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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LIST OF ACRONYMS

Acronym	Definition
AFDM	Ash-Free Dry Mass
ASCI	Algal Stream Condition Index
BMI	Benthic Macroinvertebrate
CDFW	(California) Department of Fish and Wildlife
CPOM	Coarse Particulate Organic Matter
CSBP	California Stream Bioassessment Procedure (historic sampling protocol)
CSCI	California Stream Condition Index (for benthic macroinvertebrates)
DI	Deionized water
DO	Dissolved Oxygen
EMAP	Environmental Monitoring and Assessment Program (of the U.S. EPA)
EPA	Environmental Protection Agency (of the United States)
GPS	Global Positioning System
LRBS	Log Relative Bed Stability (excess sediment index)
MCM	Margin-Center-Margin (sampling configuration)
NAD	North American Datum
NBO	Neutrally Buoyant Object
NRSA	National Rivers and Streams Assessment (of the U.S. EPA)
PCN	Particulate ratios of carbon and nitrogen
PHab	Physical Habitat
QA	Quality Assurance
QAPrP	Quality Assurance Program Plan (of SWAMP)
RBP	Rapid Bioassessment Procedures
RWB	Reachwide Benthos (sampling configuration)
SCP	Scientific Collecting Permit
SCCWRP	Southern California Coastal Water Research Project
SOP	Standard Operating Procedures
SWAMP	Surface Water Ambient Monitoring Program (State Water Resources Control Board)
TRC	Targeted Riffle Composite (sampling configuration)
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 (SWAMP). These procedures 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 and are recognized by the US Environmental Protection Agency (EPA) as California's standard bioassessment procedures. These methods are primarily intended for users interested in calculating California's bioassessment indices (e.g., the California Stream Condition Index [CSCI] or the Algal Stream Condition Indices [ASCIs]; instructions for calculating these indices are on the SWAMP website). The procedures also produce standardized measurements of instream and riparian habitat and ambient water chemistry that support interpretation of 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 algal biomass based on benthic chlorophyll a, particulate ratios of benthic carbon and nitrogen (PCN), and benthic ash-free dry mass (AFDM)
- Stream physical habitat (PHab) data (geomorphology and riparian condition)
- Water chemistry samples

1.1. Previous SOPs

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

- **Ode (2007)**, which focused on stream BMI sampling and associated PHab data collection and replaced the California Stream Bioassessment Procedure (CSBP, Harrington 2002)
- **Fetscher et al. (2009)**, which focused on stream benthic algae and biomass sampling, and associated PHab data collection
- **Ode et al., (2016)**, which updated and combined the previous SOPs into an integrated protocol

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.

Table 1. Summary of changes from the previous SOP (Ode 2016), indicating changes, additions of new material and new locations for old information.

Section	Category	Current Protocol	Difference from prior version
1.3	Scope and applicability	The index period for the South Coast has been revised to begin in March, rather than May (map updated).	The South Coast index period started in May.
1.3	Scope and applicability	Additional guidance provided on flow conditions required for proper application of the SOP.	New material added
2	Notable field conditions	Notable field conditions - new guidance for scour and wildfire. Added "Other" section so field crews can record additional notable information to field sheets. New "Step 3" provides guidance on dry channel conditions. Under "Step 5", additional guidance on the intended use of noted field conditions is provided.	New material added
4.4, 4.5	Algae sample collection	Soft-bodied algae sampling is now optional, and collection methods are moved to Appendix A6.	Sampling methods were described in the main text
4.5, 5.2	Algae sample collection	Ash-free dry mass sampling is now optional. Guidance on measuring particulate ratios of benthic carbon and nitrogen (PCN) added.	AFDM was required whenever algae were sampled. New material added.
5.2	Processing quantitative benthic algal taxonomy and biomass samples	Quantitative soft bodied algae samples should be preserved with Lugol's solution.	Quantitative algae samples were preserved with glutaraldehyde or formalin.

Section	Category	Current Protocol	Difference from prior version
5.2	Processing quantitative benthic algal taxonomy and biomass samples	Methods for processing benthic samples for molecular (e.g., DNA) methods added.	New material added
6	Transect-based measurements	Bank stability transect measurements have been dropped.	Bank stability was recorded at every transect (Section 6.5, Module G)
6.4	Algal and macrophyte cover (OPTIONAL)	Macroalgae, microalgae, and macrophyte presence measured at each location in the pebble count is optional.	Macroalgae, microalgae, and macrophyte presence (measured at each location in the pebble count) was required.

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 multi-metric index based on community composition). Contact your regional SWAMP coordinator for guidance on modifying the protocol for programs with limited resources (e.g., community-based monitoring groups).

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 also Appendix A1 for suggestions for maximizing 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 reach, or up to 6 hours in a complex stream reach. These estimates include only the time spent at the site, not time getting to the site (which varies widely). Table 2 provides a rough breakdown of time requirements per module.

Table 2. Sample and data collection modules for BMI and algal bioassessment with estimates for the average time an experienced crew will take for each task. 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	Use 150m reach length if wetted width ≤ 10 m or 250 m if wetted width > 10 m
	Temperature, pH, specific conductance, salinity, DO, alkalinity, turbidity	15	Alkalinity, conductance, pH, turbidity 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
	Notable field conditions	5	
	Water chemistry for laboratory analysis	15	Total phosphorus and nitrogen required by SWAMP when algae are sampled
	BMI sampling for Taxonomic IDs	45	
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	Algal sampling for Taxonomic IDs and biomass assessment	45	
	Depth and pebble Count + CPOM	35	Make 5 point substrate size, depth, and CPOM records at all 21 transects and inter-transects
	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)		Optional measure: Attached/ unattached macroalgae presence/ absence
	Bankfull dimensions	10-20	
	Wetted width (5 min)	5	
	Human influence	5	
	Riparian vegetation	5	

Survey Task	Module	Time	Notes
Continued…BIOTIC ASSEMBLAGE/ ALGAL BIOMASS AND PHAB SAMPLING AT CROSS-SECTIONAL TRANSECTS	Instream habitat complexity	5	
	Stream shading	10	Take 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	Record proportion of habitat classes in each inter-transect zone
	Slope (%) (25 min for autolevel method; 15 min for clinometer method)	15-25	Average slope calculated from 10 transect-to- transect slope measurements. Use autolevel for slopes ≤ 1% (clinometer acceptable for steeper gradients); time requirements increase considerably in complex streams
	Sinuosity	10	Record compass readings between transect-to-transect centers
	Excess sediment transect measures	40-90	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 NBO method)	10-15	Velocity-Area Method (VAM; preferred) or Neutrally Buoyant Object Method (NBO), 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	Channel alteration, sediment deposition, epifaunal substrate

Survey Task	Module	Time	Notes
	Photo documentation	5	At a minimum, take Upstream (Transects A, F), Downstream (Transects F, K) pictures, but ideally add one or more of the overall setting

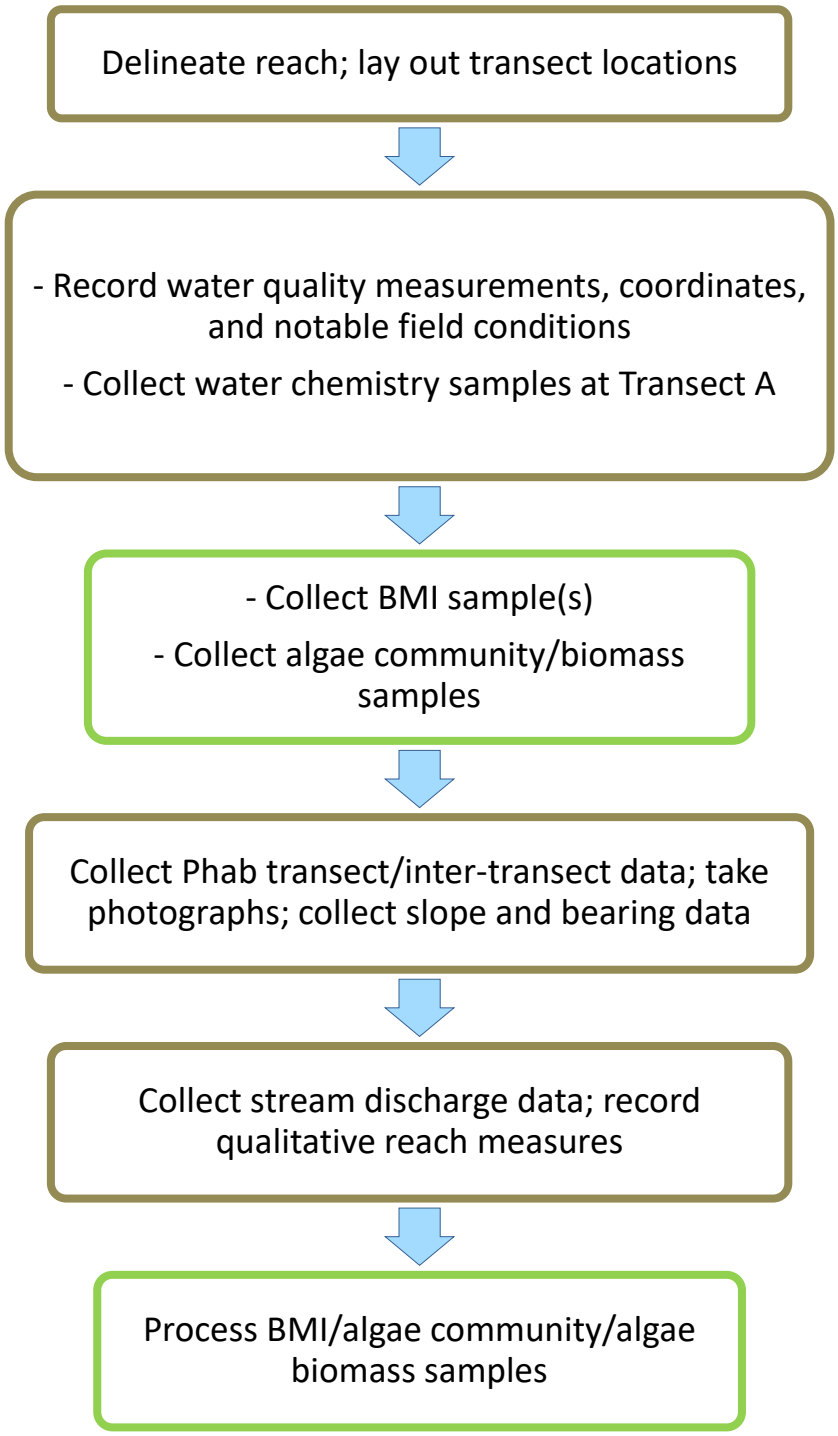

Figure 1. Recommended workflow for conducting SWAMP bioassessments.

1.3. Scope and Applicability

Waterbody types

This SOP is intended for use in California's wadeable freshwater streams that are flowing at the time of assessment. The SOP is also appropriate to use in canals, ditches, and other artificial waterways as long as sampleability requirements are met.

