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SWAMP Reachwide Benthos Method for Stream Algae Sampling and Associated Physical Habitat Data Collection

Version 6

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ACKNOWLEDGEMENTS

This Standard Operating Procedures (SOP) manual represents the contributions of a wide range of researchers and field crews. The algal specimen collection methodology presented represents a modification of the U.S. Environmental Protection Agency's (EPA's) Environmental Monitoring and Assessment Program (EMAP) multihabitat sampling protocol (Peck *et al*. 2006). Point-intercept estimation of macroalgal cover has been adapted from the U.S. Geological Survey's (USGS's) National Water Quality Assessment (NAWQA) pilot procedures (J. Berkman, *pers comm*.), and assessment of microalgal thickness has been adapted from Stevenson and Rollins (2006). The physical habitat (PHab) methods are identical to those presented in the SWAMP Bioassessment protocol of Ode (2007), with the exception of the point-intercept method for determining algal cover, which is an add-on to the PHab pebble count procedure. The PHab procedures are, in turn, minor modifications of those used in EMAP and developed by EPA's Office of Research and Development (ORD, Peck *et al*. 2006).

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LIST OF ABBREVIATIONS AND ACRONYMS

AFDM	Ash-Free Dry Mass
BMI	Benthic Macroinvertebrate
chl a	Chlorophyll a
CPOM	Coarse Particulate Organic Matter
DO	Dissolved Oxygen
EMAP	Environmental Monitoring and Assessment Program (of the U.S. EPA)
GPS	Global Positioning System
IBI	Index of Biotic Integrity
MCM	Margin-Center-Margin
NAD	North American Datum
NAWQA	National Water Quality Assessment (of the U.S. Geological Survey)
NBO	Neutrally Buoyant Object
NNE	Nutrient Numeric Endpoints
PHab	Physical Habitat
QAPrP	Quality Assurance Program Plan (of SWAMP)
ORD	Office of Research and Development (of the Environmental Protection Agency)
RBP	Rapid Bioassessment Procedures
RWB	Reachwide Benthos
SOP	Standard Operating Procedures
SWAMP	Surface Water Ambient Monitoring Program
TRC	Targeted Riffle Composite

1. INTRODUCTION

This document is the Standard Operating Procedure (SOP) for collecting and field-processing benthic stream algae for the California State Water Resources Control Board's Surface Water Ambient Monitoring Program (SWAMP). Instructions are provided for the following:

- collection of samples for taxonomic identification of benthic diatoms and soft-bodied algae
- collection of samples for determination of biomass based on chlorophyll *a* and ash-free dry mass (AFDM)
- estimation of percent algal cover

The document is designed to serve as a stand-alone SOP if algae are the only bioindicators being assessed at a given site. However, it can also serve as an add-on module to the existing SWAMP SOP for bioassessment using benthic macroinvertebrates (BMIs). Much of the procedure for collecting physical habitat (PHab) data is identical for these two assemblages. However, some PHab elements assessed in conjunction with BMI bioassessment are not included for algal bioassessment, because they are more specific to BMI habitat needs than to algae. Conversely, one PHab element for algal bioassessment (*i.e.*, point-intercept estimation of algal cover) is not part of the BMI SOP. It should also be noted that, while the standard PHab protocol associated with BMI sampling includes both a "Full" and a "Basic" (simplified) version, a distinction between basic and full protocols for algae has not been established.

For quick reference, Table 1 provides a list of elements common and distinct to the two SWAMP bioassessment assemblages. In general, if both BMIs and algae are being collected at a given site, the PHab procedure as described in Ode (2007) should be followed, with the exception of the pebble count, which should be conducted according to this SOP, because it incorporates instructions for algal cover point-intercept data collection. More specifically, if bioassessment involving the Full BMI protocol plus algae is to be implemented at a given site, practitioners should follow the **Full protocol of Ode (2007)**, and **add** only Section **3.4** (re: water chemistry), Section **5**, and Sections **6.9-6.11 from this SOP**.

Please note: This SOP (v6) has not yet been reviewed for approval by the SWAMP Roundtable.

Table 1. Sample and data collection elements included in algal and BMI bioassessment (Ode 2007; Table 1). X indicates elements included in algal bioassessment. F indicates elements that are part of the "Full" protocol for conducting BMI bioassessment, B corresponds to elements of the "Basic" BMI protocol, and O indicates elements that are "Optional".

Element	Algae[1]	BMIs
Layout of reach, marking transects, recording GPS coordinates	X	B, F
Notable field conditions	X	B, F
Temperature, pH, specific conductance, DO, alkalinity	X	B, F
Turbidity, Silica	O	O
Water chemistry for lab analysis (***see list in Section 3.4***)	X	
Algal Sampling for Taxonomic IDs	X	
Algal Sampling for Biomass Assessment	X	O
BMI Sampling for Taxonomic IDs		B, F
Wetted Width	X	B, F
Bankfull Dimensions	X	F
Depth and Pebble Count + CPOM	X	F
Percent Algal Cover (point-intercept with Pebble Count)	X	
Cobble Embeddedness	X	F
Canopy Cover	X	B, F
Gradient	X	B[2], F
Sinuosity		F
Human Influence	X	F
Riparian Vegetation		F
Instream Habitat		F
Bank Stability	X	B, F
Flow Habitat Delineation	X	B, F
Discharge	X	F
Photo documentation	X	B,F
Selected Rapid Bioassessment Procedure (RBP) visuals		F

Depending upon the requirements of the monitoring effort, different components of this SOP might be incorporated or omitted. For instance, if stream productivity in terms of algae is the primary concern of the assessment, one may wish to collect only biomass samples and algal cover point-intercept data. Alternatively, one will need to collect benthic algal assemblages (for quantification of diatom and/or soft-bodied algal taxa) in order to make more refined inferences about water quality and stream condition (*e.g.*, by applying an algal Index of Biotic Integrity (IBI)).

This SOP is organized in such a way as to facilitate the inclusion or omission of certain elements based on the goals of the monitoring effort. A list of field supplies is provided in Appendix A. It is organized according to the materials needed for each type of sampling and data collection. In

[1] A distinction between Basic and Full protocols for algae has not been established.
[2] For BMIs, a single, reachwide measurement of gradient is required for Basic, but gradient is measured at each transect for Full.

order to facilitate decisions about algal indicators to assess for program-specific needs, the introduction to Section 5 discusses what algal indicators serve which monitoring purposes.

2. GETTING STARTED

2.1 When to Sample

It is recommended that sampling for benthic stream algae be carried out during the same period as BMI sampling, generally from May through September, depending on the region. This time frame may eventually be modified (*e.g.*, expanded) based on the results of ongoing index period studies.

It should be noted that high-velocity storm flows can remove macroalgae and biofilms from the stream bottom. To be conservative, it is recommended that sampling be done at least a month after any storm event that has generated enough stream power to mobilize cobbles and sand/silt capable of scouring stream substrates. This will allow ample time for recolonization of any scoured surfaces (Round 1991; Kelly *et al*. 1998; Stevenson and Bahls in Barbour *et al*. 1999).

2.2 Before Setting Out for the Field

- ***Proper field hygiene should be practiced at all times in order to avoid transferring invasive organisms and pathogens between sites.*** This includes the implementation of effective equipment decontamination procedures. Refer to Appendix B for additional information.
- Use the equipment checklist provided in Appendix A to make sure all necessary supplies are brought along.
- Have in mind at least three sites to visit per day (target two, but plan for at least one additional site as a back up if one of the first two sites is not useable.)
- Prepare, and double check, site dossiers to make sure they are complete with maps/directions to sites and scaled aerial photo(s). Bring along county maps, atlases, and Thomas Guides to further aid location of sites. Also bring along any site access permits, passes, and/or keys, as needed (and be aware that some landowners require notice prior to each site visit).

2.3 Before Leaving Vehicle for Site

Make sure the vehicle is parked in a safe spot and there are no "No Parking" signs. Stick a business card with cell phone number in the driver's window. Be sure to display the brown administrative pass placard if you are on National Forest land (or the letter of permission that is in your site dossier, if applicable).

2.4 Determining Whether Site is Appropriate for Sampling

Make an initial survey of the potential monitoring reach from the stream banks (being sure to not disturb the instream habitat). Ensure that there is sufficient water in the stream reach to facilitate collection of algae and water samples. In order for a reach to be in appropriate condition for sampling, at least half of the reach should have a wetted width of at least 1m, and there should be no more than 3 transects that are completely dry. If there is some flexibility in terms of where to place the sampling reach, strive for as few dry transects as possible (and preferably none).

Sites should be safe to sample and legally accessible. The time required to access the sampling sites should also be a consideration in planning which sites to visit, in order to ensure that sample holding times can be met (see Table 2 on page 15 for holding-time information).

3. REACH DELINEATION AND WATER CHEMISTRY SAMPLING

Before sample and data collection can begin, the monitoring reach must be identified and delineated. This requires setting up sampling transects along the stream reach of interest. Once the reach is delineated, information about reach location and condition will need to be documented. Water chemistry parameters must also be recorded, and certain samples collected.

A set of field forms for recording information about monitoring sites, algal samples, and associated water chemistry and PHab data is provided in Appendix C. The field forms are also available in electronic version on an portable computer. It is imperative that you confirm throughout the data collection effort at each site that all necessary data have been recorded on the field forms correctly, by double-checking values, and confirming spoken values with your field partner(s). As a general practice, you should conduct a final check across all datasheets to confirm that there are no missing values before you leave the site, and rectify any blanks.

3.1 Delineating and Documenting the Monitoring Reach

To delineate the monitoring reach, you will need to scout it in its entirety in order to make sure that it is of adequate length for sampling algae. *During this process, try to stay out of the channel as much as possible, to avoid disturbing the stream bottom, which could compromise the samples and data that will be collected.*

SWAMP's standard algae (and BMI) sampling layout consists of a 150 m reach or a 250 m reach, depending upon the average wetted width of the channel. In some circumstances (see below), reach length can be < 150, but this should be avoided whenever possible. If the actual reach length is other than 150 m or 250 m, this should be noted and explained on the field forms. Under these circumstances, you will need to determine the useable length of the reach, and how to space your transects so that you can fit them into the reach at equal distances from one to the next.

The wetted channel is the zone that is inundated with water and the *wetted width* is the distance between the sides of the channel at the point where substrates are no longer surrounded by surface water. Estimate the average wetted width of the reach. If this value is ≤ 10 m, you will end up using 150 m for your monitoring reach length. If the average wetted width is > 10 m, you will use a 250 m long reach.

To set up the monitoring reach, begin a little outside of what you anticipate will be the outer boundary (based on aerials and maps) and count 150 large steps, or 250 large steps (for most adults, a large step is roughly equal to a meter), by walking along the bank. This will give a rough idea about the location of the ends of the study reach. However, keep in mind that once this is determined, the actual distances between transects and intertransects (and consequently, the reach length) will need to be more accurately measured.

As you go, identify where hydrologic inputs that could potentially modify the water chemistry environment occur along the length of the reach. If possible, there should be no tributaries or "end-of-pipe" outfalls feeding into the channel within the monitoring reach. Also, other features that should also not be present within a monitoring reach are: bridge crossings (which shade the

stream bottom and can artificially reduce or prevent algal growth), changes between natural and man-made (*i.e.*, concrete) channel bottoms, waterfalls, and impoundments (dams and weirs). If any of such features occur within the reach, and there is not enough room to accommodate a 150m-reach or 250m-reach entirely upstream or downstream of such a feature, then the reach can be somewhat < 150m. Whatever the reach length turns out to be (150 m, 250 m, or other), record it on the datasheet under "*Reach Length*".

3.2 Marking the Transects

The monitoring reach will be divided into 11 equidistant *main transects* that are arranged perpendicularly to the direction of flow. There will also be 10 additional transects (designated "*inter-transects*"), one between each pair of adjacent main transects, to give a total of 21 transects per monitoring reach. Main transects are designated "A" through "K", while inter-transects are designated by their nearest upstream and downstream main transects ("AB", "BC", *etc.*).

Once you have identified the upper and lower limits of the monitoring reach, determine the coordinates of the downstream end using a Global Positioning System (GPS) set to the North American Datum 1983 (NAD 83),[3] and record this information in decimal degrees (to five decimal places) on the datasheet under "*Reach Documentation*". Install a colored flag at water's edge on one of the banks at this location to indicate the first "main transect", or "A". Establish the positions of the remaining transects and inter-transects by heading along the entire length of the monitoring reach (again, staying out of the water/channel as much as possible) and using the transect tape or a segment of rope of appropriate length to measure off successive segments of 7.5 m (for streams of wetted width ≤ 10 m), or 12.5 m (for streams > 10 m wetted width). For monitoring reaches of non-standard length, you will divide the total, targeted length of the reach by 20 to derive the distance between the adjacent main, and inter-, transects. As you measure off the distances, always follow the virtual, mid-channel line, and not the water's edge (which may be irregular, and not reflective of the true stream curvilinear distance).

At the end of each measured segment as you head along the stream, mark the transect location on the bank with a flag. We recommend to alternate between two different flag colors (*e.g.*, *orange* could correspond to *main transects*, and *yellow* to *inter-transects*.) Determine transect orientations, and where on the banks to place the flags, by visually projecting perpendicularly from the mid-channel to the banks. Refer to Figure 1 for a visual clarification of proper transect alignment relative to the stream's direction of flow. When you have finished, the downstream-most flag will correspond to main transect "A", and the upstream-most flag (the 21st in the entire series of main and inter- transects) will correspond to main transect "K".

[3] Be aware that some GPS units re-set themselves to factory default settings when the batteries are changed. This can include the datum. Therefore, anytime you remove batteries from your unit, double check that the unit is still set to the NAD83 datum after the batteries have been replaced.

