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Wyoming Department of Environmental Quality, Water Quality Division  
Watershed Program

## Reachwide and Riffle Water Surface Slopes

### Introduction

The reachwide water surface slope is the average slope of the water surface over the length of the entire sample reach (approximately two meander cycles of 20 bankfull widths). Reachwide water surface slope is calculated as the elevation difference from the top of a riffle at the upstream end of the reach to the top of a riffle at the downstream end of the reach, divided by the channel length between the measured tops of riffles. The riffle water surface slope is the water surface slope of an individual riffle feature and is calculated as the elevation difference from the top of the riffle to the bottom of the same riffle, divided by the length of the riffle. Measuring water surface slopes require basic surveying skills and equipment. Survey basics such as establishing benchmarks, foresights, positioning the level, turning points and others are not covered here. For more information on survey basics consult Harrelson et al. (1994).

### Quality Control

Following the process described in Harrelson et al. (1994), no measurement of reachwide or riffle water surface slope is complete without checking the accuracy of the survey with a survey closure. To close the survey, take a foresight reading at the benchmark, compute the elevation, and compare the difference to the original benchmark elevation at the start of the survey. Typically a closure of no more than 0.05 feet is acceptable when conducting stream surveys. The survey closure error shall be documented on the datasheet.

### Field Procedure

Required equipment and supplies for measuring water surface slopes include:

<input type="checkbox"/> Calculator <input type="checkbox"/> Datasheet or log book <input type="checkbox"/> Laser level with tripod <input type="checkbox"/> Pens	<input type="checkbox"/> Range pins <input type="checkbox"/> Survey rod with rod level and laser receiver <input type="checkbox"/> Measurement tapes with 0.1' increments (several 300')
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### Reachwide Water Surface Slope

- 1) Establish a representative reach of a wadeable stream, generally at least two meander cycles of 20 bankfull widths in length. All reaches shall begin and end at the top of a riffle.
- 2) Beginning at the upstream end of the reach, stretch the tapes along either the left or right bank as close to the edge of the channel as possible. Where possible, the tape should be threaded through riparian vegetation or other obstructions to get as close to the channel edge as possible. Tape(s) can be secured to the ground with range pins, vegetation, or rocks. Stationing of features will be obtained from the longitudinal profile tape.
- 3) Following the process described by Harrelson et al. (1994), establish a benchmark and height-of-instrument.
- 4) Starting at the upstream end of the reach at the top of a riffle (which should be station 0), take a rod reading at the edge of water near either the left or right bank (as you are facing downstream) and record on the datasheet.

- 5) Proceed to the downstream end of the reach at the top of a riffle and take a rod reading at the edge of water near the same bank as the previous rod reading and record on the datasheet.
- 6) Determine the distance from the longitudinal tape between the upstream top of riffle and the downstream top of riffle and record on the datasheet.
- 7) The reachwide water surface slope is calculated by dividing the difference in elevation between the water surface at the most upstream top of riffle and most downstream top of riffle by the length of stream between the two riffles. Record on the datasheet.
- 8) Close the survey according to the process described in the **Quality Control** section of this document.
- 9) Reachwide water surface slope can be calculated from data gathered as part of a longitudinal profile survey. Again, use the water surface rod readings at the top of the most upstream and downstream riffles divided by the length of stream between the two riffles.

### **Riffle Water Surface Slope**

- 1) Beginning at the upstream end of the reach, stretch the tapes along either the left or right bank as close to the edge of the channel as possible. Where possible, the tape should be threaded through riparian vegetation or other obstructions to get as close to the channel edge as possible. Tape(s) can be secured to the ground with range pins, vegetation, or rocks. Stationing of features will be obtained from the longitudinal profile tape. If a longitudinal tape has already been placed in the reach, use this tape to obtain stations for the top and bottom of a riffle.
- 2) Following the process described by Harrelson et al. (1994), establish a benchmark and height-of-instrument.
- 3) Starting at the top of the riffle, take a rod reading at the edge of water near either the left or right bank (as you are facing downstream) and record on the datasheet.
- 4) Proceed to the bottom of the riffle and take a rod reading at the edge of water near the same bank as the previous rod reading and record on the datasheet.
- 5) Determine the distance from the tape between the top and bottom of the riffle and record on the datasheet.
- 6) The riffle water surface slope is calculated by dividing the difference in elevation between the water surface at the top and bottom of the riffle by the length of riffle. Record on the datasheet.
- 7) Close the survey according to the process described in the **Quality Control** section of this document.
- 8) Riffle water surface slope can be calculated from data gathered as part of a longitudinal profile survey. Again, use the water surface rod readings at the top and bottom of the selected riffle divided by the length of riffle.

### **References**

Harrelson, C.C., C.L. Rawlins and J.P. Potyondy. 1994. Stream channel reference sites: an illustrated guide to field technique. General Technical Report RM-245. U.S. Department of Agriculture, Forest Service, Rocky Mountain Forest and Range Experiment Station. Fort Collins, CO.

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Rosgen, D.L. 2008. River Stability Field Guide. Wildland Hydrology. Fort Collins, CO.

## Stream Channel Cross Section Survey

### Introduction

Data obtained from cross-sections provide the main channel dimension parameters used in Rosgen stream classification and geomorphic assessments of streambed stability and sediment supply. Dimensional parameters include bankfull cross-sectional area, bankfull width, mean bankfull depth, maximum bankfull depth, width/depth ratio, floodprone width and entrenchment ratio. All dimensional parameters are referenced to the bankfull elevation. Cross-section locations can be used to measure stream discharge, particle size distributions, channel aggradation/degradation and other morphological variables. Cross-sections require basic surveying skills and equipment. Survey basics such as establishing benchmarks, foresights, positioning the level, and turning points are not covered here. For more information on survey basics consult Harrelson et al. (1994).

### Quality Control

Following the process described in Harrelson et al. (1994), no cross-section survey is complete without checking the accuracy of the survey with a survey closure. To close the survey, take a foresight reading at the benchmark, compute the elevation, and compare the difference to the original benchmark elevation at the start of the survey. Typically a closure of no more than 0.05 feet is acceptable when conducting stream surveys. The survey closure error shall be documented on the cross-section datasheet.

### Field Procedure

Required equipment and supplies for a cross-section survey include:

<input type="checkbox"/> Calculator <input type="checkbox"/> Camera <input type="checkbox"/> Datasheet or log book <input type="checkbox"/> End caps <input type="checkbox"/> Flagging tape <input type="checkbox"/> Hammer (2-lb or heavier) <input type="checkbox"/> Laser level with tripod	<input type="checkbox"/> Pens <input type="checkbox"/> Pocket rod with 0.1' and 0.01' increments <input type="checkbox"/> Range pins <input type="checkbox"/> Rebar (3 or 4' X ½" or ¾") <input type="checkbox"/> Survey rod with rod level and laser receiver <input type="checkbox"/> Measurement tape or tag line with 0.1' increments (100, 200 or 300' length)
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- 1) Cross-sections can be located at any of the four main features (riffle, run, pool or glide) of a stream, depending on the objective of the site visit. If the objective is only for Rosgen stream classification, the cross-section should be placed at a riffle. For all other objectives, cross-sections can be placed at any of the four features. Cross-sections can also be placed at multiple locations of the same feature within a reach, depending on objectives.
- 2) Following the process described under **Bankfull Elevation–Field Identification**, identify bankfull elevation in the reach.
- 3) Establish a benchmark and height-of-instrument as described by Harrelson et al. (1994).
- 4) Determine the location of the cross-section and its corresponding endpoints. Cross-sections should not be placed over riffles or other features that have been substantially disturbed by biological sampling, animal or human activity or similar causes. Avoid placement of the cross-section at the top or bottom of a feature except in the case of glides. In streams with

active physical degradation and/or aggradation, features may migrate longitudinally within the reach from one year to another. Place the cross-section across the mid-point of the feature to increase the likelihood that the facet type you measure will be the same type you measure in subsequent years. Glide cross-sections should be placed at the bottom of the glide to ensure accurate representation of the associated cross-sectional area. Make sure that the cross-section is perpendicular to the direction of flow at bankfull. Where possible, cross-section endpoints should be located above the bankfull elevation and preferably above the floodprone elevation (twice the maximum bankfull depth).

- 5) Establish permanent markers at the cross-section endpoint locations by driving rebar vertically in the ground, leaving one-half inch above the ground if acceptable to the landowner. Attach either plastic or metal end caps on the tops of rebar for identification.
- 6) Stretch the measurement tape or tag line (tape) across the channel with zero always beginning on the left bank as you are facing downstream. The zero mark on the tape should be placed over the left cross-section endpoint. The tape can be secured to the ground with range pins. Make sure to stretch and secure the tape tight between both endpoints; sagging tapes are unacceptable. During windy conditions, flagging ribbon can be secured at regular intervals on the cross-section tape to minimize tape “waving”.
- 7) Record the location of the cross-section along the longitudinal profile tape (if present, see **Longitudinal Profile**) and sketch the cross-section location as part of the site map with associated landmarks on the datasheet. Photograph the cross-section with at least one photo looking upstream, documenting the entire cross-section and one photo from each endpoint to the opposite endpoint. If desired, triangulate the location of the cross-section between the benchmark, the nearest cross-section endpoint to the benchmark and another permanent feature and record on the datasheet. GPS locations of each endpoint can be used in place of triangulation. Document as much information as possible to the cross-section location on the datasheet so it can be relocated for future surveys.
- 8) Starting with the top of the left endpoint at 0, begin the cross-section survey. Record the rod reading at the top and base of the left endpoint as foresights on the datasheet. Proceed with rod readings at breaks in slope; record important features such as terraces, top of bank, low bank, bankfull, edge of water, inner berm, thalweg, and top and bottom of toe pins (if present). If undercuts are present, use a combination of the survey rod and pocket rod to accurately characterize the undercut. Otherwise, take survey readings at regular intervals of generally one foot. Record any features along the cross-section tape in the notes section of the datasheet. Complete the survey by taking rod readings at the base and top of the right endpoint. Record all features on the datasheet next to their corresponding rod readings.
- 9) Close the survey according to the process described in the **Quality Control** section of this document.

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Harrelson, C.C., C.L. Rawlins and J.P. Potyondy. 1994. Stream channel reference sites: an illustrated guide to field technique. General Technical Report RM-245. U.S. Department of Agriculture, Forest Service, Rocky Mountain Forest and Range Experiment Station. Fort Collins, CO.

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## Sample Processing for Large Volume Benthic Samples

The combining of 8 Surber samples into a single composite sample may result in an unacceptably large volume of material, particularly in sand-dominated streams. Large sample volumes can result in poor preservation and damaged specimens, as well as inefficient subsampling by the contract laboratory. Consequently, samples can be field processed in a manner that results in a sample component that fits into one or two 1-L sample containers. Sample volume reductions are accomplished by removing large debris and elutriating to remove gravel, sand and inorganic sediments. Field processing can be applied to individual Surber samples or to the entire composite sample.

Field processing begins with the removal of large rocks and organic debris, such as leaves and twigs from the sample. These materials are discarded after visual inspection to ensure that all organisms are removed. Material such as filamentous algae, mosses, and macrophytes should not be removed from the sample. The remaining sample is elutriated onto a 500 um sieve to separate the lighter organic material from the heavier sand and gravel. Elutriation is accomplished by placing the sample in a deep bucket or tub filled one-fourth to one-half with water. The contents are stirred by hand to suspend as much material as possible. The bucket is picked up, swirled, and gently decanted into the sieve while closely watching the advancing sediment front. Sieving effectiveness is increased and clogging is decreased if the sieve is kept in constant motion while the sample material is being decanted. Decanting is stopped when the sediment front reaches the lip of the bucket and a small amount of the heavier sand and gravel falls into the sieve. A backup container should be placed under the sieve to catch any sample material that may spill over the edge of the sieve during elutriation. After each elutriation the water (and spilled sample, if applicable) in the backup container can be poured back into the bucket containing the sample to repeat the elutriation process. If more water is needed to complete the elutriation process, streamwater is filtered through the Surber net into the sample bucket. The elutriation process should be repeated a minimum of four times. After the fourth elutriation, a small amount of the elutriate material should be placed in a shallow white tray and visually examined for invertebrates, particularly case-building caddisflies and small mollusks. If *one* or more invertebrates are found in the elutriate the process should be repeated until no invertebrates are observed in the elutriate material. When the elutriate is considered “clean” the material in the white tray should be placed in a small Nalgene bottle, labeled “elutriate” along with the sample number and site name. Elutriate samples may be sent to the contract laboratory to determine effectiveness of elutriation.