This SOP is not intended for the following types of waterbodies:

- Non-wadeable rivers (defined as streams with depths over 1 m for at least half the reach length)
- Ephemeral streams (i.e., streams that only flow in response to rain events, typically for no more than 2 continuous weeks)
- Tidally influenced streams
- Lakes, ponds, and other non-flowing waterbodies

The SOP may be used in perennial and non-perennial streams as long as flow conditions are appropriate. Samples should be collected during baseflow conditions, meaning that they are not influenced by storm events or direct inputs of surface water. Sampling during the prescribed index periods (described below and in Figure 2) will increase the likelihood of encountering baseflow conditions, but additional considerations must be taken at the time of sampling to determine if flows are appropriate.

1.4. Timing of sampling

It is recommended that biotic sampling be carried out during the period from March through September, depending upon both the region (i.e., toward the earlier end of this range in southern California, and later in the range for higher latitudes) and seasonal precipitation amounts (e.g., late spring/early summer in southern California in high rainfall El Niño years; late summer/early fall in the Sierra in high snowpack years; see Figure 2).

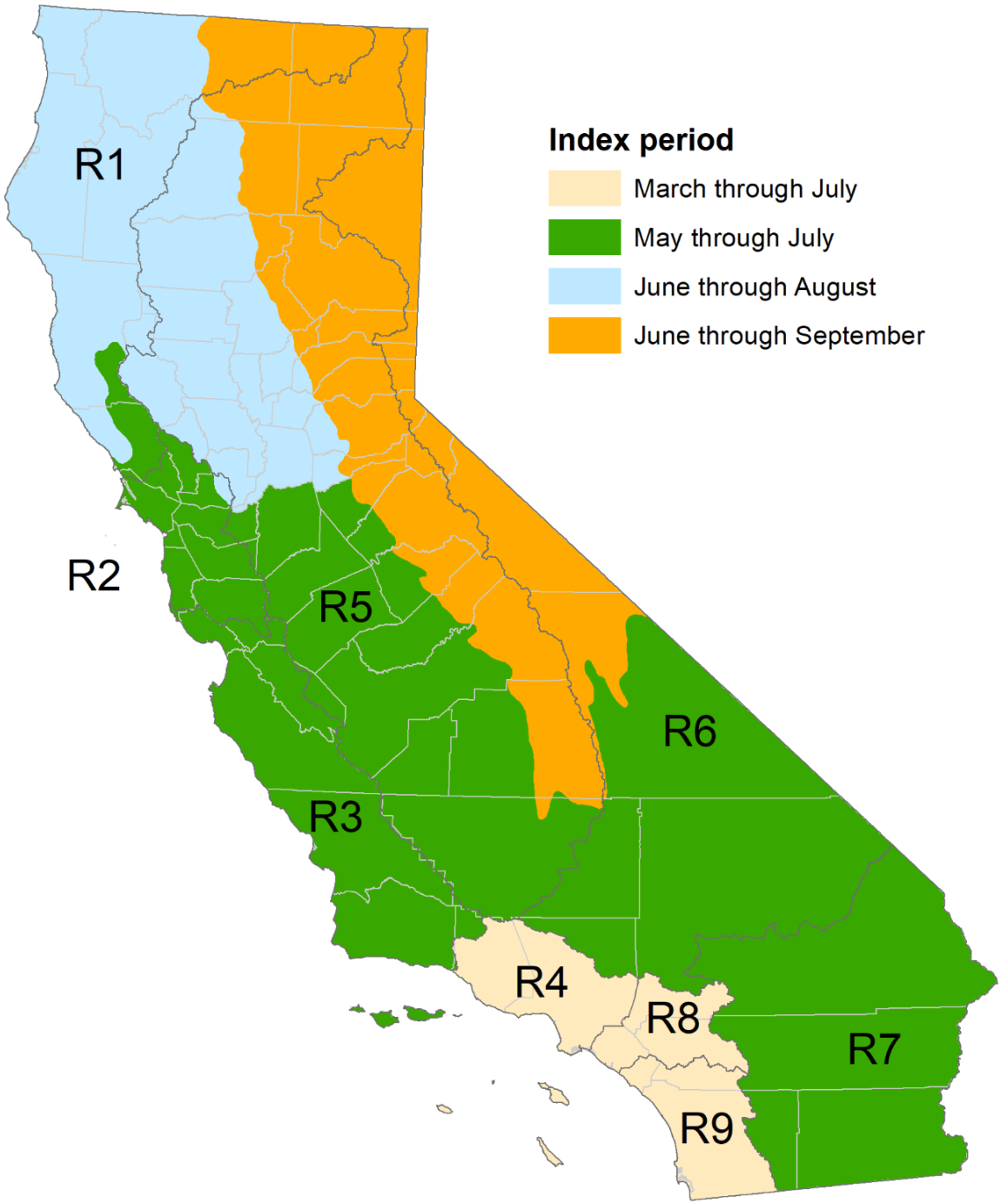

Figure 2. Map of standardized sampling periods (i.e., index periods) for different regions of California. These regions are derived in part from Omernik Level III Ecoregions developed by the US EPA, which reflect major gradients in climate, elevation, and hydrology. Labels indicate Regional Board jurisdictions. County lines are provided for reference.

Avoid Sampling After Storm Events - Sampling of streams following storm events should be avoided because sudden flow increases can displace benthic organisms from the stream bottom and dramatically alter local community abundance and composition. This can cause artificially low benthic community index scores, which can result in false positives of impaired stream condition.

To be conservative, sampling should be conducted at least three weeks after any storm event that has generated enough stream power to mobilize and scour stream substrates. See Section 2 (Step 5) below for tips on how to evaluate a site for recent scour. Two to three weeks will usually allow time for BMI and algae assemblages to return to pre-storm conditions (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 (swamp@waterboards.ca.gov) in questionable cases.

Avoid Sampling During Stream Drying - Intermittent streams exhibit drying as part of a natural hydrologic regime. This drying may occur annually, or only during years with low precipitation. Perennial streams may also experience drying events during droughts. Drying may result from natural processes (e.g., driven by climatic conditions) or human activity (e.g., diversions, dam operations). Regardless of the cause, drying can greatly affect the diversity and abundance of aquatic organisms, and thereby complicate the interpretation of bioassessment indices. Generally speaking, the SOP should not be used to collect samples from streams in the late stages of drying. Sampleable streams must meet these five criteria:

1. Although reaches may be shortened to avoid dry portions, the assessed reach must be at least 100 m long. Surface flow may be discontinuous along the length of the reach, but there should be no more than 3 dry transects within the reach.
2. The wetted width should be greater than 0.3 m (i.e., the width of a D-frame net) for at least half the reach.
3. There should be detectable surface flow at least at one location within the reach. That is, the SOP should not be used if the entire reach is stagnant, even if all other criteria are met.
4. The depth should be sufficient for proper use of a D-frame net. That is, the depth should be at least 5 cm for more than half the reach.

1.5. Training

Procedures described here are designed to produce repeatable, quantitative measures of 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 (only offered to Water Board staff currently). If any training course is available for the public, it will be announced via the SWAMP email subscription list.

In addition, regular (e.g., yearly) field audits of sampling crews, conducted by an experienced individual, are strongly 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 also 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 DFW permits can be found in Appendix A12. Likewise, for streams supporting species listed as sensitive under the State or Federal Endangered Species Acts (including, but not limited to, California red-legged frog, least Bell's vireo, southwestern willow flycatcher, arroyo toad, and salmonids), sampling may not 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/.

Landowner permission may also need to be obtained in order to access many sites. This may add a significant amount of time that needs to be accounted for before the field season. The DFW's ABL has significant experience with this process and can be consulted for advice.

1.7. Avoiding the Transfer of Invasive Species and Pathogens

Proper field hygiene must be practiced at all times in order to avoid transferring invasive organisms or pathogens between sites (e.g., 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 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 Appendix A1.

1.8. SWAMP Requirements

The "reachwide benthos" (RWB) sampling procedure, 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 with long-term monitoring projects is needed or for other site-specific situations. Additional information on the TRC method can be found in Appendix A4. The project manager must have the approval of the SWAMP Bioassessment Program Lead Scientist and the SWAMP Quality Assurance Officer before the use of methods that deviate from this SOP will be accepted. For other projects and/or programs desiring SWAMP comparability, deviations should be approved by the project manager and project QA officer in consultation with SWAMP.

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, see Figure 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 Stormwater Monitoring Coalition 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

Appendix A provides more detailed information about conducting the modules described here, including how to measure certain PHab field variables, instructions for making specialized equipment and suggestions for how to deal with special circumstances that may be encountered during stream bioassessments.

Optional measures to supplement this SOP may be included in stream assessments according to program needs (Appendix A). Examples include environmental DNA (eDNA) sampling, and deployment of water quality sondes or flow loggers. Care should be taken when incorporating additional measures to ensure any supplemental measurements do not disturb the stream prior to conducting benthic macroinvertebrate and algae sampling.

2. REACH DELINEATION AND RECORDING 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 should be documented, water chemistry parameters should 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 https://www.waterboards.ca.gov/water_issues/programs/swamp/bioassessment/sops.html.

Field crews using paper forms must designate someone other than the field recorder to review the forms for completeness[1] 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 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 Appendix A2.

Step 1. Upon arrival at the site, fill out the "Reach Documentation" section of the field forms. Record the StationCode following SWAMP formats[2]. 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. 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 probabilistic survey 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 errors. 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 GPS recording location must be noted on the field data sheets.

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

[2] 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.

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. 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 is ≤ 10 m, delineate a 150 m reach for sampling. If the average wetted width is > 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 (i.e., Transects A and K). 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 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 the length on the datasheet under "Actual Reach Length".

Step 3. Another way to avoid problematic features mentioned in Step 2 or if dry channel conditions are encountered at the end of the reach, is to "slide" the reach. The reach should be moved in one direction before starting the procedures outlined in this manual and no more than a reach length (150 m or 250 m) upstream or downstream from the original reach[3].

[3] Field crews should consult with the project manager in advance to decide whether such reaches should be moved up or down.

Step 4. 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). Use markers (e.g., wire-stemmed flags) to indicate locations of transects and inter-transects. 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 5. Under "Notable Field Conditions", record evidence of recent rainfall, flooding, scour, wildfire, or other notable information that might influence bioassessment samples. The objective of recording these observations is so that persons who submit data to the SWAMP database know whether to flag applicable analytes (especially final bioassessment index scores) as having potentially been biased by such conditions, thus assisting managers in future data interpretation and in development of associations between biological measures and natural stressors. Your observations are extremely valuable, but determinations can be subjective, so make sure to document whatever cues were used to make the assessment. If unaware of recent rainfall, flooding, scour or wildfire 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 channel[5].

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[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 situation, 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".

