# Stream Algae, Invertebrate, and Environmental Sampling Associated with Biological Water Quality Assessments

# Field Protocols

Charles Hawkins[1], Jeff Ostermiller[1], Mark Vinson[2],
R. Jan Stevenson[3], and John Olson[1]

[1]Department of Aquatic, Watershed, & Earth Resources
Western Center for Monitoring and Assessment of Freshwater Ecosystems
[2] BLM National Aquatic Monitoring Center
Utah State University
Logan, Utah 84322-5210

[3]Department of Zoology
Michigan State University
East Lansing, Michigan 48824-1115

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# Preface

The sampling procedures described in this document were designed to provide data sufficient to characterize the algal and invertebrate assemblages for the purpose of biological assessment. In addition to describing methods for sampling algae and invertebrates, we also describe methods for characterizing the main chemical and physical conditions occurring at a stream site. We designed these procedures with an eye toward standardizing sampling methods used in the biological assessments of stream water quality. The invertebrate protocols were modified from the designs used by the States of Oregon and Washington and the BLM's National Aquatic Monitoring Center. The algae sampling protocol was modified from EPA Rapid Biological Assessment procedures. Procedures for collection of reach and site environmental data were modified from a variety of sources. These procedures produce biological data that are comparable with data derived from EPA's Western Pilot Stream Project. These collection procedures allow analysis of biotic data with either multimetric (e.g., B-IBI) or predictive model (e.g., RIVPACS) methods. Use of these procedures will allow users to share data, express their data in terms of standardized bioassessment measures, and thus directly compare their results with all other parties using these methods. As such, these sampling methods provide a foundation on which to establish region-wide monitoring and assessment programs for quantifying stream resource condition. Their general use does not preclude use of other sampling procedures that may be needed to address the objectives of specific projects.

# Sequence Of Data Collection

Data collection at each site consists of 12 steps summarized here. Separate sections describe details of the procedures used.

1. Check field equipment against list before leaving (page 3).

2. Read General Notes (page 5).

3. Complete the headers of the Site Data Form and copy this information to the Field Notebook (page 6).

4. Conduct site evaluation (pages 6 - 8).

5. Measure initial temperature, conductivity, and alkalinity (pages 9).

6. Collect water chemistry samples (pages 9 - 10).

7. Collect algae sample (pages 11 - 13).

8. Sample invertebrates (pages 13 - 15).

9. Conduct site measurements - physical features and plant cover (pages 16 - 18).

10. Characterize the channel type (page 19).

11. Record an ending time and temperature.

12. Mark the location of the study site on a map.

# Field Equipment Check List

General:

Field data forms, field notebooks (Rite-in-the-Rain™ paper).

Aluminum clipboard (Tatum).

GPS to identify geographic location.

Black medium-tip permanent marker (e.g., Sharpee®).

Pencils (#2 lead).

Wading shoes/boots with waders where needed.

Pump sprayer and wash bottles for washing insects and algae off of rocks, pans, and nets.

7.5' USGS Quadrangle Maps or other maps describing the study area.

Camera.

Photographic film (number canisters prior to leaving for field).

Water Chemistry and Temperature:

Thermometers (2).

Conductivity meter.

Field alkalinity test kit.

Two (2) 125 ml plastic collection bottles (acid washed) marked for:

Filtered (Frozen) and Unfiltered ($H_2SO_4$).

One (1) 50 mL acid washed sample tube marked for Filtered ($H_2SO_4$).

One (1) 15 mL vial with 0.5 mL of trace metal or optima grade nitric acid.

One (1) new 3 mL syringe in wrapper.

Two (2) 0.5 mL ampules of 96% $H_2SO_4$ (Sulphuric Acid).

One (1) 60 mL syringe.

Ten (10) disposable Millipore filter units for syringes.

Label tape for outside of bottles.

One (1) ~2 L jug of Deionized Water for blanks

One (1) vial of prepared nutrient spike

Algae:

Rubber sampling strap with $12cm^2$ (4cm diameter) hole for delineating the surface area of rocks to be sampled.

Petri dish and spatula without slits or holes (for samples on soft-bottomed sediment).

PVC pipe for samples on large substratum (12 – 16 cm length, 4 cm diameter) fitted with a rubber collar at one end with pump and collection vial.

Stainless steel spoon, screw driver, toothbrush, and nailbrush for scraping algae.

Turkey baster.

One 1L Nalgene jar for mixing composite samples permanently marked at 50 mL intervals.

One 50 ml sample container marked at 40mL.

Labeling materials (preprinted Rite-in-the-Rain™ labels).

10% formalin.

Invertebrates:

10-sided die for generating random sample locations.

0.09 $m^2$ Surber or D-frame sampler with 500 µm mesh net (net should be ~1 m long net to prevent backwashing.

500 µm brass sieve with protective cover and bottom pan.

14 liter bucket.

White plastic wash tub.

Ten (10) 500 ml plastic sample jars.

Small spoons (2) for transferring samples to jars.

Forceps (2 pairs).

Labeling materials (preprinted Rite-in-the-Rain™ labels).

Buffered ($CaCO_3$) Formalin or 95% EtOH.

Channel:

100 m tape.

Depth measuring stick in 5 cm increments.

Gravelometer or ruler to measure substrate size.

Angular densiometer to measure channel shading.

Clinometer for measuring stream slope.

Dye for measuring current speed and 500 mL bottle for mixing.

Stop watch for measuring current speed.