Method reference: Cuffney, T.F., M.E. Gurtz, and M.R. Meador. 1993. Methods for collecting benthic invertebrate samples as part of the National Water-Quality Assessment Program. U.S. Geological Survey Open-File Report 93-406. 66 pages.

**Riffle Embeddedness**  
**(Applies only to gravel- and cobble-bed streams)****Quality Control**

Duplicate measurements are collected at ten percent (10%) of wadeable stream monitoring sites to check variability between field samplers. Each replicate consists of a minimum of 100 streambed particle measurements obtained from the same riffle where macroinvertebrate samples are collected. If the available riffle area is insufficient to collect the required number of measurements, the remainder may be collected from representative riffles upstream. Reason(s) for doing so are noted in the Field Data Sheet and measurements not obtained from the sample riffle are identified. All embeddedness measurements **must be made prior** to entering or disturbing the channel bed to avoid sampling bias.

**Definition and Relevance**

Embeddedness is a measure that quantifies the degree to which larger streambed particles (gravels, cobbles and small boulders) are embedded, buried or surrounded by fine sediment (sand, silt and clay particles <2 mm in diameter). The measure was initially used to quantify stream sedimentation (Klamt, 1976; Kelly and Dettman, 1980) and to evaluate the quality of over-wintering juvenile salmonid habitat (Munther and Frank, 1986; Burns and Edwards, 1987; Torquemada and Platts, 1988; Potyondy, 1988). It has since undergone modification by others to assess both physical and biological functions in gravel- and cobble-bed streams. Several embeddedness measurement techniques and guidelines are summarized by MacDonald, Smart, and Wissmar (1991), Bunte and Abt (2001) and Sylte and Fischenich (2002).

Embeddedness reduces bed roughness, which correspondingly, alters stream channel morphology and hydraulics. Sediment loads that exceed a stream's sediment transport capacity can cause localized aggradation and initiate predictable channel adjustments (Leopold, Wolman and Miller, 1964). Typically, an increase in sediment load alone accelerates streambank erosion with concomitant increases in channel width, width-depth ratio, gradient and meander wavelength, and decreases in mean channel depth and sinuosity (Lane, 1955; Schumm, 1969). These physical changes can disrupt or destroy habitat used by aquatic life and place additional stresses on other designated water uses.

Fine sediment deposits that are not periodically mobilized from coarser bed material eventually fill interstitial voids and reduce living space for fish and macroinvertebrates and limit the attachment area for periphyton (Barbour et al., 1999). Shifts in streambed particle size distributions from coarse-to-fine sediment can reduce the diversity and density of aquatic life (Lenat, Penrose and Eagleson, 1981; Chapman and McLeod, 1987). Embedded streambeds reduce gravel permeability and inter-gravel dissolved oxygen levels (Moring, 1982; Chapman, 1988; Platts et al., 1989; Rinne, 1990), ultimately affecting fish embryo survival, fry emergence and fry size (Tappel and Bjornn, 1983;

Young, Hubert and Wesche, 1990). The percolation of water through cobbles and gravels is also needed to flush metabolic waste products of fish embryos from redds, and for thermal attenuation, nutrient transport and decomposition processes (Young, Hubert and Wesche, 1990; Bjornn and Reiser, 1991). Long term sediment deposition may limit streambed substrate surface area utilized by juvenile fish for cover and macroinvertebrates for shelter and egg incubation (Merritt and Cummins, 1996).

## Procedure

The purpose of this SOP is to evaluate the extent to which gravel, cobble or boulders are covered or surrounded by fine sediment (defined herein as clay, silt and sand particles <2 mm in diameter) in gravel- or cobble-bed channels. A percent embeddedness value is calculated from at least 100 individual particle measurements collected from riffle or run habitat. MacDonald, Smart, and Wissmar (1991) state that using individual particles as the sample unit may be more suitable for characterizing a single habitat type where the variability is likely lower than what is expected for characterizing an entire stream reach. The intent of the evaluation is to provide information on sediment movement and/or deposition, and relate the measure to biological data collected from riffle/run habitat. The measure is used as one means to assess the effects of natural and human-induced stressors on streams.

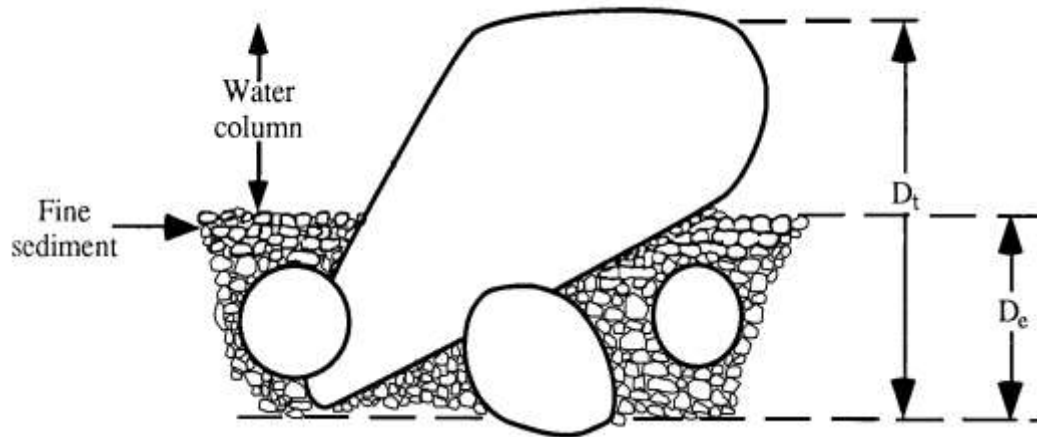
The embeddedness measurement procedure presented herein is a modified version of the procedure described by MacDonald, Smart, and Wissmar (1991). It is most applicable to channels with gravel- or cobble-dominated beds. It may have limited, if any, use in high-energy, steep gradient channels where fine sediment deposition is unlikely. It may not be as appropriate in basins where the sediment load is mostly comprised of silts and clays, and in low gradient reaches that lack the coarse particles needed to measure embeddedness.

Embeddedness is evaluated at the same time when, and in the same riffle/run habitat where, the macroinvertebrate surber samples are collected (see Macroinvertebrate Sampling SOP). Measurements are normally made prior to scrubbing rocks in the surber frame. The channel bed upstream and within the riffle/run habitat should not be disturbed prior to making measurements.

- 1) Data are collected for a minimum of 100 particles in the size range of  $\geq 10$  mm to  $\leq 300$  mm median diameter. Areas, regions or “pockets” of homogenous fine sediment that cover gravels and cobbles are defined as 100% embedded. Hardpan and bedrock are by definition 0% embedded (consider the applicability of embeddedness measures for these bed materials).
- 2) Individual particles are selected from the streambed near the predetermined random locations where surber samples are collected. Particles are selected from the “wetted” or “active” bed of the channel. The field sampler first places the surber frame on the streambed, and then selects particles either downstream or to the sides of the surber frame to prevent benthic organisms from drifting into the surber net when the particle is removed. The particles are “blindly” selected by looking away from the selection site and extending an index finger to the first particle touched on the streambed. Before the particle is removed from the bed, its top and sides are

closely examined to determine if it is covered or embedded by fine sediment. A piece of plexiglass may be used to break the water surface and provide a clearer view of the particle. This is done to verify that stain lines on the particle are not the result of past sedimentation or periphyton growth on the upper surface.

- 3) Remove the particle from the stream bed while retaining its spatial orientation to measure and record both its total vertical height ( $D_t$ ) and embedded height ( $D_e$ ) perpendicular to the bed surface. A stain line may be noticeable to differentiate the embedded portion from the portion that is above the plane of embeddedness. The particle's median or intermediate diameter ( $D_m$ ) is measured and recorded after  $D_t$  and  $D_e$  are measured.



- 4) The number of particles to be collected near each surber location may require some pre-planning, depending on the size of the riffle and the relative proximity of each randomly determined surber location. If less than 100 particles are available for embeddedness measurements in the riffle (i.e., short/narrow riffle), the remainder can be collected in the next upstream, representative riffle.
- 5) The individual  $D_t$  and  $D_e$  values for all 100 particles are summed, and a percent embeddedness value is calculated for the riffle/run habitat from the formula:

$$\text{Percent Mean Embeddedness} = 100 \left( \frac{\sum D_e}{\sum D_t} \right)$$

#### Method Considerations and Limitations (taken directly from Sylte and Fischenich, 2002)

- ▶ Cobble embeddedness exhibits high spatial and temporal variability in both natural and disturbed streams. Sampling must be intensive within streams or stream reaches to detect changes (Potyondy, 1988).
- ▶ Embeddedness measurements are most applicable in granitic watersheds or other geologies where sand is an important component of the annual sediment load and substrate. In basalts and other geologies where fines are predominantly silts and clays, low embeddedness values have high impact on fish (Chapman and McLeod, 1987).

- ▶ Cobble embeddedness is best applied to streams where embeddedness levels are suspected or known to be limiting to salmonid rearing.
- ▶ Repeat monitoring must be conducted at the same site because of high instream variability (Munther and Frank, 1986; Potyondy, 1988).
- ▶ Application of the method in streams < 6.1 m (20 ft) wide may destroy sites for future monitoring (Potyondy, 1988).
- ▶ Cobble embeddedness is most appropriate for stream-to-stream comparisons of similar reaches or for measuring temporal changes in the same reach.
- ▶ Cobble embeddedness is usually expressed as a percentage. However, this value does not reflect the amount of exposed rock, which is the critical component of the habitat for aquatic organisms. Cobble embeddedness expressed as a percent is not as sensitive to changes in sediment over time. Rocks that become completely buried in sediment are no longer part of the measurable population. Consequently, the lost “living space” is not reflected in the percent embeddedness figure.
- ▶ When the objective is to monitor changes in stream sediment over time, it is better to calculate the amount of vertically exposed rock ( $\sum (D_t - D_c)$ ). This “living space” and embeddedness can be calculated from the same field measurements. The choice may depend on the study objectives (e.g., evaluating fish or insect habitat) and whether changes over time or differences between streams are being determined. Preliminary evaluation of the vertically exposed rock parameter indicates that it also has a good correlation with the percentage and number of free matrix particles.
- ▶ Burns (1984) suggested that free matrix particles might offer a measure more sensitive than embeddedness percentages in conditions from 0 to 50 percent embeddedness. Munther and Lilburn (1988), Potyondy (1988), and Torquemada (1993) found significant correlation between percent cobble embeddedness and percent free matrix. Potyondy (1988) suggested simplifying the sampling technique to a system by tallying free matrix particles, assuming that errors on the order of  $\pm 7$  percent are acceptable. (Some studies correlate number of free matrix rocks, while others used a percentage of the embedded measured rocks. Strictly interpreting the original definition, percent free matrix is a proportion of the total measured rocks.)
- ▶ Results from the Boise National Forest annual summary, Potyondy (1988), and computer simulations by Kramer (1989) found that increased sedimentation can result in decreased embeddedness. Kramer (1989) found no use for the methodology because of this “flaw.”

## References

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**Stream Channel Longitudinal Profile Survey****Introduction**

The longitudinal profile documents the existing water surface, bankfull, low bank, terraces and thalweg elevations of a stream reach. Longitudinal profile data is used to calculate average bankfull and water surface slopes of a reach, along with maximum, minimum and average slopes of features such as riffles, runs, pools and glides. Water surface slopes of individual features such as riffles, runs, pools and glides are also referred to as facet slopes. Maximum, minimum and average bankfull depths among features and spacing are obtained from longitudinal profile data. These data are useful in geomorphic assessments of streambed stability and sediment supply and may be useful for design objectives. Longitudinal profiles require basic surveying skills and equipment. Survey basics such as establishing benchmarks, foresights, positioning the level, turning points and others are not covered here. For more information on survey basics consult Harrelson et al. (1994).

