



**SWAMP Bioassessment
Procedures** 2016

Standard Operating Procedures (SOP) for the Collection of Field Data for Bioassessments of California Wadeable Streams: Benthic Macroinvertebrates, Algae, and Physical Habitat

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ABBREVIATIONS AND ACRONYMS

AFDM	Ash-Free Dry Mass
BMI	Benthic Macroinvertebrate
chl <i>a</i>	Chlorophyll <i>a</i>
CPOM	Coarse Particulate Organic Matter
CSBP	California Stream Bioassessment Procedure
DI	Deionized water
DO	Dissolved Oxygen
DFW	(California) Department of Fish and Wildlife
EMAP	Environmental Monitoring and Assessment Program (of the U.S. EPA)
EPA	Environmental Protection Agency (of the United States)
GPS	Global Positioning System
IBI	Index of Biotic Integrity
LRBS	Log Relative Bed Stability
MCM	Margin-Center-Margin
NAD	North American Datum
NBO	Neutrally Buoyant Object
NNE	Nutrient Numeric Endpoints
NRSA	National Rivers and Streams Assessment (of the U.S. EPA)
PHab	Physical Habitat
QA	Quality Assurance
QAPrP	Quality Assurance Program Plan (of SWAMP)
RBP	Rapid Bioassessment Procedures
RWB	Reachwide Benthos
SOP	Standard Operating Procedures
SCCWRP	Southern California Coastal Water Research Project
SWAMP	Surface Water Ambient Monitoring Program (of the California State Water Resources Control Board)
TRC	Targeted Riffle Composite
VAM	Velocity-Area Method (for determining stream discharge)

1. INTRODUCTION

This document describes the Standard Operating Procedures (SOP) for bioassessment of wadeable streams for the California State Water Resources Control Board's Surface Water Ambient Monitoring Program (SWAM). These procedures are recognized by the US Environmental Protection Agency (EPA) as California's standard bioassessment procedures and are designed to support general assessment of the ecological condition of wadeable streams and rivers based on the composition of the benthic macroinvertebrate and benthic algal assemblages. The procedures also produce standardized measurements of instream and riparian habitat and ambient water chemistry to support interpretation of the biological data.

Instructions are provided for collection of the following:

- samples for taxonomic analysis of benthic macroinvertebrate (BMI) assemblages
- samples for taxonomic analysis of benthic algal assemblages (diatoms & non-diatom (soft) algae (including cyanobacteria))
- samples for determination of biomass based on benthic chlorophyll *a* and benthic ash-free dry mass (AFDM)
- stream physical habitat (PHab) data
- water chemistry samples

1.1 Previous SOPs

This document represents a consolidation of two closely related previous SOPs, and supersedes them:

- **Ode (2007)**, which focused on stream BMI sampling and associated PHab data collection and replaced previous bioassessment protocols referred to as the California Stream Bioassessment Procedure (CSBP, Harrington 1995, 1999, 2002), and
- **Fetscher et al. (2009)**, which focused on stream benthic algae and biomass sampling, and associated PHab data collection.

Most of the methods described here are close adaptations of those developed by the EPA's Environmental Monitoring and Assessment Program (EMAP) and currently used by the EPA's National Rivers and Streams Assessment (NRSA) surveys. Table 1 provides a summary of the major changes to field procedures since the previous SOPs.

Summary of Changes

Table 1 Summary of Changes

Section	Category	Current Protocol	Previous Versions (Ode 2007 & Fetscher et al. 2009)
General	General	For SWAMP, the "Full" set of PHab modules must be carried out, even if just collecting algae (and not BMIs) as the biotic assemblage.	Previously, modules such as Riparian Vegetation and Instream Habitat Complexity were not required if only algae were

			being collected for bioassessment.
1.4	Diagnosing Recent Scour	Guidance is now provided for diagnosing recent scour, which may be of concern under the rare circumstance in which sampling must occur shortly following a large storm or discharge release (e.g., from a dam); field sheets now include a place to mark for scour so that applicable analytes are flagged in the database.	No previous guidance provided for diagnosing scour; no data flags for influence of recent scour.
1.8	QA	For SWAMP, duplicate sampling of BMIs and benthic algae is required at 10% of study sites.	No previous requirement for duplicate sampling.
2	Notable Field Conditions	Field forms and database now allow users to mark whether or not the sampling reach lies within an engineered channel.	No place for recording this information was previously available.
3	Water Chemistry	For SWAMP, TN and TP are now required if collecting algae for bioassessment.	No previous requirement for TN/TP.
4.5	Algae sample collection - sediment	Delimiter (coring device) to collect sediment is now properly termed “ABS delimiter”.	Was previously (erroneously) called “PVC delimiter”.
5.2	Soft Bodied Algae Processing	If there appears to be more than one type of macroalgae (i.e., obviously different species based on color/texture) in the sample, separate cylinders should be made for each one.	Previous version had all soft algae rolled together into a single cylinder.
5.2	Processing Quantitative Benthic Algal Taxonomy and Biomass Samples	The final concentration of glutaraldehyde required for the fixed (quantitative) soft-algae sample is now 2% (qualitative samples are still to be left <i>unfixed</i>). This change will be realized by using a more dilute (20%) stock solution of glutaraldehyde, rather than changing the volume of stock fixative added to the soft-algae sample.	The final concentration of glutaraldehyde required in the fixed (quantitative) soft-algae sample was previously 2.5%. The previous concentration of stock solution for glutaraldehyde was 25%.
5.2	Processing Quantitative Benthic Algal Taxonomy and Biomass Samples	The final concentration of formalin required in the diatom sample is now 1%; also, the formalin used no longer needs to be buffered, <i>but</i> if it is, then phosphate buffer, NOT BORAX should be used; COCs should indicate whether phosphate buffer has been added to the formalin or not. This change will be realized by using a more dilute (5%) stock solution of formalin, rather than changing the volume of stock fixative added to the diatom sample.	The final concentration of formalin required in the diatom sample was previously 2% and the formalin was buffered with borax. The previous concentration of stock solution for formalin was 10%.
6.2	Pebble Count	In the Pebble Count, users must now circle “D” (dry) for CPOM and Macrophytes when they correspond to a point that is not submerged/moist.	Those fields were previously left blank when the point was dry.
6.2	Pebble Count	In the Pebble Count, SWAMP now requires that users measure pebbles rather than simply putting them into bins.	Previously, users reporting to SWAMP had the option to bin or measure the

		However, binning is still allowed when, for some reason, particles cannot be measured.	pebbles.
6.2	Pebble Count	For SWAMP, presence/absence of macroalgae is recorded during the pebble count, even if only BMIs (and not algae) are being sampled.	No previous requirement for recording macroalgae presence/absence if only collecting BMIs for bioassessment.
6.4	Pebble Count; Coarse particulate organic matter	Size for coarse particulate organic matter has been changed to those which are >1 mm in size, but no larger than 10.	Previous version had no maximum size.
6.4 , 6.8	Pebble Count; Instream Habitat Complexity	Mosses are explicitly not included in macrophytes (regardless of the module).	In the previous BMI SOP (Ode 2007) mosses were included in the macrophytes.
6.5	Bank Stability	Bank stability is now assessed along the imaginary line running from where the transect ends meet the wetted margin, to the bankfull boundary.	Previously, bank stability was estimated in the area between the upstream and downstream inter-transects.
6.8	Instream Habitat Complexity	For instream habitat complexity, estimates should include only those features within the stream's wetted margin.	Previous guidance was that estimates should include features within the banks and outside the wetted margins of the stream.
6.9	Stream shading	For SWAMP, 6 densiometer readings (four in the center of the stream and one at each bank) are now required in streams > 10 m wide.	Previously, users reporting to SWAMP could collect only the four center-stream densiometer readings, with the bank readings optional.

1.2 Sampling Overview

This SOP describes methodology for biotic sampling procedures as well as for assessing instream and riparian habitats and ambient water chemistry associated with biotic assemblage samples (Table 2). The sampling layout described in this SOP provides a framework for systematically collecting a variety of biotic, physical, and chemical data. The biotic sampling methods are designed to nest within the overall framework for assessing the biotic, physical, and chemical condition of a reach. The physical habitat characterization methods can be implemented for a stand-alone evaluation or in conjunction with a bioassessment sampling event. This information can be used to characterize stream reaches, associate physical and chemical condition with biotic condition, and explain patterns in the biotic data. Measurements of instream and riparian habitat and ambient water chemistry are essential to interpretation of bioassessment data, and must always accompany bioassessment samples for SWAMP projects.

Because bioassessment data requirements vary widely across different applications, this document describes the component measures of instream and riparian habitat as independent “modules”, which may be implemented as needed for each application. For instance, if the goal is to evaluate stream primary production, one may wish to collect only biomass samples and algal cover point-intercept data, and exclude modules focusing on instream habitat complexity. Alternatively, one may need to collect BMI and/or algal taxonomic samples in order to make more refined inferences about stream condition (e.g., by applying a multimetric index based on community composition). Recommendations for modules to include in a reduced-effort (“Basic”) version of this SOP, e.g., for citizen monitoring groups on a limited budget, are provided in the Guidance Document.

In order to ensure high-quality bioassessment data, certain tasks must be carried out prior to others. A work-flow diagram depicting the order in which tasks should be undertaken is provided in Figure 1 (see Guidance Document for suggestions to maximize efficiency).

Assuming an adequate crew size, the total time required to carry out the full suite of field procedures described in this SOP is approximately 2 to 4 hours in a typical stream, or up to 6 hours in a complex stream. These estimates include only the time spent at the site, not travel time (which varies widely). Table 2 provides a rough breakdown of time requirements per module.

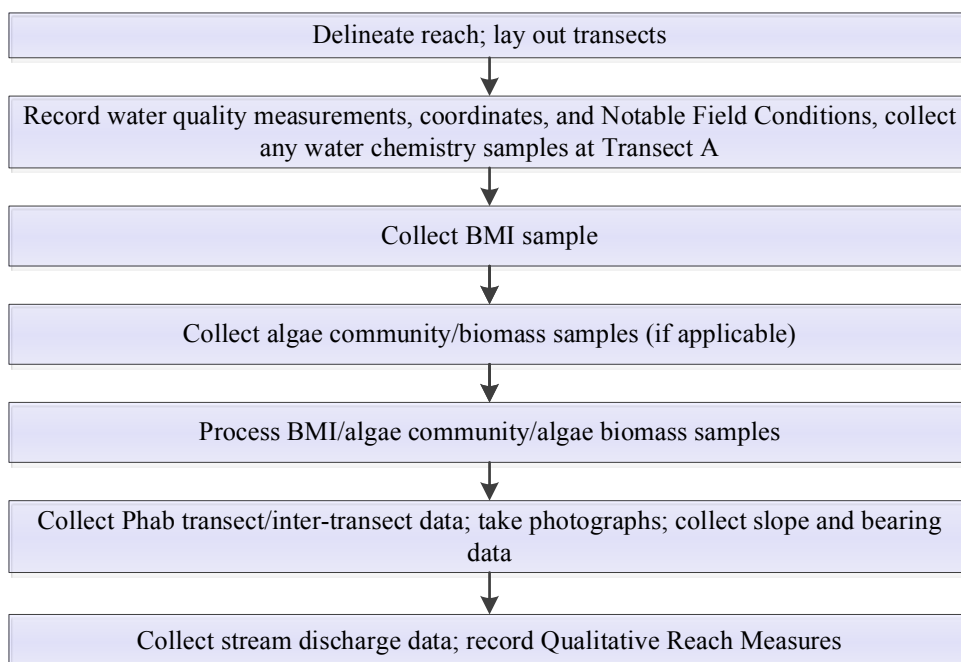


Figure 1. Recommended work flow (order of tasks) for conducting stream bioassessment.

1.3 Scope and Applicability

This SOP is intended for use in ambient monitoring of California wadeable streams that are flowing at the time of assessment, meaning that it may be used in both perennial and nonperennial streams as long as sampleability criteria are met¹. A reach is considered “sampleable” with this protocol if at least half of the reach has a wetted width of at least 0.3 m (the width of a D-frame net) and there are no more than three transects that are completely dry within the monitoring reach at the time of assessment. If more than three transects are completely dry, then the stream reach should not be sampled for biota; however, if the monitoring program allows it, the reach may be shifted in order to reduce the number of dry transects, thus allowing biota to be sampled (for more details, see Section 2 on reach delineation and transect placement). The wadeability limitation is determined by the practical ability to safely obtain a consistent sample of the benthic community from a reach. In general, a reach is considered wadeable if it is less than one meter deep for at least half the length of the reach.

It is recommended that biotic sampling be carried out during the period from May through September, depending upon the region (i.e., toward the earlier end of this range in southern California, and later in the range for higher latitudes). See Figure 2. Samples intended for ambient bioassessments are generally collected when streams are at or near base flow (i.e., not influenced by storm runoff), as sudden flow increases can displace benthic organisms from the

¹ The sampleability criteria defined here are intended to ensure comparability of data collected for ambient monitoring or regulatory compliance monitoring. Less restrictive criteria may be acceptable for other uses.

stream bottom and dramatically alter local community composition. To be conservative, it is strongly recommended that sampling be carried out at least two, and preferably three, weeks after any storm event that has generated enough stream power to mobilize cobbles and sand/silt capable of scouring stream substrates. See Section 1.4, below, for tips on how to evaluate a site for recent scour. Two to three weeks will usually allow time for benthic fauna and algae to recolonize scoured surfaces (Round 1991; Kelly *et al.* 1998; Stevenson and Bahls in Barbour *et al.* 1999). Ultimately, the time of delay from a scouring event to the acceptable window for sampling will depend on environmental setting and time of year. The project manager should consult with the SWAMP bioassessment coordinator in questionable cases.

1.4 Diagnosing Recent Scour

As mentioned above, ideally, a stream reach should **not** be sampled for bioassessment shortly following a scour event that has mobilized bed materials and potentially disrupted benthic communities. However, for certain applications (e.g., wet-weather monitoring), sampling may need to occur under such circumstances. When this happens, a note must be made in the field sheets and the database that flags applicable analytes as having potentially been subjected to recent scour conditions. If a suspected recent scour has occurred, mark “Yes” in the **Notable Field Conditions** section of the bioassessment field form that says, “Site is affected by recent scouring event”. High-flow/scour indicators that can be assessed to make the determination include:

- Lack of slime/color coating on the streambed (this may be inferred by a high frequency [i.e., near 100%] of microalgal cover scores of “0”; see Section 6.4)
- Lack of macroalgal mats, OR if present, mats displaced, as indicated by being “unnaturally” bunched up against fixed objects within the stream (like tree roots, large boulders) away from centroid of flow
- Non-rigid instream vegetation (e.g., emergent macrophytes like cattails and tules) bent over or lying down within the stream
- Absence of leaves and other detritus in pools, despite riparian cover

Following the sampling visit, under “Field Notes/Comments” on the field sheet, field crews or the project manager can add the size of, and actual time since, storms or discharge releases.

Figure 2. Index Period by Ecoregion

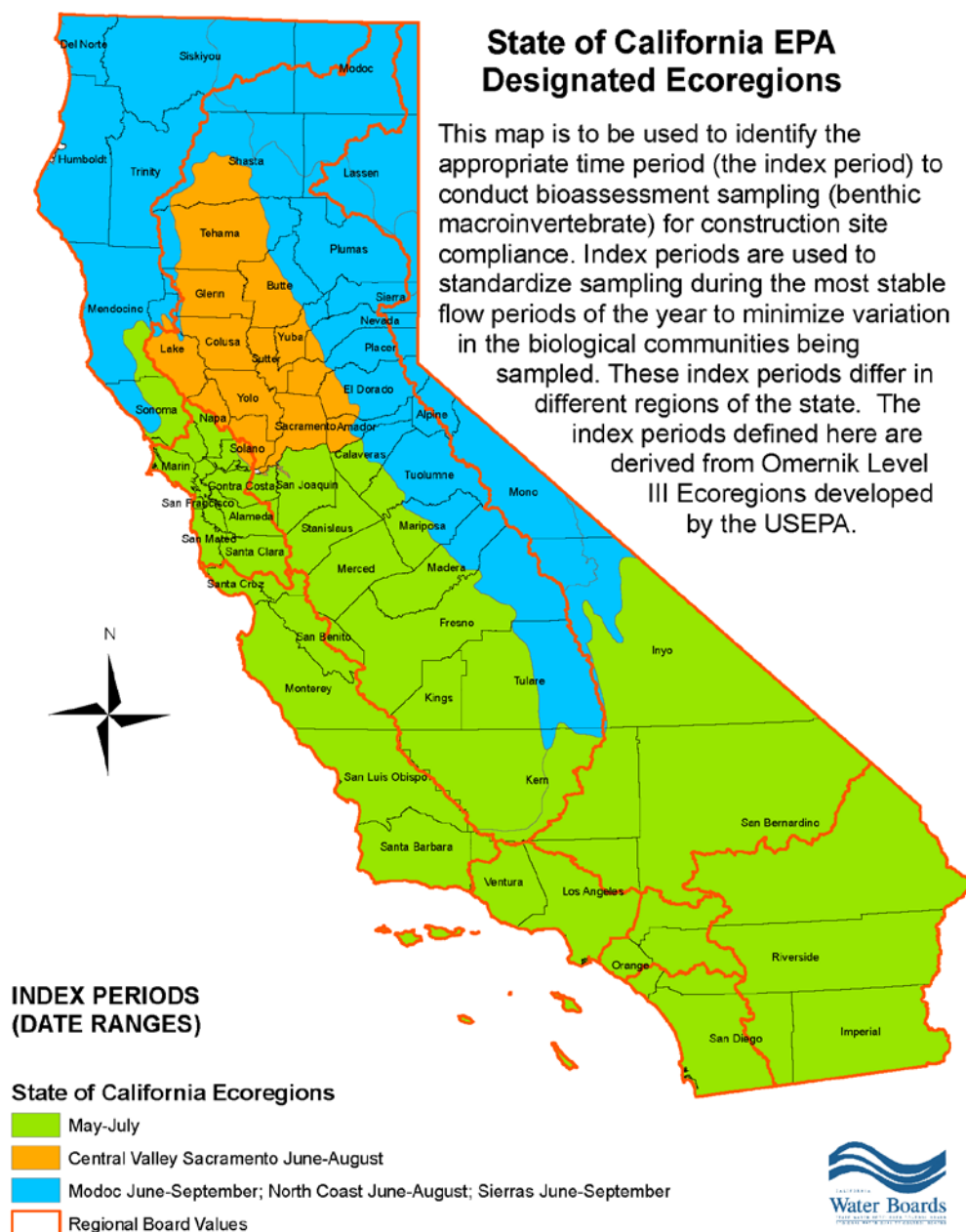


Table 2. Sample and data collection modules for BMI and algal bioassessment. The estimated time each task takes on average is provided after each Module name in parentheses. Very experienced crews may be faster in some settings.

Survey Task	Module	Time	Notes
REACH DELINEATION and WATER QUALITY Conducted before entering stream to sample biota or collect PHab data	Layout of reach, marking transects, recording GPS coordinates	15 min	Use 150m reach length if wetted width ≤ 10 m or 250m if wetted width > 10 m
	Temperature, pH, specific conductance, salinity, DO, alkalinity	10 min	Alkalinity, conductance, pH, and salinity may be measured in the laboratory from collected samples if SWAMP holding times are met whereas DO and temperature must be measured in the field
	Turbidity	5 min	Use test kit/meter or collect samples for laboratory analysis
	Notable field conditions	5 min	
	Water chemistry for laboratory analysis (total phosphorus and total nitrogen)	15 min	Required by SWAMP when algae are sampled
BIOTIC ASSEMBLAGE/ ALGAL BIOMASS AND PHAB SAMPLING AT CROSS-SECTIONAL TRANSECTS Measurements (BMIs, algae, PHab) at 11 main transects (A – K), or 21 transects (11 main plus 10 inter-transects for wetted width, substrate size, algal cover, and flow habitat)	BMI Sampling for Taxonomic IDs	45 min	
	Algal Sampling for Taxonomic IDs and biomass assessment	45 min	
	Depth and Pebble Count + CPOM	35 min	5-point substrate size, depth, and CPOM records at all 21 transects and intertransects

BIOTIC ASSEMBLAGE/ ALGAL BIOMASS AND PHAB SAMPLING AT CROSS- SECTIONAL TRANSECTS (Continued)	Cobble Embeddedness (incl. in “Pebble Count” time)		Include all cobble-sized particles in pebble count. Supplement with “random walk” if needed for 25, total
	Percent Algal Cover (part of pebble count)		Attached/unattached macroalgae presence/absence; microalgal thickness codes
	Bankfull Dimensions (10-20 min)	60-70 min	
	Wetted Width (5 min)		
	Bank Stability (5 min)		
	Human Influence (5 min)		
	Riparian Vegetation (5 min)		
	Instream Habitat Complexity (5 min)		
	Stream Shading (10 min)		6 densiometer readings required at streams where mean wetted width is > 10m; the 4 center points are sufficient in narrower streams
	Flow Habitat Delineation (15 min)		Record proportion of habitat classes in each inter-transect zone
	Slope (%) (25 min for autolevel method; 15 min for clinometer method)	15-25 min	Average slope calculated from 10 transect-to- transect slope measurements. Use autolevel for slopes $\leq 1\%$ (clinometer acceptable for steeper gradients); time requirements increase considerably in complex streams
	Sinuosity	10 min	Record compass readings between transect-to-transect centers
	Excess Sediment Transect Measures		Optional measure: Bankfull width and height, bank angles; Large woody debris counts (tallies of woody debris in several size classes); thalweg profile (100 equidistant points along thalweg); refer to NRSA SOP for details.

DISCHARGE TRANSECT	Discharge measurements (15 min for velocity-area method; 10 min for neutrally-buoyant-object method)	10-15min	Velocity-Area Method (VAM; preferred) or Neutrally Buoyant Object Method, somewhere within, or very near to, the monitoring reach; VAM may not be feasible in all streams
REACH-SCALE MEASUREMENTS	Qualitative Reach Measures (subset of Rapid Bioassessment Procedure, RBP, visuals)	5 min	Channel alteration, sediment deposition, epifaunal substrate
	Photo documentation	5 min	Upstream (Transects A, F), Downstream (Transects F, K) at minimum, but ideally add an overview picture

1.5 Training

Procedures described here are designed to produce repeatable, quantitative measures of a stream's BMI and algal assemblages and physical/habitat condition. *It is important to note that in order to generate usable data, formal field training of sampling crews is required, and Quality Assurance (QA) measures must be implemented throughout the field season.* Training courses are made available by the Water Boards Training Academy. Courses are posted regularly at: http://www.waterboards.ca.gov/water_issues/programs/academy/home.htm.

In addition, regular (e.g., yearly) field audits of sampling crews, conducted by an experienced individual, are highly recommended, with additional training and follow-up auditing carried out as necessary depending upon audit outcomes. Annual intercalibration events involving multiple crews with experience in different regions of California are strongly recommended. Contact the Department of Fish and Wildlife's Aquatic Bioassessment Laboratory to participate in intercalibration events.

1.6 Permitting

Collection of benthic samples in California waterbodies without a valid California Department of Fish and Wildlife (DFW) Scientific Collection Permit is illegal. Prior to the onset of fieldwork, a Scientific Collecting Permit (for sampling of stream biota) MUST be acquired from DFW for at least one member of the field crew. Additional information on requirements and how to obtain permits can be found in the Guidance Document. Likewise, for streams supporting species listed as sensitive under the State or Federal Endangered Species Act (including, but not limited to, California red-legged frog, least Bell's vireo, southwestern willow flycatcher, arroyo toad, and salmonids), sampling cannot be conducted at certain times of the year, or a permitted escort may be required to supervise sampling activities to ensure that resident sensitive species are not impacted. More information can be found at <http://www.fws.gov/ENDANGERED/permits/index.html> and https://www.dfg.ca.gov/wildlife/nongame/research_permit/.

1.7 Avoiding the Transfer of Invasive Species and Pathogens Amongst Sites

Proper field hygiene must be practiced at all times in order to avoid transferring invasive organisms or pathogens between sites. Examples include, but are not limited to, New Zealand mud snail and chytrid fungus. Before approaching any stream, precautions must be taken to ensure that all equipment that will come into contact with the stream or its immediate surroundings has been properly decontaminated. Such equipment includes, but is not limited to, footwear, D-frame net, algae sampling devices, water chemistry sample fill bottle, transect tape, flags, stadia rod, flow meter, water chemistry probes, and autolevel tripod. Furthermore, under no circumstances shall stream water (e.g., from water bottles used for algae sample processing) or other material collected at one site be introduced into another stream. Detailed information on acceptable decontamination procedures is provided in the Guidance Document.

1.8 SWAMP Requirements

The “reachwide benthos” (RWB) sampling procedure, as described in this SOP, is the required sampling method for ambient bioassessment under the SWAMP program. However, other sampling methods (e.g., Targeted Riffle Composite (TRC)) may be desirable if data comparability within long-term monitoring projects that have historically used other methods is sought. In general, SWAMP-funded projects must adhere to the directives of the SWAMP Quality Assurance team as detailed in: *Amendment to SWAMP Interim Guidance on Quality Assurance for SWAMP Bioassessments 9-17-08*. This memo can be found in the Guidance Document. The project manager must have the approval of the SWAMP Bioassessment Program Lead Scientist and the SWAMP Quality Assurance Officer **before** the use of alternative methods that deviate from this SOP and the above-referenced memo will be accepted. For other projects and/or programs desiring SWAMP comparability, deviations should be approved by the project manager and project QA officer.

SWAMP requires that duplicate sampling of BMIs and benthic algae occur at 10% of study sites (preferably at the same set of sites, when both assemblages are being sampled together). The recommended location for collecting duplicates is at adjacent positions along the sampling transects (described in Section 4). In addition, regular (e.g., yearly) field audits of sampling crews should be conducted by an authorized individual (e.g., qualified personnel of DFW). Note also that SWAMP requires 5% field duplicates for water chemistry measurements. In general, the SWAMP Quality Assurance Program Plan (QAPrP) in place at the time of monitoring or subsequent revisions to that QAPrP and the SMC Bioassessment QAPP (2009) should be followed for quality assurance procedures, when applicable. For more information, refer to: http://www.waterboards.ca.gov/water_issues/programs/swamp/tools.shtml#qa

SWAMP participants collecting water-quality and water-chemistry measurements may reference the California Department of Fish and Wildlife - Marine Pollution Studies Laboratory SOP: *Collections of Water and Bed Sediment Samples with Associated Field Measurements and Physical Habitat in California. Version 1.1, updated March-2014*. This procedure may be used to collect samples for a number of analyses covered by the SWAMP

Quality Assurance program. Use of this procedure is a recommendation and not a requirement for SWAMP projects. Prior to sample collection, participants using this procedure shall check its requirements against the latest SWAMP *Quality Control and Sample Handling Guidelines*.

SWAMP is planning to develop additional guidance for bioassessment quality assurance and control procedures. This may 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 (to avoid actions that would result in instrument damage or compromised samples), and interferences (regarding consequences of not following the SOP).

1.9 Supplemental Guidance

A companion document, SWAMP Bioassessment Supplemental Guidance (herein referred to as the “Guidance Document”), is referenced throughout this SOP. It provides more detailed information on the various applications of the modules described here, as well as recommendations for where, when, and/or how to implement the procedures. It also provides suggestions for how to deal with special circumstances that may be encountered during stream bioassessment sampling and more detailed information to aid in interpretation of PHab field indicators. The Guidance Document is a “living” supplement to the field sampling protocol, in the sense that it is regularly updated (unlike this SOP, which is static between versions) and serves as a repository for implementation advice. The Guidance Document is posted on the SWAMP website at http://www.waterboards.ca.gov/water_issues/programs/swamp/bioassessment/sops.shtml. Please check this site regularly in order to review the most recent information on execution of the SOP.

2. REACH DELINEATION AND SCORING NOTABLE FIELD CONDITIONS

Before biotic sample and PHab data collection can begin, the monitoring reach must be identified and delineated, information about reach location and condition is to be documented, water chemistry parameters are to be recorded, and water samples may also be collected. A set of field forms for recording information about monitoring sites, biotic samples, and associated water chemistry and PHab data is available on the SWAMP website at http://www.swrcb.ca.gov/water_issues/programs/swamp/tools.shtml#methods. Field crews using paper forms must designate someone (other than the field recorder) to review the forms for completeness² and legibility. It is imperative to 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 field partner(s). All SWAMP data management tools including an electronic data entry interface of the field forms are available

² If parameters cannot be measured for some reason, "NR" (i.e., “Not Recorded”) should be entered in the corresponding field.

from the SWAMP website for use on a portable field computer. Please visit the SWAMP Data Management Resources website for webinar training, tools, templates, and more.
http://www.waterboards.ca.gov/water_issues/programs/swamp/data_management_resources/index.shtml A list of supplies needed for sampling and data collection is provided in the Guidance Document.

Step 1. Upon arrival at the site, fill out the “Reach Documentation” section of the field forms. Record the Station Code following SWAMP formats³. Record the geographic coordinates of the **downstream end** (Transect A) of the reach (in decimal degrees to at least five decimal places) with a Global Positioning System (GPS) receiver and record the datum setting (preferably NAD83) of the unit. Coordinates are to be averaged based on procedures outlined in the GPS device manual. This average is recorded as actual coordinates on field sheet. Target coordinates need to be determined before the field sampling, and should be placed on a map (paper or digital) for visual orientation in case the GPS is not functioning in the field (e.g., in steep canyons or in mountainous regions). Sampling locations for probability sites can be moved up or downstream as much as **300 m** from the target location for reasons such as avoiding obstacles, mitigating issues regarding safety or permission to access, and GPS error. If for some reason the GPS measurements for the actual site assessed are not taken at Transect A (e.g., if no GPS signal was available at Transect A), then the actual site location must be noted on the field data sheets.

For probabilistically selected sites “target coordinates” are selected at random. Because GIS information about stream locations is imperfect, the target coordinates may not fall exactly on a streambed, but rather nearby, requiring a geospatial shift in order to correspond to the nearest streambed. The potential discrepancy between the target coordinates and where sampling actually occurs makes it essential to record the actual field coordinates on the field sheet.

