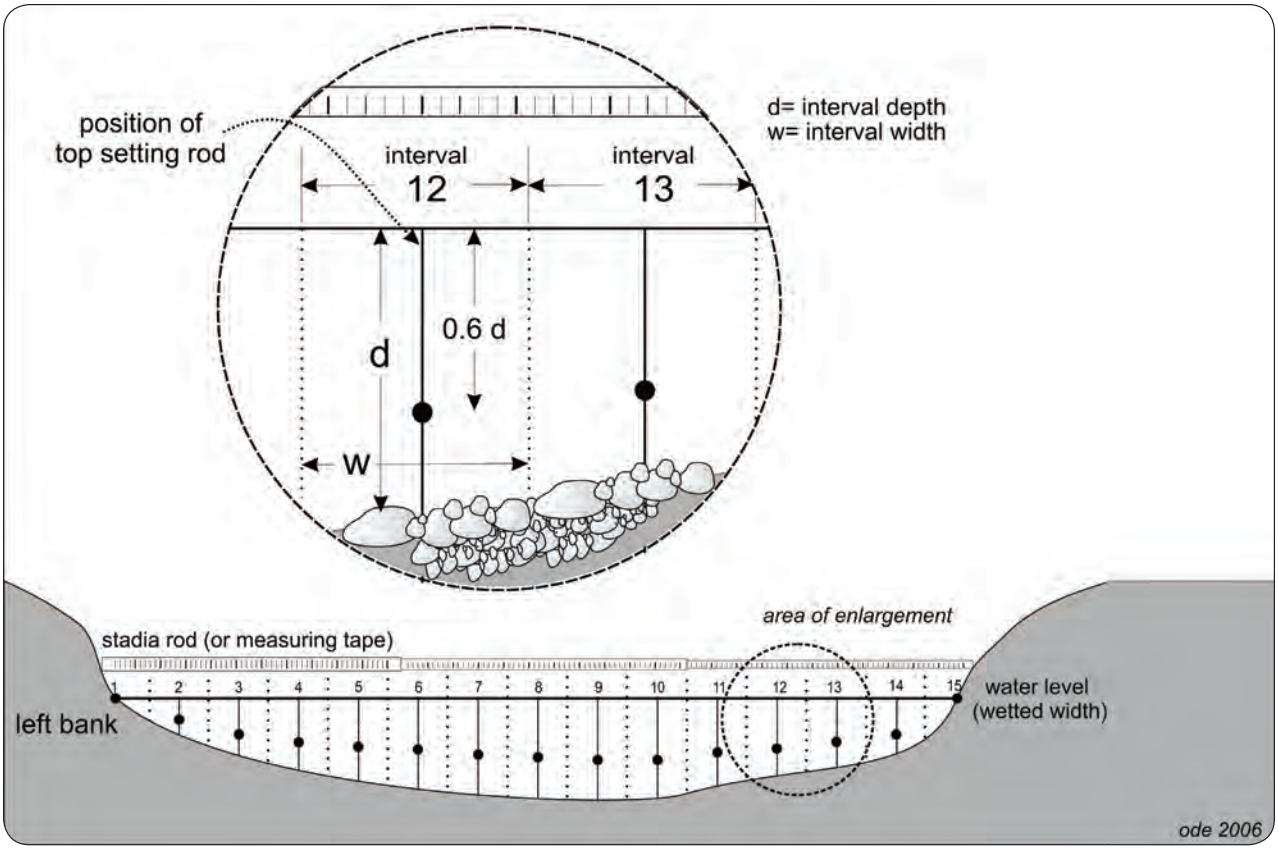


Figure 13. Diagram of layout for discharge measurements under the velocity-area method showing proper positions for velocity probe (black dots; reprinted from Ode 2007).

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.

REFERENCES

Davies A.L. and J.H.R. Gee. 1993. A simple algae sampler for algal biomass estimates in streams. *Freshwater Biology* 30: 47-51.

Fetscher, A.E. and K. McLaughlin. 2008. Incorporating Bioassessment Using Freshwater Algae into California's Surface Water Ambient Monitoring Program (SWAMP). Prepared for the California State Water Resources Control Board. California State Water Resources Control Board, Sacramento, CA.

Kelly, M.G., A. Cazaubon, E. Coring, A. Dell'Uomo, L. Ector, B. Goldsmith, H. Guasch, J. Hürlimann, A. Jarlman, B. Kawecka, J. Kwandrans, R. Laugaste, E.-A. Lindstrøm12, M. Leitao, P. Marvan, J. Padis´ak, E. Pipp, J. Prygie, E. Rott, S. Sabater, H. van Dam, and J. Vizinet. 1998. Recommendations for the routine sampling of diatoms for water quality assessments in Europe. *Journal of Applied Phycology* 10: 215–224.

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.

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.

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.

Round, F.E. 1991. Diatoms in river water-monitoring studies. *Journal of Applied Phycology* 3:129-145.

Stevenson, R.J. and L.L. Bahls. 1999. Periphyton protocols. Chapter 6 in: M.T. Barbour, J. Gerritsen, B.D. Snyder, and J.B. Stribling (eds.), Rapid bioassessment protocols for use in streams and wadeable rivers. Second Edition. EPA/841-B-99-002. US Environmental Protection Agency, Office of Water. Washington, DC.

Stevenson, R.J. and S.L. Rollins. 2006. Ecological assessments with benthic algae. *In*: Hauer, F.R. And G.A. Lamberti, eds. *Methods in Stream Ecology*. Pp. 785-803.

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.

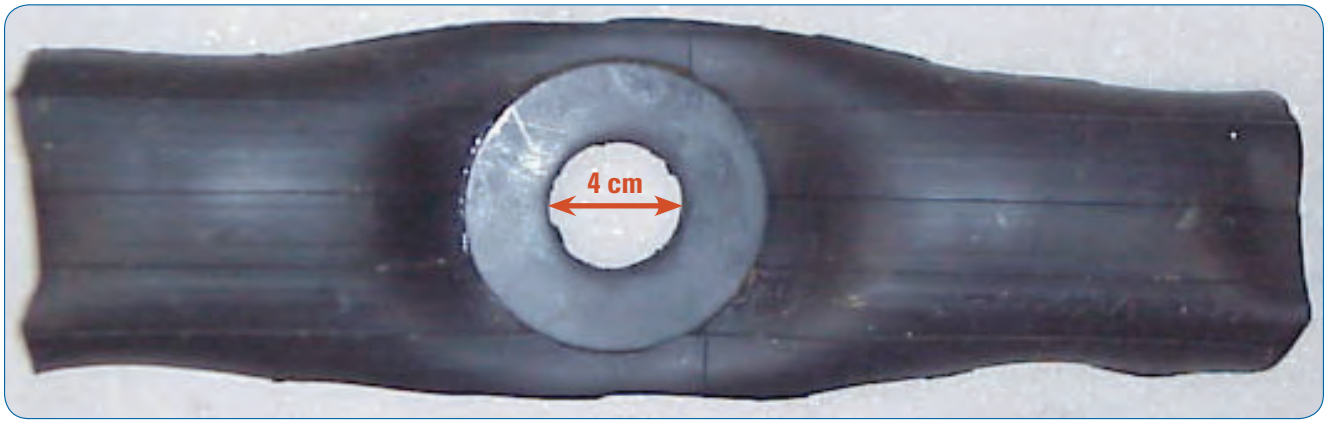
Schisler, G.J., N.K.M. Vieira, and P.G. Walker. 2008. Application of Household Disinfectants to Control New Zealand Mudsnails. North American Journal of Fisheries Management 28:1171-1176.

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.

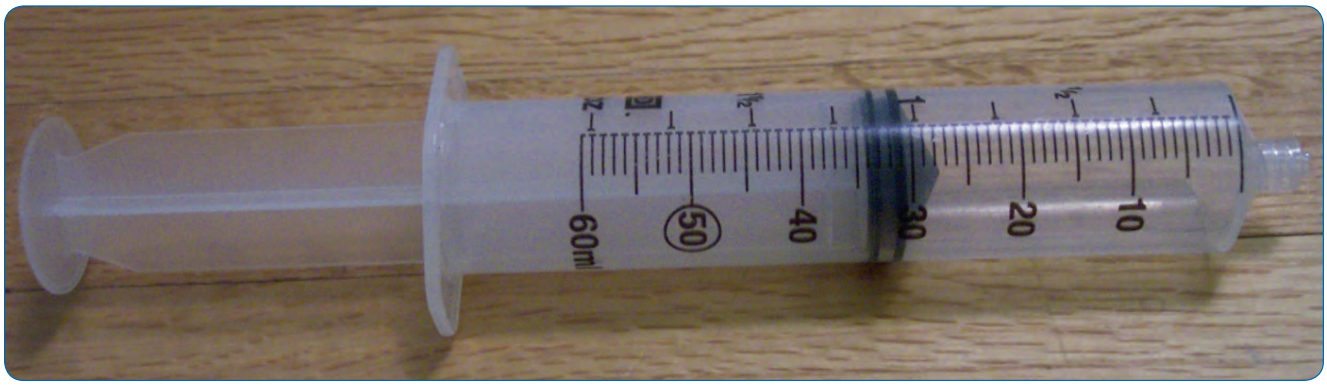


2. PVC DELIMITER

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.



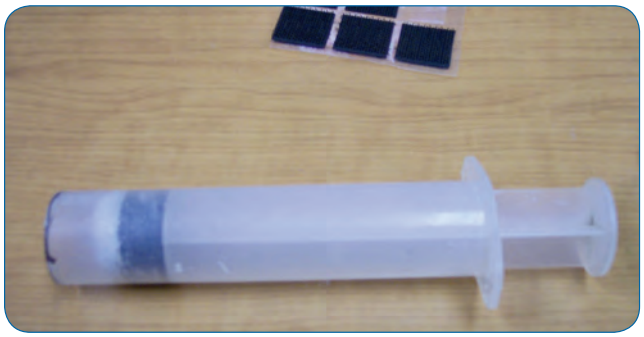
You will need a 60-mL plastic syringe for each sampler you want to make. Remove the plunger and saw the conical end off the plastic syringe, then sand the bottom so it is flat all the way around and fits tightly against a flat surface.

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.

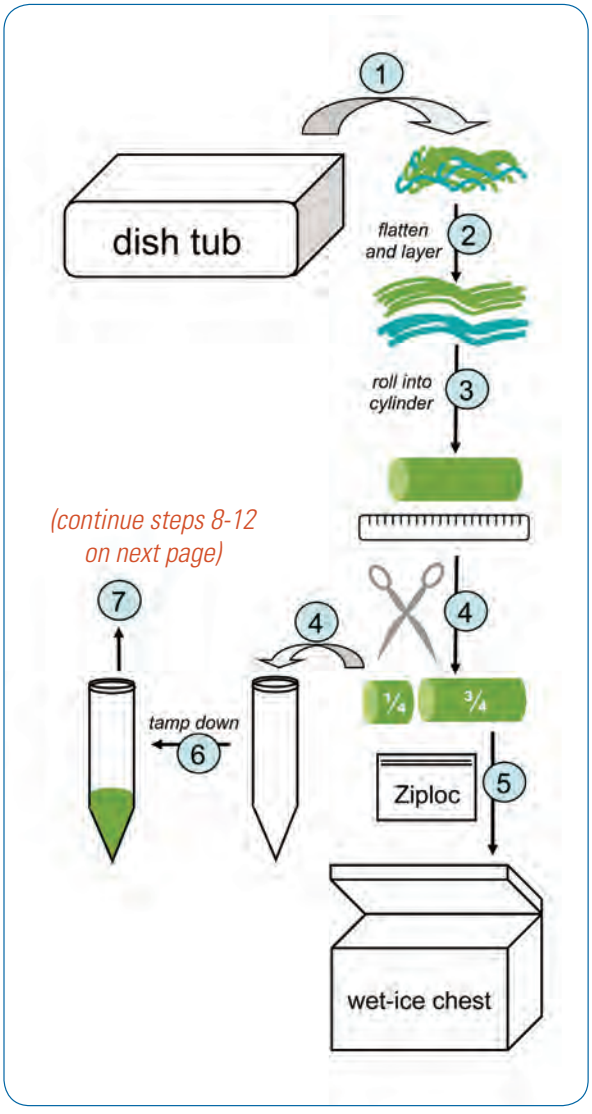
REFERENCE

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.

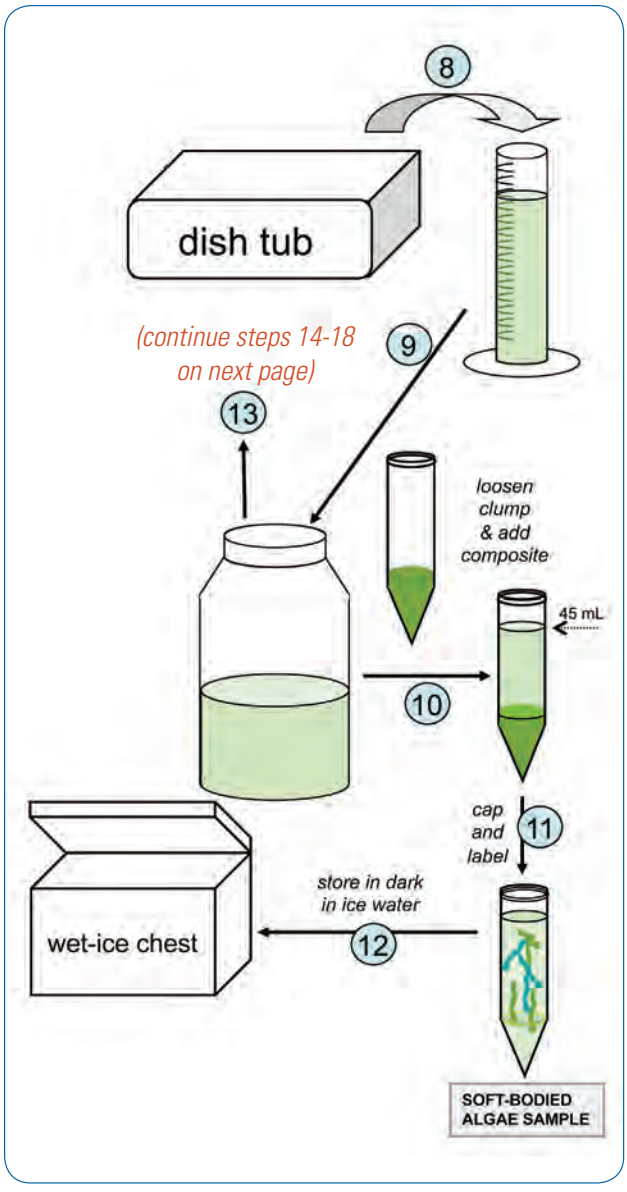
4) Measure and cut off ¼ of the cylinder and place that piece into the empty soft-bodied algae taxonomic ID sample tube.