**Quality Control**

Following the process described in Harrelson et al. (1994), no longitudinal profile is complete without checking the accuracy of the survey with a survey closure. To close the survey, take a foresight reading at the benchmark, compute the elevation, and compare the difference to the original benchmark elevation at the start of the survey. Typically a closure of no more than 0.05 feet is acceptable when conducting stream surveys. The survey closure error shall be documented on the longitudinal profile datasheet.

**Field Procedure**

Required equipment and supplies for a longitudinal profile survey include:

<input type="checkbox"/> Calculator	<input type="checkbox"/> Pens
<input type="checkbox"/> Camera	<input type="checkbox"/> Pocket rod with 0.1' and 0.01' increments
<input type="checkbox"/> Datasheet or log book	<input type="checkbox"/> Range pins
<input type="checkbox"/> End caps	<input type="checkbox"/> Rebar (3 or 4' X 1/2" or 3/4")
<input type="checkbox"/> Hammer (2-lb or heavier)	<input type="checkbox"/> Survey rod with rod level and laser receiver
<input type="checkbox"/> Laser level with tripod	<input type="checkbox"/> Measurement tapes with 0.1' increments (several 300')

- 1) Establish a representative reach of a wadeable stream, generally at least two meander cycles or 20 bankfull widths in length. Begin and end all reaches at the top of a riffle.
- 2) Beginning at the upstream end of the reach, stretch the tapes along either the left or right bank as close to the edge of the channel as possible. Where possible, the tape should be threaded through riparian vegetation or other obstructions to get as close to the channel edge as possible. Tape(s) can be secured to the ground with range pins, vegetation, or rocks. Stationing of features will be obtained from the longitudinal profile tape.
- 3) If desired, establish permanent markers at the beginning and end of the longitudinal profile tape by driving rebar vertically in the ground, leaving one-half inch above the ground if acceptable to the landowner. Attach either plastic or metal end caps on top of the rebar for identification.

- 4) The position of the longitudinal profile tape should be included on the site map along with associated landmarks, stream channel cross-sections, and other relevant features. Photo document the longitudinal profile where applicable. If desired, triangulate the top and bottom of the longitudinal profile between the benchmark and another permanent feature and record on the datasheet. GPS locations of the top and bottom of the longitudinal profile can be used in place of triangulation. Document as much information as possible to the longitudinal profile tape location on the datasheet so it can be relocated for future surveys.
- 5) Follow the process described under **Bankfull Elevation – Field Identification** to identify bankfull elevation in the reach.
- 6) Follow the process described by Harrelson et al. (1994) to establish a benchmark and height-of-instrument.
- 7) Begin the longitudinal profile survey with a thalweg measurement at station 0 on the longitudinal profile tape. Obtain the rod reading and record the value as a foresight on the datasheet. Record (at a minimum) rod readings of water surface, bankfull and low bank (if greater than bankfull) perpendicular to the longitudinal profile tape at station 0. Only take rod readings of bankfull and low bank where indicators are present. Record the quality of the bankfull indicator(s) (good, fair, etc.) and the type of feature in the notes column of the datasheet.
- 8) Continue the same sequence as in step 7, working downstream, collecting readings at the top, mid-point and bottom of each feature (riffle, run, pool and glide), along with any other major bed features (dams, weirs, etc). For streams with long features or a homogeneous bed take rod readings at regular intervals, generally spaced no more than one bankfull width.
- 9) Note the stationing of all cross-section locations (if present) on the longitudinal profile tape and record on the datasheet. Take rod readings at the tops of all cross-section endpoints located along the bank with the longitudinal profile tape and record on the datasheet.
- 10) Close the survey according to the process described in the **Quality Control** section of this document.

## References

- Harrelson, C.C., C.L. Rawlins and J.P. Potyondy. 1994. Stream channel reference sites: an illustrated guide to field technique. General Technical Report RM-245. U.S. Department of Agriculture, Forest Service, Rocky Mountain Forest and Range Experiment Station. Fort Collins, CO.
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## Macroinvertebrate Sampling - Method Selection

### Introduction

This standard operating procedure describes the criteria used to select the appropriate method (e.g. **Targeted Riffle Method** or **Multi-habitat Method**) for sampling of benthic macroinvertebrates in wadeable streams and rivers of Wyoming. Implementation of the **Targeted Riffle Method** and **Multi-habitat Method** are described elsewhere in their associated standard operating procedures. Methods for collecting benthic macroinvertebrate samples on unwadeable streams and rivers are beyond the scope of this procedure.

### Site Selection

Establish a representative (study) reach of wadeable stream at or near baseflow, generally at least two meander cycles or 20 bankfull widths in length. All reaches shall begin and end at the top of a riffle. The representative reach should be of sufficient length to encompass multiple riffles/runs (for the **Targeted Riffle Method**) or all microhabitats such as channel substrate, banks, vegetation and woody debris (for **Multi-habitat Method**) from which to produce a single representative benthic macroinvertebrate sample. The location of the benthic macroinvertebrate collection within the study reach should be representative of the typical habitat conditions that are within the representative reach.

### Method Selection

The **Targeted Riffle Method** is the preferred method of sampling benthic macroinvertebrates in wadeable stream and rivers of Wyoming because 1) riffle/run habitat generally offers the most diverse and abundant assemblage of benthic macroinvertebrates; 2) it is a quantitative procedure whereby accurate estimates of richness, relative abundance and density can be derived from the results; 3) it has greater reproducibility in that generally the same riffle habitat can be sampled repeatedly over time and 4) Wyoming's two primary biological indicators for determining aquatic life use support - Wyoming Stream Integrity Index (WSII) and the Wyoming River InVertebrate Prediction And Classification System (WY RIVPACS) – were developed exclusively using, and for analysis of, benthic macroinvertebrate data collected with the **Targeted Riffle Method**. However, in the absence of suitable riffle/run habitat, the **Multi-habitat Method** is an acceptable alternative for the collection of a representative benthic macroinvertebrate sample. The major drawbacks to the **Multi-habitat Method** is 1) the procedure is semi-qualitative whereby accurate estimates of relative abundance and density cannot be derived from the data and 2) the WSII and WY RIVPACS cannot be used to evaluate aquatic life use support from benthic macroinvertebrate data collected with the **Multi-habitat Method**. In all cases, the appropriate sampling method should be selected based on habitat availability and not whether the stream is influenced by human perturbations. Lastly, consistency in benthic macroinvertebrate collection method is imperative when conducting targeted assessments to reduce sampling variability between monitoring sites.

There will however be situations where macroinvertebrate sampling is being conducted at multiple sites on the same stream as part of a targeted assessment, though one or more of the following **Targeted Riffle Method** selection criteria are not met at one or more of the sites. Under these circumstances, the default is to implement both the **Targeted Riffle** and **Multi-habitat Methods** at all sites as part of the targeted assessment. This approach provides additional biological information

for a targeted assessment that could be used to discriminate between stable versus unstable habitats, determine whether chemical toxicity or physical degradation is influencing the benthic biota, evaluate similarities between targeted versus multi-habitat assemblages and determine the types of taxa expected to occur, all of which can be used as additional lines of evidence in the determination of designated aquatic life use support for that stream.

#### Targeted Riffle Method Selection Criteria

The **Targeted Riffle Method** can be used to obtain a representative benthic macroinvertebrate sample when all of the following criteria are met:

- Riffles or riffle/run segments are typical within the reach. This does not mean riffles and runs need to be the dominant habitat feature within the reach, only that they are expected for the stream type considering its natural watershed characteristics. Riffles comprised of artificially placed substrate are unacceptable.
- A defined riffle exists with sufficient length where eight individual Surbers can be sampled (see **Targeted Riffle Method**). If riffles in the reach are of insufficient length, then a suitable length of riffle/run can be used as an alternative, such as in Rosgen E channels. In low-elevation pool-dominated streams where riffles are short features between long pools, then a combination of two or more of these riffles can be used to collect the required number of eight individual Surbers.
- There is perceptible and adequate flow over the riffle or riffle/run segment to ensure that organisms disturbed from the substrate will be carried into the Surber net
- There are no barriers to Surber collections in the channel such as abundant emergent or floating aquatic macrophytes, debris jams, or artificial obstructions.
- The channel substrate is dominated by, or contains a mixture of, boulder, cobble, gravel, sand and/or silt. Channel substrate dominated by bedrock or hard-pan clay is unacceptable.
- Mean depth within the riffle or riffle/run segment is generally  $\leq 1$  foot. Flows at depths deeper than 1 foot could result in backwash within the Surber net and/or inefficient capture of organisms disturbed from the substrate.
- Riffle or riffle/run segment width is generally  $\geq 1$  foot.

#### Multi-habitat Method Selection Criteria

The **Multi-habitat Method** can be used as an alternative to obtain a representative benthic macroinvertebrate sample when one or more of the above **Targeted Riffle Method** selection criteria are not met.

**References**

Barbour, M.T., J. Gerritsen, B.D. Snyder and J.B. Stribling. 1999. Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish, 2<sup>nd</sup> Edition. EPA 841-B-99-002. U.S. Environmental Protection Agency, Office of Water, Washington, D.C.

Rosgen, D.L. 1996. Applied River Morphology. Wildland Hydrology. Fort Collins, CO.

Wyoming Department of Environmental Quality, Water Quality Division  
Watershed Program

### Macroinvertebrate Sampling – Multi-habitat Method

Low gradient (slope <1%) are a common feature throughout many Wyoming landscapes, particularly in the interior basin and plains regions of the State and are often characterized by dominant fine sediment (i.e., sand, silt, clay) substrates. To effectively sample benthic macroinvertebrates from these systems requires the use of a proportion-based multi-habitat sampling method. This method focuses on a multi-habitat scheme designed to sample the major habitats in proportional representation within a representative sampling reach. The following multi-habitat sample method is modified from that described in Barbour et al. (1999). Samplers should utilize this multi-habitat method when the existing characteristics of the stream preclude the use of the Surber sampler described in the Macroinvertebrate Sampling SOP.

#### Quality Control

A minimum of ten per cent of all macroinvertebrate samples must be collected in duplicate. Duplicate sampling consists of a second sampler collecting 20 “jabs” or “kicks” in the same habitat locations as the primary sample. Duplicate samples will be identified with a different location name, either real or fabricated, and **not** identified as a duplicate. These will serve as a blind QC check for the analytical laboratory as well as the field samplers. Samples are collected during the index period of July 15 through October 31 for plains and basin streams and the period of August 1 through October 31 for mountain and foothill streams. Ongoing sample collection at the same site each year is performed  $\pm$  2 weeks of the original sampling date in order to avoid skewing the results by incorporating seasonal variability. After sampling at each site is complete, all brushes, nets, and equipment will be rinsed thoroughly, examined carefully, picked free of debris, and allowed to dry. The equipment must be re-examined before being used at the next sampling site and re-cleaned if necessary.

#### Required Equipment

- ◆ Rectangular Dip Net (500  $\mu$  open mesh, frame dimensions- 0.5 m X 0.3 m).
- ◆ Sieve. 500  $\mu$  open mesh.
- ◆ Field Tape and Range Pins (if needed).
- ◆ Preservative Solution (Isopropanol or Ethyl Alcohol).
- ◆ Sample Containers and Labels.
- ◆ 2 Plastic Buckets (at least two gallon volume each).
- ◆ Large Tub.
- ◆ Field Data and Macroinvertebrate Chain of Custody Forms.