# General Notes

IMPORTANT - DO NOT LEAVE THE SITE UNTIL EVERY FIELD OF THE SITE DATA FORM HAS AN ENTRY!

FAILURE TO FILL OUT A SITE DATA FORM COMPLETELY MEANS THE TRIP TO THE SITE WAS WASTED AT A COST OF SEVERAL HUNDRED DOLLARS.

When recording data and writing notes, be neat and legible!

Include all requisite information. If a situation occurs that does not seem to fit within the protocol, discuss the problem with the rest of the crew. Make a note in the field notebook and under the comments section of the site data form describing the issue.

Be consistent. Fatigue, boredom, time constraints, and many other factors can affect the quality of information recorded in the field. If you believe anything is causing a change in the way the data are being collected, discuss the situation with the other crew members and make a decision together of how to remedy the problem.

Important: Rivers can be noisy places and it is easy to misunderstand what is being said. When recording data, the recorder should always repeat the information and the collector should verbally (and loudly!) confirm what was said (or correct it), and both people must agree before the information is recorded.

Recording Information

Information collected at each site should be recorded on the following forms:

- Site Data Form - for scoring reach condition and recording site measurements.
- Site Summary Notebook - for listing site names, ID numbers, and associated photographs, drawing site maps and recording general comments.

Definition of Terms

| | |
|---|---|
| Study Site | The section of stream beginning at the bottom of the first habitat unit sampled and ending at the top of the last habitat unit sampled. |
| Habitat Unit | An area of stream that differs in flow and morphological characteristics from adjoining areas of the stream, for example, a riffle bordered by a pool upstream and a run downstream. |
| Riparian Area | The area from the top of the bank to the point where there is a distinctive change in upland vegetation or hillslope. If there is no clear transition, consider the area within 10 - 15 m of the bank when answering riparian related questions. |

# Header Information

The following information should be included in the Site Summary Notebook and on the Site Data Form (see Appendix). Site Name, Site ID number, and Photographic Documentation information should be recorded in the Site Summary Notebook.

| | |
|---|---|
| Site Name | Record the full name of the stream. Include the river or creek designation. If an unnamed tributary is sampled, record the name of larger stream and indicate that it was a tributary (e.g. West Tributary of Fish Creek). Be sure the stream is marked on the topographic (or other) map so its location can be confirmed later. |
| Site Code | Assign a unique site ID number to each site sampled. Site numbers are recorded on the Site Data Form, Site Summary Notebook, and the Water Chemistry, Algae, and Invertebrate Sample Labels. |
| Date | Record as day, month (spell it out), year. (e.g. 04 July 1998) |
| Crew | Record the crew's name or code. |
| Vehicle | Record the license plate of the field vehicle and its general description (e.g., 12345EX, Blue Explorer) |
| Time and Temperature Measurements | The first and last pieces of information collected at each site are the temperature (°C) and time (military time). Record temperature to $0.5^{0}$ precision. |
| Location | Record longitude and latitude from the GPS for each site (middle of study section). Record in decimal degrees using the NAD83 datum. Record elevation from a 7.5' topographic map and/or the GPS. |
| Land Owner | Record the name of the land owner (Forest, Park, State, Private, etc.). |
| Crew Members | Record the full names and responsibilities of each crew member at every site. The Collector is the person who takes the samples. The Recorder is the person who writes the information into the field data form and notebooks. Crew members should alternate being collector and recorder throughout the field season. |

# Site Evaluations

Site evaluations will be used to help determine the suitability of Reference Sites and the degree or type of degradation occurring within Test sites. Upon arrival at the Study Site, walk a 300 m section of the stream and score the Site Evaluation questions, identify the type of reach, and determine management activities. Portions of this evaluation were adapted from Montana Riparian and Wetland Research Program Protocols - http://www.rwrp.umt.edu/findb.html).

Site condition is assessed by scoring each of the 6 attributes as outlined below. These assessments should be completed independently by each crew member and the average reported. If scores differ substantially, reconcile your assessment and arrive at a consensus.

1. Vegetative Cover - Describe the % of the riparian area covered by mature perennial plants (tress, shrubs, or grass). Do not include the active channel in this assessment. Do not consider the 'quality' of the vegetation, e.g., native versus exotic or healthy versus stressed.

   4 = > 95%

   3 = 85 - 95%

   2 = 75 - 85%.

   1 = < 75%

2. Erosional deposition into stream from surrounding hillslopes - Scan the hillsides on both sides of the stream for evidence of active erosion. Do not include streambanks in this assessment.

   4 = No erosional deposition is apparent.

   3 = Some signs of erosional deposition are apparent, but these areas are confined to specific, limited locales along the stream (e.g., gulleys, washes, slumps, roads).

   2 = Obvious signs of erosional deposition from the hillslopes are apparent.

   1 = Mass wasting is evident on hillslopes. Stream deposition is significant enough to cause obvious changes in stream flow (e.g., debris avalanche, torrent tracks).

3. Consumption of trees & shrubs in the riparian area and on banks by livestock - Estimate the average amount of vegetation that has been consumed in the study reach.

   4 = 0 - 5%

   3 = 5 - 25%

   2 = 25 - 50%

   1 = > 50%

4. Stream incisement - Estimate the degree of vertical incisement for the channel in the study reach. Incisement refers to the amount of vertical drop from the top of the bank to the stream channel. Incised channels have rectangular cross-sectional profiles.