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

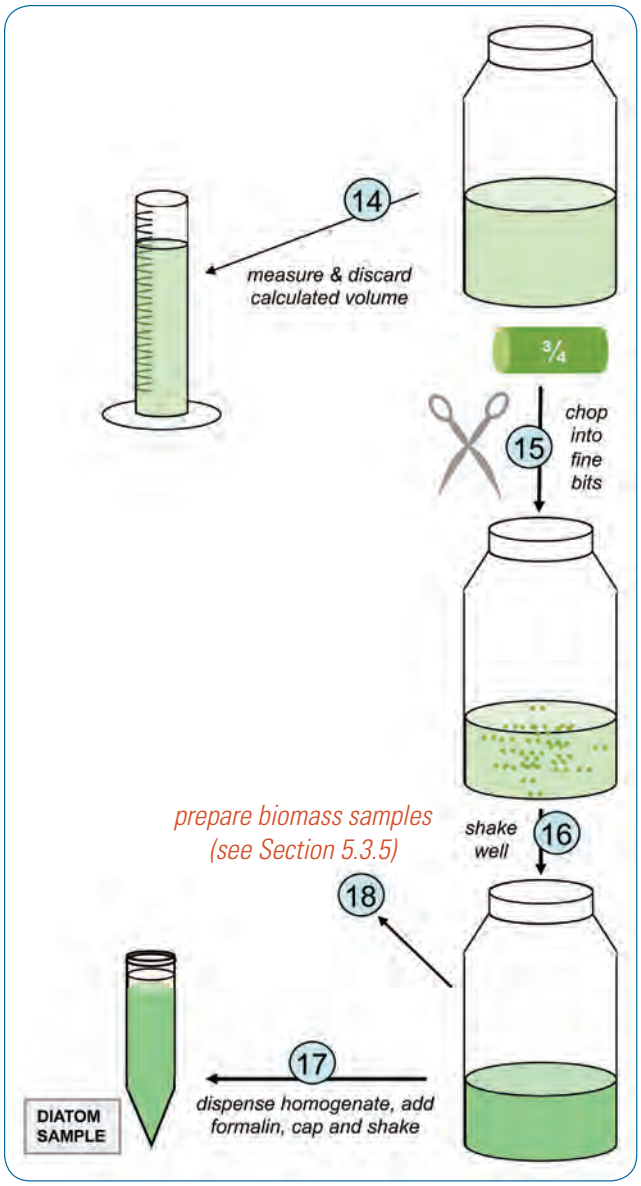
10) Loosen the macroalgae in the sample tube a little so it is no longer a dense clump lodged in the bottom and then pour freshly-agitated composite liquid into sample tube up to the 45 mL mark.

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

CALIFORNIA
Water Boards

www.waterboards.ca.gov/water_issues/programs/swamp/

Southern California Bight
2008 Regional Marine Monitoring Survey
(Bight'08)

Estuarine Eutrophication Assessment Field Operations Manual

Customized for the Ventura River Estuary Algae TMDL Comprehensive Monitoring Plan

Customization Prepared By:
Ventura County Watershed Protection District

Version 9

Prepared by:
Bight'08 Coastal Ecology Committee
Wetlands Sub-Committee

Prepared for:
Commission of Southern California Coastal Water Research Project
3535 Harbor Blvd, Suite 110
Costa Mesa, CA 92626

DRAFT

January 14, 2009 (Modified for Algae TMDL June 27, 2014)

SOUTHERN CALIFORNIA BIGHT 2008 EUTROPHICATION ASSESSMENT

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

II. OVERVIEW OF FIELD SURVEY

D. Definitions

The definition of estuary used to identify the target population follows that of the State Water Resources Control Board:

> ***ESTUARIES*** *are waters, including coastal lagoons, located at the mouths of streams that serve as areas of mixing for fresh and ocean waters. Coastal lagoons and mouths of streams that are temporarily separated from the ocean by sandbars shall be considered estuaries. Estuarine waters shall be considered to extend from a bay or the open ocean to a point upstream where there is no significant mixing of fresh water and seawater.*
>
> ***SEGMENT*** *is a subsection of an estuary that is hydrogeologically distinct from other subsections (e.g. segments may have different water residence times, different freshwater sources, etc.). For very small estuaries, the entire system represents one segment; however, for larger, more fragmented systems, the estuary can be divided into several segments. Symptoms of eutrophication may vary between estuarine segments.*

Estuarine classes designated in the eutrophication assessment are defined as follows (EPA 2007):

> **Seasonally Tidal Lagoon**- These estuaries are dominated by shallow subtidal and intertidal habitat, with a long residence time due to a seasonally restricted width of mouth or mouth closure. They support fresh to brackish submerged aquatic vegetation and emergent marsh for part of the year when the mouth is closed.
>
> **River Mouth Estuary**- This class of estuaries is the terminus of high flow, perennial river systems as they enter the coast. The estuarine portion is the mixing zone at the mouth of the river. These systems are characterized by 1) ebb-dominated flows, 2) estuarine mixing zone found within the channel during dry season, and 3) continuous disturbance of flats discourages growth of emergent vegetation during average flow years

VI. PRIMARY PRODUCER COMMUNITIES

A. Purpose and Approach

Aquatic primary producer communities targeted for this survey include macroalgae and phytoplankton.

Macroalgae and water column phytoplankton will be measured in three transects within the estuary. Optimally, these transects may be located in the same area of the estuary. Decision on the precise locations will depend on existing data on the presence and distribution of each primary producer type. Water column samples will be collected in conjunction with the transect sampling. Monitoring primary producer biomass typically requires 2 people.

B. Sample Schedule

Surveys should be scheduled around the low tide during the spring tides during the day light hours when the maximum amount of mudflat area is exposed.

C. Location of Primary Producer Transects

General Guidance
In general, the location of the primary producer transects will be driven by the goal of capturing spatial variability in biomass and/or percent cover. The three primary producer transects should be spaced throughout the system along spatial gradients. There are three major spatial gradients of interest within an estuary that should be considered:

- Salinity gradient/Hydrology
- Grain size
- Nutrient loading

The observed gradients in these three variables may co-occur or be diametrically opposed.

Macroalgal Transects
Within each index area, three transects must be laid out in the intertidal area for macroalgae biomass and percent cover, parallel to the water's edge and along the same elevational contour. These transects should each be 30 meters long, unless the total length of the bank is less than 50 meters in which case each transects should be 10 meters long. Areas with extensive and accessible mudflats where algae is known to accumulate should be targeted. If wind conditions consistently blow algae to one particular section of the estuary, transect locations should target this area. Areas with sensitive habitat (nesting grounds) and cut-banks should be avoided. Transects should be laid out during spring-low tides (when mudflat area is most exposed), approximately ¾ of the distance upslope from the water's edge at the mean lowest low water line (MLLW), approximately 1 to 2 feet above MLLW. To establish this transect as a permanent area, the distance to the start of the transect from the edge of the emergent vegetation (demarcated by a permanent stake of grey PVC pipe) should be measured

and recorded. If water-levels in seasonally tidal system exceed 30 cm at the transect location, sampling should be aborted or rescheduled. Transects should be located to capture the major areas of macroalgal accumulation. Macroalgal mats should recover between sampling events so transect sites can be the same for each sampling period. Sampling of this area has been demonstrated to be representative of macroalgae on intertidal channels and mudflats (Kennison et al. 2003).

Water Column Chlorophyll a
Samples for water column chlorophyll *a* and ambient nutrients should only be collected at one transect in each estuary (transect nearest to data logger location). These samples should be collected from the surface that is approximately 30 cm depth, co-located near the end of the macroalgae transects.

Floating Macroalgae
In systems where floating macroalgae is present (defined for this study as algae that is floating on the surface to a depth of ~5 cm, algae may be attached to the sediment or extend to a depth greater than 5 cm~~.; however the floating macroalgae sample will be cut at 5 cm depth), p~~ Percent cover ~~and biomass~~ will be estimated co-incident with the water column sampling in water that is 30 cm deep. Percent cover ~~and biomass~~ will be estimated from two quadrats floated on the surface at each water column sampling locations (typically down-slope of the start and end of the macroalgae transect line).

D. Recommended Sampling Order

The following sampling order is recommended in order to minimize disturbance of samples:

1. Macroalgae transect (~~biomass and~~ percent cover)
2. Water column sampling (oceanward end, transect 1 only)
3. Floating algae sampling at sediment sampling location
4. Water column sample processing

Every effort should be made to process the water sample as quickly as possible. The transect from which the water column sample is collected should be the last transect conducted and water should be processed as quickly as possible after collection to minimize biological alteration of the sample in the bottle.

E. Macroalgae Transects, Sediment and Water Column Sampling

Transects for macroalgae percent cover ~~and biomass~~ (on the mudflats), floating macroalgae percent cover ~~and biomass~~ (not attached to the sediment and floating on the water surface), and water column chlorophyll *a* will be co-located. See Figures 1 and 2 for how transects and sampling locations should be laid out.

Transects for macroalgae will be located on the mid- to upper-mudflat. Water column sampling will be located down-slope of the macroalgae transects in the subtidal area. Floating

macroalgae sampling will be co-located with water column sampling when floating algae is present.

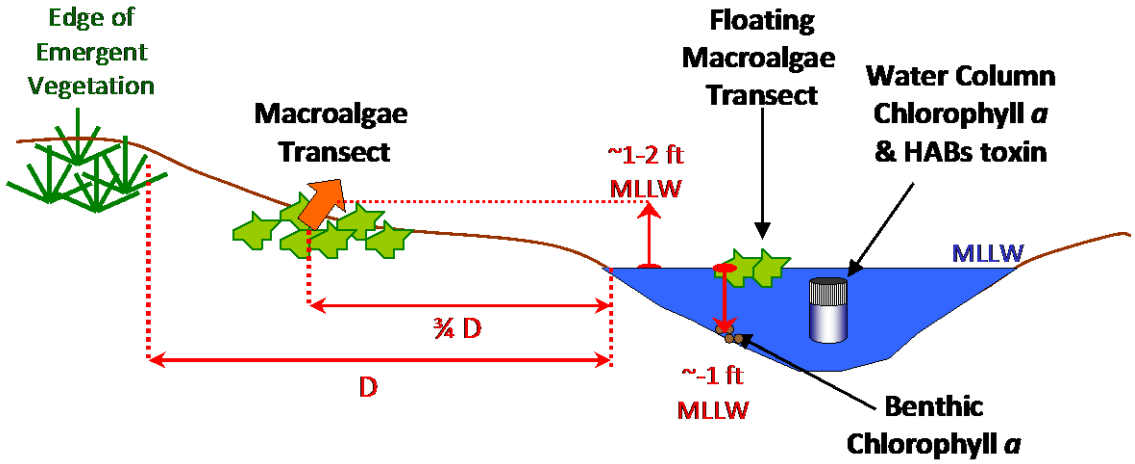
Macroalgae transects will be the same for each sampling period. A piece of grey PVC pipe will be inserted into the emergent vegetation and the distance from the pipe to the start of the macroalgae transect will be recorded and used to find the location from month to month. The location of water collection is not fixed relative to the PVC piping. Water should always be collected in water that is 30 cm (1 ft) deep. Mark on the data sheet where the sampling site is with respect to the grey PVC pipe. For seasonally tidal estuaries, when water-level within the estuary rises when the mouth is closed, every effort should be made to return to the same macroalgae site. If the macroalgae transect is under water, proceed with collection and record the depth of water at each site along the transect. If water levels rise above 30 cm at the macroalgae transect site, sampling should be aborted or rescheduled.

If no macroalgae is present at the transect site, but is present on the mudflat above or below the transect line, do not move the transect to accommodate the location of the macroalgae. Note in the comments box that algae was present along the mudflats but not on the transect.

Equipment

- General equipment:
  - Hand-held GPS unit (accurate to sub-meter)
  - Hand-held temperature/salinity meter
  - Transect tape
  - Short ruler
  - Digital camera (Q-p Card, Length Scale)
  - Data sheets, clipboard, and pencil
    - Water Column Data Sheet
    - Macroalgae Transect Data Sheet
  - Boots, waders or wet suit
  - Breakfast Table and Bench Paper
  - Gloves
  - Cooler and ice
  - Chain of Custody Form
  - QA checklist
- Macroalgae ~~biomass and~~ percent cover:
  - Quadrat- 0.5 m by 0.5 m PVC pipe strung with two orthogonal sets of 7 equally spaced taut strings
  - ~~Biomass surface area delineator (4 inch diameter plastic cylinder) and end cap~~
  - ~~Scissors or shears~~
  - ~~Labels, sample bags, and sharpie~~
- Water column chlorophyll *a* and nutrient samples:
  - Hand-held temperature/salinity meter
  - 1 liter amber sample bottle (or sample bottles supplied by laboratory)
  - Snap-closure Petri dishes with labels and sharpie

- Aluminum foil
- Whatman GF/F filters (47mm)
- Squirt bottle filled with DI water
- Forceps
- 250 mL plastic graduated cylinder
- Filter apparatus
- Hand pump
- 1 liter bottle of double-distilled water for blanks
- Luer-lok 60 mL syringes
- 0.45 µm MCE filters for dissolved nutrients and TDN/TDP
- 1 dissolved nutrients bottle, 1 TDN/TDP bottle, 1 TN/TP bottle
- Bottle tape



- Aluminum foil

Figure 1. Field view of macroalgae biomass and percent cover transects and sampling for water column chlorophyll *a* [Note: Figure includes constituents that are not part of the Ventura River Algae TMDL CMP, e.g. benthic chlorophyll *a* and HABs toxin].

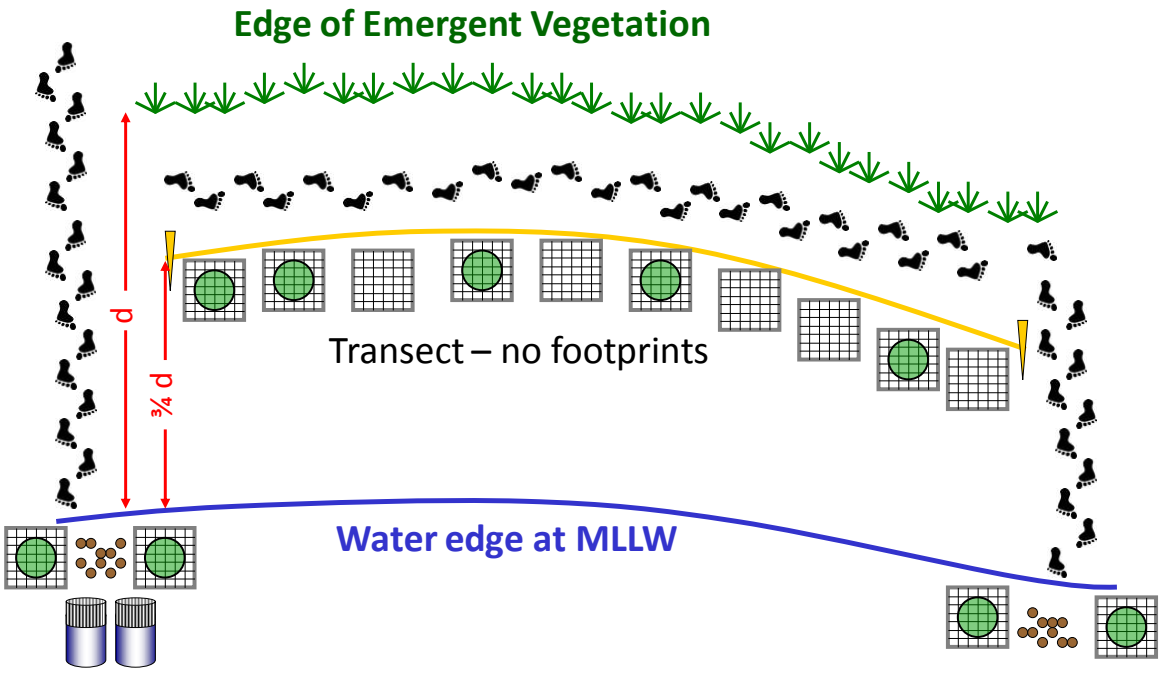


Figure 2. Plan view of an example of ~~how~~ macroalgae biomass and percent cover transects and location for sampling for water column chlorophyll *a* [note: ~~[and~~ sediment samples~~chlorophyll *a*, grain size and nutrient content, which~~ are not part of the Ventura River Algae TMDL CMP]. Two water column samples should be collected at the oceanward end of transect 1 only. Floating macroalgae will only be assessed when present.