#### Sampling Procedure

- ◆ Select a contiguous 300 ft representative reach of the stream. The reach should be at least 100 ft upstream of any road or bridge crossing and there should be no major tributaries discharging to the stream within the reach.
- ◆ Before sampling, the sampler should investigate the reach from the bank and will record the presence and relative percentage of the major habitat

types on the field data sheet from the list below:

- ◆ Covered Bank-Undercut (CU)
  - ◆ Covered Bank-No Undercut (CNU)
  - ◆ Uncovered Bank-Undercut (UU)
  - ◆ Uncovered Bank-No Undercut (UNU)
  - ◆ Bedrock, Cobble, Gravel (C)
  - ◆ Sand, Silt, Clay, Detritus (F)
  - ◆ Snags or Woody Debris (W)
  - ◆ Artificial Structure (A)
  - ◆ Macrophytes (M)
  - ◆ Other- Describe
- ◆ Multiply the relative percentage of each habitat type and multiply by 20 – this equates to the number of individual “kicks” or “jabs” that must be performed for each habitat type. Record the required number of “kicks” or “jabs” for each habitat type on the field data sheet. A total of 20 “kicks” or “jabs” will be conducted over the 300 ft length of representative reach.
  - ◆ Begin sampling at the downstream end of the reach and proceed upstream. Sample locations located along the banks will alternate between left and right banks, where possible. Sample locations within the channel shall be collected within or near the thalweg, where possible. Make sure that, within reason, sampling of each habitat type should be distributed throughout the 300 ft representative reach. In other words, if 5 individual samples are to be collected from a covered bank-undercut (CU) habitat type, do not collect all 5 samples within the first 50 ft of the reach when this habitat type is distributed throughout the 300 ft reach.
  - ◆ A sample will be taken approximately every 15 ft. If the 15 ft linear length lands in a habitat transition, the sampler should select the appropriate habitat to arrive at the representative proportions of total habitats that will be sampled in the reach. Record the distance along the 300 ft representative reach where the “kick” or “jab” was collected for each the 20 samples.
  - ◆ A “kick” is a stationary sampling in channel bed habitats where the dip net is placed on the substrate and the sampler thoroughly disturbs (vigorous kicking for 30 seconds) the substrate for an area approximately equal to the area of the dip net sampler (0.15 m<sup>2</sup>) upstream of the net. The dip net is then removed from the water with a quick upstream motion to wash organisms to the bottom of the net.

- ◆ A “jab” is normally collected in bank or woody debris habitats where the “jab” consists of thrusting the dip net *once* into an approximate 0.15 m<sup>2</sup> area (0.5 m X 0.3 m) of the substrate or brushing the surface of submerged large woody debris that would approximate 0.15 m<sup>2</sup>. It is important to only thrust the dip net once to minimize the amount of collected material. The initial “jab” is followed by 2-3 sweeps through the water containing the disturbance drift. The net is then removed with a quick up-stream motion to wash organisms to the bottom of the net.
- ◆ Macrophyte beds are sampled with a variation of the “jab”. Macrophyte beds are sampled by drawing the dip net in an upstream direction *once* through the vegetation. The initial “jab” is followed by 2-3 sweeps through the water containing the disturbance drift, directly downstream of the macrophyte bed.
- ◆ The dip net will be emptied into a rinsed large tub, and the net rinsed (500µ screened water) into the large tub generally every 5 “kicks” and/or “jabs.” The sampler should empty and rinse the net sooner if it is felt that contents of a previous sample will be lost in the subsequent “kick” or “jab.”
- ◆ Examine, wash and discard large pieces of vegetation, woody debris, pebbles, etc. making sure to remove and retain all aquatic invertebrates observed. Wash and discard all vertebrates. Retain all finer plant material and detritus.
- ◆ Sieve the composited 20 samples and transfer contents to sample container(s).
- ◆ Duplicates will be collected at the same time as the sample, but at a different stream width location (i.e. when the sample is collected along the right bank, the duplicate will be collected along the left bank, where possible). Two large tubs will be necessary when duplicates are collected.

**Sample Identification:** The sampler’s initials, year, Julian date and sample number will be written in water and alcohol resistant permanent ink or marker on a label affixed to the bottle. A second label in pencil with the same information is placed **inside** the sample container. The sample number must include: the sampler’s initials as shown on the inside front cover of the field log book (refer to SOP for Field Log Book); the year as 2 digits; the Julian day of the year as 3 digits (example: April 27 is day 117 of 1999); the day’s sample number (1, 2, 3, etc. for the first, second, third sample collected that day and the letter K for multi-habitat method). An example of complete and correct label information is: JAC-04-117-1K.

**Chain of Custody Form:** The form is required and must accompany the samples (refer to SOP for Chain of Custody). Sample identification information is also entered on this form. There are separate forms for macroinvertebrates and periphyton.

**Macroinvertebrate Sample Collection Data Form:** Macroinvertebrate Collection Observations: Enter the number of Surber samples collected and record whether duplicate samples were collected. Record the Surber sampler net mesh size. Circle type of preservative used as a reminder to preserve the sample. Record the official initials or name of the sampler.

**Method Reference** Barbour, M.T., J. Gerritsen, B.D. Snyder and J.B. Stribling. 1999. Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish, 2<sup>nd</sup> Edition. EPA 841-B-99-002. U.S. Environmental Protection Agency, Office of Water, Washington, D.C.

**Macroinvertebrate Sampling – Targeted Riffle Method****Required Equipment:**

Random number list or random number table  
Elbow or shoulder length water proof gloves  
Soft-bristed brush  
Forceps, pencil, or other pointed object  
Surber sampler: 1-foot (ft) x 1-ft frame with extended 3-ft length, 500 micron ( $\mu\text{m}$ ) mesh net  
Current velocity meter  
Sieve or sieve bucket with 500 micron ( $\mu\text{m}$ ) openings  
2 Plastic buckets (at least two gallon volume each)  
Large tub  
Plastic low density polypropylene wide mouth jars, Nalgene™, 500 and 1,000 ml  
Ethyl Alcohol (70% or greater)  
Labels and labeling tape  
Pencils and alcohol and water resistant pens such as Sharpie™  
Macroinvertebrate Chain of Custody form (refer to SOP for Chain of Custody)  
Box, wooden container, or cooler for sample storage

**Composite Samples:** Eight macroinvertebrate samples will be composited for each riffle location using a 1-ft x 1-ft frame Surber sampler with extended 3-ft length net with 500 micron ( $\mu\text{m}$ ) mesh. A mesh size other than 500 micron ( $\mu\text{m}$ ) may not be used **except** if required for direct comparison to historical macroinvertebrate collections. If the required eight samples are not collected and composited, that information must be noted on the Field Data sheet and in the field log book.

**Random Sampling Locations:** Select eight random numbers in sequence from a preassembled list. The random numbers should be four digits, organized in sets of eight, and ranked from low to high. The first two digits represent the distance in feet on the tape used to measure the riffle looking upstream. The second two digits represent the percentage of the stream width where the sample will be collected. For example, the number 5675 represents a sample location 56 feet up the tape and 75 percent of the distance across the stream width. If the riffle is less than 100 feet in length, convert the previously selected random numbers to accommodate the length of riffle encountered. For example, if the riffle is 76 feet in length, the random number 9670 would be converted to 7370. This is accomplished by multiplying  $0.76 \times 96$  (length of riffle/100  $\times$  the first pair of numbers representing the length along the tape). Adjust each of the eight random numbers in this manner so that they fall within the length of the riffle.

If the sample cannot be collected at the randomly selected location due to boulders, depth, high velocity ( $>3.3$  feet per second), debris jams, or other obstructions, look both ways across the stream and select the site nearest the intended sampling location. Record this procedure variance on the Field Data sheet. Sample from downstream to upstream, following the ranked random numbers, until eight samples have been collected. It may not be possible to collect eight samples from streams with very short riffles. In these cases, the eight samples may be collected from more than

one riffle, provided that each riffle sampled possesses similar physical (bed and bank) and riparian shading characteristics. It is also possible to sample a riffle/run segment of sufficient length to collect the eight Surber samples. Record the reasons for collection of the eight samples from multiple riffles or a riffle/run segment. Collect as many samples as possible and record the reasons for the discrepancy on the Field Data sheet.

### **Riffle Margins**

In any stream, avoid collecting samples from silty/sandy stream margins, backwater or slough habitats and pocket pools by collecting the eight (or fewer, if necessary) Surber samples within the actively flowing portion of the riffle.

**Biosurvey Observations:** Determine the relative abundance for each of the following biological groups while performing initial location reconnaissance and macroinvertebrate sampling.

Filamentous algae: green strands or clumps of algae often slick to the touch.

Floating macrophytes: examples: duckweed or plant material (leaves, etc.) detached from upstream areas

Rooted macrophytes: examples: cattails, reeds, coontail, sedges, rushes

Periphyton: visible algae and other organisms attached to stream substrate and other submerged objects; extensive periphyton growth will be visible to the naked eye and should be observed while performing substrate and embeddedness measurements.

Slimes: may be observed near areas receiving animal wastes, organic and nutrient enrichment

Fish: Observations of fish (and species, if known) should be recorded

**Sampling Procedure:** Cobble and coarse gravels (greater than 2.5 inches in diameter) lying, all or in part, within one inch of the surface of the bed and within the sample frame will be gently rubbed by hand (to remove loosely attached or clinging invertebrates), then with a soft-bristled brush. Particles that are completely visible and are greater than 50% within the frame will be sampled in their entirety, whereas particles that are completely visible with less than 50% within the frame will be discarded downstream of the Surber sampler. The exposed portions of large, partially buried particles that lie within the Surber frame shall be sampled. In some cases it may be necessary to hand scrub smaller gravels if encrusted with precipitate providing crevice habitat for invertebrates. Each particle should be visually examined to ensure removal of all organisms. It may be necessary to use a forceps or other pointed object to remove organisms that are firmly attached or that reside in crevices of rocks. Remove as much algae and periphyton attached to substrate as possible since macroinvertebrates reside on these materials. When free of organisms and algae, rocks are discarded outside and behind the sampler. Remaining substrate within the sampler will be thoroughly agitated, if possible, to a depth of at least two though no more than three inches for a period 30 seconds. The substrate should not be agitated in a way that “scoops” material into the net, but rather stirred or

kneaded in a way that dislodged organisms will be swept into the net by flowing water and collection of sediment is minimized.

Repeat the procedure at the remaining randomly selected locations until eight samples have been collected. If eight locations cannot be sampled due to limited riffle length or width, record the reason for the discrepancy on the Field Data Sheet.

Excess sediment and detritus (algae, leaves, plant material, etc.) retained in the sampler serve as a visual warning of the potential for net clogging. Empty the Surber sampler into a tub between sample locations if indications of net clogging (backwash out the front of the sampler) are visible. The eight Surber samples may be collected and composited in the net without emptying the sampler if net clogging is not observed.

Hold the net upright, splash water on the outside of the Surber sampler netting to wash organisms and detritus to the bottom of the net. Holding the net over a tub, invert the net and gently pull the net inside out. Using stream water **previously filtered** through a U.S. Standard No. 35 (500 $\mu$ m) sieve, rinse, and then examine the net to ensure that all organisms are removed. Unfiltered stream water may be poured through the inverted net to remove organisms and debris. Cobbles and large gravels are removed from the tub after close examination. Remove all fish and other vertebrates from the tub. Pour tub contents into a U.S. Standard No. 35 sieve. If the volume of inorganic material in the tub exceeds the capacity of the sieve, the inorganic fraction (primarily sand) may be reduced through the process of elutriation. See SOP for Elutriation. Inorganic material should comprise no more than 10% of the total sample volume.

**Important Note: Do not leave the samples in direct sun or subject them to elevated temperatures.** Macroinvertebrates decompose very quickly when exposed to direct sunlight. If the sample cannot be preserved quickly (less than 10 minutes) they must be put in a shaded area.

Transfer the sample from the sieve to the sample container. The bulk of the material may be transferred by hand if held over the tub. Rinse remaining material from the sieve through a funnel and into the sample container using ethyl alcohol. Fill each sample container **not more than 2/3 full** to allow room for the preservative. Add 70% or greater ethyl alcohol preservative. Use more than one bottle if the entire sample volume exceeds that of one container.

Wipe the bottle threads (and the cap if necessary) to remove any sand or dirt so that the cap will tighten properly, tighten the screw cap, then gently invert the container 3 to 4 times so the preservative will penetrate into all of the organisms. Any liquid leaking from the bottle cap with the bottle inverted indicates an incomplete seal, most likely due to dirt or debris in the bottle or cap threads. Label the bottles and place them in a box, wooden container or cooler for transport to the laboratory. Samples with a large amount of organic material will have to be decanted and re-preserved 2-4 times. Decant and re-preserve within two days of collection, and again within 1 week of collection. All samples (regardless of organic content) should be checked periodically to ensure decomposition is not occurring.

**Sample Identification:** The sampler's initials, year, Julian date and sample number will be written in water and alcohol resistant permanent ink or marker on a label affixed to the bottle. A second label in pencil with the same information is placed **inside** the sample container. The sample number must include: the sampler's initials as shown on the inside front cover of the field log book (refer to SOP for Field Log Book); the year as 2 digits; the Julian day of the year as 3 digits (example: April 27 is day 117 of 1999); the day's sample number (1, 2, 3, etc. for the first, second, third sample collected that day and the letter M for macroinvertebrate). An example of complete and correct label information is: JAC-04-117-1M.

