Appendix C

SWAMP Bioassessment Protocols

SWAMP
Surface Water Ambient Monitoring Program

SWAMP Bioassessment Procedures 2007

Standard Operating Procedures for Collecting Benthic Macroinvertebrate Samples and Associated Physical and Chemical Data for Ambient Bioassessments in California

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Standard Operating Procedures for Collecting Benthic Macroinvertebrate Samples and Associated Physical and Chemical Data for Ambient Bioassessments in California

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ACKNOWLEDGEMENTS A

The protocols described here represent the contributions of a wide range of researchers and field crews. Most of the physical habitat methods are close modifications of those used in the U.S. Environmental Protection Agency's (EPA's) Environmental Monitoring and Assessment Program (EMAP) and developed by EPA's Office of Research and Development (ORD, Peck et al. 2004). The benthic macroinvertebrate collection methods are based on EMAP methods (EPA's targeted riffle methods were derived in turn from methods developed at Utah State University; Hawkins et al. 2003).

The current version of these protocols was established by Peter Ode (Department of Fish and Game's (DFG's) Aquatic Bioassessment Laboratory (ABL)) and David Herbst (UC Santa Barbara's Sierra Nevada Aquatic Research Laboratory) with significant contributions from staff at the ABL (Jim Harrington, Shawn McBride, Doug Post, Andy Rehn, and Jennifer York), the Surface Water Ambient Monitoring Program (SWAMP) Quality Assurance (QA) Team, Thomas Suk and other members of the SWAMP bioassessment committee (Mary Adams, Lilian Busse, Matt Cover, Robert Holmes, Sean Mundell, and Jay Rowan) and three external reviewers: Chuck Hawkins, Dave Peck, and Phil Kaufmann.

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SWAMP GUIDANCE SG

SWAMP GUIDANCE FOR MACROINVERTEBRATE FIELD PROTOCOLS FOR WADEABLE STREAMS

Background: The SWAMP Bioassessment Committee met in December, 2004, and agreed that the SWAMP Quality Assurance Management Plan (QAMP) should be amended to provide greater consistency in bioassessment sampling protocols for wadeable streams. The Committee's recommendations were reviewed and accepted by the full SWAMP Roundtable[1] in February, 2005 (some of the key considerations are contained in Appendix A).

The current guidance for macroinvertebrate sampling under the SWAMP program is as follows:

1. For ambient bioassessment monitoring of wadeable streams in California, two methods are to be used at sites with riffle habitats (i.e., one "multihabitat" sample, and one sample that targets the "richest" habitat):
   - **For sites with sufficient riffle habitat,** the two samples shall be: (1) the reachwide benthos (RWB) method (also known as "multihabitat" sampling.); and (2) the targeted-riffle composite (TRC) method.
   - **For low-gradient sites that do not have sufficient riffle habitat**, the RWB method is the standard method, but we also recommend the option of collecting a sample with (2) the "Margin-Center-Margin" (MCM) method until ongoing methods comparisons are completed (see Appendix A).
   - **Notes:** (1) The protocols for each method are provided in this document; (2) Other appropriate method(s) will be allowed if the specific monitoring objectives require use of alternative method(s). (See Item #2, below.); (3) The protocol recommendations specified above will be reevaluated as results become available from ongoing methods comparison studies. (See Appendix A for more information.)

2. The SWAMP QAMP allows flexibility in sampling methods so that the most appropriate method(s) may be used to address hypothesis tests and project-specific objectives that differ from program objectives. Such situations may include, but are not necessarily limited to, special studies (e.g., evaluation of point source discharges, above/below comparisons where statistical replication is needed), stressor identification investigations, and long-term monitoring projects where consistent data comparability is desired and an alternative method is needed to achieve that comparability. In addition, in some rare cases where funding limitations would make it cost-prohibitive to complete a project in compliance with the protocols listed in #1, above, the project proponent may request to complete laboratory analysis of only one sample, and "archive" one of the macroinvertebrate samples (i.e., the RWB sample in streams with riffles) to reduce lab costs. Deviations from the protocols specified in #1 above may be granted by the SWAMP Bioassessment Coordinator or the full SWAMP Roundtable.

1. The SWAMP Roundtable is the coordinating entity for the program. Participants include staff from the State and Regional Water Boards, USEPA, the Department of Fish and Game, the Marine Pollution Studies Laboratory, Moss Landing Marine Laboratories, contractors, and other interested entities.

SECTION 1 INTRODUCTION

This document describes two standard procedures (TRC and RWB) for sampling benthic macroinvertebrate (BMI) assemblages for ambient bioassessments. This document also contains procedures for measuring instream and riparian habitats and ambient water chemistry associated with BMI samples. These sampling methods replace previous bioassessment protocols referred to as the California Stream Bioassessment Procedure (CSBP, Harrington 1995, 1999, 2002).

These procedures can produce quantitative and repeatable measures of a stream's physical/habitat condition and benthic invertebrate assemblages, but they require field training and implementation of QA measures throughout the field season.

The sampling layout described here provides a framework for systematically collecting a variety of physical, chemical, and biological data. The biological sampling methods are designed to nest within the overall framework for assessing the biotic, physical, and chemical condition of a reach. The layout used in these procedures and most of the physical habitat methods are close modifications of those used in EPA's EMAP and developed by EPA's ORD (Peck et al. 2004). Data collected using this methodology are generally directly comparable to equivalent EMAP data, except for the difference in reach length. Other exceptions are noted in the text.

The following steps are presented in an order suggested for efficient data collection. The specific order of collection for the physical parameters may be modified according to preferences of field crews, with the caveat that care must always be taken to not disturb the substrates within the streambed before BMI samples are collected.

PHYSICAL HABITAT METHODS

The physical habitat scoring methods described here can be used as a stand-alone evaluation or used in conjunction with a bioassessment sampling event. However, measurements of instream and riparian habitat and ambient water chemistry are essential to interpretation of bioassessment data and should always accompany bioassessment samples. This information can be used to classify stream reaches, associate physical and chemical condition with biotic condition, and explain patterns in the biological data.

Because bioassessment samples can be collected to answer a variety of questions, this document describes the component measures of instream and riparian habitat as independent modules. Although individual modules can be added or subtracted from the procedure to reflect specific project objectives, a standard set of modules will normally accompany bioassessment samples. This document describes two standard groupings of modules that represent two different levels of intensity for characterizing the chemical and physical habitat data (Table 1). The BASIC physical habitat characterization represents a minimum amount of physical and chemical data that should be taken along with any ambient BMI sample, the FULL physical habitat characterization represents the suite of data that should be collected with most professional level bioassessment samples (e.g., SWAMP regional monitoring programs). In addition to these data, we also briefly introduce additional data modules (e.g., excess sediment, periphyton) that can be collected as supplements to the full set (OPTIONAL). Table 1 lists the physical and chemical variables that should be measured under the different levels.

Note: *SWAMP intends to develop guidance for selecting appropriate physical habitat modules to the intended uses of data. Until this guidance is available, users of these protocols should consult with representatives of the Regional Water Quality Control Boards (Regional Boards) or the SWAMP Bioassessment Coordinator when selecting modules.*

FIELD CREW SIZE AND TIME ESTIMATES

These methods are designed to be completed by either two or three (or more) person field crews. A very experienced field crew can expect to complete the full suite of physical habitat measurements and the two BMI sampling protocols in approximately two hours. Less experienced crews will probably take closer to three or four hours to complete the work depending on the complexity of the reach. Note that this estimate includes only time at the site, not travel time between sites.

Equipment and Supplies

Recommended equipment and supplies are listed in Table 2.

Table 1. Summary of physical habitat and water chemistry and proposal for basic, full, and optional levels of effort.

Survey Task	Parameter(s)	Basic	Full	Option	Comments
REACH DELINEATION and WATER QUALITY [Conducted before entering stream to sample BMIs or conduct any habitat surveys]	Layout reach and mark transects, record GPS coordinates	X	X		Use 150-m reach length if wetted width ≤10 m; Use 250-m reach length if wetted width > 10 m
	Temperature, pH, specific conductance, DO, alkalinity	X	X		Multi-meter (e.g., YSI, Hydrolab, VWR Symphony)
	Turbidity, Silica			X	Use test kit or meter
	Notable field conditions	X	X		Recent rainfall, fire events, dominant local landuse
CROSS-SECTIONAL TRANSECTS BASIC Measurements at main 11 transects only FULL Measurements at 11 main transects (A, B, C, D, E, F, G, H, I, J, K) or 21 transects (11 main plus 10 inter-transects) for substrate size classes only	Wetted width	X	X		Stadia rod is useful here
	Flow habitat delineation	X	X		Record proportion of habitat classes in each inter-transect zone
	Depth and Pebble Count + CPOM		X		5 -point substrate size, depth and CPOM records at all 21 transects
	Cobble embeddedness		X		All cobble-sized particles in pebble count. Supplement with "random walk" if needed for 25
	Slope (%)	See reach scale	X		Average slope calculated from 10 transect to transect slope measurements. Use autolevel for slopes ≤ 1%; clinometer is OK for steeper gradients
	Sinuosity		X		Record compass readings between transect centers
	Canopy cover	X	X		Four densiometer readings at center of channel (facing L bank R bank, Upstream +Downstream)
	Riparian Vegetation		X		Record % or categories
	Instream Habitat		X		
	Human Influence		X		
	Bank Stability	X	X		Eroding / Vulnerable / Stable
	Bankfull Dimensions		X		
	Excess Sediment Transect Measures (optional)				
	Bankfull width and height, bank angles			X	
	Large woody debris counts			X	Tallies of woody debris in several size classes
	Thalweg profile			X	100 equidistant points along thalweg