Table 8. Distances along macroalgae transect for percent cover quadrats and biomass sample collection for each month. (Distances from random number generator)

May										
Quadrat #	1	2	3	4	5	6	7	8	9	10
30 m - distance along transect (m)	2.2	3	4.3	6.4	7.3	9.3	17.5	18.6	25.5	27.3
10 m - distance along transect (m)	0.5	1.4	2.7	3.3	4.9	5.7	6.2	7.3	8.3	9.2
Biomass Collection	X		X			X		X		X
June										
Quadrat #	1	2	3	4	5	6	7	8	9	10
30 m - distance along transect (m)	0.6	3.9	5.6	6.1	9.6	11.7	13	22.1	23.7	29.9
10 m - distance along transect (m)	0.9	1.2	1.8	3.7	4.5	6.1	6.6	7.6	8.3	9
Biomass Collection			X		X		X	X	X	
July										
Quadrat #	1	2	3	4	5	6	7	8	9	10
30 m - distance along transect (m)	4.7	8.4	9.8	14.7	16.2	17.6	26	27.7	28.7	29.8

10 m - distance along transect (m)	1.7	2.2	2.7	3.8	4.6	5.2	6.6	7.6	8.1	10
Biomass Collection	X	X		X	X				X	
August										
Quadrat #	1	2	3	4	5	6	7	8	9	10
30 m - distance along transect (m)	5.4	6.9	9.6	12.3	17.3	18.2	20.3	22.4	23.9	28.6
10 m - distance along transect (m)	1	1.5	2	3.7	4.5	5.7	6.4	8	8.5	9.4
Biomass Collection	X			X			X		X	X
September										
Quadrat #	1	2	3	4	5	6	7	8	9	10
30 m - distance along transect (m)	0.7	7.1	8.9	13.3	17.4	20.7	23.8	25.3	26.7	28.2
10 m - distance along transect (m)	1.2	1.9	3.5	4.3	4.8	6.3	7.7	8.5	9	9.8
Biomass Collection	X	X	X	X						X

Approaching the sites:

- Transect locations and routes to the sampling are to be determined by the Field Crew prior to the first day of sampling, as changes in Ventura River flow and sand berm status may affect transect location.
    - Transects will be 30 meters long unless the total length of the bank in the estuary is less than 50 meters in which case transects will be 10 meters long.
- Approach general area for the first transect by foot or boat.
- Stop when you are ~1.5 meters from the start of the transect.
- Take care not to leave any footprints within the designated transect.
- At the edge of the emergent vegetation stake in a small piece of grey PVC pipe and record the GPS coordinates on the data sheet.
- Using guidance in Figure 2, determine the location of the transect with respect to the edge of the emergent vegetation (and PVC pipe)
    - The start of the macroalgae transect should be located approximately ¾ of the distance upslope from the water's edge at MLLW. Typically, approximately 1 m from the edge of emergent vegetation and approximately 1 to 2 feet above mean lowest-low water, on the mid- to upper- mudflat (Figures 1 and 2). Actual dimensions may vary depending on estuarine geomorphology.
- Record the GPS coordinates for the start of transect on the Data Sheet. Transect start and end points must be recorded with a GPS unit. Also record the distance in meters from the PVC pipe.
- The oceanward location of each transect is designated as distance 0 m along the transect.
- Stake one end of the transect tape in the mud ~0.25 m upslope from where you intend to lay your quadrats.
- Carefully lay the transect tape out to 30 m or 10 m (depending on the total length of the bank greater than 50 m or less than 50 m respectively) and stake in the landward end.
- When laying out the transect, only leave footprints on the up-slope side.
- Take care not to leave any unnecessary footprints between the transect line and the water's edge (see Figures 1 and 2).

- Once the tape is laid out, take a digital photograph of the transect site from the oceanward end.

Sampling at the oceanward location:
Sampling for ambient nutrients and water column chlorophyll *a* will only occur at **one** of the three macroalgae transect locations (the transect nearest to the data logger location). The transect at which the water sample is collected should be done last to minimize the amount of time the water sample sits before being processed. Sampling for suspended chlorophyll *a* can occur either before or after the macroalgae percent cover ~~and biomass~~ sampling depending on when the tide is lowest. Water samples can be collected into an amber HDPE bottle and placed on ice and filtered at a later time (but not more than 2-3 hours after collection). It can take between 5 and 20 minutes to filter a single sample depending on how much suspended material is in the water column.

- From the 0 meter end of the transect tape (downstream/ocean-ward end), walk down to the edge of the water.
- Put on Nitrile gloves.
- Wade to a water depth of 30 cm.
- Record the local temperature and salinity on the data sheets using a hand-held meter.
- Fill one 1-liter amber high-density polyethylene bottles (HDPE) full of surface water at ~10 cm depth from the surface for surface water chlorophyll *a*.
    - Try not to disturb the sediments when you are collecting water, water should not be overly turbid when you collect the water column samples.
    - Place closed pre-labeled, 1 Liter Amber HDPE sample bottle under the water surface and open the bottle underwater at approximately 10 cm (one hand span) below the surface.
    - Close the bottle underwater and bring to surface.
    - Shake and discard water downstream (this is your first rinse).
    - Fill, rinse and discard stream water three times total.
    - After the third rinse, open bottle under water, fill, close underwater and return the bottle to surface.
    - Chlorophyll *a* and ambient nutrient samples should be collected during every primary producer sampling survey.
- Conduct floating macroalgae surveys as described below.
- Take the water sample bottles up to the edge of the vegetation.
- Find a shady place to begin filtering the water column samples or store water on ice until samples can be cleanly filtered.

Filtering for Water Column Chlorophyll a

- Wear gloves when filtering for chlorophyll *a*.
- Take the water column sample bottles back to the bank to filter the samples.
    - The procedure to filter chlorophyll samples should be carried out within 6 hours of sample collection, and in the shade as much as possible, to minimize exposure of the sample to light, which degrades chlorophyll.

- Chlorophyll *a* will be measured in duplicate for 10% of samples, so the procedure below will be conducted twice in June of odd years (e.g. 2015, 2017, 2019 etc.) to provide two filters total.

- Open up the breakfast tray and put on gloves
- Rinse filter apparatus three times with a small amount of DI water
- Using clean filter forceps, center a glass-fiber filter (Whatman GF/F) onto the mesh platform of a clean filtering tower apparatus.
  - Avoid touching the filters with hands or anything other than clean forceps.
- Start with the blanks
  - Rinse graduated cylinder 3 times with distilled-deionized blank water
  - Carefully measure 250 mL of distilled-deionized water into a clean graduated cylinder
  - Pour the blank water into the filter reservoir and place the reservoir cap back on the tower top.
  - To filter the sample, create a gentle vacuum with the hand pump (300-400 mm/Hg).
  - Keep up the vacuum until all the water has filtered through
  - Break the vacuum by removing the pump
  - Unscrew the reservoir from the base
  - Use forceps to fold the filter in half on the mesh screen
  - Remove the filter and place it inside a clean, labeled, snap-top Petri dish.
  - Place the petri dish inside a small square of aluminum foil, label the outside of the foil as well.
  - Fill in all required information on the labels. This is the field blank (FB)
  - Note on the data sheet that a field blank was collected (when relevant).
  - Discard the filtered blank water.
- Once the blank has been collected you can start filtering sample water.
- Rigorously shake the sample bottle and rinse the graduated cylinder with a small amount of sample water (repeat for three rinses total); discard rinse water
- Carefully measure 250 mL from the sample using the rinsed graduated cylinder.
- Pour the measured sample into the filter reservoir and place the reservoir cap back on the tower top.
- To filter the sample, create a gentle vacuum with the hand pump (300-400 mm/Hg).
  - Proceed slowly, careful not to exceed 400 mm/Hg on the hand pump gauge, until all of the liquid in the sample is passed through the filter.
  - If it becomes impossible to filter all 250 mL of the sample and remove the water efficiently, discard the filter and try again with a smaller volume (*e.g.*, 100 mL).
  - If there is no color on the filter and the water passes through the filter easily, try increasing the volume to 500 mL to insure sufficient pigment for analysis.
- The filter should be left slightly moist, in order to avoid applying excessive pressure to the sample, which could cause algal cells to burst and consequent loss of chlorophyll.
- Unscrew the reservoir from the base

- Use forceps to fold the filter in half on the mesh screen so that no biomass will fall off the filter
- Remove the filter and place it inside a clean, labeled, snap-top Petri dish.
- Place the petri dish inside a small square of aluminum foil, label the outside of the foil as well
- Fill in all required information on the labels. This is replicate #1.
- Check on the Data Sheet that the samples were collected
    - Chlorophyll
    - Total volume sampled (volume should be recorded on the sample label and on the data sheet).
- Place the Petri dish into the ice in a cooler.
- Rigorously shake the sample bottle again and measure out the volume for your duplicate chlorophyll samples.
- Rinse top part of the filter apparatus with DI water and begin to filter the duplicate sample from the same bottle of sample water as directed above. This sample is Replicate #2.
    - 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.
- Discard remaining filtrate.
- Place the filters and sample bottles on ice in the dark.

Filtering samples for nutrient analysis

- Wear gloves.
- Start with the Field Blanks (FB)
    - Open Fisher Brand MCE 0.45 μm filter (or equivalent)
    - Pull plunger out of a clean 60 mL syringe
    - Affix MCE filter on the end of the syringe
    - Fill the syringe will a few milliliters of distilled deionized water (DDI) and rinse the syringe and the rubber stopper of the plunger. Rinse a total of three times.
    - Fill the syringe with DDI water
    - Put the plunger back in the syringe, take off the filter to push out the air trapped in the syringe.
    - Push ~10 mL through the filter and discard the rinse water
    - Open the TDN/TDP field blank (FB) bottle
    - Rinse the bottle and the cap three times with ~5 mL of blank water, discarding the rinse water each time
    - Fill TDN/TDP FB bottle no more than 2/3 full with blank water.
    - Open the dissolved nutrients FB bottle
    - Rinse three times with blank water, discarding rinse water each time
    - Fill dissolved nutrients FB bottle no more than 2/3 full with blank water.
    - Refill the syringe with DDI water as necessary
    - Remove the MCE filter from the syringe

- Open the TN/TP sample bottle
- Rinse the TN/TP bottle 3 times with unfiltered DDI blank water, discarding the rinse each time
- Fill TN/TP bottle no more than 2/3 full with blank water.
- Store all the field blanks on ice in the dark.
- Note on the Data Sheet that the blanks were collected.

- You can now start preparing the nutrient samples.
  - Rigorously shake amber water column sample bottle.
  - Open Fisher Brand MCE 0.45 μm filter (or equivalent)
  - Pull plunger out of a clean 60 mL syringe
  - Affix MCE filter on the end of the syringe
  - Put a few milliliters of sample water into the syringe, rinse and discard rinse water; repeat for a total of 3 rinses
  - Rinse the rubber stopper end of the plunger with sample water too.
  - After the third rinse, fill syringe with sample water from the sample bottle
  - Put the plunger back in the syringe
  - Remove the filter (do not touch the end of the filter where the water comes out) and push the plunger into the syringe all the air is expelled.
  - Put the filter back on the syringe.
  - Push approximately 10 mL through the filter discarding this water to rinse the filter.
  - Open a 30 mL HDPE bottle pre-labeled for TDN/TDP (total dissolved nitrogen and total dissolved phosphorus)
  - Expel 5-10 mL of sample water into the bottle, rinse bottle and cap, discard rinse water; repeat for 3 rinses total
  - Slowly add ~15-20 mL of water into the TDN/TDP bottle.
    - Bottle should only be no more than two-thirds full. This is necessary because water will expand when frozen.
  - Cap tightly, record all information on the sample bottle label and data sheet
  - Cover label with plastic tape, and place in the cooler on ice.
  - Remove filter from syringe and refill syringe with sample water following procedure above.
  - Attach a new MCE filter to the end of the syringe
  - Push approximately 10 mL through the filter discarding this water to rinse the filter.
  - Open a 30 mL HDPE bottle pre-labeled for dissolved nutrients (or other size as needed by laboratory)
  - Expel 5-10 mL of sample water into the bottle, rinse bottle and cap, discard rinse water; repeat for 3 rinses total
  - Slowly add ~15-20 mL of water into the nutrient bottle.
    - Bottle should be no more two-thirds full. This is necessary because water will expand when frozen.
  - Cap tightly, record all information on the sample bottle label and data sheet
  - Cover label with plastic tape, and place in the cooler on ice.

- Remove filter from syringe and refill syringe with sample water
- You do not need to open a new filter to add water to the syringe, reuse the MCE filter from the nutrient sample.
- Remove the filter from the end of the full syringe.
- Open a 30 mL HDPE bottle pre-labeled for TN/TP (total nitrogen and total phosphorus)
- Expel 5-10 mL of UNFILTERED sample water into the bottle, rinse bottle and cap, discard rinse water; repeat for 3 rinses total
- Slowly add ~15-20 mL of unfiltered water into the TN/TP bottle.
    - Bottle should only be no more than two-thirds full. This is necessary because water will expand when frozen.
- Cap tightly, record all information on the sample bottle label and data sheet
- Cover label with plastic tape, and place in the cooler on ice.
- Discard any remaining water in the amber bottles.
- Needed volume may vary depending on requirements from laboratory.

Macroalgae percent cover ~~and biomass~~ transect:

- Macroalgae percent cover will be determined using the point intercept method.
- Ten distances along the transect tape have been randomly selected for percent cover analysis~~ and five of those same distances will be randomly selected for biomass collection~~.
    - Distances along the transect tape that should be used for each sampling month are provided in Table 8.
- At each designated location carefully lay the quadrat down parallel to the transect tape, with the center of the quadrat aligned with the distance designated, careful to place it where there have been no recent human footprints or disturbance.
- Record the location along the transect (in meters) on the Data Sheet.
- [NOTE: Recording the different species of macroalgae is not specified as a requirement by the TMDL. Only % cover is listed.
- Record ~~the type of~~algae cover directly below each intercept (crosshair) of the quadrat by placing a hash-mark in the appropriate box~~es~~ (Figure 3).
    - ~~Common categories are provided on the data sheet; include algae that has been rafted in on the tide (e.g. macrocystis).~~
        - If no algae is encountered, mark in the “absent” row
        - ~~Photos of commonly encountered species are provided in Appendix 7~~
    - ~~If the cover does not match one of the provided categories, use Other 1 and Other 2 and provide names or detailed descriptions.~~
        - ~~If you cannot identify the cover, after you have finished measuring the quadrat place a sample of it in a Ziploc bag and label it with the site ID, transect number, distance along the transect tape, quadrat number, and whether it was “other 1” or “other 2”.~~
    - ~~The first time any given macroalgae species is encountered, take a digital photograph of the sample (include in the frame of the image a metric scale bar~~

~~for size reference and a Q-Pac Card for color reference) and collect a sample into a pre-labeled Ziploc bag as a voucher specimen (the voucher specimen does not have to be from the quadrat)~~

- ~~Note on the sample bag, the species you believe it is and the photo number.~~
- ~~Fill in a water-proof label and place it into the sample bag with the biomass sample.~~
- ~~Store the voucher specimen samples on ice in the cooler and ship to SCCWRP with Biomass samples.~~
- Record data for each quadrat in the designated columns ~~1 through 10~~ on the Data Sheet.
  - ~~Primary producer cover underneath each intercept on the quadrat must be recorded on the Data Sheet.~~
  - The sum of hash-marks in each column must equal ~~at least~~ 49~~, totals above 49 are possible if more than one category is recorded for an intercept~~.