**Chain of Custody Form:** The form is required and must accompany the samples (refer to SOP for Chain of Custody). Sample identification information is also entered on this form. There are separate forms for macroinvertebrates and periphyton.

**Macroinvertebrate Sample Collection Data Form:** Macroinvertebrate Collection Observations: Enter the number of Surber samples collected and record whether duplicate samples were collected. Record the Surber sampler net mesh size. Circle type of preservative used as a reminder to preserve the sample. Record the official initials or name of the sampler.

#### Method Reference

Barbour, M.T., J. Gerritsen, B.D. Snyder and J.B. Stribling. 1999. Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish, 2<sup>nd</sup> Edition. EPA 841-B-99-002. U.S. Environmental Protection Agency, Office of Water, Washington, D.C.

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**Pebble Counts – Reachwide and Cross-Sections****Introduction**

Channel substrate composition of a reach influences Rosgen stream type, channel form and hydraulics, erosion rates and sediment supply and also influences the survival, growth and reproduction of aquatic life. Channel substrate composition to be used in geomorphic analyses is collected using two different methods of pebble counts. To determine Rosgen stream type, Rosgen's (1996) modification of Wolman's (1954) reachwide pebble count is used. A reachwide pebble count entails measurement of a total of 100 random particles, proportionally sampled from the channel bed across pool and non-pool features within the bankfull width. For hydraulic and sediment competency calculations, 100 particles are randomly sampled within the active width of the channel bed at a particular feature (riffle, run, pool, glide) (Rosgen 1996).

**Quality Control**

Samplers follow the SOP

**Field Procedure**

Required equipment and supplies for pebble counts include:

<input type="checkbox"/> Calculator <input type="checkbox"/> Datasheet or log book <input type="checkbox"/> Pens	<input type="checkbox"/> Ruler (in metric units) <input type="checkbox"/> Sand card
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**Reachwide Pebble Count**

- 1) Establish a representative reach of a wadeable stream, generally at least two meander cycles or 20 bankfull widths in length.
- 2) Following the process described under **Bankfull Elevation–Field Identification**, identify bankfull elevation in the reach.
- 3) Where applicable, conduct the reachwide pebble count after all cross-section and/or longitudinal surveys have been completed.
- 4) Walk the entire length of the reach and record pool and non-pool lengths on the datasheet. Pool and non-pool lengths may also be obtained from a longitudinal profile if available.
- 5) Compute the percentage of the total length of the reach that is pool and non-pool and record on the datasheet.
- 6) Use the percentage of pool and non-pool features to proportionally sample the pool and non-pool features within the reach. For example, if 60% of the reach is pool, then 60 of the 100 total particles will be sampled from pool features. Likewise, if 40% of the reach is non-pool, then 40 of the 100 total particles will be sampled from non-pool features.
- 7) Distribute the particles proportionally among an appropriate number of features within the reach. Generally, no more than 10 particles should be collected from any one feature. Using the same example, if 60 particles are to be collected from pool features, then six individual pool features will be used with a total of 10 particles collected per feature.

- 8) Begin the pebble count at either the top or bottom of the reach.
- 9) Particles are randomly collected at evenly spaced intervals across the entire bankfull channel at each pool and non-pool feature. Particles can be collected either perpendicular or diagonally across the feature. Maintain consistency in how particles are collected throughout the reach.
- 10) At each interval, a particle is “blindly” selected by looking away from the selection site and extending an index finger to the first particle touched on the channel bed at either the tip of the boot or the mid-point between the legs, is selected. Maintain consistency with the particle selection method used.
- 11) Measure the intermediate or median diameter of the particle ( $D_m$ ) (see **Riffle Embeddedness**) with a ruler and record on the datasheet in millimeters. If a sand card is used, then record the narrative description (i.e., coarse sand, fine sand, etc.) on the datasheet. If a particle is too large to be moved, conduct an estimate and record ‘est.’ next to that data point on the datasheet. If the particle size falls at the division point between two size classes, always record the particle size in the smallest size class. For example, if the  $D_m$  is 4 mm, then record that value under the very fine gravel class (2-4 mm) rather than the fine gravel class (4-5.7 mm).
- 12) To prevent over-sampling of bank material generally take only one bank sample at every other feature. This provides a 5% representative sample of the bank material within the bankfull width.
- 13) Continue throughout the reach with the pebble count, alternating between pool and non-pool features until 100 particles are measured.

### Cross-Section Pebble Count

- 1) Conduct the cross-section pebble count after the cross-section has been surveyed.
- 2) One hundred particles are randomly collected at evenly spaced intervals along a transect, across the entire active width of the channel, immediately below the surveyed cross-section. The active channel is the portion of channel, which may or may not incorporate bankfull, that receives periodic scour and/or fill during during bankfull events. If the active width is small, then more than one transect may be sampled to obtain 100 particles, provided the particles are within the bed of the surveyed cross-section.
- 3) At each interval, the first particle touched with a finger, at either the tip of the boot or the mid-point between the legs, is selected. Maintain consistency with the particle selection method used.
- 4) Measure the intermediate or median diameter of the particle ( $D_m$ ) (see **Riffle Embeddedness**) with a ruler and record on the datasheet in millimeters. If a sand card is used, then record the narrative description (i.e., coarse sand, fine sand, etc.) on the datasheet. Measure particles too large to be moved in place.
- 5) Continue along the transect(s) until 100 particles are measured.

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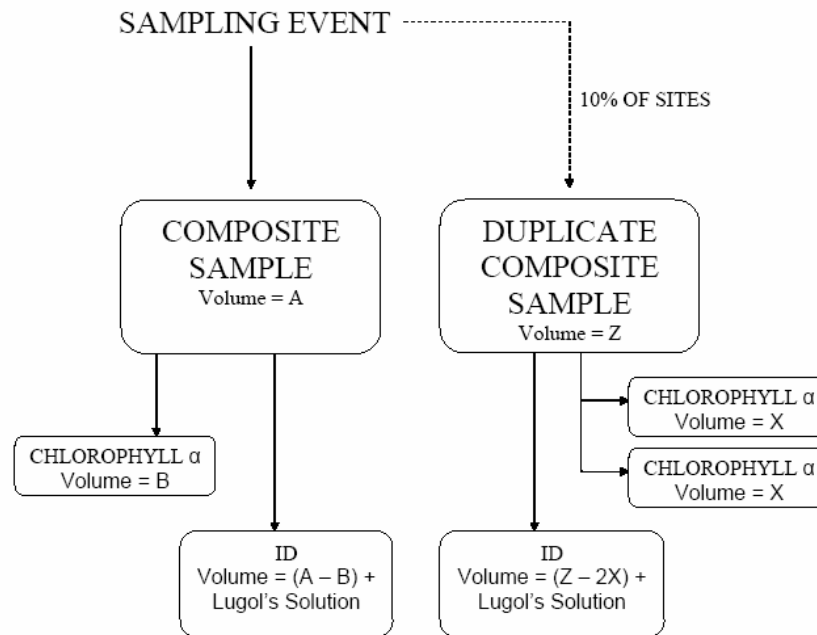
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## Periphyton: Sampling Methods and Subsample Processing for Chlorophyll $\alpha$ Analysis and Taxonomic Identification

### Quality Control

Following the processes described under **Sampling Methods-Field Procedures**, at least ten percent (10%) of all collected composite samples must consist of duplicate composite samples (e.g., 2 duplicates for 11 to 20 samples, 3 duplicates for 21 to 30 samples, etc.). Duplicate composite sampling consists of two samplers each with the same equipment, collecting simultaneously alongside 1) randomly selected locations for Epilithic samples, 2) woody snag locations for Epidendric samples 3) shallow depositional locations for Episammic/Epipellic samples or 4) locations of emergent or submerged vegetation for Epiphytic samples.

Following the processes described under **Subsample Processing Procedures**, the sampler that collected the duplicate composite sample, extracts two chlorophyll  $\alpha$  subsamples from the duplicate composite sample. The remaining duplicate composite sample volume will be used for the duplicate ID subsample. Duplicate composite samples are collected to check the variability between field samplers while the two duplicate chlorophyll  $\alpha$  subsamples provide an indication of precision and the quality of the duplicate composite sample homogenization. An illustration of the duplicate composite sample/subsample processes is provided below:



## Introduction

Periphyton are benthic algae that live attached or in close proximity to various substrates associated with the stream bottom. The structure, diversity and abundance of periphyton is highly dependent on the diversity and availability of substrates in the stream. Periphyton algae often form visible filaments or colonies in the form of mats or biofilms attached to substrate. Two basic types of periphyton are found in Wyoming streams: diatoms (Division Chrysophyta, Class Bacillariophyceae) and soft-bodied algae. Soft-bodied algae are represented by four major divisions: green algae (Chlorophyta), blue-green algae (Cyanophyta), gold/brown algae (Chrysophyta) and occasionally red algae (Rhodophyta).

Periphyton are important primary producers and chemical modulators in stream ecosystems. As such, periphyton can be more sensitive to certain stressors such as nutrients, salts, sediment and temperature compared to other aquatic organisms. Measures of periphyton structure, diversity and density are useful in the assessment of biological condition for surface waters. For more information on periphyton and their use in bioassessments, refer to Barbour et al. (1999) and Stevenson et al. (1996).

## Sampling Time - Index Period

The recommended sample period for periphyton follows the sample period for benthic macroinvertebrates (see **Macroinvertebrate Sampling Index Period** Standard Operating Procedure (SOP)). It may be necessary to sample outside the recommended index period to coincide with flows in ephemeral, intermittent or dewatered streams.

## Sampling Methods - Field Procedure

The field procedure(s) for collecting periphyton will vary depending on the chosen targeted habitat. The targeted habitat represents the most common and stable habitat in the stream reach. Field selection of the targeted habitat where samples are collected will be based on the following prioritization: 1) riffles with dominant coarse substrate (Epilithic habitat); 2) woody snags in streams with dominant fine-grained substrate (Epidendric habitat); organically rich 3) pea gravel/sand (Episammic habitat) or 4) silt (Epipellic habitat) depositional areas along stream margins, and 5) emergent or 6) submerged vegetation (Epiphytic habitat).

Ensure that all equipment and supplies needed to conduct the periphyton sampling and subsequent subsample processing are assembled and ready for use. Required items include:

<input type="checkbox"/> Aluminum foil <input type="checkbox"/> Battery powered stirrer (optional) <input type="checkbox"/> Digital caliper <input type="checkbox"/> Distilled or deionized water <input type="checkbox"/> Dry ice (or wet if necessary) <input type="checkbox"/> Envelopes <input type="checkbox"/> Filtration apparatus that includes hand pump (with gage), tubing, filter base and filter funnel <input type="checkbox"/> Forceps <input type="checkbox"/> Funnel <input type="checkbox"/> Glass microfiber filters (47 mm @ 0.7 micron) <input type="checkbox"/> Graduated cylinders <input type="checkbox"/> Hand saw (folding) <input type="checkbox"/> Knife (pocket knife preferable) <input type="checkbox"/> Labels <input type="checkbox"/> Lugol's solution <input type="checkbox"/> Pens and permanent markers	<input type="checkbox"/> Plastic beaker (500 mL) <input type="checkbox"/> Plastic petri dishes (47 mm) <input type="checkbox"/> Plastic sample bottles (500 & 1000 mL Nalgene®) <input type="checkbox"/> Plastic tape (electrical preferable) <input type="checkbox"/> Plastic trays <input type="checkbox"/> Pocket calculator <input type="checkbox"/> Pruning shears <input type="checkbox"/> Rinse bottle <input type="checkbox"/> Ruler (with metric increments) <input type="checkbox"/> Scissor <input type="checkbox"/> Sealable plastic bags <input type="checkbox"/> Spatula <input type="checkbox"/> Serological volumetric pipettes (10 mL disposable) with rubber bulb <input type="checkbox"/> Toothbrush (soft and firm bristled) <input type="checkbox"/> Top-setting or survey rod <input type="checkbox"/> Velocity meter
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### **Sampling Method for Epilithic (Coarse Substrate) Habitats**