Step 2. To delineate the monitoring reach, first scout it to ensure it is of adequate length for sampling biota. The length to use depends upon the average “wetted width” of the stream reach. The “wetted channel” is the zone that is inundated with water, and “wetted width” is the distance between the sides of the channel at the point where substrates are no longer surrounded by surface water. If the average wetted width ≤ 10 m, delineate a 150 m reach for sampling. If the average wetted width > 10 m, delineate a 250 m reach. When delineating the reach, *stay out of the channel as much as possible* to avoid disturbing the stream bottom, which could compromise the water and biotic samples, and PHab data, that will subsequently be collected.

Starting at one end of the reach, walk along the stream bank, taking large steps (for most adults, a large step is roughly equal to a meter) and count the steps until reaching 150 m (or 250 m for larger streams). This will give a rough idea about the location of the ends of the sampling reach. If the monitoring program affords flexibility in terms of where the sampling reach can be placed, scout for any features that should ideally be excluded (e.g., tributaries, “end-of-pipe” outfalls feeding into the channel, bridge crossings, major changes between natural and artificial channel structures, waterfalls, and impoundments). If any such features are near the target sampling location, and there is not enough room to accommodate a full 150 m reach or 250 m reach

³ Before going in the field, a station code needs to be assigned to each of the sampling sites. For SWAMP-funded projects, please contact the SWAMP database management team for station codes.

entirely upstream or downstream of the feature(s), then the reach may be shortened (to as little as 100 m) in order to exclude them. Record on the datasheet under “Actual Reach Length” the length of the reach that has been delineated.

Step 3. Use markers (e.g., wire-stemmed flags) to indicate locations of transects and intertransects. The standard sampling layout consists of 11 “main” transects (A-K) interspersed with 10 “inter-transects”, all of which are arranged perpendicularly to the primary direction of stream flow (usually the thalweg), and placed at equal distances from one to the next (Figure 3). The first flag should be installed at water’s edge on one bank at the downstream limit of the sampling reach to indicate the first main transect (“A”). The positions of the remaining transects and inter-transects are then established by heading upstream along the bank and using the transect tape or a segment of rope of appropriate length to measure off successive segments of 7.5 m (if sampling reach is 150 m), or 12.5 m (if it is 250 m).⁴

Step 4. Under “Notable Field Conditions”, record evidence of recent flooding, fire, or other disturbances that might influence bioassessment samples, such as scour, for which specific guidance is provided in Section 1.4, above. These are subjective determinations, so use whatever cues are available to make the call. If unaware of recent fire or rainfall events, select the “no” option on the form. Also, to the best of your ability, record the dominant land use and land cover in the area surrounding the reach (*i.e.*, evaluate land cover within 50 m of either side of the stream reach). Use a scaled aerial photograph of the site and vicinity as an aid. Finally, mark whether or not the sampling reach occurs within an engineered channel⁵.

⁴ Although it is usually easiest to establish transect positions from the banks (this also prevents disturbance to the stream channel), this can result in uneven spacing of transects in complex stream reaches. To avoid this, estimate transect positions by projecting from the mid-channel to the banks. Refer to Figure 3 for a visual clarification of proper transect alignment relative to the stream’s direction of flow. For monitoring reaches of non-standard length (*i.e.*, < 150 m; see Step 2 above), divide the total length of the reach by 20 to derive the distance between the adjacent main, and inter-, transects. Alternating between two different flag colors (e.g., orange and yellow, or blue), to demarcate main- vs. inter-transects is recommended, as well as writing the transect/inter-transects names on the flags.

⁵ Engineered channels include streams that have been straightened or armored (with riprap, rocks, grout, concrete, or earthen levees) on the banks, streambed, or floodplain of the channel. Partially armored channels (e.g., armored only at bridge abutments) are considered to be “engineered”.

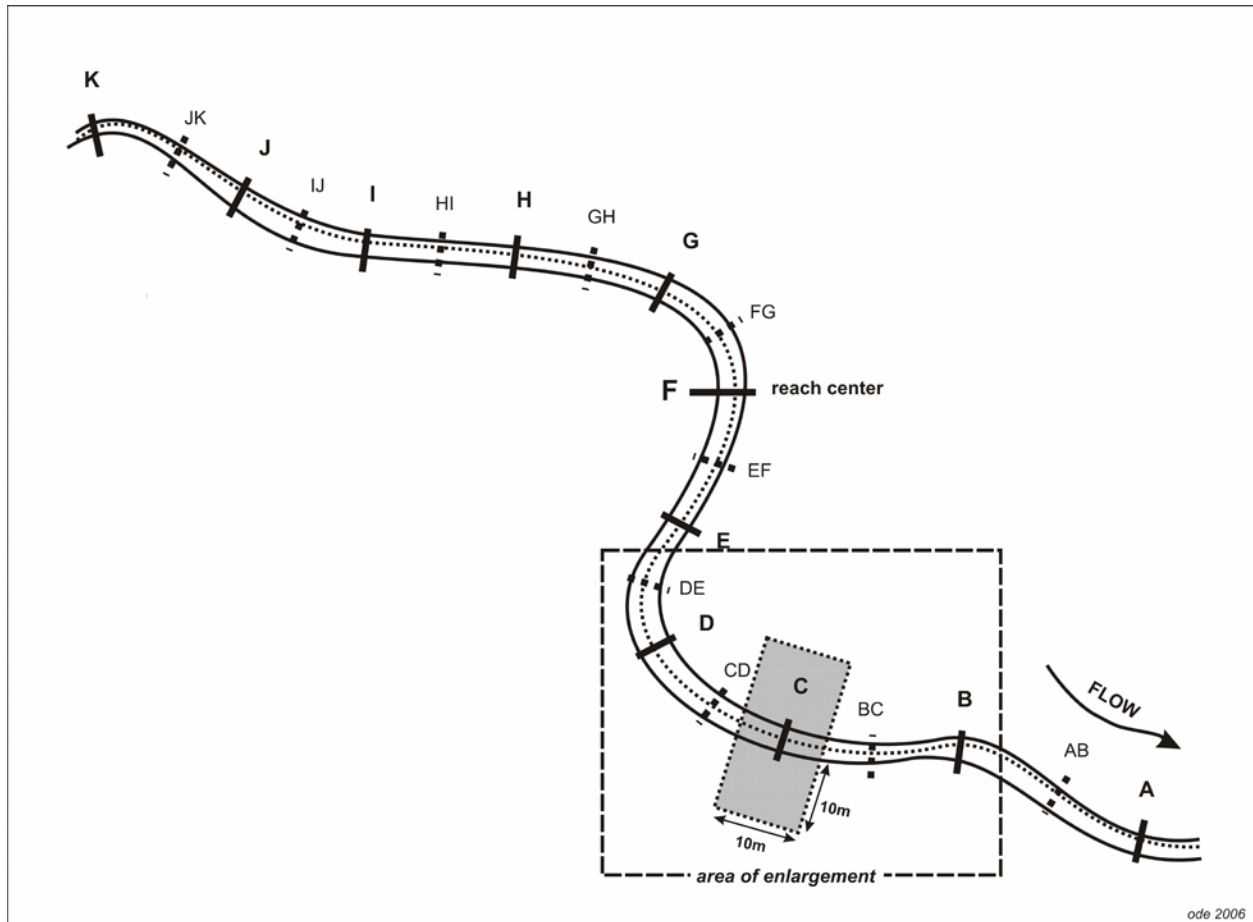


Figure 3. Reach layout geometry for physical habitat (PHab) and biotic sampling showing positions of 11 main transects (A-K) and the 10 inter-transects (AB-JK). The “area of enlargement” highlighted in the figure is expanded in Figure 17. *Note:* reach length = 150 m for streams ≤ 10 m average wetted width, and reach length = 250 m for streams > 10 m average wetted width.

3. WATER CHEMISTRY SAMPLING

Before entering the stream to sample water, remember to adhere to proper field hygiene practices (see Section 1.7 for more details) at all times. In addition, be sure to sample water in such a way that it does not interfere with subsequent 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 the consequent introduction of this matter into the water chemistry samples). All water chemistry/toxicology samples should be collected prior to stepping in the water anywhere upstream of the water/toxicology sampling spot and should not be collected in a location where subsequent biotic samples or PHab data are to be collected. Sampling water chemistry just downstream of Transect A, the same general location as where the GPS coordinates were taken⁶, and before any other sampling activities take place, achieves both of these goals.

Step 1. Calibrate probes as necessary (some require daily calibration) and record the calibration date on the field form. For calibration procedures, follow the SWAMP QAPrP in place at the time of monitoring or subsequent revisions to that QAPrP (http://www.waterboards.ca.gov/water_issues/programs/swamp/tools.shtml#qa), or the manufacturer's guidelines, whatever is more stringent. Field measurements in this SOP are typically taken with a handheld water-quality meter (e.g., YSI, Hydrolab), but field test kits (e.g., Hach) may provide acceptable information as well.

Step 2. Measure and record common ambient water-chemistry parameters⁷:

- Turbidity (NTU)
- Water temperature (°C)
- Specific conductivity (µS/cm)
- Salinity (ppt)
- Alkalinity (mg/L)
- pH
- Dissolved oxygen (mg/L and % saturation)

Because it may be affected by disturbance of the streambed that occurs during sampling, measure turbidity (if applicable) first. If water samples are also to be collected, such sampling should also occur at this location and time, and collection should also precede probe measurements. Measurements and water chemistry sample collection should take place in areas with flowing water, avoiding depositional zones (e.g., pools), if possible.

⁶ If, for whatever reason, measurements are not taken at Transect A before biotic sampling in the reach has begun, they should be taken immediately upstream of Transect K (the most undisturbed transect), and this change of sampling location should be noted on the field sheet.

⁷ SWAMP-required ambient water chemistry parameters measured in the field are: pH, DO, specific conductivity, salinity, alkalinity, and water temperature. Samples for all other ambient water chemistry should be analyzed in the laboratory (except for silica, which can be measured in the field with kits *or* in the laboratory). Turbidity and silica are optional measurements for SWAMP purposes.

Turbidity can be measured with a multi-probe (e.g., YSI) or a turbidimeter, or it can be analyzed in the laboratory. If using a portable meter, collect approximately 250 mL of water for turbidity measurements approximately 10 cm below the water surface (if possible), and take two separate readings from subsamples of the same grab sample and report the average. Likewise, all probe measurements should be made 10 cm below the water surface.

Alkalinity (mg/L) may be measured with a field test kit (e.g. Hach AL-AP #2444301) or in the laboratory. A digital titrator (e.g., Hach) using low-concentration acid (such as 0.16N H₂SO₄) as the titrant is recommended for determining alkalinity in low-alkalinity streams (i.e., < ~100 mg/L CaCO₃). If algae samples are being collected, SWAMP requires that samples also be collected for analysis of water-column total nitrogen (TN) and total phosphorus (TP); nitrate-nitrite, and orthophosphate are also recommended. TN/TP samples should not be filtered. Sample holding times, field preparation, bottle types, and recommended volumes for each water-chemistry analyte can be found in the Quality Control and Sample Handling Guidelines⁸ (http://www.waterboards.ca.gov/water_issues/programs/swamp/tools.shtml#field). Greater detail on field sampling methods for water chemistry can be found at: http://www.waterboards.ca.gov/water_issues/programs/swamp/docs/final_collect_water_sed_phys_habitat.pdf.

⁸ Crews can opt to collect water at the end of sampling for holding time purposes, in which case sampling should be conducted in undisturbed water.

4. BIOTIC COMMUNITY SAMPLING

Once the transects have been laid out and water sampling is complete, the biotic samples (BMIs and/or algae) can be collected. On a transect-by-transect basis, any biotic sampling should occur before PHab data are collected, and BMIs should always be collected before algae because BMIs are often highly motile and could be flushed by the algae sampling activity.

4.1 The Reachwide Benthos (RWB) Method for Biotic Sample Collection

The RWB procedure employs an objective method for selecting subsampling locations that is built upon the layout of the 11 main transects that will be also used for physical habitat measurements. This 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-samples may fall in a variety of “erosional”⁹ or “depositional”¹⁰ habitats.

For the RWB method, the sub-sampling position alternates between left, center, and right portions of the main 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”) across the wetted width in most systems. The left and right sides of the stream are determined when facing downstream.

SWAMP programs should employ a modified version of the RWB method, called the Margin-Center-Margin (MCM) method when all three of the following stream conditions are met: 1) very low slope (generally $< \sim 0.3\%$); 2) uniform sandy/fine-substrate; and 3) stable habitat at stream margins. The MCM protocol modification is to collect subsamples at 0%, 50%, and 100% of wetted width instead of 25%, 50%, and 75%, to ensure collection of biota from marginal habitats. There is no hard rule for using the MCM variation, but in general it should be reserved for reaches where the bulk of the streambed consists of unstable habitat (e.g., shifting sands), and the only stable microhabitats (e.g., macrophytes, algae) are restricted to the margins and would otherwise be missed. The type of sampling method used (RWB, MCM, or TRC) should be circled on the field sheet under “collection method”.

The recommended method for collecting duplicate biotic samples is at adjacent positions along the sampling transects according to the scheme depicted in Figure 3 (the duplicates are shown in light grey, with dashed-line outlines). Both samples should be collected at each transect before moving on to the next transect.

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 Conventionally, “left bank” has been defined as the left bank when facing *downstream* (i.e., in the direction of the current).

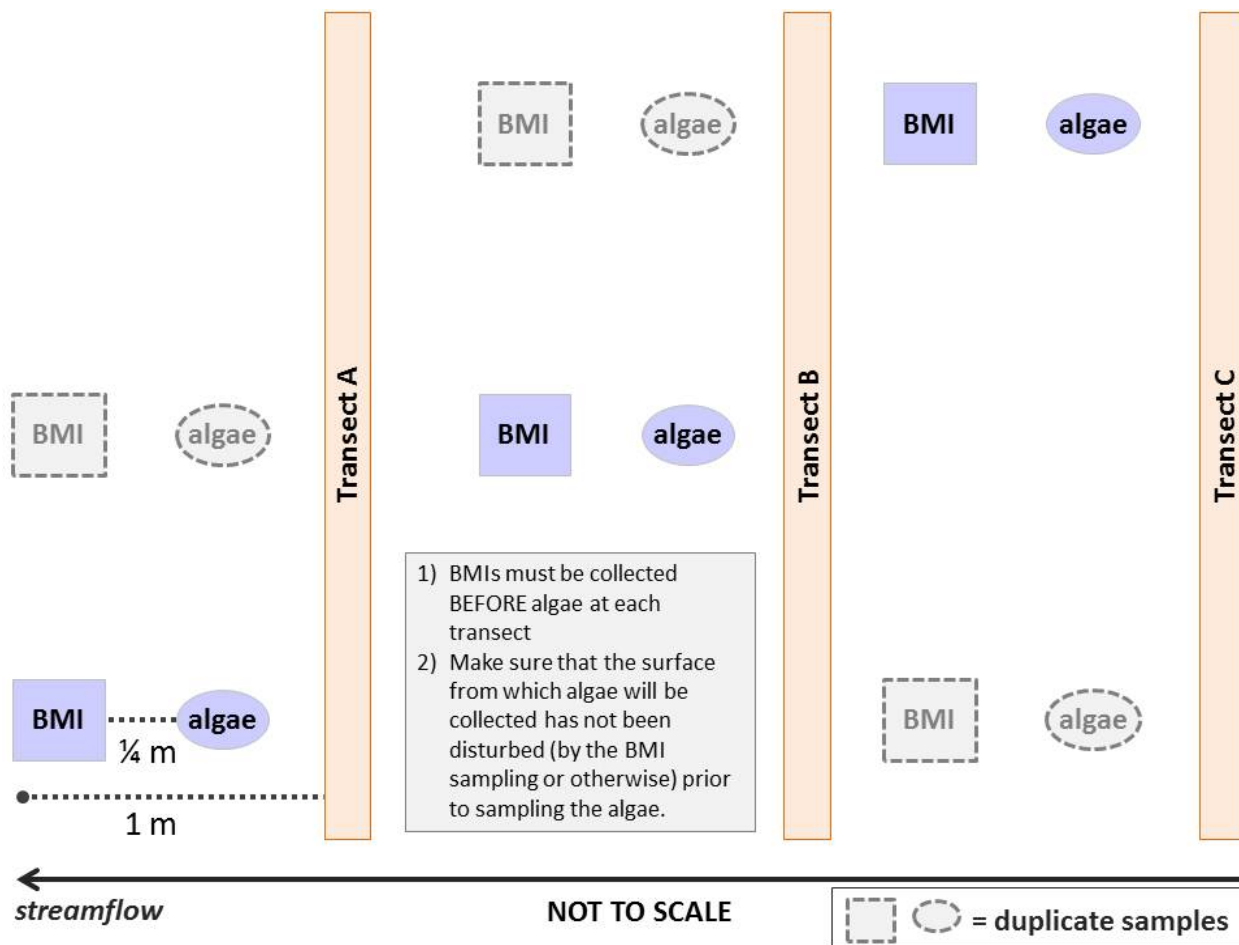


Figure 4. Sampling array for collection of BMIs, algae, and duplicate samples (outlined with dashed lines) for each assemblage. The lower left corner of diagram shows distances between BMI and algae sampling points relative to a transect (i.e., one sample collected at the Left location while the duplicate is collected at the Center). For convenience, only Transects A through C of the sampling reach are shown, but the same pattern of placement should be rotated across all 11 transects.

4.2 General Considerations for Sampling BMIs

While TRC sampling for BMIs may be considered useful for some programs, RWB is the required procedure for SWAMP programs. The following section describes only the RWB method. Supplemental information on TRC can be found in the Guidance Document.

Before sampling BMIs at any given site, be sure to thoroughly inspect the D-frame net to ensure that no organisms are carried over from previous sites, which could contaminate the sample.

4.3 Module A: RWB Sampling Procedure for BMIs

Step 1. Starting with the downstream transect (Transect A), identify a point that is 25% (or 0% for the MCM modification) of the stream width from the left bank. If it is not possible to collect

a sample at the designated point because of deep water, obstacles, or unsafe conditions, adjust the sampling spot while keeping the point as close as possible to the designated position. Always be as objective as possible when identifying the sampling spot; resist the urge to sample the “best looking” or most convenient area of the streambed.

Step 2. Once the sampling spot is identified, place the 500- μ m D-frame net in the water 1 m downstream of the target transect. In order to avoid affecting subsequent PHab data collection, do not sample directly on the transect. Position the net so its mouth is perpendicular to, and facing into, the flow of the water. If there is sufficient current in the area at the sampling spot to fully extend the net, use the normal D-net collection technique (as described in steps 3-6 below) to collect the sub-sample.¹²

Step 3. Holding the net in position on the substrate, visually define a square shape (a “sampling plot”) on the stream bottom upstream of the net opening, approximately one net-width wide and one net-width long. Because standard D-nets are 12 inches wide, the area within this plot is 1 ft² (0.09 m²). Restrict sampling to within that area.

Step 4. Working backward from the upstream edge of the sampling plot, check the sampling plot for heavy organisms such as mussels, caddis cases, and snails. Remove these organisms from the substrate by hand and place them into the net. Carefully pick up and rub stones directly in front of the net to remove attached animals. Pick up and clean all of the rocks larger than a golf ball within the sampling plot such that all the organisms attached to them are washed downstream into the net. Set these rocks outside the sampling plot after they have been cleaned. Large rocks that protrude less than halfway into the sampling area should be pushed aside. If the substrate is consolidated, bedrock, or comprised of large, heavy rocks, kick and dislodge the substrate (with the feet) to displace BMIs into the net. If a rock cannot be removed from the stream bottom, rub it with your hands or feet (concentrating on cracks or indentations), thereby loosening any attached insects. While disturbing the plot, let the water current carry all loosened material into the net. Do not use a brush to dislodge organisms from substrates.

Step 5. Once the coarser substrates have been removed from the sampling plot, dig through the remaining underlying material with fingers or a digging tool (e.g., rebar or an abalone iron) to a depth of about 10 cm (less in sandy streams), where gravels and finer particles are often dominant. Thoroughly manipulate the substrates in the plot to encourage flow to dislodge any resistant organisms. Note: the sampler may spend as much time as necessary to inspect and clean larger substrates, but should take a standard time of 30 seconds for the digging portion of this step. To the extent practical, reduce the amount of sand particles in the net, as they damage organisms and degrade taxonomic data quality.

¹² When sampling in slack water and flow volume is insufficient to use a D-frame net to capture dislodged BMIs drifting downstream, spend 30 seconds hand picking a sample from 1 ft² area of substrate at the sampling location. Then stir up the substrate with gloved hands and use a sieve with 500- μ m mesh size to collect the organisms from the water in the same way the net is used in larger pools to wash the organisms to the bottom of the net.

For slack-water habitats, vigorously kick the remaining finer substrate within the plot using the feet while dragging the net repeatedly through the disturbed area just above the bottom. Keep moving the net so that the organisms trapped in the net will not escape. Continue kicking the substrate and moving the net for 30 seconds. For vegetation-choked sampling points, sweep the net through the vegetation within a 1-ft² (0.09 m²) plot for 30 seconds. After 30 seconds, remove the net from the water with a quick, upward motion to wash the organisms to the bottom of the net.

Step 6. Let the water run clear before carefully lifting the net. Dip the lower portion of net in the stream several times to remove fine sediments and to concentrate organisms into the end of the net, while being careful to prevent water or foreign material from entering the mouth of the net. *Be particularly careful to avoid “backflow” situations, in which collected material restricts flow through the net and the resulting turbulent flow causes collected material to escape the net; this is a major potential source of loss of BMIs during sampling.*

Step 7 Move on to the next transect to repeat the sampling process across all 11 main transects. The sampling position within each transect is alternated between the left, center, and right positions along a transect (25%, 50%, and 75% of wetted width, respectively, for standard RWB, or 0%, 50%, and 100% if using the MCM collection method), then cycling through the same order over and over again while moving upstream from transect to transect. Ultimately, you will collect from the left and center 4 times each, and the right 3 times.¹³

Step 8. Fill and label sample jars. Once all 11 subsamples have been collected, proceed to Section 5.1 “Processing Benthic Macroinvertebrate Samples”.

4.4 General Considerations for Sampling Benthic Algae

The following is a short introduction to 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 fieldwork and laboratory work. Percent algal cover, for instance, is a rapid means of estimating algal primary production that can be carried out entirely in the field and is conducted in tandem with the PHab pebble count. Therefore, the 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 (Tetra Tech 2006). Higher resolution taxonomic information about algal assemblages can be used in algal Indices of Biotic Integrity (IBIs; *e.g.*, Fetscher *et al.* 2014), and offers more in-depth insight into water quality. For this type of data, algal specimens

¹³Care should be taken in transporting samples between reaches. The use of a reachwide sample bucket can help minimize any possible sample loss. Samples from each transect can be placed in the bucket for transport. This method would be similar to the reach wide sample bucket used for algae sampling.

must be collected quantitatively (and qualitatively, in the case of soft-bodied algae). The quantitative samples are fixed (preserved) and both quantitative and qualitative samples are 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 algal data described in this SOP require RWB or MCM sampling of algal specimens in a manner analogous to that which is carried out for BMIs.

With the exception of the qualitative soft-algae sample, all of the algae samples described in this SOP can be obtained from a single “composite sample” (Figure 5) generated by the RWB (or MCM) 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 3 provides a summary of their attributes.

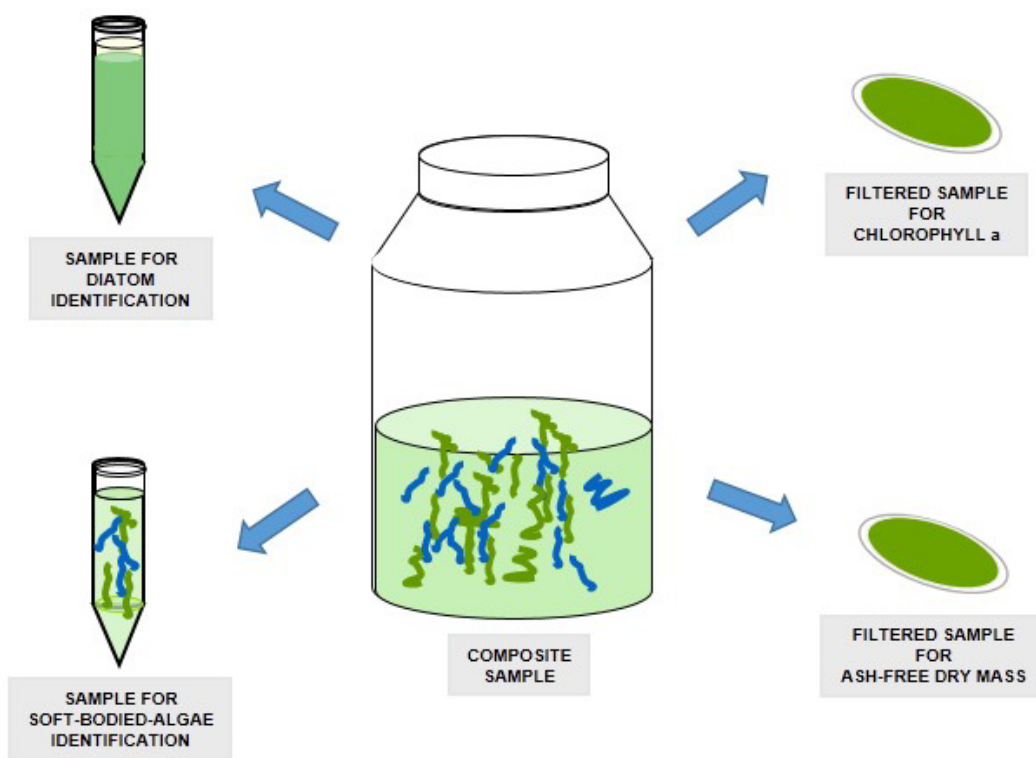


Figure 5. The four sample types that can be prepared from the algae “composite sample”.

Table 3. Types of algal indicators and considerations for their assessment.

	Algal indicator for	Collection method	Collection vessel	Preservation / fixation method / holding times
Percent Algal Cover	Stream primary production measured as algal abundance	Point-intercept component of the PHab pebble count	N/A	N/A
Chlorophyll <i>a</i>	Stream primary production measured as algal biomass; key indicator for the Nutrient Numeric Endpoints (NNE) framework	RWB or MCM sample collection	Glass-fiber filter	Filter, wrap in foil, store on wet ice in the field, but freeze (pref. -80°C) within 4h of collection; analyze within 28d
AFDM	Stream primary production measured as biomass of organic matter, including algae; indicator for the NNE framework	RWB or MCM sample collection	Glass-fiber filter (pre-combusted ¹⁴)	Filter, wrap in foil, store on wet ice in the field, but freeze (pref. -80°C) within 4h of collection; analyze within 28d
Diatoms	Indicative of factors such as trophic status, organic enrichment, low DO, siltation, pH, metals.	RWB or MCM sample collection	50 mL centrifuge tube	Add 5% formalin for a 1% final concentration immediately after collection; keep dark and away from heat; fixed samples can be stored for at least 2 years
Soft-bodied algae <u>quantitative</u> sample¹⁵	Indicative of factors such as nitrogen limitation/ trophic status; siltation; pH; temperature, light availability, nuisance/ toxic algal blooms	RWB or MCM sample collection	50 mL centrifuge tube	Keep unfixed samples in dark on wet (not dry) ice; add glutaraldehyde (to a 2% final concentration) <i>under a fume hood</i> , as soon as possible, but no later than 96 hours after sampling; after fixing, refrigerate and keep in dark; fixed samples can be stored for at least 2 years
Soft-bodied algae <u>qualitative</u> sample	Used for IBI calculation as well as to help laboratory identify specimens in the quantitative sample (above)	By hand	Whirl-Pak™ bag	No fixative; keep fresh sample on wet ice (or refrigerated) and in the dark; tally species present within 2 weeks of collection (preferably much sooner)

¹⁴ Pre-combustion removes any possible residual organic matter from the filter.

¹⁵ For the purposes of this SOP, the soft-bodied assemblage includes cyanobacteria

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 the decay of soft-bodied algae (especially for the fresh qualitative samples; see Section 4.6, “Procedure for Collecting and Storing Qualitative Benthic Algal Samples”).

4.5 Module B: RWB Sampling Procedure for Benthic Algae – Quantitative Samples

As with the RWB and MCM methods for BMIs, a quantitative subsample of benthic algae is collected at each of the 11 main transects, and these are combined into a single composite sample. Up to four aliquots are then drawn from the composite sample, and these can be used for analysis of the following: diatom assemblage, soft-bodied algae assemblage, benthic chlorophyll *a* concentration, and benthic AFDM concentration. A qualitative sample of soft bodied algae is collected in addition to the quantitative sample (see Section 4.6, below). Also, as with BMIs (see Section 4.3, Step 1; and Fig. 4), algae sample collection should begin at Transect A and proceed upstream to Transect K, rotating through the “left”, “center”, “right”, “left”, etc. positions along the 11 main transects. At each transect, BMIs must be collected before algae in order to minimize the chances of disturbing BMIs (potentially causing some to flee the area) during collection of algae. It is likewise important to make sure that the surface from which algae will be collected has not been recently disturbed (by the BMI sampling, or otherwise) prior to sampling the algae.

After the BMIs are collected at a given spot, the algae sample should be taken ¼ m upstream from the center of the upper edge of the scar in the stream bottom left from the BMI sampling, according to the schematic in Figure 3. The best way to guarantee that BMI sampling does not interfere with algae sampling is for the person sampling algae to witness exactly where the BMI collector is disturbing the stream bottom in the process of sampling the BMIs. One should not rely upon guessing where the BMIs were collected in order to determine this. Sometimes the “scar” where BMIs were collected will be obvious, but often it will not. If only algae (and not BMIs) are being collected, then the specimens should be collected 1 m downstream of the transects. If only algae (and not BMIs) are being collected in a low-slope reach in which the MCM method is employed, the collection location should be 1 m downstream of the main transect and, for each of the “margin” positions, at a distance of 15 cm (i.e., ½ the width of a D-frame net) inward from the wetted margin of the bank.