   4 = No incisement.

   3 = Old incisement, i.e., banks still relatively high above channel, but banks are no longer vertical.

   2 = Deep incisement with new floodplain development, i.e., tops of incised banks have laterally eroded to produce a small floodplain, which may be bounded by old banks.

   1 = Deep incisement with active downcutting.

5. Percent of stream with active lateral cutting - Lateral cutting refers to bank erosion in which the stream is actively eroding the side of a bank. Lateral erosion is evident by the presence of bare soil or rock. Do not count deeply undercut, but stable, banks as active lateral cutting.

   4 = 5% or less of the streambank shows active lateral cutting.

   3 = 5 - 15% of the streambank shows active lateral cutting.

   2 = 15 - 35% of the streambank shows active lateral cutting.

   1 = > 35% of the streambank shows active lateral cutting.

6. Percent of streambank with deep, binding root mass - Root masses will be typically associated with trees and shrubs but may include roots of some grasses and other vegetation.

4 = > 85% of the bank with deep, binding root mass.

3 = 65 - 85% of the bank with deep, binding root mass.

2 = 35 - 64% of the bank with deep, binding root mass.

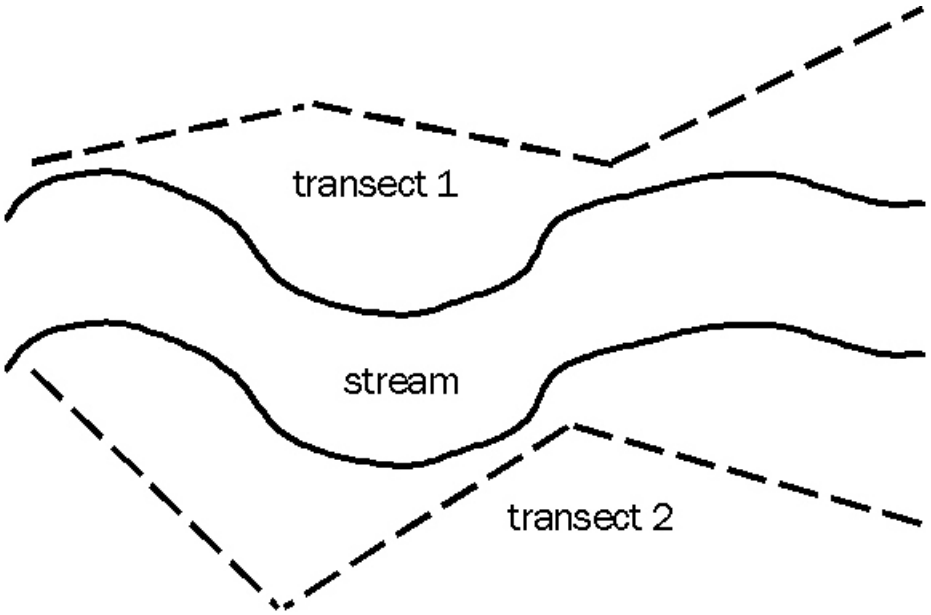
1 = < 35% of the bank with deep, binding root mass.

Management Activities

Rank all of the management activities, as described on the site data form, that occur along the Study Site according to their relative impact on the stream system. A ranking of 1 represents the most significant impact. Tie scores are possible. Also note any specific impacts to the stream system.

Livestock Use Index

We calculate a livestock use index by counting the number of cow and sheep droppings (feces) observed along two 75m, zig-zag transects, 1 located on each side of the stream. Start each transect at the lower end of the study area. Each transect should zig-zag 3 times over the 75m total length. Start at stream's edge and locate a landmark ~ 25m away and angling at ~ 30-45° away from the stream. Walk directly toward the landmark and count all cattle and sheep droppings within 1m on either side of you (i.e., a 2m wide band). Keep counts for cattle and sheep separate. Keep counts of 'new' and 'old' droppings separate as well. New droppings cannot be turned over intact with a stick; old droppings can. Use a 25m tape or string to locate the end of the first 25m part of the transect. After completing the first 25m of the transect, locate another landmark ~ 25 meter away back toward the stream channel. Count droppings along this transect. The 3rd portion of the transect should angle away from the stream again. Repeat this procedure on the other side of the stream.



# Temperature, Conductivity, and Alkalinity

Temperature – Place the thermometer into the main flow. After 10 seconds, read and record the temperature in degrees Celsius to the nearest $0.5^0$.

Conductivity – Turn on the conductivity meter and place the probe into the main flow. When the reading stabilizes, record the conductivity as micro-Siemens / $cm^2$ ($\mu S/cm^2$). Note that you may need to change the sensitivity settings on the meter to obtain accurate measurements, but record all values in common ($\mu S/cm^2$) units (not $mS/cm^2$).

Alkalinity – Use the test kit to determine both the phenophalain and total alkalinity of the water. Record values as mg/L (= ppm) $CaCO_3$.

# Water Chemistry Samples

Note: This procedure requires that you handle and add acid to water samples. Be extremely careful when pipetting and breaking the acid ampules into the bottles. Wear safety goggles and gloves, and have water readily available for rinse any acid spilled on you. The nutrient samples must be frozen when stored or transported, so this requires using dry ice. Because dry ice sublimates CO2, ensure that the cooler chest can vent so pressure doesn't build up. Because CO2 can become poisonous, be sure the car is well ventilated. Do not allow bare skin to contact the dry ice as this will cause frostbite.