1. Randomly select eight sampling locations within the riffle. If also sampling for macroinvertebrates using a Surber sampler, samples will be collected in close proximity to (but not within) the randomly selected Surber sample locations. See **Macroinvertebrate Sampling** SOP for description of selecting random sample locations.
2. Carefully remove 1 or 2 rocks from each of the eight randomly selected sample locations while retaining the rock's orientation as it occurred in the stream to avoid loss of periphyton. Rocks should be relatively flat and range in size from about 4 cm (coarse gravel) to 10 cm (small cobble) in diameter. Collect only one rock per randomly selected sample location if the diameter of the first rock selected is equal to or exceeds 7.5 cm. If the diameter of the first rock selected is less than 7.5 cm, select a second rock. If possible, select rocks that are similar with respect to size, depth and exposure to sunlight. A total of 8 to 16 rocks are collected at each sample site. Gently place the rocks (as they were oriented in the stream) in a plastic tray; do not stack rocks upon one another. Transport the tray to a convenient sample-processing area. Where possible, process the sample out of direct sunlight to minimize degradation of chlorophyll.
3. Measure water depth and velocity at each of the eight locations using a top-setting rod and velocity meter and record on the datasheet. *NOTE: Additional measurements of depth and velocity are not required if the sampler is already measuring these parameters for the macroinvertebrate sample.* Assuming the sun is directly overhead, determine the relative degree of riparian shading (e.g. shaded, partial, or full sun) at each randomly selected sample location and record on the datasheet.
4. Scrub only the upper surface of each rock with a firm-bristled toothbrush using a circular motion. In circumstances where rocks are much greater than 10 cm

(medium to large cobbles), firmly brush only a portion of the upper rock surface around 10 cm in diameter. Do not brush the sides or bottom of rocks. If needed, remove any filamentous algae and mosses by scraping with a knife and place in a separate plastic tray. Use a knife or scissor to cut algal filaments or moss into roughly 2 to 3 mm segments. Gently brush other larger plant material that may be attached to the rocks but do not collect the plants. Rinse the sampled rock surface, attached plants and toothbrush bristles with a rinse bottle containing deionized or distilled water. Use rinse water sparingly, but be thorough. Collect rinsate in the plastic tray containing any filamentous algae or mosses. Repeat for the remaining rocks. Keep the sample volume less than 500 mL. After sample processing is complete, measure and record the total rinsate volume (now considered the *composite sample volume*) on the datasheet and pour the rinsate through a funnel into a 500 mL Nalgene® sample bottle.

5. For each rock processed, cover the surface with a sheet of aluminum foil. Either trim the foil with a knife or fold the foil to match the area sampled. Place the trimmed/folded foil templates into a labeled collection envelope and attach to the field data sheets.
6. Process the composite sample following steps described in **Subsample Processing Procedures** to extract subsamples for chlorophyll  $\alpha$  analysis and taxonomic identification.

#### **Sampling Method for Epidendric (Woody Snag) Habitats**

Collecting quantitative microalgal periphyton samples from epidendric habitats presents a challenge because they generally have an irregular surface and are difficult to remove without loss of periphyton biomass. Use the following method to address these difficulties when sampling epidendric habitats:

1. Select a total of eight pieces of woody snag material from the same number of different locations throughout the reach. Select pieces greater than 1 cm in diameter that have likely been submerged for most of the year to allow for sufficient periphyton colonization but which are not smothered by bottom sediments.
2. Carefully remove an approximately 10 to 20 cm long section of each woody snag with pruning shears or a hand saw and place in a plastic tray. Transport the tray to a convenient sample-processing area. Where possible, process the sample out of direct sunlight to minimize degradation of chlorophyll.
3. Measure water depth and velocity at the point where each of the eight woody snags were removed using a top-setting rod and velocity meter and record on the datasheet. Assuming the sun is directly overhead, determine the relative degree of riparian shading (e.g. shaded, partial, or full sun) at each of the eight sample locations and record on the datasheet.

4. Scrub the entire surface of the woody section with a firm-bristled toothbrush. If needed, remove any filamentous algae and mosses by scraping with a knife and place in a separate plastic tray. Use a knife or scissor to cut algal filaments or moss into roughly 2 to 3 mm segments. Rinse the toothbrush and the section of wood with a rinse bottle containing deionized or distilled water. Use rinse water sparingly, but be thorough. Collect rinsate in the plastic tray containing any filamentous algae or mosses. Set the section of wood aside. Repeat for the remaining woody sections. Keep the sample volume less than 500 mL. After sample processing is complete, measure and record the total rinsate volume (now considered the *composite sample volume*) on the datasheet and pour the rinsate through a funnel into a 500 mL Nalgene® sample bottle.
5. Measure the length and diameter (take an average of three diameter measurements) of each cleaned woody section and calculate the total sampled surface area by using the following formula (assumes a cylinder):

$$\text{Total Sampled Area (cm}^2\text{)} = \sum_{l=1}^n (\pi)(di)(li)$$

Where,

$n$  = number of discrete collections

$\pi$  = 3.1416

$di$  = mean diameter of each woody section, in centimeters

$li$  = length of each woody section, in centimeters

Alternatively, a foil template can be used (see Epilithic habitat method) for irregularly shaped woody sections. Record the sampled surface areas on the datasheet.

6. Process the composite sample following the steps described in **Subsample Processing Procedures** to extract subsamples for chlorophyll  $\alpha$  analysis and taxonomic identification.

#### **Sampling Method for Episammic (Pea gravel/Sand) and Epipellic (Silt) Habitats**

Quantitative microalgal periphyton samples are collected from the upper 5 to 7 mm layer of episammic (pea gravel  $\leq$  5 mm/sand) and epipellic (silt) habitat in organically-rich depositional areas of the reach. Use the following method to sample episammic or epipellic habitats:

1. Select a total of five different locations, in shallow organically-rich depositional zones that consist of either pea gravel, sand or silt substrate. *NOTE: All five locations must be from the same type of habitat, either pea gravel/sand or silt.*
2. At each location, hold the lid of a plastic Petri dish (47 mm diameter) upside down in the water; gently stir/shake the lid to remove air bubbles without disturbing the substrate.

3. With the lid still submerged, turn the inside of the lid toward the substrate that will be sampled without disturbing the substrate.
4. Carefully and slowly press (in cookie cutter fashion) the lid into the substrate.
5. Slide the lid onto a spatula to enclose the discrete collection. Holding the Petri dish firm against the spatula, carefully wash extraneous sediment from the spatula and lift out of the water.
6. Transport the Petri Dish and spatula to a convenient sample-processing area. Where possible, process the sample out of direct sunlight to minimize degradation of chlorophyll.
7. Invert the lid and remove the spatula. Be careful not to lose any of the discrete sample still adhering to the spatula.
8. Rinse the substrate from the lid and spatula with a rinse bottle containing deionized or distilled water into a 500 mL Nalgene<sup>®</sup> sample bottle. Use rinse water sparingly, but be thorough. Combine all five discrete sample collections in the 500 mL Nalgene<sup>®</sup> sample bottle. Repeat at the remaining sample locations. Keep the sample volume less than 500 mL. After sample processing is complete, measure and record the total rinsate volume (now considered the *composite sample volume*) on the datasheet.
9. The total sample surface area for all five discrete samples collected with a 47 mm Petri dish is 85 cm<sup>2</sup>. Record the sampled surface area on the datasheet.
10. Measure water depth and velocity at the point where each of the five discrete collections were removed using a top-setting rod and velocity meter and record on the datasheet. Assuming the sun is directly overhead, determine the relative degree of riparian shading (e.g. shaded, partial, or full sun) at each of the five sample locations and record on the datasheet.
11. Process the composite sample following the steps described in **Subsample Processing Procedures** to extract subsamples for chlorophyll  $\alpha$  analysis and taxonomic identification.

### **Sampling Method for Epiphytic (Emergent Vegetation) Habitats**

Sampling emergent macrophytes is appropriate only when it is not possible to sample other preferred targeted habitats (e.g., epilithic, epidendric, episammic, and epipellic) due to their absence or rare occurrence in the reach. Collecting quantitative microalgal periphyton samples from emergent vegetation presents a challenge because of varying sizes and shapes in vegetation and the care needed to remove the vegetation without loss of periphyton biomass. Use the following method to address these difficulties when sampling epiphytic (emergent vegetation) habitats:

1. All samples should be collected from live specimens of the same emergent species or group of closely-related emergent species that are common in the reach to facilitate sampling of the same species/group in the future. This will allow for representative comparisons of periphyton data over time. Groups of closely-related emergent species such as *Carex* (sedges), *Juncus* (rushes), *Polygonum* (smartweed) and *Typha* (cattails) are all suitable.
2. Record the species or group of closely-related species that will be sampled on the field datasheet.
3. Select a total of five sections of emergent vegetation from the same number of different locations throughout the reach. Each section represents a stem or leaf (no roots), however, all five sections must be of the same type. *NOTE: Do not cause unneeded disturbance to the emergent vegetation or excessive wave-action when wading; epiphytic periphyton are often loosely attached and easily dislodged.*
4. Prior to sampling a section, the un-submerged portion of the selected emergent vegetation should be removed with pruning shears or a scissor at water level and discarded. Select a large diameter/width section of stem or leaf from the submerged portion of the emergent vegetation just below water level. Select sections that have likely been submerged for most of the growing season to allow for sufficient periphyton colonization but which are not smothered by bottom sediments.
5. Carefully remove an approximately 10 to 20 cm long section of each stem or leaf with pruning shears or a scissor and place in a plastic tray. Transport the tray to a convenient sample-processing area. Where possible, process the sample out of direct sunlight to minimize degradation of chlorophyll.
6. Measure water depth and velocity at the point where each of the five sections of emergent vegetation were removed using a top-setting rod and velocity meter and record on the datasheet.
7. Gently brush the entire surface of the stem or both sides of a leaf section with a soft-bristled toothbrush. If needed, remove any filamentous algae and mosses by brushing and place in a separate plastic tray. Use a knife or scissor to cut algal filaments or moss into roughly 2 to 3 mm segments. Rinse the toothbrush and the stem or leaf section with a rinse bottle containing deionized or distilled water. Use rinse water sparingly, but be thorough. Collect rinsate in the plastic tray containing any filamentous algae and mosses. Set the stem or leaf section aside. Repeat for the remaining stem or leaf sections. Keep the sample volume less than 500 mL. After sample processing is complete, measure and record the total rinsate volume (now considered the *composite sample volume*) on the datasheet and pour the rinsate through a funnel into a 500 mL Nalgene® sample bottle.
8. For cylindrical-shaped stem or leaf samples, use a digital caliper to measure the length and diameter (take an average of three diameter measurements) of each

cleaned stem/leaf section and calculate the total sampled surface area by using the following formula (assumes a cylinder):

$$\text{Total Sampled Area (cm}^2\text{)} = \sum_{l=1}^n (\pi)(di)(li)$$

Where,

$n$  = number of discrete collections

$\pi$  = 3.1416

$di$  = mean diameter of each cylindrical stem section, in centimeters

$li$  = length of each cylindrical stem section, in centimeters

9. For triangular stem samples, use a digital caliper to measure the width of all three sides (measure the width of each side and take an average) and length for each cleaned stem section. Calculate the total sampled surface area by using the following formula (assumes an equilateral triangle):

$$\text{Total Sampled Area (cm}^2\text{)} = \sum_{l=1}^n 3(wi)(li)$$

Where,

$n$  = number of discrete collections

$wi$  = mean width of each triangular stem section, in centimeters

$li$  = length of each triangular stem section, in centimeters

10. For non-cylindrical leaf samples, place each cleaned leaf section on a sheet of aluminum foil. With a permanent marker or pen, trace the shape of the leaf section to match the area sampled. For large leaf sections, either trim the foil with a knife or fold the foil to match the area sampled. Mark "X 2" on each leaf template to note that both sides of the leaf section were sampled. Place the marked or trimmed/folded foil templates into a labeled collection envelope and attach to the field data sheets.
11. Process the composite sample following steps described in **Subsample Processing Procedures** to extract subsamples for chlorophyll  $\alpha$  analysis and taxonomic identification.