To ensure that samples of the stream’s algal community and algal biomass concentration are representative of the sampling reach, samples should always be collected by centering the sampling device on the specific point indicated in the above guidelines (*i.e.*, resisting the urge to subjectively choose where to sample). This is particularly important for yielding a representative biomass sample, because subjectively choosing or avoiding spots with high or low levels of algal growth can easily bias the results.

Because in the RWB and MCM methods, subsample locations are objectively defined by the transect layout, the position of individual subsampling points may fall within a variety different types of habitats, each of which has implications for the type of substrate likely to be encountered and therefore the type of algae sampling device to use. When confronted with a

situation in which an algae sampling location straddles two substratum types, overlay a sampling device (e.g., the rubber delimiter) centered on the sampling spot and determine which substrate occupies the majority of the area inside the delimiter, then shift the sampling spot the minimal distance necessary for that substrate type to be entirely within the delimiter, and sample there. Three devices are possible: a syringe scrubber (for hard, immobile surfaces, such as bedrock), a rubber delimiter (for hard, mobile surfaces, such as cobbles and small boulders), and an ABS delimiter (for soft, particulate substrates, such as sand). As the subsamples are collected, a tally must be taken of the number of times each of the classes of sampling device is used: 1) delimiter (either ABS or rubber), and 2) the syringe scrubber. The tallies are used to estimate the total surface area sampled (i.e., 12.6 cm² for each use of the rubber or ABS delimiter and 5.3 cm² for each use of the syringe scrubber). The tallies are recorded in the “Algae Samples” field form under “Collection Device”. The total surface area is used to estimate the soft-bodied algal total biovolume and the chlorophyll a and AFDM values. Instructions for making all algae-sampling devices are provided in the Guidance Document.

The recommended method for collecting duplicate algae samples is analogous to that described for BMIs: at adjacent positions along the sampling transects according to the scheme depicted in Figure 3. Both the sample and the duplicate should be collected at each transect before moving on to the next transect.

Before sampling, the dish tub or bucket that will contain the material to be collected must be scrubbed with a *stiff-bristled brush or scouring pad* and thoroughly rinsed with stream water from the site to be sampled, 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 apparatus (e.g., toothbrushes, graduated cylinders, delimiters, trowels, syringe scrubbers, turkey basters).¹⁶

4.5.1 Collecting from Cobbles, Large Gravel, and Wood Using the Rubber Delimiter

Step 1. If the substrate type corresponding to the algae sampling point is located on a large piece of hard substrate that can be easily removed from the stream (e.g., a cobble, a piece of wood, or a piece of large gravel), use the rubber delimiter. These substrates typically occur in erosional habitats, such as riffles and runs. Carefully lift the substrate, moving slowly to avoid disturbing its top surface as much as possible, and remove it from the water. Always collect the algae sample from the substrate that is most exposed to the sun. If a sampling point is covered by a thick mat of macroalgae, the “substrate” collected at that point would be macroalgae itself (see Section 4.5.3), not the material that lies beneath it.

Step 2. Hold the substrate over a dish tub or bucket and wrap a rubber delimiter (Figure 6) around the piece to expose the sun-exposed surface through the hole. Center the hole on the exact point on the cobble that had been identified as the “algae sampling point” for that transect, and avoid subjectively choosing the spot that is easiest to sample or has the most algae.

¹⁶ Scrubbing of the collection bucket/tub can be done prior to arriving at the site but must be checked upon arrival.

Step 3. Dislodge attached algae from this area by brushing it with a clean, firm-bristled toothbrush. If there is a thick mat of attached algae on the piece of substrate, or the algae is firmly encrusted on its surface, use forceps or a razor blade first to scrape the larger algal matter and place this in the dish tub. Then scrub the area with the brush. Collect only algal material that is visible within the area defined by the hole, as the algal filaments are laying down on the surface of the substrate and within the delimiter. Portions of algae filaments that extend beyond the opening of the hole are not part of the sample. Make sure that the entire surface within the delimiter has been scrubbed well in order to remove all the algae in that area.



Figure 6. Rubber delimiter

Step 4. Fill a wash bottle or turkey baster with stream water from the current site. Using as small a volume of water as possible, rinse the scrubbed algae from both the toothbrush and the sample area on the piece of substrate 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 point directly into the tub rather than flowing along the (non-target) sides of the substrate. Use water sparingly for each piece of substrate, because ideally less than 500 mL water, total, should be used for the full set of 11 samples collected along the transects; this includes any water used for rinsing algae off of sampling devices into the dish tub. The scrubbed part of the substrate should feel relatively rough, indicating that most of the algae have been removed. Several rounds of scrubbing and toothbrush-rinsing may be required in order to achieve this state. After thoroughly scrubbing and rinsing the sampling area on the piece of substrate, return it to the stream.

4.5.2 Collecting from Sediment

Step 1. If the substrate type that falls under the sampling point is made of particulate matter, such as silt and fine gravel, use the ABS delimiter. Typically, this occurs in depositional habitat, such as pools. The ABS delimiter is a plastic corer with an internal diameter of 4 cm (Figure 6). Quantitatively isolate sand/silt/gravel, centered on the sampling point, by pressing into the top 1 cm of sediment with the delimiter. A brightly colored line painted around the periphery of the delimiter, at 1 cm above the lip of the opening, is helpful for confirming insertion depth.



Figure 7. ABS delimiter, showing pink line at 1cm depth mark

Step 2. Gently slide a pointed, flat masonry trowel beneath the delimiter, being careful to keep the collected sediment contained within the area demarcated by the delimiter. Lift the delimiter,

keeping a tight seal between the delimiter and trowel to prevent the water inside from leaking out, resulting in loss of sample material.

Step 3. Remove sediment around the outside of the delimiter, and then empty the entire delimiter's contents into the dish tub. Using water sparingly, rinse any leftover sediment from the trowel into the tub.

4.5.3 Collecting a Mass of Macroalgae Using the ABS delimiter

Step 1. If the target substrate 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), position the trowel directly under the macroalgae and press the ABS delimiter into the algae to define a 12.6 cm² area. Note: when collecting a mass 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, feel under the mat to determine where the bottom is, slide the trowel down to that spot, and then press the ABS delimiter downward slowly to “sandwich” the targeted section of macroalgae between the delimiter and the trowel. The goal is to collect a representative sample of the algae, by stream bottom area, as it exists in the stream.

Step 2. Use a sharp razor blade or knife to cut away and discard algae material from around the edges of the delimiter. Do not pull filaments without cutting them, and do not bunch the macroalgae up nor stretch it out during this process.

Step 3. Add the macroalgal specimen that was isolated by the ABS delimiter to the dish tub.

4.5.4 Collecting from Macrophytes

Step 1. If the material to be sampled is part of a submerged, living macrophyte, or old, dead leaves settled at the bottom of a pool, use the ABS delimiter/trowel combination to isolate a 12.6 cm² section of macrophyte that has been exposed to the surface of the stream.

Step 2. As with the macroalgae (Section 4.5.3), cut away and discard the extra material that falls outside the delimiter.

Step 3. Add the macrophyte specimen that was isolated by the ABS delimiter to the dish tub.

4.5.5 Collecting from Hard, Submerged, Anchored Substrates: Concrete, Bedrock, and Boulders

Step 1. If the substrate at a sampling point cannot be removed from the water (as in the case of bedrock, a large or deeply embedded boulder, a concrete channel bottom, or hardpan), use a “syringe scrubber” device (Davies and Gee 1993; Figure 7) to collect a sample underwater. To use this device, affix a fresh, white scrubbing pad circle onto the bottom of the syringe



Figure 8. Syringe scrubber.

plunger using the Velcro hooks on the end of the plunger. Submerge the device in the stream and work the plunger up and down a couple times to lubricate it. Then press the plunger down so that the bottom of the scrubbing pad is flush with the bottom of the barrel.

Step 2. Submerge the syringe in the stream again, this time pressing the syringe bottom firmly against the substrate, centered on the sampling point. Once a good seal with the substrate is achieved, rotate the syringe scrubber completely 3 times in order to collect the biofilm from the substrate surface onto the pad. If the surface of the substrate where the sampling point fell is not flat enough to allow for a tight seal with the syringe barrel, move the collection point to the nearest area that is sufficiently flat and collect the sample there.

Step 3. After rotating the syringe scrubber, and before removing it from the substrate, gently retract the plunger slightly (e.g., <5 mm), so that the pad is no longer touching the substrate, but not so much that a lot of water enters the barrel. Carefully slide the trowel under syringe barrel, slightly tilting the barrel to allow the trowel to enter. If there is a strong current, lift the downstream side of the barrel. Then pull the instrument back out of the water with the trowel still firmly sealed against the syringe-barrel bottom.

Step 4. Hold the syringe scrubber over the dish tub and remove the trowel, allowing any water that was between the trowel and the scrubber pad to fall into the tub (but discard the water inside the plunger-handle end of the barrel—there is no need to add this water to the dish tub, as it does not contain sample material and will only serve to dilute the sample).

Step 5. 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 filled with stream water from the current site, and wringing the pad into the dish tub before discarding it. 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. If there are filaments of algae entrained within the pad, remove these using pointed-tip forceps, and place these in the dish tub, before wringing the pad out. It is recommended that a fresh (new) pad be used each time a sample is collected, even within the same stream reach. After completing sampling at a site, discard all used pads—they should never be reused between sites.

4.5.6 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 deemed 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).

As with BMIs, after collecting at each sampling spot, move on to the next transect to repeat the sampling process across all 11 main transects. The sampling position within each transect is alternated between the left, center, and right positions along a transect (25%, 50% and 75% of wetted width, respectively, or corresponding to the 0%, 50%, and 100% points across the stream if using the MCM protocol for BMI sampling), then cycling through the same order over and over again while moving upstream from transect to transect. Once all 11 subsamples have been collected, proceed to Section 5.2, “Processing Quantitative Benthic Algal Taxonomy and Biomass Samples”.

4.6 Module B (continued): Procedure for Collecting and Storing Qualitative Soft Algae Samples

Whenever quantitative soft algae samples (Section 5.2) are collected for taxonomic analysis, a “qualitative” soft algae sample must also be collected. The qualitative sample consists of a composite of all types of soft-bodied algae observed within the reach. This sample is required for calculation of some of the metrics for the IBIs that use soft algae data, such as “H20” and “S2” (Fetscher et al. 2014). The qualitative sample 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. In addition, if the qualitative sample is kept cool and in the dark, and is delivered to the laboratory in a timely manner (*i.e.*, within two weeks of collection), there is a possibility of culturing live specimens, which is sometimes essential for standard taxonomic effort-level identifications.

Collection of the qualitative soft-bodied algae sample can be conducted at any time during the field visit, as long as its collection does not in any way interfere with the water chemistry, biotic, and PHab sampling/data collection (*i.e.*, by kicking up sediment, displacing BMIs, and/or disturbing the stream bottom). It helps to have the collection bag on hand at all times so that it can be used for spontaneous grabs of specimens that are spotted during the course of the other fieldwork (*e.g.*, conducting PHab data collection). However, the entire sampling reach should be visually scoured at least one time during the course of the day’s fieldwork in an effort to see, and collect samples from, all patches of distinct soft-bodied-algal specimens therein.

Step 1. Using a thick, waterproof marker, label a Whirl-Pak™ bag with the Station Code, Date, and Sample ID.

Step 2. Hand-pick specimens of all visibly 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). The Guidance Document includes photos that will help collectors develop an eye for the variety of types of algae that may be encountered in streams. A few helpful tips:

- Some algae (*e.g.*, species of *Chara*, *Paralemanea*, and *Vaucheria*) look like submerged macrophytes or mosses.
- Algae come in many colors, and may be green, dark-brown, golden, red, black, or bluish-green.
- Some cyanobacteria, such *Nostoc* spp., look like gelatinous globules or “deflated” sacs, ranging in size from smaller than a pea to larger than a lime.

- Collect from as many distinct locations as possible throughout the reach so as to capture as much of the apparent diversity as possible.
- Include any holdfast structures that had attached the macroalgae to the substrate, as these structures can be useful for taxonomic identification.
- Since these samples are merely qualitative, it is not necessary to collect them in a manner that is representative of their relative abundances within the reach.
- When in doubt as to whether a candidate specimen qualifies as “algae”, add it to the sample; final determinations will be made by the taxonomist.
- A qualitative sample should be collected at *every* site for which soft-bodied algae are being sampled, whether or not macroalgae are visible in the reach. In the absence of macroalgae, rock scrapings, substrate particles, and CPOM should still be collected (as described above).
- Macroalgae growing within 10 m of the reach may also be added to the qualitative sample.

Step 3. Fill the bag with a total volume of up to 100 mL of qualitative algae sample + stream water. Purge extra air from the bag, and seal with the wire tabs by twisting them together (not just folding them over, as this can result in leakage). Tuck the ends of the wire tabs inward so that they cannot poke holes in the bag. Collect as many bags as needed, based on the variety of algae visible in the stream reach. If multiple bags are collected, number them accordingly (e.g., “bag 2 of 4”) so that the laboratory will know how many bags to process for that site.

Step 4. Double-bag the qualitative samples, and slip a filled-out (with pencil) label (Figure 9) printed on waterproof paper into the outer bag. Store in cool, dark conditions (i.e., in the wet ice cooler, not on dry ice). Do not let the bags touch ice (or “blue-ice” packs) directly, which could cause the samples to freeze, thus destroying them. Do not add any fixative to these samples.

Step 5. Refrigerate the qualitative samples immediately upon return to the laboratory. 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 rapidly. Coordinate beforehand with the receiving laboratory, as necessary, in order to ensure that samples are processed in a timely fashion.

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

Figure 9. Label for soft-bodied algae qualitative sample.

5. BIOTIC SAMPLE PROCESSING

5.1 Module A (continued): Processing Benthic Macroinvertebrate Samples

Step 1. Once all BMI subsamples (11 for RWB or MCM) have been collected and composited, transfer the composited sample to one or more 500-mL wide-mouth plastic sample jar, preferably one with straight edges. Never fill a jar more than halfway with sampled material; use as many jars as necessary in order to prevent this.

Samples with a lot of organic material (e.g., plants, algae, leaf litter) tend to contain a lot of water that may inhibit sample preservation. Gently squeeze out as much water as possible (through the mesh of the D-frame net) before placing the sample in the jar, to prevent diluting the alcohol too much. Approach this task gingerly, so as not to damage invertebrates during this process.

Invert the contents of the D-frame net into the sample jar. Perform this operation over a large, white tray to avoid loss of any sampled material and make recovery of spilled organisms easier. If possible, remove the larger twigs and rocks by hand after carefully inspecting for clinging organisms. Use forceps to remove any organisms clinging to the net and place these in the sample jar. All samples should be completely transferred to the sample jar without elutriation.

If the samples contain a lot of fine particles, confirm that the sampling procedure is being executed correctly (i.e., use care to disturb the substrate as gently as possible and avoid kicking).¹⁷

Step 2. Place a date/locality label (Figure 10), filled out in pencil, on the inside of the jar and completely fill the jar with 95% ethanol¹⁸. To ensure proper preservation of BMIs, gently rotate jars that contain mostly mud or sand so that the ethanol is well distributed. Affix a second waterproof label on the outside of the jar. It is recommended that the label for the outside of the jar be printed with a laser printer (with alcohol-proof toner); otherwise, fill the label out by hand in pencil. Tape the label with transparent tape. Make sure all samples have both internal and external labels.

¹⁷ Samples with an abundance of sand or organic material should be processed expeditiously at the lab, as specimens in these samples can degrade quickly. Therefore, the presence of these kinds of samples should be communicated to the taxonomy lab as soon as possible and they should not be stored for a long time before delivering to the taxonomy lab for processing. See Woodard et al. 2012 for details

¹⁸ Note that the target concentration of ethanol is 70%, but 95% ethanol is used in the field to compensate for dilution from water in the sample. Final concentration of ethanol can be confirmed in the laboratory upon receipt of samples.

Contract/ Billing Code: _____		
Project: _____	Date: _____	Time: _____
Site Code: _____		Sample ID: _____
Repl #: _____	Jar #: _____	of _____
Stream Name: _____		
County: _____	Collector: _____	
Sampling method (circle one): RWB / MCM / TRC		

Figure 10. Example date/locality label for BMI samples.

If field crews do not ship samples directly to the laboratory, then section 2.3 of the SOP for laboratory processing and identification of benthic macroinvertebrates in California (Woodard et al. 2012; http://www.swrcb.ca.gov/water_issues/programs/swamp/docs/bmi_lab_sop_final.pdf) should be followed for long-term storage of the samples.

5.2 Module B (continued): Processing Quantitative Benthic Algal Taxonomy and Biomass Samples

After having sampled substrates across the monitoring reach, there should be material from all 11 transects in the dish tub. Depending on the types of habitats in the stream and substrates encountered, the dish tub may contain stream water with suspended microalgae, and silt, and/or sand, and/or fine gravel, and/or small pieces of wood or macrophyte. The algae clinging to these substrates must be detached and suspended into the water to form a “composite sample”.

Step 1. Any pieces of macrophyte (i.e., vascular plants, not algae), twigs, or dead leaves that had been collected with the ABS delimiter should be massaged thoroughly between the fingers and rinsed into the tub in order to remove the algae coating them. These vascular plant fragments can then be discarded. If there are any clumps of macroalgae in the dish tub, there is a special step required for processing them. The procedure is described in detail below.

Step 2. Systematically massage all the sand and/or silt in the dish tub between the fingers to dislodge clinging microalgae (to be thorough, try to make contact with “every grain” while doing this). For pieces of gravel, use a toothbrush to remove algal material from surfaces. Rinse toothbrush and brushed gravel into the tub. 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 *liquid* in the dish tub will be measured before the algal taxonomic and biomass samples are prepared. To do this, the liquid in the tub will be separated from the rinsed sediment such that the volume measured does not include sediment (see below). After the liquid

sample has been retrieved and measured, the rinsed sediment will be discarded back into the stream. Whereas a single sample type is collected for BMIs, 4 different types of quantitative¹⁹ laboratory samples may be prepared from the composite sample when collecting algae (Figure 4):

- for taxonomic ID/enumeration
 1. diatoms
 2. soft-bodied algae
- for biomass
 3. chlorophyll *a* (“chl *a*”)
 4. ash-free dry mass (“AFDM”)

The general process for sample preparation is as follows. The taxonomic ID/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 with glutaraldehyde in a laboratory under a fume hood within 96 hours of collection. The chl *a* and AFDM biomass 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 must occur within 28 days of collection. If the filters will not be brought to the laboratory freezer on the same day they were collected, they should be kept on dry ice. The taxonomic ID samples are kept on wet ice until they are fixed, and then stored in the refrigerator (never frozen).

Algae sample labels are shown in Figure 11. Recorded on each sample label are the volume of the composite sample (see below), as well as the volume aliquoted (for the taxonomic ID samples) or filtered (for the chl *a* and AFDM samples). All of these volumes are 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 (Station Code, Date, stream name, etc.) is filled out.

¹⁹ Qualitative samples are also collected, when soft-bodied algae are to be analyzed (Section 4.6)

Quantitative Algae Taxonomic ID samples:

Contract/ Billing Code: _____	<small>circle one:</small> diatoms soft
Project: _____ Date: _____ Time: _____	
Site Code: _____ Sample ID: _____	
Repl #: _____ Vol Aliquotted (mL): _____	
Composite Vol (mL): _____	
# Delimiter Grabs (Rub.+ABS): <input type="checkbox"/> # Syringe: <input type="checkbox"/>	
Fixative Added (<i>buffered?</i>): _____	
Stream Name: _____	
County: _____ Collector: _____	
Sampling method (circle one): RWB / MCM	

Biomass samples:

Contract/ Billing Code: _____	<small>circle one:</small> chl a AFDM
Project: _____ Date: _____ Time: _____	
Site Code: _____ Sample ID: _____	
Repl #: _____ Vol Filtered (mL): _____	
Composite Vol (mL): _____	
# Delimiter Grabs (Rub.+ ABS): <input type="checkbox"/> # Syringe: <input type="checkbox"/>	
Stream Name: _____	
County: _____ Collector: _____	
Sampling method (circle one): RWB / MCM	

Figure 11. Labels for algae quantitative taxonomic identification (left) and biomass samples.

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 algae 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. Figure 12 provides a decision tree for how to proceed with the algal sample-processing steps.

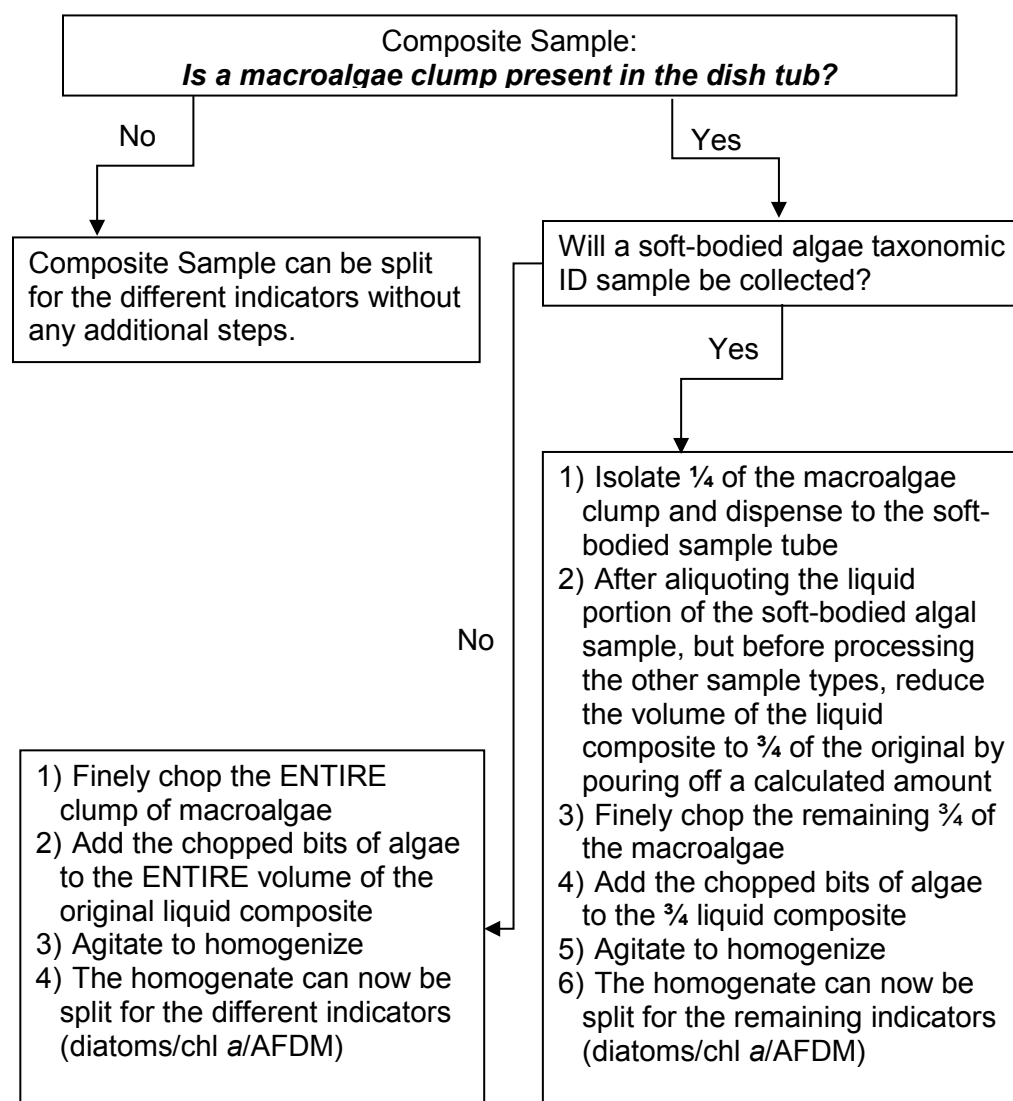


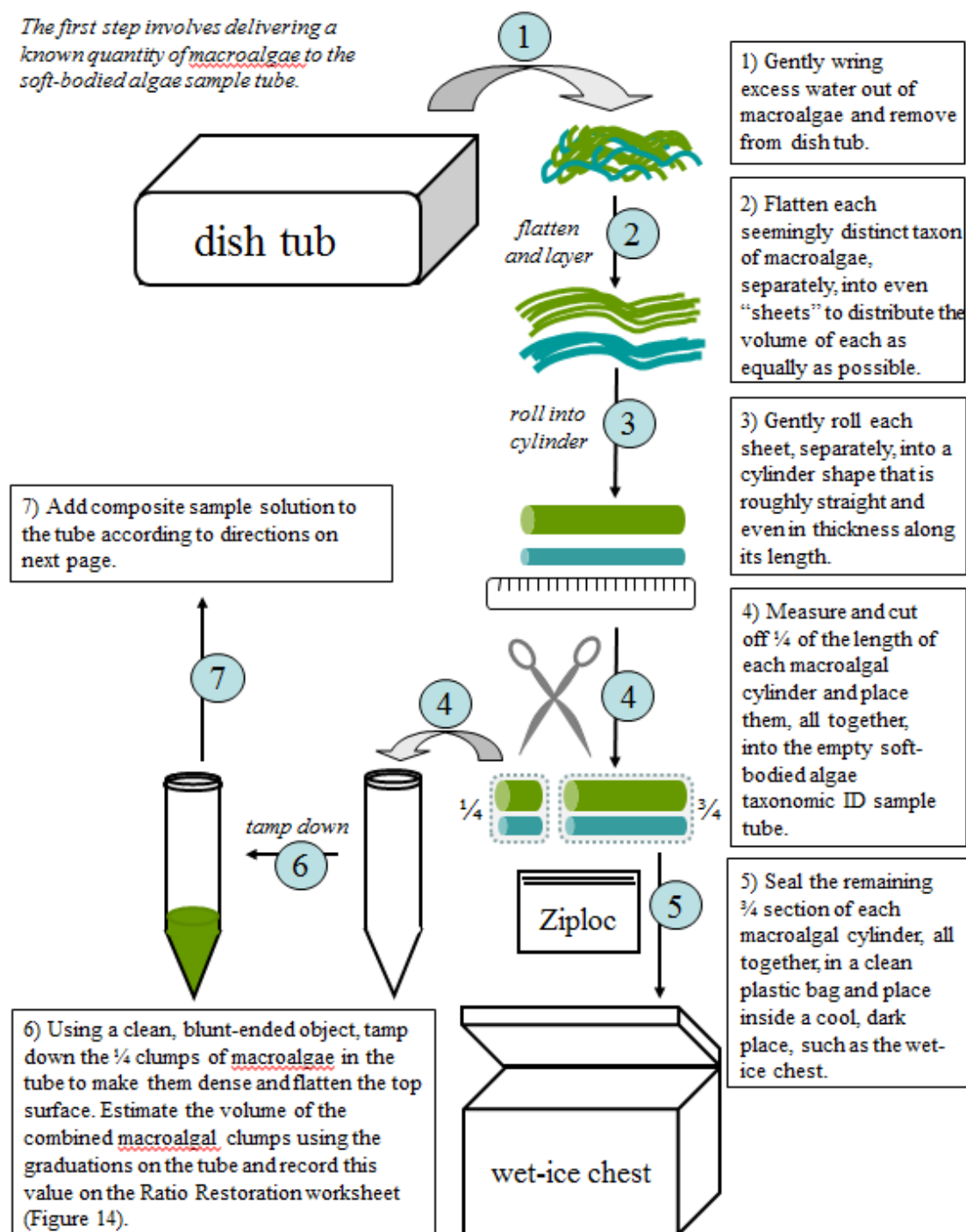
Figure 12. Summary of major sample-processing decision points based on presence of macroalgal clump(s) and need to prepare a soft-bodied algal sample.

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 Figure 13. *It is recommended that this flowchart be printed in color, laminated (if possible) or printed on water-proof paper, and brought along to the field for a quick reference on handling macroalgal clumps in the composite sample.*

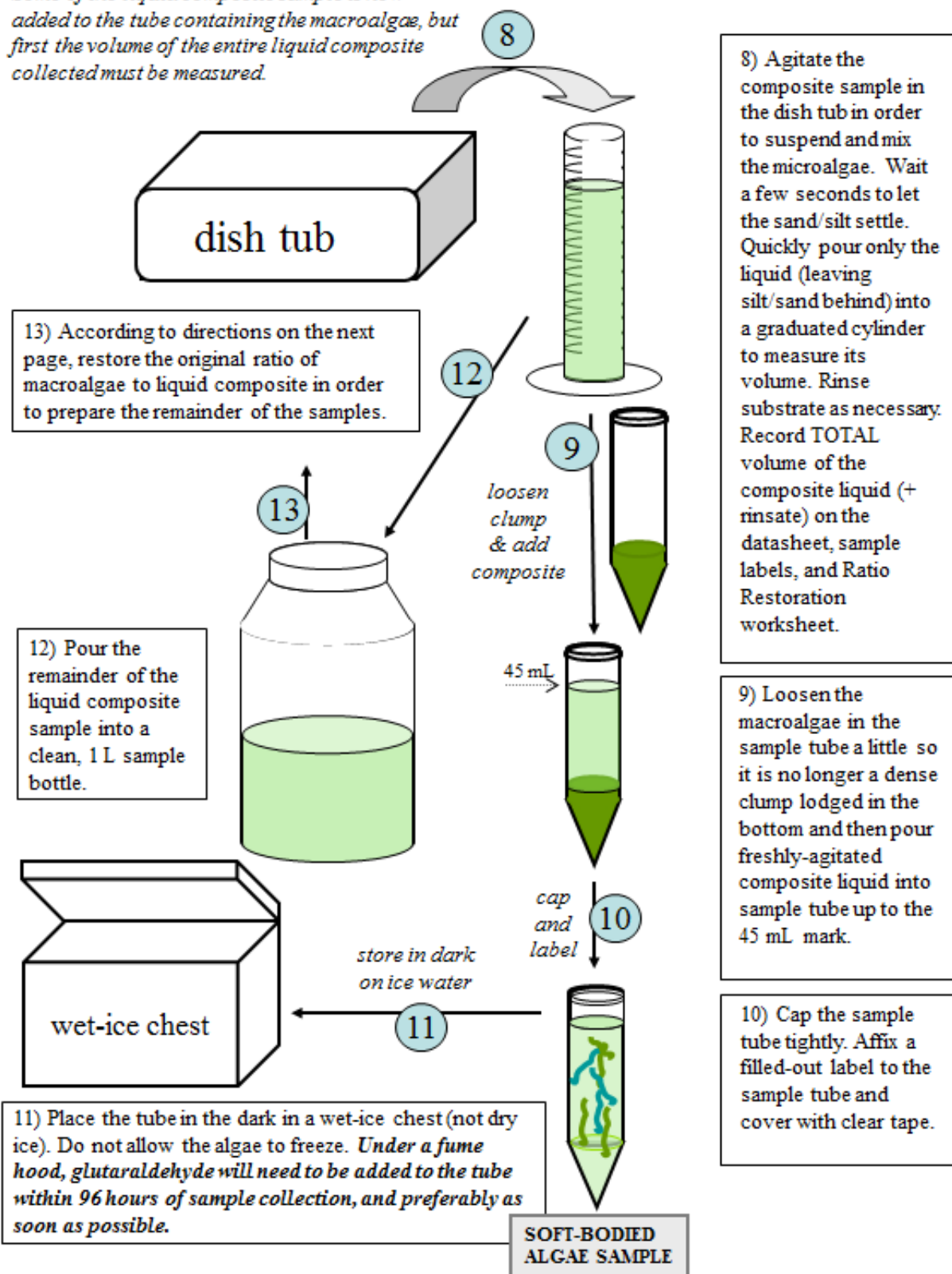
Note: It is unlikely that the $\frac{1}{4}$ macroalgal clump will occupy all the space in the soft-bodied algae quantitative sample tube, but if it does, a second tube will be needed in order to accommodate all the sample material plus liquid. If such an action is taken, it should be noted in the Comments section of the field sheets and the tubes should be clearly identified as belonging to the same sample, for record-keeping purposes. Do not fill either tube so full that there will not be enough room for the fixative.