Also, be extremely careful that you treat and label the water samples exactly as described below. Do not breathe into the bottles. Do not place your fingers into the water in the bottles. Do not allow anything to fall into the bottles. Do not put labels inside of these bottles. Contaminated samples or mislabeled samples will result in unusable data.

Choose an area to take the chemistry samples that is up stream of both your sampling reach and any disturbance you may have caused in the stream. The sampling area should be an area where the water is well mixed (e.g. at a riffle or a run, not a pool or a slow moving lateral margin).

Rinse the first empty 250 mL bottle three times, filling it with 10-20 mL of water by submerging in the stream, and then capping, shaking, and emptying. Then fill the bottle a fourth time leaving ~ 5 mL extra space. Carefully break an $H_2SO_4$ ampule into the bottle, cap, and shake well. Label the outside of this bottle as "Unfiltered/$H_2SO_4$" using a permanent marker.

Rinse the 50 mL syringe three times with stream water by filling completely and emptying downstream of your sample area. Then, fill the syringe with stream water and place a filter on the end of the syringe. Do not let anything touch the end of the filter at anytime. Rinse all filters by passing 10-20 mL through them before rinsing bottles or collecting water for samples.

Rinse the 50 mL tube three times by pushing 10-20 mL of water through the filter, and then capping, shaking, and emptying the tube. Once the syringe is empty, remove the filter

(placing it back in its package to keep it clean), refill the syringe with stream water, and reattach the filter without touching the end. After the tube has been rinsed three times, begin to push water through the filter to fill the tube, refilling the syringe as necessary, until the tube is nearly full. Carefully break an $H_2SO_4$ ampule into the tube, cap, and shake well. Label the outside of this tube as "Filtered/$H_2SO_4$".

Rinse the second empty 250 mL bottle three times with filtered water using the same procedure as for the 50 mL tube. Again, fill the bottle by pushing water through the filter until only 10-20 mL of space remains. Do not add anything to this bottle and label “Filtered/Frozen". Do not leave this bottle in sunlight for extended periods (hours). Upon returning to the vehicle, place this sample on dry ice and keep frozen until delivered to lab.

Remove a 15 mL vial with 0.5 mL $HNO_3$ from the 250 mL Nalgene bottle used to transport the vials. Fill a new (wrapped) 3 mL (3cc) syringe with stream water. Attach a filter to the end of the syringe and rinse the filter by pushing water through it until only 0.5 mL remains. Place this remaining 0.5 mL in the vial with the acid. Take the filter off, refill the syringe, replace the filter, and filter the entire 3 mL into the vial. Repeat this procedure twice more until you have filtered a total of 9.5 mL of stream water into the vial bringing the total volume in the vial to 10 mL (water + acid). Ensure all volume measurements are as accurate as possible. Cap the vial and shake it well. Label the outside of this bottle as "Filtered/$HNO_3$", including .

Place the used acid ampules in a secure container with hard water.

If you mess up a sample, start over. It is better to take some extra time than to leave the site with samples of uncertain quality. Once you secure all four samples, tape the lids to ensure that the caps will not loosen and allow the water samples to leak. Label each bottle/tube with the site number, site name, date, wether it is filtered or unfiltered and how it is preserved ($HNO_3$, $H_2SO_4$, or frozen). Cover each label with clear packing tape to prevent labels from rubbing off.

Field blanks. At every 10th station, collect a set of field blank water samples. These will be used to measure the amount of contamination that occurs in the field, in transport and in the lab. Use Deionized water (DI) as you would stream water in the process above. Using a small amount of DI water from a jug stored for the trip, rinse the four bottles/tube, the syringe, and the filter. Keep the jug of DI away from areas of blowing dust or leaves to prevent the DI from becoming contaminated. Fill one bottle with unfiltered water. Fill the other two bottles and the tube with filtered DI water. Preserved the unfiltered water with $H_2SO_4$ as above and label the outside of the bottle "Unfiltered/$H_2SO_4$". Preserve the filtered water in the tube with $H_2SO_4$ and label “Filtered/$H_2SO_4$. Place the other bottle on dry ice and keep frozen and label the outside of the bottle "Filtered/Frozen". Empty the water filled bottle, rinse, fill and preserve with $HNO_3$ as above and label the outside of the bottle "Filtered /$HNO_3$". All bottles/tubes are labeled with their own site number (in sequence), site name, and date. Record the site number and name in the field notebook, noting that these were blanks, and record that field blanks were collected on the data sheet.

Field Spikes. Starting on the first day, and then every four day in the field afterward, create a field spike sample so any degradation of nutrients can be measured. Prepare a single bottle

of filtered water as for field blanks, add spike, and freeze on dry ice. Label with date and "Field Spike".

## Algae (Periphyton)

The following procedures outline a stepwise approach for collecting periphyton samples from each of the 4 fast-water habitat units established for the reach.

Based on the type of substrate available, select one sampling method for the reach. The method should be selected based on the following priority: rock, bedrock/boulder, snag, sand, silt.

Rocks – Collect 4 pieces of substrate (5 – 25 cm diameter) from each of the 4 fast-water habitat units (i.e. riffles) for a total of 16 samples. When collecting from each habitat unit, visually bisect the unit both longitudinally and laterally to create 4 equal-area rectangles. Randomly select a particle from each of the rectangular quadrants. Make sure to keep track of the upper surface of each piece of substratum. Where possible, all of these samples should be taken from a depth of approximately 15 - 20 cm (if the stream is <15cm deep sample at the deepest point). If the substrates of the required size do not occur in all 4 habitat units, collect substrates from those habitat units that do have suitable substrate. If substrate within the 5 - 25 cm diameter size-classes is unavailable, refer to the procedures for sampling bedrock/boulder, snag, or loose sediments below.