- ~~After data for percent cover is collected, record the average thickness (top of the mat to the sediment surface) of the macroalgae mat in centimeters using the short ruler.~~
- Record the general condition of the macroalgae on the field data sheet by circling the appropriate code on the data sheets:
  - Dead- for Desiccated/Dead
  - Frsh- for Fresh/Healthy
  - Int- for Intermediate
  - See Appendix 8 for comparison photos
- Carefully remove the quadrat from the mud.
- ~~Macroalgal biomass will be collected at five, randomly chosen quadrat locations designated in the percent cover section above.~~
  - ~~On the Data Sheet, if biomass is collected at a quadrat mark a "Y" in the biomass row, if biomass is not collected mark an "N". Not every site will be occupied by macroalgae during each sampling, so the actual biomass samples will be dependent on seasonal patterns.~~
  - ~~Where applicable, place the biomass delineator where the center of the quadrat had been.~~
  - ~~Quantitatively remove all biomass from within the delineator and place into a pre-labeled Ziploc bag. Use scissors to cut biomass inside the delineator away from biomass outside.~~
  - ~~Fill in a water-proof label and place it into the sample bag with the biomass sample.~~
- Once percent cover ~~and biomass (if applicable) have~~ has been ~~collected~~recorded, move the quadrat to the next designated distance and repeat until all 10 locations have been assessed.
- ~~Store all biomass samples in separate sample bags ice in a cooler.~~

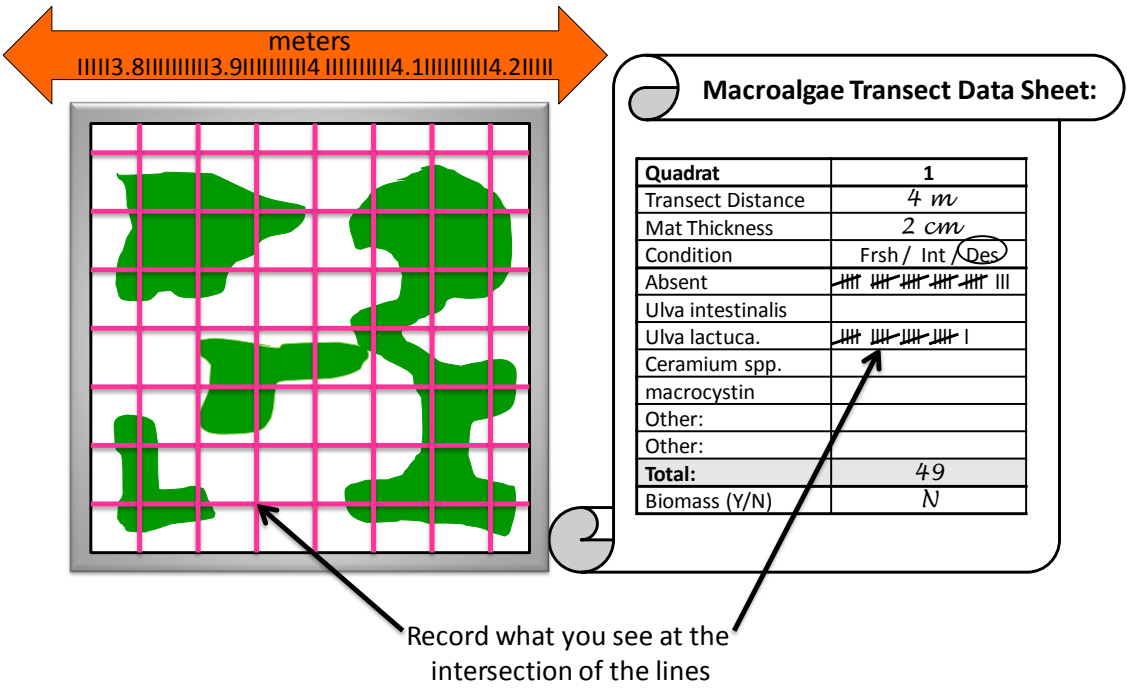
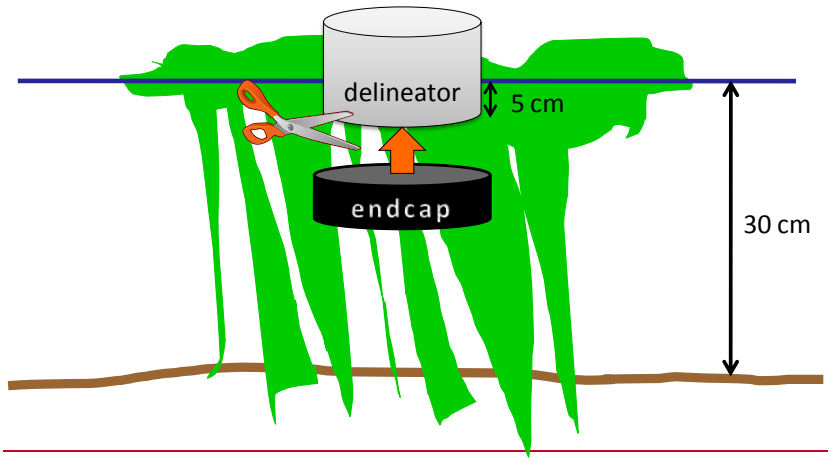


Figure 3. Record percent cover data from underneath the intersection of the two lines as hash-marks on the data sheet. ~~**Total hash-marks should be greater than or equal to 49.**~~ **[Note: VR Algae TMDL-specific data sheet does not require collection of all data (e.g. algae classification) included in this full protocol.]**

Floating Macroalgae

- If floating macroalgae is present in the system, percent cover ~~and biomass~~ will be assessed at the landward and oceanward locations in the subtidal area (co-located with water column sampling); if no floating algae is present, mark "absent" in the floating algae section of the data sheet. Floating algae can be assessed at the same time water is collected.
- Wade into the system to a depth of 30 cm.
- Standing on the approximate location where water column was sampled, turn to face ocean-ward (downstream) and float the quadrat on the surface of the water
- Floating algae for this study is defined as any algae that reaches the surface; it may be attached to the sediment or freely floating.
- ~~Record the type of cover directly below each intercept of the quadrat by placing a hash-mark in the appropriate boxes (Figure 3).~~
- Record data for each quadrat in the designated columns ~~1 through 4~~ on the Data Sheet.
    - Primary producer cover underneath each intercept on the quadrat must be recorded on the Data Sheet.
    - The sum of hash-marks in each column must equal at least 49, totals above 49 are possible if more than one category is recorded for an intercept.
- Record the general condition of the macroalgae on the field data sheet
    - Score condition as "Dead", "Fresh", or "Intermediate" as for the macroalgae transects

- ~~Macroalgal biomass will be collected each floating algae quadrat. Floating macroalgae biomass is defined here as algae floating on the surface collected to a depth of ~5cm (line on side of biomass delineator). Algae that extends deeper than 5 cm from the surface will be cut away from the "surface" biomass (Figure 4).~~
    - ~~Place the biomass delineator where the center of the quadrat had been.~~
    - ~~Cut surface biomass away from deeper biomass using scissors~~
    - ~~Place the endcap (screen on bottom) under the water surface away from the quadrat and bring up to the surface underneath the delineator~~
    - ~~Remove all biomass from within the delineator and place into a pre-labeled bag using scissors or shears to cut away biomass inside the delineator.~~
    - ~~Fill in a water-proof label and place it into the sample bag with the biomass sample.~~
- Once percent cover ~~and biomass have~~has been collected, turn and face the upstream (landward) direction and repeat the percent cover ~~and biomass~~ collection procedure~~s~~.
- Once both percent cover and biomass collection at two locations has been completed at the oceanward end, proceed to the landward end of the macroalgae transect and repeat for two quadrats.
- Do not assess floating macroalgae percent cover ~~and biomass~~ until ~~sediment and water~~all other ~~column~~ sampling has been completed.
- ~~Store all biomass samples separately on ice in a cooler.~~



~~Figure 4. Floating Macroalgae Collection Procedure~~

The end of the transect

- No water column samples need to be collected at the landward end of the transect.
- After collection of percent cover ~~and biomass~~ along the transect, conduct surveys of floating macroalgae percent cover ~~and biomass~~ as described above.
- At the end of the transect, take a digital photograph of the transect area.

~~*How to Deal with Obvious Vertical Zonation of Macroalgae Communities*~~

- ~~For estuaries with obvious vertical zonation of algae along the mudflat, maintain the established protocols as described above but mark "Y" in the appropriate box on the field sheet to note the existence of zonation.~~
- ~~If time permits in the field do a semi-quantitative analysis of the zonation:~~
  - ~~Starting at the edge of emergent vegetation and moving perpendicular to the edge of the water note the distance to the first zone~~
  - ~~Estimate the relative distance spanned by the band of macroalgae and identify the species of algae.~~
  - ~~Estimate the distance to the next band of algae and identify the species and the relative distance spanned by the band~~
  - ~~Continue until you reach the water's edge.~~
  - ~~Note the "band" sampled in the macroalgae data sheet and its relative position in the zonation.~~

At the End of the Sampling

- A rough sketch of macroalgae cover (both floating and on the mudflats) should be drawn on ~~the backs of~~ each field data sheet); note on the sketch if floating algae was rafted to a location outside the designated transect areas and/or if winds have pushed floating algae to one side of the estuary.
- Field leader should check that the data sheets have been completely filled out and should sign the bottom of the sheets.
- Field leader should check the cooler to ensure that all samples have been collected and placed on ice.
- Check the number of samples against the Chain of Custody form.
- Prepare the Chain of Custody form, sign and date, for delivery to the laboratory at the end of sampling.

G. Data Sheets

Example data sheets for the macroalgae transects are given in ~~Appendix 4~~VR Algae TMDL CMP Appendix B. A new data sheet should be used for each new transect. All assessments for a single transect can be recorded on the same data sheet. Record date and time of sampling and all site observations. Use the comments box to record any observations or field notes.

H. Safety Issues

Due to safety concerns Primary Producer Community Assessment should be conducted in teams of two. If the mud is particularly unconsolidated, one team member must remain on vegetated or consolidated sediment. In the event that one team member is stuck, the second should be in position to throw a line, etc.

To prevent the introduction of New Zealand mudsnail or other invasive species, follow the protocols outlined in ~~Appendix 6~~VR Algae TMDL CMP Appendix D3 to properly clean all of your equipment before and after sampling in the watershed.

The estuary may have sensitive nesting habitat which must be avoided during sampling. R~~See Appendix 5 to determine if your estuary has sensitive habitat and if so, be sure to specified routes. These r~~outes should ~~have been~~ be outlined to avoid all sensitive areas, do not under any circumstances take shortcuts to the transect locations.

Adverse weather conditions may result in a rescheduling of primary producer transect monitoring. In case of a large storm, it is possible to delay sampling; however, macroalgal surveys will need to be rescheduled around sufficient low tides, if applicable.

I. Holding times

Due to the rapid degradation of the samples collected and to ensure that the best possible data is collected, samples should be delivered to the laboratory within specified holding times. shipping all samples types together within 48 hours is preferable.

Table 9. Holding times for Primary Producer Samples

Sample	Holding Time	Delivery to Laboratory
Watercolumn chlorophyll *a*~~Macroalgal Biomass~~	30 days~~48 hours~~	24 hours*~~24 hours~~
~~Watercolumn chlorophyll *a*~~	~~30 days~~	~~24 hours~~

*Or as specified in laboratory method.

J. Sample Delivery

- ~~Biomass samples and c~~Chlorophyll *a* samples must be delivered to the laboratory as soon as possible
    - Photocopies of the field data sheets and the original chain of custody form(s) should be signed and given to the delivery person.
- In the event that samples cannot be delivered to the laboratory on the day of collection, please follow the following preservation protocols:
    - Chlorophyll *a* filters should be frozen
    - ~~Biomass samples should be refrigerated.~~
        - ~~Once samples have been received by the laboratory, each individual bag from a transect will weighed wet individually, and then composited.~~
        - ~~They will then be rinsed to remove salts and sorted by species.~~
        - ~~Excess water will be shed from the samples using a salad spinner. Composite sample wet weight will be recorded. Samples will dry overnight at 60 °C and the composite sample dry weight will be recorded.~~

- If shipping a cooler to a laboratory~~In the event that a laboratory pick-up person cannot be arranged~~, samples should be packed on blue ice in coolers and shipped priority overnight ~~using FEDEX~~.
  - ~~As much as possible, ship all samples from all of your sites together to save on shipping costs.~~
  - Photocopies of Field Data Sheets and Chain of Custody forms should be placed in a Ziploc Bag inside each cooler.
  - The laboratory should be contacted as soon as samples are shipped so that they will know to expect it and sample processing can begin immediately upon arrival.
- All original hardcopies of the field data sheets should be kept on file.

K. Quality Assurance/ Quality Control (QA/QC)

Data QA/QC

Field Data Sheets are used to record QA information and flags for questionable data. Site observations/information collected on the Data sheet include:

- Current weather conditions
- ~~Position of the tide gate during sampling~~
- Condition of the ocean inlet during sampling
- Time of low tide and tidal height, if estuary is open
- Description of mudflats and subtidal area
- Latitude and longitude/ or distance along transect where each sample is collected

These data will help provide context for spurious data (e.g. overcast day may result in lower than expected water column chlorophyll results).

All data collected on the data sheet should be entered clearly. All hash-marks for percent cover should be clear and should not overlap with other marks. Field crews should double check that all 25 intercepts have been analyzed by adding up the number of hash-marks. Team Leaders should also double check that all samples have been collected and that the Data sheets have been completely filled out before leaving a transect site.

VII. FLOW

D. Channel Velocity and Cross-section Measurements

Channel discharge is the volume of water that moves past a point in a given amount of time. Stream discharge can be estimated via water levels by developing a rating curve which relates water level, wetted cross-sectional area of the stream, and velocity to 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. Where possible, the velocity-area method will be used. If the velocity-area method cannot be used, record the reason on the field data sheet and use the buoyant object method instead. Similarly, if flow is too low to measure, or is ponded or dry, then record it on the field data sheet.

Channel velocity and cross-sectional geometry should be collected coincident with primary producer sampling (Table 7).

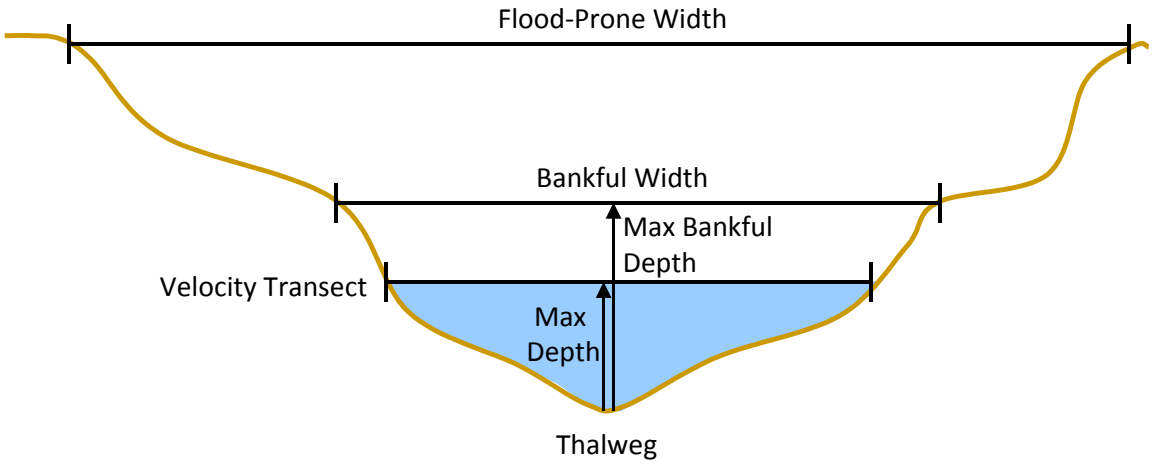


Figure 8. Diagram of channel cross-section

Channel Profile:

- Place the transect tape across the channel and record the bankfull width on the Data Sheet
  - Facing downstream, the left bank should represent the 0 m.
- Keeping the measuring tape tight across the channel at bankfull stage, measure and record the bankfull depth at the maximum bankfull depth at the channel thalweg.

Stream Velocity Transect:

- Select the best location for measuring discharge and lay the transect tape across the wetted width, staking in each side of the tape so it remains tight.

- Document the latitude and longitude of the site, as repeated measurements will occur at this fixed location.
- 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.