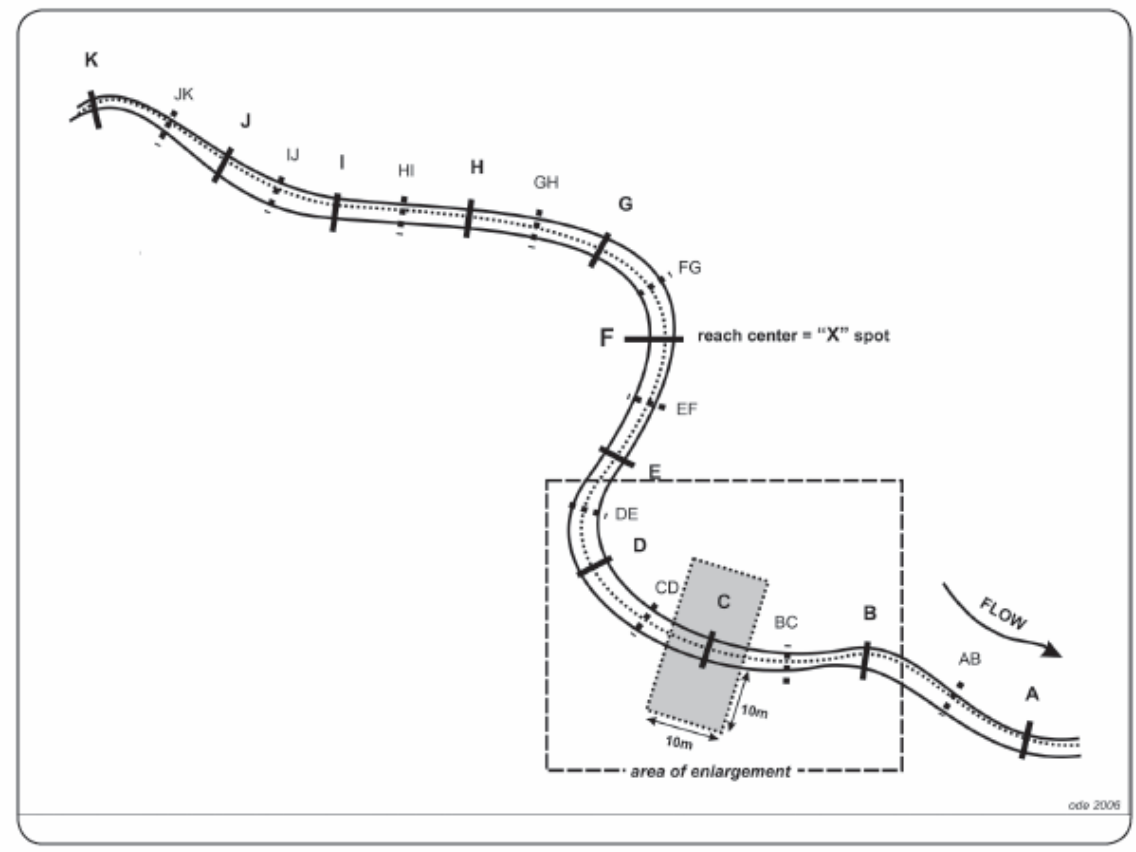


Figure 1. Reach layout geometry for physical habitat (PHab) and biological sampling showing positions of 11 main transects (A-K) and the 10 supplemental inter-transects (AB-JK). The area highlighted in the figure is expanded in Figure 11. *Note*: reach length = 150 m for streams ≤ 10 m average wetted width, and reach length = 250 m for streams > 10 m average wetted width (reprinted from Ode 2007).

3.3 Notable Field Conditions

Record under "*Notable Field Conditions*" any evidence of recent flooding, fire, or other disturbances that might influence algae samples. Especially note if flow conditions have been affected by recent rainfall, which can cause significant under-sampling of algal biomass and diversity. If you are unaware of recent fire or rainfall events, select the "no" option on the forms. Record the dominant land use and land cover in the area surrounding the reach by evaluating land cover within 50 m of either side of the stream reach. You can use a scaled aerial photograph of the site and vicinity to guide you. Note that before heading out to the field, it is convenient to add a 150 m (or 250 m) line adjacent the stream to be sampled in order to get an idea about the anticipated approximate upstream and downstream boundaries of the monitoring reach.

3.4 Water Chemistry

Measure and record common ambient water chemistry measurements (pH, dissolved oxygen (DO), specific conductance, alkalinity, and water temperature) just outside of the reach, at the downstream end, near the same location that the GPS coordinates were taken. This should be done in such a way that it does not interfere with biotic sampling and PHab data collection, but

also in such a way that water samples are not compromised by other sampling activities upstream (*e.g.*, by suspension of matter from the stream bottom into the water column, and consequently the introduction of this matter into the water chemistry samples).

Water chemistry measurements are typically taken with a handheld, water-quality meter (*e.g.*, YSI, Hydrolab), but field test kits (*e.g.*, Hach) can provide acceptable information if they are properly calibrated. For appropriate calibration methods and calibration frequency, consult the current SWAMP Quality Assurance Program Plan (QAPrP)[4], or follow manufacturer's guidelines. *Note 1:* If characteristics of the site prohibit downstream entry, measurements may be taken at other points in the reach. In all cases, ambient chemistry measurements should be taken at the start of the survey (*i.e.*, before algae sampling and PHab data collection). *Note 2:* Programs should consider collecting lab samples for sites with low-ionic-strength waters, as alkalinity test kits may not perform well at such sites (consult the SWAMP QAPrP for more detailed information).

A suite of analytes must also be evaluated to aid in interpretation of the algal data. These are listed below. Consult the SWAMP QAPrP for specific instructions on the proper techniques for collecting, preserving, and storing these water samples until analysis.

- Nitrate as N (NO_3)
- Nitrite as N (NO_2)
- Ammonia as N (NH_3)
- Nitrogen, Total (TN)[5]
- Orthophosphate as P (dissolved; SRP)
- Phosphorous as P (total; TPHOS)
- Dissolved Organic Carbon (DOC)
- Chloride (CL)

- Silica as SiO_2, dissolved (*Note:* this analyte is recommended for research purposes, but is not part of the standard algae protocol)

[4] This document is available online from the SWAMP website:
http://www.swrcb.ca.gov/water_issues/programs/swamp/docs/qapp/swamp_qapp_master090108a.pdf
[5] TKN (Total Kjehldahl Nitrogen can be calculated from TN and NO_3.

4. REACHWIDE BENTHOS SAMPLING OF ALGAE

The following is a short introduction of several types of algal indicators that can be monitored as part of a bioassessment effort. For a more detailed discussion, see Fetscher and McLaughlin (2008). The most appropriate indicators to include in a given program will ultimately depend upon that program's goals, because the various indicators provide information at varying levels of resolution and applicability to different uses. Likewise, the various indicators require different levels of investment in terms of fieldwork and lab work. Percent algal cover, for instance, is a rapid means of estimating algal primary productivity that can be carried out entirely in the field and is conducted in tandem with the PHab pebble count. Other estimators of algal biomass include chlorophyll *a* and AFDM, which involve quantitative collection of algae, preservation, and subsequent laboratory analysis. Algal biomass is a key component of the California Nutrient Numeric Endpoints (NNE) framework. Higher resolution information about algal assemblages can be used in algal IBIs, and offers more in-depth insight into water quality. For this type of data, algal specimens must be collected quantitatively (and qualitatively, in the case of soft-bodied algae). The quantitative samples are then fixed/preserved carefully and subjected to taxonomic analysis.

While the percent algal cover data are recorded in conjunction with standard PHab procedures, and do not require the collection of samples, all the other types of data described in this protocol require reachwide benthos (RWB) sampling of algal specimens in a manner analogous to that which is carried out for BMIs.

All four of the algal samples described in this SOP: chlorophyll *a*, AFDM, diatom assemblage, and soft-bodied algal assemblage, can be obtained from a single composite sample generated by the RWB method. Which combination of these samples to prepare and submit to laboratory processing will depend on the needs of the monitoring program. To aid in the selection of algal indicators, Table 2 provides a summary of their attributes.

Table 2. Types of algal indicators and considerations for their assessment.

	Algal indicator for	Collection method	Collection vessel	Preservation / fixation method / holding times	Qualitative live sample required?
Percent Algal Cover	Stream productivity measured as algal abundance	Point-intercept add-on to the PHab pebble count	N/A	N/A	N/A
Chlorophyll a[6]	Stream productivity measured as algal biomass; key indicator for the Nutrient Numeric Endpoints (NNE) framework	RWB sample collection	Glass-fiber filter	Wet ice, dark (foil-wrapped); Freezing within 4h, and filter analysis within 28d	N/A
AFDM	Stream productivity measured as biomass of organic matter (including algae); indicator for the NNE framework	RWB sample collection	Glass-fiber filter (pre-combusted[7])	Wet ice, dark (foil-wrapped); Freezing within 4h, and filter analysis within 28d	N/A
Diatoms	Used in IBIs. Indicative of factors such as trophic status; organic enrichment; low DO; siltation; pH; metals	RWB sample collection	50 mL centrifuge tube	Add 10% buffered formalin for a 2% final concentration immediately after collection; keep dark and away from heat	Optional
Soft-bodied algae[8]	Used in IBIs. Indicative of factors such as nitrogen limitation/ trophic status; siltation; pH; temperature, light availability, nuisance/ toxic algal blooms	RWB sample collection	50 mL centrifuge tube	Keep unfixed samples in dark on wet (NOT DRY) ice; add glutaraldehyde (to a 2.5% final concentration) as soon as possible, but no later than 4 days after sampling; after fixing, keep dark and away from heat	Required

[6] It is valuable to assess both chlorophyll *a* **and** phaeophytin *a* (the degradation product of the former) content of benthic algal samples, as this may provide a more robust assessment of algal biomass.
[7] Precombustion is recommended in order to remove any possible residual organic matter from the filter.
[8] For the purposes of this SOP, the soft-bodied assemblage includes cyanobacteria (an explanation of the rationale for this is provided in Fetscher and McLaughlin 2008)

4.1 General Considerations for Sampling Benthic Algae

This SOP describes the RWB method for collecting benthic algae. It employs an objective approach for selecting sub-sampling locations that is built upon the 11 main transects described in the previous section. This approach is analogous to the SWAMP procedure for BMI sampling (Ode 2007), and is ultimately based on EPA's Environmental Monitoring and Assessment Program (EMAP; Peck, *et al.* 2006). After collection, the 11 sub-samples are composited into a single sample per site (sampling reach).

The RWB method can be used to sample any wadeable stream reach since it does not target specific habitats. Because sampling locations are defined by the transect layout, the position of individual sub-sampling spots may fall within a variety of "erosional[9]" or "depositional[10]" habitats, each of which has implications for the type of substrate likely to be encountered and therefore the type of sampling device to use.

For the RWB method, the sub-sampling position alternates between left, center, and right portions of the transects, as one proceeds upstream from one transect to the next. These sampling locations are defined as the points at 25% ("left[11]"), 50% ("center") and 75% ("right") of the wetted width in high-gradient systems, and at "margin-center-margin" (MCM) positions in low-gradient systems. The RWB-MCM method should be only used in low-gradient streams where channel substrates are nearly uniform, resulting in low diversity within the channel. The interim cut-off between "low" and "high" gradient is 1%. Best professional judgment can be used to estimate whether the stream reach should be treated as low- or high-gradient. However, if there is uncertainty about the gradient, it should be measured prior to collecting the biotic assemblage samples. See Section 4.2 for specific instructions about where algae sampling locations should be positioned at the margins of low-gradient sampling reaches.

Algae should be sampled prior to PHab data collection (described in Sections 6-8), so as not to disturb the algae by trampling the transects, as occurs during the PHab process. Furthermore, to avoid disturbing the transects for eventual collection of PHab data, as with BMIs, algae should be collected at a location that is systematically offset from each transect (see Section 4.2 below).

4.2 Collection of Algae in Conjunction with Benthic Macroinvertebrates

If only algae (or only BMIs) are being collected for bioassessment, then the specimens should be collected 1 m downstream of the transects. If *both* assemblages are being sampled, then the algae should be collected above the spot where the BMIs are collected, according to the schematic in Figure 2. BMIs must be collected BEFORE algae at each of the transects, in order to minimize the chances disturbing BMIs during algal collection. After the BMIs are collected at each spot,

[9] Erosional – habitats in the stream that are dominated by fast-moving water, such as riffles, where stream power is more likely to facilitate erosion (suspension) of loose benthic material than deposition; examples of "erosional" substrates include cobbles and boulders.
[10] Depositional – habitats in the stream that are dominated by slow-moving water, such as pools, where deposition of materials from the water column is more likely to occur than erosion (or (re)suspension) of bed materials; examples of "depositional" substrates include silt and sand.
[11] For our purposes, "left" is defined as the left bank when facing *downstream*.

the algae sample should be taken ¼ m upstream from the center of the upper edge of the "scar" in the stream bottom left from the BMI sampling. *It is important to make sure that the surface from which algae will be collected has not been disturbed (by the BMI sampling, or otherwise) prior to sampling the algae*.

Note that if only algae (and not BMIs) are being collected in a low-gradient reach, the collection location should be 1 m downstream of the transect and, for each of the "margin" positions, at a distance of 15 cm from the wetted margin of the bank. Fifteen centimeters is chosen because it is approximately ½ the width of a D-frame net. Remember: never use a brush as sampling device for BMIs.

If duplicates are to be sampled (of either or both assemblages), locations for sampling them should be arranged as depicted in Figure 2 (the duplicates are shown in light grey). Note that for convenience, only Transects A through C are shown, but the same pattern of placement should be rotated across all 11 transects.

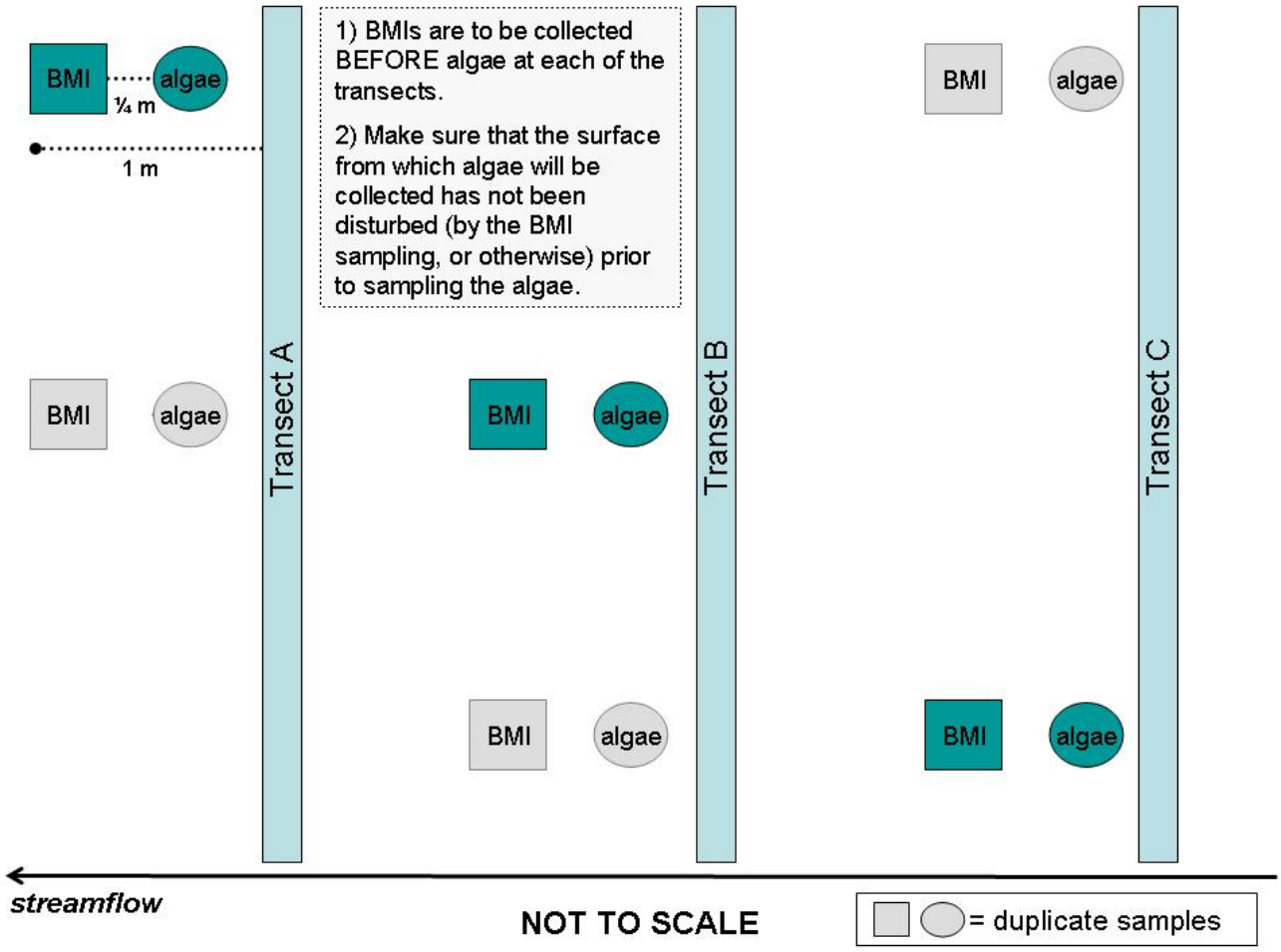


Figure 2. Sampling array for collection of algae, BMIs, and duplicates of each assemblage. For convenience, only Transects A through C are shown, but the same pattern of placement should be rotated across all 11 transects.