Figure 13. Processing Soft-Bodied Algal and Diatom Samples When Macroalgal Clumps are in the Sample

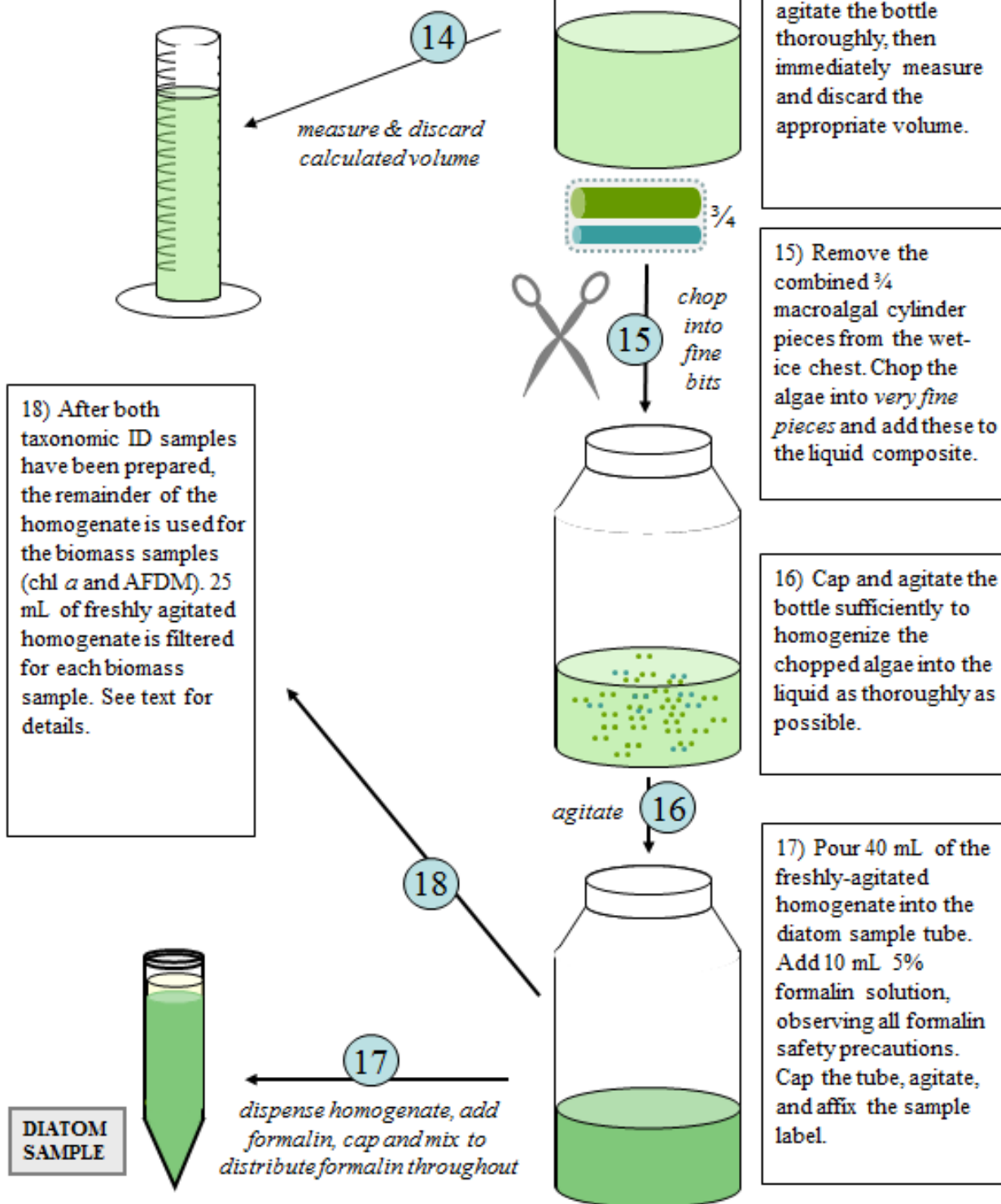
The first step involves delivering a known quantity of macroalgae to the soft-bodied algae sample tube.



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.



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.



Step 3. If one or more macroalgal clumps are present in the dish tub, first remove them from the dish tub, wring them out gently into the tub, and roll them into cylinder shapes that are relatively even in thickness along their length. If there appears to be more than one type of macroalgae (i.e., obviously different species based on color/texture) in the sample, separate cylinders should be made for each one.

Step 4. Measure the length of the cylinder(s) with a ruler and cut a quarter off of each one, lengthwise, with scissors. Place all the quarter pieces together into the (still empty) soft-bodied algae ID sample (50 mL centrifuge) tube. Push the clump of combined macroalgal specimens down into the sample tube, and flatten the top so that the volume 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 13).

Step 5. Place the remaining three-quarters length of the cylinder(s) in a Whirl-Pak™ bag. Seal and label the bag and store it in the wet ice cooler.

Step 6. Once algal specimens have been removed from all the substrates (sand, gravel, cobble, wood, leaves) in the dish tub, according to the procedure described in Steps 1 and 2 at the beginning of Section 5.2, 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 sediment (silt, sand) behind. Transfer the measured liquid into a clean 1L plastic bottle. Rinse the sediment once or twice until it appears that little to no additional suspended material (microalgae) is coming off because the rinsate is clear (or nearly clear). 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. *Note: use water sparingly, because the total sample volume plus rinsate should be no more than about 400-500 mL.* Because as much of the silt and sand as possible is being left behind, the final volume should ideally reflect only the liquid component of the sample. On the field sheet, under the Algae Samples section, record the total volume of all the liquid that had been in the dish tub, plus the water used for rinsing the substrates and sampling devices. This is the “**composite volume**”. Record this value on *all* algae sample labels (biomass and taxonomic samples).

Step 7. 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, if present) up to the 45 mL mark. If no macroalgal clumps had been collected during sampling, simply pour the liquid sample into the empty soft-algae sample tube to the 45 mL mark. Midway through pouring, swirl the composite sample some more (first clockwise, then counter-clockwise) to ensure that the microalgae are still fully suspended. Cap the tube tightly. 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.

Glutaraldehyde is necessary for fixing soft-bodied algae samples in order to preserve fine morphological features and pigment colors, as both can be crucial characters for taxonomic determination. *However, glutaraldehyde is a hazardous substance that poses a number of safety risks.* As such, it must be handled only in a fume hood, by trained personnel wearing appropriate protective gear. Refer to the Guidance Document for an SOP on handling glutaraldehyde.

To fix the soft-bodied algae sample: working under a fume hood, add glutaraldehyde to the tube to a final concentration of 2%. This can be achieved, for example, by adding 5 mL of 20% glutaraldehyde to 45 mL of sample. Distribute the glutaraldehyde throughout the sample by inverting the tightly closed tube repeatedly. Once the samples are fixed, they must be stored in the dark in a refrigerator. Wrap the tubes in foil, if necessary, to maintain darkness.

If no fume hood is available, arrangements should be made for the glutaraldehyde to be added to the samples by personnel with access to a hood (e.g., the taxonomy lab). In the meantime, the unfixed samples must be kept in the dark and on wet ice (but not allowed to freeze), and must be fixed within 96 hours of collection (and preferably sooner). Therefore, if the taxonomy laboratory or another party will be adding the fixative, it is imperative to plan ahead to arrange for this to be done in a timely manner, and also to clearly mark which tubes will need to have fixative added to them.

Step 8. In the field, after the (unfixed) soft-bodied algal sample has been prepared, and before preparing the diatom sample (and biomass samples, which will be discussed in the next steps), *if* a macroalgal clump had been present in the dish tub, then the volume of the remaining composite liquid must be reduced to equal $\frac{3}{4}$ of the original volume. This is necessary because $\frac{1}{4}$ of the macroalgal clump was taken out of the composite sample but a full $\frac{1}{4}$ was not removed from the liquid portion. As such, the original ratio between liquid and macroalgae must be restored before further sample preparation. The following procedure is used to reduce the volume of liquid composite to $\frac{3}{4}$ of the original. For convenience, the following formula (Equation 1) can be used to calculate how many mL to pour off and discard from the composite:

Equation 1. Adjusting the volume of composite sample. (Be sure to honor the rules governing algebraic “order of operations” in calculating the volume to pour off.)

$$\text{volume (mL) of composite to pour off} = (0.25 * C) - 45 + A$$

where “C” is the original composite volume and “A” is the approximate volume of the (combined) clump(s) of macroalgae placed in the soft-bodied algae sample tube (tamped down and flattened). A copy of the Ratio Restoration worksheet shown in Figure 14 can be used to calculate the amount of composite to pour off.

Liquid portion of composite sample: _____ mL = C
Volume of ¼ macroalgal chunk: _____ mL = A
<p>Volume of liquid composite to pour off:</p> $(0.25 * \underset{\substack{\uparrow \\ C}}{\text{_____}}) - 45 + \underset{\substack{\uparrow \\ A}}{\text{_____}}$ <div style="text-align: center; margin-top: 10px;"> = _____ mL </div>

Figure 14. Ratio Restoration worksheet. Be sure to honor the rules governing “order of operations” in calculating the volume to pour off.

As always whenever pouring off aliquots, be sure to agitate the composite liquid adequately in order to resuspend any settled microalgae before pouring off the calculated volume.

Step 9. Once the required amount of composite liquid has been discarded, the remaining ¾ of the macroalgal cylinder (from the bag in the wet ice cooler) is cut with scissors into fine pieces (resulting in strands that are no more than ~3 mm long), 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 such that distinct fragments of macroalgae are not easily discernible, because the goal is to “homogenize” the macroalgae into the liquid as much as possible. If a macroalgal clump was present in the dish tub, but no sample is to be prepared for analysis of the soft-bodied algal community, then ALL of the macroalgal clump should be finely chopped into the full volume of measured composite liquid. In this case, there would be no need to discard ¼

of the composite volume before introducing the (full amount of) chopped macroalgal into the liquid.

Step 10. After introducing the finely chopped macroalgae into the composite liquid, cap the composite bottle and agitate sufficiently to homogenize the tiny bits of algae into the liquid as much as possible, while not agitating so hard as to risk busting cells and releasing chl *a*.

Step 11. 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 algae suspended. Add 10 mL of the 5% formalin to the sample (for a final concentration of 1%). *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 the Guidance Document for instructions on preparing the buffered formalin solution and for an SOP on handling formalin.

Step 12. Cap the tube tightly and invert it several times 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 heat. The remaining composite sample homogenate can be used to prepare the chl-*a* and AFDM filters as described below.

If no algal taxonomic data are required for the project at hand, and only biomass data are needed, finely chop *all* macroalgae (if present) directly into the *entire* volume of liquid (which must still be measured and recorded). Then proceed to Step 13.

Step 13. Now the biomass samples can be prepared. The procedure to filter chl *a* samples should be carried out quickly, and in the shade as much as possible, to minimize exposure of the sample to light/heat, thus minimizing chl *a* degradation. Use clean filter forceps to center a glass fiber filter (47 mm, 0.7 µm pore size) onto the mesh platform of a clean filtering apparatus, and rinse the filter a little with DI water to seat it well into the mesh before attaching the filter chamber on top. Never touch the filters with hands or anything other than clean forceps. Agitate the sample homogenate to resuspend all the macroalgal fragments and microalgal material. Measure 25 mL using a small, clean graduated cylinder. 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 into the filter chamber. Once empty, rinse the graduated cylinder with a few mL of DI water, and add this to the filter chamber.

Step 14. 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 chl *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 chamber 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, pointed forceps, being careful not to remove any algae in the process. Remove the filter from the filtering device. Always thoroughly rinse the sides of the filter chamber and the interface between the mesh filter seating and the screw-on part of the apparatus with DI water between samples.

Step 15. Fold the filter in half (with the sample material on the inside, like a taco) 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, 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 by twisting its wire tabs *together*, so that water in the cooler will not be able to enter the bag. Shove the sample packet down into the ice in the cooler to make sure it stays submerged and does not float to the top. This may be achieved by sealing the sample bags in a large Ziploc™ bag with a rock in it. Keep chlorophyll *a* filters as cold as possible and place them in the freezer (-80°, if available) or on dry ice as soon as possible (and within four hours of collection); the analytical holding time for the chl *a* filters is 28 days from collection, when kept frozen.

Step 16. For the AFDM samples, use glass-fiber filters (47 mm, 0.7 µm pore size) that have been pre-combusted²⁰. Never touch the filters with hands or anything other than clean forceps. Follow the same process as that used for chl-*a* sample filtering. Record the volume filtered for the AFDM sample. Keep AFDM filters as cold as possible until the samples can be frozen back at the laboratory that evening, or place on dry ice until they can be stored in the laboratory freezer. The analytical holding time for the AFDM samples is 28 days from collection, when kept frozen.

²⁰ Check with the laboratory that will be analyzing the samples about obtaining pre-combusted filters.

6. PHYSICAL HABITAT TRANSECT-BASED MEASUREMENTS

After all biotic samples have been collected at a given transect, PHab data collection may begin. These data are designed to characterize a stream reach's physical habitat, knowledge about which can aid interpretation of the biotic data. In some cases, however, PHab data may be desired for a site assessment even when biotic/biomass samples are not being collected.

The majority of PHab measurements in this SOP are gathered relative to the 11 main transects (Figure 3), and data for the PHab parameters described in this section are entered on transect-specific field sheets (and in the case of the "Pebble Count" data, also on the inter-transect field sheets). PHab data collection starts at the downstream transect (Transect A) and proceeds working upstream along the monitoring reach. Some programs (*e.g.*, citizen monitoring efforts) may elect to collect a less-intensive subset of PHab data than the full suite described here. To this end, the Guidance Document provides suggestions for a "Basic" protocol.

6.1 Module C: Wetted Width and Bankfull Dimensions

Step 1. Measure the *wetted width* associated with the transect and record this (in meters) in the box at the top of the transect form. 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 (Figure 15). The wetted width can include emergent, unvegetated sandbars or boulders in the middle of a channel, but should not include emergent, vegetated "islands" (defined as features that are not flooded during average year high-water events). When a transect crosses an island, subtract the width of the island from the distance between the wetted margins.

Step 2. Scout beyond the wetted channel along the stream reach to identify the location of the *bankfull* margins on either bank by looking for evidence of annual or semi-annual flood events. The bankfull channel is the zone of maximum water inundation in a normal-flow year (*i.e.*, one- to two-year flood events; see Figure 15 and the Guidance Document for a depiction of wetted width and bankfull dimensions). Because most channel-formation processes are believed to act when flows are within this zone (Mount 1995), bankfull dimensions provide a valuable indication of stream power during high-flow events and therefore relative size of the water body.

Examples of evidence for bankfull location include topographic, vegetation, and geologic cues (changes in bank slope, changes from annual to perennial vegetation, changes in the size distribution of surface sediments, location of water stains on concrete and bedrock channels, etc.). Although it is tempting to use the position of drift material caught in vegetation to identify bankfull location, it only indicates the discharge height during extreme recent flow events, and should not be used as an indicator by itself. Note that, perhaps more than any other component of PHab assessment, identification of bankfull location requires extensive experience in multiple ecoregions and stream types, and *training in the field under the supervision of experienced bioassessment practitioners is essential.*

It is helpful during the initial reach delineation to investigate the entire reach when attempting to interpret evidence for bankfull location, because the true bankfull margin may be obscured at various points along the reach. However, bear in mind also that bank dimensions may change in the middle of a sampling reach.

Step 3. Stretch a tape or stadia rod from bank to bank at the bankfull position along the transect. Record this distance (in meters) as bankfull width at the top of the transect form. If using flexible tape, make sure the tape is taut before taking a reading.

Step 4. Record bankfull height (in meters) as the vertical distance between the water surface and the height (Figure 15) of the bank at bankfull position. This can be done by standing at the wetted edge or transect center holding a meter stick vertically from the water surface to the stretched tape to measure the height.

Step 5. Carry out the above steps at each of the 11 main transects.

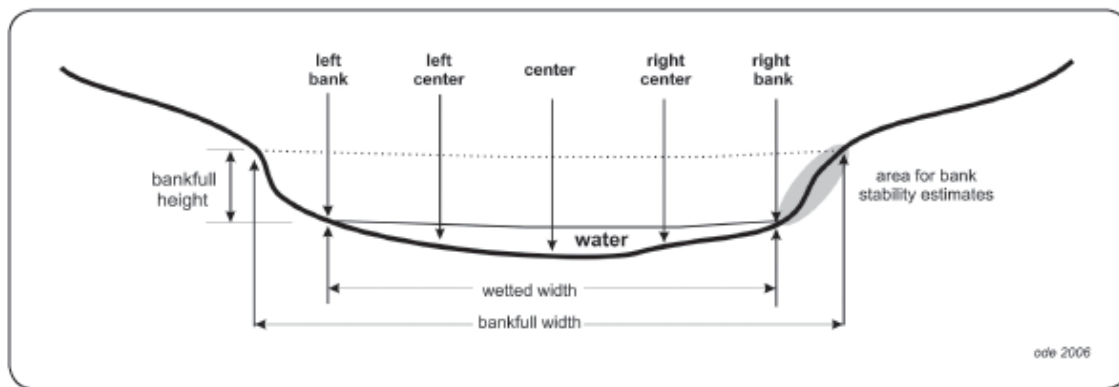


Figure 15. Cross sectional diagram of a typical stream channel showing locations of wetted and bankfull width measurements, substrate measurements, and bank stability visual estimates.

6.2 Module D: Substrate Size, Depth, and Coarse Particulate Organic Matter (CPOM)

Particle size frequency distributions often provide information about instream habitat conditions that affect BMI distributions, and may also reflect the stream's ability to accrue algal biomass. Changes in particle size distributions often accompany stream disturbances, and may be a key source of stress to benthic organisms.

The Wolman "pebble count" technique (Wolman 1954) is a widely used and cost-effective method for estimating the particle-size distribution that produces data that correlate with costly, but more precise, bulk-sediment samples. The method described here follows the NRSA protocol (which is a version of the Wolman count) and records sizes of 105 particles in a reach: five particles, equidistant from one another, along each of the 11 main transects and 10 inter-transects. Depth refers to the depth of surface water in the stream at each of these points. Coarse particulate organic matter (CPOM; small particles of organic material, such as leaves/twigs, that are >1 mm in size, but no larger than 10 mm) is an indicator of the amount of allochthonous

organic matter available at a site. Because CPOM is food resource for certain benthic macroinvertebrates, its abundance can provide information about the quality of the food web in a stream reach. Pebble count, depth, and CPOM are all measured in tandem at each of the 105 points along the sampling reach.

Step 1. At each transect (and inter-transect), use a stadia rod or tape measure to divide the wetted stream width by four to get the distance between the five points (Left, Left Center, Center, Right Center and Right; Figure 15) and locate the positions of these points along the transect. Once the positions are identified, lower a graduated rod (e.g., a waterproof meter stick) straight down through the water column to identify the particle located at the tip of the rod

Step 2. Measure the depth from the water surface to the top of the particle with the graduated rod and record to the nearest cm.

Step 3. Record the presence or absence of CPOM within 1 cm from the edge of the rod.

Step 4. Remove the particle from the streambed and measure and record the length of its intermediate axis (Figure 16) to the nearest mm. Actual measurements should always be recorded, whenever possible (i.e., for the fine gravel through large boulder-sized bed materials). If a direct measurement is impossible (e.g., the particle is deeply embedded or in a deep pool), an approximate size may be designated by assigning a particle size classes listed in Table 4 based on visual estimation. Regardless of the method, all particles < 0.06 mm should be recorded as fines, and all particles between 0.06 mm and 2.0 mm recorded as sand. “Wood” applies to woody material, living or dead. “Hardpan” applies to consolidated fines, where individual particles cannot be easily separated or dispersed. Substrates (e.g., trash, macrophytes, live tree roots, and any other substrate not captured by the other available categories) that do not fall into any of the categories should be recorded as “other” (OT).

Record particle measurement (or size class) on the transect sheet under “mm/size class” in the “Transect Substrates” portion of the form. If recording particle size class, use only the standard codes in Table 4 to record the information.

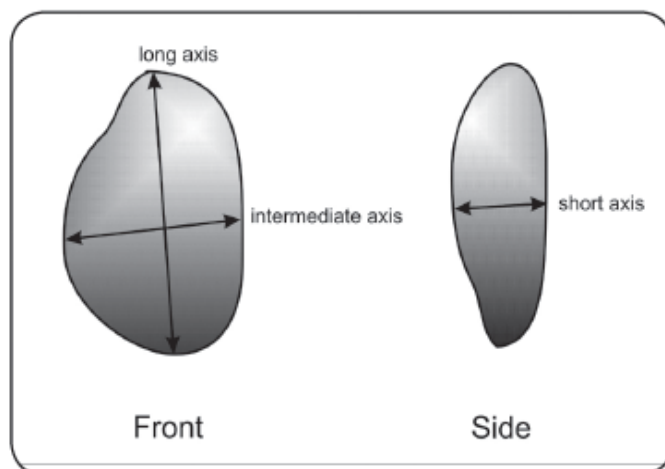


Figure 16. Diagram of three major perpendicular axes of substrate particles. The intermediate axis is recorded for pebble counts.

Table 4. Particle size class codes, descriptions, and measurements. SWAMP requires that actual measurements be recorded, whenever possible (i.e., for the fine gravel through large boulder-sized bed materials).

Size Class Code	Size Class Description	Intermediate Axis Common Size Reference	Size Class Range
RS	bedrock, smooth	larger than a car	> 4 m
RR	bedrock, rough	larger than a car	> 4 m
RC ²¹	concrete/ asphalt	larger than a car	> 4 m
XB	boulder, large	meter stick to car	1 - 4 m
SB	boulder, small	basketball to meter stick	250 mm - 1 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		
OT	other		

Step 5. If the particle is cobble-sized (64 - 250 mm diameter), record to the nearest 5% the percent of the cobble surface that had been embedded by fine particles (< 2 mm diameter; see Cobble Embeddedness measurement procedure, Section 6.3, below).

²¹ Only continuous sections of concrete (e.g., concrete channel) should be coded as "RC". Concrete agglomerations smaller than 4 m should be treated as a single particle, and measured accordingly.

Sometimes points with dry (not submerged or moist) substrates are encountered during the course of PHab data collection along transects/inter-transects. 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 and primary-producer 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 well as Macrophytes and CPOM, as “D” for “dry”

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 toward the active channel), as well as the presence of dried algae, water stains, micro-topographic transitions, changes in substrate composition, soil cracks, and others.

6.3 Module E: Cobble Embeddedness

The degree to which fine particles fill interstitial spaces in the streambed has a significant impact on the ecology of benthic organisms and fish, but techniques for measuring this impact vary greatly (this is summarized by Sylte and Fischenich 2002, <http://stream.fs.fed.us/news/streamnt/pdf/StreamOCT4.pdf>). Here we define embeddedness as the percent of the surface area (not volume) of cobble-sized particles (64 - 250 mm) that is buried by fines or sand particles (< 2.0 mm diameter). Ideally, at least 25 cobbles are assessed for embeddedness in each sampling reach: Embeddedness is determined for each cobble that is measured for particle size, up to a total of 25 cobbles. If < 25 cobbles are encountered during the pebble count, the remainder are “made up” by assessing cobbles that lie outside of the PHab data collection transects (see Step 3, below). In certain streams, it may not be possible to find 25 cobbles.

Step 1. Every time a cobble-sized particle is encountered during the pebble count, remove the cobble from the stream bed and visually estimate the percentage of the cobble's surface area that had been buried by fine particles. If removal of the cobble is impossible, approximate embeddedness to the best extent possible. In the rare circumstances that multiple sample points

land on the same cobble, do not take a second embeddedness measurement. Once embeddedness has been assessed for 25 cobbles, no more need be assessed.

Step 2. Record the embeddedness values for the first 25 cobble-sized particles encountered during the pebble count in the “% Cobble Embed” field in the “Transect Substrates” portion of the transect sheet.

Step 3. If 25 cobbles are not encountered during the pebble count by the time Transect K has been sampled, supplement the data by conducting a “random walk”²². Starting at a random point in the reach, follow a line from one bank to the other at a randomly chosen angle, recording embeddedness of any cobbles encountered (that were not previously recorded) along the way. Upon arriving at the other bank, reverse the process with a new randomly chosen angle. Spend a maximum of 10 minutes on the random walk, even if 25 cobbles have not been encountered by that time. Embeddedness for any cobbles encountered outside of the pebble count locations should be recorded in the “Additional Cobble Embeddedness” section of the field sheets.²³

6.4 Module F: Algal and Macrophyte 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)²⁴. It is a reflection of stream primary production and has implications for the health of food webs as well as the damaging effects of eutrophication stimulated by excess nutrients in concert with other environmental co-factors (*e.g.*, loss of canopy cover).

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 5 points along the transects associated with the pebble count. If the point corresponding to each pebble in the pebble count intercepts algae, then algae is recorded as “present” at that point.

Step 1. 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 ruler can be used for measurement. For layers too thin to measure, use the indicators listed in the last column of Table 4. Note that these thickness codes refer only to microalgal film, not macroalgal mats (macroalgal thickness is not assessed in this protocol).

²² It is preferable to wait until the rest of the PHab transect/inter-transect measures are complete before doing this, so as not to trample any as-yet unsampled transects in the course of the random walk.

²³ An easy way to ensure that 25 embeddedness measurements are taken is to put an X in one of the boxes on the first data sheet each time a cobble is encountered during normal transect measurements. Then, after all transects are complete, fill in the remaining boxes with embeddedness estimates.

²⁴ Refer to the glossary for comprehensive definitions of microalgae and macroalgae and the Guidance Document for photos

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 obscuring) the stream bottom. Microalgal species can grow as epiphytes upon macroalgal filaments and mats, coating them with a slimy, brown-tinted film. The Guidance Document provides some additional information to help distinguish between microalgae and macroalgae.

Table 5. Microalgal thickness codes and descriptions (modified from Stevenson and Rollins 2006).

Code	Thickness	Indicators
0	No microalgae present	The surface of the substrate is not at all 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	(see explanation in text)
D	Dry point	

Sometimes, due to the nature of the substrate, it can be difficult to discern whether a microalgal layer is present. For example, deposits of very fine sediments might obscure the diagnostic color of a microalgal layer, and the slipperiness of very fine silt may make tactile determination of microalgae impossible. If presence/absence of a microalgal layer cannot be determined with confidence, score microalgal thickness as “UD”.

Step 2. 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 (Guidance Document) in order to see below the water’s surface). 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, current, 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 at the time of the assessment, and the algae is freely floating at or near the water’s surface. The data-collection point does not need to intercept attached algae at its point of attachment in order for it to be scored as “Attached”; all that is required is for the algae to be attached to the streambed somewhere, even if the attachment occurs far from the sample point. 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.²⁵

If any portion (above- or underwater) of 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. Macrophytes are defined as herbaceous, vascular plants rooted or floating within the stream’s wetted channel, such as sedge, cattail, knotweed, *Arundo donax*, watercress, water-primrose, duckweed, etc. Our definition of aquatic macrophytes excludes trees, root mats, shrubs, mosses, and algae. This is the same as the definition of macrophytes used for Module J (Instream Habitat Complexity).

6.5 Module G: Bank Stability

The vulnerability of stream banks to erosion is often of interest in bioassessment because of its direct relationship with sedimentation. For each transect, record a visual assessment of bank vulnerability along an imaginary line between the wetted width and bankfull width of the stream channel (Figure 15)²⁶. Choose one of three vulnerability states: *eroded* (evidence of mass wasting), *vulnerable* (unprotected banks), or *stable*. All three states may be evident in a single reach at both natural and highly modified streams. The following indicators help describe the states:

- Eroded: Exposed tree roots, obvious bank slumps, fallen trees.
- Vulnerable: Sparse vegetation
- Stable: Bank armoring, robust vegetation, few exposed tree roots

6.6 Module H: Human Influence

The influence of human activities on stream biota is a central question in bioassessment analyses. Quantification of human activities is used to evaluate stress and to identify minimally disturbed reference sites. Reach-scale observations provide a crucial supplement to data provided by aerial imagery and GIS analysis.