- The layout for discharge measurements is illustrated in Figure 6.
- Flow velocity should be measured with a flow meter.
- Record the wetted width on the data sheet and divide this into 10 to 20 equal distances, you will be measuring velocity at each of these distances (including at each of the banks).
  - 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.
  - Facing downstream, the left bank should represent the 0 m mark.
- Record the distance from the bank to the first location. Using the top-setting rod that comes with the flow velocity meter, measure the water depth at the first location.
- 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 first location, at 0.6 of the total water depth (this position generally approximates average velocity in the water column), and perpendicular to the transect tape (facing upstream). See Figure 7 for positioning detail.
- 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.
- Streams deeper than 0.5 m, move the velocity probe to the surface and record the velocity of the surface water on the data sheet in addition to the measurement recorded above (two velocities at each distance for these sites).
- Move to the next interval location and record the distance from the bank and the surface and depth flow measurements.
- Continue until all intervals have been sampled.
  - Note: The first and last intervals usually have depths and velocities of zero.

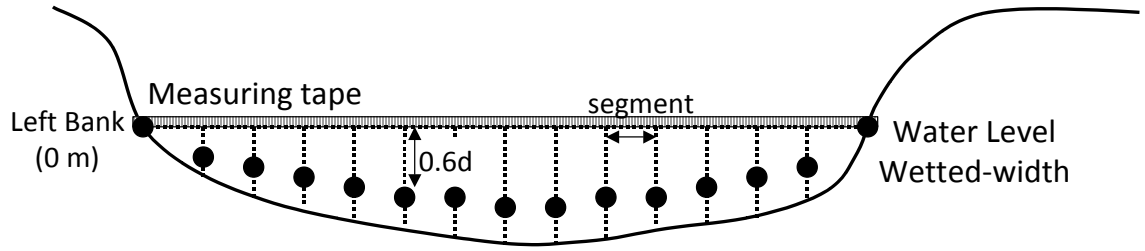


Figure 9. Diagram of layout for discharge measurements under the velocity-area method showing proper positions for velocity probe (black dots).

E. Continuous Depth Measurements

Equipment:
HOBO logger
Notebook computer with HOBO software installed
USB base station
Protective latex sleeves (aka condoms)
Tap water
PVC casing
Cable ties

HOBO

Connecting the logger to a laptop computer:

- The logger requires an USB-Optic Base Station to connect to the computer. The computer must have the HOBO software installed.
- Plug the base station cable into a USB port on the computer.
- Unscrew the black plastic end from the logger by turning it counter-clockwise.
- Insert the logger with the flat on the logger and the base station aligned. Gently twist to make sure it is fully seated in the base station.
- Use the logger software to launch the logger.
- Program the logger to record a depth measurement every 6 minutes.
- The HOBO pressure gauge records absolute pressure which is converted to water level by the HOBO software. You will need to download barometric pressure from a nearby Meteorological station (within 3 miles of the HOBO station). If you do not have a local Met station, we will need to deploy a second HOBO to record the ambient air pressure.

- Use software to upload data as necessary. Save data files with the estuary abbreviation, name of freshwater source and date.
- Check the battery voltage; if a battery falls below 3.1 V the logger will record "bad battery" in the datafile. If the battery is failing, it will need to be replaced by the manufacturer. In this event, contact Karen McLaughlin to obtain a substitute HOBO.
- The HOBO will need to be serviced approximately every 30 days. Servicing can be coordinated with the YSI servicing. If sedimentation rates are high, the HOBO may need to be serviced more frequently so that it doesn't get buried and the depth of the HOBO relative to the channel bottom is well characterized.

Operation:

- A light in the communication window of the logger confirms logger operation. The following table explains when logger blinks during operation:

When:	**The light:**
The logger is logging	Blinks once every one to four seconds (the shorter the logging interval, the faster the light blinks); blinks when logging a sample
The logger is awaiting a start because it is launched in Start At Interval or Delay Start mode	Blinks once every eight seconds until logging begins

Deploying the logger:

- A piece of PVC pipe will be installed at each flow monitoring location and will serve as a mounting platform for the HOBO. The mounting platform will have a hole drilled at a specific distance off the bottom of the stream. Record this distance on the data sheet each time you go to service the HOBO.
- Insert the hobo in a protective latex sleeve (condom). Add a little fresh water (from the tap—do not use deionized water) to the condom and tie-off the end.
- Place the HOBO into the PVC casing. Align the holes on each of the end caps with the holes on the casing and secure them with cable ties. Place a third cable tie through the holes in the casing, around the HOBO to gently secure it in the center of the casing.
- Attach the casing to the PVC pipe.
- Periodically inspect the logger for fouling. Biological growth on the face of the sensor will throw off the sensor's accuracy.
- The logger can be damaged by shock and may lose accuracy if dropped. If your HOBO is dropped or otherwise damaged, contact Karen McLaughlin for a replacement.
- To obtain the highest level of accuracy, the logger should be allowed to come to full temperature equilibrium (approximately 30 min) before any reference levels are recorded.
- Fill in appropriate data on the Freshwater Loading data sheet.

G. Data Sheets

An example data sheet for the Freshwater Nutrient Loading component is given in Appendix 4. A new data sheet should be used for each freshwater source into an estuary. Field data sheets record the discharge measurements made with the flow meter, records that samples were collected for TN/TP, and logs any maintenance to the HOBO sensordata logger, if applicable. Flow measurements do not need to be recorded if a U.S.G.S. gauging station is present. Record date and time of sampling and all site observations. Use the comments box to record any observations or field notes.

All data from the Field Data Sheets must be transferred to the electronic data sheets by the field team member who recorded the data within one week of collection. Data sheets must be uploaded to SCCWRP's FTP site within one week of sampling. See Data Delivery below.

H. Safety Issues

Depending on safety concerns at each Mass Loading Sitethe site, field work can should be conducted in teams of at leastone or two. If possible, freshwater monitoring should be conducted coincident with primary producer community monitoring or maintenance to the continuous monitoring sondes. Because measurements of discharge must be collected by wading into the streams, care should be taken for discharge and TN/TP collection following storm events. Safety of the field team is the number one priority and streams should not be entered if conditions are too dangerous (swift currents, large debris, etc.). In the event of dangerous conditions, do not collect discharge measurements and attempt to collect the TN/TP sample from a bridge or overpass instead of by wading.

New Zealand mudsnail and other invasive species are a concern for some of the sitesin Ventura County, so (See Appendix 5 for special concerns for each estuary). Check the list to see if invasive species are a concern for your specific estuary and if so, follow the protocols outlined in Appendix 6 to properly clean all of your equipment before sampling a new site.

Some watersheds have sensitive nesting habitat that must be avoided during sampling. Plan routes and transects to avoid these locations See Appendix 5 to determine if your estuary has sensitive habitat and if so, be sure to specified routes. These routes have been outlined to avoid all sensitive areas,and do not travel through these areas under any circumstances take shortcuts to the transect locations.

I. Sample Delivery

Wherever possible, samples for nutrient concentration should be delivered with biomass, sediment and water column samples from the primary producer surveys, via the SCCWRP runner. If freshwater loading studies are decoupled from the primary producer community assessments, samples should be delivered to SCCWRP within the time frame outlined in Table

9. If a SCCWRP runner is not availableIf shipping samples to the laboratory, samples should be packed on blue ice in coolers and shipped priority overnight. using FEDEX. Use FEDEX account number 122793168 and reference number W3249-2. SCCWRP will ship coolers back to organizations within one week of delivery. Photocopies of Field Data Sheets and Chain of Custody forms should be placed in a Ziploc Bag inside each cooler. All original hardcopies of the field data sheets should be maintained by each organization. See section VIII for descriptions of labeling, sample tracking, and chain of custody forms. An email should be sent to the Field Coordinator (karenm@sccwrp.org)laboratory as soon as samples are shipped so that she they will know to expect it and sample processing can begin immediately upon arrival.

J. Quality Assurance/ Quality Control (QA/QC)

Scientific Team Training

SCCWRP will provide training for all field teams prior to the start of the sampling period. This training will include how to operate the flow meters and the HOBO depth loggers, and how to collect samples for TN/TP. Training will occur in the field and will also serve as an intercalibration exercise to insure that all field teams are collecting data in the same way. Following initial training, each team will explore their specific systems with wildlife specialists appointed by the landowners (Fish and Game, etc.) to identify areas of sensitive habitat that should be avoided during sampling. These meetings will be set up to determine specific routes to sampling areas that can be adhered to during each sampling event. The Lead Scientist from each organization will be responsible for ensuring that their field personnel have been trained properly on all field methods and procedures that will be used during the survey. It will be their responsibility to review the Field Operations Manual with their field crews, and to make sure that each person understands that these procedures must be followed during the survey. Personnel that cannot perform a required operation will not participate in conducting that operation.

Field Audits

Pre-survey field audits will be conducted in an attempt to ascertain each organization's adherence to primary producer community assessment protocols. These audits will be conducted by SCCWRP's QA officer or his/her representative. During the field audits, an Auditor will observe the field teams perform the required procedures, and as necessary, provide corrective instruction. The Auditor may also conduct subsequent audits for all participants during the primary producer assessment to ensure that data is being collected in a uniform manner and that all required information is recorded by the field crews.

Data QA/QC

Field Data Sheets are used to record QA information and flags for questionable data. Site observations/information collected on the Data sheet include:

- Current weather conditions
- Condition of the stream
- Presence/absence of large debris
- Presence of a gauging station

These data will help provide context for spurious data or missing data (e.g. stream too swift for discharge measurements).

All data collected on the data sheet should be entered clearly. Team LeadersThe Team Leader should double check that all samples have been collected and that the Data sheets Sheets have been completely filled out before leaving the site.

Information collected on the Data Sheets will be transferred to an electronic template back in the lab. This transfer should be conducted by the individual who recorded the data in the field. Templates will be set up in Microsoft Excel. Most fields will possess a dropdown menu or look-up list from which the user can select appropriate values. This will insure that all organizations enter data uniformly into the database. User QA/QC checks that should be conducted before data is sent to SCCWRP are as follows:

- Ensure all columns are properly labeled,
- Ensure all fields are populated with correct data in the proper format
- Ensure the template has not been altered.

Database software for the Bight 08 project requires all field operators be spelled correctly. Furthermore, the software is case sensitive. Files uploaded in the wrong format may be rejected and users will be asked to correct the problem and resubmit.

K. Data Delivery

All field data should be transferred from hard copies to the electronic field data template within one week of collection. The individual who recorded all data in the field should be the person who enters the data into the template. Electronic templates should be uploaded to the Bight 08 Data Submission website within one week of sampling:
www.sccwrp.org → Data tab → Data Submission
Enter all relevant fields and use "browse" tab to select files to upload.
An email should be sent to Karen McLaughlin (karenm@sccwrp.org) to let her know that the data has been transferred to the FTP site so that QA/QC can begin.

Following data submittal, SCCWRP personnel will remove file from FTP site each week for additional QA/QC. After sample processing and analysis, data for total nitrogen and total phosphorus and calculations of nitrogen and phosphorus loading will be added to each template. QA flags for any exceedences of measurement quality objectives (MQO's) or other QA flags relating to sample collection and processing will also be added to the templates. Copies of completed templates will be emailed to each organization. Each month QA'ed field and laboratory data will be uploaded into the Bight 08 database.

VIII. LABELING AND SHIPPING OF SAMPLES AND FIELD DATA SHEETS

A. Sample Labels/ Tracking

Each sample will be identified and tracked by the ~~estuary code~~site ID, parameter, ~~volume or surface area collected,~~ and date and time sampled~~, and split number if applicable. Sample log numbers will be handled by SCCWRP for the samples shipped.~~

B. Labels

All containers ~~provided by SCCWRP will be pre-~~should be labeled and field teams will be responsible for filling in the required fields. Bottles should be labeled with weather proof labels~~Avery™ Weather Proof White Labels 1" x 2⅝" (Avery # 5520).~~ with d~~D~~ates ~~will be~~ reported as day/month/year. Following example sampling external labels should be covered with clear postal tape to prevent them from falling off the container. Below follows a description of the fields for each sample type:

Water Column Chlorophyll a (example):

~~Bight 08 Eutrophication Assessment~~Ventura River Estuary Algae TMDL
~~Estuary~~Site ID:~~_____~~ TMDL-Est~~_____~~ ~~Sampling 1~~

~~*Macroalgal Biomass (example):*~~

Bight 08 Eutrophication Assessment
Estuary_____ Sampling 1
Date:_____ Time:_____
Macroalgae Biomass: MAT1Q2
Transect #1 Quadrat # 2

~~*Floating Macroalgal Biomass (example):*~~

Bight 08 Eutrophication Assessment
Estuary_____ Sampling 1
Date:_____ Time:_____
Floating Macroalgae: FMAT1OO
Transect #1 Oceanward end-Ocean

Estuarine ~~Ambient~~ Nutrients (example):

Ventura River Estuary Algae TMDL
~~**Bight 08 Eutrophication Assessment**~~
Site ID: TMDL-Est
~~Estuary_____ Sampling 1~~
Date:_____ Time:_____

Freshwater Nutrients (example):

Ventura River Estuary Algae TMDL~~Bight 08 Eutrophication Assessment~~
Site ID: TMDL-Est
~~Estuary_____ Sampling 1~~

C. Field Data Sheets and Field Logs

Field data sheets for the ~~primary producer biomass and~~ percent cover estimates and field logs for continuous monitoring should be maintained by all field teams. Team leaders should review all data sheets and logs before leaving a site to ~~insure~~ ensure that all data has been collected and all QA/QC guidelines have been met. Copies of the field data sheets should be kept in either hard copy or electronic copy format.~~sent as hard-copies to SCCWRP with the samples they accompany. Alternatively, hard-copies of field data sheets may be scanned and emailed to karenm@sccwrp.org as PDF files. Field logs can be sent to SCCWRP as hard-copies at the end of the monitoring period. Organizations~~Samplers will be expected to keep paper copies on file. Examples of data sheets and logs are in Appendix 4.

D. Shipping Samples

~~All biomass samples must be shipped to SCCWRP within 24 hours of collection. This is to insure that the samples do not degrade significantly by the time we sort them. Biomass samples should be kept refrigerated (or on ice in the field) until they are shipped. Samples should be shipped on blue ice.~~

Water column chlorophyll a filters~~, HABs toxin filters, benthic chlorophyll a composite samples,~~ and total nitrogen/total phosphorus samples must be shipped to ~~SCCWRP~~ the laboratory within 24 hours of collection. Filters ~~and sediment sample containers~~ must be wrapped in foil to keep the pigments from degrading and should be kept frozen (or on ice in the field) until shipment.

E. Chain of Custody Forms

Chain of custody forms are to be filled out whenever samples are collected~~shipped to SCCWRP~~. These forms detail the transfer of samples from the organization that collected them to ~~SCCWRP~~ the laboratory where they will be processed. The sample and container type is to be included on the forms to identify the samples being transferred. This form should be signed by ~~either the Team Leader or the Scientific Lead before shipment~~the person who collected the samples prior to shipment. A copy of the form must be retained by the ~~organization~~ monitoring consultant and the original with signatures will accompany the samples~~. If the samples are shipped by courier, a copy of the chain of custody form is to be faxed or emailed to the Field Coordinator for tracking purposes. An example chain of custody form is in Appendix 4.~~.

IX. SAFETY

A. General Safety Concerns

Sample collection in estuaries is inherently hazardous and this danger is greatly compounded in bad weather. Thus, the safety of the field teams and equipment is of paramount importance throughout the project. Each person working in the field during the project should take personal responsibility for their own safety.

Safety awareness by the Team Leaders and all team members is the greatest single factor that will reduce accidents. Each field team should follow all established rules and provisions within their respective organization's safety program. Sampling should be canceled or postponed during hazardous weather conditions. The final decision shall be made by the Science Lead. As with any field program, the first priority is the safety of the people, followed by the safety of the equipment, and then the recovery of the data.