Survey Task	Parameter(s)	Basic	Full	Option	Comments
DISCHARGE TRANSECT	Discharge measurements		X		Velocity-Area Method or Neutrally Buoyant Object Method
REACH SCALE MEASUREMENTS:	EPA-RBP visual scoring of habitat features	*		X	*Used for citizen monitoring and comparison with legacy data
	Selected RBP visuals:		X		Channel alteration, sediment deposition, epifaunal substrate (redundant if doing EPA-RBP scoring)
	Slope (%, not degrees)	X	See transect scale		Single measurement for entire reach only for BASIC. Use autolevel for slopes ≤ 1%, clinometer is OK for higher gradients
	Photo documentation	X	X		Upstream (A, F, K) Downstream (F)
OTHER OPTIONAL COMPONENTS					
FOOD RESOURCE QUANTIFICATION	Periphyton (3 replicates)			X	Qualitative characterization of diatom growth and filamentous algal growth, quantification of biomass (AFDM, chl-a)
	CPOM & FPOM (3 replicates)			X	CPOM field measure of wet mass >1 mm particles, FPOM as 0.25 – 1 mm fraction (AFDM in lab)

Table 2. Field equipment and supplies

Physical Habitat	BMI Collection	General/ Ambient Chemistry
• GPS receiver • topographic maps • measuring tape (150-m) • small metric ruler or gravelometer for substrate measurements • digital watch, random number table or ten-sided die • stadia rod • clinometer • autolevel (for slopes < 1%) • handlevel (optional) • current velocity meter • stopwatch for velocity measurements • convex spherical densitometer • flags/ flagging tape • rangefinder	• D-frame kick net (fitted with 500-µ mesh bag) • standard # 35 sieve (500-µ mesh) • wide-mouth 500-mL or 1000 mL plastic jars • white sorting pan (enamel or plastic) • 95% EtOH • fine tipped forceps or soft forceps • waterproof paper and tape for attaching labels • 10-20-L plastic bucket for sample elutriation • preprinted waterproof labels (e.g., Rite-in-the-Rain™) • disposable gloves/ elbow length insulated gloves	• sampling SOP (this document) • hip or chest waders, or wading boots/shoes • field forms printed on waterproof paper (e.g., Rite-in-the-Rain™) • clip board and pencils • digital camera • centigrade thermometer • pH meter • DO meter • conductivity meter • field alkalinity meter • water chemistry containers • calibration standards • spare batteries for meters • first aid kit

SECTION 2
REACH DELINEATION AND WATER QUALITY

REACH LAYOUT AND GENERAL DOCUMENTATION

The systematic positioning of transects is essential to collecting representative samples and to the objective quantification of physical habitat measures. The standard sampling layout consists of a 150-m reach (length measured along the bank) divided into 11 equidistant transects that are arranged perpendicular to the direction of flow (Figure 1, Figure 2). Ten additional transects (designated "inter-transects") located between the main transects give a total of 21 transects per reach. Main transects are designated A through K while inter-transects are designated by their nearest upstream and downstream transects (e.g., AB, BC, etc.). In extreme circumstances, reach length can be shorter than 150 m (e.g., if upstream and downstream barriers preclude a 150-m reach), but this should be avoided whenever possible. If the actual reach length is other than 150 m or 250 m this should be noted and explained on the field forms.

Note 1: *The standard reach length differs from that used in the EMAP design, in which reach length was defined as 40x stream width, with a minimum reach length of 150 m. The EMAP reach length approach is used to ensure that enough habitat is sampled to support accurate fish assemblage estimates and relatively precise characterization of channel characteristics (e.g., residual pool volumes and woody debris estimates, which that are critical for relative bed stability estimates). Programs wishing to sample fish assemblages or produce relative bed stability estimates should strongly consider adopting the EMAP guidance for setting reach length.*

Note 2: *Streams > 10 m wetted width should use a reach length of 250 m. Some very large streams (i.e., > 20-m wetted width) may not be adequately represented even by a 250-m reach. In these cases, field crews should define a reach length that is representative of the larger stream segment being studied (i.e., attempt to include two to three meander cycles, or four to six riffle-pool sequences when possible).*

Note 3: *When the exact reach location is not restricted by the sampling design, attempt to position reaches upstream of bridges to avoid this influence.*

Step 1. Upon arrival at the sampling site, fill out the reach documentation section of the field forms (site and project identification, stream and watershed name, crew members, and date/time). If known at the time of sampling, record the Site Code following SWAMP site code formats. Determine the geographic coordinates of the downstream end of the reach (preferably in decimal degrees to at least four decimal places) with a GPS receiver and record the datum setting of the unit (preferably NAD83/ WGS84).

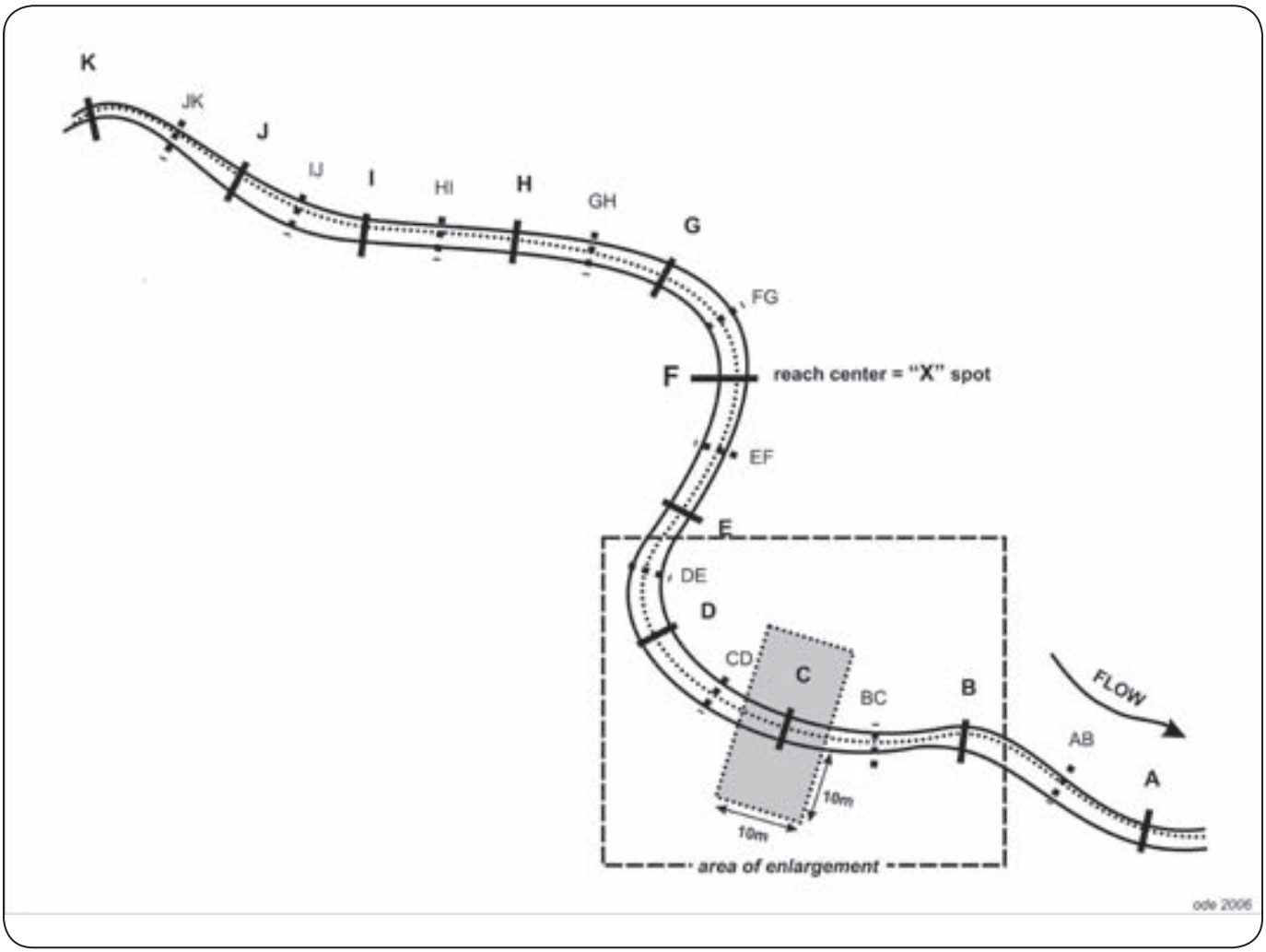

Figure 1. Reach layout geometry for physical habitat and biological sampling showing positions of 11 main transects (A – K) and the 10 supplemental inter-transects (AB- JK). The area highlighted in the figure is expanded in Figure 2. Note: reach length = 150 m for streams ≤ 10-m average wetted width, and reach length = 250 m for streams > 10-m average wetted width.

Step 2. Once a site has been identified, make an initial survey of the reach from the stream banks (being sure to not disturb the instream habitat). If TRC samples will be collected, identify all riffle habitats suitable for sampling (see Section IIIa for suitable habitat types) and note their positions so that a subset can be identified for sampling.

Step 3. Determine if the average wetted width is greater or less than 10 m. If the average wetted width ≤ 10 m, use a 150-m reach length. If the average wetted width > 10 m, use a 250-m reach length.

Step 4. Starting at one end of the reach, establish the position of the 11 main transects (labeled A-K from downstream to upstream) by measuring 15 m (25 m for streams > 10 m wetted width) along the bank from the previous transect. The 10 inter-transects should be established equidistant from the adjacent main transects (i.e., 7.5 m from main transects for 150-m reaches, 12.5 m for 250-m reaches). Since the data collection will start at the downstream end, is often easiest to establish transects starting from the upstream end. For easy setup and breakdown, mark the main transects with easily removable markers (e.g., large washers tied with strips of flagging, surveyor's flags).

Note 1: *While it is usually easiest to establish transect positions from the banks (this also reduces disturbance to the stream channel), this can result in uneven spacing of transects in complex stream reaches. To avoid this, estimate transect positions by projecting from the mid-channel to the banks.*

Note 2: *Flagging of a single bank is recommended to reduce mistakes caused by missed markers.*

Step 5. Measure and record common ambient water chemistry measurements (pH, DO, specific conductance, alkalinity, water temperature) at the downstream end of the reach (near same location as the GPS coordinates were taken). These are typically taken with a handheld water quality meter (e.g., YSI, Hydrolab), but field test kits (e.g., Hach) can provide acceptable information if they are properly calibrated. For appropriate calibration methods and calibration frequency, consult the current SWAMP QAMP (Appendix F), or follow manufacturer's guidelines.