Anthropogenic features and activities associated with each main transect (for a distance of 5 m upstream and 5 m downstream from the transect, totaling a width of 10 m centered on the transect; Figure 17) are recorded in terms of zones based on how close they are to the wetted margins.²⁷ The area in which human influence is measured extends outward 50 m in both directions from the bank along the entire reach.

²⁵ Because pebble counts span the “wetted width” of each transect, pebbles at the margin positions will often 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. Algal cover should therefore be recorded at all five observation points along each transect.

²⁶ Note that sandbars are not considered part of the bank.

²⁷ The relative distance between the wetted and bankfull margins can complicate the assessment of human influence. If the wetted edge and the bankfull margin are at the same point, then land uses between the wetted edge and bankfull margin are not present, and that location cannot be scored. Conversely, in some streams, the bank and the wetted edge may be many meters apart. In that situation, the wetted edge should be used as a consistent point for defining the area.

For each human disturbance feature/activity class, circle “Y” if it is present between the wetted margins; otherwise, circle “N”, and then assess each side of the stream as follows: If the feature/activity is present between the wetted edge and bankfull margin, circle “B”; if it is outside within 10 m of the bank circle “C”; if it is within 50 m of the bank, circle “P”; otherwise, circle 0. The relative distance between the wetted and bankfull margins can If the wetted edge and the bankfull margin are at the same point, then land uses between the wetted edge and bankfull margin are not present, and that location cannot be scored. Conversely, in some streams, the bank and the wetted edge may be many meters apart. In that situation, the wetted edge should be used as a consistent point for defining the area.

For each feature/activity, the most proximal category takes precedence and therefore is the distance at which that feature/activity should be scored. For example, if a feature/activity is observed within the channel, as well as on the banks, circle “Y” to denote the channel, and move on to scoring the next feature/activity class. Note that certain features (e.g., parks) are not applicable within the channel, and for these, “B” would represent the most proximal location possible.

Table 6 provides definitions of Human Influence features/activities. Circle only the closest location for each impact that applies, being careful not to double-count any human influence observations.²⁸

²⁸ Double counts are prevented by SWAMP electronic forms.

Table 6. Definitions of Human Influence features/activities.

Feature/Activity	Description/Indicators
Walls/Rip-rap/Dams	Artificial stone, concrete, or cement structures that are built into the stream, including check dams
Buildings	(self explanatory)
Pavement/Cleared lot	Vacant land with disturbed soil or ruderal vegetation, or paved
Roads or Railroads	Includes unpaved roads and high use trails
Pipes (inlet/outlet)	A physical structure discharging into, or withdrawing from, the stream; does not need to be active and can include pipes within the banks
Landfill/Trash	Garbage; can include large, stable (e.g., cars) items, as well as ephemeral (candy wrappers)
Park/Lawn	Managed active or passive recreation areas; often irrigated.
Row crops	Agricultural fields; generally includes annual crops that are replanted each season or year
Pasture/Range	Areas where cattle, sheep, or other livestock are actively grazed; evidence includes manure, hoof prints, terracing of hillslopes, and reduced vegetation
Logging operations	Places where trees are cut down; evidence includes stumps, clearcuts, woodchips, slash, flumes
Mining activity	Tailings, borrow-pits, spoils, prospecting mines, sluices
Vegetation Management	Removal or reduction of vegetation for purposes (e.g., flood control, fuel reduction) other than logging; lawn maintenance should be covered under park/lawn
Bridges/Abutments	(self explanatory)
Orchards/Vineyards	Agricultural fields with woody vegetation that is infrequently replanted

6.7 Module I: Riparian Vegetation

Riparian vegetation has a strong influence on the composition of stream communities through its roles in directly and indirectly controlling the food base, moderating sediment inputs, and acting

as a buffer between the stream channel and the surrounding environment. These methods provide a cursory survey of the condition of the riparian corridor²⁹. Observations are made in the same 10 m x 10 m riparian area, on either side of the wetted channel, used for assessing human influence “C” zone (Figure 17).

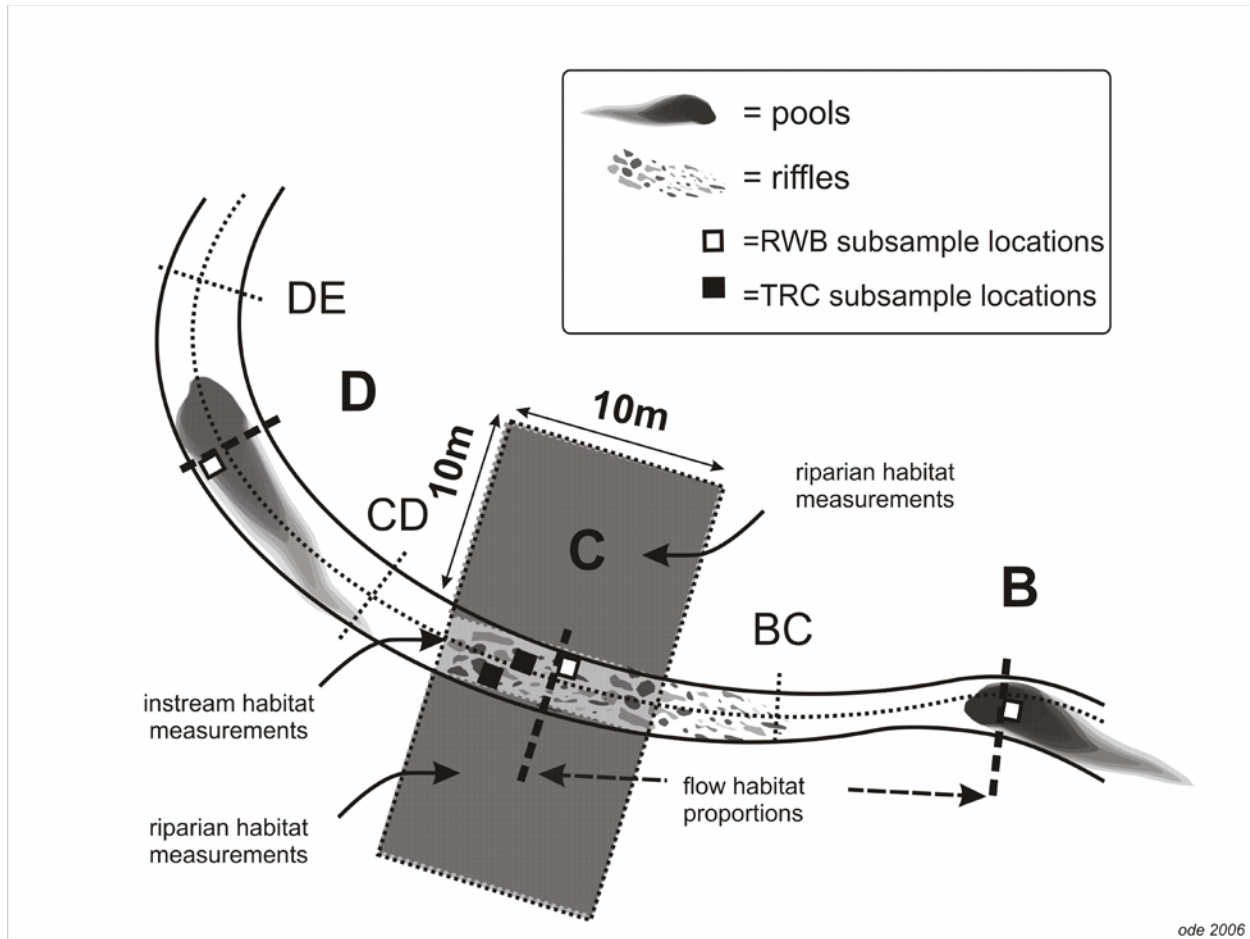


Figure 17. Section of the standard reach expanded from Figure 1 showing the appropriate positions for collecting riparian habitat and flow habitat proportion measurements. Also shown here is the human-influence zone corresponding to the area within 10m of the wetted width (i.e., zone “C”).

Step 1. Mentally divide the riparian area into three elevation zones relative to the ground surface:

- Ground cover (< 0.5 m high)
- Lower canopy (0.5 m - 5 m)
- Upper canopy (> 5 m).

Within each zone, record the density of the following riparian classes:

²⁹ Programs may want to consider adding the California Rapid Assessment Method for wetlands (CRAM; <http://www.cramwetlands.org/>) to their stream bioassessment data collection efforts in order to obtain more comprehensive information on riparian condition of monitoring sites.

- Upper Canopy: Trees and Saplings
- Lower Canopy: Woody Shrubs and Saplings
- Ground cover:
 - Woody Ground Cover
 - Herbaceous Ground Cover
 - Barren, Bare Soil and Duff (artificial banks, rip-rap, concrete, asphalt, etc. should be recorded as “barren”).

An individual plant may contribute to multiple elevation zones. However, low-hanging canopy vegetation should not contribute to groundcover.

Step 2. Indicate the areal cover (i.e., shading) by each riparian vegetation class as either: 1) absent, 2) sparse (< 10%), 3) moderate (10-40%), 4) heavy (40-75%), or 5) very heavy (> 75%).

Each of the elevation zones (upper canopy, lower canopy, and ground cover) should be evaluated independently of the others. All together, they do not need to total to 100%. However, the total for the three ground cover categories (Woody Ground Cover; Herbaceous Ground Cover; Barren, Bare Soil and Duff Ground Cover) should equal 100%.

6.8 Module J: Instream Habitat Complexity

The instream habitat complexity measure was developed by the EMAP program to quantify fish concealment features in the stream channel, but it also provides valuable information about the general condition and complexity of the stream channel for other fauna. Estimates should include only those features that are found between the stream’s wetted margins.

Record the category (Table 7) best approximating percentage of areal cover of nine different instream (wetted channel) features within a zone 5 m upstream and 5 m downstream of the transect (Figure 17). Indicate the areal cover of each feature as either: 1) absent, 2) sparse (< 10%), 3) moderate (10-40%), 4) heavy (40-75%), or 5) very heavy (> 75%). Note that the sum of the percentages of the different features does not necessarily need to equal 100%.

Table 7. Instream Habitat Complexity components and descriptions.

Component	Description and Comments
Filamentous algae	<ul style="list-style-type: none"> • Visible growths of macroalgae. • Do not include non-filamentous macroalgae (e.g., <i>Nostoc</i> spp.)
Aquatic macrophytes and emergent vegetation	Herbaceous plants rooted or floating within the stream's wetted channel, such as sedge, cattail, knotweed, watercress, water-primrose, duckweed, etc.; our definition of aquatic macrophytes excludes trees, shrubs, mosses, and algae
Boulders	Intermediate axis ≥ 25 cm (Figure 16)
Small woody debris	< 30 cm diameter
Large woody debris	≥ 30 cm diameter
Undercut banks	<ul style="list-style-type: none"> • Banks providing sufficient cover for an item at least the size of a fist. • Estimate as an areal (not linear) feature: % of streambed area covered by undercut banks.
Overhanging vegetation	<ul style="list-style-type: none"> • Vegetation within 1 m of the surface of the water. • Estimate as an areal (not linear) feature: % of streambed area covered by overhanging vegetation.
Live tree roots	(self-explanatory)
Artificial structures	<ul style="list-style-type: none"> • Any items with an anthropogenic origin. • In concrete channels, do not count the channel itself. • In restored channels, do not count natural items introduced as part of restoration activities (e.g., root wads) • Include stable trash items (e.g., cars, tires, shopping carts) expected to remain in place after a typical storm, but do not include ephemeral trash items (e.g., soda cans, candy wrappers, diapers)

6.9 Module K: Stream Shading (Densimeter Readings)

The amount of sunlight that can reach the stream is important because it influences stream temperature as well as primary productivity, which in turn affects food webs and constrains eutrophication. Using a convex spherical densimeter, stream shading is estimated in terms of percent cover of objects (vegetation, buildings, etc.) that block sunlight. The method described uses the Strickler (1959) modification of a densimeter to correct for over-estimation of stream shading that occurs with unmodified readings. Taping off (Figure 18) the lower left and right portions of the mirror emphasizes overhead structures over foreground structures (the main source of bias in stream shading measurements).

The densiometer is read by counting the number of line intersections on the mirror that are obscured by overhanging vegetation or other features that prevent sunlight from reaching the stream. All densiometer readings should be taken at 0.3 m above the water surface, and with the bubble on the densiometer leveled. 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, when the densiometer is oriented so that the "V" of the tape is closest to the observer's face.

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. The observer should revolve around the densiometer (i.e., the densiometer pivots around a point) over the center point of the transect (as opposed to the densiometer revolving around the observer).

For sites with a mean wetted width > 10 m, two additional readings must be taken: one at the left bank and one at the right, standing at the water's edge and facing away from the stream, toward the floodplain. These additional readings are useful in the case of larger streams and rivers, where the center of the channel does not provide adequate information about the degree to which shading is affecting the stream. For smaller streams, these additional two measures are recommended, but optional.

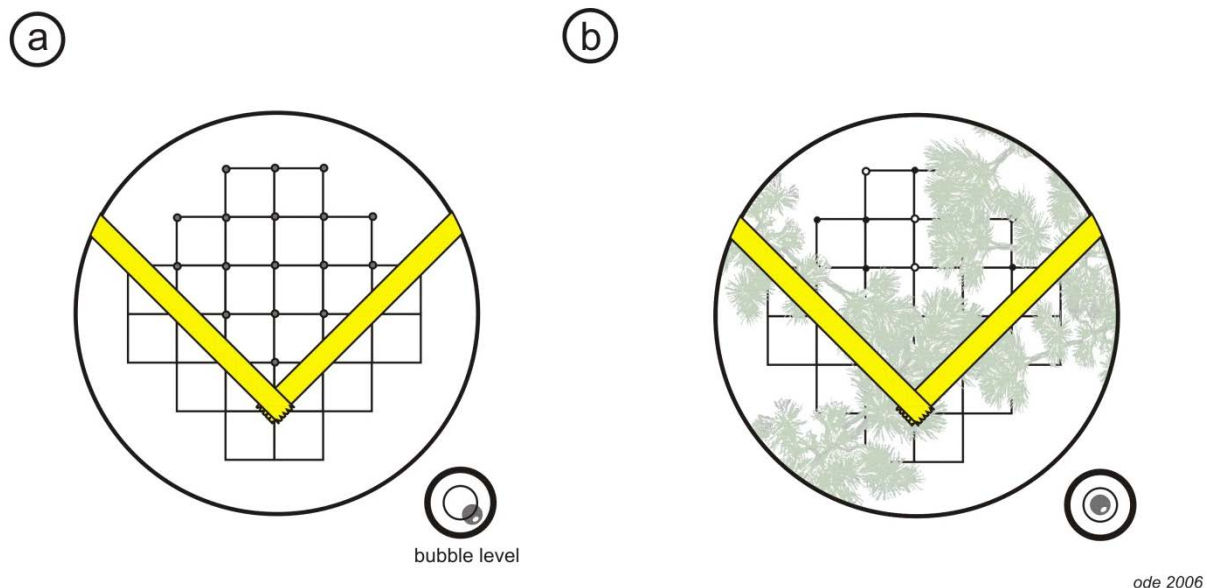


Figure 18. Representation of the mirrored surface of a convex spherical densiometer showing the position for taping the mirror and the intersection points used for the densiometer reading. The score for the hypothetical condition in (b) is 9 covered intersection points out of 17 possible (within the "V" formed by the two pieces of tape). Note the position of the bubble in (b) which indicates that the densiometer is leveled, as opposed to (a), which indicates it is not leveled.

6.10 Module L: Slope and Sinuosity

The slope of a stream reach is one of the major stream classification variables, being a primary determinant of potential water velocities and stream power, which are in turn important controls on aquatic habitat and sediment transport within the reach. The slope of a stream reach is often strongly correlated with many biotic metrics and other PHab measures, and is therefore very useful when interpreting biotic data.

The “Full” PHab method described in this SOP uses transect-to-transect measurements to calculate the average slope through a reach. Although this is more time-intensive than the reach-scale transect measures outlined in the “Basic” protocol (see Guidance Document), it results in more precise slope determination and affords the ability to quantify slope variability within a reach. Sinuosity (calculated as the ratio of the length of the flow path between the ends of the reach and the straight line distance between the ends of the reach; Kaufmann et al. 1999) is measured at the same time as slope. These two measurements work best with two people: one taking the readings at the upstream transect (“backsighting”) and the other holding a stadia rod at the downstream transect (Figure 19).³⁰

In small, highly sinuous or densely vegetated streams, it may not be possible to obtain a clear line of sight from one transect to the next. If the midpoint of the next transect is not visible from the starting point, divide the inter-transect distance into sub-sections, using the “Supplemental Sections” (indicating the proportion of the total length represented by each section) on the field sheet. Otherwise, leave Supplemental Sections blank. Do not measure slope across dry land/meanders in the stream.

³⁰ Slope measurements can be measured from a point on the transect at water’s edge, but sinuosity measurements should be taken from mid channel. If water depth or obstructions prevent this, attempt to estimate the correct bearing.

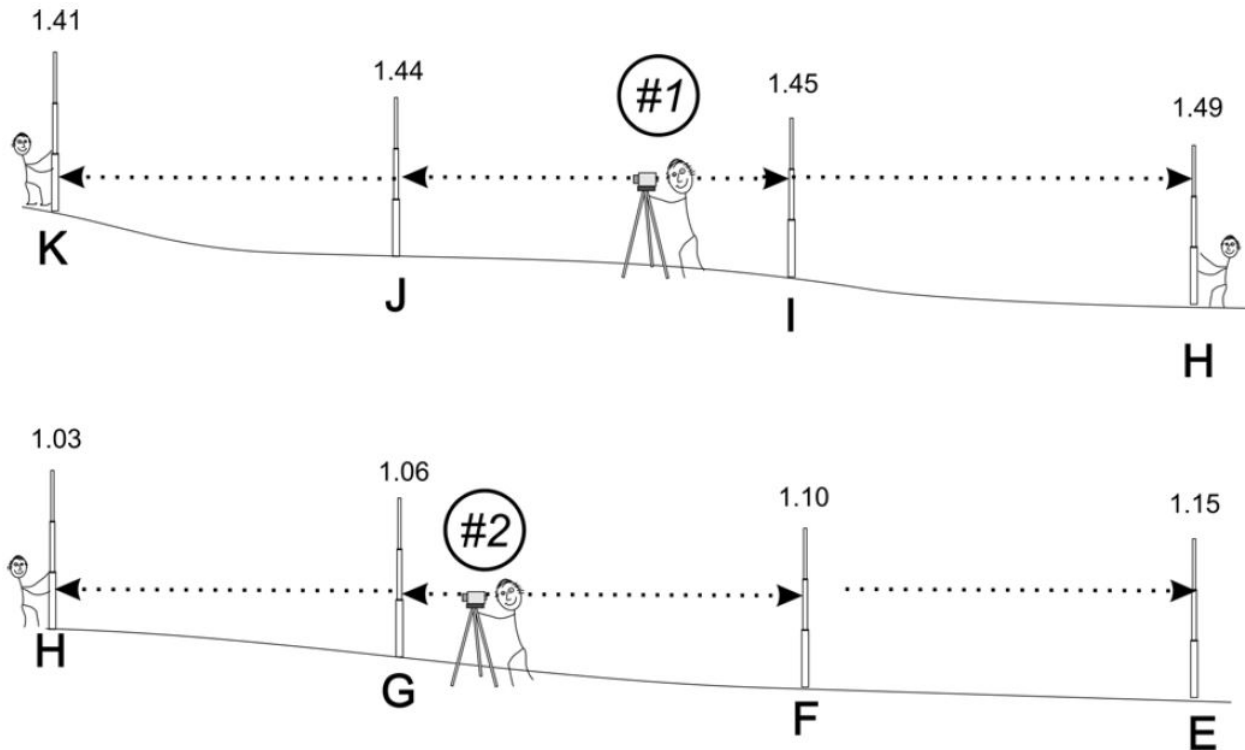


Figure 19. Use of an autolevel to measure slope of sampling reach.

Although slope and sinuosity are measured independently, always record the two data points at each location.

An autolevel should always be used for reaches with a slope of ≤ 1 . Either a clinometer or an autolevel may be used for reaches with a slope of $> 1\%$, and sometimes (*e.g.*, in steep areas that are also heavily vegetated) a clinometer is preferable for logistical reasons. If a reach is visually estimated to be close to 1% , use the autolevel. An autolevel or hand level measures the elevation difference (rise) between transects; the distance between transects (run) is also required for a slope calculation. Conversely, if a clinometer is used, the percent slope is recorded directly.

Do not measure slope across dry land (*e.g.*, across a meander bend).

6.10.1 Slope - autolevel method

Step 1. Identify a good spot to set up the autolevel (ideally near the middle of the reach, if there is good visibility from this location to both Transects A and K). The autolevel should be positioned on stable, and preferably flat, ground. Set the height of the autolevel to comfortable eye level for the operator. Level the plane of view of the autolevel by centering its bubble. Start by adjusting placement and length of the tripod legs, and then fine-tune the adjustment using the knobs on the autolevel.

Step 2. Begin “shooting” the change in elevation of the water level of the stream from transect to transect. Try to start with one of the outer transects (like K)³¹. Have a crew member at Transect K 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 record, 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 on the “Slope and Bearing Form” on the field sheet³², and then have the stadia rod holder proceed to the next transect (*e.g.*, Transect J), 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. If executed correctly, the bubble should still be centered while in this new orientation, without any further height adjustments to the autolevel or tripod. If the autolevel is displaced from its original position, it will no longer be possible to take a height measurement of Transect J’s water surface, relative to that of Transect K, to determine the slope between the two transects. In this case, the elevation must be measured anew (see Step 3).

Step 3. 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 (or if the length of the stadia rod is exceeded in a steep reach, or if the autolevel is bumped out of position before all the measurements are done), a new location must be set up for the autolevel. In order to maintain a relationship with 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, the remaining transects can be sighted from the new position. On the Slope and Bearing Form corresponding to autolevel use, indicate the transect at which the autolevel’s position has been changed (*i.e.*, list the transect that was measured from the original and the new positions twice on the datasheet: once for the original position, and once for the new).

Also indicate the segment lengths or distance between main transects (*i.e.*, 15 m, 25 m or other). These data will later be used to determine the slopes between transects and for the reach as a whole.

6.10.2 Slope - clinometer method

Step 1. Stand erect next to the stadia rod (held perpendicularly to the ground) on level ground and tie a highly visible piece of flagging around the rod at eye level. Then, beginning with the upper transect (Transect K), stand where the wetted margin intersects with the transect, and have a second person hold the flagged stadia rod perpendicularly to the ground at the wetted margin of the next downstream transect (Transect J).

Step 2. Use the clinometer to measure the percent slope of the water surface between the upstream transect and the downstream transect by sighting to the flagged position on the stadia

³¹ It does not matter if the measurements of slope and/or elevation difference are determined starting at the upstream or downstream end of the reach, but they must be reported as positive numbers.

³² Only the elevation difference (cm) will be recorded in the database. “Raw” stadia rod readings can be written on the hard copy sheets for reference and calculations but they will not be stored in the database.

rod, and record the value in the "Slope and Bearing Form" section of the field sheets. The clinometer gives both percent slope and degree of the slope (the measurements differ by a factor of ~2.2), so be careful to read and record **percent slope** rather than degrees slope. Percent slope is read from the scale on the right hand side when looking through most clinometers (but confirm this with the owner's manual for your own model).

Step 3. Continue measuring slope at each one of the transects. Note that when moving from transect to transect, the clinometer reader must stand exactly where the stadia rod had been placed during the previous reading.

Step 4. If the stream reach geometry makes it difficult to sight a line between transects, divide the distance into two or three sections and record the slope and the proportion of the total segment length between transects for each of these sections in the appropriate boxes on the slope form ("Supplemental Segment").

6.10.3 Sinuosity

Step 1. Take a compass reading from the center of each main transect to the center of the next main transect downstream and record this bearing to the nearest degree in the "Slope and Bearing Form" section of the field sheet. Bearing measurements should always be taken from the upstream to downstream transect.

Step 2. Proceed downstream to the next transect pair (I-J) and continue to record slope and bearing between each pair of transects until measurements have been recorded for all transects.

6.11 Module M: 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, albeit optional, to take a photograph at Transect A, facing downstream and Transect K, facing upstream to document conditions immediately adjacent to the reach. Use digital photographs. Record the image numbers on the front page of the field form under "Photographs". 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 station code and date) for that site prior to taking the series of photos.

7. PHYSICAL HABITAT INTER-TRANSECT-BASED MEASUREMENTS

Although most measures are taken near the main transects, a few measures are also recorded at the “inter-transects” located at the midpoint between main transects. These measures are: 1) Wetted Width, 2) Substrate Measurements (“Pebble Count”)/Depth/CPOM/Cobble Embeddedness/Algal and Macrophyte Cover, and 3) Flow Habitats.

7.1 Module C (part two): Inter-transect Wetted Width

Measure wetted width the same way it was measured for the main transects.

7.2 Modules D, E, and F (part two): Substrate Measurements, Depth, CPOM, and Algal/Macrophyte Percent Cover

Collect particle size measurements, water depth, CPOM, embeddedness and algal and macrophyte cover data the same way they were collected for the main transects.

7.3 Module N: Flow Habitats

Because many BMIs and algae prefer specific flow and substrate microhabitats, the proportional representation of these habitats in a reach is often of interest in bioassessments. Like the riparian and instream PHab measures, this procedure produces a semi-quantitative measure consisting of 10 transect-based visual estimates. A description of flow habitat types used for this SOP is provided in Table 7. These flow habitat types are products of geology, slope, and discharge, and one habitat type may change into another as water levels increase or decrease; therefore, the habitat types should be recorded at the time of sampling.

On the inter-transect field sheet, record to the nearest 5% percentages of the various flow habitats present within the region between the upstream inter-transect and downstream inter-transect bracketing each main transect (the total percentage of flow habitats for each stream section must total 100%). Although these definitions differ from geomorphological definitions presented in other hydrologic references, they were developed to produce more easily standardized and objective categories that improve data comparability. Please adhere to the definitions used in this text when employing this SOP.

Table 7. Flow habitat types

Type	Description
cascade/falls	Short, high-slope drops in stream bed elevation often accompanied by boulders and considerable turbulence. In high-slope streams, cascades and falls are often associated with step-pools. To qualify for this category, water must drop > 0.5 m in height within a short longitudinal distance (< 0.5 m).
rapid	Sections of stream with deep (>0.5 m), swiftly flowing (>0.3 m/s) water and considerable surface turbulence. Rapids tend to have larger substrate sizes than riffles.
riffle	“Shallow/fast” (< 0.5 m deep, > 0.3 m/s); riffles are shallow sections where the water flows over coarse stream bed particles that create mild to moderate surface turbulence.
runs/step-runs	“Deep/fast” (> 0.5 m deep, > 0.3 m/s); long, relatively straight, low-slope sections without flow obstructions. The streambed is typically even and the water flows faster than it does in a pool. Unlike rapids, runs have little surface turbulence.
glide	“Shallow/slow” (< 0.5 m deep, < 0.3 m/s); sections of stream with little or no turbulence, but faster velocity than pools. Includes still or slow-moving shallow backwaters and shallow margins of pools.
pool	“Deep/slow” (> 0.5 m deep, < 0.3 m/s); a reach of stream that is characterized by deep, low-velocity water and a smooth surface.
dry	Any surface area within the channel’s wetted width that is above water (e.g., mid-channel point bars). When assessing dry habitats, only count areas with particulate substrate; do not count tops of emergent rocks and boulders.

8. PHYSICAL HABITAT REACH-BASED MEASUREMENTS

8.1 Module O: 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. Discharge affects the concentration of nutrients, fine sediments, and pollutants, and its measurement is 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 temporal scales (diurnal, seasonal, inter-annual), it can also be very useful for understanding variation in stream condition.

For this SOP, a single discharge measurement is conducted in order to estimate discharge through the sampling reach. There is no prescribed point in the reach where the measurement should be taken; rather, it is up to the discretion of the field crew, depending upon streambed morphology and flow. It is preferable to take the discharge measurement in a section where flow velocities are > 0.15 m/s and most depths are > 15 cm, but slower velocities and shallower depths can be used, if necessary. If flow volume is sufficient for a transect-based “velocity-area” discharge calculation (Section 8.1.1), this is the preferred method. If the velocity meter probe cannot be submerged, but there is visible flow, the following two options are available: 1) use of the Neutrally Buoyant Object approach (which is the second most preferred method to measure flow) OR 2) a visual estimation of the velocity based on best professional judgment. In small, shallow streams with complex substrate, it may still be difficult to accurately measure discharge, even where water movement is obvious. If visual estimation is used, the velocity measurement must be denoted with a “visual estimate” flag in the data base.

Data for this parameter are entered in the “Discharge Measurements” section of the field sheet.

8.1.1 Velocity Area Method

The layout for discharge measurements under the velocity-area method is illustrated in Figure 20. Flow velocity should be measured with either a Swiffer Instruments propeller-type flow meter or a Marsh-McBirney inductive probe flow meter with a top-setting rod. Refer to the manufacturer instrument manual for calibration procedures.

Step 1. Select the best location (cross-section) in the reach to place a transect across which to measure discharge. This does not need to coincide with any of the main or inter-transects where other PHab measurements were taken, however it should lie within, or very near, the stream reach being assessed. Choose a cross section with flow that is as uniform as possible (i.e., hydraulically smooth), and with the simplest possible cross-sectional geometry. It is helpful to move bed material or other obstacles to create a more uniform cross-section before beginning the discharge measurements, but this cannot be done after measurements have begun, or it will skew results.

Step 2. Measure the wetted width of the discharge transect and divide this into 10 to 20 equal segments. The use of more segments gives a better discharge calculation, but is impractical in small channels. At least 10 intervals should be used when stream width permits, but interval width should not be < 15 cm.

Step 3. Record the distance from the bank to the end of the first interval. Using the top-setting rod, measure and record the median depth of the first interval (Figure 20).