4.3 Procedure for Collection of Quantitative Algal Samples

During all phases of algae sampling and processing, in order to preserve specimen integrity, every attempt should be made to keep the sample material out of the sun, and in general, to *protect the algae from heat and desiccation*, as much as possible. This is necessary in order to reduce the risk of chlorophyll *a* degradation, limit cell division post-collection, and curb senescence/decay of live soft-bodied algae (especially for the qualitative samples; see Section 5.4). The need to maintain the integrity of the algal samples during collection and processing should always be borne in mind when planning the sampling scheme for a given site.

In addition, before sampling at any given site, *the dish tub that will contain sample material must be scrubbed with a stiff-bristled brush or scouring pad and thoroughly rinsed* with stream water, so that no algal material is carried over from the previous site to contaminate the current sample. The *same applies to all other algae sampling apparati* (toothbrushes for scrubbing, graduated cylinders, turkey basters, PVC and rubber delimiters, spatulas, syringe scrubbers, *etc.*).

4.3.1 Identifying the Sampling Locations

As with BMIs, algae sample collection should begin at Transect A and proceed upstream to Transect K. Except in circumstances in which the substrate to be sampled cannot be removed from the stream, a single sample of substrate material that corresponds to the objectively determined sampling point is gathered at each transect and placed in the plastic dish tub[12]. Proceeding from transect to transect with the dish tub, the sample collector rotates through the three collection positions in the following order: left at the first transect ("A"), center at the next transect ("B"), right at the next transect ("C"), then back to the left side ("D"), and so on through Transect K.

As substrates are gathered, a tally is taken of the number of samples that correspond to each of the classes of sampling device based on the surface area they sample: 1) *12.6 cm*^{2} for the PVC or rubber delimiters, and 2) *5.3 cm*^{2} for the syringe scrubber. The tallies are recorded in the *Algae Samples* field form under *Collection Device*. This information will ultimately be used to determine total stream surface area sampled at each site, which in turn will be used to calculate the soft-bodied algal biovolume and the biomass values. It may be helpful to use a tally meter in order to avoid having to carry a datasheet during substrate collection.

4.3.2 Collecting Erosional Substrates

If the substrate type that falls under the sampling spot is in erosional habitat and can be removed from the stream (*e.g.*, a cobble, a piece of wood, or a piece of coarse gravel with an exposed surface area of at least 12.6 cm^2), carefully lift the substrate, moving slowly in an effort to disturb its top surface as minimally as possible, and remove it from the stream. Then wipe any excess sand, silt, or BMIs, if present, off the ***bottom*** of the piece of substrate, and place it in the dish tub. It is helpful to place the substrate in such a way that makes it obvious what surface was facing upward when it was removed from the stream. Eventually, when you isolate a sample of algae from this substrate, you will want to obtain your sample from the portion of the substrate that had been exposed to the surface of the stream (and not buried) during the period leading up

[12] If preferred, a white, plastic 5-gallon bucket could be used instead of the dish tub.

to the sampling event. *For pieces of substrate with an exposed surface area that is < 12.6 cm^2, the PVC delimiter should be used (Section 4.3.3).*

Be sure to place the substrate (e.g., cobble) in the dish tub in such a way that surfaces covered with non-target algae are not rubbing against anything, which could cause non-target algae to slough off into the tub, thus artificially inflating the amount of algae collected. To avoid this problem, and especially if a large number of cobbles are likely to be sampled across a given stream reach, one may choose to isolate the algal specimen from each cobble as it is selected, rather than collecting all the cobbles into the dish tub and then isolating the algal specimens from them after all transects have been sampled. See Section 4.3.9 for further elaboration on this alternative approach.

4.3.3 Collecting Depositional Substrates

If the substrate type that falls under the sampling spot is removable and is in depositional habitat (*e.g.*, silt, sand, fine gravel), and/or has an exposed surface area per particle that is < 12.6 cm^2, you will use a PVC delimiter. This is a plastic coring device with an internal diameter of 4 cm (Figure 3). Instructions for making a PVC delimiter are provided in Appendix D.

Figure 3. PVC Delimiter

Isolate a specific quantity of sand/silt/gravel, centered on the sampling spot, by pressing *into the top 1 cm* of sediment with a PVC delimiter. Gently slide a masonry or kitchen spatula beneath the delimiter, being careful to keep the collected sediment contained within. Pull the PVC delimiter out of the water (with the spatula still in place) and remove any extra sediment from the spatula around the outside of the delimiter. Transfer the contents held in the delimiter by the spatula to the dish tub. *Be sure not to pour the sediment sample on top of any cobbles that may be in the dish tub, as this could result in the sloughing of non-target algae from the cobbles into the dish tub, thus artificially inflating the amount of algae collected.*

4.3.4 Collecting Sections of Macroalgae

If the substrate you hit on a given transect is a mass of macroalgae (including an unattached, floating mat that is believed to be native to the reach being sampled, and not imported from upstream), position the spatula directly under the macroalgae and press the PVC delimiter into the algae to define a 12.6 cm^2 area. Use a razor blade to remove and discard any extra material from around the edges of the delimiter and then add the macroalgal specimen that was isolated by the PVC delimiter to the dish tub.

4.3.5 Collecting Sections of Macrophytes

If the substrate to be sampled is part of an immersed macrophyte, or old, dead leaves settled at the bottom of a pool, use the PVC delimiter/spatula combination to isolate a 12.6 cm^2 section of substrate that has been exposed to the surface of the stream. As with the macroalgae (Section 4.3.4), cut away and discard the extra material that falls outside the delimiter using a razor blade.

4.3.6 Collecting from Concrete, Bedrock, and Boulders

If the substrate falling under a sampling spot cannot be removed from the water (as in the case of bedrock, a boulder, or a concrete channel bottom), use a "syringe scrubber" device (Davies and Gee 1993; Figure 4) to collect an algae sample underwater. Instructions for making a syringe scrubber are provided in Appendix D.

Figure 4. Syringe Scrubber.

To use this device, affix a fresh, white scrubbing pad circle onto the bottom of the syringe plunger using the Velcro hooks on the end of the plunger. Press the plunger down so that the bottom of the scrubbing pad is flush with the bottom of the barrel. Then submerge the instrument, press the syringe firmly against the substrate, and rotate the syringe scrubber 3 times in order to collect the biofilm from the substrate surface onto the scrubbing pad. If the surface of the substrate where your sampling point fell is not flat enough to allow for a tight seal with the syringe barrel, objectively choose whatever sufficiently flat area on the exposed face of the substrate is *closest* to where the original point fell, and sample there.

After sampling, and before removing the syringe scrubber from the substrate, gently retract the *plunger* just slightly, so it is not up against the substrate anymore, but not so much that it pulls a lot of water into the barrel. Carefully slide the spatula under syringe barrel (which should be pulled just slightly away from the substrate on one side to allow the spatula to slide under), trying not to allow too much water to rush into the barrel. Then pull the instrument back up out of the water with the spatula still firmly sealed against the syringe-barrel bottom.

Hold the syringe scrubber over the dish tub and then remove the spatula, allowing any water to fall into the tub. Carefully detach the pad from the plunger and hold the pad over the tub. Using rinse water *sparingly*, remove as much algal material from the pad as possible by rinsing it off with the wash bottle, or a turkey baster, filled with stream water (from the current site—never carried over from a previous site), and wringing it into the dish tub before discarding the used pad. Start this process by rinsing from the backside of the pad (the side that had been affixed to the plunger) to "push" the collected algae forward out of the front surface of the pad.

It is recommended that a fresh (new) pad be used each time a sample is collected, even within the same stream reach. Under no circumstances should the same pad be used at more than one site.

4.3.7 Collecting from Other Substrate Types

If other substrate types are encountered, they can be sampled from as long as there is good reason to believe that they were not recently introduced into the stream (*e.g.*, by flowing from the upstream regions, or by recently falling into the stream), as they would then not be representative of the local instream environment. Use the collection instrument you deem to be most appropriate to sample the substrate and, as with any substrate, be sure to account for the surface area sampled (in this case, using the "*Other"* box on the *Collection Device* portion of the field forms).

4.3.8 Removal of Algae from Collected Substrates

After having sub-sampled substrates across the monitoring reach, there should be 11 transects' worth of material in the dish tub. Depending on the types of habitats in the stream and substrates encountered, the tub may contain cobbles, and/or sand, and/or gravel, and/or small pieces of wood or macrophyte. Now a measured quantity of the algae clinging to these substrates must be removed and suspended in water to form a "composite sample" according to the instructions in the following sections.

For erosional substrate types that were removed from the stream (*e.g.*, cobbles and small pieces of wood), use a rubber delimiter to isolate a 12.6 cm^2 area from which algae will be removed. A rubber delimiter can be made from a mountain bike tire with a hole cut out and reinforced with an appropriately sized rubber washer (Figure 5). Appendix D describes the procedure for making a rubber delimiter.

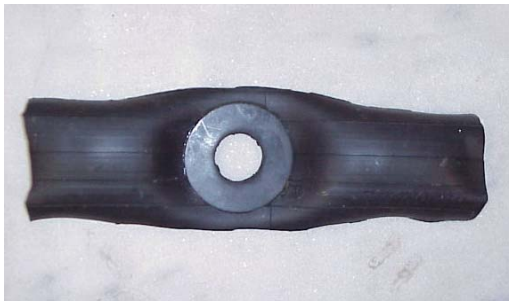


Figure 5. Rubber Delimiter

Wrap the rubber delimiter around the substrate to expose the desired sampling surface through the hole. *Take care to ensure that the surface that will be scrubbed is truly the **upper** (generally at least somewhat "slimy") surface of the substrate as it had been oriented in the stream.* Dislodge attached algae from this area by brushing it with a firm-bristled toothbrush (*remember that this toothbrush must first have been thoroughly rinsed since the previous site to avoid contamination with algal specimens from other streams*). If there is a thick mat of algae, or the algae is firmly encrusted on the surface of the substrate, use forceps or a razor blade first to dislodge the larger matter and put this in the dish tub. Then scrub the area with the brush.

Make sure that the entire surface within the delimiter has been scrubbed well in order to remove all the algae in that area. Fill a wash bottle or turkey baster with stream water from the current site (*never carried over from a previous stream*). Using as minimal a volume of water as possible, rinse the scrubbed algae from the sample area into the dish tub. Take care to squirt water only on the surface that is showing through the hole in the delimiter, and not anywhere else on the substrate's surface. It is helpful to invert the rock when rinsing so that the target surface is facing down toward the dish tub, and the rinsate drips off the sampling spot directly into the tub rather than flowing along the (non-target) sides of the substrate. *Use water sparingly for each piece of substrate, because you should attempt to use no more than 400-500 mL **total** for the full suite of 11 samples collected along the transects (this includes any water used for rinsing algae off of sampling devices into the dish tub).* After scrubbing is complete, rinse the delimiter and the brush into the dish tub, also. The scrubbed part of the substrate should feel relatively rough when you have finished, meaning that essentially all of the algae have been removed. After the sampling area on the piece of substrate has been thoroughly scrubbed and rinsed, the piece of substrate can be returned to the stream.

For depositional samples (*e.g.*, silt, sand, or gravel), there is no need to isolate a specific area of the substrate within the dish tub, because the sample area was pre-isolated by using the PVC

delimiter during collection. Simply massage all the sand and/or silt in the dish tub thoroughly between the fingers to dislodge any clinging algae. For pieces of gravel, use a toothbrush to remove algal material from surfaces.

Rinse the sediment thoroughly (but as sparingly as possible) with stream water so as to create a suspension of the dislodged microalgae (*i.e.*, the sample). The final volume of the sample *liquid* in the dish tub will be measured before the algal taxonomic and biomass samples are prepared (described below). To do this, the liquid in the tub will be separated from the rinsed sediment such that the volume measured does not include sediment. After the liquid sample has been retrieved and measured, the rinsed sediment will be discarded back into the stream.

Other types of substrate, like pieces of macrophyte or dead leaves that had been collected with the PVC delimiter, should also be massaged between the fingers and rinsed into the tub in order to remove the algae coating them.

For macroalgal clumps there is a special step required for processing the samples. This procedure is described in detail in the next section.

4.3.9 Alternative Approach: Processing Samples at Each Transect

It is also acceptable to isolate the algal specimens from each "piece" of substrate collected before moving on to the next transect. This approach has the disadvantage of requiring that all algae sampling/scraping tools be carried along with the collector as s/he proceeds up the stream, and that s/he pause to isolate the algae several times across the stream reach rather than one time at the end of all the transects. However, it limits the amount of substrate material that needs to be carried in the dish tub, thus making it lighter. This could be particularly important if a large number of cobbles are encountered across sampling points, such that it could be difficult or impossible to carry them all to Transect K, or to carry them in such a way that non-target algae can easily be prevented from sloughing off into the tub *via* abrasion. For convenience, one may elect to wear a fisherman's vest to facilitate carrying all the algae sampling/scraping tools that will need to be brought along on the substrate sampling trip if employing this alternative approach.

5. ALGAL SAMPLE PROCESSING

Four different types of laboratory samples may be prepared from the composite sample:

- ID/enumeration samples
    1. diatoms
    2. soft-bodied algae
- biomass samples
    3. chlorophyll *a* ("chl *a*") sample
    4. ash-free dry mass ("AFDM")

5.1 General Considerations for Processing Benthic Algal Samples

The general process for sample preparation is as follows. The ID/enumeration samples are each aliquoted into 50-mL centrifuge tubes and chemically fixed (preserved). Diatom samples are fixed in the field with formalin immediately following collection, and soft-bodied algae samples are fixed in a laboratory with glutaraldehyde (within four days of collection). The chlorophyll *a* and AFDM samples are collected on filters in the field and stored on wet ice, and then frozen as soon as possible after returning from the field (and within four hours of collection). The filters are kept frozen until analysis, which should occur within 28 days of collection. If the field crew is spending the night in a hotel, it is necessary to buy dry ice to freeze the biomass filters upon finishing the day's fieldwork, and to keep them on dry ice until the samples can be transferred to the freezer back at the lab.

Algae sample labels are shown in Figure 6. Recorded on each sample label are the volume of the composite sample (composite sample described in Sections 5.2.1 and 5.3.2), as well as the volume of *sample* aliquoted (for the taxonomic ID samples) or filtered (for the chlorophyll *a* and ADFM samples). All of these volumes should be recorded on the field forms, as well, under the *Algae Samples* section. On the sample labels, the sample type: "chl *a*", "AFDM", "diatoms", or "soft" is circled, and all the remaining information on each label, like Site Code, Date, and site coordinates is filled out.