B. Sensitive Habitat

In some estuarine systems, there are restrictions on access due to sensitive nesting habitat. These areas must be avoided during nesting season. ~~. Specific concerns for each estuary are listed in Appendix 5. Designated routes around each sensitive area are indicated on the maps in Appendix 2.~~ Team Leaders are expected to know the locations of all sensitive habitat in their systems and convey the importance of avoiding such areas to their field teams.

C. Construction in Estuaries

~~During the course of the Eutrophication Assessment, some~~ The estuary may~~ies will~~ experience construction on infrastructure within the system (e.g., construction to existing bridges and railroad trestles, installation of culverts, etc.). Science Leads should make every effort to determine if there are plans for construction within their estuaries during the assessment. If construction does occur concurrently with the assessment (planned or unplanned), Science Leads should discuss potential impacts (either to team safety or data quality) with the Field Coordinator and Project Manager and determine whether or not sites should be moved or removed from the assessment.

X. LITERATURE CITED

Bates, S., et, and al. 1989. Pennate diatom Nitzschia pungem as the primary source of domoic acid, a toxin in shellfish from eastern Prince Edward Island, Canada. Canadian Journal of Fisheries and Aquatic Sciences 46:1203-1215.

Bates, S. S., A. S. W. DeFreitas, J. E. Milley, R. Pocklington, M. A. Quilliam, J. C. Smith, and J. Worms. 1991. Controls on domoic acid production by the diatom Nitzschia pungens f. multiseries in culture: nutrients and irradiance. Canadian Journal of Fisheries and Aquatic Sciences 48:1136-1144.

Borsuk, M. E., C. A. Stow, J. R. A. Luettich, H. W. Paerl, and J. L. Pinckney. 2001. Modelling Oxygen Dynamics in an Intermittently Stratified Estuary: Estimation of Process Rates Using Field Data. Esturine, Coastal and Shelf Science 52:33-49.

Burkholder, J., E. Noga, C. Hobbs, and H. Glasgow. 1992. New 'phantom' dinoflagellate is the causative agent of major estuarine fish kills. Nature 358:407-410.

Cloern, J. E. 1982. Does the benthos control phytoplankton biomass in South San Francisco Bay? Marine Ecology Progress Series 9:191-202.

Cloern, J. E. 1991. Tidal stirring and phytoplankton bloom dynamics in and estuary. Journal of Marine Research 49:203-221.

Cloern, J. E. 1996. Phytoplankton bloom dynamics in coastal ecosystems: A review with some general lessons from sustained investigation of San Francisco Bay, California. Reviews of Geophysics 34:127-168.

Cloern, J. E. 1999. The relative importance of light and nutrient limitation of phytoplankton growth: a simple index of coastal ecosystem sensitivity to nutrient enrichment. Aquatic Ecology 33:3-16.

Cloern, J. E. 2001. Our evolving conceptual model of the coastal eutrophication problem. Marine Ecology-Progress Series 210:223-253.

Cloern, J. E., B. E. Cole, R. L. J. Wong, and A. E. Alpine. 1985. Temporal dynamics of estuarine phytoplankton: A case study of San Francisco Bay. Hydrobiologia 129:153-176.

Diaz, R. J. 2001. Overview of Hypoxia around the World. Journal of Environmental Quality 30:275-281.

Fong, P., K. E. Boyer, and J. B. Zedler. 1998. Developing and indicator of nutrient enrichment in coastal estuaries and lagoons using tissue nitrogen content of the opportunistic algal, Enteromorpha intestinalis (L.Link). Journal of Experimental Marine Biology and Ecology 231:63-79.

Hernandez, I., G. Peralta, J. L. Perez-Llorens, and J. J. Vergara. 1997. Biomass and dynamics of growth of *Ulva* species in Palmones River Estuary. Journal of Phycology 33:764-772.

Johnson, M. R., S. L. Williams, C. H. Lieberman, and A. Solbak. 2003. Changes in the abundance of the seagrasses Zostera marina L. (eelgrass) and Ruppia maritima L. (widgeongrass) in San Diego, California, following an El Nino event. Estuaries 26:106-115.

Kamer, K., K. A. Boyle, and P. Fong. 2001. Macroalgal bloom dynamics in a highly eutrophic southern California estuary. Estuaries 24:623-635.

Kamer, K., and E. Stein. 2003. Dissolved oxygen conentration as a potential indicator of water quality in Newport Bay: A review of scientific research, historical data, and criteria development. Southern California Coastal Water Research Project, Westminster, CA.

Kennison, R., K. Kamer, and P. Fong. 2003. Nutrient dynamics and macroalgal blooms: A comparison of five southern California estuaries. Southern California Coastal Water Research Project, Westminster, CA.

McGlathery, K. 2001. Macroalgal blooms contribute to the decline of seagrass in nutrient-enriched coastal waters. Journal of Phycology 37:453-456.

Rabalais, N. N., and D. Harper, editors. 1992. Studies of benthic biota in areas affected by moderate and severe hypoxia. NOAA Coastal Ocean Program, Texas A&M Sea Grant, College Station, TX.

Stanley, D. W., and S. W. Nixon. 1992. Stratification and Bottom-Water Hypoxia in the Pamlico River Estuary. Estuaries 15:270-281.

Sutula, M., C. Creager, and G. Wortham. 2007. Technical Approach to Develop Nutrient Numeric Endpoints for California Estuaries. Southern California Coastal Water Research Project, TetraTech, Costa Mesa.

Sutula, M., K. Kamer, and J. Cable. 2004. Sediments as a non-point source of nutrients to Malibu Lagoon, California (USA). Southern California Coastal Water Research Project, Westminster, California.

Trainer, V., B. Hickey, and R. Horner. 2002. Biological and physical dynamics of domoic acid production off the Wasington Coast. Limnology and Oceanography 47:1438-1446.

Turner, R. E., N. Qureshi, N. N. Rabalais, Q. Dortch, D. Justic, R. F. Shaw, and J. Cope. 1998. Fluctuating silicate: nitrate ratios and coastal plankton food webs. Proceedings of the National Academy of Sciences 95:13,048-013.

Twilley, R. R. 1985. The exchange of organic carbon in basin mangrove forests in a southwest Florida estuary. Estuarine Coastal and Shelf Science 20:543-557.

Valiela, I., K. Foreman, M. LaMontagne, D. Hersh, J. Costa, P. Peckol, B. DeMeo-Andreson, C. D'Avanzo, M. Babione, C. Sham, J. Brawley, and K. Lajtha. 1992. Couplings of Watersheds and Coastal Waters: Sources and Consequences of Nutrient Enrichment in Waquoit Bay, Massachusetts. Estuaries 15:433-457.

Valiela, I., J. McClelland, J. Hauxwell, P. J. Behr, D. Hersh, and K. Foreman. 1997. Macroagal blooms in shallow estuaries: Controls and ecophysiological and ecosystem consequences. Limnology and Oceanography 42:1105-1118.

Appendix 1: List of Acronyms

CHL-	Chlorophyll
CoC-	Chain of Custody
DA-	Domic Acid
DFG-	Department of Fish and Game
DDI-	Distilled-DeIonized (water)
CSUCI-	Cal State University, Channel Islands
FB-	Field Blank
FTP-	File Transfer Protocol
GPS-	Global Positioning System
HAB-	Harmful Algal Bloom
HDPE-	High Density Polyethylene
IM-	Information Management
MCE-	Mixed Cellulose Ester
MLLW-	Mean Lowest Low Water
MQO-	Measurement Quality Objective
NH_4-	Ammonium
NO_2-	Nitrite
NO_3-	Nitrate
QAPP-	Quality Assurance Protocol Plan
QA/QC-	Quality Assurance/ Quality Control
PDF-	Portable Document Format
PES-	Polyethersulphone
PVC-	Poly-Vinyl Chloride (piping)
RCDSMM-	Resource Conservation District of the Santa Monica Mountains
SAV-	Submerged Aquatic Vegetation
SCB-	Southern California Bight
SCCWRP-	Southern California Coastal Water Research Project
SMBRC-	Santa Monica Bay Restoration Commission
SRP-	Soluble Reactive Phosphorus (mostly ortho-phosphate)
TDN-	Total Dissolved Nitrogen
TDP-	Total Dissolved Phosphorus
TJNERR-	Tijuana National Estuarine Research Reserve
TMDL-	Total Maximum Daily Load
TN-	Total Nitrogen
TOC-	Total Organic Carbon
TP-	Total Phosphorus
USFWS-	United States Fish and Wildlife Service
USGS-	United States Geological Survey

~~**Appendix 2: Maps**~~

~~**Maps of estuaries with index areas highlighted**~~

Appendix 4: Example Data Sheets and Sample ID Template

Continuous Monitoring Field Report

Station ID	Field Team Name(s):
Site Name	
Data Sonde ID Number:	
Date deployed:	Date recovered:
Time deployed:	Time recovered:

Site Observations:

Days since last rainfall in deployment period:	Current Weather: Clear / Partly Cloudy / Overcast / Rainy / Foggy (circle one)	
Tide gate position: open / closed / N/A (circle one)	Ocean Inlet Condition: Open / Restricted / Closed / N/A	
Direction of Tide: Ebb / Flood / Slack	Time Low Tide	Time High Tide:
Floating debris/Wrack on Housing: Y / N	Water clarity:	
Damage to housing: Y / N	Damage to sonde: Y / N	
Biofouling: None / Mild / Moderate / Severe		
Recent Human Activity in/near site: Water activity / intertidal activity / activity near sonde		
Comments:		

Recovery Check List:

Inspection:		Functionality:	
Casing damage: Y / N		Communicates with computer: Y / N	
Sonde Damage: Y/ N	Probe damaged: Y / N	Data logging failure: Y / N	
Data File Name:		File size:	

Calibration:

Date of Calibration:	Date of Drift Check:	
Calibration solution	Reading at Drift Check	Reading after Calibration
pH buffer (7.00)		
pH buffer (10.00)		
Conductivity (10 mS/cm)		
Turbidity (0 NTU)		
Turbidity (100 NTU)		
Dissolved Oxygen (100%)		
Chlorophyll (0 ug/L)		

Pre-deployment Check List:

Inspection:		Reporting:	
Battery case sealed		Date m/d/y	
Probes clean		Time hh:mm:ss	
Casing clean and undamaged		Temp C	
Wipers		SpCond mS/cm	
Functionality:		Sal ppt	
Communicates with computer		DO sat%	
Battery Life		DO mg/L	

Amount Free Memory		pH	
Correct Date/Time		pH (mV)	
Start sonde logging		Turbid+NTU	
Verify logging (15 min intervals)		Chl ug/L	
		Battery volts	

Field Lead Signature:_____

Primary Producer Assessment Field Data Sheet: Sediment and Water Column Sampling

Station ID:		Field Team Name(s):	
Site Name:			
Transect Number:			
Date:		Start Time:	End Time:
Oceanward Latitude:		Landward Latitude:	
Oceanward Longitude:		Landward Longitude:	

Site Observations

Days since last rainfall in deployment period:		Tide gate position: Open / Closed / N/A	
Weather: Clear / Partly Cloudy / Overcast / Rainy / Foggy		Ocean Inlet: Open / Restricted / Closed / N/A	
Time low tide:	Time high tide:	Direction of Tide: Ebb / Flood / Slack / N/A	
Oceanward Water Temp (°C):	Oceaward Salinity (ppt):	Landward Water Temp (°C):	Landward Salinity (ppt):
Comments:			

Water Column Sampling – Oceanward End Only

Rep	Sample ID	Distance from PVC	Chl *a* Vol. Filtered (mL)	NO_2 NO_3 NH_4 SRP	TDN/ TDP	TN/ TP	Domoic Acid Vol. Filtered (mL)	Microsystin Vol. Filtered (mL)
1								
2								
FB								

Sediment Sampling

Location	Sample ID	# plugs:	Remove algae?	Sediment Description
Oceanward				Average Grain Size:_____ Color:_____
Landward				Average Grain Size:_____ Color:_____

Floating Macroalgae

Quadrat	Oceanward – Facing Ocean	Oceanward – Facing Land	Landward – Facing Ocean	Landward – Facing Land
Mat Thickness (mm)	Est: Y/N	Est: Y/N	Est: Y/N	Est: Y/N
Condition	Frsh / Int / Des/ Dd	Frsh / Int / Des/ Dd	Frsh / Int / Des/ Dd	Frsh / Int / Des/ Dd
Absent				
Ulva intestinalis				

~~Ulva lactuca~~				
~~Macrocystis spp.~~				
~~Duck weed~~				
~~Filamentous algae~~				
~~Other 1:~~				
~~Other 2:~~				
~~Total:~~				
~~Biomass (Y/N)~~				
~~Comments on location of floating algae~~				

~~Field Lead Signature:_____~~

Primary Producer Assessment Field Data Sheet: Macroalgae Transect

Station ID:	Field Team Name(s):	
Site Name:		
Transect Number:		
Date:	Start Time:	End Time:
Start Latitude:	Start Longitude:	
End Latitude:	End Longitude:	
PVC Latitude:	PVC Longitude:	

Site Observations

Days since last rainfall in deployment period:	Tide gate position: Open / Closed / N/A
Weather: Clear / Partly Cloudy / Overcast / Rainy / Foggy	Ocean Inlet: Open / Restricted / Closed / N/A
Time low tide: Time high tide:	Direction of Tide: Ebb / Flood / Slack / N/A
Photo oceanward:	Photo landward:
Vertical zonation of macroalgae? Y/N Describe:	
Comments:	

Macroalgal Transect - Distance from PVC (at oceanward end):

Quadrat	1	2	3	4	5	6	7	8	9	10
Distance (m)										
Mat Thick (mm)										
Estimated?	Y / N	Y / N	Y / N	Y / N	Y / N	Y / N	Y / N	Y / N	Y / N	Y / N
Condition	Frsh / Int / Des/ Dd	Frsh / Int / Des/ Dd	Frsh / Int / Des/ Dd	Frsh / Int / Des/ Dd	Frsh / Int / Des/ Dd	Frsh / Int / Des/ Dd	Frsh / Int / Des/ Dd	Frsh / Int / Des/ Dd	Frsh / Int / Des/ Dd	Frsh / Int / Des/ Dd
Bare										
Ulva intestinalis (string-like)										
Ulva lactuca (sheet-like)										
Ceramium										
Gracilaria										
Filamentous algae										
Ruppia (spp.)										
Macrocystis Wrack: Y / N										
Phyllospadix Wrack: Y / N										
Decayed and Unidentifiable										
Other 1:										

Other 2:										
Total:										
Biomass (Y/N)										

Field Lead Signature: _____

Primary Producer Community Assessment Field Data Sheet: SAV Transect

Station ID:	Field Team Name(s):	
Site Name:		
Transect Number:		
Date:	Start Time:	End Time:
Start Latitude:	Start Longitude:	
End Latitude:	End Longitude:	
PVC Latitude:	PVC Longitude:	

Site Observations

Days since last rainfall in deployment period:		Tide gate position: Open / Closed / N/A
Weather: Clear / Partly Cloudy / Overcast / Rainy / Foggy		Ocean Inlet Condition: Open / Restricted / Closed / N/A
Time low tide:	Time high tide:	Direction of Tide: Ebb / Flood / Slack / N/A
Total channel width:		Maximum Channel Depth:
Right hand photo:		Left hand photo:
Comments:		

Floating Macroalgae Transect

Quadrat	1	2	3	4	5
Distance from Bank (m)					
Water Depth (m)					
Mat Thickness (mm)					
Condition	Frsh / Int / Des/ Dd	Frsh / Int / Des/ Dd	Frsh / Int / Des/ Dd	Frsh / Int / Des/ Dd	Frsh / Int / Des/ Dd
Absent					
Ulva intestinalis					
Ulva lactuca					
Filamentous algae					
Other 1:					
Other 2:					
Total:					
Biomass (Y/N)					