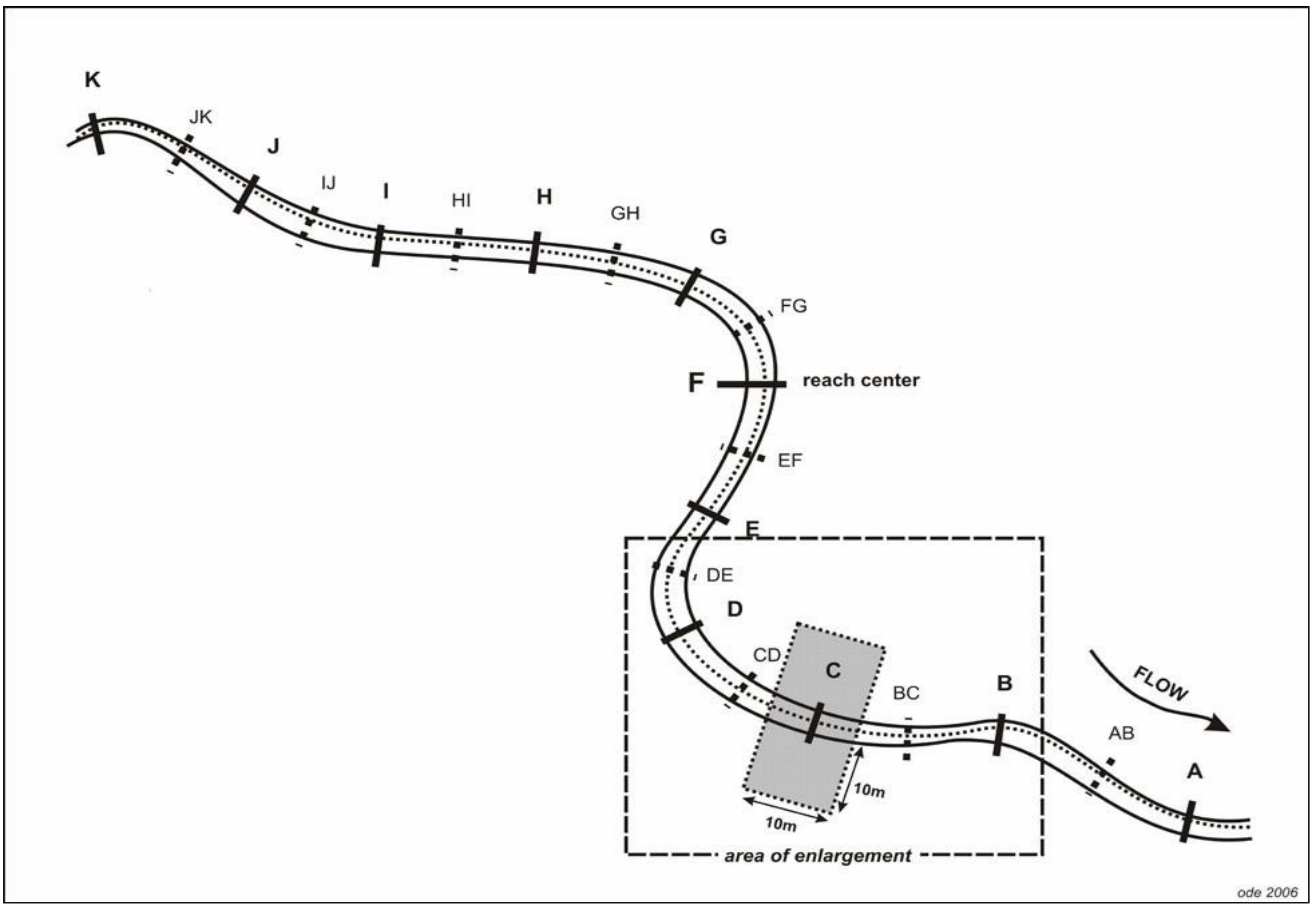

Figure 3. Reach layout geometry for physical habitat (PHab) and biotic sampling showing positions of 11 main transects (A-K) and the 10 inter-transects (AB-JK). The "area of enlargement" highlighted in the figure is expanded in Figure 14. Reach length = 150 m for streams ≤ 10 m average wetted width and reach length = 250 m for streams > 10 m average wetted width.

2.1. Detecting Recent Scour Events

A stream reach should not be sampled for bioassessment for three or more weeks 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. 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:

- Large recent deposits of leaf litter/thatch/wrack are clumped against the base of riparian vegetation above the low-flow wetted channel and/or above the bankfull channel in the floodplain; smaller clumps of such material may be trapped in branches of riparian vegetation well above the wetted channel and oriented with the direction of flow
- Channel bars consist of fresh deposits of poorly sorted coarse substrate (gravel size and larger that is unvegetated); sand and silt is loosely deposited among larger particles like cobble and boulders so that the larger particles are not touching (i.e., their weight is supported by the fine particles instead of the fines filling in the interstices between them); larger particles are lying haphazardly (i.e., not lying with flat sides horizontal and stacked like shingles)
- Non-rigid instream vegetation (e.g., emergent macrophytes like cattails and tules, or even younger willows on stream banks and channel bars) is bent over or lying down within the stream and/or bankfull channel
- Absence of leaves and other detritus in pools, despite riparian cover
- Lack of slime/color coating on the streambed (this may also 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

It is important to note that if a significant scour event has recently occurred, several of the above indicators will likely occur together, and ideally, multiple lines of evidence will be present to inform whether you should indicate on the field sheets that recent scour has occurred. You should only rely on single lines of evidence if they are very compelling. For example, clumps of wrack can persist in the lower riparian canopy for months after a storm event, so if that is the only indication of scour, it would be best not to indicate that a recent scour has occurred.

Documenting recent storm events (optional, but recommended) – 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. If dates are known before sampling record the time a relative size of recent storms; if it is not known, look up storm information for the area and record after returning from the field.

2.2. Indications of recent Fire Events[6]:

< 1 year

Wildfires that have occurred within one year prior to sampling should be relatively easy to detect in most situations, especially if the fire occurred only weeks or months prior to sampling If a suspected recent wildfire has occurred, mark the "< 1 year" box in the Notable Field Conditions section of the bioassessment field form that says, "Evidence of fire in reach or immediately upstream (<500 m)".

In high-intensity burn areas, all three vegetation layers (ground, middle, canopy) will be affected in the riparian zone and in the surrounding landscape: ground cover will likely be absent, soils exposed and piles of ash apparent where shrubs, fallen limbs and downed trees have burned; mid-layer shrubs will be absent, or present only as blackened stick-like remains bare of foliage; larger canopy trees will have entire trunks blackened with fresh char, foliage burned away, and many may have collapsed and fallen into the stream channel. Shading will be minimal due to the lack of mid- and upper-canopy foliage.

In lower intensity burns, ground cover will likely be absent, but mid-sized shrubs may have only partially burned with some portions still living. Canopy trees will show fresh charring only on the lower trunk, with foliage on lower branches browned, shriveled and dead, but mid- and upper- canopy branches will still have growing foliage. *Note: you are **not** being asked to distinguish or estimate burn intensity; these are just guidelines for detecting recent fire events that should be recorded as occurring < 1 year prior to sampling.*

If sampling occurs an entire season after a wildfire (i.e., after winter rains have likely caused surface runoff events that eroded exposed soils), but still within 1 year of the fire, the channel may be choked with fine sediment (mostly sand) such that the bed is flat overall, with a uniform gradient, lacking well-defined channel pools, or with pools uncommon and irregularly spaced (note: extreme flood events where large boulders become entrained can also occur when heavy rains fall on exposed mountain slopes). Fast-growing annuals (grasses, forbs, wildflowers) will likely have returned as ground cover in the riparian zone and surrounding area, but woody mid-layer shrubs will show only partial regrowth, and canopy trees and shading may still be lacking

[6] Following the sampling visit, under "Field Notes/Comments" on the field sheet, we recommend that field crews or the project manager add the actual time since fire, and even the name of the fire that affected the site. This information can be determined in the office either prior to sampling or at the end of the sampling week using California Fire Perimeter Maps available from CalFire. This is an increasingly important component of physical habitat data entry as wildfires become more frequent and extreme.

depending on burn severity.

< 5 years

Estimating time since fire can be more difficult as years pass after the event, but if there is evidence that a wildfire occurred at the site more than a year ago but less than 5 years ago, mark the "< 5 year" box in the Notable Field Conditions section of the bioassessment field form that says, "Evidence of fire in reach or immediately upstream (<500 m)".

At sites where fire was less recent, the area surrounding the site will likely have herbaceous ground cover re-established. Mid-layer woody shrubs will show partial regrowth, but signs of fire described above will still be evident (e.g., the new growth will surround and be somewhat shorter than the old blackened stick-like remains which are bare of foliage). Larger canopy trees may still be completely absent depending on burn severity. As a stream channel begins to equilibrate in years following a fire, channel pools will become present, the spacing between pools and riffles will tend to be regular and the bed will not be flat and choked with fine sediment. Channel bars will consist of fairly well-sorted bed material (smaller grain size on the top and downstream end of the bar, larger grain size along the margins and upstream end of the bar). Because of the post-fire lack of shading where canopy trees were burned, regrowth of riparian vegetation (e.g., willows, young alders) may be extremely dense in the first few years after a fire, with thickets forming along the low-flow channel margins within the bankfull channel.

2.3. Other Notable Conditions to Document:

This section of the field form is to be used by sampling crews to document other notable conditions observed that could be influencing the aquatic benthic community during the time of sampling. These observations can assist data managers when interpreting bioassessment data and index scores.

Examples of observations to document under "Other" include:

- Signs of grazing by domesticated animals (e.g., feces, hoof prints, trampled vegetation on hillsides) near or within the stream
- Observed discharges and discharge types (e.g., flowing storm drain outfall, flows from irrigation return water ditch)
- Stream flow diversions or evidence of diverted flows or withdrawals
- Notable drops in stream flow during sampling
- Evidence of recent stream drying, such as dried algal mats along the margins
- Significant off road vehicle activity within or across the stream
- In-stream mining (e.g., suction dredging)

3. WATER CHEMISTRY SAMPLING

Sample water chemistry just downstream of Transect A, the same general location as where the GPS coordinates were taken[7], and before any other sampling activities take place. 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. **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.**

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, 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 parameters[8]:

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

[7] If 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.

[8] 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.

Measure turbidity first, if applicable, because it may be affected by disturbance of the streambed that can occur during chemistry sampling. 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. Field probe measures and water chemistry sample collection should take place in areas with flowing water, avoiding depositional zones (e.g., pools), if possible.

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 H_2SO^4) as the titrant is recommended for determining alkalinity in low-alkalinity streams (i.e., < ~100 mg/L $CaCO_3$). 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 (NO_2+NO_3), orthophosphate, and particulate ratios of benthic carbon and nitrogen (PCN) 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 Guidelines[9]. Greater detail on field sampling methods for water chemistry can be found at the Water Boards SWAMP webpage.