Step 4. Stand downstream of the transect and off to the side of the probe in order to avoid interfering with the flow measurement. Set the probe of the flow meter at the midpoint of the first interval along the discharge transect, facing upstream perpendicularly to the direction of flow. If necessary, a thin piece of flagging tape can be attached to the top-setting rod and submerged to identify the direction of flow and thus inform proper angling of the probe. Determine the depth of the water and adjust the top-setting rod accordingly, such that the probe is held at a depth of 0.6 of the total stream depth. This position generally approximates average velocity in the water column. See Figure 20 for positioning detail. Refer to the top-setting rod owner's manual for further instructions on positioning of probe height.

Step 5. Allow the flow velocity meter to equilibrate for at least 15 seconds, and then record velocity to the nearest ft/s. If the option is available, use the flow-averaging setting on the flow meter³³. Record the flow velocity. Under very low flow conditions, flow velocity meters may register readings of zero even when there is noticeable flow. In these situations, record the appropriate ResQualCode (ND, Not Detected) and QACode (FLV, Velocity too low to be measured) and leave the Result field blank in the database. The Instrument Detection Limit (IDL) should be noted for the instrument used. In areas that are too shallow to measure velocity, use the Neutrally Buoyant Object method.

If the flow is moving upstream (such as near banks or in an eddy), point the probe into the flow and record the velocity with a negative symbol on the field sheet. Record an "NG" QA flag with this result in the database in order to identify the result as a negative value.

Step 6. Complete Steps 3 through 5 on the remaining intervals. Frequently, the first and last intervals have depths and velocities of zero.

³³ Set the averaging interval to at least 15 seconds (30 seconds if velocity is > 2 ft/s) and record the 15secondaverage velocity measurement for each segment.

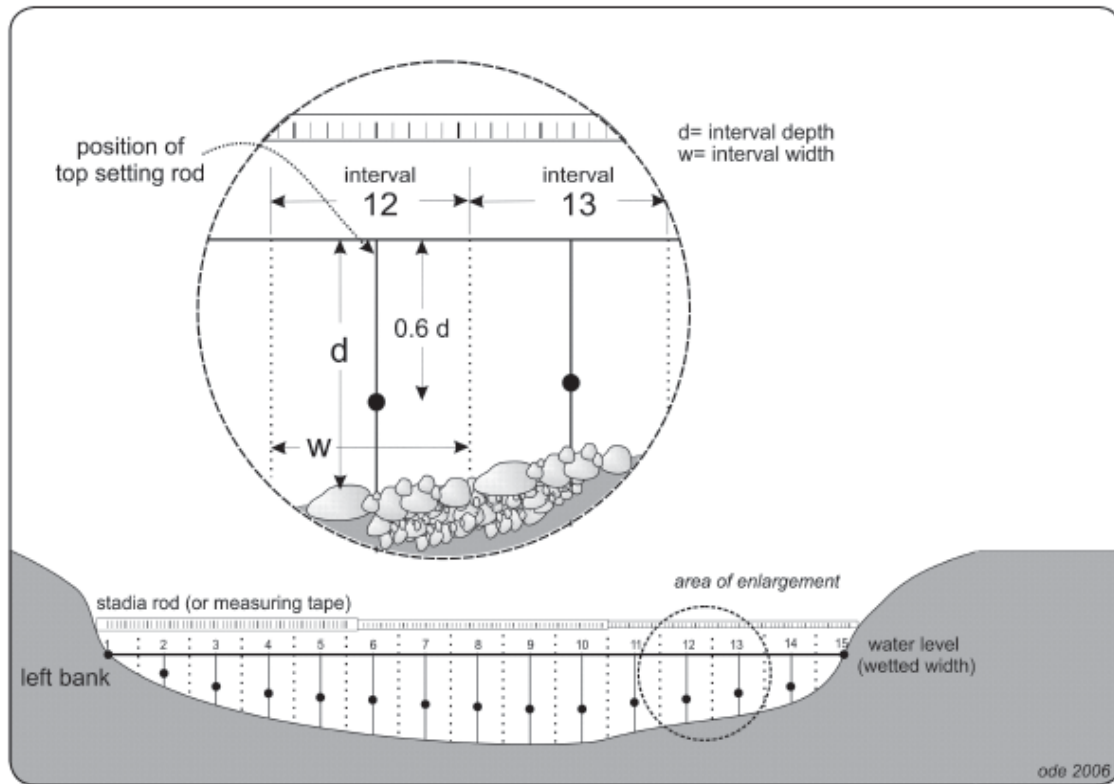


Figure 20. Diagram of layout for discharge measurements under the velocity-area method showing proper positions for velocity probe (black dots).

8.1.2 Neutrally Buoyant Object Method

If the reach is too shallow to use a flow velocity meter, the neutrally buoyant object (NBO) method can be used to measure flow velocity. However, since this method is less precise than the flow velocity meter, it should be used only if the velocity-area method will not work. The movement of an NBO (one whose density allows it to just balance between sinking and floating) will approximate that of the water it floats in better than a light object. Examples of NBOs include a large piece of fresh orange peel, a rubber ball, and a moderately heavy piece of wood.

To estimate the flow velocity, three transects are used to measure the cross-sectional areas within the test reach, and three flow velocity estimates are used to measure average velocity of water passing through it. To improve precision in velocity measurements, the test reach should be long enough for the float time to last at least 10-15 seconds. This will allow for an average of the instantaneous variation in flow and minimize the influence of error in the stopwatch timing. The use of longer times is recommended, when possible.

Step 1. Identify a sufficiently long test reach that has relatively uniform flow and a uniform cross-sectional shape. (The same criteria for selection of a discharge reach apply to selecting a test reach for the NBO method.)

Step 2. Record the length of the test reach.

Step 3. Measure the cross sectional area of the test reach in three places (an “Upper Section”, a “Middle Section” and a “Lower Section”). Three evenly-spaced cross sections are preferred, but a single one may be used if the cross section through the test reach is uniform (*e.g.*, in a concrete channel). On the “Float Reach Cross Section” of the field sheet, record the width once, and the depth at five equally-spaced positions, across each of the three cross sections of the test reach.

Step 4. Place the NBO in the water upstream of the test reach and record the length of time (in seconds) that it takes for the object to pass between the reach’s upstream and downstream boundaries. Repeat this twice more for a total of three timed “floats”.

8.2 Module P: Post-Sampling Observations: Qualitative Reach Measures

EPA’s Rapid Bioassessment Procedures (RBPs, Barbour et al. 1999) include a set of 10 visual criteria for assessing instream and riparian habitat. The RBP has been used in the CSBP since its first edition (1995), and thus this information is often valuable for comparison to legacy datasets. The criteria also have a useful didactic role, since they help force the user to quantify key features of the physical environment where bioassessment samples are collected. The full suite of RBP stream habitat visual estimates are not covered in this SOP because they are generally replaced by more quantitative measurements of similar variables. However, three of the RBP measures (“Epifaunal Substrate/Cover”, “Sediment Deposition”, and “Channel Alteration”) have been found to be reasonably repeatable and thus are included.

Record observations in the “Additional Habitat Characterization” section of the field sheet.

8. OPTIONAL SUPPLEMENTAL MEASURES

Optional measures to supplement this SOP may be included in stream assessments according to program needs. These include the excess sediment index (sometimes referred to as log relative bed stability, LRBS) and additional measurements collected for the LRBS calculations (Kaufmann et al. 1999), such as tallies of woody debris and thalweg. The [NRSA Field Operations Manual \(USEPA 2009\)](#) provides more details on collecting these data types.

Large woody debris (logs, snags, branches, etc.) that is capable of obstructing flow when the channel is at bankfull (i.e., just short of flood) stage contributes to the “roughness” of a channel. The effect of this variable is to reduce water velocity and thereby reduce the stream’s competence to move substrate particles. The NRSA (Section 6.2.4.2) protocol tallies all woody debris with a diameter > 10 cm (~4”) into one of 12 size classes based on the length and width of each object. Tallies are conducted in the zone between the main transects.

A stream’s thalweg is a longitudinal profile that connects the deepest points of successive cross-sections of the stream. The thalweg defines the primary path of water flow through the reach. Thalweg measurements (NRSA; Section 5.2.7) perform many functions in the NRSA protocols, producing measurements for the excess sediment calculations (residual pool volume, stream size, channel complexity) and flow habitat variability.

9. REFERENCES

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10. GLOSSARY

Aliquot – a measured portion of a sample, or subsample

Allochthonous – derived from a source external to the stream channel (e.g., riparian vegetation as a source of organic matter) as opposed to autochthonous, which indicates a source inside the stream channel (e.g., algae or macrophytes rooted in the stream)

Ambient bioassessment – monitoring that is intended to describe general biotic condition as opposed to a diagnosis of sources of impairment

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 is often used as a surrogate for algal biomass

Bankfull – the bankfull channel is the zone of maximum water inundation in a normal flow year (one- to two-year flood events)

Benthic algae – algae that are attached 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

Benthic macroinvertebrates (BMI) – bottom-dwelling invertebrates large enough to be seen with the unaided eye

Biofilm – a matrix/film adhering to stream substrates and consisting of microorganisms (e.g., algae, fungi, bacteria, protozoans) and detritus

Chlorophyll *a* – primary light receptor/photosynthetic pigment in algae and cyanobacteria and higher plants; the concentration of this pigment per stream surface area sampled provides an estimate of algal biomass

Coarse particulate organic matter (CPOM) – particles of decaying organic material, such as leaves and twigs, that are between 1 and 10 mm in diameter and suitable for consumption by BMIs in the “shredder” functional feeding group

Cobble embeddedness – The percent of surface area of cobble-sized particles (64-250 mm) buried by fine particles (<2.0 mm diameter)

Composite sample - volume of all the liquid material amassed during sampling, including water used for rinsing substrate and sampling devices.

Cyanobacteria – historically referred to as “blue-green” algae, but actually chlorophyll-*a* containing prokaryotes that are capable of photosynthesis and co-occur with “true” (i.e., eukaryotic) benthic algae in streams; useful as a bioindicator, and field-sampled and laboratory-processed as 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

Diatom – a unicellular golden-brown alga (Bacillariophyta) that possesses a rigid, silicified (silica-based) cell wall in the form of a “pill box”

Elutriation – the process of using a liquid (water) to separate denser material (e.g., stream sediments) from lighter materials (organic particles and benthic organisms). -.

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

Fines – substrate particles < 0.06 mm diameter (not gritty to the touch)

Guidance Document – a companion document to this SOP that provides more information on the various applications of the indicators described herein, as well as recommendations for where and when to use this SOP. It also provides more detailed information on how to deal with special circumstances that may be encountered during bioassessment sampling.

Homogenate – mixture of algae liquid composite sample and finely chopped fragments of macroalgae that comprises the quantitative sample for the diatom taxonomic ID, chlorophyll a, and AFDM subsamples

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, or ecological health, of the environments they occupy (*e.g.*, algae or benthic macroinvertebrates)

Inter-transects – transects established at points equidistant between the main transects

Macroalgae – soft bodied algae that form macroscopically discernible filaments, mats, or globose structures

Macrophyte, aquatic – herbaceous, vascular plant rooted or floating within the stream's wetted channel, such as sedge, cattail, knotweed, watercress, water-primrose, duckweed, etc.; our definition of aquatic macrophytes excludes trees, shrubs, mosses, and algae

Microalgae – diatoms and microscopic soft-bodied algae (can co-occur with other microorganisms in a biofilm)

Prospecting mine – a hand-excavated, hard-rock mining hole that is open to the surface (common in the Sierra Nevada)

Reach – a linear segment of the stream channel

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

Riparian – an area of land and vegetation adjacent to a stream that has a direct effect on the stream by providing shade, habitat for wildlife, contributing allochthonous organic matter, modulating water levels via evaporative transpiration, etc.

Sinuosity – the ratio of the length of the flow path between the ends of the reach and the straight line distance between the ends of the reach (Kaufmann et al. 1999)

Soft-bodied algae – non-diatom algal taxa; for the purposes of this SOP, cyanobacteria are included in this assemblage

Substrate – the composition of a streambed, including both inorganic and organic particles

Target coordinates – the nominal or tentative location of a sampling site, which may differ from the actual location from which samples are collected

Thalweg – the thalweg defines the primary path of water flow through the reach; it is often inferred by depth for practical purposes, but is not always the deepest point

Transects – lines drawn perpendicular to the path of flow used for standardizing biotic sampling and data collection locations

Wadeable stream – a stream that can be sampled by field crews wearing chest waders (generally < 1 meter deep for at least half the reach)

Wetted width – the width of the channel containing water (the active channel), defined as the distance between the sides of the channel at the point where substrates are no longer surrounded by surface water



**SWAMP Bioassessment
Procedures** 2016

Supplemental Guidance for the SWAMP Bioassessment Field Protocol

*Standard Operating Procedures
for the Collection of Field Data
for Bioassessments
of California Wadeable Streams:
Benthic Macroinvertebrates, Algae,
and Physical Habitat*

**March 2016 v2
(unformatted)**



www.waterboards.ca.gov/swamp

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1. Field Guidance

1.1 Labor division for efficiency in the field

To maximize efficiency in the field, a workable division of labor is as follows (where Team "1" and "2" could consist of one or more people): Team 1 collects water chemistry samples and data, and measures discharge along a "flow transect" (as long as a good one can be located near, but downstream of, Transect A), while Team 2 lays out the transects for the sampling reach (remember: those setting up the transects must stay out of the stream). Then Team 1 begins collecting biota (one person focuses on BMIs and the other focuses on algae). Once Team 1 is done collecting at Transect A, Team 2 begins collecting PHab data at that Transect, then follows Team 1 as they proceed from transect to transect. Team 2 collects slope and sinuosity data while Team 1 processes the biotic samples.

1.2 Memo: Amendment to SWAMP Interim Guidance on Quality Assurance for SWAMP Bioassessments

Surface Water Ambient Monitoring Program Quality Assurance Program Memorandum *(Approved by the Interim SWAMP Coordinator)*

To: SWAMP Round Table

From: Beverly H. van Buuren, SWAMP Quality Assurance Officer and
Peter R. Ode, SWAMP Bioassessment Lead Scientist

Date: September 17, 2008

Re: Amendment to SWAMP Interim Guidance on Quality Assurance for
SWAMP Bioassessments

On May 21, 2007, the SWAMP Quality Assurance (QA) Officer and SWAMP Bioassessment Coordinator issued interim guidance for all SWAMP-funded bioassessment projects defining QA requirements for several key elements of SWAMP's Bioassessment Program. This memorandum, effective September 17, 2008, amends the May 2007 document with updated guidance on macroinvertebrate sample collection.

If you have any questions regarding this guidance, please contact the SWAMP QA Officer, Beverly H. van Buuren at (206) 297-1378, or via email at bvanbuuren@mlml.calstate.edu or the SWAMP Bioassessment Lead Scientist, Peter Ode, at (916) 358-0316, or via email at pode@ospr.dfg.ca.gov.

Macroinvertebrate Sample Collection

The previous SWAMP policy (Ode 2007, <http://www.swrcb.ca.gov/swamp/biocalstreams.html>) was to collect two field methods under most circumstances: a targeted habitat method (“targeted riffle composite”, or TRC) and a systematic representation of multiple habitats in a reach (“reachwide benthos”, or RWB). Recent published and unpublished analyses (Gerth and Herlihy 2007, Rehn et al. 2007, Mazor et al. 2008) provide evidence that RWB and TRC methods can produce generally comparable results across a broad range of settings within California. Based on these analyses, SWAMP is now adopting a single field method in MOST streams statewide. However, because the comparability data are equivocal in some settings, SWAMP policy includes two specific exceptions.

SWAMP-funded bioassessments shall adhere to the following guidelines:

1. In most regions of the state (see exceptions listed in 2 and 3 below), SWAMP funded programs shall collect bioassessment samples using the RWB method (sometimes referred to as multi-habitat or MH).
2. SWAMP programs shall continue to collect both TRC and RWB samples in environmental settings where method comparability results are equivocal. While SWAMP will define specific criteria for these settings in a future guidance memo, the current interim policy is to collect both methods at high elevation pool-dominated streams (>2000 m elevation, >80% pool reaches, boulder cascades).
3. SWAMP programs shall employ a modified version of the RWB in large, low-gradient streams dominated by sandy bottoms (e.g., low gradient coastal streams, large Central Valley streams). The modification is to collect subsamples at 0%, 50%, and 100% of stream width instead of 25%, 50%, and 75% of stream width) to ensure collection of marginal habitats.
4. SWAMP programs may choose (at their discretion) to continue to collect a second method at any site where additional sampling data is likely to produce complementary information.

The SWAMP Bioassessment Program will establish a Technical Advisory Committee (TAC) to provide additional guidance on the following topics:

1. *Refined criteria for defining when the exceptions described in 2 and 3 above should be applied*
2. *Analytical considerations for combining RWB and TRC datasets or applying data collected with one method to an indicator (e.g., index of biotic integrity (IBI) or observed/expected (O/E) model) derived from another method*

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1.3 Note for delineating BMI sample area

If desired, a 1ft² wire frame can be placed on the stream bottom in front of the D-frame net to delineate the area to be sampled for BMIs, but it is often sufficient to visually estimate the prescribed sampling area (based on the net dimensions) in order to keep sampling effort consistent between transects and between sites.

1.4 Applying the Basic SOP and recognizing situations in which modifications to procedures may be made

The SOP is geared toward ambient assessment (i.e., monitoring a large number of probabilistically selected sites in order to arrive at regional condition estimate with a known level of confidence). Other applications, such as site-specific compliance monitoring, may benefit from augmentation to aspects of this protocol. For example, if the monitoring goal is to determine, with high precision, the amount of algal biomass in a particular stream reach in order to understand nutrient impacts, a higher spatial density of algae sampling (such as 33, instead of 11, subsamples to create the composite sample) may be desirable.

Although individual modules can be added or eliminated from the SOP to reflect project objectives requiring different levels of intensity for characterizing the chemical and physical habitat data, a core set of PHab measures is recommended to accompany all bioassessment samples. The following “**Basic**” list of PHab measurements reflects the minimum amount of physical and chemical data that should be taken along with any ambient bioassessment sample.

- ☐ Layout of reach, marking transects, recording GPS coordinates
- ☐ Temperature, pH, specific conductance, salinity, DO, alkalinity

- ☐ Notable field conditions
- ☐ Wetted Width
- ☐ Stream Shading
- ☐ Bank Stability
- ☐ Percent Algal Cover
- ☐ Flow Habitat Delineation
- ☐ Slope (%): conducted at *reach* scale (i.e., a *single* slope measurement for the *entire* reach, as opposed to one for each transect as described in the SOP)
- ☐ Photo documentation

However, note that the "Full" PHab characterization (i.e., the full suite of Modules described in the SOP) represents the data that should be collected with most professional-level bioassessment sampling efforts (and Full PHab is required for SWAMP regional monitoring programs). A single set of field sheets is used in conjunction with this SOP, regardless of whether the Basic or Full level of effort for the PHab measures and water chemistry is carried out. For the Basic level, fill in "NR" (for "Not Recorded") for the PHab measures that are not taken.

When there are some dry transects in the sampling reach, bioassessment practices for the two assemblages (BMIs and algae) are different. In the case of algae, the crew should collect the subsample at the closest wet place (i.e., the goal is to achieve 11 subsamples for the composite, even if sampling locations must be shifted to some degree). For BMIs, crews should skip the dry transect and make a note of how many subsamples were ultimately collected (i.e., some number < 11).

Note that it is acceptable to sample biota in natural pools, because a reach-wide benthos (RWB) method is being used to collect samples. If it is necessary to shorten the sampling reach, note that if it is < 100 m long, the "representativeness" of the sample may be compromised.

1.5 Information resources for avoiding introduction of invasive species and pathogens into streams

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., Schisler et al., 2008).

There are several online sources of information regarding invasive species, including information on cleaning and disinfecting gear, such as the Whirling Disease Foundation (www.whirling-disease.org), the USDA Forest Service (*Preventing Accidental Introductions of Freshwater Invasive Species*, available from http://www.fs.fed.us/invasivespecies/documents/Aquatic_is_prevention.pdf), and the California Department of Fish and Wildlife (Hosea and Finlayson 2005 and <https://www.dfg.ca.gov/invasives/>). General information about freshwater invasive species is available from the U.S. Geological Survey Nonindigenous Aquatic Species website (<http://nas.er.usgs.gov>) and the *Protect Your Waters* website (<http://www.protectyourwaters.net/hitchhikers>) that is co-sponsored by the U.S. Fish and Wildlife Service. The California State Water Resources Control Board Aquatic Invasive Species website (http://www.swrcb.ca.gov/water_issues/programs/swamp/ais/) should also be consulted regularly for updates.

YouTube videos are also available for viewing. These include the Invasive Species Playlist:

<https://www.youtube.com/playlist?list=PLMSa5d-iIl6Nyoo0daTJLeXOan3dj7OCi>

and Cleaning and Treating Water Quality Monitoring Field Gear for Freshwater Aquatic Invasive Species:

<https://www.youtube.com/watch?v=YMI4hgeMMAw&index=2&list=PLMSa5diIl6Nyoo0daTJLeXOan3dj7OCi>

A pamphlet for prevention of the spread of New Zealand Mudsnail prepared by SeaGrant Oregon State University can be accessed here:

<http://seagrant.oregonstate.edu/sgpubs/onlinepubs/g10001.pdf>

The following procedures for decontamination of equipment to prevent the spread of chytrid fungus is taken from the SWAMP depressional wetlands SOP (Fetscher et al. 2014)

Chytrid fungus has been decimating amphibian populations worldwide, including causing declines in mountain yellow-legged frogs in the Sierra Nevada. Consult the decontamination SOP from USFS Region 4, which has an excellent summary table of viable methods for multiple types of AIS (USFS 2013;

http://www.fs.fed.us/r4/resources/aquatic/guidelines/2011techguidelines_fire_AIS.pdf).

A 5% Quat 128 solution requires 30 seconds of soak time to kill chytrid fungus.

However, New Zealand mud snail (NZMS), although unlikely to be found in wetlands, could nonetheless be present, and requires 10 minutes of soak time at the same concentration. Because lakes and reservoirs may have NZMS and be sampled with this protocol, a 10 min soak time for all gear in 5% Quat 128 solution or similar Quat-related product should prevent movement of all potential invasive species, including aquatic diseases. If a non-chemical solution is preferred, waders can be fully cleaned of mud and debris, then exposed to sun for three hours and allowed to rest completely dry for 48 hrs. Please refer to the USFS guidance for specifics. *Note: freezing gear alone will not kill chytrid fungus, so this is not an acceptable method of decontamination for wetland sampling.*

An alternative method for decontaminating gear for chytrid fungus is via the use of a bleach solution, along with freezing to kill any NZMS that may be present. In consideration of the difficult logistics of field decontamination, a potential approach would be to have 1 pair of waders available for each crew member per site, until the waders are decontaminated. Be sure to keep any used waders in closed, heavy-duty garbage bags during transit, in order to avoid contaminating field vehicles. At appropriate intervals, all of the waders can be cleaned with a brush, rinsed, and treated with the appropriate concentration of bleach for the prescribed time (USFS 2013;

http://www.fs.fed.us/r4/resources/aquatic/guidelines/2011techguidelines_fire_AIS.pdf), dried, and then frozen. After each site visit, the syringe or water grabber can be thoroughly scrubbed and then treated with bleach (as described above for waders), rinsed well, and allowed to dry to promote evaporation of any residual bleach, or a new syringe can be used at each site.

References

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¹ Test strips are available to test the concentration.

1.6 Targeted riffle composite procedure

The Targeted Riffle Composite (TRC) method is designed for sampling BMIs in wadeable streams that contain fast-water (riffle/run) habitats and is not appropriate for water bodies without fastwater habitats. The Regional Board protocol should be used in these situations. Riffles are often used for collecting biological samples (e.g., the old CSBP methods) because they often have the highest BMI diversity in wadeable streams. This method expands the definition to include other fast water habitats, however care should be taken when attempting to apply this method in low gradient streams. ***Note:** Since all streams (even low gradient streams) have variation in flow habitats within the channel, this guidance should not be interpreted as including areas within low gradient streams that are only marginally faster than the surrounding habitats. The Regional Board protocol should be applied in these situations.*

The TRC was developed by the Western Center for Monitoring and Assessment of Freshwater Ecosystems (www.cnr.usu.edu/wmc) in Logan, Utah (Hawkins et al. 2003) and slightly modified by the EPA program (Peck et al. 2004). The TRC has been widely used in California by the US Forest Service (USFS) and the EMAP Western Pilot, and in the interest of methodological consistency between state and federal water resource agencies, has been adopted as the standard riffle protocol for bioassessment in California. The version described here is the EMAP modification, which distributes the sampling effort throughout the reach.

Sampling Locations – Acceptable Habitat Types

Riffles are the preferred habitat for TRC sampling, but other fast water habitats are acceptable for sampling if riffles are sparse. Common flow-defined habitat types are listed in the table below in decreasing order of energy. Most streams contain some or all of the following fast water habitat types: 1) cascades/falls, 2) rapids, 3) riffles, 4) runs. All of these are acceptable for TRC sampling if riffles are not available. ***Note:** Because the common habitat types are arranged on a continuum of high to low energy environments, the categories grade into each other continuously and are not discrete. Thus, determination of habitat types requires somewhat subjective decision-making.*

Sampling Locations – Selecting Habitat Units

A TRC sample is a composite of eight individual kick samples of 1 ft² (0.09 m²) of substrate each. During your initial survey of the reach, take a mental note of the number and position of the main riffles in a reach (and other fast water habitats if needed). Randomly distribute the eight sub-samples among the fast water habitats in the reach, giving preference to riffles where possible (Figure 1). Unless you are sampling in small streams, try to avoid very small riffle units (i.e., <5 ft²). If fewer than eight riffles are present in a reach, more than one sample may be taken from a single riffle, especially if the riffles are large.

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

Table 1. Common habitat types in stream channels, arranged in decreasing order of energy

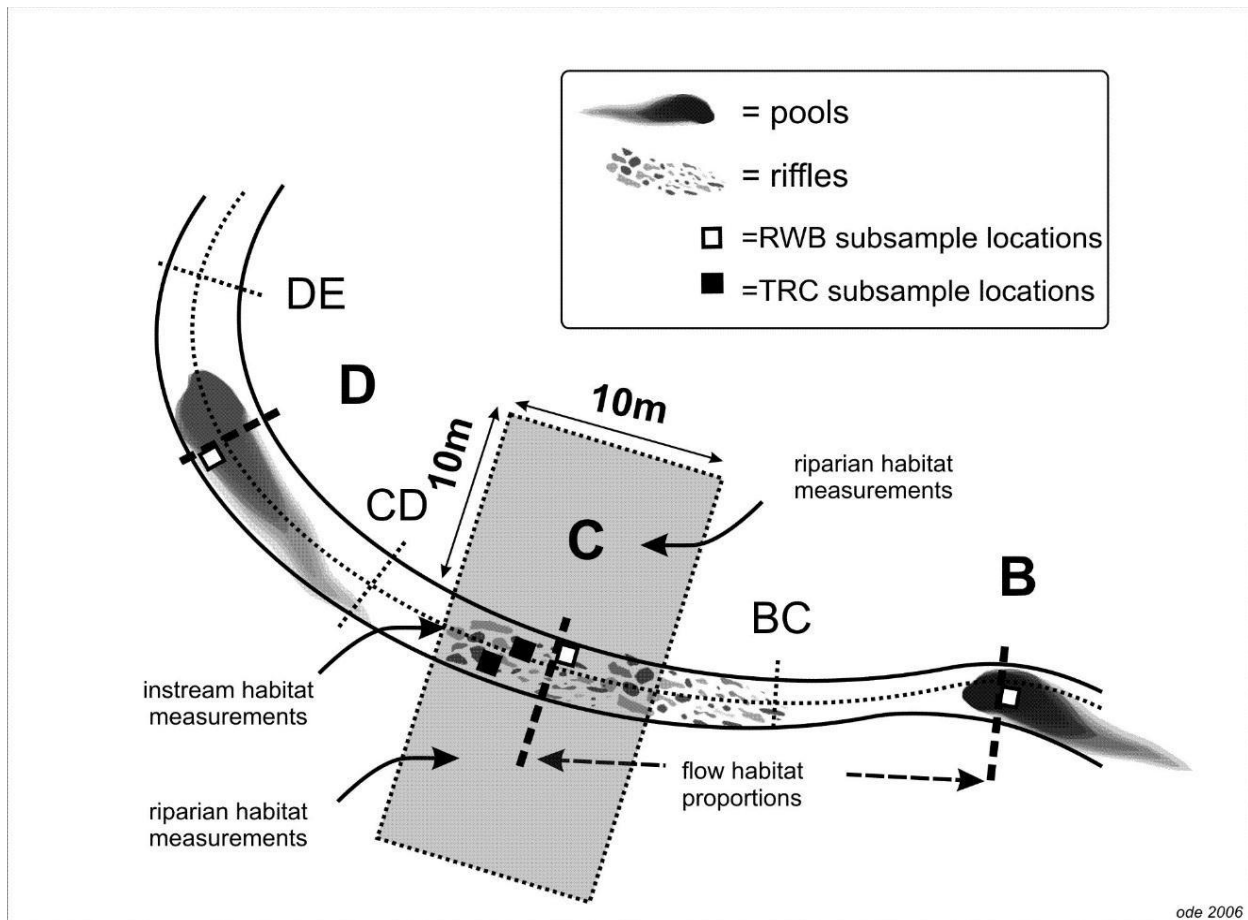


Figure 1. Section of the standard reach expanded from Figure 2 (in the SOP) showing the appropriate positions for collecting benthic macroinvertebrate samples, instream and riparian habitat measurements and flow habitat proportion measurements.

Sampling Procedure

Begin sampling at the downstream end of the reach at the first randomly selected riffle and work your way upstream.

TRC-Step 1. Determine net placement within each habitat unit by generating a pair of random numbers between 0 and 9. Examples of convenient random number generators include the hundredths place on the stopwatch feature of a digital watch, a 10 sided die and a random number chart. The first number in each pair (multiplied by 10) represents the percent upstream along the habitat unit's length. The second number in each pair represents the percent of the riffle width from right bank. For example, if the two generated random numbers are 4 and 7, you will walk upstream 40% of the distance of the riffle and then go 70% of the distance across the riffle. This position is the center of the 1 ft² (0.09 m²) sampling quadrat for that riffle. If you are unable to sample this location because it is too deep or it is occupied by a large boulder, select a new pair of random numbers and pick a new spot.