Note 1: *If characteristics of the site prohibit downstream entry, measurements may be taken at other points in the reach. In all cases, ambient chemistry measurements should be taken at the beginning of the reach survey.*

Note 2: *Alkalinity test kits may not perform well in low ionic strength waters. Programs should consider collecting lab samples for these sites (see SWAMP QAMP for guidance on collecting water chemistry samples).*

Step 6. Take a minimum of four (4) photographs of the reach at the following locations: a) Transect A facing upstream, b) Transect F facing upstream, c) Transect F facing downstream, and d) Transect K facing downstream. It may also be desirable to take a photograph at Transect A facing downstream and Transect K facing upstream to document conditions immediately adjacent to the reach. Digital photographs should be used when possible. Record the image numbers on the front page of the field form.

Note 1: *When possible, photograph names should follow SWAMP coding conventions ("StationCode_yyyy_mm_dd_uniquecode"). The unique code should include one of the following codes to indicate direction: RB (right bank), LB (left bank), BB (both banks), US (upstream), DS (downstream). SWAMP suggests using unique codes created by the camera to facilitate file organization. Example: 603WQLB02_2004_03_20_RBDS1253.*

Step 7. Record the dominant land use and land cover in the area surrounding the reach (evaluate land cover within 50 m of either side of the stream reach).

Step 8. At the bottom of the form, record evidence of recent flooding, fire, or other disturbances that might influence bioassessment samples. Especially note if flow conditions have been affected by recent rainfall, which can cause significant under-sampling of BMI diversity (see note in the following section). If you are unaware of recent fire or rainfall events, select the "no" option on the forms.

SECTION 3 COLLECT BENTHIC MACROINVERTEBRATES

MULTIPLE HABITAT AND TARGETED RIFFLE PROTOCOLS

Note 1: *BMI samples intended for ambient bioassessments are generally collected when streams are at or near base flow (i.e., not influenced by surface runoff) as sudden flow increases can dramatically alter local community composition.*

Note 2: *Guidance for choosing among TRC sampling, RWB sampling or both will be provided in a separate document (see Appendix A for current guidance for sampling under SWAMP).*

Once the reach transects have been laid out, the biological samples (BMIs and algae if included) should be collected before any other physical habitat measures so that substrates are not disturbed prior to sampling. Both TRC and RWB methods use 500-µ mesh D-frame nets (see list of BMI sampling equipment in Table 2). The two samples can be collected at the same time by carrying two D-nets and compositing the material from the two samples in their respective nets. If a two person field crew is responsible for both the physical habitat data and benthic invertebrate samples, it is generally best to collect the benthos at each transect, then immediately record the physical habitat data before moving to the next transect. Obviously, this requires especially careful handling of the D-nets during the course of sampling to avoid loss or contamination of the samples. It can be helpful to clearly label the two D nets as RWB and TRC. Larger field crews may choose to split the sampling between biological team and a physical habitat team and have the biological team go through the reach first. The positions of the TRC and RWB subsampling locations are illustrated in Figure 2.

SECTION III A. TARGETED RIFFLE COMPOSITE PROCEDURE

The TRC method is designed for sampling BMIs in wadeable streams that contain fast-water (riffle/run) habitats and is not appropriate for waterbodies without fastwater habitats. The RWB protocol should be used in these situations. Riffles are often used for collecting biological samples (e.g., the old CSBP methods) because they often have the highest BMI diversity in wadeable streams. This method expands the definition to include other fast water habitats, however care should be taken when attempting to apply this method in low gradient streams.

Note: *Since all streams (even low gradient streams) have variation in flow habitats within the channel, this guidance should not be interpreted as including areas within low gradient streams that are only marginally faster than the surrounding habitats. The RWB protocol should be applied in these situations.*

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

Sampling Locations – Acceptable Habitat Types

Riffles are the preferred habitat for TRC sampling, but other fast water habitats are acceptable for sampling if riffles are sparse. Common flow-defined habitat types are listed in Table 3 in decreasing order of energy. Most streams contain some or all of the following fast water habitat types: 1) cascades/falls, 2) rapids, 3) riffles, 4) runs. All of these are acceptable for TRC sampling if riffles are not available.

Note: *Because the common habitat types are arranged on a continuum between high to low energy environments, the categories grade into each other continuously and are not discrete. Thus, determination of habitat types requires somewhat subjective decision-making.*

Table 3. Common habitat types in stream channels, arranged in decreasing order of energy

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

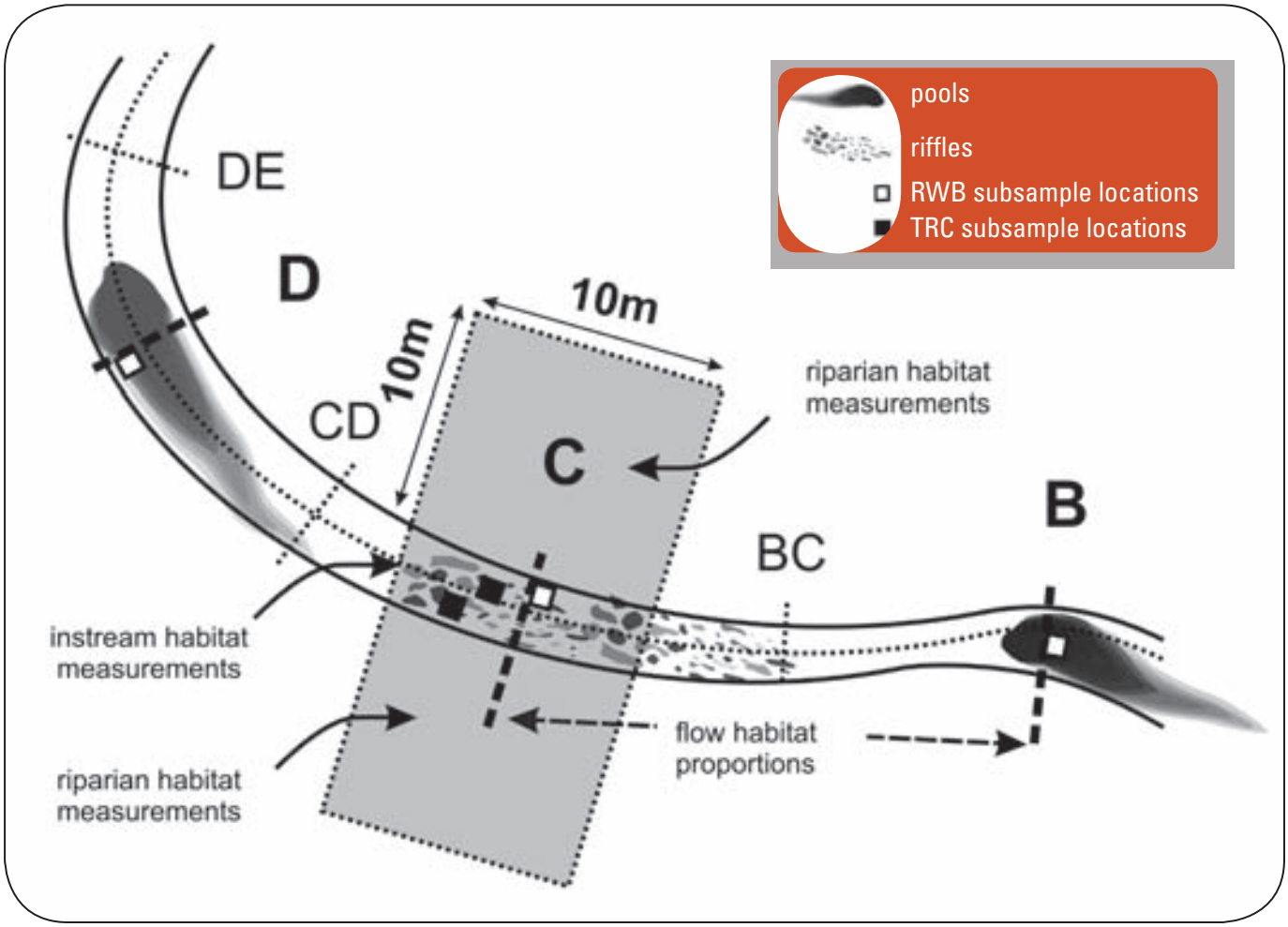

Figure 2. Section of the standard reach expanded from Figure 1 showing the appropriate positions for collecting benthic macroinvertebrate samples, instream and riparian habitat measurements and flow habitat proportion measurements.

Sampling Locations – Selecting Habitat Units

A TRC sample is a composite of eight individual kick samples of 1 ft^2 (0.09 m^2) of substrate each. During your initial layout of the reach, take a mental note of the number and position of the main riffles in a reach (and other fast water habitats if needed). Randomly distribute the eight sub-samples among the fast water habitats in the reach, giving preference to riffles where possible. Unless you are sampling in small streams, try to avoid very small riffle units (i.e., < 5 ft^2). If fewer than eight riffles are present in a reach, more than one sample may be taken from a single riffle, especially if the riffles are large.

Sampling Procedure

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

TRC-Step 1. Determine net placement within each habitat unit by generating a pair of random numbers between 0 and 9. Examples of convenient random number generators include the hundredths place on the stopwatch feature of a digital watch, a 10 sided die and a random number chart. The first number in each pair (multiplied by 10) represents the percent upstream along the habitat unit's length. The second number in each pair represents the percent of the riffle width from right bank. For example, if the two generated random numbers are 4 and 7, you will walk upstream 40% of the distance of the riffle and then go 70% of the distance across the riffle (see Figure 3). This position is the center of the 1 ft^2 (0.09 m^2) sampling quadrat for that riffle. If you are unable to sample this location because it is too deep or it is occupied by a large boulder, select a new pair of random numbers and pick a new spot.

TRC-Step 2. Position a 500-µ D-net (with the net opening perpendicular to the flow and facing upstream) quickly and securely on the stream bottom to eliminate gaps under the frame. Avoid, and if necessary remove, large rocks that prevent the sampler from seating properly on the stream bottom.