[9] Crews may opt to collect water at the end of sampling for holding time purposes, in which case sampling should be conducted in undisturbed water. (see also footnote #6)

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 mobile and could be flushed by the algae sampling activity. BMI samples are much more likely to be affected by algae sampling than *vice versa*.

4.1. General Considerations for Biotic sampling (RWB)[10]

The Reachwide Benthos (RWB)[i] procedure employs a systematic method for selecting subsampling locations that is built upon the layout of the 11 main transects. 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[11]" or "depositional[12]" 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[13]"), 50% ("center") and 75% ("right") across the wetted width.

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[10] RWB is the required procedure for SWAMP programs. The following section describes only the RWB method. Supplemental information on the TRC method can be found in Appendix A4.

[11] 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; cobbles and boulders are common.

[12] 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; silt and sand are common.

[13] By convention, "left bank" is defined as the left bank when facing downstream (i.e., in the direction of the current).

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 < ~ 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 concentrated near 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."

Collecting duplicate samples - The recommended method for collecting duplicate biotic samples is at adjacent positions along the sampling transects according to the scheme depicted in Figure 4 (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. In small streams, it may also be appropriate to move duplicate sampling locations depicted below slightly upstream of the transect for situations where the first BMI and/or algae sampling has resulted in disturbance of the adjacent duplicate sampling location.

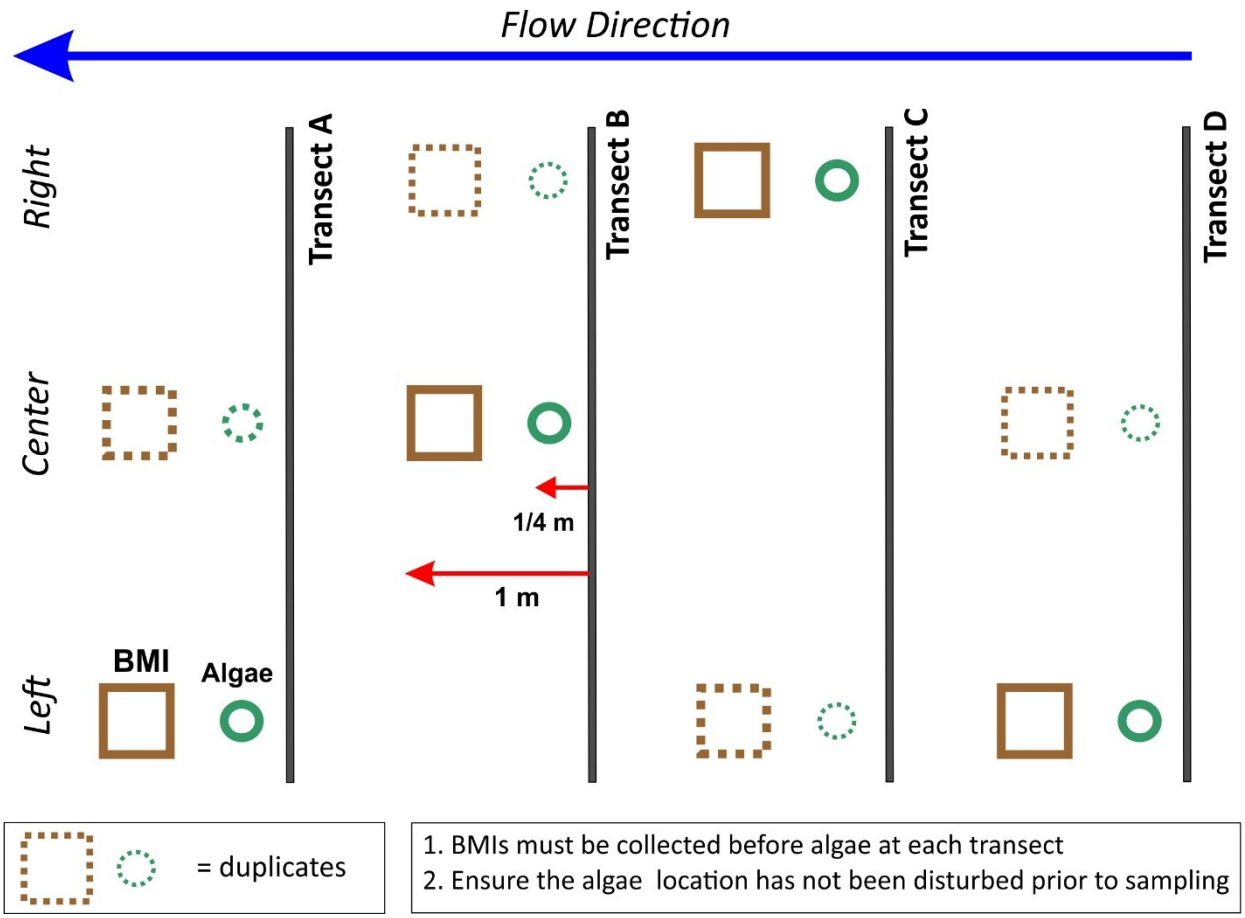

Figure 4. Sampling array for collection of BMIs, algae, and duplicate subsamples (outlined with dashed lines) for each assemblage. The lower left corner of diagram shows distances between BMI and algae sampling points relative to a transect (i.e., one sample collected at the Left location while the duplicate 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 continued for transects D-K. *Not to scale.*

Collecting samples from dry transects - When there are dry transects in the sampling reach, bioassessment practices for the two assemblages (BMIs and algae) are different.

For BMIs, crews should skip the dry transect and record on the field forms the number of subsamples that were ultimately collected (i.e., some number < 11). Care should be taken to avoid sampling a stream at or near the cessation of baseflow, which can result in a sample with erroneously low biointegrity index scores. For algae, samples should be collected from the nearest wet place to the intended sampling location. The goal is to collect algae from 11 unique locations for the composite, even if sampling locations must be shifted to some degree.

4.2. 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 location 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-µm D-frame net[14] 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[15].

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 $1ft^2$ ($0.09\ m^2$). Restrict sampling to within the plot.

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.

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[14] 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.

[15] 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 $1ft^2$ area of substrate at the sampling location. Then stir up the substrate with gloved hands and use a sieve with 500-µm 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.

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 can damage organisms and degrade taxonomic data quality.

For slack-water habitats, vigorously kick the remaining finer substrate within the plot while dragging the net repeatedly through the disturbed area just above the bottom. Keep moving the net so 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-ft^2 (0.09 m^2) 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[16].

[16] Care 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.

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

4.3. 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 each 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.

Higher resolution taxonomic information about algal assemblages can be used in calculating algal biological indices (e.g., Theroux et al. 2020), and offers more in-depth insight into water quality. For this type of data, algal specimens must be collected quantitatively preserved and subjected to taxonomic analysis. While the percent algal cover data are (optionally) 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 collection of algal samples.

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.

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 Appendix A7).

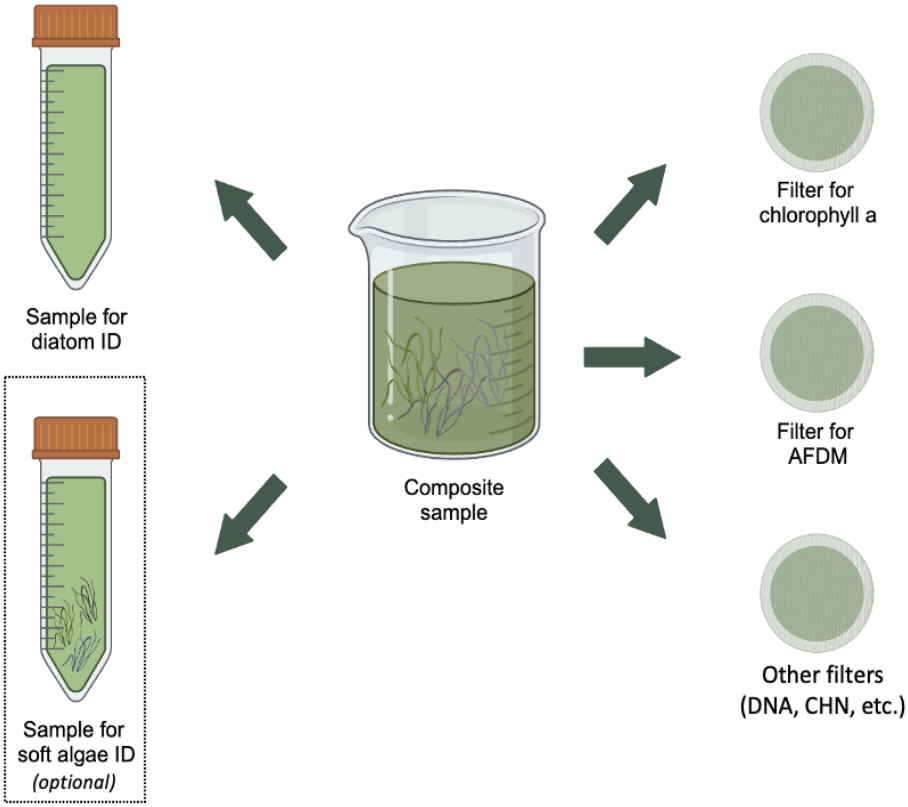

Figure 5. Many sample types can be prepared from the algae “composite sample.”

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

Measurement	Algal Indicator	Collection Method	Collection Vessel	Preservation / Fixation Method / Holding Times
Percent algal cover (optional)	Stream primary production measured as algal abundance; key indicator of aesthetic impacts, but less direct relationship to aquatic life.	Point-intercept component of the PHab pebble count	N/A	N/A
Chlorophyll *a*	Stream primary production measured as algal biomass; key eutrophication indicator.	RWB or MCM sample collection	Glass-fiber filter	Filter, wrap in foil, store on wet ice in the field, but freeze (pref. -80ºC) within 4h of collection; analyze within 28d
AFDM (optional*)	Stream primary production measured as biomass of organic matter, including algae; key eutrophication indicator.	RWB or MCM sample collection	Glass-fiber filter (pre-combusted[17])	Filter, wrap in foil, store on wet ice in the field, but freeze (pref. -80ºC) within 4h of collection; analyze within 28d
Particulate carbon and nitrogen (optional*)	Using benthic N and C:N ratios to assess eutrophication linked to nutrient loading	RWB or MCM sample collection	Glass-fiber filter (pre-combusted)	Filter, wrap in foil, freeze at -20°C. See EPA Method 440.
Diatoms	Indicative of factors such as trophic status, organic enrichment, low DO, siltation, pH, metals. Used to calculate ASCIs.	RWB or MCM sample collection	50 mL centrifuge tube	Add 1 mL of 50% Lugol's to 45 mL of sample; add preservative same day of collection; keep dark and cold (4 ˚C, do not freeze); fixed samples should be analyzed as soon as possible. Samples can be stored for up to 12 months.

[17] Pre-combustion removes residual organic matter from the filter.