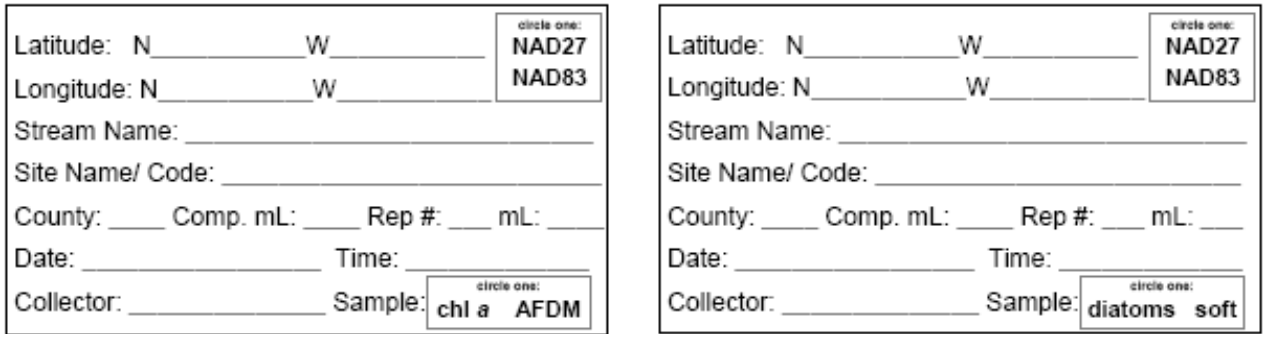


Figure 6. Labels for biomass and taxonomic identification samples.

Before preparing the algae samples it is necessary to determine two things:

- **Are there any clumps of macroalgae in the composite sample (as opposed to just microalgae suspended in liquid)?**

AND

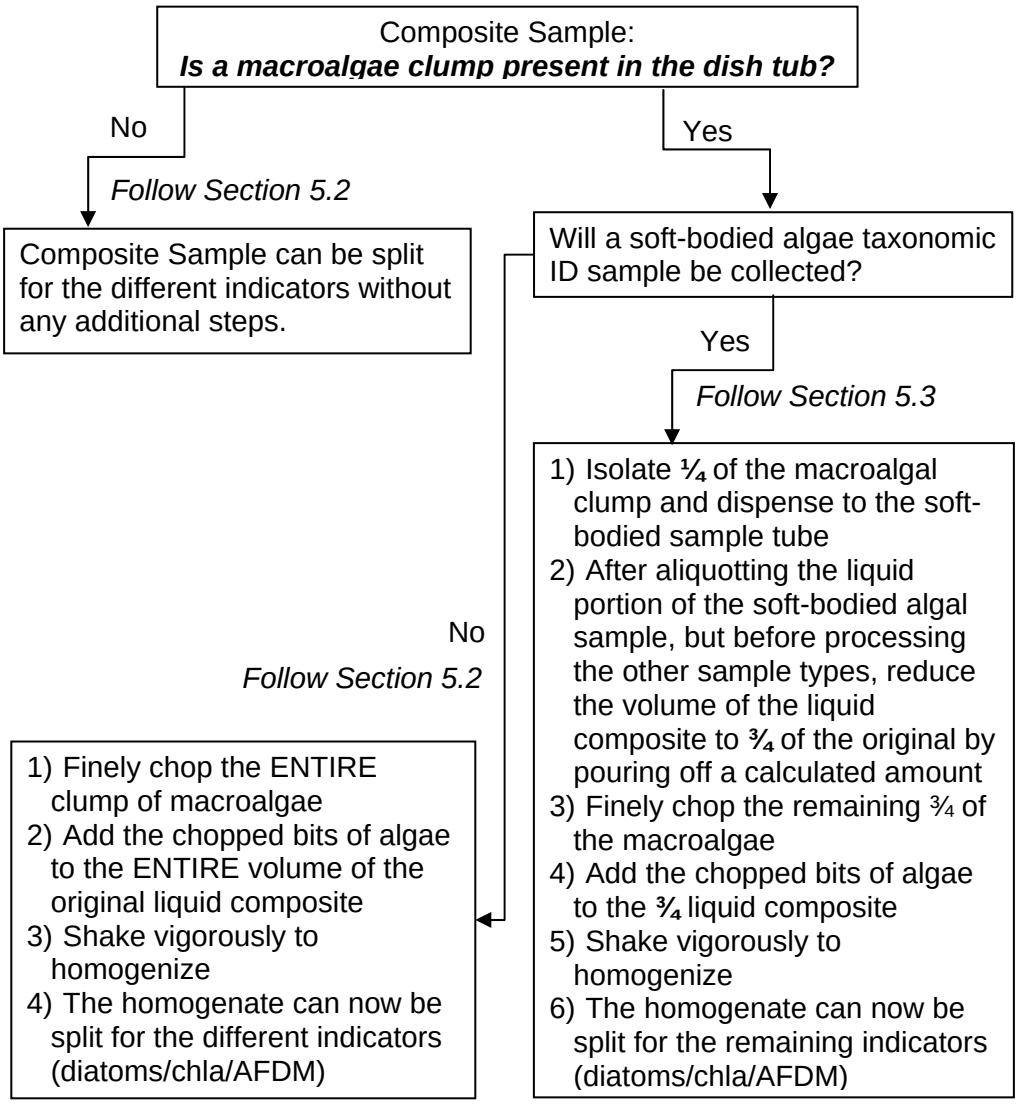
- **Is a soft-bodied algal taxonomic sample going to be prepared?**

The answers to these questions will determine the course of action for preparing the algae samples for a given site:

- If there is no macroalgal clump, liquid composite sample will simply be added to each taxonomic ID sample tube (40 mL for diatoms and 45 mL for soft-bodied algae). Biomass samples will also be prepared using the liquid composite sample, as is.
- If there is a macroalgal clump present, but no soft-bodied sample will be prepared, the entire clump will be chopped into fine bits and incorporated directly into the liquid portion of the composite sample, and the mixture will be shaken to homogenize it before preparing the diatom and/or biomass samples.
- If there is a macroalgal clump AND a soft-bodied algal taxonomic ID sample is to be prepared, then a more complex procedure must be employed in order to properly process the macroalgae before preparing the various samples.

Table 3 provides a summary of the various sample-processing steps that are involved, and the following sections describe the procedures in detail. Use Section 5.2. if there is NO macroalgal clump present in the dish tub OR soft-bodied algal sample will be NOT be prepared. Use Section 5.3 if there IS a macroalgal clump present AND a soft-bodied algal sample will be prepared.

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



5.2 Sample Processing when there is no Macroalgal Clump OR when no Soft-Bodied Sample is being Prepared

This section describes the sample-processing procedure for the situation in which there was either 1) no macroalgal clump in the dish tub containing the composite sample material, or 2) no soft-bodied algal sample will be prepared. If there was no macroalgal clump but both soft bodied algae AND other sample types are to be prepared, follow all the instruction in this section with the exception of the final portion of Section 5.2.1 that is in <brackets> and discusses how to process macroalgae when preparing only diatom and/or biomass samples.

If there is a macroalgal clump, but no soft-bodied algae sample is to be collected, follow the instructions in this section, including the bracketed portion of Section 5.2.1, and skip Section 5.2.2, which deals with soft-bodied algal sample processing.

5.2.1 Measuring the Composite Liquid Volume

Once algal specimens have been removed from all the substrates (*e.g.*, sand, gravel, cobble, wood, leaves) in the dish tub, according to the procedure described in Section 4, thoroughly agitate the liquid to get as much as possible of the microalgae into suspension, and then immediately pour the liquid into a CLEAN graduated cylinder to measure its volume. Try to leave all substrate material (*e.g.*, silt, sand) behind. Transfer the measured liquid into a CLEAN 1L plastic bottle. Using a minimal amount of stream water, rinse the substrate once or twice with stream water until it appears that little to no additional suspended material (microalgae) is coming off. Add this rinsate to the graduated cylinder to measure it also. If necessary, repeat this process (regularly agitating the dish tub) until all the liquid has been measured and transferred to the sample bottle. *Use water sparingly, because the total sample volume (plus rinsate) should be no more than about 400-500 mL.*

Because you are leaving as much as possible the silt, sand, and any large substrate material behind, the final volume should reflect only the liquid component of the sample plus rinse water. Record the total volume of all the liquid that had been in the dish tub, including any that was used for rinsing the substrates and sampling devices, on the field sheet under the *Algae Samples* section. This is the **COMPOSITE VOLUME**. This value will also be recorded on all algae sample labels (*i.e.*, for the diatom and soft-bodied algae taxonomic ID samples, the chlorophyll *a*, and the AFDM).

< Note: if no soft-bodied algae sample is to be prepared, but there is a macroalgal clump in the sample, separate the clump from the liquid portion of the sample, measure and record the composite volume of the liquid (as described above), then cut the macroalgal clump into very fine pieces with CLEAN scissors and add these pieces to the composite liquid. The pieces should be chopped small enough so that they practically "blend" into the liquid (*i.e.,* distinct fragments of macroalgae are not easily discernible), because the goal is to "homogenize" the macroalgae into the liquid as much as possible. Shake vigorously to homogenize the macroalgal fragments into the liquid. Then proceed to Section 5.2.3 and beyond to prepare the diatom and/or biomass samples.**>**

5.2.2 Preparing the Soft-Bodied Algae Taxonomic ID Sample

Pour freshly-agitated liquid composite sample into the soft-bodied algae sample tube to the 45 mL mark. Midway through pouring, the composite sample should be swirled some more (first clockwise, then counter-clockwise) to ensure that the microalgae are still fully suspended. Cap the tube tightly. Completely fill out a sample label and affix it to the sample tube. Cover the label completely with clear plastic tape to prevent the writing on the label from smearing. Place the tube in the wet ice chest to keep it in the dark and as cold as possible, but make sure it is never allowed to freeze.

As soon as possible, and no longer than four days after collection of the sample, glutaraldehyde (to a final concentration of 2.5%) must be added to the tube and distributed throughout the sample by agitation and turning the tube upside down repeatedly. Glutaraldehyde is necessary for fixing soft-bodied algal samples in order to preserve fine morphological features and the color of pigments, as both can be crucial characters for taxonomic determination. *Glutaraldehyde is a hazardous substance that poses a number of safety risks.* As such, it should be handled in a fume hood by trained personnel wearing appropriate gear. Refer to Appendix E for an SOP for the use of glutaraldehyde.

Members of the field crew can either have the glutaraldehyde added to the samples back at their own lab, or arrange for the glutaraldehyde to be added to the samples by the taxonomy lab. In either case, the unfixed samples must be kept in the dark and on wet ice (but not allowed to freeze), and must be fixed within four days of collection (and preferably sooner). If the taxonomy lab will be adding the fixative, it is imperative to plan ahead to arrange for this to be done, and also to clearly mark which tubes will need to have fixative added to them. Once the samples are fixed, it is no longer imperative to store them on wet ice. Following fixation, they can simply be stored in a cool, dark place.

5.2.3 Preparing the Diatom Taxonomic ID Sample

Diatom samples should be fixed as soon as possible after collection to reduce the possibility of cell division post-sampling. A 10% solution of buffered formalin is used to fix diatoms, and instructions for preparing this solution are provided in Appendix D.

To prepare the diatom sample, aliquot 40 mL of freshly-agitated composite liquid into the diatom ID sample tube, swirling the composite sample bottle again midway through pouring to keep the microalgae suspended. Add 10 mL of the 10% buffered formalin to the sample. This can be done using a small syringe or bulb pipette. Alternatively, if preferred, the centrifuge tubes for the diatom samples can be pre-loaded with 10 mL of the 10% buffered formalin and 40 mL of sample can be added carefully to the fixative, to avoid having to dispense the fixative in the field.

Fixatives such as formalin must be used with great care. Be sure to wear formalin-safe gloves and safety goggles when using the fixative, as it should never be touched with bare hands or allowed to splash onto skin or into eyes. Also make sure it is used only in a very well-ventilated place and avoid breathing in any fumes. Minimize the amount of time that vessels containing formalin are open. Fixative added to the sample must not be allowed to ooze outside the vessel

that contains it, including the sample tubes. Refer to Appendix F for an SOP for the use of formalin.

Cap the tube tightly and shake it to mix the formalin into the sample. Fill out a sample label and affix it to the sample tube. Cover the label completely with clear plastic tape to prevent the writing on the label from smearing. Keep the fixed diatom samples in the dark and away from extreme heat.

5.2.4 Preparing the Biomass Samples

The remaining composite sample liquid can be used to prepare the chlorophyll-*a* and AFDM filters as described below.

Chlorophyll *a* samples: The procedure to filter chlorophyll *a* samples should be carried out quickly, and in the shade as much as possible, to minimize exposure of the sample to light, and minimize chlorophyll *a* degradation thereby. For the chlorophyll *a* samples, use CLEAN filter forceps (rinsed with DI water three times) to center a glass fiber filter (47 mm, 0.7 µm pore size) onto the mesh platform of a CLEAN filtering tower apparatus (rinsed with DI water three times), and rinse the filter a little with DI water to seat it well into the mesh before attaching the filter reservoir on top. Never touch the filters with hands or anything other than clean forceps.

Agitate the composite sample to resuspend all the microalgal material. Carefully measure 25 mL using a small, CLEAN graduated cylinder (rinsed with DI water three times). Midway through pouring the 25 mL, swirl the composite sample again to ensure that the material is still fully suspended. Pour the remainder of the 25 mL, and then pour the measured sample into the filter reservoir. Once empty, rinse the graduated cylinder with a few mL of DI water, and add this to the reservoir.

To filter the sample, create a *gentle* vacuum with the hand pump. Be sure to proceed very slowly, and pump only one stroke at a time until all of the liquid in the sample is passed through the filter. Pressure on the sample should never exceed 7 psi, as this could cause cells to burst and release contents, including chlorophyll *a*, into the filtrate and be lost. If it becomes impossible to filter a whole 25 mL of the sample and remove the water efficiently, discard the filter and try again with a smaller volume (*e.g.,* 10 mL). It is not necessary to collect on multiple filters to try to achieve a total volume of 25 mL. Simply filter as much as possible on a single filter, up to 25 mL, and then use that filter as the sample. Be sure to record the volume of the composite sample that was actually filtered, both on the datasheet, and on the sample label.

Rinse the sides of the filter reservoir with a few mL of DI water, and continue filtering until the water is drawn down. The filter should not be sucked dry, but rather left slightly moist, in order to avoid applying excessive pressure to the sample, which could cause algal cells to burst. After the liquid has passed through, check the filter to see if there are any bits of non-algal plant matter (like tiny seedlings or bits of leaves). If so, remove them with a clean forceps, being careful not to remove any algae in the process. If possible, rinse the removed items with DI water onto the filter before discarding them. Remove the filter from the filtering device. *Note: always thoroughly rinse the sides of the filter reservoir and the interface between the mesh filter seating and the screw-on part of the reservoir with DI water between samples.*

Being careful not to remove any of the collected material from the filter, fold the filter in half (with the sample material on the inside) using the forceps, and place it inside a clean, snap-top Petri dish[13]. Envelop the Petri dish completely within a small sheet of aluminum foil in order to prevent any light from reaching the filter. Place the covered Petri dish and its corresponding, completely filled-out sample label (face outward) into a 100 mL Whirl-pak bag[14], purge as much of the air out of the bag as possible, "whirl" it shut, and seal it tightly with its wire tabs, so that water in the cooler will not be able to enter the bag. Note that if the Whirl-pak bags contain a lot of air, they will float on top of the ice water in the cooler, and they then run the risk of not being kept cold enough. Shove the sample packet down into the ice in the cooler. Note: a clean, clear plastic centrifuge tube is also an acceptable container in which to store the filter. It must also be properly labeled, wrapped in aluminum foil, and kept submerged in wet ice.