Prior to collecting substrates, thoroughly rinse the pan, brush, and spatula to remove any traces of algae from previously visited sites. One-at-a-time, place each piece of substratum over the white pan. Wrap the rubber sampling strap around a rock so that the opening is not stretched and is placed over the top surface of the rock. The opening in the strap delineates an area on the upper surface of each piece of substratum that should be scraped, brushed and then rinsed into the white pan. After removing any conspicuous caddis cases, snails, or other attached invertebrates, scrape loose algae from the delineated area on the rock with a small spatula. Then brush the area (brushing dense periphyton clogs up the brush). If the substrate is soft or if the surface of the rock is travertine (precipitated $CaCO_3$), do not scrape or brush more than 0.5mm into the rock surface. Rinse the material into the white pan, but be careful to only rinse algae from the delineated area into the pan (not from other parts of the rock). Repeat for each piece of substratum rinsing the material into the pan to create 1 composite sample consisting of all 16 samples. Rinse the material from the white pan into the 1L Nalgene bottle.

Carefully examine all of the tools used for dislodging the periphyton and rinse any remaining material into the composite sample using a minimal amount of stream water.

Dilute the composite sample to the nearest 50ml increment (use the smallest possible level). Record the level of dilution on the data sheet, the interior sample label, and the exterior of the sample container.

Close the 1L bottle containing the composite sample, shake vigorously until all material is fully suspended and homogenized (clumps of algae broken up). Use the turkey baster to continue mixing and extract ~10 mL and place in the 50 mL periphyton sample container. Extract additional aliquots until 40 mL has been transferred to the sample container (as indicated by the 40 mL mark on the side of the container). Preserve with 2mL of 10% formalin.

Label these samples as "Rock" (inside and outside) and seal the cap with tape. Make sure that the date, stream name, and identification number are identical to what was recorded on the data sheets and on the macroinvertebrate samples.

**Periphyton Sample**

Date: 5 July 1998
Site ID #: PNW98 - 046
Site Name: Fun Creek
Sample Type: Rock

To avoid contamination of subsequent samples, thoroughly rinse all of the sampling tools before leaving the field.

Sampling from Unusual Substrates

The following sampling procedures should only be used in situations where substrate sizes of 5 - 25 cm diameter are unavailable.

Bedrock/Boulders (Large, Unremoveable Substrates) – Place the PVC pipe with the rubber collar over a piece of substratum that is submerged at a depth of approximately 15 - 20 cm (if the stream is <15cm deep sample at the deepest point) and press down with sufficient pressure to create a seal. Dislodge the algae located within the pipe with a screwdriver. Place the rubber stopper, hoses, and vacuum pump assembly on the PVC pipe. Pump the algae-water solution from the pipe into the attached sampling container. Pour that sample into the 1L Nalgene sample homogenization jar.

Repeat these steps for 16 total substrate surfaces (4 from each habitat unit) and combine all samples in the same 1L jar to create a composite sample.

Remove, preserve and label a 40 mL subsample from the 1L composite sample container by following steps outlined in the sampling protocols for "rocks".

Record "Bedrock/Boulder Sample" on the sample jar labels and within the field notes.

Snags – Remove small logs or branches that have been in the water for long times (months) and scrape periphyton from a known area into the white pan. Use the small spatula, toothbrush and squirt bottle to remove algae from the snags and rinse them into the white pan. Use the rubber sampling strap to delineate an area on the snag (or measure the area with a tape measure) from which periphyton are scraped from each snag. Mark down the total area on the sample labels and all areas in the field notebook. Sample 16 snags. If 16 different snags are not available, then take multiple samples from the larger logs, but not more than 3 from any given snag.

Rinse sample from the white pan to the 1 L Nalgene bottle.

Remove, preserve and label a subsample (40mL) from the 1L composite sample container by following steps outlined in the sampling protocols for “rocks.”

Record “Snag Sample,” sample volume, and sample area on the sample jar labels and within the field notes.

Gravel and Sand – Invert the petri dish over a portion of sediments submerged at a depth of approximately 15-20cm (if the stream is <15cm deep sample at the deepest point) and trap the sediments by inserting the spatula under the dish. Transfer the sample to the 1L Nalgene sample homogenization container. Repeat this step for 16 samples, 4 from each habitat in the reach.

Pour a small amount (~20 - 30 mL) of stream water over the gravel and sand in the 1L Nalgene bottle, cover, and shake and swirl vigorously to remove algae from gravel and sands. Allow 10 seconds for sands and gravels to settle and pour algal-water suspension into the white pan. Repeat this step eight times so that algae is removed from the gravel and sands and rinsed into the white pan. Rinse the gravel and sand out of the 1L Nalgene sample homogenization container. Rinse that container with stream water. Then pour the algal-water suspension from the white pan into the 1L Nalgene container.

Remove, preserve and label a 40mL subsample from the 1L composite sample container by following the steps outlined in the sampling protocols for “rocks.”

Record “Sand Sample”, sample volume, and sample area on the sample jar labels and within the field notes.