### **Sampling Method for Epiphytic (Submerged Vegetation) Habitats**

Sampling submerged macrophytes is appropriate only when it is not possible to sample other preferred targeted habitats (e.g., epilithic, epidendric, episammic, epipellic or epiphytic-emergent vegetation) due to their absence or rare occurrence in the reach. Collecting quantitative periphyton samples from submerged macrophytes can be a challenge because several submerged macrophytes have small or finely dissected leaves, which present difficulties for accurately calculating the surface area of periphyton colonization. Furthermore, care is needed to remove the vegetation without the loss of periphyton biomass. Use the following method to address these difficulties when sampling epiphytic (submerged vegetation) habitats:

1. All samples should be collected from live specimens of the same submerged species or group of closely-related submerged species that are common in the reach to facilitate sampling of the same species/group in the future. This will allow for representative comparisons of periphyton data over time. Groups of closely-related submerged species such as *Ceratophyllum* (coontail), *Myriophyllum* (milfoil), *Najas* (water-nymph), and *Potamogeton* (pondweed) are all suitable.
2. Record the species or group of closely-related species that will be sampled on the field datasheet.
3. Select a total of five samples of submerged vegetation from the same number of different locations throughout the reach. Each sample should consist of a single stem plus associated branches of the plant from the lowest healthy leaves to the tip. Submerged leafless stems should not be included. Select samples that have likely been submerged for most of the growing season to allow for sufficient periphyton colonization but which are not smothered by bottom sediments. *NOTE: Do not cause unneeded disturbance to the emergent vegetation or excessive wave-action when wading; epiphytic periphyton are often loosely attached and easily dislodged.*
4. Carefully remove the sample with pruning shears or a scissor and gently place in a plastic tray. Transport the tray to a convenient sample-processing area. Where possible, process the sample out of direct sunlight to minimize degradation of chlorophyll.
5. Measure water depth and velocity at the point where each of the five samples of submerged vegetation were removed using a top-setting rod and velocity meter and record on the datasheet.
6. Use a knife or scissor to cut the sample's stem and branches into 10 to 20 cm segments, preferably at the plant nodes. Do not cut through the leaves. *NOTE: For samples with small or finely dissected leaves, the sampler has the option of discarding the leaves and only processing the stems and branches. If leaves are discarded, sampler must note this on the datasheet.* Use a knife or scissor to cut any algal filaments or moss into roughly 2 to 3 mm segments. Repeat for remaining samples. Within the plastic tray, separate the sample segments from the algal/moss segments.
7. Fill a 1000 mL Nalgene<sup>®</sup> bottle with 300 mL of deionized or distilled water. Place sample segments in the Nalgene<sup>®</sup> bottle and cap the bottle. Shake the

Nalgene<sup>®</sup> bottle vigorously for 30 seconds to dislodge attached periphyton. Open the Nalgene<sup>®</sup> bottle, remove individual segments and rinse segments with a bottle containing deionized or distilled water. Use rinse water sparingly, but be thorough. Collect rinsate in the 1000 mL Nalgene<sup>®</sup> bottle. Repeat for remaining sample segments. Set aside rinsed sample segments. Once all sample segments have been processed, place algal/moss segments in the 1000 mL Nalgene<sup>®</sup> bottle. Any dislodged periphyton remaining in the plastic tray should be inserted through a funnel into the 1000 mL Nalgene<sup>®</sup> bottle. Keep the sample volume less than 500 mL. After sample processing is complete, measure and record the total rinsate volume (now considered the *composite sample volume*) on the datasheet.

8. For cylindrical-shaped stems, branches and leaf samples, use a digital caliper to measure the length and diameter of each cleaned stem, branch or leaf segment<sup>1</sup>. Calculate the total sampled surface area by using the following formula (assumes a cylinder):

$$\text{Total Sampled Area (cm}^2\text{)} = \sum_{l=1}^n (\pi)(di)(li)$$

Where,

$n$  = number of discrete collections

$\pi$  = 3.1416

$di$  = mean diameter of each cylindrical stem section, in centimeters

$li$  = length of each cylindrical stem section, in centimeters

<sup>1</sup>For samples with  $\geq 10$  cylindrical-shaped leaves, take an average of the length and width measured from each of the randomly-selected leaf segments. Use these mean values and the above equation to obtain a surface area and then multiply by the total number of leaf segments in the sample. This alternative method may also be used to calculate the area for cylindrical-shaped branches when the sample contains  $\geq 10$  branches. In all cases, the stem must be measured in its entirety.

9. For non-cylindrical or broad-shaped leaf samples, place each cleaned leaf segment on a sheet of aluminum foil<sup>2</sup>. With a permanent marker or pen, trace the shape of the leaf segment to match the area sampled. For large leaf segments, either trim the foil with a knife or fold the foil to match the area sampled. Mark “X 2” on each leaf template to note that both sides of the leaf segment were sampled. Place the marked or trimmed/folded foil templates into a labeled collection envelope and attach to the field data sheets.

<sup>2</sup>For samples with  $\geq 10$  non-cylindrical or broad-shaped leaves, randomly select three leaf segments and follow the above foil template procedure. Record the total number of leaves on the datasheet. Take the average surface area from the

three measured leaf segments and multiply by the total number of leaf segments in the sample. Record the total area on the datasheet.

10. Process the composite sample following steps described in **Subsample Processing Procedures** to extract subsamples for chlorophyll  $\alpha$  analysis and taxonomic identification.

### Subsample Processing Procedures

Each composite sample processed in the field is used to extract subsamples for chlorophyll  $\alpha$  analysis and taxonomic identification. Successful execution of subsample processing procedures described here is dependent on measuring and tracking the various volumes as the composite sample is processed. One subsample is extracted from each composite sample for the purpose of determining chlorophyll  $\alpha$  in the laboratory. The remaining volume of the composite sample is considered the ID subsample and is preserved for taxonomic identification. Subsampling processing procedures for periphyton composite samples are as follows:

1. In an area out of direct sunlight, assemble the filtration apparatus by attaching the filter base with rubber stopper to the filtration flask. Join the flask and a hand-operated vacuum pump (with pressure gage) using a section of tubing.
2. Place a 47 mm 0.7 micron glass microfiber filter (for example, Whatman<sup>®</sup> GF/F) on the filter base and wet with deionized or distilled water. *NOTE: Wetting the filter will help it adhere to the base in windy conditions.* Attach the filter funnel to the filter base.
3. Prior to subsample extraction, homogenize the composite sample by vigorously shaking or using a battery-powered stirrer for 30 seconds.
4. Extract one 10 mL aliquot of homogenized composite sample using a disposable serological volumetric glass pipette and dispense onto the middle of the wetted glass microfiber filter.
5. Filter the aliquot with the vacuum pump using 7 to 10 psi.
  - a. Examine the filter. An adequate amount of periphytic biomass for analysis is indicated by the green or brown color of material retained on the filter. If needed, extract additional 5 mL aliquots and filter until a green or brown color on the filter is apparent. *NOTE: For composite samples with abundant organic material and/or fine sediment, filtration of a 10 mL aliquot may not be possible. In these circumstances, filter one 5 mL aliquot. If no difficulties were apparent when filtering the first 5 mL aliquot, proceed with filtering a second 5 mL aliquot.*
  - b. The filtered aliquot(s) represent the chlorophyll  $\alpha$  subsample. Determine the number of aliquots filtered and record the chlorophyll  $\alpha$  subsample volume on the datasheet. For example, 2 aliquots x 5 mL/aliquot = 10 mL subsample volume.

- c. Rinse the sides of the filter funnel with deionized or distilled water, allow the water to be vacuumed completely before releasing the vacuum from the filtering apparatus.
  - d. Using forceps, fold the filter into quarters with the filtered biomass inside. Remove the filter from the funnel base with forceps and wrap in a small piece of aluminum foil. Place the aluminum foil wrapped filter in a separate 47 mm Petri dish.
  - e. Seal the sides of the Petri dish with plastic tape and label the Petri dish with the following required information:
    - i. Site name
    - ii. Sample ID
    - iii. Collection date (mm-dd-yyyy)
    - iv. Collection Time (24 hr.)
    - v. Composite sample volume (mL)
    - vi. Subsample volume (mL)
  - f. Repeat the aliquot extraction and filtration processes if necessary for quality control duplicates.
  - g. Insert the labeled Petri dish(s) in a resealable plastic bag and place in a cooler containing dry ice. About 4.5 kg (10 pounds) of dry ice is needed for a small cooler (< 2 gal). Insulate the cooler with newspaper to minimize sublimation of dry ice. *NOTE: Wet ice can be used if dry ice is not available. Make a note on the data sheet when wet ice is used.*
  - h. Coolers should be shipped within a few days after the subsamples have been prepared because of a 25 day holding time limit. Subsamples can be temporarily stored in a freezer (at -20°C) at the field office over weekends. Contact laboratory personnel to make them aware of plans to ship (via overnight shipping service) coolers containing dry ice and frozen subsamples. Make sure you disclose to the carrier the amount of dry ice in the cooler prior to shipping.
6. Measure the volume of the remaining composite sample (which represents the ID subsample volume) and record on the datasheet.
  7. Preserve the ID subsample with 5 to 10 percent Lugol's solution (see **Sample Preservative-Lugol's Solution** for preparation). Five percent should be sufficient for most samples, although up to 10 percent can be used for samples rich in organic matter. Record the preservative volume on the datasheet. The quantities of Lugol's solution required for selected sample volumes are:

500 mL ID subsample, add 25 mL Lugol's solution

400 mL ID subsample, add 20 mL Lugol's solution  
 250 mL ID subsample, add 12 mL Lugol's solution

8. Label the ID subsample with the following required information:
  - a. Site name
  - b. Sample ID
  - c. Collection date (mm-dd-yyyy)
  - d. Collection time (24 hr.)
  - e. ID subsample volume (mL) [ID subsample + preservative]

### Sample Preservative-Lugol's Solution

Prepare Lugol's solution by dissolving 20 g potassium iodide (KI) and 10 g iodine crystals in 200 mL distilled water containing 20 mL glacial acetic acid. Store Lugol's solution in an opaque plastic bottle.

### References

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## **Bankfull Elevation – Field Identification**

### **Introduction**

Bankfull discharge is a frequently occurring peak flow whose corresponding stage or elevation often represents the incipient point of flooding associated with a return period of 1-2 years. Bankfull elevation (and its associated discharge) serves as a consistent reference point which can be related to the formation, maintenance and dimensions of the channel as it exists under the current climatic regime. Bankfull elevation often represents the breakpoint between processes of channel and floodplain formation. Correctly identifying bankfull elevation is crucial and serves as the foundation for all subsequent geomorphic methods used in the determination of channel classification, dimension, pattern and profile.

Bankfull discharge in Wyoming generally occurs in the late spring or early summer which coincides with snow-melt or the period of frequent and/or intense precipitation events. However, bankfull discharge can conceptually occur at any time during the year. Because site visits are often not conducted during a bankfull event, bankfull indicators must be relied on to correctly identify bankfull elevation. There are several bankfull indicators though no one indicator is suitable in all circumstances.

### **Quality Control**

Appropriate use of bankfull elevation indicators requires adherence to the following principles which can also serve as quality control for this method:

- 1) Seek indicators appropriate for specific Rosgen stream types.
- 2) Know the recent flood and drought history of the area to avoid being misled by spurious indicators. This includes conducting site reconnaissance during bankfull discharge events.
- 3) Use multiple indicators wherever possible as reinforcement of a common stage or elevation.
- 4) Never identify bankfull elevation in reaches of the stream that are subject to frequent inundation caused by beaver dams, diversion structures, etc.
- 5) Bankfull elevation above and below hydrologic anomalies that influence the entire active channel such as natural controls (boulders, bedrock), headcuts, dams and similar features will likely be different. These breaks in bankfull elevation should be accounted for at all site visits.
- 6) Except in cases noted above, bankfull indicators should be at a consistent elevation along an individual stream reach.
- 7) Reachwide bankfull slope should be similar to the reachwide water surface slope, assuming both variables were measured on the same day and rapid aggradation or degradation is not occurring.

- 8) Bankfull indicators along pools, particularly along the outside of meander bends, may be at a higher elevation than indicators at riffles. However, there should still be consistency in elevation of bankfull indicators along the entire reach.
- 9) Where possible, calibrate field-determined bankfull stage elevation and corresponding bankfull channel dimensions to known recurrence interval discharges at gage stations and/or applicable regional curves.
- 10) Persistent long-term drought conditions may create a false “bankfull” elevation that does not correspond to the actual bankfull elevation under the current climatic regime. See step 9.