Keep chlorophyll *a* filters as cold as possible and place in the freezer or dry ice as soon as possible (and within four hours of collection); the holding time for the chlorophyll *a* filters is 28 days from collection, when kept frozen.

Ash-free dry mass (AFDM) samples: For the AFDM samples, you should use glass-fiber filters (47 mm, 0.7 μm pore size) that have been precombusted. Never touch the filters with hands or anything other than a CLEAN forceps (rinsed with DI water three times). The filters to use should be labeled "for AFDM", and stored in aluminum sleeves. Follow the same process as that used for chlorophyll-a sample filtering. After all the liquid has passed through, check the filter to see if there are any pieces of non-algal plant matter (such bits of leaves or wood). If so, remove them with a clean forceps (rinsed with DI water three times), being careful not to remove any algae in the process. The goal with AFDM, for the purposes of this SOP, is to target the ALGAL portion of the organic matter in the sample, and therefore field crews should do their best to remove non-algal contributors of organic matter from the sample. Remove the filter from the filtering device. *Note: always thoroughly rinse the sides of the filter reservoir and the interface between the mesh filter seating and the screw-on part of the reservoir with DI water between samples.*

Use the forceps to fold the AFDM filter in half (with the sample material on the inside) and wrap it loosely in a small sleeve of clean aluminum foil. Be careful not to squeeze the filter, which could cause the sample to ooze from the filter onto the aluminum sleeve. Store the filter in a sealed 100 mL Whirl-pak bag containing a completely filled-out sample label, including the volume that was filtered (*i.e*., 25 mL or otherwise). As with the chlorophyll-*a*, purge as much of the air out of each bag as possible, "whirl" it shut, and seal tightly with the wire tabs. Shove the sample packet down into the ice in the cooler. Note: a clean, clear plastic centrifuge tube is also an acceptable container in which to store the filter. It must also be properly labeled and kept submerged in wet ice.

[13] It may be beneficial to write the Site Code or sample ID code on the Petri dish itself, in addition to filling out the full sample label.
[14] Other bag types are acceptable only if they are water-tight (note that Ziploc bags often leak when submerged).

Keep AFDM filters as cold as possible until the samples can be frozen back at the lab that evening, or place on dry ice until they can be stored in the lab freezer. The holding time for the AFDM samples is 28 days from collection, when kept frozen.

5.3 Processing Soft-bodied and Other Sample Types when a Macroalgal Clump is Present

The following is a description of how to proceed when a soft-bodied algal taxonomic ID sample is to be prepared AND macroalgal clump(s) are present in the sample in the dish tub. A flowchart of this procedure is provided in Appendix G. *It is recommended that this flowchart be printed out in color, laminated (if possible) or printed out on water-proof paper, and brought along to the field for quick reference on handling macroalgal clumps in the composite sample.* The reason for the extra step in the processing of the macroalgae for the purposes of the soft-bodied algae sample is that it maintains larger, more intact macroalgal specimens for examination in the laboratory, rather than chopping up all of the macroalgal specimens before sending them to the lab. This is important, because availability of intact specimens greatly improves the chances that the taxonomist will be able to identify the soft-bodied algae to low taxonomic levels.

5.3.1 Isolating and Dividing the Macroalgal Clump

For this procedure, the macroalgal clump is first removed from the dish tub, wrung out gently, and rolled into a cylinder shape that is relatively even in thickness along its length. If there is more than one type of macroalgae in the sample, the various types should be layered on top of one another lengthwise so that they are represented in roughly constant proportions across the length of the "cylinder". The cylinder is measured with a ruler and a quarter of its length is cut off with scissors and put into the (still empty) soft-bodied algae ID centrifuge tube[15]. The clump is pushed down into the tube, and the top is flattened, so that the volume of the clump can be estimated using the graduations on the tube. The estimated volume of this clump will be used in a calculation (see Equation 1 and Figure 8). The remaining three-quarters length of cylinder is set aside in the shade/cool. It is recommended that this section be placed in a Ziploc bag, sealed, and put in the wet ice cooler.

5.3.2 Measuring the Composite Liquid Volume

Once algal specimens have been removed from all the substrates (*e.g.*, sand, gravel, cobble, wood, leaves) in the dish tub, according to the procedure described in Section 4, gently agitate the dish tub to suspend the microalgae in the liquid, and then start pouring this suspension into a CLEAN graduated cylinder to measure the volume of the liquid. Try to leave all substrate material (*e.g.*, silt, sand) behind. Transfer the measured liquid into a CLEAN 1L plastic bottle. Using a minimal amount of stream water, rinse the substrate once or twice with stream water until it appears that little to no additional suspended material (microalgae) is coming off. Add this rinsate to the graduated cylinder to measure it also. If necessary, repeat this process

[15] It is unlikely that the ¼ macroalgal clump will occupy all the space in the sample tube, but if it does, a second tube will be needed in order to accommodate all the sample material plus liquid. If such an action is taken, it should be noted in the Comments section of the field sheets and the tubes should be clearly identified as belonging to the same sample, for record keeping purposes.

(regularly agitating the dish tub) until all the liquid has been measured and transferred to the sample bottle. *Use water sparingly, because the total sample volume plus rinsate should be no more than about 400-500 mL.*

Because you are leaving as much of the silt, sand, and any large substrate material behind as possible, the final volume should reflect only the liquid component of the sample plus rinse water. Record the total volume of all the liquid that had been in the dish tub, including any that was used for rinsing the substrates and sampling devices, on the field sheet under the *Algae Samples* section. This is the **COMPOSITE VOLUME**. This value will also be recorded on all algae sample labels (*i.e.*, for the diatom and soft-bodied algae taxonomic ID samples, the chlorophyll *a*, and the AFDM).

5.3.3 Preparing the Soft-Bodied Algae Taxonomic ID Sample

Pour freshly-agitated liquid composite sample from the 1-L bottle into the soft-bodied algae sample tube (on top of the clump of macroalgae) up to the 45 mL mark. Midway through pouring, the composite sample should be swirled some more (first clockwise, then counter-clockwise) to ensure that the microalgae are still fully suspended. Cap the tube tightly. Completely fill out a sample label and affix it to the sample tube. Cover the label completely with clear plastic tape to prevent the writing on the label from smearing. Place the tube in the wet ice chest to *keep it in the dark and as cold as possible, but make sure it is never allowed to freeze.*

As soon as possible, and no longer than four days after collection of the sample, glutaraldehyde (to a final concentration of 2.5%) must be added to the tube and distributed throughout the sample by agitation and turning the tube upside down repeatedly. Glutaraldehyde is necessary for soft-bodied algal samples in order to preserve fine morphological features and the color of pigments, as both can be crucial characters for taxonomic determination. *Glutaraldehyde is a hazardous substance that poses a number of safety risks.* As such, it should be handled in a fume hood by trained personnel wearing appropriate gear. Refer to Appendix E for an SOP for the use of glutaraldehyde.

Members of the field crew can either have the glutaraldehyde added to the samples back at their own lab, or arrange for the glutaraldehyde to be added to the samples by the taxonomy lab. In either case, the unfixed samples must be kept in the dark and on wet ice (but not allowed to freeze), and must be fixed within four days of collection (and preferably sooner). If the taxonomy lab will be adding the fixative, it is imperative to plan ahead to arrange for this to be done, and also to clearly mark which tubes will need to have fixative added to them. Once the samples are fixed, it is no longer imperative to store them on wet ice. Following fixation, they can simply be stored in a cool, dark place.

5.3.4 Preparing the Diatom Taxonomic ID Sample

After the soft-bodied algal sample has been prepared, and before preparing the diatom sample (and biomass samples, which will be discussed in the next section), the volume of the remaining

composite liquid must be reduced to equal ¾ of the original volume[16]. This is necessary because ¼ of the macroalgae clump was taken out of the composite sample but a full ¼ was not removed from the water portion. As such, the original ratio between water and macroalgae must be restored before further sample preparation.

The follow procedure is used to reduce the volume of liquid composite to ¾ of the original. For convenience, you can use this formula to calculate how many mL to pour off and discard from the composite:

Equation 1. Adjusting the volume of composite sample

volume (mL) of composite to pour off = (0.25 * C) – 45 + A

where "**C**" is the original composite volume and "**A**" is the approximate volume of the clump of macroalgae that was placed in the soft-bodied algae sample tube (tamped down and flattened). You may wish to fill out a copy of the Ratio Restoration worksheet shown in Figure 8 to calculate the amount of composite to pour off.

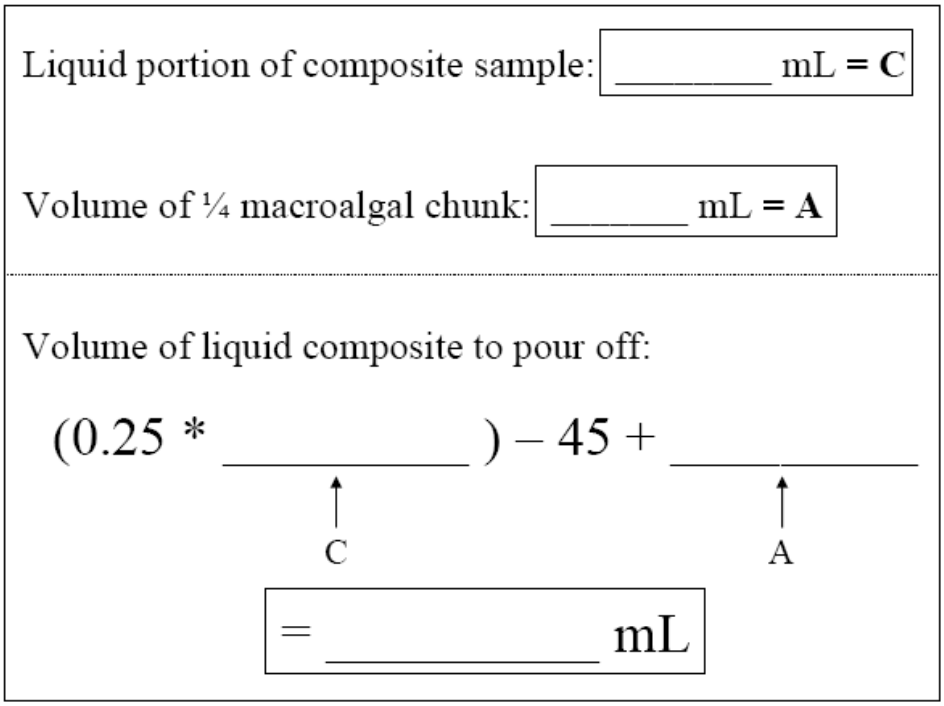


Figure 8. Ratio Restoration worksheet.

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

[16] For example, if the original composite volume was 480mL, you will be discarding enough composite liquid to get down to 360 mL.

Once the required amount of composite liquid has been discarded, the remaining ¾ of the macroalgal clump ("cylinder") is cut into **very** fine pieces with a scissors, and these are added to the reduced-volume composite liquid. The pieces should be chopped small enough so that they practically "blend" into the liquid (*i.e.,* distinct fragments of macroalgae are not easily discernible), because the goal is to "homogenize" the macroalgae into the liquid as much as possible. Now the ratio of macroalgae to liquid from the original sample in the dish tub is restored. Cap the composite bottle and shake vigorously to homogenize the bits into the liquid as much as possible, while not agitating so hard as to risk busting cells and releasing chlorophyll.

Diatom samples should be fixed as soon as possible after collection to reduce the possibility of cell division post-sampling. A 10% solution of buffered formalin is used to fix diatoms, and instructions for preparing this solution are provided in Appendix D.

To prepare the diatom sample, aliquot 40 mL of freshly-agitated sample homogenate into the diatom ID sample tube, swirling the composite sample bottle again midway through pouring to keep the microalgae suspended. Add 10mL of the 10% buffered formalin to the sample. This can be done using a small syringe or bulb pipette. Alternatively, if preferred, the centrifuge tubes for the diatom samples can be pre-loaded with 10 mL of the 10% buffered formalin and 40 mL of sample can be added carefully to the fixative, to avoid having to dispense the fixative in the field.

Fixatives such as formalin must be used with great care. Be sure to wear formalin-safe gloves and safety goggles when using the fixative, as it should never be touched with bare hands or allowed to splash onto skin or into eyes. Also make sure it is used only in a very well-ventilated place and avoid breathing in any fumes. Minimize the amount of time that vessels containing formalin are open. Fixative added to the sample must not be allowed to ooze outside the vessel that contains it, including the sample tubes. Refer to Appendix F for an SOP for the use of formalin.

Cap the tube tightly and shake it to mix the formalin into the sample. Fill out a sample label and affix it to the sample tube. Cover the label completely with clear plastic tape to prevent the writing on the label from smearing. Keep the fixed diatom samples in the dark and away from extreme heat.

5.3.5 Preparing the Biomass Samples

The remaining composite sample homogenate can be used to prepare the chlorophyll-*a* and AFDM filters as described below.

Chlorophyll *a* samples: The procedure to filter chlorophyll *a* samples should be carried out quickly, and in the shade as much as possible, to minimize exposure of the sample to light, and minimize chlorophyll *a* degradation thereby. For the chlorophyll *a* samples, use a CLEAN filter forceps (rinsed with DI water three times) to center a glass fiber filter (47 mm, 0.7 µm pore size) onto the mesh platform of a CLEAN filtering tower apparatus (rinsed with DI water three times), and rinse the filter a little with DI water to seat it well into the mesh before attaching the filter reservoir on top. Never touch the filters with hands or anything other than a clean forceps.

Agitate the composite sample homogenate to resuspend all the macroalgal fragments and microalgal material. Carefully measure 25 mL using a small, CLEAN graduated cylinder (rinsed with DI water three times). Midway through pouring the 25 mL, swirl the homogenate again to ensure that the material is still fully suspended. Pour the remainder of the 25 mL, and then pour the measured homogenate into the filter reservoir. Once empty, rinse the graduated cylinder with a few mL of DI water, and add this to the reservoir.

To filter the sample, create a *gentle* vacuum with the hand pump. Be sure to proceed very slowly, and pump only one stroke at a time until all of the liquid in the sample is passed through the filter. Pressure on the sample should never exceed 7 psi, as this could cause cells to burst and release contents, including chlorophyll *a*, into the filtrate and be lost. If it becomes impossible to filter a whole 25 mL of the sample and remove the water efficiently, discard the filter and try again with a smaller volume (*e.g.,* 10 mL). It is not necessary to collect on multiple filters to try to achieve a total volume of 25 mL. Simply filter as much as possible on a single filter, up to 25 mL, and then use that filter as the sample. Be sure to record the volume of the composite sample that was actually filtered, both on the datasheet, and on the sample label.