TRC-Step 3. Holding the net in position on the substrate, visually define a square quadrat that is one net width wide and one net width long upstream of the net opening. Since D-nets are 12 inches wide, the area within this quadrat is 1ft^2 (0.09 m^2). Restrict your sampling to within that area. If desired, a wire frame of the correct dimensions can be placed in front of the net to help delineate the quadrat to be sampled, but it is often sufficient to use the net dimensions to keep the sampling area consistent.

TRC-Step 4. Working backward from the upstream edge of the sampling plot, check the quadrat for heavy organisms such as mussels, snails, and stone-cased caddisflies. Remove these organisms from the substrate by hand and place them into the net. Carefully pick up and rub stones directly in front of the net to remove attached animals. Remove and clean all of the rocks larger than a golf ball (~3 cm) within your sampling quadrat such that all the organisms attached to them are washed downstream into your net. Set these rocks outside your sampling quadrat after you have cleaned them. If the substrate is consolidated or comprised of large, heavy rocks, use your feet to kick and dislodge the substrate to displace BMIs into the net. If you cannot remove a rock from the stream bottom, rub it (concentrating on cracks or indentations) thereby loosening any attached insects. As you are disturbing the plot, let the water current carry all loosened material into the net.

Note 1: *Brushes are sometimes used in other bioassessment protocols to help loosen organisms, but in the interest of standardizing collections, do not use a brush when following this protocol.*

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

TRC-Step 5. Once the coarser substrates have been removed from the quadrat, dig your fingers through the remaining underlying material to a depth of about 10 cm (this material is often comprised of gravels and finer particles). Thoroughly manipulate the substrates in the quadrat.

Note: *The sampler may spend as much time as necessary to inspect and clean larger substrates, but should take a standard time of 30 seconds to perform Step 5.*

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

TRC-Step 7. Move upstream to the next randomly selected habitat unit and repeat steps one through six, taking care to keep the net wet but uncontaminated by foreign material when moving the net from riffle to riffle. Sometimes, the net will become so full of material from the streambed that it is no longer effective at capturing BMIs. In these cases, the net should be emptied into sample jars as frequently as necessary, following guidelines described below in the "Preparation of BMI Sample Jars" section. Continue until you have sampled eight 1ft^2 (0.09 m^2) of benthos.

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

Figure 3. Example showing the method for selecting a subsampling position within a selected riffle under the TRC method. In this example, the random numbers 4 and 7 were selected

SECTION III B. REACHWIDE BENTHOS (MULTIHABITAT) PROCEDURE

The RWB procedure employs an objective method for selecting subsampling locations that is built upon the 11 transects used for physical habitat measurements. The RWB procedure can be used to sample any wadeable stream reach since it does not target specific habitats. Because sampling locations are defined by the transect layout, the position of individual sub-samples may fall in a variety of erosional or depositional habitats.

Note: *Sampling locations should be displaced one meter downstream of the transects to avoid disturbing substrates for subsequent physical habitat assessments.*

RWB -Step 1. The sampling position within each transect is alternated between the left, center and right positions along a transect (25%, 50% and 75% of wetted width, respectively) as you move upstream from transect to transect. Starting with the downstream transect (Transect

A), identify a point that is 25% of the stream width from the right bank (note that the right bank will be on your left as you face upstream). If you cannot collect a sample at the designated point because of deep water obstacles or unsafe conditions, relocate the point as close as possible to the designated position.

Note: *A modification to this procedure is currently being investigated by SWAMP. This "margin-center-margin" (MCM) modification replaces the samples at 25% and 75% of wetted width with samples of the marginal habitats (including emergent and submergent vegetation).*

RWB -Step 2. Place a 500-µ D-net in the water so the mouth of the net is perpendicular to and facing into the flow of the water. If there is sufficient current in the area at the sampling point to fully extend the net, use the normal D-net collection technique to collect the sub-sample (TRC-Step 3 through TRC-Step 6 above). If flow volume and velocity is not sufficient to use the normal collection technique, use the sampling procedure for "slack water" habitats (RWB-Step 3 through RWB-Step 7 below).

RWB -Step 3. Visually define a 1 ft^2 (0.09 m^2) quadrat that is one net-width wide and one net-width long at the sampling point.

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

RWB -Step 5. Vigorously kick the remaining finer substrate within the quadrat with your feet while dragging the net repeatedly through the disturbed area just above the bottom. Keep moving the net all the time so that the organisms trapped in the net will not escape. Continue kicking the substrate and moving the net for 30 seconds. For vegetation-choked sampling points, sweep the net through the vegetation within a 1ft^2 (0.09 m^2) quadrat for 30 seconds.

Note: *If flow volume is insufficient to use a D- net, spend 30 seconds hand picking a sample from 1ft^2 of substrate at the sampling point, then stir up the substrate with your gloved hands and use a sieve with 500-µ mesh size to collect the organisms from the water in the same way the net is used in larger pools.*

RWB -Step 6. After 30 seconds, remove the net from the water with a quick upstream motion to wash the organisms to the bottom of the net.

RWB -Step 7. PROCEED to Section IIIc: Filling and Labeling BMI Sample Jars

SECTION III C. FILLING AND LABELING BENTHIC MACROINVERTEBRATE SAMPLE JARS

Step 1. Once all sub-samples (eight for TRC, 11 for RWB) have been collected, transfer benthos to a 500-mL or 1000-mL wide-mouth plastic sample jar using one of the following methods.

Note: *Field elutriation should only be used by well-trained field crews who are proficient at removing all benthic organisms from the discarded inorganic material. Training in the recognition of aquatic invertebrates is highly recommended.*

Step 1a. Complete Transfer of all Sampled Material – Invert the contents of the kick net into the sample jar. Perform this operation over a white enameled tray to avoid loss of any sampled material and make recovery of spilled organisms easier. If possible, remove the larger twigs and rocks by hand after carefully inspecting for clinging organisms, but be sure not to lose any organisms. Use forceps to remove any organisms clinging to the net and place these in the sample jar.

Step 1b. Field Elutriation of Samples – Empty the contents of the net into a large plastic bucket (10-20 L is sufficient). Use forceps to remove any organisms clinging to the net and place these in the bucket. Add stream water to the bucket and gently swirl the contents of the bucket in order to suspend the organic material (being certain to not introduce entrained organisms from the source water). Pour the organic matter from the bucket through a 500-µ sieve (or use the 500-µ net). Repeat this process until no additional material can be elutriated (i.e., only inorganic material is left in the bucket). If possible, remove the larger twigs and rocks by hand after carefully inspecting for clinging organisms, but be sure not to lose any organisms. Transfer all of the material in the sieve (invertebrates and organic matter) into the sample jar. Carefully inspect the gravel and debris remaining in the bottom of the bucket for any cased caddisflies, clams, snails, or other dense animals that might remain. Remove any remaining animals by hand and place them in the sample jar.

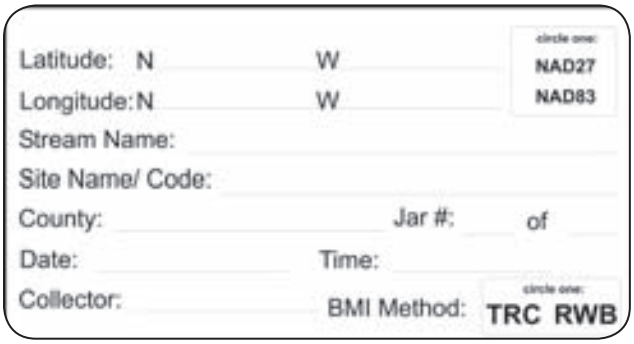
Latitude: N W
Longitude: N W
circle one: NAD27 NAD83
Stream Name:
Site Name/ Code:
County: Jar #: of
Date: Time:
Collector: BMI Method: circle one: TRC RWB

Figure 4. Example date - locality label for all BMI samples.

Step 2. Place a completed date/locality label (see Figure 4) on the inside of the jar (use pencil only as most "permanent" inks dissolve in ethanol) and completely fill with 95% ethanol. Place a second label on the outside of the jar. Note that the target concentration of ethanol is 70%, but 95% ethanol is used in the field to account for dilution from water in the sample. If organic and inorganic material does not accumulate in the net quickly, it may be possible to transfer all the material in the net into one jar. Otherwise, divide the material evenly among several jars

(being careful to clearly label them as part of a set). To ensure proper preservation of benthic macroinvertebrates it is critical that the ethanol is in contact with the BMIs in the sample jar. Never fill a jar more than 2/3 full with sampled material, and gently rotate jars that contain mostly mud or sand to ensure that the ethanol is well distributed. If jars will be stored for longer than a month prior to processing, jars should not contain more than 50% sample material.

SECTION 4 MAIN CROSS-SECTIONAL TRANSECT MEASURES

SECTION IVA. PHYSICAL MEASURES

The majority of physical habitat measurements in this protocol are made relative to the main cross-sectional transects (Figure 5). All the measures taken relative to each transect are recorded on forms specific to that transect. Start with the downstream transect (Transect A) and repeat steps 6-15 for all 11 main transects.

Module A. Transect Dimensions: Wetted Width and Bankfull Dimensions

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

Bankfull Width and Depth – The bankfull channel is the zone of maximum water inundation in a normal flow year (one to two year flood events). Since most channel formation processes are believed to act when flows are within this zone (Mount 1995), bankfull dimensions provide a valuable indication of relative size of the waterbody.

Note: *Bankfull dimensions are notoriously difficult to assess, even by experienced field crews (see Heil and Johnson 1995). It is often useful to discuss the interpretation of bankfull locations among the field crew members to reach a consensus. The USFS Stream Team provides a good set of instructional videos for improving consistency in accurate bankfull measurements (http://www.stream.fs.fed.us/publications/videos.html).*

Step 1. Scout along the stream margins to identify the location of the bankfull margins on either bank by looking for evidence of annual or semi-annual flood events. Examples of useful evidence includes topographic, vegetative, or geologic cues (changes in bank slope, changes from annual to perennial vegetation, changes in the size distribution of surface sediments). While the position of drift material caught in vegetation may be a helpful aid, this can lead to very misleading measurements.