Measurement	Algal Indicator	Collection Method	Collection Vessel	Preservation / Fixation Method / Holding Times
Soft-bodied algae quantitative sample (optional*)[18]	Indicative of factors such as nitrogen limitation/ trophic status; siltation; pH; temperature, light availability, nuisance/ toxic algal blooms. Used to calculate ASCIs.	RWB or MCM sample collection	50 mL centrifuge tube	Keep unfixed samples in dark on wet (not dry) ice; add 5 ml Lugol's (10% potassium iodide, 5% iodine) 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
Cyanobacteria, diatoms, soft-bodied algae molecular (DNA) sample (optional*)	Same as above	RWB or MCM sample collection		Keep unfiltered composite sample in the dark on wet (not dry) ice; filter in the field or lab as soon as possible, but no later than 6 hours after sampling; after filtering put filter in a 2 ml vial with preservation solution and keep in -80°C freezer; frozen samples can be stored for at least 2 years
Soft-bodied algae qualitative sample (optional[19])	Used for historic IBI calculation, to help laboratory identify specimens in the quantitative sample, culturing for gene sequencing (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)

[18] For the purposes of this SOP, the soft-bodied assemblage includes cyanobacteria.

[19] Optional collection at the discretion of the program. See Appendix A6 for specific processing instructions.

4.4. 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. Aliquots are then drawn from the composite sample, and these can be used for analysis of the following: diatom assemblage, soft-bodied algae assemblage (optional), benthic chlorophyll a concentration, benthic AFDM concentration (optional), particulate carbon and nitrogen (optional), and DNA samples. 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, the algae sample should be taken ¼ m upstream from the center of the upstream edge of the scar in the stream bottom left from the BMI sampling, according to the schematic in Figure 4. 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.

Individual subsampling points may fall on a variety of different substrates, which require different sampling devices. When confronted with a situation in which an algae sampling location straddles two substrate 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 minimum 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 cm^2 for each use of the rubber or ABS delimiter and 5.3 cm^2 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 Appendix A5.

The recommended method for collecting duplicate algae samples is identical to that described for BMIs: at adjacent positions along the sampling transects according to the scheme depicted in Figure 4. Both the sample and the duplicate should be collected at each transect before moving on to the next transect.

Before sampling, the sampling vessel[20]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 devices (e.g., toothbrushes, graduated cylinders, delimiters, trowels, syringe scrubbers, turkey basters).[21]

If soft-bodied algal samples are collected for microscopy-based taxonomic analysis, collecting a qualitative sample is also recommended. Instructions for collecting qualitative samples are provided in Appendix A7.

[20] A wide mouth jar, bucket, or dish tub would be suitable as a sampling vessel. Key characteristics include: easily washable, sturdy, able to be easily transported while sampling.

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

4.4.1. Collecting from Hard Surfaces 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.

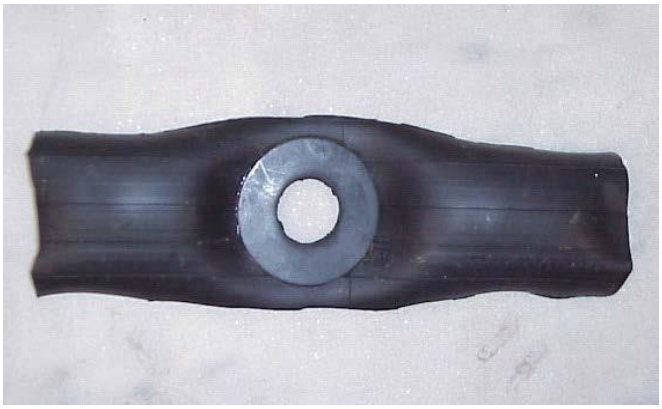

Figure 6. Rubber delimiter (see Appendix A5 for assembly instructions).

Step 2. Hold the substrate over the sampling vessel 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.

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 sampling vessel. 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.

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 sampling vessel, 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 sampling vessel. 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.

4.4.2. Collecting from Sediment using the ABS delimiter

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 7). 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.

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 sampling vessel. Using water sparingly, rinse any leftover sediment from the trowel into the tub.

Figure 7. ABS delimiter, showing pink line at 1 cm depth mark. See Appendix A5 for assembly instructions.

4.4.3. Collecting a Mass of Algae 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 cm^2 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 sampling vessel.

4.4.4. Collecting from Macrophytes using the ABS delimiter

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 cm^2 section of macrophyte after raising it to the surface.

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 sampling vessel.

4.4.5. Collecting from Hard, Submerged and Anchored Substrates using a syringe scrubber

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 8) to collect a sample underwater. To use this device, affix a fresh, white scrubbing pad circle onto the bottom of the syringe plunger using the Velcro hooks on the end of the plunger. 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.

Figure 8. Syringe scrubber (see Appendix A5 for assembly instructions).

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 the 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 bottom of the syringe barrel.

Step 4. Hold the syringe scrubber over the sampling vessel 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 sampling vessel, 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 sampling vessel 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 sampling vessel, 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.

4.4.6. 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 most appropriate collection instrument to sample the substrate being sure to account for the surface area sampled (in this case, use 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".

5. BIOTIC SAMPLE PROCESSING

5.1. MODULE A: 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 empty 500-mL wide-mouth plastic sample jar(s), 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., take care to disturb the substrate as gently as possible and avoid kicking).[22]

[22] 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.

Step 2. Place a date/locality label (Figure 9), filled out in pencil, on the inside of the jar and completely fill the jar with 95% ethanol[23]. 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 (avoid using pens as most inks will dissolve in ethanol). Tape the label with transparent tape. Make sure all samples have both internal and external labels.

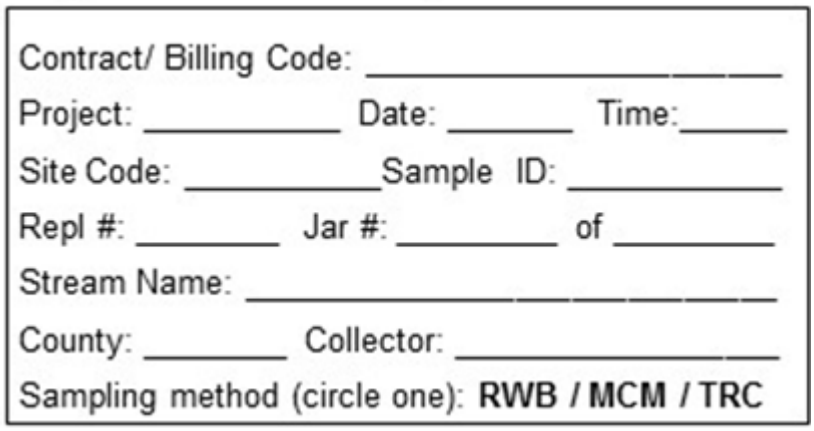

Figure 9. Example date/locality label for BMI samples.

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.

[23] 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.

5.2. MODULE B: Processing Quantitative Benthic Algae Sample for Diatom Taxonomy and Biomass Analyses

After collecting benthic algae samples, there should be material from all 11 transects in the sampling vessel. Depending on the types of habitats in the stream and substrates encountered, the sampling vessel may contain stream water with suspended microalgae, and silt, and/or sand, and/or fine gravel, and/or small pieces of wood or macrophytes. The algae clinging to these substrates must be detached and suspended into the water to form a "composite sample". Whereas a single sample type is collected for BMIs, many different types of quantitative laboratory samples may be prepared from the composite sample when collecting algae (Figure 10).

The six standard sample types derived from the composite sample are:

- For molecular taxonomic analysis
    - composite of diatoms and soft bodied algae (including cyanobacteria)
- For traditional (microscopy-based) taxonomic analysis
    - diatoms
    - soft-bodied algae (optional)[24]
- For biomass
    - chl a
    - AFDM, optional analysis
    - PCN, optional analysis

Creating the algae composite sample

Step 1. Clean and discard large organic material. 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 necessary to view all material, dump contents of the sampling vessel into a tray or dish tub.** If there are any clumps of macroalgae in the tub, there is a special process required for them (Steps 4 and 5, Figure 10).

[24] If soft-bodied algal samples are collected for microscopy-based taxonomic analysis, you must use the sample processing instructions provided in Appendix A6.

Step 2. Clean inorganic sediments. 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). Rinse water is ideally stream water that has been collected in a separate squirt bottle to aid in cleaning[25].

Step 3. Measure the composite sample volume - Once algal specimens have been removed from all the substrates (sand, gravel, cobble, wood, leaves) in the dish tub (Steps 1 and 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 the sediment (silt, sand) behind. Transfer the measured liquid into a clean 1L plastic bottle. Rinse the sediment once or twice until it appears that 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 was 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).

Before proceeding to the algae sample preparation steps, it is necessary to determine two things:

1. **Are there any clumps of macroalgae in the composite sample (as opposed to just microalgae suspended in liquid)?** If YES, complete Step 4 and Step 5. If NO, proceed to Step 6.
2. **Is a non-molecular soft-bodied algae taxonomic sample going to be prepared? If** YES, consult Appendix A6 for instructions.

[25] Deionized water may be used if stream water is not available but is not preferred due to potential to burst algae cells.

Step 4. Process macroalgae clumps. If one or more macroalgal clumps are present in the dish tub, first remove them from the dish tub (Step 4a), flatten them and wring them out gently into the tub (Step 4b), and roll them into cylinder shapes that are relatively even in thickness along their length (Step 4c, see Figure 10). If there are no macroalgae clumps, proceed to Step 6.

Step 5. Cut up and return processed macroalgae to sample. Cut the macroalgal cylinder with scissors into fine pieces (resulting in strands that are no more than ~3 mm long) and add these to the 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.

Step 6. Homogenize the composite sample. 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*. **The composite sample is now ready for preparing algae samples.**

Step 7. Diatom sample: Diatom samples are preserved with diluted Lugol's solution[26, 27]. The diatom taxonomic ID sample is kept in the dark on wet ice until fixed and then stored in the refrigerator (do not freeze).

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Step 7a. Aliquot 45 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 1 - 5 mL of the 50% Lugol's solution to the sample to preserve[28].

Step 7b. Cap the tube tightly and invert it several times to mix the Lugol's 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. Store samples in a cool and dark location.

Diatom samples can be preserved in Lugol's solution for up to 12 months depending on the Lugol's concentration and amount of organic material in the sample. Lugol's is not suitable for long-term preservation. Formalin should be used for long-term archival of algal samples.

Step 8. The remaining composite sample homogenate can be used to prepare the chl-*a*, AFDM, PCN, and molecular filters.

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[26] Prepare a 1:1 dilution of Lugol's solution (10% potassium iodide, 5% iodine) with distilled water. Fixatives such as Lugol's must be used with care: be sure to wear appropriate gloves and safety goggles when using fixative. Plastic containers are safe for transporting Lugol's in the field.

[27] Lugol's is a DEA-regulated substance. Some groups may have difficulty acquiring Lugol's due to lab restrictions on controlled substances. For more information, visit http://www.gpo.gov/fdsys/

[28] Add between 1 and 5 ml of 50% Lugol's depending on the preference of the taxonomy lab. Higher concentrations of Lugol's are preferred by some taxonomists because lower concentrations of preservative allow the proliferation of fungi and bacteria. Contact your diatom taxonomist prior to sampling to confirm the preferred final fixative concentration, including for any archived samples.