Silt and Fine Loose Sediments – Invert the petri dish over a portion of sediments submerged at a depth of approximately 15-20cm (if the stream is <15cm deep sample at the deepest point) and trap the sediments by inserting the spatula under the dish.

Holding the spatula with the fines trapped under the dish, rinse all the sediments within the dish into the 1L Nalgene sample homogenization container. Repeat above steps to collect 16 total sediment samples within the reach.

Remove, preserve and label a 40mL subsample from the 1L composite sample container by following steps outlined in the sampling protocols for “rocks.”

Record “Sediment Sample,” sample volume, and sample area on the sample jar labels and within the field notes.

## Invertebrate Sampling

We describe 2 different types of sampling, the choice of which will depend on specific project goals and objectives. In general, we recommend use of a 0.72 $m^2$ fixed-area sample from a standard riffle or run habitat. Alternatively, some projects might be best served by collecting a 10-minute, fixed-time sample from multiple habitats (the secondary sample). In both cases, a 500 µm mesh net should be used to collect the samples.

Fixed-Area Sample – This sample should be taken from 4 different fast-water (e.g. riffles,

runs) habitat units. Two separate 0.09 $m^2$ fixed-area collections should be taken from each unit for a total of 8 collections. If no fast-water habitats occur, take the 8 collections from shallow, slow-water habitat units. The 8 individual collections should be composited into a single sample that will be used to represent the study area. This composite sample should be preserved in 1 or more sample jars depending on the amount of material collected.

*Sampling Locations for Fixed-Area Samples* – Sampling should begin at the first fast-water habitat encountered at the site and continue upstream with the next 3 fast-water habitat units. Determine net placement within each habitat unit by generating 2 pairs of random numbers between 0 and 9. The first number in each pair (multiplied by 10) represents the percent upstream along the habitat unit's length. The second number in each pair represents the percent of the stream's width from bank left. Repeat this process to locate the second sampling location. Take samples where the length and width distances intersect (estimate by eye). If it is not possible to take a sample at one or both of these locations (log in the way, too deep, cannot seal bottom of net, etc.), draw additional random numbers until you can.

*Sampling Locations for Deep or Fast Streams* – If the stream or individual habitat unit is too deep to sample without snorkeling, or the current is too fast to maintain stable footing, then samples should be taken from areas near the bank. If practical, sample both banks. Delimit habitat units as above, unless channel morphology is homogeneous (e.g., one unit > 100m long). In this case, define 4 sample areas as contiguous 25m sections of bank. Chose random sample locations based on the dice as above but consider the % width coordinate as the % distance away from the bank of that area that can be sampled. Usually the 50% width coordinate will be approximately knee deep. Record approximate widths from bank for each habitat unit on the map that is sketched in the field note book.

*Taking the Samples* – Place the sampler so the mouth of the net is perpendicular to and facing into the flow of water. If there is no detectable flow, orient the net to most easily facilitate washing benthic material into the net. Collect invertebrates from within the 0.09 $m^2$ sampling frame in front of the net. If no sampling frame is used, visually imagine the square sampling plot in front of the net and restrict your sampling to within that area. Work from the upstream edge of the sampling plot backward and carefully pick up and rub stones directly in front of the net to remove attached animals. Quickly inspect each stone to make sure you have dislodged everything and then set it aside. If a rock is lodged in the stream bottom, rub it a few times concentrating on any cracks or indentations. After removing all large stones, disturb small substrates (i.e. sand or gravel) to a depth of about 10 cm by raking and stirring with your hands. Continue this process until you can see no additional animals or organic matter being washed into the net. After completing the sample, hold the net vertically (cup down!) and rinse material to the bottom of the net. If a substantial amount of material is in the net, empty the net into the 14 liter bucket for processing before continuing to the next sample location. Otherwise, move to the next sample location and repeat the above procedure.

*Fixed-Time Sample* – This sample consists of a 10-minute, fixed-time sample taken from the different habitat types in the reach. In cases where the 4 habitat units are homogeneous and contiguous, but other habitats occur immediately up or down stream, these habitats can be included in the timed sample. Visually appraise the area and estimate the proportion of

different habitat types. The amount of time spent sampling each habitat type should be allocated in proportion to the occurrence of each habitat type in the study reach. Do not sample more or less than 10 minutes. This sample will be preserved in a separate jar or jars.

Field Processing, Preservation, and Labeling of the Sample

Field processing requires a 14-liter bucket, a white plastic wash tub, and a 500 µm sieve. The bucket will be used for decanting animals from inorganic substrates into the sieve. The wash tub will be used to transfer stream water to the bucket and then to visually inspect inorganic residue for heavy animals that were not decanted.

After taking a sample, empty the net's contents into the 14 liter bucket. If the net has a cup at the end, remove the cup over the top of the bucket and wash it out. The crew member not taking the sample should then begin processing the sample while other samples are being taken. Add water to the bucket with the sample and decant invertebrates and organic matter from the sample by mixing the contents of the bucket with your hand and then pouring suspended material through the 500 µm sieve. Repeat this process until no additional material can be decanted. Transfer the material in the sieve (invertebrates and organic matter) into the sample jar(s) with a small spoon and then wash any remaining material in the sieve into the jar with a wash bottle. Place the inorganic residue remaining in the bucket into the plastic wash tub and cover with water to about 1 cm in depth. Inspect the gravel on the bottom of the tub for any cased caddisflies or other animals that might remain. Remove any remaining animals by hand and place in the sample jar. Once the tub has been picked by one crew member, it should be checked by the other member and then the gravel discarded. Once the last of the 8 constant-area samples have been taken, make sure you thoroughly wash any remaining animals from the net by vigorously pouring water down the sides of the net and into the cup. You may also need to use the pump sprayer or your fingers to dislodge tenacious clingers. Use this same procedure at the end of processing the fixed-time sample.