Note: *The exact nature of this evidence varies widely across a range of stream types and geomorphic characteristics. It is helpful to investigate the entire reach when attempting to interpret this evidence because the true bankfull margin may be obscured at various points along the reach. Often the bankfull position is easier to interpret from one bank than the other; in these cases, it is easiest to infer the opposite bank position by projecting across the channel. Additionally, height can be verified by measuring the height from both edges of the wetted channel to the bankfull height (these heights should be equal).*

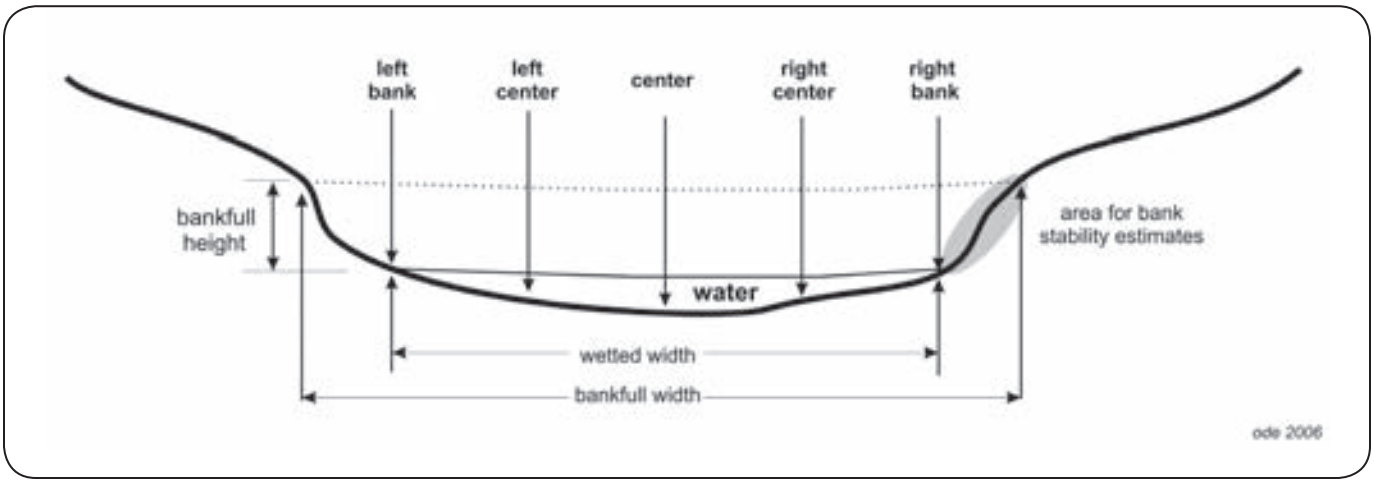

Figure 5. Cross sectional diagram of a typical stream channel showing locations of substrate measurements, wetted and bankfull width measurements, and bank stability visual estimates.

Step 2. Stretch a tape from bank to bank at the bankfull position. Measure the width of the bankfull channel from bank to bank at bankfull height and perpendicular to the direction of stream flow.

Step 3. Measure bankfull height (the vertical distance between the water height of the water and the height of the bank, Figure 5) and record.

Module B. Transect Substrate Measurements

Particle size frequency distributions often provide valuable information about instream habitat conditions that affect BMI distributions. The Wolman pebble count technique (Wolman 1954) is a widely used and cost-effective method for estimating the particle size distribution and produces data that correlates with costly, but more quantitative bulk sediment samples. The method described here follows the EMAP protocol, which records sizes of 105 particles in a reach (five particles from each of 11 main transects and 10 inter-transects).

Note: *The size cutoff for the finest particle sizes in the EMAP protocol (< 0.06 mm) differs from that used by the Sierra Nevada Aquatic Research Laboratory (SNARL) program (0.25 mm), although the narrative description for this cutoff is the same (the point at which fine particles rubbed between one's fingers no longer feel gritty).*

Coarse particulate organic matter (CPOM, particles of decaying organic material such as leaves that are greater than 1.0 mm in diameter) is a general indicator of the amount of allochthonous organic matter available at a site, and its measurement can provide valuable information about the basis of the food web in a stream reach. The presence of CPOM associated with each particle is quantified at the same time that particles are measured for the pebble counts.

Step 1. Transect substrate measurements are taken at five equidistant points along each transect (Figure 5). Divide the wetted stream width by four to get the distance between the five points (Left Bank, Left Center, Center, Right Center and Right Bank) and use a measuring device to locate the positions of these points (a stadia rod is especially helpful here). Once the positions are identified, lower a graduated rod (e.g., a marked ski pole) though the water column perpendicular to both the flow and the transect to objectively select the particle located at the tip of the rod.

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

Step 3. Record the presence or absence of CPOM >1mm within 1 cm of the particle.

Step 4. If the particle is cobble-sized (64-250 mm), record the percent of the cobble that is embedded by fine particles (<2 mm) to the nearest 5% (see cobble embeddedness text below).

Step 5. Remove the particle from the streambed, then measure and record the length of its intermediate axis to the nearest mm (see Figure 6). Alternatively, assign the particle to one of the size classes listed in the bottom of the transect form. Particle sizes classes can be estimated visually or with a quantitative measuring device (e.g., pass/ no-pass template, "gravelometer"). Regardless of the method, all particles less than 0.06 mm should be recorded as fines, all particles between 0.06mm and 2.0 mm recorded as sand. Field crews may want to carry vials containing sediment particles with these size ranges until they are familiar with these particles.

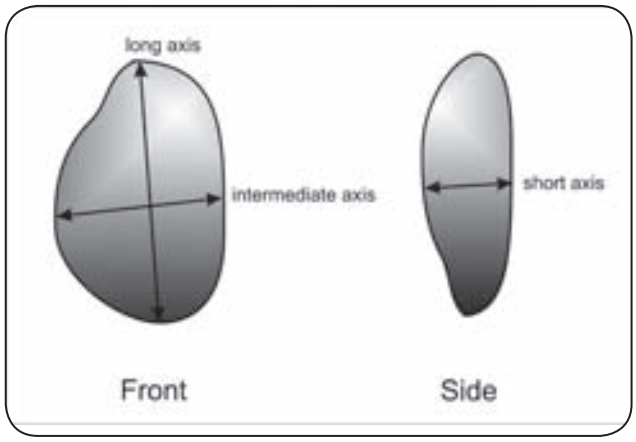

Figure 6. Diagram of three major perpendicular axes of substrate particles. The intermediate axis is recorded for pebble counts.

Module C. Cobble Embeddedness

The quantification of substrate embeddedness has long been a challenge to stream geomorphologists and ecologists (Klamt 1976, Kelley and Dettman 1980). It is generally agreed that the degree to which fine particles fill interstitial spaces has a significant impact on the ecology of benthic organisms and fish, but techniques for measuring this impact vary greatly (this is summarized well by Sylte and Fischenich 2002, http://stream.fs.fed.us/news/streamnt/pdf/StreamOCT4.pdf). Here we define embeddedness as the volume of cobble-sized particles (64-250 mm) that is buried by fine particles (<2.0 mm diameter).

Note: *This method differs from the EMAP method for measuring embeddedness, which measures embeddedness of all particles larger than 2 mm.*

Table 4. Size class codes and definitions for particle size measurements

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

Step 1. Every time a cobble-sized particle is encountered during the pebble count, remove the cobble from the stream bed and visually estimate the percentage of the cobble's volume that has been buried by fine particles. Since visual estimates of volume and surface area are subject to large amounts of observer error, field crews should routinely calibrate their estimates with each other and with other field crews.

Step 2. In the spaces to the right of the pebble count data, record the embeddedness of all cobble-sized particles encountered during the pebble count.

Note: *The cobble embeddedness scores do not correspond with the specific particles in the pebble count cells to the left, but are merely a convenient place to record the data.*

Step 3. If 25 cobbles are not encountered during the pebble count, supplement the cobbles by conducting a "random walk" through the reach. Starting at a random point in the reach, follow a transect from one bank to the other at a randomly chosen angle. Once at the other bank reverse the process with a new randomly chosen angle. Record embeddedness of cobble-sized particles in the cobble embeddedness boxes on the transect forms until you reach 25 cobbles. If 25 cobble-sized particles are not present in the entire reach, then record the values for cobbles that are present.

Module D. Canopy Cover

This method uses the Strickler (1959) modification of a convex spherical densiometer to correct for over-estimation of canopy density that occurs with unmodified readings. Read the densiometer by counting the number of line intersections that are obscured by overhanging vegetation (see Figure 7). Taping off the lower left and right portions of the mirror emphasizes overhead vegetation over foreground vegetation (the main source of bias in canopy density measurements). All densiometer readings should be taken with the bubble leveled and 0.3 m (1 ft) above the water surface.

Step 1. Using a modified convex spherical densitometer, take and record four 17-point readings all taken from the center of each transect: a) facing upstream, b) facing downstream, c) facing the left bank, d) facing the right bank.