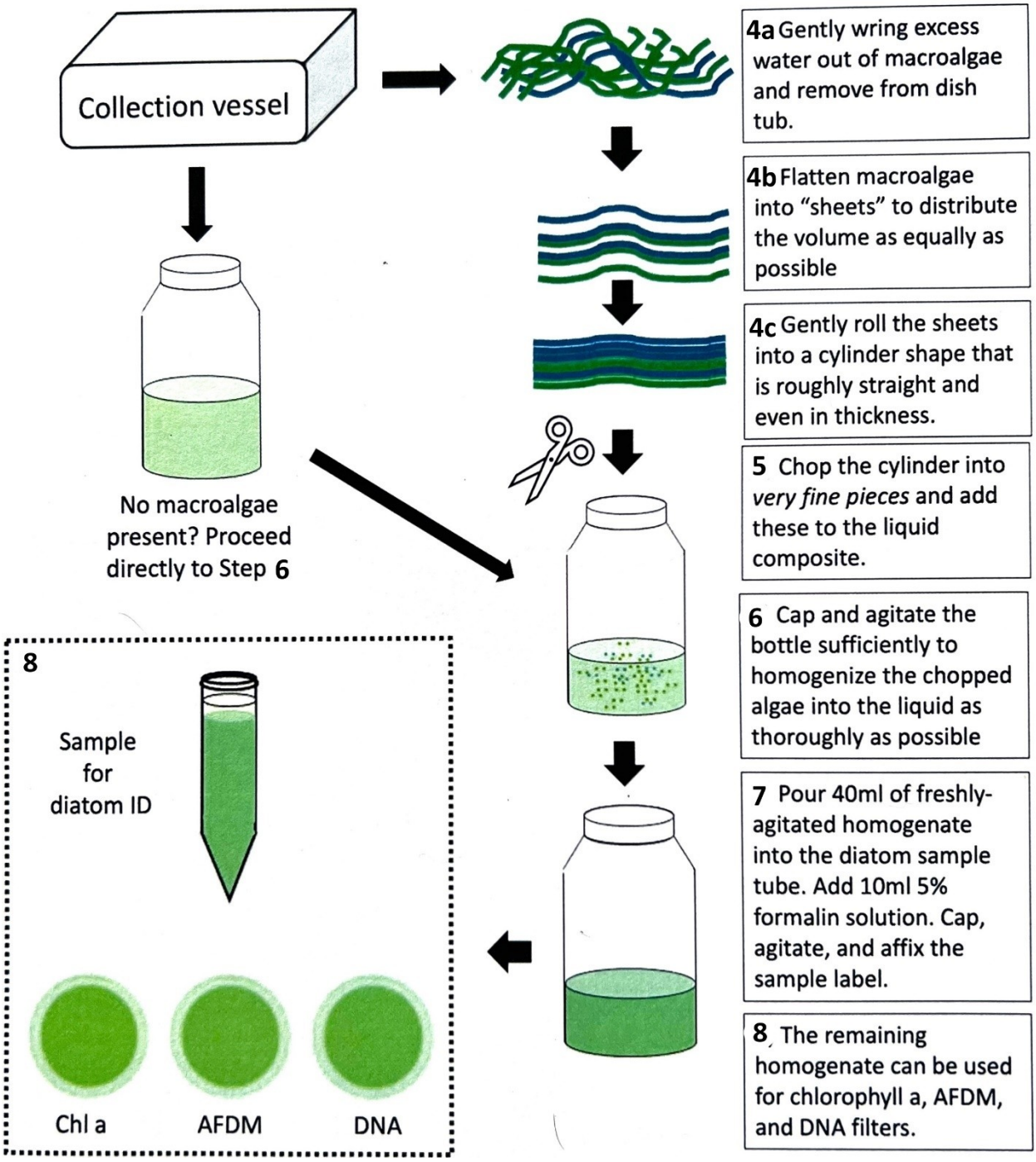

Figure 10. Processing steps for creating the composite sample and preparing macroalgae and diatom samples (Steps 4-8). *Note that diatom samples are now 45 ml and fixed with Lugol's and not formalin as listed in Step 7. See text for volumes and instructions.*

Chlorophyll a: 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.

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 inches Hg (~3.5 psi)*[29]*, 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 be 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 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.

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 100mL 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.

[29] Prior versions of this SOP specified a threshold of 7 psi, which is too high

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 (-80º, if available) or on wet or 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**.

DNA (optional): For the molecular sample, store the composite material on blue ice and out of the sun until filtration. Filtration of **unpreserved** samples **must** happen in the field or the lab within 6 hours of sample collection.

For molecular (DNA) filters, use a sterile cellulose nitrate or mixed cellulose ester filter with a pore size of <0.45um. Filter 25ml of composite material; if the filter becomes clogged before the 25ml is completed, record the total volume filtered. Remove the filter using sterile forceps and fold the filter at least twice. Place the filter in a 2ml Eppendorf tube pre-filled with preservation solution. Invert the tube five times to mix preservation solution and submerge the filter. Store tubes in a -20ºC or -80ºC freezer. Filters in preservation solution are stable for one year. Additional guidance on collecting algal DNA samples can be found in Appendix A9.

AFDM (optional): For the AFDM samples, use pre-combusted[30] glass-fiber filters (47 mm, 0.7 µm pore size). 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.

PCN (optional, sometimes called "CHN"): For the PCN samples, use pre-combusted glass-fiber filters (25 mm preferred, 47 mm possible). Follow the same process used for chl-*a* and AFDM sample filtering, though a syringe Swinnex-style filtration unit is recommended for 25 mm filters. 25 mm filter target volumes are 1-5 ml of algae composite and up to 10 ml for 47 mm filters. Filtered material should be enough to cover the filter without forming a thickened layer. Record the volume filtered for the PCN sample. Keep PCN filters as cold as possible until the samples can be frozen at the laboratory. The analytical holding time for the PCN samples is 100 days from collection, when kept frozen.

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

Example 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 taxonomic ID samples) or filtered (for the chl a, and ADFM, PCN, and DNA samples). All of these volumes are also recorded on the field forms 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.

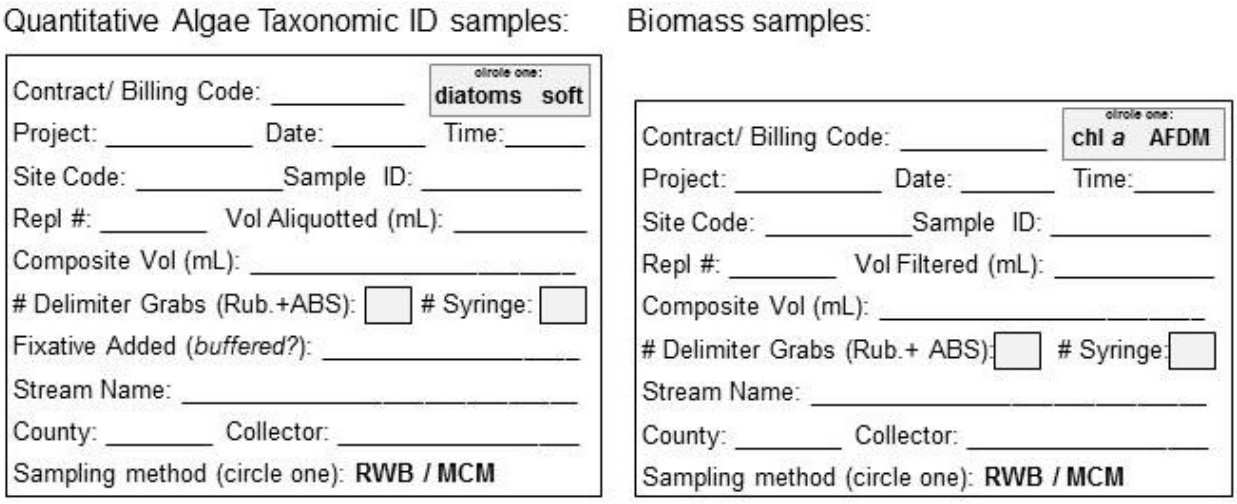

Figure 11. Example labels for algae quantitative taxonomic identification (left) and biomass samples.

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 and riparian condition, 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.

6.1. MODULE C: Wetted Width and Bankfull Dimensions

Step 1: If the channel is dry at the location of a given transect, check the box that says "Dry Channel" at the top of the transect form near where wetted width would normally be recorded**.** This information may be used to flag certain analytes and index scores in the SWAMP database. If the transect is dry, do not record the Transect Substrates or Instream Habitat Complexity sections of the transect form.

Step 2. Measure the wetted width associated with the transect and record (in meters) 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 12). 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).

Step 3. 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 12 and Appendix A3 for a depiction of wetted width and bankfull dimensions). Because most channel-formation processes act when flows are within this zone (Mount 1995), bankfull dimensions provide a valuable indication of stream power during high-flow events and the relative size of the water body.

Examples of evidence for bankfull location include topographic, vegetative, 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 across 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 4. 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 5. Record bankfull height (in meters) as the vertical distance between the water surface and the height (Figure 12) 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 6. Carry out the above steps at each of the 11 main transects.

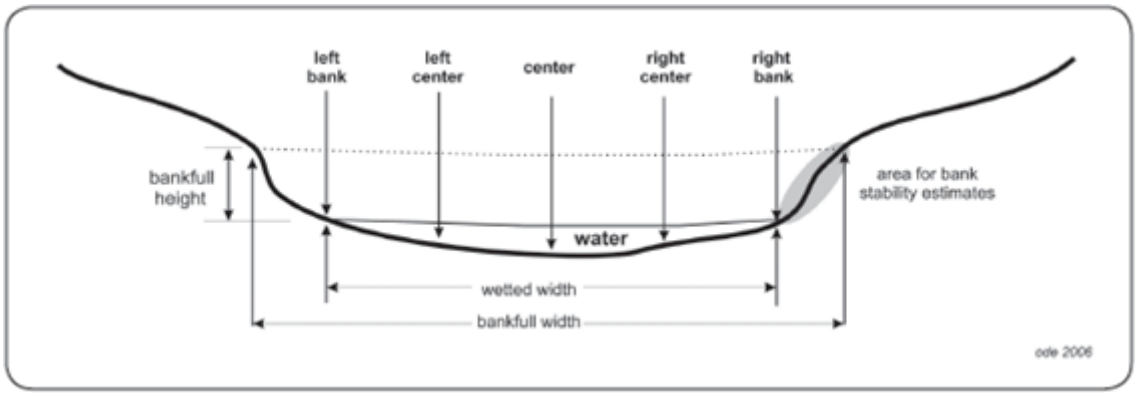

Figure 12. Cross sectional diagram of a typical stream channel showing locations of wetted and bankfull width measurements and substrate measurements.