Brackish Water Submerged Aquatic Vegetation Transect

Quadrat	1	2	3	4	5
Distance from Bank (m)					
Water Depth (m)					
Condition (circle one)	Frsh / Int / Des/ Dead	Frsh / Int / Des/ Dead	Frsh / Int / Des/ Dead	Frsh / Int / Des/ Dead	Frsh / Int / Des/ Dead
Estimated Percent Cover					
Bare					
Chara spp.					
Ruppia spp.					
Other:					
Other:					

Total:	100%	100%	100%	100%	100%
Biomass (Y/N)					

Condition Designations:

Frsh : Fresh	Int : Intermediate	Des: Desiccated	Dd or Dead : Dead

Freshwater Loading Field Data Sheet

Field Lead Signature:_____

Station ID:	Field Team Name(s):
Site Name:	
Date:	Time:
Latitude:	Latitude:

Site Observations:

Days since last rainfall in deployment period:	Weather: Clear / Partly Cloudy / Overcast / Rainy / Foggy
Gauging station: Y / N	HOBO: Y / N
Stream Flow: stagnant / low / moderate / high	Large Debris: Present / Absent
Comments:	

Nutrient Samples

Rep	Sample ID	TN/ TP
1		
2		
FB		

Channel Cross-section

Bankfull Width:	Maximum Bankfull Depth:

Velocity Measurements

Wetted Width:									
#	Distance from Bank (cm)	Water Depth (cm)	Surface Flow (m/s)	Depth Flow (m/s)	#	Distance from Bank (cm)	Water Depth (cm)	Surface Flow (m/s)	Depth Flow (m/s)
1					11				
2					12				
3					13				
4					14				
5					15				
6					16				
7					17				
8					18				
9					19				
10					20				

Stream Bottom Type (check one or more):

Muddy Bottom	Sandy Bottom	Gravel Bottom	Cobble Bottom	Vegetated Bottom	Concrete

HOBO

Days Since Last Maintenance		Wrack on Casing (Y/N)	
Damage to Casing (Y/N)		Biofouling: None / Mild / Moderate / Severe	

Damage to Hobo (Y/N)			
Hobo Logging (Y/N)		Distance from Sediment to Hobo at recovery (cm)	
Battery Volts		Distance from Sediment to Hobo at redeployment (cm)	
File Upload: Y / N		File Name:	

Field Lead Signature:_____

Q/A Checklist for Primary Producer and Freshwater Loading Assessment

Estuary:_____ Organization:_____

- ☐ **Conduct three (3) macroalgae transects**
  - ☐ Ten (10) percent cover estimates at each transect (30 total)
  - ☐ ~~Five (5) macroalgae biomass samples from transect at each transect (15 total)~~
  - ☐ Four (4) floating macroalgae percent cover estimates; two (2) at the oceanward size and two (2) at the landward side of each tranect (12 total)
  - ☐ ~~Four (4) floating macroalgae biomass samples; two (2) at the oceanward size and two (2) at the landward side of each tranect (12 total)~~
  - ☐ ~~Two (2) sediment composites with a minimum of 10 sediment plugs each from the oceanward and landward sides of the macroalgae transects (6 total)~~
  - ☐ Water column sampling from one of the transect sites
    - ☐ Duplicate chlorophyll a filters plus one field blank (3 filters total)
    - ☐ Duplicate TN/TP bottles plus one field blank (3 bottles total)
    - ☐ Duplicate TDN/TDP bottles plus one field blank (3 bottles total)
    - ☐ Duplicate dissolved nutrient bottles plus one field blank (3 bottles total)
  - ☐ Measure temperature and salinity at each transect site
- ☐ ~~**Conduct three (3) submerged aquatic vegetation transects (if applicable)**~~
  - ☐ ~~Five (5) (if channel is >50 m) or three (3) (if channel is <50m) percent cover estimates at each transect (15 or 9 total)~~
  - ☐ ~~Five (5) (if channel is >50 m) or three (3) (if channel is <50m) SAV biomass samples from transect at each transect (15 or 9 total)~~
  - ☐ ~~Five (5) (if channel is >50 m) or three (3) (if channel is <50m) floating macroalgae percent cover estimates; two (2) at the oceanward size and two (2) at the landward side of each tranect (15 or 9 total)~~
  - ☐ ~~Five (5) (if channel is >50 m) or three (3) (if channel is <50m) floating macroalgae biomass samples~~
  - ☐ ~~Measure temperature and salinity at each transect site~~
- ☐ ~~**Conduct assessment of freshwater loading**~~
  - ☐ ~~Capture flow information with flow meter~~
  - ☐ ~~Collect TN/TP sample from channel thalweg~~
- ☐ **Fill in all data sheets and forms:**
  - ☐ Macroalgae Transect Data Sheet
  - ☐ ~~Sediment and Water Column Sampling Data Sheet~~
  - ☐ ~~SAV Transect Data Sheet~~
  - ☐ ~~Freshwater Loading Data Sheet~~
  - ☐ Chain of Custody Form

Specific Measures to Avoid Critical or Sensitive Habitat

This section includes specific measures to avoid critical or sensitive habitat. Habitats surveyed in the assessment include: 1) subtidal water column and surface sediments, 2) brackish water submerged aquatic vegetation, and 3) intertidal mudflat. At times, access via established paths or ruderal habitat may occur in order to reach the habitats targeted for sampling. Table 3 summarizes the measures have been established to avoid critical or sensitive habitat for threatened and endangered species for this assessment in these habitats.

Table A5.2. Measures planned to avoid critical or sensitive habitat.

Species	Habitat Type	Measures to Avoid Disturbance
Light-footed Clapper Rail	Low marsh	No surveys will be conducted in marsh habitat, but surveys of macroalgae will be conducted on the mudflat adjacent to low marsh. All survey work will be done at a minimum of 1 m, and optimally 3 m distance from the edge of the emergent marsh. Areas in which a 3 m buffer is not possible are generated disturbed and muted estuaries that do not have Clapper Rail populations. Access to the site will avoid when possible traversing the marsh plain. When necessary, a line of entry will be chosen in consultation with Resource agencies that utilizes ruderal habitat.
Belding's Savannah Sparrow	Pickleweed Marsh	
Tidewater Goby and Steelhead Trout	Subtidal surface water, SAV and sediments	All habitats were steelhead trout or tidewater goby are present will be accessed by canoe or kayak to minimize disturbance of surface waters, SAV, and sediments. When harvesting brackish water SAV with a rake, SAV will be gentled pushed to drive away any fish. SAV harvested will be collected in a sorting tray and any fish incidentally captured will be immediately released. Surficial sediments collected for benthic microalgae (top 1 cm) is not expected to impact critical habitat for either species, but as a precautionary measure, coarse grained sediments will be avoided and water column above the sediment will be gently agitated to make sure no fish are present.

Appendix 6: New Zealand Mudsnail Equipment Decontamination Protocol

Preferred decontamination is via freezing of any equipment that has come into contact with water or wet mud over the course of sampling (including clothing, transect tapes, etc.) Equipment should be frozen (<0°C) for 24 to 48 hours to assure that adult and larval individuals are killed. For sensitive equipment such as probes that could be damaged by freezing, a disinfecting solution of copper sulfate can be used. Note that whenever possible, freezing is the preferred alternative due to greater efficacy.

Cleaning solution:
Copper sulfate
Dissolve copper sulfate pentahydrate crystals (99.1% purity)
Dilution rate:
3.785 g/gallon
37.85 g/10 gallon
18.925 g/5 gallon
56.775 g/15 gallon
75.7 g/20 gallon

1) Immersion procedure

- Remove wading gear/other gear, turn right side out, remove insoles
- Place all gear cleaning solution so that it is completely covered. Weigh down if necessary
- Allow to remain in solution for at least 5 minutes
- Remove each piece from cleaning solution, and inspect to make sure debris is removed. Use brush if necessary
- Rinse with clean water (not stream water!)

2) Spray bottle procedure

- Remove all gear, turn right side if necessary, remove internal items (insoles)
- Place in an empty container
- Spray to the point of saturation/runoff
- Allow to set for 5 minutes
- Rinse

California Department of Fish and Wildlife
Aquatic Invasive Species Decontamination Protocol

The California Department of Fish and Wildlife (CDFW) is committed to protecting the state's diverse fish, wildlife, and plant resources, and the habitats upon which they depend. Preventing the spread of aquatic invasive species (AIS) in both CDFW's activities, as well as those activities CDFW permits others to conduct is important to achieving this goal. The protocols outlined below are a mandatory condition of your CDFW authorization to work in aquatic habitats. They are intended to prevent the spread of AIS, including New Zealand mudsnail (*Potamopyrgus antipodarum*), quagga mussel (*Dreissena rostriformis bugensis*) and zebra mussel (*Dreissena polymorpha*). Information about New Zealand mudsnails and quagga and zebra mussels is summarized in Attachments A and B. For complete information on the threats of AIS and aids to their identification, please visit the Department's Invasive Species Program webpage at www.dfg.ca.gov/invasives or call (866) 440-9530.

Many AIS are difficult, if not impossible to see in the environment and can be unknowingly transported to new locations on equipment. Therefore, decontamination is necessary to prevent the spread of AIS between collection locations. Equipment shall be decontaminated between each use in different waterbodies. All equipment, including but not limited to, wading equipment, dive equipment, sampling equipment (e.g., water quality probes, nets, substrate samples, etc.), and watercraft, must be decontaminated using one or more of the protocols listed below. As an alternative to decontaminating on-site, you may wish to have separate equipment for each site and to decontaminate it all at the end of the day. Listed below are three options for equipment decontamination. Use your judgment and field sampling needs to select the method(s) that are appropriate for your equipment and schedule. **Because there are currently no molluscicides registered with the California Department of Pesticide Regulation that have been demonstrated to be effective for these three species, CDFW cannot recommend chemical decontamination.** If you would like training on implementing these protocols please contact the Invasive Species Hotline at (866) 440-9530 or e-mail invasives@wildlife.ca.gov

General field procedures to prevent the spread of AIS:

- If decontamination is not done on site, transport contaminated equipment in sealed plastic bags and keep separate from clean gear.
- When practical, in flowing water begin work upstream and work downstream. This avoids transporting AIS to non-infested upstream areas.
- For locations know to be infested with AIS, use dedicated equipment that is only used in infested waters. Store this equipment separately.

Equipment Decontamination Methods

Option 1: Dry

- Scrub gear with a stiff-bristled brush to remove all organisms. Thoroughly brush small crevices such as boot laces, seams, net corners, etc.
- Allow equipment to thoroughly dry (i.e., until there is complete absence of moisture), preferably in the sun. Keep dry for a minimum of 48 hours to ensure any organisms are desiccated.

Option 2: Hot water soak

- Scrub gear with a stiff-bristled brush to remove all organisms. Thoroughly brush small crevices such as boot laces, seams, net corners, etc.
- Immerse equipment in 140° F or hotter water. If necessary, weigh it down to ensure it remains immersed.
- Soak in 140° F or hotter water for a minimum of five minutes.

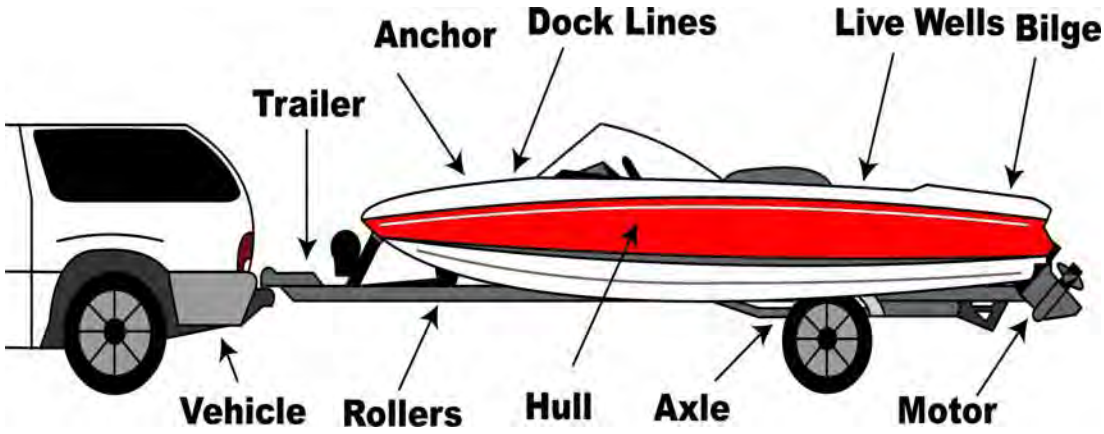
Option 3: Freeze

- Scrub gear with a stiff-bristled brush to remove all organisms. Thoroughly brush small crevices such as boot laces, seams, net corners, etc.
- Place in a freezer 32°F or colder for a minimum of eight hours.

Watercraft Decontamination

- Prior to leaving the launch area, remove all plants and mud from your watercraft, trailer, and equipment. Dispose of all material in the trash.
- Prior to leaving the launch area drain all water from your watercraft and dry all areas, including motor, motor cooling system, live wells, bilges, and lower end unit.
- Upon return to base facilities, pressure wash the watercraft and trailer with 140° F water*, including all of the boat equipment (i.e. ropes, anchors, etc.) that came into contact with the water.
- Flush the engine with 140° F water for at least 10 minutes and run 140° F water through the live wells, bilges, and all other areas that could contain water.

*To ensure 100% mortality the water needs to be 140° F at the point of contact or 155° F at the nozzle.



Reporting Aquatic Invasive Species

If you suspect you have found New Zealand mudsnail, quagga and zebra mussels, or other AIS, please immediately notify the CDFW Invasive Species Program at (866) 440-9530 or e-mail invasives@wildlife.ca.gov. Please provide your contact information, specific location of discovery, and digital photographs of the organisms (if possible).

Attachment A

New Zealand Mudsnail

The threat posed by New Zealand mudsnails (NZMS):

- NZMS reproduce asexually therefore it only takes a single NZMS to colonize a new location.
- NZMS are prolific, and a single NZMS can give rise to 40 million snails in one year.
- Densities of over 750,000 NZMS per square meter have been documented.
- NZMS out-compete and replace native invertebrates that are the preferred foods of many fish species and alter the food web of streams and lakes.

Identifying NZMS:

- NZMS average 1/8 inch in length, but young snails may be as small as a grain of sand. Adults bear live young.
- See the photos, below, for assistance identifying NZMS. Expert identification will be necessary to confirm identification.

NIOSH

Fast Facts

Protecting Yourself from

Poisonous Plants

Any person working outdoors is at risk of exposure to poisonous plants, such as poison ivy, poison oak, and poison sumac. When in contact with skin, the sap oil (urushiol) of these plants can cause an allergic reaction. Burning these poisonous plants produces smoke that, when inhaled, can cause lung irritation.

Workers may become exposed through:

- Direct contact with the plant
- Indirect contact (touching tools, animals, or clothing with urushiol on them)
- Inhalation of particles containing urushiol from burning plants

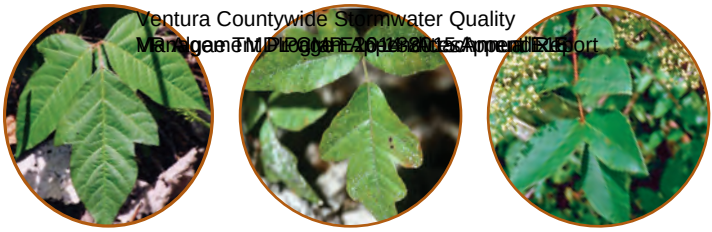
Symptoms of Skin Contact

- Red rash within a few days of contact
- Swelling
- Itching
- Possible bumps, patches, streaking or weeping blisters
  NOTE: Blister fluids are not contagious

First Aid

If you are exposed to a poisonous plant:

- Immediately rinse skin with rubbing alcohol, poison plant wash, or degreasing soap (such as dishwashing soap) or detergent, and lots of water.
  - Rinse frequently so that wash solutions do not dry on the skin and further spread the urushiol.
- Scrub under nails with a brush.
- Apply wet compresses, calamine lotion, or hydrocortisone cream to the skin to reduce itching and blistering.
  - Oatmeal baths may relieve itching.
- An antihistamine may help relieve itching.
  - NOTE: Drowsiness may occur.
- In severe cases or if the rash is on the face or genitals, seek professional medical attention.
- Call 911 or go to a hospital emergency room if you have a severe allergic reaction, such as swelling or difficulty breathing, or have had a severe reaction in the past.