Note: *This method deviates slightly from that of EMAP (in which two additional readings are taken at the left and right wetted edges to increase representation of bank vegetation).*

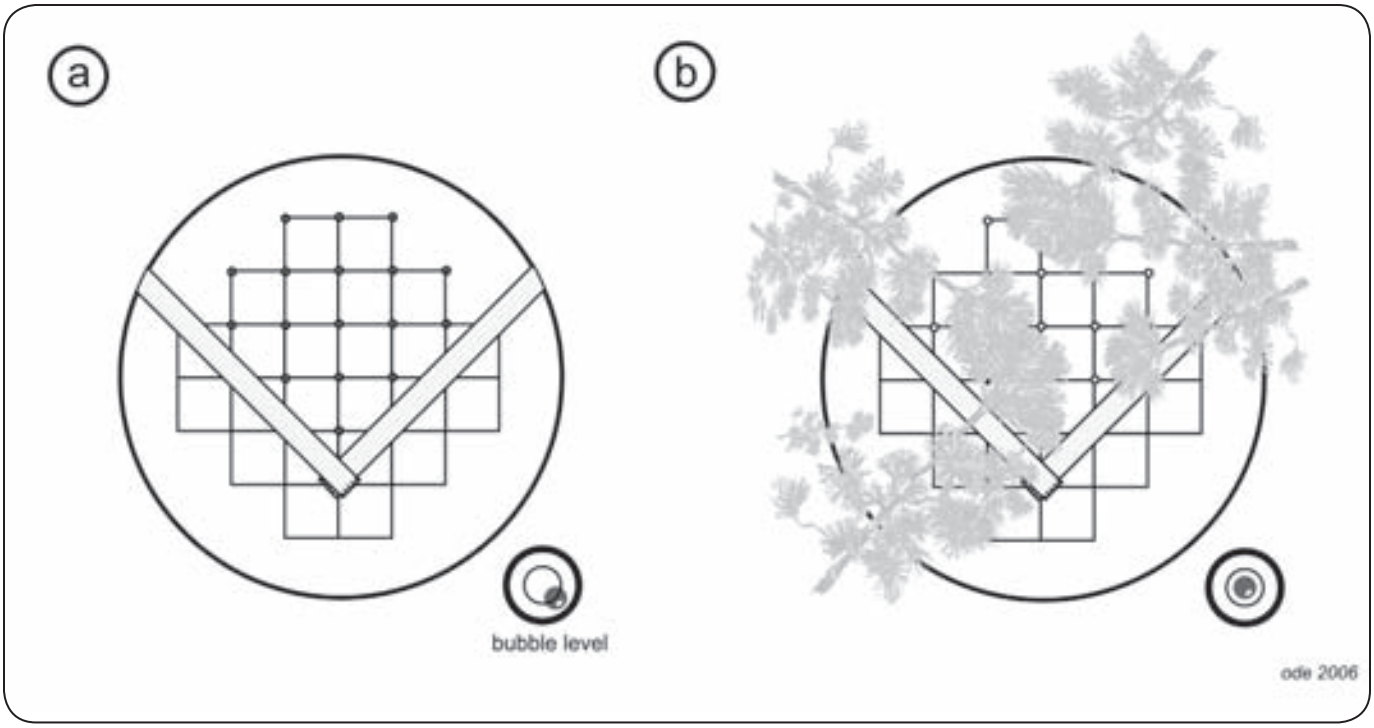

Figure 7. Representation of the mirrored surface of a convex spherical densiometer showing the position for taping the mirror and the intersection points used for the densiometer reading. The score for the hypothetical condition in (b) is 10 covered intersection points out of 17 possible. Note the position of the bubble level in (b) when the densiometer is leveled.

Module E. Gradient and Sinuosity

The gradient of a stream reach is one of the major stream classification variables, giving an indication of potential water velocities and stream power, which are in turn important controls on aquatic habitat and sediment transport within the reach. The gradient (slope) of a stream reach is often strongly correlated with many BMI metrics and other physical habitat measures and is therefore very useful when interpreting BMI data.

The "full" physical habitat method uses 10 transect to transect measurements to calculate the average slope through a reach. Although this is a little more time intensive than the reach-scale transect measures used in the "basic" protocol, it results in more precise slope determination and the ability to quantify slope variability within a reach. Sinuosity (calculated as the ratio of the length of the flow path between the ends of the reach and the straight line distance between the ends of the reach, Kaufmann et al. 1999) is measured at the same time as slope. These two measurements work best with two people, one taking the readings at the upstream transect ("backsighting") and the other holding a stadia rod at the downstream transect. If you cannot see the mid point of the next transect from the starting point, use the supplemental sections (indicating the proportion of the total length represented by each section). Otherwise, leave these blank.

Note 1: *An auto level should be used for reaches with a percent slope of less than or equal to 1%. All methods (clinometer, hand level, or auto level) may be used for reaches with a percent slope of greater than 1%. The following description is for clinometer-based slope measurements, but the same principles apply to use of an auto or hand level.*

Note 2: *In reaches that are close to 1%, you will not know whether you are above or below the 1% slope cutoff before taking readings. In these cases, default to use of an autolevel.*

Step 1. Beginning with the upper transect (Transect K), one person (the measurer) should stand at the water margin with a clinometer held at eye level. A second person should stand at the margin of the next downstream transect (Transect J) with a stadia rod flagged at the eye level of the person taking the clinometer readings. Be sure you mark your eye level while standing on level ground! Adjust for water depth by measuring from the same height above the water surface at both transects. This is most easily accomplished by holding the base of the pole at water level.

Note: *An alternative technique is to use two stadia rods pre-flagged at the eye-height of the person taking the readings.*

Step 2. Use a clinometer to measure the percent slope of the water surface (not the streambed) between the upstream transect and the downstream transect by sighting to the flagged position on the stadia rod. The clinometer reads both percent slope and degree of the slope. Be careful to read and record percent slope rather than degrees slope (these measurements differ by a factor of ~ 2.2). Percent slope is the scale on the right hand side as you look through most clinometers (e.g., Suunto models).

Note: *If an auto level or hand level is used, record the elevation difference (rise) between transects and the segment length (run) instead of the percent slope.*

Step 3. If the stream reach geometry makes it difficult to sight a line between transects, divide the distance into two or three sections and record the slope and the proportion of the total segment length between transects for each of these sections in the appropriate boxes on the slope form (supplemental segments).

Note: *Never measure slope across dry land (e.g., across a meander bend).*

Step 4. Take a compass reading from the center of each main transect to the center of the next main transect downstream and record this bearing to the nearest degree on the slope and bearing section of the form. Bearing measurements should always be taken from the upstream to downstream transect.

Step 5. Proceed downstream to the next transect pair (I-J) and continue to record slope and bearing between each pair of transects until measurements have been recorded for all transects.

SECTION IVB. VISUAL ESTIMATES OF HUMAN INFLUENCE, INSTREAM HABITAT, AND RIPARIAN VEGETATION

The transect-based approach used here permits semi-quantitative calculations from visual estimates even though most are categorical data (i.e., either presence/ absence or size classes) because we can calculate the percentage of transects that fall into different categories. These modules are adapted directly from EMAP protocols with some modifications as noted.

Module F. Human Influence

The influence of human activities on stream biota is of critical concern in bioassessment analyses. Quantification of human activities for these analyses is often performed with GIS techniques, which are very useful but are not capable of accounting for human activities occurring at the reach scale. Reach scale observations are often critical for explaining results that might seem anomalous on the basis of only remote mapping tools.

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

Step 2. Record the presence of any of the 11 human influence categories in the stream channel within a zone 5 m upstream and 5 m downstream of the transect.

Module G. Riparian Vegetation

Riparian vegetation (vegetation in the region beyond the bankfull margins) has a strong influence on the composition of stream communities through its direct and indirect roles in controlling the food base, moderating sediment inputs and acting as a buffer between the stream channel and the surrounding environment. These methods provide a cursory survey of the condition of the riparian corridor. Observations are made in the same 10 x 10 m riparian area used for assessing human influence (see Figure 2).

Note: *Riparian vegetation measurements should only include living or recently dead vegetation.*

The riparian vegetation categories used here were condensed from the EMAP version, which further breaks the canopy classes into different components. However, because we have consolidated EMAP categories into fewer categories rather than creating new categories, existing EMAP data can be easily converted to this format simply by combining the appropriate categories.

Step 1. Divide the riparian zone into three elevation zones: 1) ground cover (< 0.5 m), 2) lower canopy (0.5 m - 5 m), and 3) upper canopy (> 5 m). Record the density of the following riparian classes: 1) Upper Canopy–Trees and Saplings, 2) Lower Canopy–Woody Shrubs and Saplings, 3) Woody Ground Cover–Shrubs, Saplings, 4) Herbaceous Ground Cover–Herbs and Grasses, and 5) Ground Cover–Barren, Bare Soil and Duff. Artificial banks (e.g., rip-rap, concrete, asphalt) should be recorded as barren.

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

Module H. Instream Habitat Complexity

Instream habitat complexity was developed by the EMAP program to quantify fish concealment features in the stream channel, but it also provides good information about the general condition and complexity of the stream channel. Estimates should include features within the banks and outside the wetted margins of the stream.

Step 1. Record the amount of nine different channel features within a zone 5m upstream and 5m downstream of the transect (see Figure 2): 1) filamentous algae (long-stranded algal forms that are large enough to see with the naked eye), 2) aquatic macrophytes (include mosses and vascular plants), 3) boulders (> 25 cm), 4 and 5) woody debris (break into two classes- larger and smaller than 30 cm diameter), 6) undercut banks, 7) overhanging vegetation, 8) live tree roots and 9) artificial structures (includes any anthropogenic objects including large trash objects like tires and shopping carts). Indicate the areal cover of each feature as either: 1) absent, 2) sparse (< 10%), 3) moderate (10-40%), 4) heavy (40-75%), or 5) very heavy (> 75%).

SECTION 5 INTER-TRANSECT MEASURES

While most measures are taken at or relative to the main transects, a few measures are recorded at transects located at the midpoint between main transects. These are called "inter-transects".

Module B (Part 2) Pebble Counts (same as for transects, but no cobble embeddedness measures)

Step 1. Divide the wetted stream width by four to get the distance between the five points (Left Bank, Left Center, Center, Right Center and Right Bank) and use a measuring device to locate the positions of these points (a stadia rod is especially helpful here, see Figure 5). Once the positions are identified, lower a graduated rod through the water column perpendicular to both the flow and the transect to objectively select the particle located at its tip.

Step 2. With the graduated rod, measure the depth from the water surface to the top of the particle and record to the nearest cm.

Step 3. Remove the particle from the streambed, then measure and record the length of its intermediate axis to the nearest mm (see Figure 6). Alternatively, assign the particle to one of the size classes listed in the bottom of the transect form (see Table 3 for a list of size classes). Particle size classes may be estimated visually or with a quantitative measuring device (e.g., pass/ no-pass template, gravelometer). Regardless of the method, all particles less than 0.06 mm should be recorded as fines, while all particles between 0.06 mm and 2.0 mm should be recorded as sand. Field crews may want to carry vials containing sediment particles with these size ranges until they are familiar with these particle size classes.