Rinse the sides of the filter reservoir with a few mL of DI water, and continue filtering until the water is drawn down. The filter should not be sucked dry, but rather left slightly moist, in order to avoid applying excessive pressure to the sample, which could cause algal cells to burst. After all the liquid has passed through, check the filter to see if there are any bits of non-algal plant matter (like tiny seedlings or bits of leaves). If so, remove them with a clean forceps, being careful not to remove any algae in the process. Remove the filter from the filtering device. *Note: always thoroughly rinse the sides of the filter reservoir and the interface between the mesh filter seating and the screw-on part of the reservoir with DI water between samples.*

Fold the filter in half (with the sample material on the inside) using the forceps, and place it inside a clean, snap-top Petri dish. Envelope the Petri dish completely within a small sheet of aluminum foil in order to prevent any light from reaching the filter. Place the covered Petri dish and its corresponding, completely filled-out sample label (face outward) into a 100 mL Whirl-pak bag, purge as much of the air out of the bag as possible, "whirl" it shut, and seal it tightly with its wire tabs, *so that water in the cooler will not be able to enter the bag*. Note that if the Whirl-pak bags contain a lot of air, they will float on top of the ice water in the cooler, and they then run the risk of not being kept cold enough. Shove the sample packet down into the ice in the cooler.

Keep chlorophyll *a* filters as cold as possible and place in the freezer or dry ice as soon as possible (and within four hours of collection); the holding time for the chlorophyll *a* filters is 28 days from collection, when kept frozen.

Ash-free dry mass (AFDM) samples: For the AFDM samples, you should use glass-fiber filters (47 mm, 0.7 μm pore size) that have been precombusted. Never touch the filters with hands or anything other than a clean forceps (rinsed with DI water three times). The filters to use should be labeled "for AFDM", and stored in aluminum sleeves. Follow the same process as that used for chlorophyll-a sample filtering. After all the liquid has passed through, check the filter to see if there are any bits of non-algal plant matter (such as bits of leaves or wood). If so, remove them

with a clean forceps (rinsed with DI water three times), being careful not to remove any algae in the process. Remove the filter from the filtering device. *Note: always thoroughly rinse the sides of the filter reservoir and the interface between the mesh filter seating and the screw-on part of the reservoir with DI water between samples.*

Use the forceps to fold the AFDM filter in half (with the sample material on the inside) and wrap it loosely in a small sleeve of clean aluminum foil. Be careful not to squeeze the filter, which could cause the sample to ooze from the filter onto the aluminum sleeve. Store the filter in a sealed 100 mL Whirl-pak bag containing a completely filled-out sample label, including the volume that was filtered (*i.e.*, 25 mL or otherwise). As with the chlorophyll-*a*, purge as much of the air out of each bag as possible, "whirl" it shut, and seal tightly with the wire tabs. Shove the sample packet down into the ice in the cooler.

Keep AFDM filters as cold as possible until the samples can be frozen back at the lab that evening, or place on dry ice until they can be stored in the lab freezer. The holding time for the AFDM samples is 28 days from collection, when kept frozen.

5.4 Procedure for Collecting Qualitative Algal Samples

If your program calls for the collection of soft-bodied taxonomic ID samples, then you will also need to collect a "qualitative" sample at every monitoring reach. The qualitative samples consist of a composite of all types of soft-bodied algae visible within the reach. This is of value because it can provide a fairly exhaustive list of soft-bodied algal taxa present at the site and can also aid identification of taxa captured in the RWB sampling, since it allows larger, more intact specimens to be collected than those that may end up in the more heavily processed quantitative sample (described above). In addition, if the qualitative sample is kept cool and in the dark, and is delivered to the lab in a timely manner (*i.e.*, as quickly as possible, and within two weeks of collection), the live specimens can be cultured, which can also aid in identification. For example, some taxa in the Zygnematales cannot be identified to species level unless they are in a sexual phase during examination. If asexual at the time of collection (which is the typical situation), live specimens could be induced to a sexual phase in the lab. Collection of a qualitative *diatom* sample is optional, and is typically not needed for general bioassessment purposes.

For qualitative soft-bodied algal samples, collect specimens of all obviously different types of macroalgal filaments and mats, microalgae (in the forms of scrapings using a razor blade or knife), and depositional samples (suctioned from along the surface of sediments using a clean turkey baster). Collect from as many distinct locations as possible throughout the reach so as to capture as much of the apparent diversity in the reach as you can. Also, when possible, try to grab part of the holdfast structures that attached the macroalgae to the substrate, as these structures can be useful for taxonomic identification.

Since these samples are merely qualitative, and not quantitative, you need not worry about collecting them in a manner that is representative of their relative abundances within the reach. Note, however that if there is only a small amount of macroalgae in the stream, it should be allocated preferentially to the soft-bodied algae laboratory sample, as opposed to the diatoms (if a diatom qualitative sample is being collected), because it is primarily needed for the soft-bodied

algal identification work (although diatoms can live as epiphytes on macroalgae, so macroalgal samples are also of values for the diatom work).

Using a thick, waterproof marker, label a Whirl-pak bag with the Site Code, Date, Sample ID, and "soft" (or "diatom", if also collecting a diatom sample). Fill the bag with a total volume of up to 100 mL of qualitative algae sample + water. Purge any extra air from the bag, seal with the wire tabs by twisting them together (not just folding them, as this can result in leakage), tuck the ends of the wire tabs inward so that they cannot poke other bags, and store in the cooler on wet ice in the field. Be careful not to place the bags right up against ice or frozen blue-ice bags, because this could cause the algae to freeze and thus destroy the sample. Unlike with the quantitative samples, **do not add glutaraldehyde or formalin** (or *any* other fixative) to these qualitative samples. Keep the qualitative samples on wet ice and refrigerate immediately upon return to the lab. Because they are not preserved, these samples should be examined by a taxonomist as soon as possible (and within two weeks, at most), as they can decompose fairly rapidly. Decomposition is of particular concern for the soft-bodied algae sample.

If it is impossible to get the soft-bodied qualitative samples to a taxonomist within two weeks of sample collection, then split the qualitative samples in half, transfer one half to a 50 mL centrifuge tube and preserve it with glutaraldehyde (to a 2.5% final concentration) and leave the other half un-fixed (but continue to store in the cold/dark until examination by a taxonomist). This should be done in order to preserve part of the sample for morphological identification, but still maintain some possibility of keeping some specimens alive, in case culturing is necessary. *Glutaraldehyde is a hazardous substance that can pose health and safety risks. Add glutaraldehyde in a fume hood, wearing safety goggles and glutaraldehyde-safe gloves.* Refer to Appendix E for more detailed instructions on the safe handling of glutaraldehyde.

5.5 Algal Sampling Quality Assurance / Quality Control

The SWAMP bioassessment group is currently developing guidelines for quality assurance and quality control for bioassessment procedures. Future revisions to this document will include more specific information covering personnel qualifications, training and field audit procedures, procedures for field calibration, procedures for chain of custody documentation, requirements for measurement precision, health and safety warnings, cautions (actions that would result in instrument damage or compromised samples), and interferences (consequences of not following the SOP).

It is recommended that duplicate sampling of benthic algae occur at 10% of study sites. The recommended method for collecting duplicates is at adjacent positions along the sampling transect according to the scheme depicted in Figure 2. Both samples should be collected at each transect before moving on to the next transect.

6. PHYSICAL HABITAT TRANSECT-BASED MEASUREMENTS TO ACCOMPANY ALGAL BIOASSESSMENT

Once all algae samples have been collected at a given transect, PHab data collection can begin there. PHab data are designed to assess the physical habitat conditions of the stream reach being sampled. Knowledge about the PHab parameters can aid interpretation of the biotic assemblage data collected. Data for the following PHab parameters will be entered on transect-specific datasheets (corresponding to each of the 11 main transects along the monitoring reach). These datasheets are provided in Appendix C.

It should be noted that the data collection procedures for the parameters below reflect those that are described in the SWAMP BMI Bioassessment SOP (Ode 2007). With respect to PHab assessment, the only deviation between this SOP and that of Ode (2007) is in terms of omission of certain parameters. However, where there is overlap in parameters between the two SOPs, they are assessed in exactly the same manner. The one exception to this is the addition, in this SOP, of percent algal cover determination to the pebble count as described in Ode (2007). Also, note that because the datasheets in Appendix C are multi-purpose datasheets, developed for both BMIs and algae, they include some PHab parameters that are not a part of this SOP. Specifically, the following PHab data that appear on the datasheets are not collected when only algae are being sampled: 1) Riparian vegetation, and 2) Instream habitat complexity. As such, these sections are not filled out on the datasheets when only algae samples are being collected.

6.1 Wetted Width

The wetted channel is the zone that is inundated with water and the wetted width is the distance between the sides of the channel at the point where substrates are no longer surrounded by surface water. Measure the wetted stream width and record this in the box at the top of the Transect data form.

6.2 Bankfull Width

The bankfull channel is the zone of maximum water inundation in a normal flow year (one-to-two year flood events). Since most channel formation processes are believed to act when flows are within this zone, bankfull dimensions provide a valuable indication of relative size of the waterbody.

Scout along the stream margins to identify the location of the bankfull margins on either bank by looking for evidence of annual or semi-annual flood events. Examples of useful evidence include topographic, vegetative, or geologic cues (changes in bank slope, changes from annual to perennial vegetation, changes in the size distribution of surface sediments). While the position of drift material caught in vegetation may be a helpful aid, this can lead to very misleading measurements. Note: The exact nature of this evidence varies widely across a range of stream types and geomorphic characteristics. It is helpful to investigate the entire reach when attempting to interpret this evidence because the true bankfull margin may be obscured at various points along the reach. Often the bankfull position is easier to interpret from one bank than the other; in these cases, it is easiest to infer the opposite bank position by projecting across the channel.

Additionally, height can be verified by measuring the height from both edges of the wetted channel to the bankfull height (these heights should be equal).

Stretch a tape from bank to bank at the bankfull position. Measure the width of the bankfull channel from bank to bank at bankfull height and perpendicular to the direction of stream flow.

6.3 Bankfull Height

Measure bankfull height (the vertical distance between the **water surface** and the height of the bank, Figure 4) and record in the boxes at the top of the Transect data form under "*Bankfull Width*" and "*Bankfull Height*".

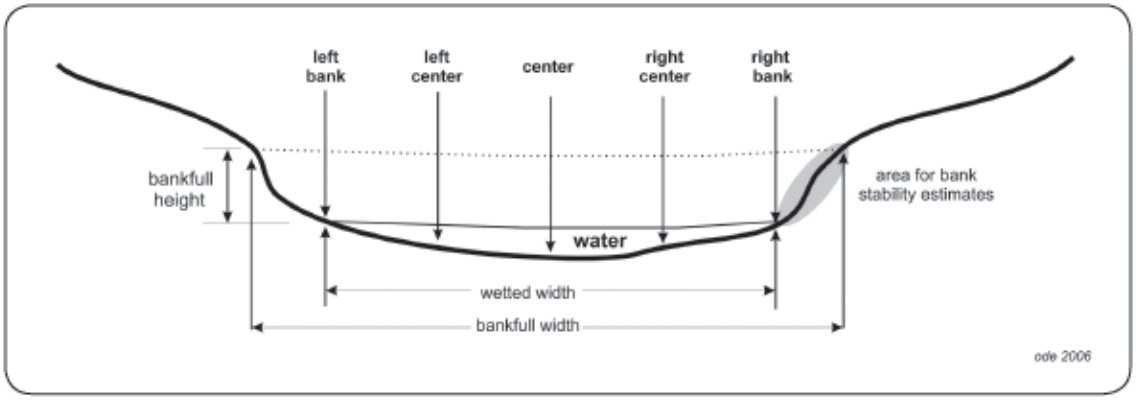


Figure 9. Cross sectional diagram of a typical stream channel showing locations of substrate measurements, wetted and bankfull width measurements, and bank stability visual estimates (reprinted from Ode 2007).

6.4 "Pebble Count": Transect Substrates

Particle size frequency distributions often provide valuable information about instream habitat conditions that affect benthic communities. The Wolman pebble count technique is a widely used and cost-effective method for estimating the particle size distribution and produces data that correlate with costly, but more quantitative bulk sediment samples. Coarse particulate organic matter (CPOM, particles of organic material such as leaves that are greater than 1.0 mm in diameter) is a general indicator of the amount of allochthonous organic matter available at a site, and its measurement can provide valuable information about the basis of the food web in a stream reach. The presence of CPOM associated with each particle is quantified at the same time that particles are measured for the pebble counts.

Transect substrate measurements are taken at five equidistant points along each transect (Figure 9). Divide the wetted stream width by four to get the distance between the five points (Left Bank, Left Center, Center, Right Center and Right Bank) and use a measuring device to locate the positions of these points (*e.g.*, a stadia rod or measuring tape). Once the positions are identified, lower a folding meter stick through the water column perpendicular to both the flow and the transect to identify the particle located at the tip of the meter stick. *It is important that you are*

not subjective about selecting a particle, as this will result in failing to generate an accurate assessment of the size class distribution of particles present in that stream reach.

6.5 Depth

With the folding meter stick, measure the depth from the water surface to the top of the particle to the nearest cm and record on the datasheet.

6.6 Particle Size Class

Remove the particle from the streambed. Assign the particle to one of the size classes listed in Table 3 (these are also provided in a box on the transect form), based on its *intermediate axis length* (Figure 10). Record this information under Substrate size class.

Table 3. Particle size class codes, descriptions, and measurements (adapted from Ode 2007)

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

Be sure to use measurements or the established codes for particle size class. If the latter, confirm the 2-letter codes for the particles as you call them out to your partner recording the data to ensure you are using the correct codes.

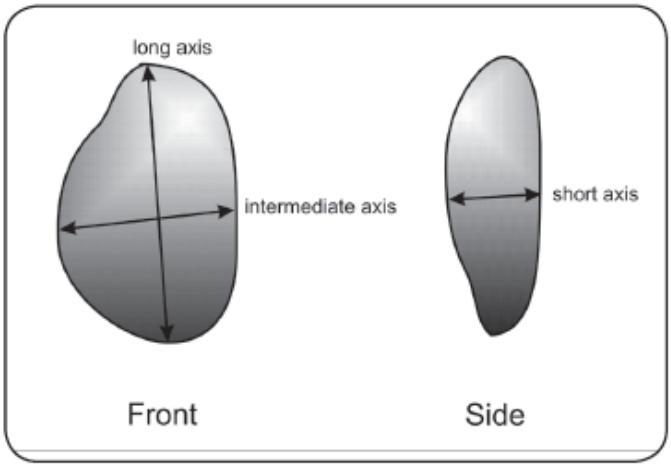


Figure 10. Diagram of three major perpendicular axes of substrate particles. The intermediate axis is recorded for pebble counts (reprinted from Ode 2007).

6.7 Cobble Embeddedness

It is generally agreed that the degree to which fine particles fill interstitial spaces has a significant impact on the ecology of benthic organisms and fish, but techniques for measuring this impact vary greatly. Here we define embeddedness as the volume of cobble-sized particles (64-250 mm) that is buried by fine and sand particles (<2.0 mm diameter).