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 equidistant particles along each transect, 11 main and 10 inter-transects).

The depth of surface water above each of these points is recorded to record variation in depth throughout the reach.

Coarse particulate organic matter (CPOM; small particles of organic material, such as leaves and smaller twigs, that are >1 mm in size) is an indicator of the amount of allochthonous organic matter available at a site. Because CPOM is a food resource for many 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 at each of the 105 points in the sampling reach.

Step 1. At each 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 12) 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 of each substrate particle.

Step 4. Remove the particle from the streambed and measure and record the length of its intermediate axis (Figure 13) 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 class 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.

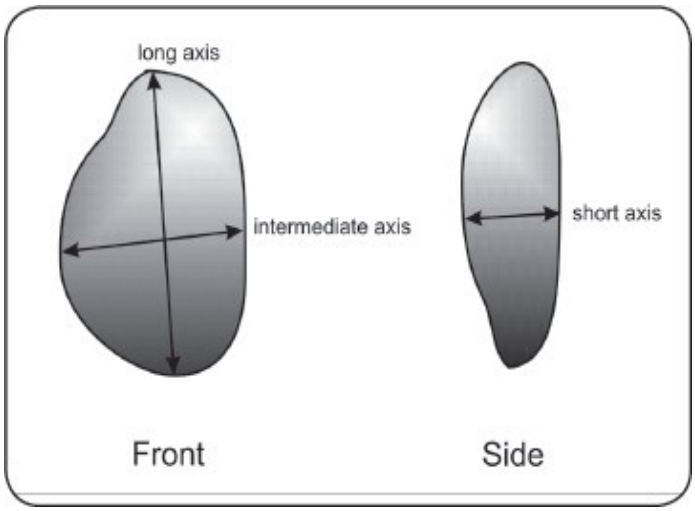

Figure 13. Diagram of three major perpendicular axes of substrate particles (the intermediate axis is recorded for pebble counts).

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[31]	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).

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., regularly inundated during storms), or whether the point is on a stable island (i.e., 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.

[31] 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.

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"

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). Here we define embeddedness as the percentage by 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 volume 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"[32]. 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[33].

Some streams such as sand dominated channels have either no cobble or small isolated pockets of cobbles. Cement lined channels have either no cobble or cobble sized particles that have been deposited there by human intervention and not natural processes. When cobbles are not encountered during the pebble count and the method described in Step 3 cannot be conducted properly, do not seek out cobbles to fulfill the minimum requirements.

6.4. MODULE F: Algal and Macrophyte Cover (Optional)

Algal cover refers to the amount of algae in the stream reach in terms of macroalgal growths (e.g., filaments, mats, globules) and microalgal coatings on stream substrates. Algal biomasss accumulation 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). Although microalgae (e.g., diatoms) can sometimes comprise a large portion of algal biomass in a eutrophic stream, nutrient enrichment more often leads to a replacement of microalgae with macroalgal species[34].

Algal cover is estimated by recording the presence/absence of both types of algae 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 has algae, then algae is recorded as "present" at that point.

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[32] 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

[33] 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.

[34] Microalgae presence and thickness are no longer required as part of this SOP.

Step 1. 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 (see Appendix A5) 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 a physical connection to something (like a cobble, boulder, or a gravel bed) lying on the bottom of the stream, whereas unattached macroalgae have no obvious physical connection with the streambed and the algae is freely floating at or near the water's surface. For each class of macroalgae (Attached and Unattached), mark "P" (for "present") if intercepted by the sampling point and "A" (for "absent") if not intercepted[35].

Step 2. 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 I (Instream Habitat Complexity).

Step 3. 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 6. 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 5. Note that these thickness codes refer only to microalgal film, not macroalgal mats (macroalgal thickness is not assessed in this protocol).

Be sure to collect microalgal thickness data from whatever substrate is topmost within the stream and therefore is most likely to be exposed to sunlight. Sometimes this substrate is not the actual pebble used in the pebble count, but rather a substrate type that occurs above the pebble, such as a thick mat of macroalgae. Microalgal species can grow as epiphytes upon macroalgal filaments and mats, coating them with a slimy, brown-tinted film. Appendix A7 provides additional information to help distinguish between microalgae and macroalgae.

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[35] 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.

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

Code	Thickness Class	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	<1 mm	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	1-5 mm	
4	5-20 mm	
5	> 20 mm	
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”.

6.5. MODULE G: 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 corridor[36]. Observations are made in a 10 m x 10 m riparian area, on either side of the wetted channel (Figure 14).

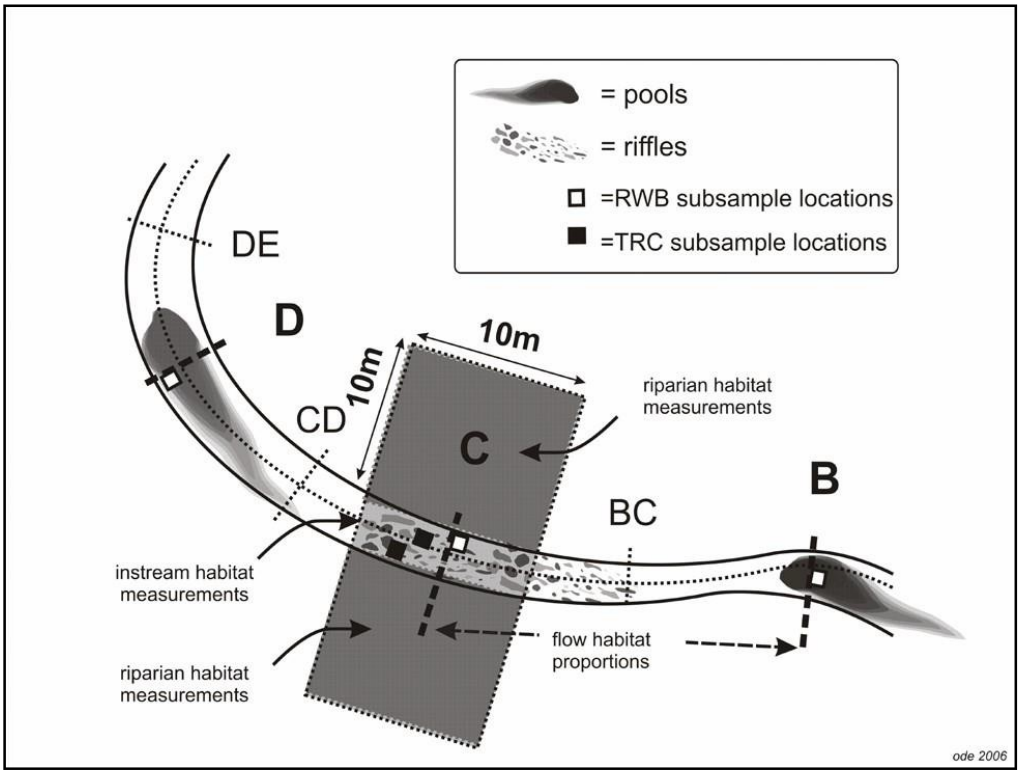

Figure 14. Section of the standard reach expanded from Figure 3 showing the appropriate positions for collecting riparian habitat and flow habitat proportion measurements. Also shown here is the human-influence zone corresponding to the area within 10m of the wetted width (i.e., zone "C").

[36] 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 the riparian condition of monitoring sites.

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:

- 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 scores.

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%.

6.6. 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) are recorded in terms of zones based on how close they are to the wetted margins[37]. The area in which human influence is measured extends outward 50 m in both directions from the stream along the entire reach.

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 within the riparian area plot (defined in Module G, Figure 14) circle "C"; if it outside the riparian area plot and within 50 m of the stream, circle "P"; otherwise, circle 0.

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. If human activities are observed, provide an explanation under "Notable field conditions", as described in Chapter 2.

Refer to Table 6 for definitions of Human Influence features and activities. Circle **only** the closest location for each impact that applies, being careful not to double-count any human influence observations[38].

[37] The wetted edge may be far from the bankfull margin in certain channels. Nonetheless, human activities should be recorded based on their proximity to the wetted edge and not their proximity to the bankfull margin.

[38] Double counts are prevented in 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 (inlets/outlets)	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: Instream Habitat Complexity

The instream habitat complexity measure was developed by the US EPA's 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 14). 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 need to equal 100%.

Table 7. Descriptions of instream habitat complexity components.

Component	Description and Comments
Filamentous algae	• Visible growths of macroalgae • Do not include non-filamentous macroalgae (e.g., *Nostoc* 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 13)
Small woody debris	< 30 cm diameter
Large woody debris	≥ 30 cm diameter
Undercut banks	• 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	• 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)

Component	Description and Comments
Artificial structures	● Any relatively permanent items with a human 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)

6.8. MODULE J: Stream Shading (Densiometer Readings)

The amount of sunlight that can reach the stream influences both stream temperature as well as primary productivity, which in turn affects food webs and the likelihood of eutrophication. Using a convex spherical densiometer, 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 densiometer to correct for over-estimation of stream shading that occurs with unmodified readings. Taping off the lower left and right portions of the mirror (Figure 15) 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 level. 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 the left bank, b) facing upstream, c) facing the right bank, d) facing the downstream. The observer should revolve around the densiometer (i.e., the densitometer 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 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.

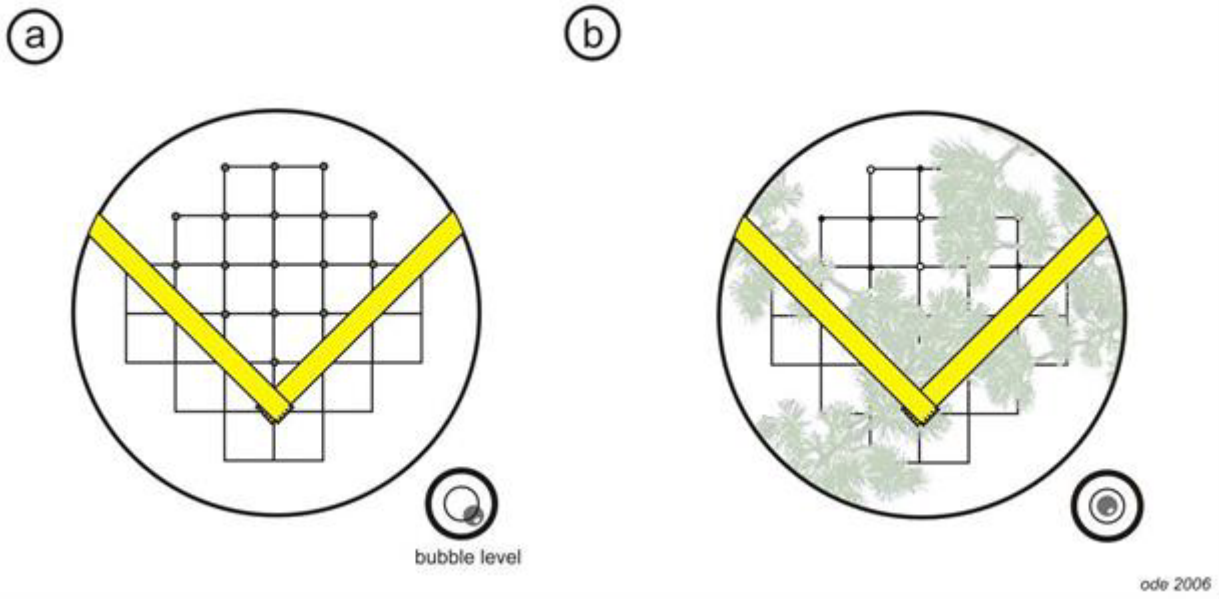

Figure 15. Representation of the mirrored surface of a convex spherical densiometer showing the position for taping the mirror and the intersection points used for the densiometer reading. The score for the hypothetical condition in (b) is 9 covered intersection points out of 17 possible (within the "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.