Step 4. Record the presence (P) or absence (A) of any CPOM within 1 cm of each particle.

Module J. Flow Habitats

Because many benthic macroinvertebrates prefer specific flow and substrate microhabitats, the proportional representation of these habitats in a reach is often of interest in bioassessments. There are many different ways to quantify the proportions of different flow habitats (for example, see text on EMAP's "thalweg profile" below). Like the riparian and instream measures listed above, this procedure produces a semi-quantitative measure consisting of 10 transect-based visual estimates.

Note: *The categories used here are based on those used in the EMAP protocol, with pools combined into one class and cascades and falls combined into another class.*

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

SECTION 6 DISCHARGE

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

This procedure (modified from the EMAP protocol) provides for two different methods for calculating discharge. It is preferable to take discharge measurements in sections where flow velocities are greater than 0.15 m/s and most depths are greater than 15 cm, but slower velocities and shallower depths can be used. If flow volume is sufficient for a transect-based "velocity-area" discharge calculation, this is by far the preferred method. If flow volume is too low to permit this procedure or if your flow meter fails, use the "neutrally buoyant object/ timed flow" method.

Note: *Programs that sample fixed sites repeatedly may want to consider installing permanent discharge estimation structures (e.g., stage gauges, wiers).*

Module K. Discharge: Velocity Area Method

The layout for discharge measurements under the velocity-area (VA) method is illustrated in Figure 8. Flow velocity should be measured with either a Swoffer Instruments propeller-type flow meter or a Marsh-McBirney inductive probe flow meter. Refer to the manufacturers' instrument manuals for calibration procedures.

VA-Step 1. Select the best location in the reach for measuring discharge. To maximize the repeatability of the discharge measurement, choose a transect with the most uniform flow (select hydraulically smooth flow whenever possible) and simplest cross-sectional geometry. It is acceptable to move substrates or other obstacles to create a more uniform cross-section before beginning the discharge measurements.

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

VA-Step 3. Record the distance from the bank to the end of the first interval. Using the top-setting rod that comes with the flow velocity meter, measure the median depth of the first interval.

VA-Step 4. Standing downstream of the transect to avoid interfering with the flow, use the top-setting rod to set the probe of the flow meter (either the propeller or the electromagnetic probe) at the midpoint of each interval, at 0.6 of the interval depth (this position generally approximates average velocity in the water column), and at right angles to the transect (facing upstream). See Figure 8 for positioning detail.

VA-Step 5. Allow the flow velocity meter to equilibrate for 10-20 seconds then record velocity to the nearest m/s. If the option is available, use the flow averaging setting on the flow meter.

Note: *Under very low flow conditions, flow velocity meters may register readings of zero even when there is noticeable flow. In these situations, record a velocity of 0.5x the minimum flow detection capabilities of the instrument.*

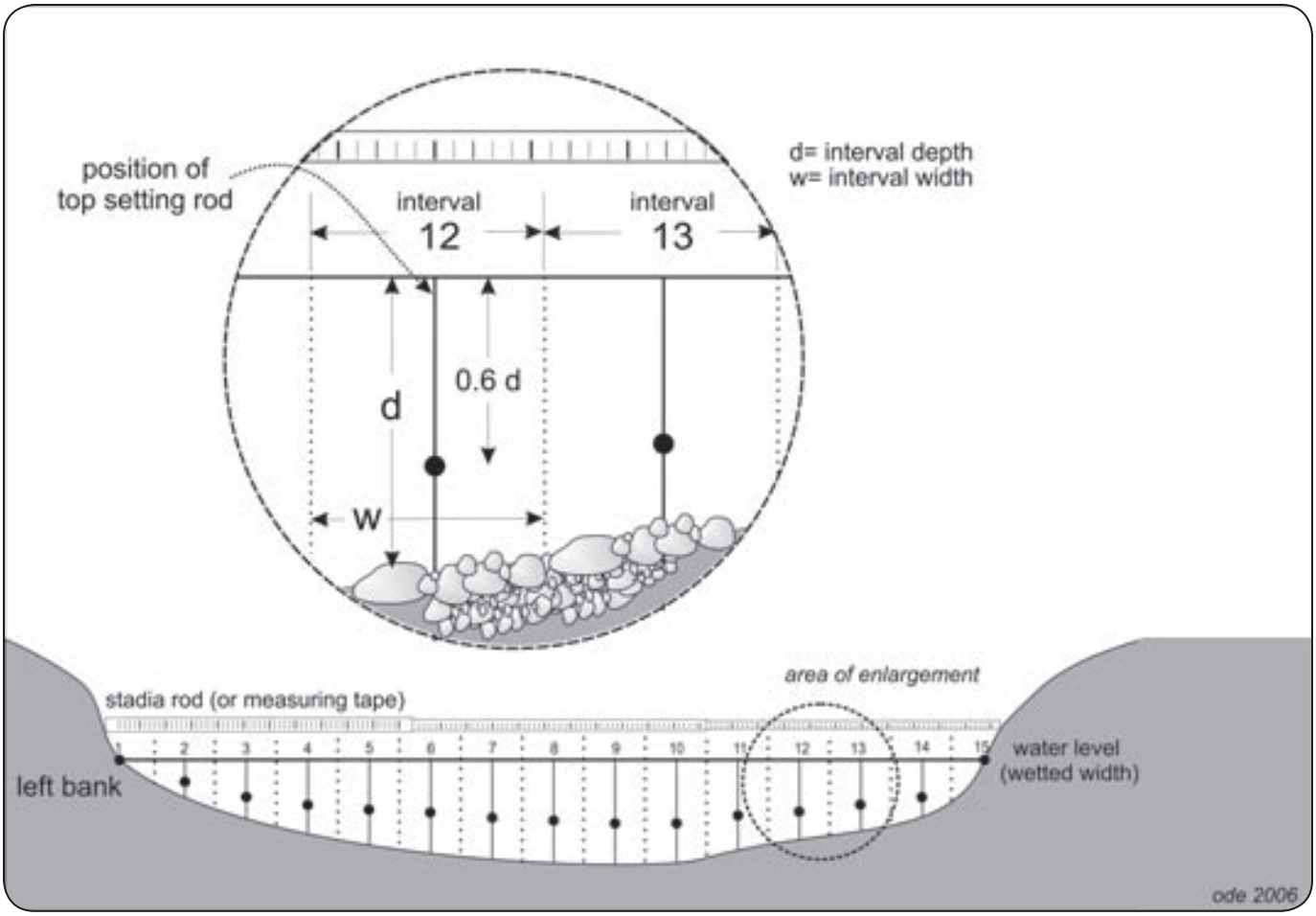

Figure 8. Diagram of layout for discharge measurements under the velocity-area method showing proper positions for velocity probe (black dots).

VA-Step 6. Complete Steps 3 through 5 on the remaining intervals.

Note: *The first and last intervals usually have depths and velocities of zero.*

Module L. Discharge: Neutrally Buoyant Object Method

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

NBO-Step 1. The position of the discharge sub-reach is not as critical as it is for the velocity-area method, but the same criteria for selection of a discharge reach apply to the neutrally buoyant object method. Identify a section that has relatively uniform flow and a uniform cross sectional shape.

NBO-Step 2. The cross sectional area is estimated in a manner that is similar but less precise than that used in the velocity area method. Measure the cross sectional area in one to three places in the section designated for the discharge measurement (three evenly-spaced cross sections are preferred, but one may be used if the cross section through the reach is very uniform). Record the width once for each cross section and measure depth at five equally-spaced positions along each transect.

NBO-Step 3. Record the length of the discharge reach.

NBO-Step 4. Place a neutrally buoyant object (e.g., orange, rubber ball, heavy piece of wood, etc.) in the water upstream of the discharge reach and record the length of time in seconds that it takes for the object to pass between the upstream and downstream boundaries of the reach. Repeat this timed float three times.

SECTION 7
POST-SAMPLING OBSERVATIONS

Module M. Rapid Bioassessment Procedures Visual Assessment Scores (for Basic Physical Habitat, or optional supplement)

EPA's Rapid Bioassessment Procedures (RBPs, Barbour et al. 1999) include a set of 10 visual criteria for assessing instream and riparian habitat. The RBP has been used in the CSBP since its first edition (1995) and thus, this information is often valuable for comparison to legacy datasets. The criteria also have a useful didactic role since they help force the user to quantify key features of the physical environment where bioassessment samples are collected.

Module N. Additional Habitat Characterization (Full Physical Habitat only)

The RBP stream habitat visual estimates described in Step 1 are not included in the Full Physical Habitat version because they are generally replaced by more quantitative measurements of similar variables. However, we have found that three of the RBP measures are reasonably repeatable and include them in the reachwide assessment portion of the Full Physical Habitat version.

Note: *This is the only case in which a measurement included in the basic procedure is not included in the full.*

Module O. Reach Slope (for Basic Physical Habitat only)

Reach slope should be recorded as percent slope as opposed to degrees slope to avoid confusion. Slope measurements work best with two people, one taking the readings at the upstream transect and the other holding a stadia rod at the downstream transect. If you cannot see the mid point of the next transect from the starting point, use the supplemental sections (indicating the proportion of the total length represented by each section).

An auto level (with a tripod) should be used for reaches with a percent slope of less than or equal to 1%. All methods (clinometer, hand level, or auto level) may be used for reaches with a percent slope of greater than 1%. In reaches that are close to 1%, you will not know whether you are above or below the 1% slope cutoff. In these cases, default to use of an autolevel.

Step 1. Divide the reach into multiple segments such that stadia rod markings can be easily read with the measuring device to be employed (this is especially a factor for clinometer and hand level readings).

Step 2. Use a clinometer, hand level, or auto level to measure the percent slope of the water surface (not the streambed) between the top and bottom of each segment. Be sure to adjust for water depth by measuring from the same height above the water surface at both transects. Also be sure to record percent slope, not degrees slope. Record the segment length for each of these sections in the appropriate boxes on the BASIC slope form.