When a cobble-sized particle is encountered during the pebble count, visually estimate the percentage of the cobble's volume that has been buried by fine/sand particles (this will likely require removing the cobble from the streambed). Record, to the nearest 5%, the embeddedness of up to 25 cobble-sized particles within the sampling reach in the corresponding "% Cobble Embed" field for each cobble.

If 25 cobbles are not encountered during the pebble count, supplement the cobbles by conducting a "random walk" through the reach. Starting at a random point in the reach, follow a line from one bank to the other at a randomly chosen angle. Once at the other bank reverse the process with a new randomly chosen angle. Enter any of these additional embeddedness values at the bottom of the first page of the set of field forms, under "ADDITIONAL COBBLE EMBEDDEDNESS MEASURES".

If 25 cobble sized particles are not present in the entire reach, then record the values for however many cobbles are present.

6.8 CPOM

Record the presence or absence of Coarse Particulate Organic Matter (CPOM) that is > 1 mm diameter, and within 1 cm of the particle.

6.9 Algal Cover

Algal cover refers to the amount of algae in the stream reach, both in terms of microalgal coatings on stream substrates and macroalgae (*e.g.*, filaments, mats, globules). Algal cover is estimated by a point-intercept approach that entails collecting information about the presence/absence of algae at each of the points along the transects associated with the pebble count. If the imaginary point corresponding to each pebble in the pebble count intercepts algae, then algae is recorded as "present" at that point. The percentage of the points across the sampling reach that have algae present yields an estimate of the percent algal cover.

To assess algal cover, for each point along the pebble count, record information about the presence of algae as follows. For any film of algae ("Microalgae" on the datasheet) coating the substrate at a given point, estimate the presence / thickness category according to the scheme in Table 4. For thicker microalgal layers, a small metal or plastic rod with demarcations at 1, 5, and 20 mm can be used for measurement. For layers too thin to measure, use the diagnostic criteria listed in the last column of Table 4.

Table 4. Microalgal thickness codes and descriptions (adapted from Stevenson and Rollins 2006).

Code	Thickness	Diagnostics
0	No microalgae present	The surface of the substrate feels rough, not slimy.
1	Present, but not visible	The surface of the substrate feels slimy, but the microalgal layer is too thin to be visible.
2	<1mm	Rubbing fingers on the substrate surface produces a brownish tint on them, and scraping the substrate leaves a visible trail, but the microalgal layer is too thin to measure.
3	1-5mm	
4	5-20mm	
5	>20mm	
Z	Cannot determine if a microalgal layer is present	(see explanation below)
D	dry point	

Note that sometimes, due to the nature of the substrate, it can be difficult to discern whether a microalgal layer is present (particularly if it is very thin). For example, in the case of very fine sediments, the dark color of the silt can obscure the diagnostic color of a microalgal layer, and the inherent "sliminess" of very fine silt may make tactile determination of microalgae impossible. Therefore, when silt is the substrate, only relatively thick layers of microalgae might be easily discernible. If presence/absence of a microalgal layer cannot be determined with certainty, score microalgal thickness as "Z".

In addition to recording the presence and thickness of microalgae on the surfaces of substrates, record the presence/absence of attached algae in the water column, as well as unattached, floating mats on the water's surface, corresponding to each pebble count sampling point. Do this by envisioning an imaginary "line" extending from the water's surface down to the stream bottom where the target "pebble" lies (particularly in turbulent water, it may be helpful to use a viewing bucket (Appendix D) in order to see below the water's surface). If this line intercepts macroalgae, either floating on the water's surface, or somewhere within the water column, the appropriate algal class(es) should be recorded as "present". Attached macroalgal filaments have an obvious physical connection to something (like a cobble, boulder, or a gravel bed) lying on the bottom of the stream, whereas for Unattached macroalgae, there is no obvious physical connection with the streambed, and the algae is just freely floating at or near the water's surface. For each class of macroalgae (Attached and Unattached), mark "P" (for "present") if intercepted by the sampling point and "A" (for "absent") if not intercepted.

Bear in mind that, because pebble counts span the "wetted width" of each transect, the expectation is that even the pebbles at the bank positions will generally be at least moist, and sometimes even submerged. As such, it is important to realize that algal cover can occur at the bank positions of the pebble count as well as intermediate positions across the stream. An exception to this is when the pebble surface is completely dry. Section 6.11 provides instructions for data collection in this situation.

6.10 Macrophytes

If a vascular plant (*i.e.*, a macrophyte) is intercepted by the imaginary line associated with the pebble count point, mark "P" for "present" under Macrophytes. Otherwise, mark "A" for absent. Examples of macrophytes include cattails, tules, rushes, sedges, monkeyflowers, speedwells, knotweed, and watercress.

6.11 Dry Substrates

To determine how to collect data at dry sampling points, it is necessary to first establish whether the dry area in question lies within the stream's active channel (*i.e.,* therefore regularly inundated during storms), or whether the point is on a stable island (*i.e.,* therefore rarely, if ever, inundated). Stable islands are typically vegetated, often with woody shrubs or trees, and have heights near or exceeding bankfull height. *Pebble counts should not be conducted on stable islands*. If the transect spans a portion of the study reach in which the channel is bifurcated such that there are two channels with an intervening island, the entire transect should be placed across the dominant channel, and all five pebble count points should be located on that side.

If the point falls on a dry surface that is within the usual active channel (*i.e.*, subject to regular disturbance by flows), then pebble count/algal cover data from the dry point should be recorded as follows:

- score **Depth** as **0**
- score particle **Size Class** and **Embeddedness as described above** for wet particles
- score all the algae variables (**Microalgae, Macroalgae Attached, and Macroalgae Unattached**) as **"D"** for "dry"
- leave **CPOM** and **Macrophytes** "**blank**" (*i.e.*, do not circle anything). These parameters will register as NR (Not Recorded) in the database.

Ordinarily, the sampling transect would span the wetted width of the channel, but when no water is present at a given transect, evidence of the typical wetted extent of the active channel will need to be used to infer appropriate transect boundaries. Such indicators can include the transition from vegetated to unvegetated area (*i.e.*, moving from banks to active channel), as well as the presence of dried algae, water stains, microtopographic transitions, changes in substrate composition, and others.

6.12 Bank Stability

The vulnerability of stream banks to erosion is often of interest in bioassessments because of its direct relationship with sedimentation.

For each transect, record a visual assessment of bank vulnerability in the region between the wetted width and bankfull width of the stream margins and between the upstream and downstream inter-transects. Choose one of three vulnerability states: eroded (evidence of mass wasting), vulnerable (obvious signs of bank erosion or unprotected banks), or stable.

6.13 Human Influence

For the left and right banks, estimate a 10 x 10 m riparian area centered on the edges of the transect (see Figure 11). In the "*Human Influence*" section of the Transect data sheet, record the presence of 14 human influence categories in three spatial zones relative to this 10 x 10 m square (between the wetted edge and bankfull margin, between the bankfull margin and 10 m from the stream, and between 10 m and 50 m beyond the stream margins): 1) walls/rip-rap/dams, 2) buildings, 3) pavement/cleared lots, 4) roads/railroads, 5) pipes (inlets or outlets), 6) landfills or trash, 7) parks or lawns (e.g., golf courses), 8) row crops, 9) pasture/ rangelands, 10) logging/ timber harvest activities, 11) mining activities, 12) vegetative management (herbicides, brush removal, mowing), 13) bridges/ abutments, 14) orchards or vineyards. Circle all combinations of impacts and locations that apply, but be careful to not double-count any human influence observations.

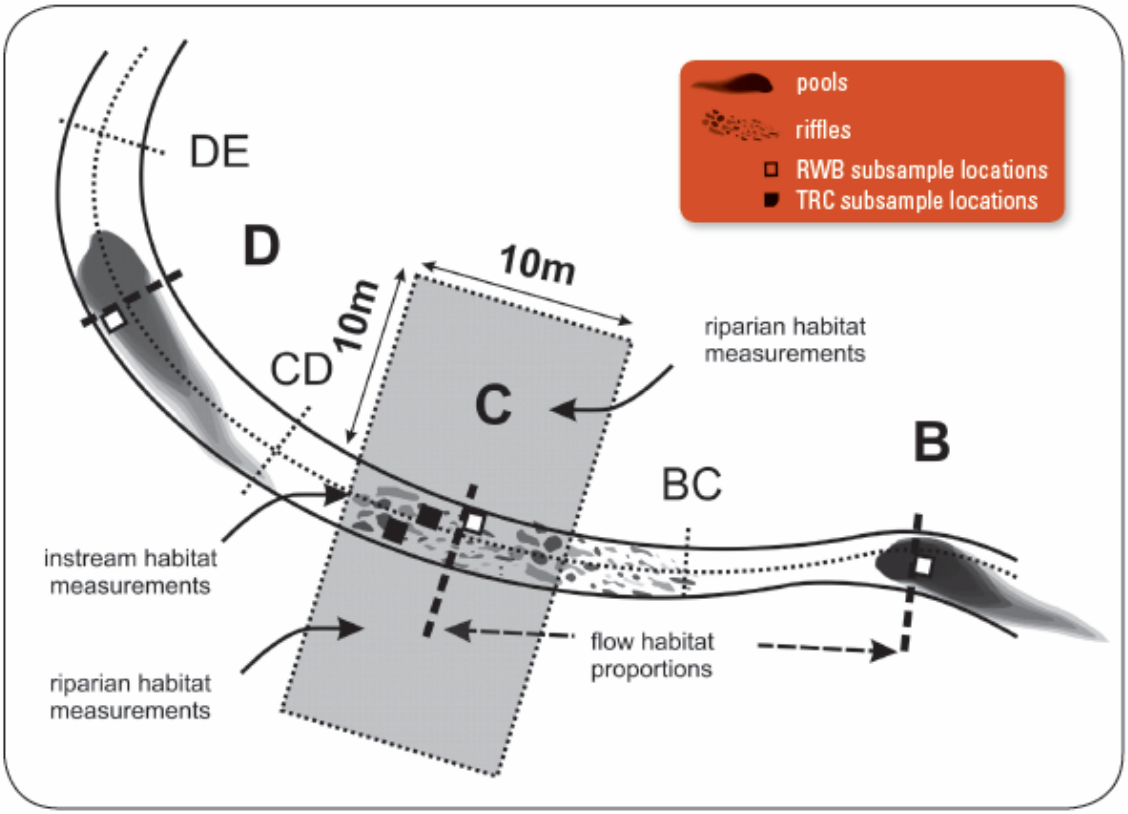


Figure 11. Section of the standard reach expanded from Figure 1 showing the appropriate positions for collecting algae samples (the white square, labeled "RWB" in the legend box) and flow habitat proportion measurements (reprinted from Ode 2007).

Record the presence of any of the 14 human influence categories in the stream channel within a zone 5 m upstream and 5 m downstream of the transect.

6.14 Densiometer Readings (Canopy Cover)

The densiometer is read by counting the number of line intersections that are obscured by overhanging vegetation. Before using, the densiometer should be modified by taping off the lower left and right portions of the mirror in order to emphasize overhead vegetation over foreground vegetation (the main source of bias in canopy density measurements; see Figure 12.)

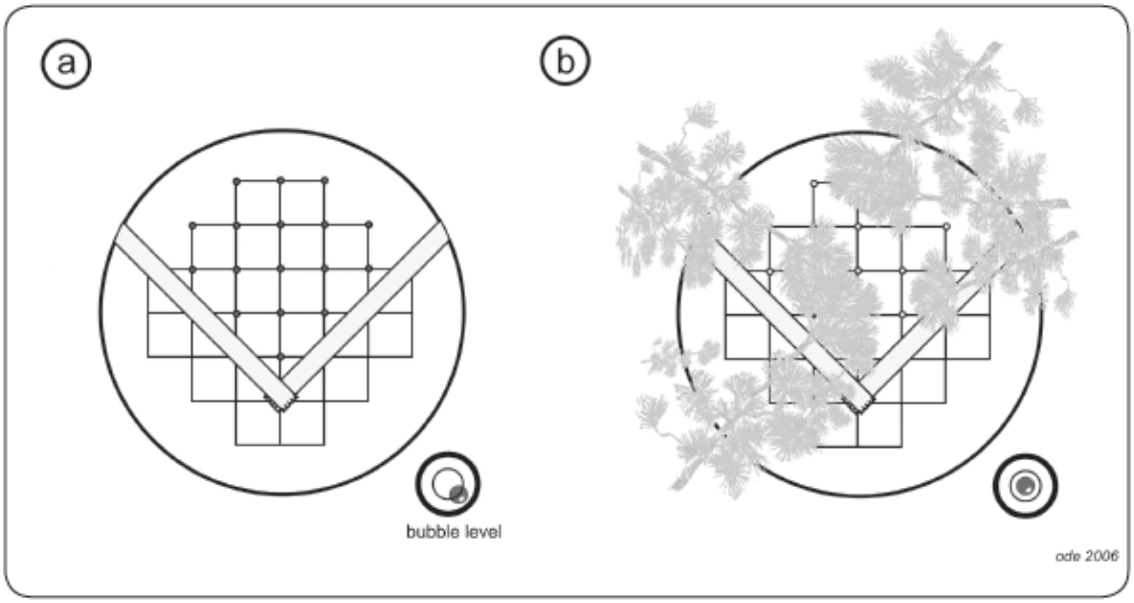


Figure 12. Representation of the mirrored surface of a convex spherical densiometer showing the position for taping the mirror and the intersection points used for the densiometer reading. The score for the hypothetical condition in (b) is 10 covered intersection points out of 17 possible. Note the position of the bubble level in (b) when the densiometer is leveled (reprinted from Ode 2007).

All densiometer readings should be taken with the bubble leveled, and 0.3 m (1 ft) above the water surface. The densiometer should be held just far enough from the squatting observer's body so that his/her forehead is just barely obscured by the intersection of the two pieces of tape.

Take and record four 17-point readings from the center of each transect: a) facing upstream, b) facing downstream, c) facing the left bank, d) facing the right bank. Optional readings can also be taken at the left and right banks (facing away from the stream, for these positions).

7. PHYSICAL HABITAT INTER-TRANSECT-BASED MEASUREMENTS

While most measures are taken at or relative to the main transects, a few measures are recorded at transects located at the midpoint between main transects. These are called "Inter-transects". The following measurements are taken relative to the Inter-transects: 1) Wetted Width, 2) Flow Habitats, and 3). "Pebble Count": Transect Substrates (including algal cover, as for the main transects).

7.1 Inter-transect Wetted Width

Measure the same way that *Transect* wetted width was measured.

7.2 Inter-transect Substrates and Percent Algal Cover

Collect these data the same way that *Transect* substrates and percent algal cover data were collected.

7.3 Flow Habitats

Because many benthic organisms prefer specific flow and substrate microhabitats, the proportional representation of these habitats in a reach is often of interest in bioassessments. There are many different ways to quantify the proportions of different flow habitats. This procedure produces a semi-quantitative measure consisting of 10 transect-based visual estimates.

At each Intertransect, identify the percentage of six different habitat types in the region *between the upstream Transect and downstream Transect*: 1) cascades, 2) falls, 3) rapids, 4) riffles, 5) runs, 6) glides, 7) pools, and 8) dry areas. Record percentages to the nearest 5% — the total percentage of surface area for each section must equal 100%.