## **Bankfull Indicators**

Use the following common bankfull indicators to identify bankfull elevation, many of which have been adapted from Rosgen (2008). In all cases, multiple bankfull indicators should be used to identify bankfull elevation. Primary indicators should always be sought out at the site; secondary indicators should be used only as supplemental information to support primary indicators. Illustrated examples of bankfull elevation and associated bankfull indicators from Wyoming streams are shown in Appendix 1.

### Primary Indicators

- 1) Floodplains – Bankfull elevation is often associated with the point at which water begins to spread out onto the floodplain. This may or may not be the top of the bank. This is one of the best indicators of bankfull elevation for use on Rosgen C, D, DA and E stream types which often have well-developed floodplains. Floodplain indicators do not apply to entrenched Rosgen A, B, F and G stream types which generally do not have floodplains. Most streams in alluvial/colluvial valleys have three distinct terraces. Do not confuse the low terrace with the floodplain, which may be close in elevation. The low terrace is an abandoned floodplain often characterized by upland or a mixture of upland and facultative riparian vegetation.
- 2) Breaks in Slope – A change in slope from a near vertical bank to a more horizontal bank is often the best indicator of the incipient point of flooding, or the transition from the bankfull channel to a floodplain. Such changes in slope often correspond to the “bankfull bench”. However, streams that have undergone physical alterations in the past or are actively degrading or aggrading can have multiple slope breaks that represent abandoned floodplains or terraces, rather than the bankfull elevation. For incised channels with near vertical banks, the first substantial break in slope (example: transitioning from 90° to 45°) at the bottom of the near vertical bank can be the bankfull elevation.
- 3) Scour Lines – A scour line at a consistent elevation along a reach that lies below an intact soil layer can represent bankfull elevation. Scour lines may or may not have exposed root hairs.
- 4) Undercuts – On bank sections where the perennial vegetation forms a dense root mat, the upper extent or top of the undercut is normally slightly below bankfull elevation. Undercuts are best used as indicators in channels lacking obvious floodplains.

- 5) Depositional Features – The elevation on top of the highest depositional feature (point bar or mid-channel bar) within the active channel is often associated with the bankfull elevation. However, in streams that have experienced recent record flood events, the tops of the highest depositional features may be above bankfull elevation. In streams that are rapidly degrading (downcutting), the tops of the highest depositional features may also be above the bankfull elevation.
- 6) Particle Size Demarcation – The point at which there is a distinct change in particle size of the active channel bed at a consistent elevation along a reach is often associated with bankfull elevation. Changes in particle size can be from coarse to fine or from fine to coarse and may also correspond to a break in slope or the top of a depositional feature.

### Secondary Indicators

- 1) Vegetation - Using vegetation to identify bankfull elevation must be done cautiously. When vegetation is used as a sole indicator, bankfull is frequently underestimated. Common riparian species such as alder (*Alnus* sp.), dogwood (*Cornus* sp.) and redtop (*Deschampsia* sp.) can be used as supplemental indicators of bankfull elevation in Wyoming streams. Generally, bankfull elevation is located at or just under the base of riparian vegetation often associated with a scour line. Willow (*Salix* sp.) and cottonwood (*Populus* sp.) should not be used as indicators as they can colonize within the bankfull channel. Mature woody species are generally found above the bankfull elevation and should not be used. Vegetation generally is not an appropriate indicator in streams where active degradation such as bank sloughing is occurring.
- 2) Lichens or Mosses – A noticeable change in color, pattern and/or species of lichens or mosses on boulders or bedrock at a consistent elevation along a reach may represent bankfull elevation.
- 3) Debris Lines - The top of a debris line consisting of leaf and woody litter, dead algae, fecal material, trash or other floating debris at a consistent elevation along a reach may represent bankfull elevation. However, do not confuse debris deposited by flow events larger than bankfull to represent bankfull elevation.
- 4) Stain Lines – The top of a noticeable stain line on boulders or bedrock at a consistent elevation along a reach may represent bankfull elevation. Generally, staining will be of a lighter color than the substrate on which it occurs.

### **Field Procedure**

Required equipment and supplies for identification of bankfull elevation include:

<input type="checkbox"/> Calculator <input type="checkbox"/> Camera <input type="checkbox"/> Datasheet or log book	<input type="checkbox"/> Pens <input type="checkbox"/> Pin flags <input type="checkbox"/> Pocket rod with 0.1' and 0.01' increments and level
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- 1) Select a representative reach of a wadeable stream, generally at least two meander cycles or 20 bankfull widths in length, to identify bankfull indicators.
- 2) Determine whether hydrologic anomalies such as natural controls (boulders, bedrock), headcuts, dams and similar features exist in the reach and account for their influence on bankfull elevation accordingly.

- 3) Using the bankfull indicators described above, walk the entire length of the reach, multiple times if needed, and identify primary and secondary bankfull indicators where applicable. Care should be taken to use only the best bankfull indicators that provide the strongest evidence of bankfull elevation.
- 4) Mark the locations of both primary and secondary bankfull indicators with pin flags.
- 5) Use a pocket rod or survey rod to measure the distance from the current water surface to the estimated bankfull elevation at each of the best bankfull indicators. Bankfull indicators should follow a generally consistent elevation throughout the reach. Distances from the current water surface to the estimated bankfull elevation should be similar among all measurements. Outlying distances will be evident and should be removed or revisited and verified.
- 6) Use a weighted (primary indicators have greater weight than secondary indicators) average distance between water surface and bankfull elevation as a reference point when conducting subsequent geomorphic survey procedures such as cross-sections and longitudinal profiles on the same day the average value was measured.
- 7) If desired for future reference, photo document the location of the bankfull elevation using the pin flags as reference points, making sure the entire bankfull channel is visible in the photograph. If a measurement tape has been stretched longitudinally along the entire reach, record the distance along the tape where the bankfull indicator in the photograph is located.

## References

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Figure 1 – Bankfull elevation associated with a defined floodplain and a break in slope or bankfull bench.

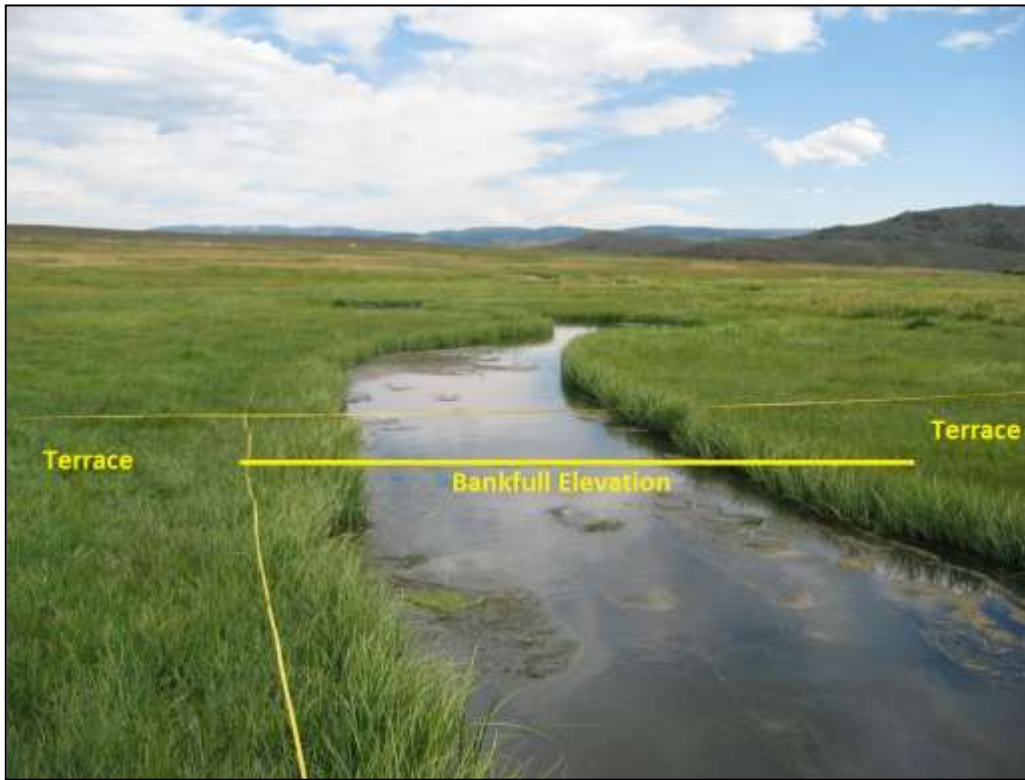


Figure 2 – Bankfull elevation associated with a defined floodplain and a break in slope or bankfull bench.

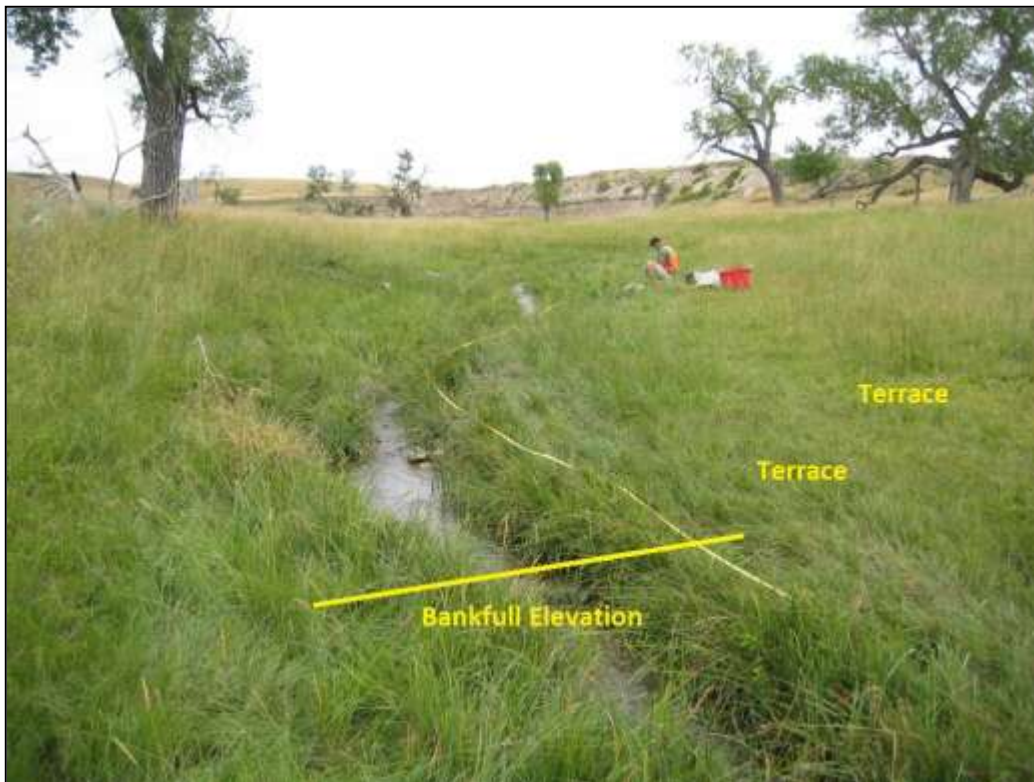


Figure 3 – Bankfull elevation associated with a break in slope, top of a point bar, intermittent scour lines and the base of redtop (*Deschampsia* sp.).



Figure 4 – Bankfull elevation associated with a break in slope, top of a point bar, a subtle floodplain, intermittent scour lines and the base of redtop (*Deschampsia* sp.).



Figure 5 – Bankfull elevation associated with the top of a point bar and a break in slope at the bottom of a vertical bank with a scour line.



Figure 6 – Bankfull elevation associated with the top of a stain line on bedrock.



Figure 7 – Bankfull elevation associated with a break in slope or bankfull bench and the base of alder (*Alnus* sp.).



Figure 8 – Bankfull elevation associated with a break in slope or bankfull bench, intermittent scour lines and the base of alder (*Alnus* sp.).



Figure 9 – Bankfull elevation associated with the top of a depositional feature, a break in slope and a bankfull bench.



Figure 10 – Bankfull elevation associated with the top of a point bar, a break in slope at the base of the vertical portion of the opposite bank, and complimentary scour lines.