TRC-Step 2. Position a 500- μ D-net (with the net opening perpendicular to the flow and facing upstream) quickly and securely on the stream bottom to eliminate gaps under the frame. Avoid,

and if necessary remove, large rocks that prevent the sampler from seating properly on the stream bottom.

TRC-Step 3. Holding the net in position on the substrate, visually define a square quadrat that is one net width wide and one net width long upstream of the net opening. Since D-nets are 12 inches wide, the area within this quadrat is 1ft² (0.09 m²). Restrict your sampling to within that area. If desired, a wire frame of the correct dimensions can be placed in front of the net to help delineate the quadrat to be sampled, but it is often sufficient to use the net dimensions to keep the sampling area consistent.

TRC-Step 4. Working backward from the upstream edge of the sampling plot, check the quadrat for heavy organisms such as mussels and snails. Remove these organisms from the substrate by hand and place them into the net. Carefully pick up and rub stones directly in front of the net to remove attached animals. Remove and clean all of the rocks larger than a golf ball within your sampling quadrat such that all the organisms attached to them are washed downstream into your net. Set these rocks outside your sampling quadrat after you have cleaned them. If the substrate is consolidated or comprised of large, heavy rocks, use your feet to kick and dislodge the substrate to displace BMIs into the net. If you cannot remove a rock from the stream bottom, rub it (concentrating on cracks or indentations) thereby loosening any attached insects. As you are disturbing the plot, let the water current carry all loosened material into the net.

***Note1:** Brushes are sometimes used to help loosen organisms, but in the interest of standardizing collections, do not use a brush when following this protocol.*

***Note 2:** In sandy-bottomed streams, kicking within run habitats can quickly fill the sampling net with sand. In these situations, follow the standard procedures but use care to disturb the substrate gently and avoid kicking.*

TRC-Step 5. Once the coarser substrates have been removed from the quadrat, dig your fingers through the remaining underlying material to a depth of about 10 cm (this material is often comprised of gravels and finer particles). Thoroughly manipulate the substrates in the quadrat. ***Note:** the sampler may spend as much time as necessary to inspect and clean larger substrates, but should take a standard time of 30 seconds to perform Step 5.*

TRC-Step 6. Let the water run clear of any insects or organic material before carefully lifting the net. Immerse the net in the stream several times to remove fine sediments and to concentrate organisms at the end of the net, but be careful to avoid having any water or foreign material enter the mouth of the net during this operation.

TRC-Step 7. Move upstream to the next randomly selected habitat unit and repeat steps one through six, taking care to keep the net wet but uncontaminated by foreign material when moving the net from riffle to riffle. Sometimes, the net will become so full of material from the streambed that it is no longer effective at capturing BMIs. In these cases, the net should be emptied into sample jars as frequently as necessary, following guidelines described below in the “Preparation of BMI Sample Jars” section. Continue until you have sampled eight 1ft² (0.09 m²) of benthos.

TRC-Step 8. PROCEED to Section IIc. Filling and Labeling BMI Sample Jars.

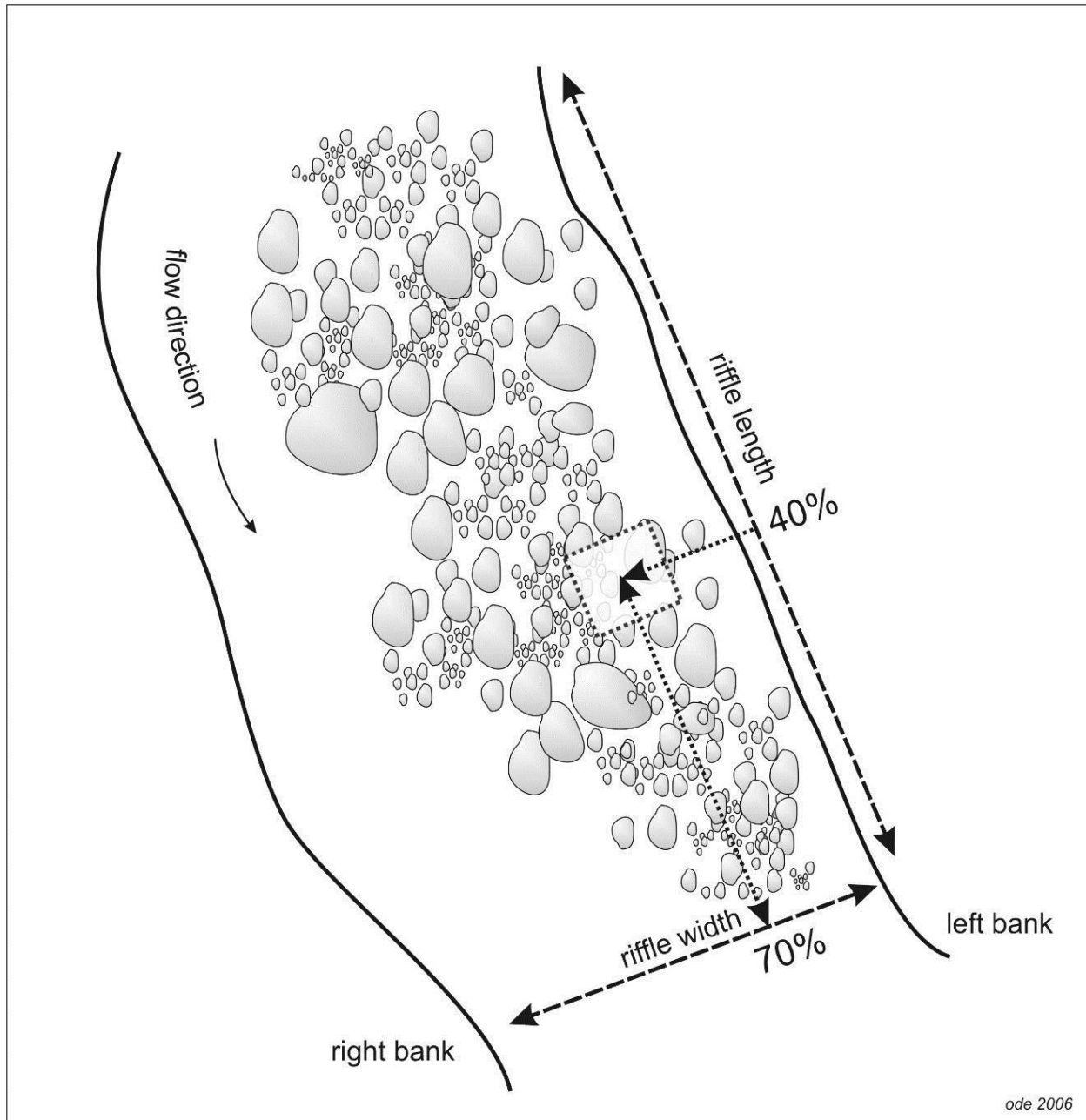


Figure 2. Example showing the method for selecting a subsampling position within a selected riffle under the TRC method. In this example, the random numbers 4 and 7 were selected.

Some additional suggestions:

- If TRC samples will be collected, while scouting the reach, also identify all riffle habitats suitable for sampling and note their positions so that a subset can be identified for sampling.
- Both reachwide benthos (RWB) and TRC for methods for BMI sampling use 500-µm mesh D-frame nets. The two samples can be collected at the same time by carrying two D-nets and compositing the material from the two samples in their respective nets. If a two-person collecting team is responsible for both the physical habitat data and benthic invertebrate samples, it is generally best to collect the benthos at each transect, then immediately record the physical habitat data before moving to the next transect. Obviously, this requires especially careful handling of the D-nets during the course of sampling to avoid loss or contamination of the samples. It can be helpful to clearly label the two D nets as RWB and TRC. Larger field crews may choose to split the sampling between biotic team and a physical habitat team and have the biotic team go through the reach first. The positions of both the TRC and RWB subsampling locations are illustrated in Figure 1.

1.7 Determining bankfull position in streams

BANKFULL: WHAT IT IS AND HOW TO LOCATE IT

WHY BANKFULL?

Several procedures in this manual require you to locate what is known as the “bankfull channel edge,” or more simply as “bankfull.” This is an important concept in understanding the workings of a stream.

HOW DOES A “BANKFULL” GET CREATED?

Most lower portions of streams in our area are alluvial, meaning that they create their own channels by moving sediment from the surrounding hillslopes and from the stream channel itself. Major episodes of such movement occur during floods and are called “channelforming events.” These events determine the size of the channel needed to convey the water. In a period of relatively stable climate and landcover, a stream system will develop an equilibrium between its flows and the size of the channel, whereby the channel is large enough to contain the stream under most flow conditions. When flows are greater than this capacity, the stream overflows its banks and flooding occurs.

In such streams, the channel is usually big enough to contain a high-flow event that recurs on an average of every 1.5 years (which we call the “1.5-year flood”). Such a frequency of inundation is frequent enough that perennial vegetation can’t grow there, either because its roots are too wet or its seedlings get swept away. So usually, what you’ll see if you look at the cross-section of a stream channel is a sort of “bowl” that contains the stream most of the time, inside which no perennial vegetation grows, and a place over the top of this bowl where the water can flow during a high-water event greater than a 1.5-year flood. This “floodplain” may be on one or both banks, depending on the site.

WHAT ARE INDICATORS OF BANKFULL?

Most stream systems are in a continual cycle of change, and every site is unique; thus, no single indicator of bankfull can always get you the “right answer.” There are several indicators which can help to identify the bankfull channel edge, and you should consider all that are present at a given site:

A. Bank slope: In stream channels with natural (undiked) riparian areas and a low, flat floodplain, the bankfull edge is located at the edge of this plain. Often the floodplain will slope down very gradually and then more abruptly. This abrupt slope-break is usually a good indicator. However, you may find such a slope-break on only one bank, or none at all, for instance if the channel has cut down into the streambed. Or the slope-break may be impossible to find on a bank that is slumping or undercut.

B. Vegetation: The bankfull edge is often indicated by a demarcation line between lower areas that are either bare or have aquatic and annual vegetation, and higher areas with perennial vegetation such as ferns, shrubs, and trees. (Keep in mind, though, that the vegetation line is always in transition, retreating during wetter periods and advancing during dryer ones. So except for ferns, you should rely most heavily on perennial vegetation which is more than 6 feet high.) One particular confusion arises from willow or alder trees growing within the bankfull channel, because the channel has migrated into them, or they fell into the stream and managed to reestablish themselves. Therefore, when you look at vegetation, you should also look at soils...

C. Soils: Look for a transition as you move up the bank, from cobble/gravel to sand/silt to soil. Above bankfull level, you should find old leaf litter forming into soil with organic matter. (Beware: this may be covered by flood deposits, so you may have to dig down.)

D. Point bars and bank undercuts: Often on the inside of meander bends, the stream will build up a bar of sediment from the eddy current created by the bend; the top of such a bar is the minimum height of bankfull. Similarly, on the outside of such bends, the stream will often undercut the bank and expose root mats. If you reach up beneath this mat, you can estimate the upper extent of the undercut. This would also be the minimum height of bankfull.

E. Lines on boulders/bedrock: If you're in a steep channel with no clear floodplain, look for the highest mineral-stain line or the lowest line of lichen or moss on stable rock.

F. Adjacent indicators: If the indicators are unclear where you're looking, try looking up or downstream to see if there is a clear bankfull line from which you can extrapolate.

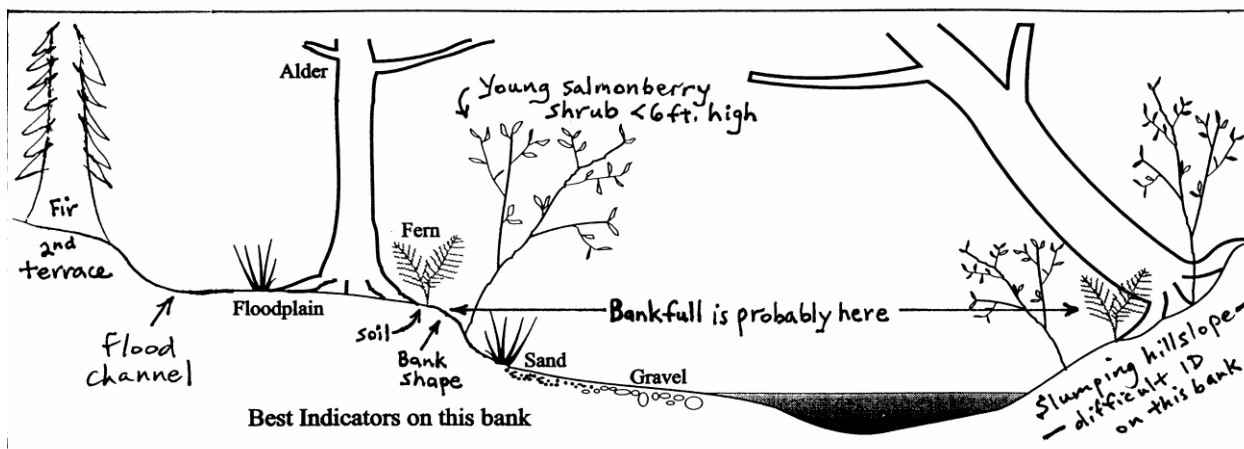


Figure 3. Typical bankfull ID situation, adapted from Pleus and Schuett-Hames, 1998.

HOW SHOULD I LOCATE BANKFULL?

The following method was found by the TFW program to maximize data precision and minimize bias toward over- or under-estimation of bankfull elevation:

1. Start on the bank with the best bankfull indicators.
 - a. Move up the bank from the channel, observing the indicators listed above. When you reach a point at which you're no longer 100% sure that you're below bankfull, mark that level with a flag or stick.
 - b. Then walk up to what is clearly dry land, and walk around, observing indicators and moving back toward the bankfull edge. When you're no longer 100% confident that you're above bankfull, mark that point.
 - c. Reassess the indicators and your confidence levels, and consult with your fellow samplers, and make adjustments as needed.
 - d. The bankfull channel edge is at the elevation point midway between these two points.
2. Now follow the same procedure on the other bank. If it is not possible to accurately identify the bankfull level on that bank (which often happens on the outside bank of a meander bend), locate it using a level line from the bankfull point on the first bank.
(Also referenced for this section: Harrelson et al., 1994.)¹

Identifying the Boundaries of the Bankfull Channel

To measure bankfull width and depth, you must first determine the edge of the bankfull channel. Unfortunately, the boundaries of the bankfull channel are not always easy to identify. Geomorphologists have used many methods to delineate the bankfull channel. None are without shortcomings, and the most accurate methods are not feasible for stream surveys on remote and engaged stream reaches because they require long-term discharge records or the use of surveying techniques (Williams, 1978).

The TFW Ambient Monitoring Program uses a combination of indicators developed by Dunne and Leopold (1978) to delineate the bankfull channels. The indicators include floodplain level, the shape of the bank, and changes in vegetation.

Floodplain indicators- In channels with natural (un-diked) riparian areas and a low, flat flood-plain, the boundary of the bankfull channel corresponds with the top of the low bank between the active channel and the floodplain. The floodplain must be frequently flooded, i.e., during floods with a recurrence interval of approximately 1.5 - 2 years.

In many streams in forested parts of the state, frequently inundated floodplains are often absent, particularly when the channel is confined between steep hillslopes or is incised into an elevated terrace deposit that is not frequently flooded. This indicator is also not appropriate for streams that have been artificially diked or channelized.

¹ Field crews may also want to view this instructional video: <http://www.youtube.com/watch?v=UuS7H2NxJIM>. Often, the bankfull position is easier to interpret from one bank than from the other; in these cases, it is easiest to infer the opposite bank's bankfull position by projecting a level line across the channel. Additionally, height can be verified by measuring the height from both edges of the wetted channel to the bankfull height (i.e., these heights should be equal).

Vegetative indicators- The bankfull channel boundary is often marked by a distinct demarcation line in the vegetation between lower areas that are either bare or have aquatic vegetation, and higher areas vegetated with perennial vegetation such as shrubs, grasses or trees. In boulder or bedrock confined channels, it may be marked by the line between bare rock and moss. Unfortunately, the vegetation line changes over time, retreating due to disturbance during large peak flow events, and advancing during periods between floods.

Identifying the bankfull channel boundary using vegetative indicators requires caution. The vegetation line can be deceptively low when moisture-tolerant species are present. Reed canary-grass, willow and sedges are examples of plants that may actively invade and colonize areas within the bankfull channel. When using vegetative indicators, use only perennial vegetation greater than 1 meter in height of species that do not invade the active channel.

Other situations- Sometimes it may be possible to identify the height of the bankfull channel on one side of the channel but not the other. For example, this occurs when there is a low floodplain with vegetative indicators on one side of the stream and a steep, eroding bank on the other. In these cases, extend a level line horizontally across the channel from the side with good indicators to determine bankfull height on the side lacking indicators.

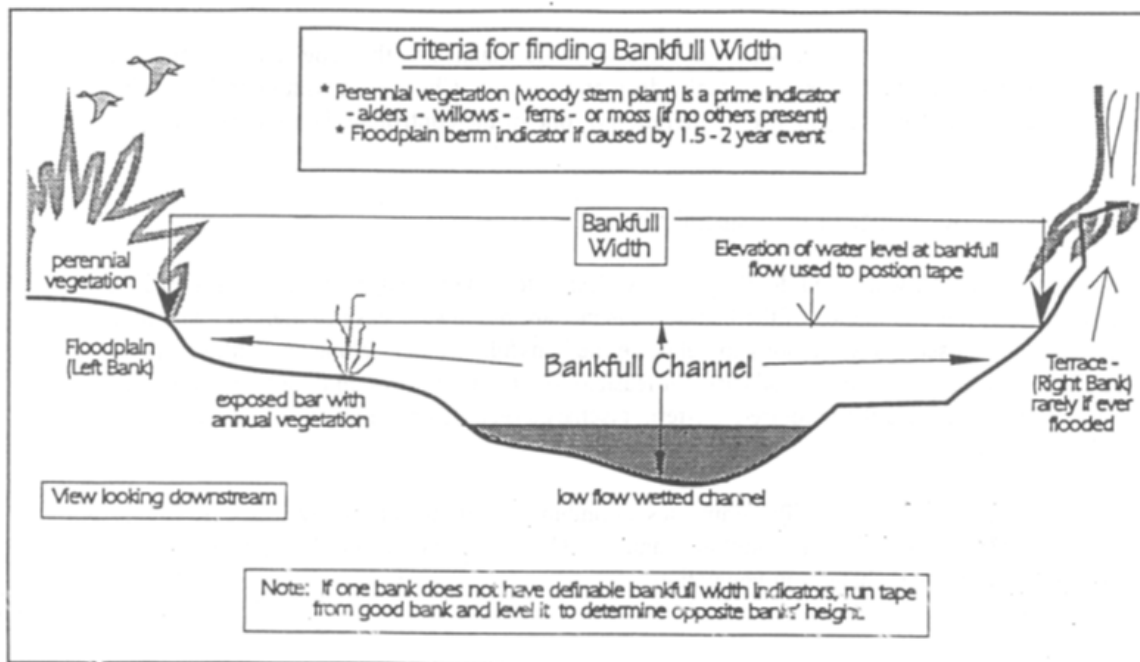


Figure 4. Determining the bankfull width of channels.

One of the most difficult situations is encountered in stream reaches where large gravel bars have been deposited by floods. It can be very difficult to determine if the tops of newly deposited bars protrude above the level of the bankfull channel. Vegetative indicators are unreliable because riparian vegetation is often disturbed during large storm events and revegetation of bars with perennial vegetation may take many years. In these cases, examine the margins of the channel for perennial riparian vegetation and extend a horizontal line across the channel to determine if the bar tops are above or below the bankfull level. If you are still in doubt after doing this, include the area within the bankfull channel.

In other cases, physical obstructions such as debris jams, undercut banks, or complete lack of indicators may make determination or measurement of bankfull dimensions impossible at the reference point. In these cases, take the measurement at the nearest place where it is feasible.

Taking Bankfull Width and Depth Measurements

To measure bankfull width, securely attach the end of the fiberglass tape measure at one boundary of the bankfull channel. Extend the tape across the channel to the other boundary of the bankfull channel. This distance is the bankfull width. If a side-channel is present, add the bankfull width of the side-channel to that of the main channel.

While the tape is stretched between these two points, determine the average bankfull depth. Bankfull depth measurements are taken at regular intervals across the stream channel. The number of measurements, and distance between them, depends on the width of the channel (Figure 4). Take

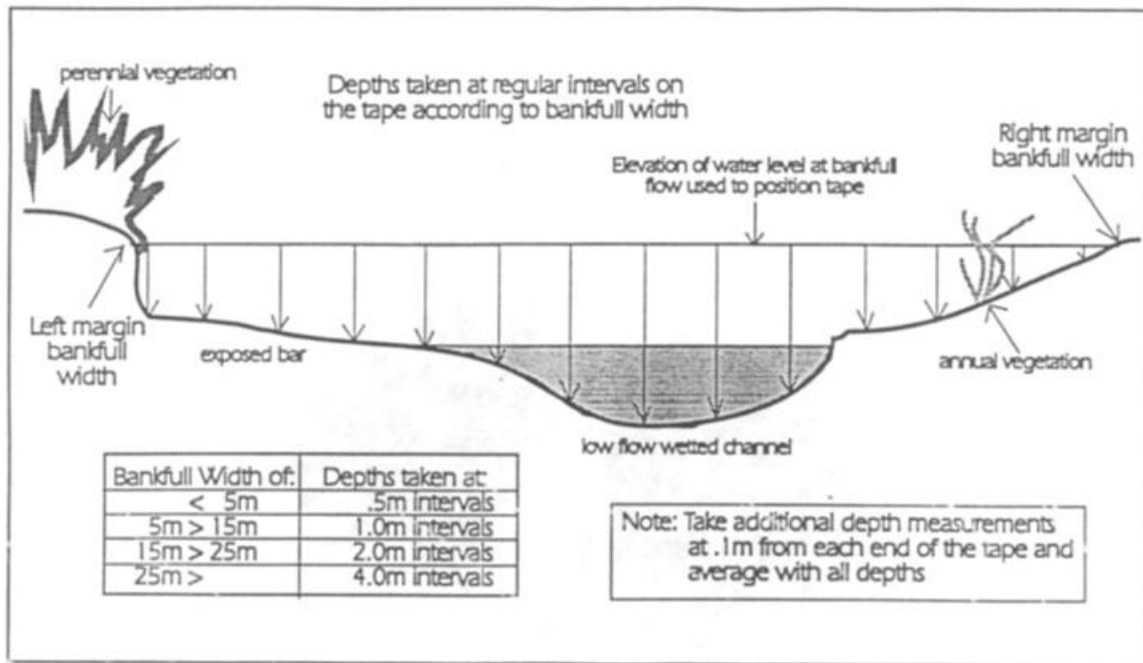


Figure 5. Measuring bankfull depth.

measurements at 0.5 meter intervals in channels less than 5 meters in width, at 1 meter intervals in channels between 5 and 15 meters in width, at 2 meter intervals for channels between 15 and 25 meters, and every 4 meters in channels greater than 25 meters in width. In addition, take an initial measurement 0.1 meter out from the starting point, and 0.1 meter before the endpoint.

Bankfull depth is the distance from the channel bed to the estimated water surface elevation at bankfull flow, represented by a tape stretched horizontally between the bankfull boundaries. The depth of water at the time of the survey, or its absence, does not affect this measurement. The sum of all depth measurements are then divided by the number of measurements taken to compute average bankfull depth.

Canopy Closure Measurement

Canopy closure measurements are taken at every reference point. To measure canopy closure, stand in the middle of the wetted portion of the channel and take four readings with the densiometer. Begin with a reading facing directly downstream. Turn clockwise 90 degrees and take a reading facing the right bank. Turn another 90 degrees clockwise and take a reading facing upstream, and finally turn clockwise another 90 degrees and take a reading facing the left bank.

To take a densiometer reading, hold the densiometer 12-18" in front of you at elbow height. Use the circular bubble-level to ensure that it is level. Look down on the surface of the densiometer, which has 24 squares etched into its reflective face. The reflection of your head should be just outside the grid. Imagine that each square is sub-divided into four additional squares, so that there are 96 smaller quarter-squares. Envision a dot in the center of each quarter-square. Count the total number.

1.8 Recommendations for Preparing for Fieldwork

- Have in mind at least three sites to visit per day (target two, but plan for at least one additional site as a backup if one of the first two sites is not useable.) Sites should be safe to sample and legally accessible. The time required to access the sampling sites should be a consideration in planning which sites to visit, in order to ensure that sample holding times can be met
- Use the equipment checklist provided below to make sure all necessary supplies are brought along.
- Check with contract laboratory on sampling containers, pre-combusted glass-fiber filters, addition of glutaraldehyde to soft-bodied algae quantitative samples, and storage and shipping of samples.
- Prepare, and double check, site dossiers to make sure they are complete with maps/directions to sites and scaled aerial photo(s). Note that 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. Bring along smartphone and/or county maps, atlases, and Thomas Guides to further aid location of sites.
- Other considerations when planning fieldwork include whether site access permits, passes, and/or gate keys are needed. Furthermore, some landowners require notice prior to each site visit, or that an on-site escort accompany the field crews during sampling, such that pre-visit coordination with the landowner is necessary.
- At the 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 the letter of permission that is in your site dossier, if applicable).

LIST OF SUPPLIES

The first column indicates what task(s) in the SOP the item is needed for: “G” = general; “W” = water quality measurements; “P” = PHab data collection; “B” = BMI sampling; “D” = diatom sampling; “S” = soft-bodied algae sampling; “C” = chlorophyll *a* sampling; “A” = AFDM sampling

Table 2. List of Supplies

Needed for:		Item	Quantity / Site	Specifications, Comments
G		Sampling SOP (this document)	1/person	
G		Equipment decontamination supplies		
G		Hip or chest waders, or wading boots/shoes (not felt-soled)	at least 1 pair/person	
G		Full set of datasheets printed on waterproof paper (e.g., Rite-in-the-Rain™)	1 full set (and spare set recommended)	
G		Fine-tipped and thick-tipped waterproof/alcohol-proof pens and markers	2 to 3, each	
G		Pencils	2 to 3, each	
G		Clipboard	2 to 3	
G		Site dossier containing site maps, aerials, etc.	1	Add a 150-m scale line to aerials adjacent to stream
G		Thomas Guide, regional maps, topographic maps	as needed	
G		First aid kit	1	
W		Centigrade thermometer	1	
W		pH meter	1	
W		DO meter and spare membrane	1	
W		Conductivity meter	1	
W		Turbidimeter and vial(s) (optional)	1	
W		Field alkalinity meter or test kit (e.g., Hach)	1	
W		Water chemistry containers	as needed	
W		Calibration standards	1 set	
W		Spare batteries, user's manuals, and spare parts for meters	as needed	
P		Digital camera & spare batteries	1	
P		GPS receiver & spare batteries	1	
P		Measuring tape; 150 m (and 250 m, optional)	1	
P		Lengths of rope (7.5 m and 12.5 m)	1 each	For measuring distance between main and inter-transsects in delineating the monitoring reach
P		Digital watch/stopwatch & spare batteries	1	For timing duration of float for NBO stream velocity measure; also can be used to generate random number for selecting locations to place net for TRC sampling

Needed for:	Item	Quantity / Site	Specifications, Comments
P	10-sided die or random number table (if no digital watch available)	1	For selecting locations to place net for TRC sampling
P	Stadia rod	1	
P	Marked ski pole (or waterproof meter stick)	1	Mark pole with cm graduations to measure water depth during pebble count
P	Clinometer	1	
P	Autolevel and tripod	1	Required for slopes <1%
P	Hand level (optional)	1	
P	Current velocity meter & top-setting rod	1	Examples: Swiffer Instruments propeller-type flow meter; Marsh-McBirney inductive probe flow meter; check battery and calibration as needed
P	Flagging tape	1 strip	To determine direction of stream flow for proper angling of the current velocity meter probe
P	Convex spherical densimeter	1	Taped to expose only 17 intersections of the grid
P	Transect flags; or large, heavy washers each tied with a strip of flagging tape	21 total	Two colors; label with main transect (11 ct.) and inter-transect (10 ct.) names
P	Small/slender rod with 1, 5, and 20 mm marks	1	For measuring microalgal thickness
P	Rangefinder & spare batteries	1	
P	Fresh orange peel OR plastic film canister partially full of water OR ice cube	1	Use as neutrally buoyant object
P	Small metric ruler or gravelometer for substrate measurements	1	
P, S	Algae viewing bucket (optional)	1	
B	D-frame kick net (fitted with 500- \square m mesh bag)	1	
B	Standard #35 sieve (500- \square m mesh)	1	
B	Wide mouth 500-mL or 1000-mL plastic jars	several	
B	White sorting pan (enamel or plastic; optional)	1	
B	95% EtOH	1 gallon	
B	Fine-tipped forceps or soft forceps	1	
B	Waterproof paper and tape for attaching labels	as needed	

Needed for:	Item	Quantity / Site	Specifications, Comments
B	Large spill tray	1	Used when transferring the BMIs from the D-frame net to the sample jar in order to avoid any loss of sample material
B	Preprinted waterproof labels (e.g., on Rite-in-the-Rain™ paper)	as needed	It is recommended that the label be printed on a laser printer using alcohol-proof ink
B	Disposable gloves/elbow length insulated gloves		
D, S, C, A	White dish tub, rectangular, plastic, 11.5 qt, OR white plastic 5-gallon bucket with lid, 5L	1	Must be white, to avoid potential interference of pigmented shards from the tub or bucket in the chlorophyll <i>a</i> analysis
D, S, C, A	Scrubbing brush or scouring pad to clean dish tub or bucket, etc.	1	
D, S, C, A	Composite sample receiving bottle (wide-mouth HDPE jar with cap, 1 L)	1	Fisher 05-719-239
D, S, C, A	Graduated cylinder, 1L, 500 mL, 100 mL, and 25 mL, plastic	1 each	e.g., 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 delimeter, 12.6 cm ² area	1	
D, S, C, A	Masonry trowel (flat, <i>pointed</i> , with a surface area > 12.6 cm ²)	1	
D, S, C, A	Rubber delimeter, 12.6 cm ² area	1	
D, S, C, A	Toothbrush, firm-bristled	1	
D, S, C, A	Syringe scrubber, 60 mL syringe, 5.3 cm ² area	1	
D, S, C, A	White (non-pigmented) scrubbing-pad circles	11 per replicate	
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	Exacto™ or Swiss-army-style knife	1	
D, S, C, A	Sample labels (printed on waterproof paper)	4 per replicate	
D, S, C, A	Clear plastic tape, 5 cm wide	Length of ~20 cm 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, C, A	Tarp, plastic, clean	1	To cover the ground at the algae processing station

Needed for:	Item	Quantity / Site	Specifications, Comments
D, C, A	Wide-mouthed measuring cup with a broad pouring spout	1	For pouring homogenate sample into the diatom sample vial, and for preparation of biomass filters
D, S	Centrifuge tubes, 50 mL, plastic	2 per replicate	Cole Parmer 06344-27
D, S	Rack for 50 mL centrifuge tubes	1	
D	5% formalin solution	10 mL per replicate	
D	Formalin-resistant gloves	1 pair	
D	Safety goggles or face shield	1	
D	Small syringe or bulb pipette	1	
D	Verniculite packing material	as needed	
S	Turkey baster	1	
S (see note)	20% glutaraldehyde solution (<i>to be dispensed by trained individual using a laboratory fume hood, and wearing appropriate safety gear</i>)	5 mL per replicate	Note: glutaraldehyde 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
S, C	Umbrella	1	To shade processing station when shade is not available at site
C, A	Filter forceps	1	Fisher 0975350
C, A	Pointed forceps	1	Fisher 08-900
C, A	Filtering chamber/tower, 47 mm, plastic	1	Hach 2254400
C, A	Hand vacuum pump	1	Fisher 13-874-612B
C, A	Deionized water	500 mL	
C, A	Dry ice (if not returning to lab immediately following the day's fieldwork)	10 lbs	
C, A	Snapping Petri dish, 47 mm	2 per replicate	Fisher 08-757-105
C	Glass fiber filter, 47 mm, 0.7 μ m pore size	1 per replicate	Fisher 09804142H
C	Aluminum foil	~100 cm ² per replicate	
A	Glass fiber filter, 47 mm, 0.7 μ m pore size; pre-combusted	1 per replicate	Check with analytical laboratory ahead of time; they should be able to supply these

2. Algae Sampling

2.1 Notes for processing soft-bodied algal and diatom samples when macroalgal clumps are in the sample

Note 1: Whenever aliquoting the homogenate, make sure that it is well mixed, and that there are no “rafts” of algal fragments coalescing at the surface or behind the lip of the bottle such that they compromise sample representativeness when aliquoting the diatom and/or biomass samples. In order to prevent this, it is recommended that the well-mixed homogenate be poured quickly from the bottle into a wide-mouthed measuring cup with a broad pouring spout. From this vessel, the sample can then be poured out more deliberately for careful measurement and maintenance of homogeneity.