6.9. MODULE K: 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. It is therefore very useful when interpreting biotic data.

The PHab method described in this SOP uses transect-to-transect measurements to calculate the average slope through a reach. This results in precise slope determination and allows can be used to calculate 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 16)[39].
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.

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).

[39] 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.

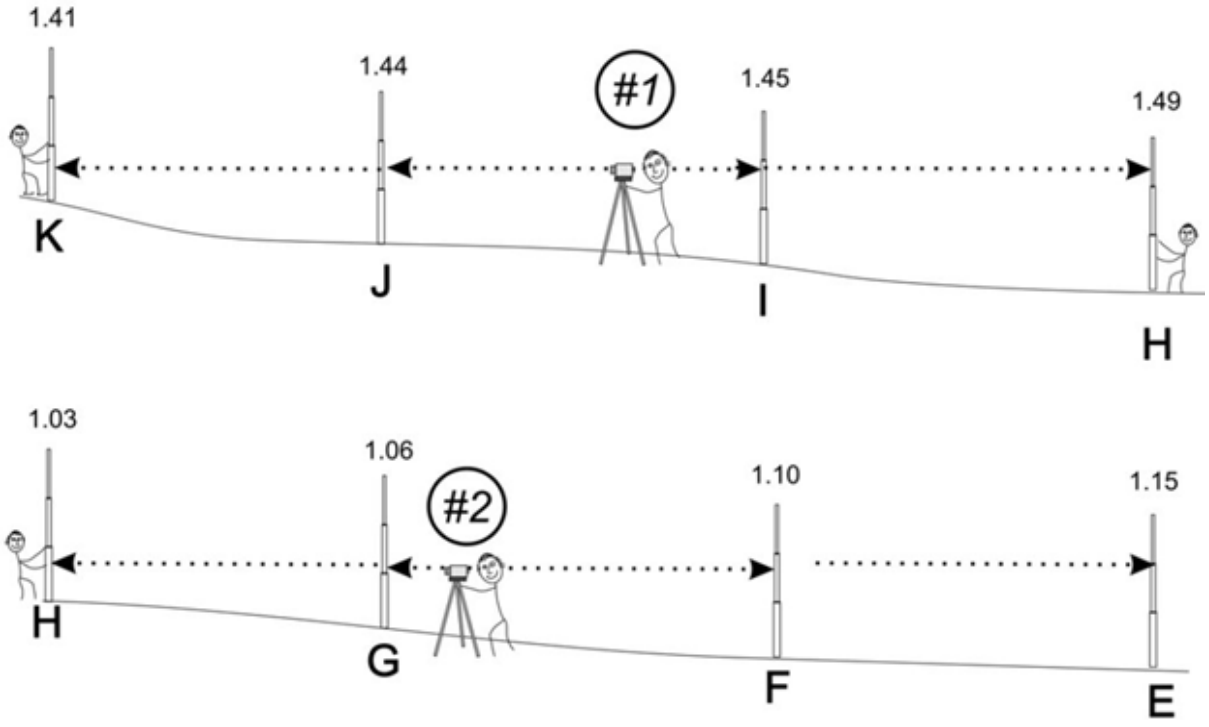

Figure 16. Use of an autolevel to measure slope of sampling reach.

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 level using the adjustment 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)[40]. 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

[40] 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.

in the viewfinder) hits. Record this information on the "Slope and Bearing Form" on the field sheet[41], 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. 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, 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 be used to determine the slopes between transects and for the reach as a whole.

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 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

[41] 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.

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). Make sure to keep both eyes open when reading measurements from the clinometer.

Step 3. Continue measuring slope at each 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").

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.

6.10. MODULE L: Photographs

Take a minimum of four (4) digital 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, but optional, to take a photograph at Transect A, facing downstream and Transect K, facing upstream to document conditions immediately adjacent to the reach. 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 MEASUREMENTS

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

7.1. Intertransect Measures (repeat Modules C, D, E, and F)

Wetted Width (see MODULE C)
Measure wetted width the same way it was measured for the main transects. If the channel is dry at the location of a given inter-transect, check the box that says "Dry Channel" at the top of the inter-transect form near where wetted width would normally be recorded**.** This information may be used to flag certain analytes and index scores in the SWAMP database. If the inter-transect is dry, do not record the Inter-Transect Substrates section of the inter-transect form.

Substrate Measurements, Depth, CPOM, Algal/Macrophytes Percent Cover (see MODULES D, E, F)
Collect particle size measurements, water depth, CPOM, embeddedness and algal and macrophyte cover data the same way they were collected for the main transects.

7.2. MODULE M: 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 8. These flow habitat types are products of geology, slope, and discharge, and one habitat type may transition 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 percentages (to the nearest 5%) of the various flow habitats present within the region between the downstream and upstream transects (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 8. 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 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. 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 completely dry. Do not count mid-channel point bars or tops of emergent rocks and boulders as dry habitat.

8. PHYSICAL HABITAT REACH-BASED MEASUREMENTS

8.1. MODULE N: 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, discharge for the sampling reach is estimated from a single transect. There is no prescribed point in the reach where the transect should be placed, but field crews should pick a transect that with relatively uniform flow and a simple cross-sectional profile. 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 (VAM, Section 8.2), this is the preferred method. If the velocity meter probe cannot be fully submerged, but there is visible flow, the following two options are available: 1) the Neutrally Buoyant Object (NBO) 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 database.

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

Velocity-Area Method (VAM)

The layout for discharge measurements under the velocity-area method is illustrated in Figure 17. Flow velocity should be measured with either a Swoffer 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 for measuring 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 17).

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 17 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 meter[42]. 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.

[42] Set the averaging interval to at least 15 seconds (30 seconds if velocity is > 2 ft/s) and record the 15 second average velocity measurement for each segment.

If the flow is moving upstream (e.g., 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.

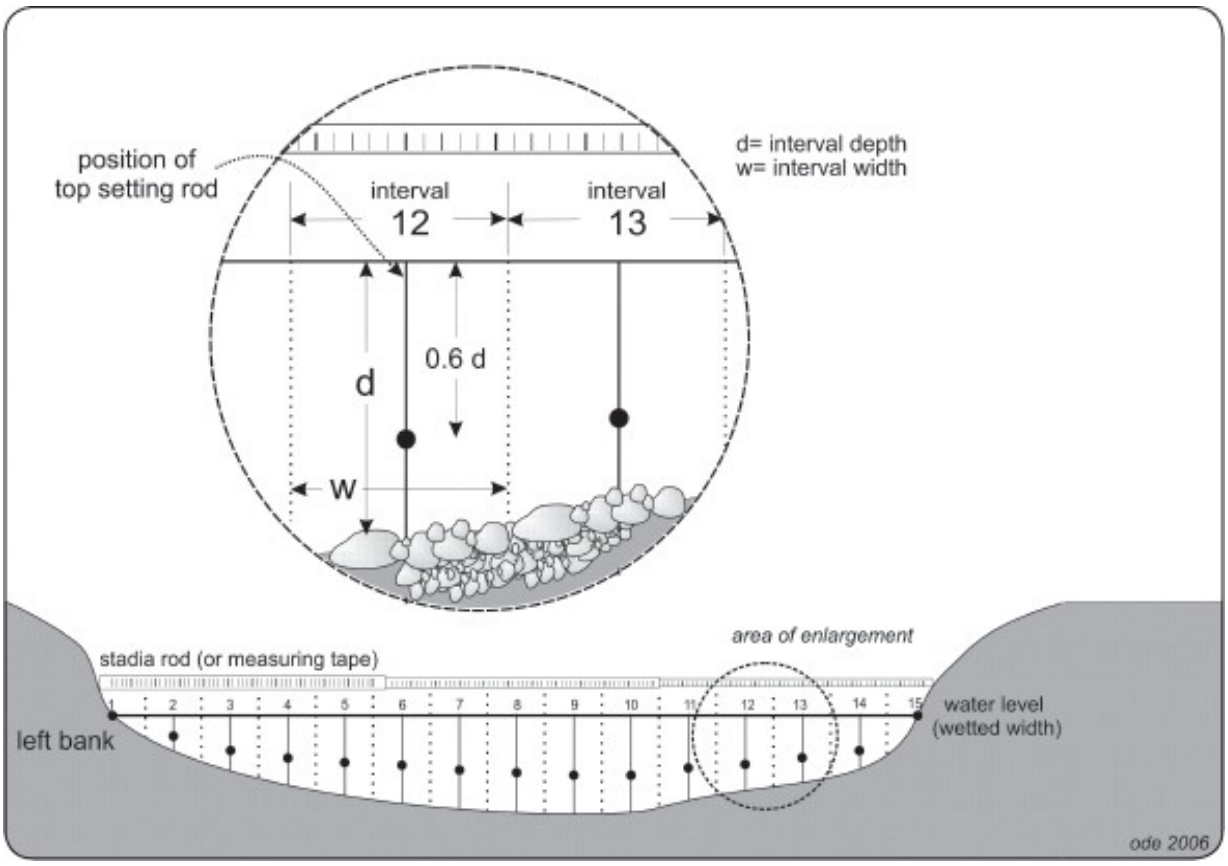

Figure 17. Diagram of layout for discharge measurements under the velocity-area method showing proper positions for velocity probe (black dots).

Neutrally Buoyant Object Method (NBO)

If the reach is too shallow to use a flow velocity meter, the neutrally buoyant object 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 4. 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 5. 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 O: 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 was included in older protocols (e.g., CSBP) and 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.

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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)

Base flow – the portion of stream flow that does not come directly from surface water inputs (as after precipitation events or snowmelt). Baseflow primarily consists of sub-surface flows and groundwater inputs.

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 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 resuspension) 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)

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)

Index Period – a standardized sampling period for conducting sampling

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 longitudinal segment of the stream channel

Reachwide benthos (RWB) – method for biotic assemblage sample collection that does not target a specific substrate type, but rather systematically selects sampling locations across the reach, allowing for any of a number of substrate types to be represented in the resulting composite sample

Riparian zone – 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

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 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 water.