Once the samples have been processed, add enough water to the 2 plastic sampling jars to completely cover the contents and then preserve the samples with 70-80 ml of buffered formalin. Immediately label the jars both INSIDE with a paper label and OUTSIDE on both the lid and bottle with a Sharpie permanent marker. The following is an example of properly completed labels:

Inside Label

**Invertebrate Sample**

**Date:** 5 July 1998
**Site ID #:** PNW98 - 046
Site Name: Fun Creek
**Sample Type:** **Fixed-Area** **10-minute**
Jar # 1 of 3

Outside Label

5 July 1998
PNW98 - 046
Fun Creek
Fixed-Area Sample
Jar # 1 of 3

# Site Measurements

Stream Slope – Use the clinometer to measure stream slope. If possible, take 1 reading from the top to the bottom of the study site. You will need your partner to stand at the other end of the study site. Both observer and partner should stand at waters edge. Estimate percent slope by aligning the cross hair of the clinometer with a point on your partner equal in height to your eye and reading the right-hand scale on the clinometer. If the stream characteristics make a top to bottom reading impossible, break the study site into 2 or more equal length sections and take separate readings. Average these readings to characterize the entire study site. Record the clinometer reading to the nearest 0.5%.

Stream Travel Time (current speed) – Mark off a 50m section of stream (25m if the stream current is very slow). Place ~ 1 ml of flouroscene dye in a 500 mL bottle. Fill with water, cap, and shake well. One person will release the dye in the thalweg of the stream at the zero (0) meter mark (upstream) and the other person will record the time it takes the leading and trailing edges of the dye plume to reach the 50 m mark. Note that the trailing edge is defined as the last portion of visible dye within the thalweg (main current) of the stream. Pockets of dye may persist in backwaters, which we will not include in our estimates.

Dominant Fast-Water Habitat Type - Identify the dominant fast-water habitat type for the study site. These criteria refer to conditions at base flow.

| Name | Code | Description | Name | Code | Description |
|---|---|---|---|---|---|
| Rapid | RA | Gradient >4% with swiftly flowing water and considerable surface turbulence. Rapids tend to have larger substrate sizes than riffles. | Run | RU | Runs are long, usually straight, low-gradient stream units without flow obstructions. The stream bottom is usually even and the water does not “pool”. |
| Riffle | RI | Gradient <4% and shallow with moderate velocity and moderate surface turbulence. | Step-Run | SR | Stepruns are a series of runs that are separated by short (<3m) riffles or flow obstructions. |

Dominant Slow-Water Habitat Type - Identify the dominant slow-water habitat type for the study site. These criteria refer to conditions at base flow. Note that these habitat units are formed by scour processes that occur during periods of high discharge.

| Name | Code | Description | Name | Code | Description |
|---|---|---|---|---|---|
| Lateral | LAT | As streams go around bends they scour laterally and lateral scour pools are created along the banks. The deepest portion is the stream margin. | Plunge | PLU | Plunge pools are formed as water drops over an object. The deepest portion of the pool is directly underneath the falling point. |
| Scour | SCO | Pool shape is often like a bowl. The deepest part of pool is in the center of the channel. | Dammed | DAM | Water backs up against an obstruction in the stream channel. The deepest portion will be against the object forming the obstruction. |

Channel Shade - Take four densiometer readings from the center of each of the 4 habitat units sampled for invertebrates. Readings should be taken facing upstream, downstream, bank left, and bank right (determined looking downstream). For each reading, place the densiometer near the surface of the stream and level it before taking a reading. Estimate shading by assigning 0 -4 points to each square on the densiometer grid and summing across grid squares. Points are assigned based on the percent of each square containing a shade object: 0 for no objects, 1 for 25% cover, 2 for 50% cover, 3 for 75% cover, and 4 for 100% cover.

Width - Starting at the downstream edge of the first fast-water habitat unit, measure wetted stream width at 10 different transects located at approximately 10 m intervals. Record to the nearest 5 centimeters.

Depth - Take 3 depth measurements at 1/4, 1/2, and 3/4 width intervals along each of the width transects. Record the resulting 30 measurements to the nearest centimeter.

Substrate - Substrate size is measured in each habitat unit.

*Visual estimate — Note this method is only being used for evaluation purposes at this time. Ignore unless participating in a project designed to compare visual estimates with quantitative measures.* Each crew member should independently estimate the median substrate size present in the 4 sampled riffles by picking up one piece of substratum that you think represents the median size. Measure the particle with the gravelometer and assign it to one of the following categories. Each crew member should also estimate the % of substrate particles in each of the substrate categories below. Base these estimates on the entire reach.

| | | | | | |
|---|---|---|---|---|---|
| Bedrock | BED | Solid rock forming a continuous surface. | Gravel | GR | Mix of rounded course material from 2-16 mm in diameter. |
| Boulder | BO | Stones over 256 mm in diameter. | Sand | SN | Small particles < 2 mm in diameter |
| Cobble | LC | Stones from 64-256 mm in diameter. | Silt / Muck | MU | Mixture of very fine inorganic and organic material |
| Pebble | PB | Stones from 16-64 mm in diameter. | Aquatic Plants | AP | Rooted macrophytes or mosses (not algae) |