Poisonous plants, from left to right: poison ivy, poison oak, poison sumac.

Images courtesy of U.S. Department of Agriculture.

Protect Yourself

- Wear long sleeves, long pants, boots, and gloves.
  - Wash exposed clothing separately in hot water with detergent.
- Barrier skin creams, such as lotion containing bentoquatum, may offer some protection.
- After use, clean tools with rubbing alcohol or soap and lots of water. Urushiol can remain active on the surface of objects for up to 5 years.
  - Wear disposable gloves during this process.
- Do not burn plants or brush piles that may contain poison ivy, poison oak, or poison sumac.
  - Inhaling smoke from burning plants can cause severe allergic respiratory problems.

When exposure to burning poisonous plants is unavoidable, employers should provide workers with:

- A NIOSH-certified half-face piece particulate respirator rated R–95, P–95, or better. This recommendation does NOT apply to wildland firefighters, who may require a higher level of protection.
- These respirators should protect against exposure to burning poisonous plants, but will not protect against all possible combustion products in smoke, such as carbon monoxide.
- Respirators must be worn correctly and consistently throughout the time they are used.
- For respirators to be effective there must be a tight seal between the user's face and the respirator.
- Respirators must be used in the context of a written comprehensive respiratory protection program (see OSHA Respiratory Protection standard 29 CFR 1910.134).
- For more information about respirators, visit www.cdc.gov/niosh/npptl/topics/respirators/

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Centers for Disease Control and Prevention
National Institute for Occupational Safety and Health

www.cdc.gov/niosh/topics/outdoor/

DHHS (NIOSH) Publication No. 2010–118

Telephone: 1–800–CDC–INFO
TTY: 1–888–232–6348
E-mail: cdcinfo@cdc.gov

NIOSH

Fast Facts

Protecting Yourself from

Stinging Insects

Flying Insects

Outdoor workers are at risk of being stung by flying insects (bees, wasps, and hornets) and fire ants. While most stings cause only mild discomfort, some may result in severe allergic reactions that require immediate medical care and may cause death.

First Aid

If a worker is stung by a stinging insect:

- Have someone stay with the worker to be sure that they do not have an allergic reaction.
- Wash the site with soap and water.
- Remove the stinger using gauze wiped over the area or by scraping a fingernail over the area.
  - Never squeeze the stinger or use tweezers.
- Apply ice to reduce swelling.
- Do not scratch the sting as this may increase swelling, itching, and risk of infection.

Protect Yourself

- Wear light-colored, smooth-finished clothing.
- Avoid perfumed soaps, shampoos, and deodorants.
  - Do not wear cologne or perfume.
- Wear clean clothing and bathe daily.
- Wear clothing to cover as much of the body as possible.
- Avoid flowering plants when possible.
- Keep work areas clean. Some insects are attracted to discarded food.
- Remain calm and still if a single stinging insect is flying around. (Swatting may cause it to sting.)
- If attacked by several stinging insects, run to get away. (Bees release a chemical when they sting, which attracts other bees.)
  - Go indoors.
  - Shaded areas are better than open areas.
  - Do not jump into water. Some insects (ex. Africanized honey bees) are known to hover above the water.
- If an insect is inside your vehicle, stop slowly, and open all the windows.
- Workers with a history of severe allergic reactions to insect bites or stings should carry an epinephrine autoinjector and wear medical ID jewelry stating their allergy.

Fire ants bite and sting. They are aggressive when stinging and inject venom, which causes a burning sensation. Red bumps form at the sting, and within a day or two they become white fluid-filled pustules.

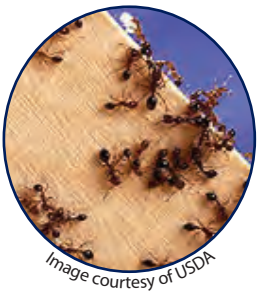


Image courtesy of USDA

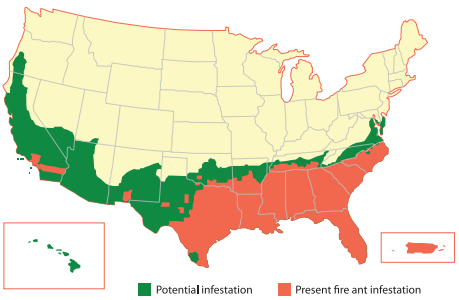
First Aid

- Rub off ants briskly, as they will attach to the skin with their jaws.
- Antihistamines may help.
  - Follow directions on packaging.
  - Drowsiness may occur.
- Seek immediate medical attention if a sting causes severe chest pain, nausea, severe sweating, loss of breath, serious swelling, or slurred speech.

Protect Yourself

- Do not disturb ant mounds.
- Be careful when lifting items (including animal carcasses) off the ground, as they may be covered in ants.
- Fire ants may be found on trees and in water, so always look over the area before starting to work.
- Tuck pants into socks or boots.
- Workers with a history of severe allergic reactions to insect bites or stings should carry an epinephrine autoinjector and wear medical ID jewelry stating their allergy.

Present and Potential Ranges of Imported Fire Ant Infestation



Source: U.S. Department of Agriculture

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Centers for Disease Control and Prevention
National Institute for Occupational Safety and Health

www.cdc.gov/niosh/topics/outdoor/

DHHS (NIOSH) Publication No. 2010–117

Telephone: 1–800–CDC–INFO
TTY: 1–888–232–6348
E-mail: cdcinfo@cdc.gov

NIOSH

Fast Facts

Protecting Yourself from

Ticks and Mosquitoes

Outdoor workers may be exposed to vector-borne diseases spread from the bites of infected ticks and mosquitoes. Ticks and mosquitoes may carry bacteria, parasites or viruses.

One of the most common tick-borne diseases in the U.S. is Lyme disease. Ticks are found in wooded areas, high grass, or leaf litter. They are most active during the spring, summer and fall, but in warmer areas may be active all year round.

One of the most common diseases carried by mosquitoes in the U.S. is West Nile virus infection. Mosquitoes may be found near standing water, or in weedy or wooded areas. They are usually most active during dawn and dusk in the warmer months.

Symptoms of Vector-borne Diseases

- Body/muscle aches
- Fever
- Headaches
- Fatigue
- Joint pain
- Rash
- Stiff neck
- Paralysis

Workplace Controls

Decrease tick populations:

- Remove leaf litter.
- Remove, mow, or cut back tall grass and brush.
- Discourage deer activity.

Eliminate standing water to decrease mosquito populations:

- Remove, turn over, cover, or store equipment.
- Remove debris from ditches.
- Fill in areas that collect standing water.
- Place drain holes in containers that collect water and cannot be discarded.

Mosquitoes (left) and ticks (front of card) may carry bacteria, parasites or viruses.

Image courtesy of U.S. Department of Agriculture

Protect Yourself

- Wear a hat and light-colored clothing (so ticks can be easily spotted), including long-sleeved shirts and long pants tucked into boots or socks.
- Use insect repellents.
  - Use repellents containing 20–50% DEET on exposed skin and clothing.
  - Reapply repellents as needed. (Always follow products labels).
- Use insecticides such as permethrin for greater protection.
  - Permethrin can be used on clothing, but not on skin.
  - One application to pants, socks, and shoes may be effective through several washings.
- Check skin and clothing for ticks daily. Check hair, underarms, and groin.
- Immediately remove ticks using fine-tipped tweezers.
  - Grasp the tick firmly, as close to your skin as possible.
  - Pull the tick's body away from your skin with a steady motion.
  - Clean the area with soap and water.
- Wash and dry work clothes using the "hot" settings to kill any ticks present.
- If you develop symptoms of a vector-borne disease, seek medical attention promptly. Tell your doctor that you work outdoors and report any ticks or mosquito bites.

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Centers for Disease Control and Prevention
National Institute for Occupational Safety and Health

www.cdc.gov/niosh/topics/outdoor/

DHHS (NIOSH) Publication No. 2010–119

Telephone: 1–800–CDC–INFO
TTY: 1–888–232–6348
E-mail: cdcinfo@cdc.gov

SAFER • HEALTHIER • PEOPLE™

NIOSH

Fast Facts

Protecting Yourself from

Heat Stress

Heat stress, from exertion or hot environments, places workers at risk for illnesses such as heat stroke, heat exhaustion, or heat cramps.

Heat Stroke

A condition that occurs when the body becomes unable to control its temperature, and can cause death or permanent disability.

Symptoms

- High body temperature
- Confusion
- Loss of coordination
- Hot, dry skin or profuse sweating
- Throbbing headache
- Seizures, coma

First Aid

- Request immediate medical assistance.
- Move the worker to a cool, shaded area.
- Remove excess clothing and apply cool water to their body.

Heat Exhaustion

The body's response to an excessive loss of water and salt, usually through sweating.

Symptoms

- Rapid heart beat
- Heavy sweating
- Extreme weakness or fatigue
- Dizziness
- Nausea, vomiting
- Irritability
- Fast, shallow breathing
- Slightly elevated body temperature

First Aid

- Rest in a cool area.
- Drink plenty of water or other cool beverages.
- Take a cool shower, bath, or sponge bath.

Heat Cramps

Affect workers who sweat a lot during strenuous activity. Sweating depletes the body's salt and moisture levels.

Symptoms

- Muscle cramps, pain, or spasms in the abdomen, arms or legs

First Aid

- Stop all activity, and sit in a cool place.
- Drink clear juice or a sports beverage, or drink water with food.
  - Avoid salt tablets.
- Do not return to strenuous work for a few hours after the cramps subside.
- Seek medical attention if you have the following: heart problems, are on a low-sodium diet, or if the cramps do not subside within one hour.

Protect Yourself

Avoid heavy exertion, extreme heat, sun exposure, and high humidity when possible. When these cannot be avoided, take the following preventative steps:

- Monitor your physical condition and that of your coworkers for signs or symptoms of heat illnesses.
- Wear light-colored, loose-fitting, breathable clothing such as cotton.
  - Avoid non-breathable synthetic clothing.
- Gradually build up to heavy work.
- Schedule heavy work during the coolest parts of day.
- Take more breaks when doing heavier work, and in high heat and humidity.
  - Take breaks in the shade or a cool area.
- Drink water frequently. Drink enough water that you never become thirsty.
- Be aware that protective clothing or personal protective equipment may increase the risk of heat-related illnesses.

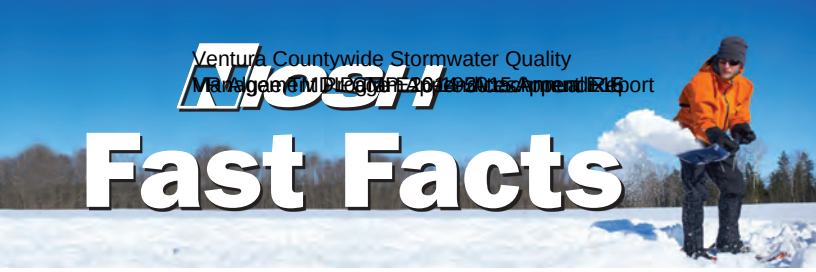
DEPARTMENT OF HEALTH AND HUMAN SERVICES
Centers for Disease Control and Prevention
National Institute for Occupational Safety and Health

www.cdc.gov/niosh/topics/outdoor/

DHHS (NIOSH) Publication No. 2010–114

Telephone: 1–800–CDC–INFO
TTY: 1–888–232–6348
E-mail: cdcinfo@cdc.gov

SAFER • HEALTHIER • PEOPLE™



Fast Facts

Protecting Yourself from
Cold Stress

Workers who are exposed to extreme cold or work in cold environments may be at risk of cold stress. Extremely cold or wet weather is a dangerous situation that can cause occupational illness and injuries such as hypothermia, frostbite, trench foot, and chilblains.

Hypothermia

A condition in which the body uses up its stored energy and can no longer produce heat. Often occurs after prolonged exposure to cold temperature.

Early symptoms

- Shivering
- Fatigue
- Loss of coordination
- Confusion and disorientation

Late symptoms

- No shivering
- Blue skin
- Dilated pupils
- Slowed pulse and breathing
- Loss of consciousness

First Aid

- Request immediate medical assistance.
- Move the victim into a warm room or shelter.
- Remove wet clothing.
- Warm the center of their body first—chest, neck, head, and groin—using an electric blanket; or use skin-to-skin contact under loose, dry layers of blankets, clothing, or towels.
- If conscious, warm beverages may help increase the body temperature. Do not give alcohol.
- Once temperature has increased keep them dry and wrapped in a warm blanket, including the head and neck.
- If no pulse, begin CPR.

Frostbite

An injury to the body that is caused by freezing, which most often affects the nose, ears, cheeks, chin, fingers, or toes.

Symptoms

- Reduced blood flow to hands and feet
- Numbness
- Aching
- Tingling or stinging
- Bluish or pale, waxy skin

First Aid

- Get into a warm room as soon as possible.
- Unless necessary, do not walk on frostbitten feet or toes.
- Immerse the affected area in warm (not hot) water, or warm the affected area using body heat. Do not use a heating pad, fireplace, or radiator for warming.
- Do not massage the frostbitten area; doing so may cause more damage.

Trench Foot

An injury of the feet resulting from prolonged exposure to wet and cold conditions that can occur at temperatures as high as 60 °F if the feet are constantly wet.

Symptoms

- Reddening of the skin
- Numbness
- Leg cramps
- Swelling
- Tingling pain
- Blisters or ulcers
- Bleeding under the skin
- Gangrene (foot may turn dark purple, blue, or gray)

First Aid

- Remove shoes/boots and wet socks.
- Dry feet.
- Avoid walking on feet, as this may cause tissue damage.

Chilblains

Ulcers formed by damaged small blood vessels in the skin, caused by the repeated exposure of skin to temperatures just above freezing to as high as 60 °F.

Symptoms

- Redness
- Itching
- Possible blistering
- Inflammation
- Possible ulceration in severe cases

First Aid

- Avoid scratching.
- Slowly warm the skin.
- Use corticosteroid creams to relieve itching and swelling
- Keep blisters and ulcers clean and covered.

Protect Yourself

- Monitor your physical condition and that of your coworkers.
- Wear appropriate clothing.
  - Wear several layers of loose clothing for insulation.
  - Tight clothing reduces blood circulation to the extremities.
- Be aware that some clothing may restrict movement resulting in a hazardous situation.
- Protect the ears, face, hands and feet in extremely cold or wet weather.
  - Boots should be waterproof and insulated.
  - Wear a hat to reduce the loss of body heat from your head.
- Move into warm locations during breaks; limit the amount of time outside.
- Carry extra socks, gloves, hats, jacket, blankets, a change of clothes and a thermos of hot liquid.
- Include chemical hot packs in your first aid kit.
- Avoid touching cold metal surfaces with bare skin.

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Centers for Disease Control and Prevention
National Institute for Occupational Safety and Health

www.cdc.gov/niosh/topics/outdoor/

DHHS (NIOSH) Publication No. 2010–115

Telephone: 1–800–CDC–INFO
TTY: 1–888–232–6348
E-mail: cdcinfo@cdc.gov