SECTION 8 OPTIONAL EXCESS SEDIMENT MEASURES

Future editions of these protocols will include supplemental modules, including a full discussion of the measurements used for calculating the excess sediment index (sometimes referred to as log relative bed stability, LRBS). However, since several of the measurements in EMAP's physical habitat protocols are interwoven into the layout of this protocol, a brief overview of the additional measurements collected for the LRBS calculations is included here for information purposes only. For detailed explanations of these measurements, consult Peck et al. 2004.

Woody Debris Tallies

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

Thalweg Measurements

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

SECTION 9
OPTIONAL PERIPHYTON QUANTIFICATION

Periphyton Quantification

Characterization of periphyton has a dual role in bioassessments, as periphyton is both a food and habitat resource for benthic macroinvertebrates and fish and an effective bioindicator on its own. Quantification of periphytic resources will be covered under a separate SWAMP bioassessment protocol, but will include procedures for qualitative characterization of diatom assemblages, documentation of filamentous algal growth, and biomass quantification (e.g., ash-free dry mass and chlorophyll a).

SECTION 10
QUALITY ASSURANCE & CONTROL PROCEDURES

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

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DEFINITIONS OF TERMS USED IN SOP D

Terms & Definitions	
TERM	**DEFINITION**
ABL	California Department of Fish and Game's Aquatic Bioassessment Laboratory
Allocthonous	Derived from a source external to the stream channel (e.g., riparian vegetation) as opposed to autochthonous, which indicates a source inside the stream channel (e.g., periphyton).
Ambient Bioassessment	Biological monitoring that is intended to describe general biotic condition as opposed to a diagnosis of sources of impairment
Bankfull	The bankfull channel is the zone of maximum water inundation in a normal flow year (one to two year flood events)
BMI	Benthic macroinvertebrates: bottom-dwelling invertebrates large enough to be seen with the unaided eye
Cobble Embeddedness	The volume of cobble-sized particles (64-250 mm) that is buried by fine particles (<2.0 mm diameter)
CPOM	Coarse particulate organic matter (CPOM, particles of decaying organic material such as leaves that are greater than 1.0 mm in diameter)
CSBP	California State Bioassessment Procedures
DFG	California Department of Fish and Game
EMAP	The U.S. Environmental Protection Agency's Environmental Monitoring and Assessment Program
EPA	The U.S. Environmental Protection Agency
Fines	Substrate particles less than 0.06 mm diameter (not gritty to touch)
Inter-transects	Transects established at points equidistant between the main transects
MCM	Margin-Center-Margin alternative procedure for sampling low gradient habitats
ORD	EPA's Office of Research and Development
QAMP	Quality assurance management plan
RBP	EPA's Rapid Bioassessment Procedures
Reach	A segment of the stream channel
Riparian	An area of land and vegetation adjacent to a stream that has a direct effect on the stream.
RWB	Reach-wide benthos composite sampling method for benthic macroinvertebrates, also referred to as multi-habitat method
SCCWRP	Southern Coastal California Water Research Project
SNARL	Sierra Nevada Aquatic Research Laboratory
Substrate	The composition of a streambed, including both inorganic and organic particles
SWAMP	The State Water Resources Control Board's Surface Water Ambient Monitoring Program
Thalweg	A longitudinal profile that connects the deepest points at successive cross-sections of the stream. The thalweg defines the primary path of water flow through the reach

TERM	DEFINITION
Transects	Lines drawn perpendicular to the path of flow used for standardizing sampling locations
TRC	Targeted riffle composite sampling method for benthic macroinvertebrates
USFS	The United States Forest Service
Wadeable Streams	Streams that can be sampled by field crews wearing chest waders (generally less that 0.5 m - 1.0 meters deep)

APPENDIX A

FACTORS TO CONSIDER WHEN RECOMMENDING/ CHANGING BIOASSESSMENT METHODS

Beyond the primary considerations of precision and accuracy, there are at least five other key issues that SWAMP has considered and should consider in the future, when recommending or changing its official methods for bioassessment. These issues include:

1. Costs of Collecting Samples via Multiple Protocols – Collecting, processing, and interpreting samples using more than one method for each indicator (e.g., algae, macroinvertebrates, fish) per site adds significant costs to bioassessment monitoring programs. SWAMP should strive to identify the minimum set of protocols necessary for each indicator. However, this should not come at the expense of sound monitoring. If more than one method is needed to interpret the biological response, then this decision should be based on a cost-benefit assessment.

2. Costs of Maintaining Multiple SWAMP Protocols – While multiple methods for monitoring a given indicator may provide additional accuracy in specific habitats, there are significant costs to maintaining multiple protocols:

a. Need to maintain method-specific infrastructure (e.g., separate reference samples, separate indices of biotic integrity (IBIs), separate O/E models, etc.).
b. May lose or impair ability to compare across sites if different methods are used (see Issue 5 below).
c. Guidance on when to use methods becomes more complex. For example, we need to define very specifically which methods to use at each water body type; and thus, which tools can be used to interpret them.

Recommendation: *SWAMP should maintain as few protocols as necessary. If we elect to add new or modified protocols it should be because we have determined that the added value is worth all of the costs listed above.*

3. Separating Physical Impairment from Water Quality Impairment – One of the original reasons for adding a multihabitat component to SWAMP bioassessment programs was the potential for distinguishing physical and water quality impairment sources (see recommendations in Barbour and Hill 2002). In regards to macroinvertebrate indicators, the conventional wisdom has been that reachwide (RW, sometimes referred to as multihabitat or MH) samples should be relatively more responsive to physical habitat alteration (i.e., fine sediment inputs) than targeted-riffle (TR) samples because it is believed that erosional habitats take longer

to respond to sediment stresses, and because pockets of riffle habitat are thought to act as refugia from habitat loss. To the extent that this is true, RW and TR samples may offer complementary information that allows us to separate these sources of impairment.

While very few studies have addressed this conventional wisdom directly, recent studies suggest that this may not be as much a factor as previously believed. In a recent comparison of TR and RW samples at nearly 200 sites statewide, the ABL found at most weak evidence to support this notion (Rehn et al. 2007). Gerth and Herlihy (2006) came to the same conclusion in their analysis of ~500 sites in the eastern and western United States. However, this issue is far from resolved and SWAMP scientists currently are not in agreement regarding this issue. Since the majority of bioassessment programs in California have emphasized targeted riffle sampling, SWAMP will undoubtedly want to evaluate this question further before making any policy decision to discontinue TR sampling.

Recommendation: *Until this issue can be evaluated further and resolved to SWAMP's satisfaction, ambient macroinvertebrate sampling should include collection of both RW samples and richest targeted habitat (TR or MCM) samples at every site. (The TR method should be used where sufficient riffles are present, and the MCM method should be used at low-gradient sites where sufficient riffle habitat is not available.)*

4. Compatibility with Previous Data – To address this issue, at least three sets of macroinvertebrate sampling method comparisons have been conducted in California.

a. **Targeted Riffle Methods –** Comparisons are complete. Samples collected under the current TR protocols are considered interchangeable with both CSBP and SNARL samples (Ode et al. 2005, Herbst and Silldorff 2006).
b. **Low Gradient Sand-Dominated Streams –** Collaborative studies are currently underway between Water Board Regions 3 and 5, the Southern California Coastal Water Research Project (SCCWRP), and ABL to compare the performance of: (1) the "low-gradient" CSBP; (2) RW samples; and (3) a modification of the RW method designed to emphasize habitats along stream margins (MCM). The results of these low-gradient methods comparisons are not yet available.
c. **Targeted Riffle vs. Reachwide Methods –** A recent comparison of RW and TR samples collected from nearly 200 EMAP/ CMAP sites is in peer review press (Rehn et al. 2007). Results demonstrate remarkably similar performance of the methods across a wide range of habitats. Gerth and Herlihy (2006) recently published a similar analysis with the same conclusions. However, the bioassessment committee has yet to carefully review and discuss these analyses and their implications for SWAMP biomonitoring.

5. Comparability Among Sites – The ability to compare biological condition across sites is a common requirement of most ambient bioassessment programs. This type of analysis is confounded if different methods are used at these sites. One of the big advantages of reachwide (i.e., multihabitat) methods is that they can be applied anywhere because they don't require a specific habitat for sampling. Statewide

bioassessments and most regional programs will require the ability to compare their bioassessment results among multiple sites (e.g., within a watershed, within a region, statewide).

INTERIM RECOMMENDATIONS FOR MACROINVERTEBRATE SAMPLING (UPDATED DECEMBER 2006):

1. Until we can reach consensus on the outstanding issues (i.e., whether a single method for macroinvertebrate sampling will meet our needs, and the outcome of RW vs. MCM comparison studies for low-gradient wadeable streams/rivers), SWAMP recommends collecting both a reachwide (i.e., multihabitat) and a targeted habitat sample at each site. In high gradient streams, this means using both the RW and TR methods. In low-gradient streams, we recommend collecting both RW and MCM samples until the results are available from the low-gradient ("non-riffle") comparison. In rare cases where monitoring objectives cannot be met following these recommendations, the SWAMP Bioassessment Coordinator may authorize deviations. For example, where project-specific objectives differ from ambient monitoring, the SWAMP Bioassessment Coordinator may authorize alternate methods. In rare cases where funding is extremely limited and the cost of following the above recommendations would be prohibitive, the SWAMP Bioassessment Coordinator may authorize cost-saving options such as collecting both samples, but archiving one of the samples for later lab analysis.

2. SWAMP should develop guidance specifying when and where different methods should be used. For example, at "low gradient" sites, what is the slope cut-off (or other channel feature criteria to use) when deciding whether to apply TR or MCM? In addition, while SWAMP may eventually choose to adopt a single method (such as RW) at most sites, some regions may determine that the value of targeted habitat sampling merits continued sampling with supplemental protocols. In the latter case, or if SWAMP determines that distinct methods are needed for different habitat types, the guidance should specify the types of waterbodies or classes of waterbodies that require different methods.

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