A description of each of these flow habitat types is provided below:

- cascades: short, high-gradient drops in stream bed elevation often accompanied by boulders and considerable turbulence
- falls: high-gradient drops in elevation of the stream bed associated with an abrupt change in the bedrock
- rapids: sections of stream with swiftly flowing water and considerable surface turbulence (rapids tend to have larger substrate sizes than riffles)
- riffles: *"shallow/fast"*; riffles are shallow sections where the water flows over coarse stream bed particles that create mild to moderate surface turbulence (< 0.5 m deep, > 0.3 m/s)
- runs: *"deep/fast"*; long, relatively straight, low-gradient sections without flow obstructions. The stream bed is typically even and the water flows faster than it does in a pool (> 0.5 m deep, > 0.3 m/s)
- glides: *"shallow/slow"*; sections of stream with little or no turbulence, but faster velocity than pools (< 0.5 m deep, < 0.3 m/s)
- pools: *"deep/slow";* a reach of stream that is characterized by deep, low-velocity water and a smooth surface (> 0.5 m deep, < 0.3 m/s)
- dry: any surface area within the channel's wetted width that is above water

After you have collected all the above Transect-, and Inter-transect-, based measurements, collect data on Gradient. Also, if you have not already done so, take photographs at specific Transects, as indicated below. After you have collected Gradient data at each Transect, and have taken photographs where indicated, remove the corresponding flag from the stream bank.

7.4 Photographs

Take a minimum of four (4) photographs of the reach at the following locations: a) Transect A facing upstream, b) Transect F facing upstream, c) Transect F facing downstream, and d) Transect K facing downstream. It is also desirable to take a photograph at Transect A facing downstream and Transect K facing upstream to document conditions immediately adjacent to the reach. Digital photographs should be used. Record the image numbers on the front page of the field form under "*Photographs*". *NOTE: An easy way to keep track of which site each series of photographs belongs to is to take a close-up of the front data sheet (containing legible site code and date) for that site prior to taking the series of photos.*

8. REACHWIDE MEASUREMENTS

8.1 Gradient

The gradient of a stream reach is one of the major stream classification variables, giving an indication of potential water velocities and stream power, which are in turn important controls on aquatic habitat and sediment transport within the reach. The data collected for gradient are recorded on the "*Slope and Bearing*" form.

Note: An autolevel should be used for reaches with a percent slope of less than or equal to 1%. Either a clinometer or an autolevel may be used for reaches with a percent slope of greater than 1%, and sometimes a clinometer is preferable in really steep areas that are also heavily vegetated. The following description is for clinometer-based slope measurements. *In reaches that are close to 1%, you will not know whether you are above or below the 1% slope cutoff. In these cases, default to use of an autolevel, which is described further below*.

Clinometer method: Transect to transect measurements taken with a clinometer are used to calculate the average slope through a reach. This measurement works best with two people, one taking the readings at the upstream transect ("backsighting") and the other holding a stadia rod at the downstream transect. If you cannot see the mid point of the next transect from the starting point, use the supplemental sections (indicating the proportion of the total length represented by each section). Otherwise, leave these blank.

Beginning with the upper transect (Transect K), one person (the measurer) should stand at the water margin with a clinometer held at eye level. A second person should stand at the margin of the next downstream transect (Transect J) with a stadia rod flagged at the eye level of the person taking the clinometer readings. Be sure you mark your eye level while standing on level ground! Adjust for water depth by measuring from the same height above the water surface at both transects. This is most easily accomplished by holding the base of the stadia rod at water level. Note: an alternative technique is to use two stadia rods pre-flagged at the eye-height of the person taking the readings.

Use a clinometer to measure the percent slope of the water surface (not the streambed) between the upstream transect and the downstream transect by sighting to the flagged position on the stadia rod. The clinometer reads both percent slope and degree of the slope. Be careful to read and record percent slope rather than degrees slope (the measurements differ by a factor of ~2.2). Percent slope is the scale on the right hand side as you look through most clinometers. Note: If an autolevel or hand level is used, record the elevation difference (rise) between transects and the segment length (run) instead of the percent slope.

If the stream reach geometry makes it difficult to sight a line between transects, divide the distance into two sections and record the slope and the proportion of the total segment length between transects for each of these sections in the appropriate boxes on the slope form (supplemental segments). Do not measure slope across dry land (*e.g.*, across a meander bend).

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

the other transect and inter-transect based measurements for PHab, you may remove the transect flags as you go.

Autolevel method (preferred): To measure gradient using an autolevel, identify a good spot to set up the autolevel, preferably somewhere around the center of the reach (if there is good visibility from this location to both the upstream and downstream ends of the reach.) Set up the autolevel on very stable, and preferably fairly flat, ground. Set the height of the autolevel to comfortable eye level for the operator. Level the plane of view of the autolevel by balancing it using the bubble. Start by adjusting the legs, and then fine-tune the adjustment using the knobs. Once balanced, begin "shooting" the change in the height of the water level of the stream from transect to transect. Try to start with one of the outer transects (like A). Have a field partner at Transect A hold the Stadia rod at water's edge and perpendicular to the ground. Viewing through the autolevel (and focusing as necessary), look at the Stadia rod and note to the smallest demarcation on the stadia rod the height at which the autolevel line of view (*i.e.*, the middle line in the viewfinder) hits. Record this information, and then have the Stadia rod holder proceed to the next transect (*e.g.*, Transect B), again holding the base of the Stadia rod at water's edge. Very carefully, rotate the head of the autolevel so that it points to the new Stadia rod location. *Do not bump the autolevel out of its position, because if this happens, you will not be able to take a height measurement of Transect B's water surface relative to that of Transect A, to determine the slope between the two transects*.

If the autolevel is bumped out of position before all the measurements are done, or if there is a point along the reach at which there is no longer a clear line of sight from the autolevel to the Stadia rod positioned at the transect, at water's edge, a new location must be set up for the autolevel. In order to maintain a relationship between water heights of the various transects already measured, it will be necessary to "re-shoot" the height of the water at the last transect for which a valid measurement was attained. From there, assuming there is no more disturbance to the position of the autolevel, you can continue cycling through the remaining transects from the new position. On the Slope and Bearing Form corresponding to autolevel use, indicate when the autolevel's position has been changed. If it is necessary to move the autolevel at some point, the transect that was measured from the original and the new position will be listed twice on the datasheet: once for the original position, and once for the new. Also indicate the distance between main transects (*i.e.*, 15 m, 25 m or other). These pieces of information will later be used to determine the slopes between transects and for the reach as a whole.

8.2 Stream Discharge

Stream discharge is the volume of water that moves past a point in a given amount of time and is generally reported as cubic feet per second (cfs) or cubic meters per second (cms). Because discharge is directly related to water volume, discharge affects the concentration of nutrients, fine sediments and pollutants; and discharge measurements are critical for understanding impacts of disturbances such as impoundments, water withdrawals and water augmentation. Discharge is also closely related to many habitat characteristics including temperature regimes, physical habitat diversity, and habitat connectivity. As a direct result of these relationships, stream discharge is often also a strong predictor of biotic community composition. Since stream volume can vary significantly on many different temporal scales (diurnal, seasonal, inter-annually), it can also be very useful for understanding variation in stream condition.

It is preferable to take discharge measurements in sections where flow velocities are greater than 0.5 ft/s and most depths are greater than 15 cm, but slower velocities and shallower depths can be used. If flow volume is sufficient for a transect-based "velocity-area" discharge calculation, this is by far the preferred method. If flow volume is too low to permit this procedure or if your flow meter fails, use the "neutrally buoyant object/ timed flow" method.

8.2.1 Discharge: Velocity Area Method

The layout for discharge measurements under the velocity-area (VA) method is illustrated in Figure 13 . Flow velocity should be measured with either a Swoffer Instruments propeller-type flow meter or a Marsh-McBirney inductive probe flow meter.

Select the best location in the reach for measuring discharge. To maximize the repeatability of the discharge measurement, *choose a transect with the most uniform flow (select hydraulically smooth flow whenever possible) and simplest cross-sectional geometry*. It is acceptable to move substrates or other obstacles to create a more uniform cross-section before beginning the discharge measurements.

Data for this parameter will be entered in the "*Discharge Measurements*" section of the datasheet with the basic site information at the top ("*Reach Documentation*"). Measure the wetted width of the discharge transect and divide this into 10 to 20 equal segments. The use of more segments gives a better discharge calculation, but is impractical in small channels. A minimum of 10 intervals should be used when stream width permits, but interval width should not be less than 15 cm.

At each interval, record the distance from the bank to the end of the interval. Using the top-setting rod that comes with the flow velocity meter, measure the median depth of the interval. Standing downstream of the transect to avoid interfering with the flow, use the top-setting rod to set the probe of the flow meter at the midpoint of the interval, at 0.6 of the interval depth (this position generally approximates average velocity in the water column), and at right angles to the transect (facing upstream). See Figure 13 for positioning detail.

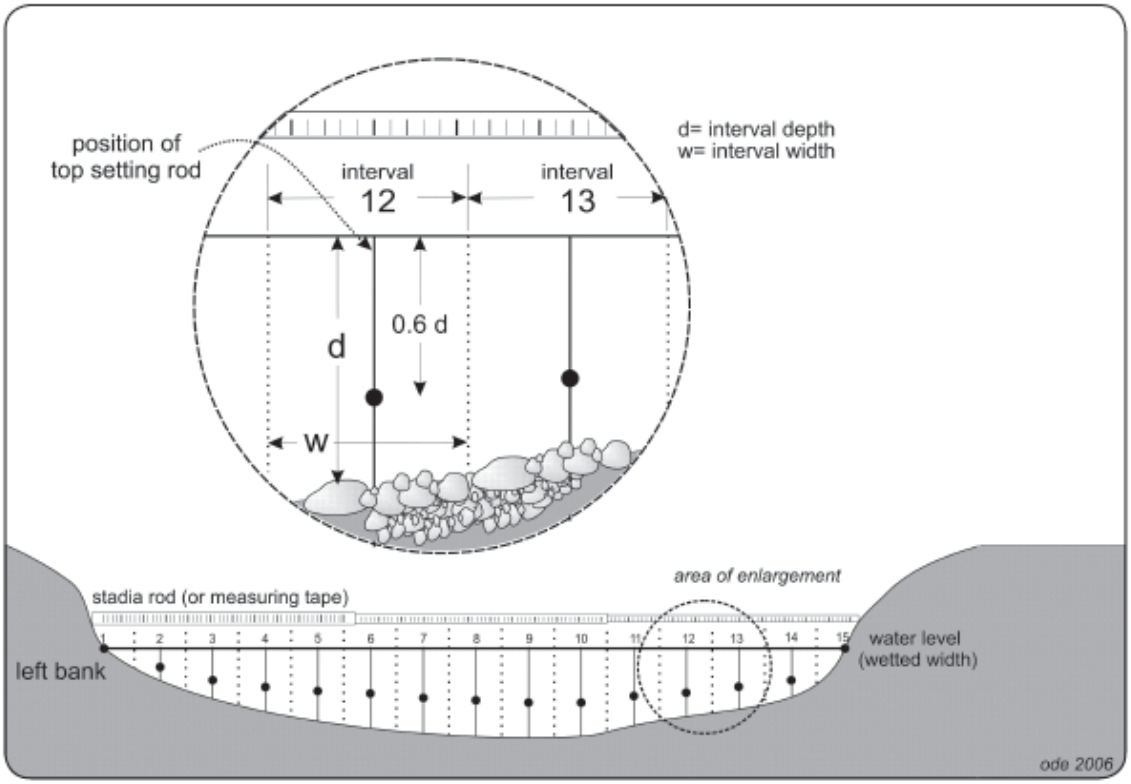


Figure 13. Diagram of layout for discharge measurements under the velocity-area method showing proper positions for velocity probe (black dots; reprinted from Ode 2007).

Allow the flow velocity meter to equilibrate for 10-20 seconds, then record velocity to the nearest **ft/s**. If the option is available, use the flow averaging setting on the flow meter. Note: Under very low flow conditions, flow velocity meters may register readings of zero even when there is noticeable flow. In these situations, record a velocity of 0.5x the minimum flow detection capabilities of the instrument. Complete these steps on each of the intervals across the stream. Note: The first and last intervals usually have depths and velocities of zero.

8.2.2 Discharge: Neutrally Buoyant Object Method

If streams are too shallow to use a flow velocity meter, the neutrally buoyant object (NBO) method should be used to measure flow velocity. However, since this method is less precise than the flow velocity meter it should only be used if absolutely necessary. A neutrally buoyant object (one whose density allows it to just balance between sinking and floating) will act as if it were nearly weightless, thus its movement will approximate that of the water it floats in better than a light object. A piece of orange peel works well. To estimate the flow velocity through a reach, three transects are used to measure the cross-sectional areas within the test section sub-reach and three flow velocity estimates are used to measure average velocity through the test reach. To improve precision in velocity measurements, the reach segment should be long enough for the float time to last at least 10-15 seconds.

The position of the discharge sub-reach is not as critical as it is for the velocity-area method, but the same criteria for selection of a discharge reach apply to the neutrally buoyant object method. Identify a section that has relatively uniform flow and a uniform cross sectional shape.

The cross sectional area is estimated in a manner that is similar but less precise than that used in the velocity area method. Measure the cross sectional area in one to three places in the section designated for the discharge measurement (three evenly-spaced cross sections are preferred, but one may be used if the cross section through the reach is very uniform). Record the width once for each cross section and measure depth at five equally-spaced positions along each transect.

Record the length of the discharge reach.

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10. GLOSSARY

Aliquot – a measured portion of a sample, or subsample

Ash-free dry mass (AFDM) – the portion, by mass, of a dried sample that is represented by organic matter; the concentration of AFDM per stream surface area sampled can be used as a surrogate for algal biomass

Benthic algae – algae that are anchored to, or have at one point been anchored to, the stream bottom, in contrast to planktonic algae which are free-floating in the water column

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

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

Composite sample - Volume of all the liquid material amassed during sampling, including water used for rinsing substrate and sampling devices. Final composite volume should not exceed 400-500 ml.

Cyanobacteria – historically referred to as "blue-green" algae, but actually bacteria that are capable of photosynthesis and co-occur with true benthic algae in streams; useful as a bioindicator, and field-sampled and laboratory-processed concurrently with soft-bodied algae

Depositional – habitats in the stream that are dominated by slow-moving water, such as pools, where deposition of materials from the water column is more likely to occur than erosion (or (re)suspension) of loose bed materials; examples of "depositional" substrates include silt and sand

Diatom – a unicellular alga that possesses a rigid, silicified (silica-based) cell wall in the form of a "pill box"

Erosional – habitats in the stream that are dominated by fast-moving water, such as riffles, where stream power is more likely to facilitate erosion (suspension) of loose benthic material than deposition; examples of "erosional" substrates include cobbles and boulders

Homogenate – mixture of liquid composite sample and finely chopped fragments of macroalgae

Index of Biotic Integrity (IBI) – a quantitative assessment tool that uses information about the composition of one or more assemblages of organisms to make inferences about condition the environment they occupy (*e.g.*, the assemblage of interest could be diatoms or benthic macroinvertebrates living in a stream)

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

Microalgae – diatoms and microscopic soft-bodied algae, including unicellular forms; can co-occur with other microorganisms in a biofilm

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

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

Wetted width – the distance between the sides of the channel at the point where substrates are no longer surrounded by surface water