Note 2: Formalin can be added to the diatom sample tube using a small syringe or bulb pipette. Alternatively, if preferred, the tubes for diatom samples can be pre-loaded (in the laboratory) with 10 mL of the 5% formalin such that 40 mL of sample can be added to the fixative (thus avoiding having to dispense the fixative in the field).

2.2 Instructions for Constructing Algae Sampling Devices

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 inner tube with rubber cement.



ABS Delimiter:

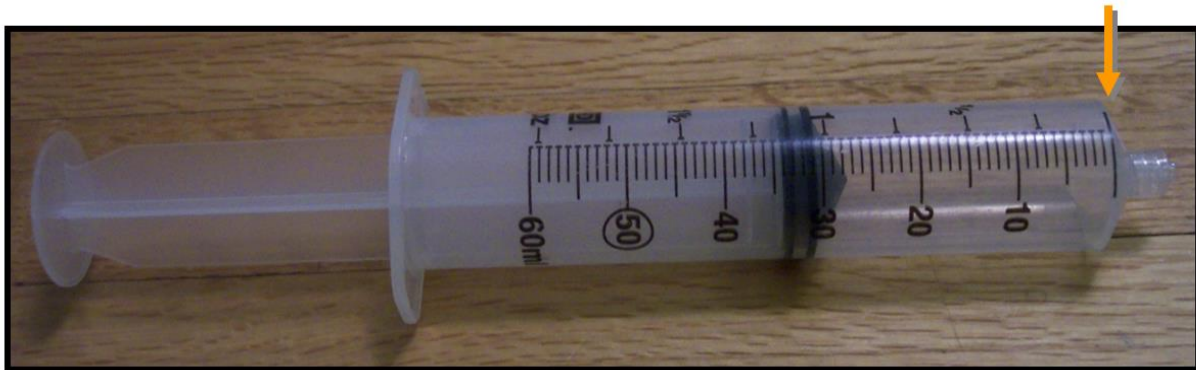
The ABS delimiter for “depositional”/soft substrates like sand, small gravel, and silt is made from a 1½” sewer cleanout, which can be found at a home-improvement or plumbing supply store. The hole in the bottom of the cleanout is 4 cm in diameter. The bottom edge of the cleanout is filed to make it 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.



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.

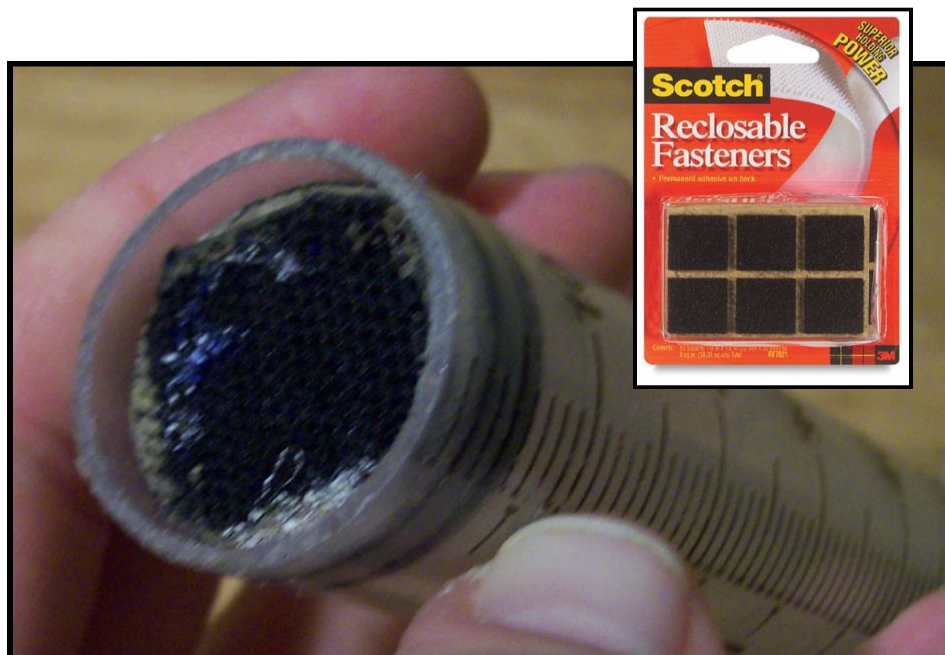
You will need a 60-mL plastic syringe for each sampler you want to make. Remove the plunger and saw the conical end off the plastic syringe, then sand the bottom so it is flat all the way around and fits tightly against a flat surface.



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.



Viewing bucket:

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.



For additional guidance, the following section is a highly detailed set of instructions for manufacture of the algal sampling devices. This write-up has been provided courtesy of David Williams of the San Francisco Bay Regional Water Quality Control Board

2.3 Tips and Tricks for Fabrication of SWAMP Algae Sampling Tools

February 2012

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This document supplements Appendix D of SWAMP's "Standard Operating Procedures for Collecting Stream Algae Samples and Associated Physical Habitat and Chemical Data for Ambient Bioassessments in California" (Fetscher *et al* 2009). Three key field sampling devices required for algae sampling are not available for purchase and must be assembled from readily available materials. The writers of the original SOP did a good job of describing how to construct the field sampling devices for experienced technicians; however, not everyone is handy with tools or savvy about glues. This document is intended to provide detailed instructions for fabrication of high-quality field equipment. It is critical for field personnel performing the SWAMP Algae Collection SOP to be using uniform, correctly fabricated sampling devices to ensure collection of robust and comparable data. Attention to detail while preparing these devices will mitigate sampling error.

The majority of the materials required for construction of the algae sampling tools may be purchased in a hardware or drug store. The rubber delimiter requires some supplies that can be purchased either at bicycle shop or sporting goods store, and the syringe scrubber requires a purchase from a laboratory supply company or sourcing from colleagues. We have suggested particular product brand names (and in some cases part numbers) because these materials proved hassle free during fabrication and withstood field operations without noticeable wear-and-tear.

I. Rubber Delimiter:

For use on large "erosional" / hard surfaces, such as cobbles and wood that can be removed from the stream, the rubber delimiter is made from a cut bicycle inner tube with a hole cut in the center. A rubber washer is affixed to the inner tube to enforce the hole. Fabrication of this device involves steps similar to patching a bicycle inner tube. Recommend two per field crew.

Required supplies (makes 2):

- 1 x 26inch diameter (mountain bike) inner tube
- 2 x 66mm outer diameter rubber washer ¹
- Rubber cement or Liquid Nails™ HOME PROJECTS REPAIR ADHESIVE (LN-201) ²
- 4cm diameter circle template (Burt's Beeswax Lip Balm Tin (.30 oz) works well)
- Razor blade or X-acto™ knife (must be very sharp)

- Coarse - Medium (40-80) grit sand paper
- Acetone or rubbing alcohol (90%)
- Coin or bicycle tire iron
- Heavy object (not made out of rubber)

¹ DO NOT use a metal washer – the washer must be rubber so that it can conform to irregular shaped surfaces. The washer will be found at a home improvement store (e.g., Home Depot) in the plumbing section. It is made for sealing the flange at the bottom of a tub or shower drain and is exactly 66mm outer diameter (OD), 41mm inner diameter (ID) and 3mm thick.

² Use a vulcanizing compound from a bike repair kit or Liquid Nails™ HOME PROJECTS REPAIR ADHESIVE (LN-201). The product can be found in a three-pack with two other types of Liquid Nails™ adhesive or purchased separately. ***Do NOT use Liquid Nails™ construction adhesive.*** Make sure the adhesive you use is designed to glue rubber to rubber; super glue won't work.

1. Cut the inner tube:
 - a. Remove the valve stem (where the air goes in) by cutting across the tube on both sides of the valve.
 - i. Discard the valve stem.
 - b. Cut the remaining tube in half to make two tubes each about 0.75m long.
 Repeat the remaining steps below for each half
 - c. Slice along one seam of the tube (the long way) to create a flat strap.
 - d. Cut the hole in the center of the strap.
 - i. Position the 4cm template (lip balm container) in the very center of the strap.
 - ii. Follow around the edge of the container with the X-acto™ knife to slice the hole. (Note: as long as the knife is very sharp this produces a much cleaner cut than tracing the hole with a pen first.)
2. Affix the rubber washer to the hole:
 - a. Sand both surfaces to be glued together until visibly scuffed.
 - i. Sand the inner tube surrounding the hole to an area slightly larger than the washer will cover.
 - ii. Sand one side of the washer and the inner/outer edges.
 - b. Clean all sanded surfaces with the acetone or alcohol. Allow to dry a couple minutes.
 - c. Apply rubber cement to both clean flat sanded surfaces.
 - i. Allow to dry just until the rubber cement feels tacky. DO NOT allow to dry completely.
 - d. Affix the washer to the inner tube around the hole. (Note: The inner diameter of the washer will be ~ 1 mm too large for the edge of the hole.)
 - i. Press the washer firmly into place and then gently rub the edge of coin or a bicycle tire iron from the inner to the outer edge of the washer, all the way around.
 - e. Put the heavy object on top of the washer and leave to dry overnight
 - f. Next morning, run a bead of rubber cement around both the inner and outer edge of the washer and allow to dry completely for at least 24 hours.

The area sampled must be correct to calculate biomass accurately and consistently, so the size of this hole is critical to your measurements.

II. ABS Delimiter:

For use in small “depositional” / soft substrates like sand, small gravel, and silt, the delimiter is made from an **ABS** sewer cleanout fitting filed or ground sharp to ease insertion into the substrate. A bright line marked around the outside surface of the delimiter, 1cm up from the bottom edge, helps facilitate consistent sampling. Creation of this device requires access to power tools. You can file the edge sharp (bevel), but this will take a long time. Always be sure to wear proper personal protective equipment.

Recommend two per field crew. This tool does not float!

Required supplies (makes 2):

- (2) 1½ inch ABS sewer cleanout fitting⁴
- (2) 1½ inch ABS cleanout fitting cap⁵
- Paint pen in bright color⁶
- Fine point permanent marker
- Acetone or rubbing alcohol (90%)
- Masking or plain Scotch™ tape
- Ruler with cm increments
- Electric sander / large coarse file
- C-clamp / Bench vise
- Eye protection

⁴ The sewer cleanout is a small black 1½ inch ABS end piece available at most hardware stores.

It has a threaded fitting (threaded on inside) on one side and tubing (to slide in or over a length of tubing) on the other side. **The inner diameter is 4cm.**

⁵ The cleanout cap screws into the threaded end of the sewer cleanout and has a square top. It may be sold separately from the cleanout so we have listed it as a separate line item. It makes a handle for the delimiter.

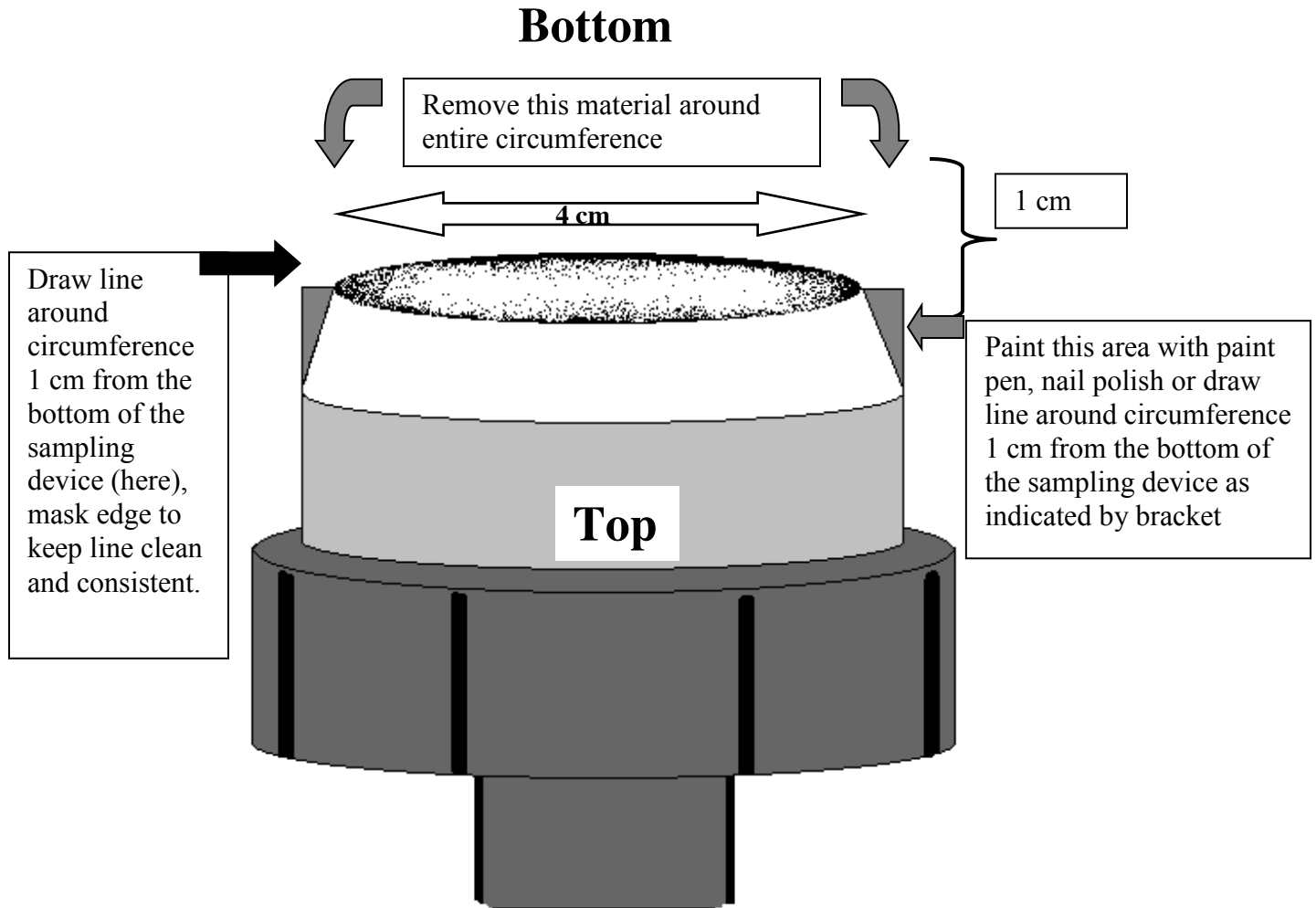
⁶ Also sold at most hardware stores in silver or gold.

1. See Figure #1 (next page)
2. Sharpen (bevel) the edge.
 - a. Using the fine point permanent marker, mark a line around the outer edge of the cleanout’s smooth end’s outer circumference (tubing connecting end, not threaded end), 1cm up from the bottom edge.
 - b. Screw the lid into the cleanout. Clamp the square end of the lid tightly into the C-clamp or bench vise.
 - c. Use the electric sander to sharpen (bevel) the bottom outer diameter of the cleanout, below the 1cm line.
 - i. Carefully remove the outer material, leaving the inner material of the inner diameter intact. Work at a shallow angle so that the bevel begins at the line marked 1cm up from the bottom edge and ends at the bottom edge. This will eventually leave the bottom inner edge sharp (please see figure 1).
 - ii. Clean the delimiter with acetone or rubbing alcohol (90%).
 - iii. Allow to dry for a few minutes.

3. Redraw the line.
 - a. Mask around the fitting 1 cm up from the sharpened bottom edge with tape. This should be where your bevel ends. Make sure to account for the angle now present. Do not measure flat against the bevel or “hypotenuse.” The line needs to be 1 cm from the edge in the plane parallel to the fitting.
 - b. Use the paint pen to draw a bright line around delimiter’s outer circumference, 1 cm up from the bottom edge.
 - c. Allow to dry completely.
 - d. Repeat if necessary.

The masked edge indicates the depth to which to insert the delimiter into the substrate when sampling. Appendix D of the SWAMP Algae Collection SOP calls for a line to be drawn here; however, free painting a consistent clean line can be difficult. Masking this line with tape, before marking / painting helps to make the line clean and consistent. Use the masked edge as the 1cm depth indicator.

The area sampled must be correct to calculate biomass accurately and consistently.



III. Syringe Scrubber:

For use on hard substrates that can't be picked up out of the stream, like submerged bedrock, concrete channel bottoms, or logs. The syringe scrubber is made from a sawed-off syringe barrel and its plunger fitted with the hook side of a hook and loop fastener (Velcro®). Small circular scrubber pads cut from large squares of disposable white scrubbing pads are affixed to the end of the plunger and used to scrub the algae from the substrate. This is the only device with materials that are not readily available for in-store purchase – allow time for shipping. Recommend two per field crew.

Required supplies:

- (2) 60-mL plastic syringe (Cole Parmer EW-07940-30) ⁷
- (Velcro®) Package of Scotch™ 3M Reclosable Fasteners, multipurpose $\frac{3}{4}$ inch squares ⁸
- Package of white scrubber pads ⁹
- Liquid Nails™ HOME PROJECTS REPAIR ADHESIVE (LN-201) ¹⁰
- Acetone or rubbing alcohol (90%)
- Super fine point permanent marker

- Medium (80-100) grit sand paper
- Sharp knife
- Cutting board
- Ruler with mm increments
- Calculator

⁷ The syringes are available from a laboratory supply company, such as Cole-Parmer. The outlet is the twist-in style. Some programs may have these syringes that have been used for water sampling and it is advisable to recycle and reuse. You can also ask around the bioassessment community to see if anyone has extra syringes lying around.

!!!!!!BE SURE TO VERIFY THAT THE INNER DIAMETER OF THE SYRINGE BARREL IS 26mm.

⁸ Other brands/types of hook and loop fasteners (Velcro®) won't stick to the scrubber pads as well as the 3M offerings.

⁹ The scrubber pads must be white (non-pigmented) because colored pads will interfere with the chlorophyll α analysis. Scotch Brite™ pads work well: they are 15cm wide by 20cm tall by ~1cm thick.

¹⁰ DO NOT use Gorilla Glue™ as it tends to foam up and throw off the alignment of the hook fastener pad.

1. Cut the outlet end of the syringe.
 - a. Fully depress the plunger. Use the bottom edge of the plunger as a template/ guide to draw or score a circle around the outside of the end of the syringe. (Note: You will be cutting below the 10ml line.)
 - b. Remove the plunger.
 - c. Roll the syringe on the cutting board with the blade of the knife, scoring around the template/ guide line.
 - i. DO NOT just saw through the end of the syringe – gently score the template line first, then follow that score around several times by rolling the syringe on the cutting board with the edge of your knife until the end comes off. Similar to the way a tubing cutter works for those of you with any plumbing experience.
 - ii. Sand the cut end of the syringe with sand paper laid on a flat hard surface. Sand until the cut you have made is even and flat all the way around, so that when the end of the syringe is set on a flat surface it sets squarely and there are no gaps.
 - d. Measure and note the inner diameter of the syringe

The SWAMP data sheets have an area sampled for the syringe scrubber of 5.3cm² in the algae samples collection devices section on page 24. The inner diameter of the barrel of your syringe **MUST HAVE** an inner diameter of 26mm for the area listed in the SWAMP data sheets, 5.3cm², to be correct. Notice that the diameter unit here is mm and the area unit is cm. So, if you want to do the calculation you can use 2.6cm for the diameter, but using a measuring device capable of measuring in millimeters is preferably used to verify the inner diameter of the syringe barrel. ***The area sampled must be correct to calculate biomass accurately and consistently.***

Remember: Circle Area = $\pi \cdot r^2 = \frac{1}{4} \cdot \pi \cdot d^2$ (d= diameter, r= radius (1/2 diameter), $\pi= 3.14159$) There are also many on-line calculators (e.g., <http://www.1728.com/diam.htm>).

2. Firmly affix the rubber tip to the plastic plunger. If your syringe has a rubber end on the plunger, you must firmly affix it to the plunger. When you are sampling with this device

you must rotate the device to “scrub” the sample surface. If the plunger spins in the rubber tip, your scrubbing pad patch will not rotate and you will not get a legitimate sample.

- a. Remove the rubber tip from the plunger.
 - b. Scuff the surfaces to be glued together (the end of the plunger and the inside of the rubber tip).
 - c. Clean the plunger and inside of the rubber tip with acetone or rubbing alcohol (90%). Allow to dry for a couple minutes.
 - d. Use Liquid Nails™ HOME PROJECTS REPAIR ADHESIVE (LN-201) to glue the rubber tip to the plunger.
 - e. Allow an hour or more for the adhesive to dry before step 3.
 3. Affix the Scotch™ pads to the plunger.
 - a. Use the hook side of the material only, not the fuzzy side. Cut the tips off all four corners of the square pieces, sized to just cover the nose of the plunger.
 - b. Use sandpaper to roughen and flatten the nose of the plunger. If the plunger is made of rubber, you can cut off the pointy tip with a razor blade or sharp utility knife before you sand. If you do need to trim off the tip of the rubber plunger be sure it is even and flat. Make sure to sand over the whole area to be glued, including the rubber.
 - c. Clean the plunger with acetone or rubbing alcohol (90%). Allow to dry.
 - d. Evenly spread Liquid Nails™ HOME PROJECTS REPAIR ADHESIVE (LN-201) over the nose of the plunger. Firmly press trimmed Scotch™ pad into place. Allow to dry.
 4. Cut white scrub pad circles for use in the field.
 - a. Use the cut end of the syringe as a template to draw circles in the scrubber pad. When you cut out the circle you MUST NOT leave any ink on the finished circle, this can interfere with the chlorophyll α analysis.
 - b. Make at least two pads for each planned site. **Do not reuse pads from site to site; this would result in sample contamination.**
- The area sampled must be correct to calculate biomass accurately and consistently.**

2.4 Standard Operating Procedures for Glutaraldehyde for the Preservation of Soft Algae

STANDARD OPERATING PROCEDURES (SOP) FOR GLUTARALDEHYDE FOR THE PRESERVATION OF SOFT ALGAE

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 Wildlife 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 does 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.

2.5 Preparation of formalin solution

Preparing a 1-L solution of 5-percent (buffered²) Formalin (modified from Moulton et al. 2002):

- Add 50 mL of formaldehyde (37-40%) to 950 mL of water in a chemically resistant, non-breakable bottle.
- Tightly seal the bottle and mix by carefully inverting the bottle several times.
- Label the outside of the bottle with "5-percent formalin," the date of preparation, and related hazardous chemical stickers. *If* the solution has been buffered with phosphate (it is not required to be buffered), indicate this on the label as well.

² Do not use borax to buffer formalin, as indicated in earlier versions. Use either unbuffered formalin or phosphate-buffered formalin. Be sure to indicate clearly on the diatom sample label and chain-of-custody form whether or not the formalin used in each sample has been phosphate-buffered.

2.6 SOP for the use of formalin for fixing diatoms (adapted from Peck, et al. 2006)

Note: Formalin must only be handled by trained individuals who understand the safe handling and use of this chemical

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 immediately 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 5% 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 5% formalin in a laboratory fume hood prior to going into the field.

The preparation of the 5% formalin stock solution should always be done by trained personnel under a laboratory fume hood while wearing protective gloves, clothing, and goggles.

2.7 Examples of macroalgae



2.8 Distinguishing macro from microalgae

Macroalgae: soft-bodied algae that form macroscopically discernible filaments, mats, or globose structures that remain cohesive when picked up



Microalgae: diatoms and microscopic soft-bodied algae that form a seemingly “amorphous”, slimy coating on substrate, like icing on a cake.

3. Information about scientific collecting permits

2013 Requirements for Conducting Routine Monitoring Using SWAMP Bioassessment Procedures

1. Go to the following link on the CDFW website to download the application and instructions:
http://www.dfg.ca.gov/wildlife/nongame/research_permit/scp/scp_aplic_procs.html
2. Fill out Section 1 if you are working by yourself and Section 2 if you are an entity. The SCP calls it the Principle Scientific Investigator or PI. The PI is responsible for the crew and must be a crew leader and not a manager or someone who will never be in the field.
3. The important part of Section 3 is indicating that you are only sampling for freshwater invertebrates. If you check any other box then there will be others besides Jim Harrington (of CDFW), who will need to approve your application.
4. Section 5 is where you describe what you will be doing and where you will be doing it. Write in this section that you will be using SWAMP protocols and submitting your data to CEDEN. Then list the sites including GPS coordinates along with the approximate dates you will be sampling.
5. When your application is submitted to Jim Harrington, who will have the SCP staff put the following requirements on your permit.
 - The individual listed in Section 1 or the Principle Scientific Investigator (PI) and field crew listed in Section 2 must have SWAMP bioassessment and algae training and/or related work experience prior to the 2013 field season. They must also meet all SWAMP training requirements throughout the life of the permit.
 - The individual listed in Section 1 or the entity listed in Section 2 must have a SWAMP Bioassessment QAPP or similar documentation describing the procedures used by them to insure the data collected was of the quality acceptable by the SWAMP program.
 - The QAPP must describe the level of taxonomic effort and subsampling size as described in SAFIT document <http://www.safit.org/ste.php>) and decontamination techniques for sampling equipment and procedures for reporting Aquatic Invasive Species (AIS).
 - Within one year of sample collection or upon the request of CDFW, all data produced under this permit must be in a format that can be uploaded to CEDEN.



State of California – Natural Resources Agency
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EDMUND G. BROWN JR., Governor
CHARLTON H. BONHAM, Director



CDFW's Aquatic Bioassessment Laboratory (ABL) is working with the Fisheries Branch to ensure that the data you collect can be used by CDFW and others to help us protect and preserve aquatic resources in California. We appreciate that you agree to use the standardized bioassessment procedure developed by the ABL for the State Water Resources Control Board's Surface Water Ambient Monitoring Program (SWAMP).

The following list of bioassessment specific conditions is a requirement of your Scientific Collectors Permit (SCP):

The individual listed in Section 1 or the Principle Scientific Investigator (PI) and field crew listed in Section 2 must have SWAMP bioassessment and algae training and/or related work experience prior to the first sampling of the year. They must also meet all SWAMP training requirements throughout the life of the permit.

The individual listed in Section 1 or the entity listed in Section 2 must have a SWAMP Bioassessment QAPP or equivalent documentation describing the procedures used by them to ensure the data collected was of the quality acceptable by the SWAMP program.

The QAPP must describe the level of taxonomic effort and subsampling size as described in the SAFIT Standard Taxonomic Effort document* and decontamination techniques for sampling equipment and procedures for reporting Aquatic Invasive Species (AIS).

GIS coordinates for the sites sampled during each field season must be sent to James.Harrington@wildlife.ca.gov no later than 14 days before they are sampled.

At the end of the field season, the final list of sites sampled must be confirmed in an e-mail to James.Harrington@wildlife.ca.gov . Within one year of sample collection or upon the request of CDFW, all data produced for the current year under this permit must be in a format that can be uploaded to the California Environmental Database Exchange Network.

* The Southwest Association of Freshwater Invertebrate Taxonomy documents are available at <http://www.safit.org/ste.html> .

SWAMP standard procedure documents and other related bioassessment technical reports related to conducting bioassessment are located at http://www.waterboards.ca.gov/water_issues/programs/swamp/tools.shtml#methods

Any questions on bioassessment procedures or with these specific conditions can be addressed to James Harrington by e-mail James.Harrington@wildlife.ca.gov or by calling (916) 358-2862.