*Particle Size Measurements and Periphyton Cover* — Measure ≥ 25 pieces of substrate and classify moss and algae cover on each substrate in each of the 4 habitat units by collecting particles along zigzag transects. In general use 3 transects per habitat unit, although you may have to vary the number of transects used depending on the width of the stream. You should measure rock size and characterize moss/algae cover on an approximately equal number of rocks at uniform intervals along each transect. We will measure these intervals in terms of boot lengths, and you will therefore need to estimate how many steps (heel to toe) you will have to take to reach each sampling point. To sample, begin at the bottom of the reach and walk (heel to toe) along the first transect. After walking the necessary number of steps to reach the 1$^{st}$ sampling point, stop, and without looking down reach down and pick

up the piece of substrate nearest your big toe.

Estimate amount of moss, macro-algae, and micro-algae cover separately. Record moss and macro-algae cover with a scale from 0-3 with separate estimates for each:

0 - no moss or macro-algae present:
1 - some (but < 5% coverage) moss or macro-algae present
2 - 5-25% cover of substratum by moss or macro-algae; and
3 - > 25% cover of substratum by moss or macro-algae.

Estimate thickness of micro-algae (periphyton) on the rock with a 0-5 thickness scale:

0 - substrate is rough with no apparent growth;
0.5 - substrate is slimy, but biofilm is not visible (tracks cannot be drawn in the film with the back of your fingernail; endolithic algae can appear green but will not scratch easily from the substratum);
1 - a thin layer of microalgae is visible (tracks can be drawn in the film with the back of your fingernail);
2 - accumulation of microalgae to a thickness of 0.5-1 mm;
3 - accumulation of microalgae from 1 mm to 5 mm thick;
4 - accumulation of microalgae from 5 mm to 20 mm;
5 - layer of microalgae > than 2 cm.

(Note that if substrate is too large to pick-up, plant growth should still be characterized.)

Characterize substrate size by placing each particle through the smallest hole possible in the gravelometer and record the particle size as that of the largest opening the particle will not pass through. Continue this process, walking along each transect until a minimum of 25 measurements have been taken. Complete each transect even though 25 particles may have been measured prior to the end of a transect.

Map Depicting the Study Site - In the field notebook, draw a map that depicts the study site. At a minimal, the map should include the following information: compass direction, basic sketch showing the shape (plan view) and size (give scale) of the stream channel, an arrow to indicate the direction of flow, major habitat units/patches, large objects obstructing the stream channel, unique channel characteristics (e.g. islands, tributaries), and directions to the site.

Photographs - Take 3 photographs at each site: one from the bottom looking up, one from the top looking down, and one from whatever vantage point provides the best overall lateral view of the site. Record the exposure number and roll number in the Site Summary Notebook and on the Field Data Form. After a roll of film is complete, wrap the film in a piece of masking tape and write the roll number on the tape. Store exposed film in a zip lock bag and in a cool, dark place.

# Channel-Type Characterization

Channel and Valley Type Classifications (adapted from Montgomery and Buffington, 1993, Channel classification, prediction of channel response, and assessment of channel condition, Report TFW-SH10-93-002, Washington State Timber/Fish/Wildlife).

The first step in classifying the channel is to establish the valley segment characteristics. Valleys are separated into 3 categories: colluvial, bedrock, or alluvial. In general, colluvial valleys are those in which colluvial fills (i.e., material from hill slopes) accumulate and are periodically excavated by the stream. In bedrock valleys, there is no contiguous alluvial bed. Some alluvial material may be temporarily stored in scour holes, or behind flow obstructions, but in general the bedrock valley channel bed lacks an alluvial cover, and there is little, if any, channel fill. In alluvial valleys, the channels are capable of sorting and transporting the load supplied to them from upslope channels, but the transport capacity is not sufficient to scour them to bedrock. Use the table below to classify channels as either colluvial, bedrock, or one of the following subcategories of alluvial channels: cascade channel, step-pool channel, plane-bed channel, pool-riffle channel, regime channel, or braided channel.

| Classification of Stream Channels | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Colluvial | Bedrock | Alluvial | | | | | |
| | | | Cascade | Step-pool | Plane-bed | Pool-riffle | Regime | Braided |
| Predominant bed material | Variable | Bedrock | Boulder | Cobble/ boulder | Gravel/ cobble | Gravel | Sand | Variable |
| Bedform pattern | Variable | Variable | None | Vertical oscillatory | None | Laterally oscillatory | Multilayered | Laterally oscillatory |
| Dominant roughness elements | Boulders, large woody debris | Streambed, banks | Boulders, banks | Bedforms (steps, pools), boulders, large woody debris, banks | Boulders and cobbles, banks | Bedforms (bars, pools) boulders and cobbles, large woody debris, sinuosity, banks | Sinuosity, bedforms (dunes, riffles, bars), banks | Bedforms (bars, pools) |
| Dominant sediment sources | Hillslope, debris flows | Fluvial, hillslope, debris flows | Fluvial, hillslope, debris flows | Fluvial, hillslope, debris flows | Fluvial, bank erosion, debris flows | Fluvial, bank erosion, inactive channels, debris flows | Fluvial, bank erosion, inactive channels | Fluvial, bank erosion, debris flows |
| Typical slope (%) | >20 | Variable | 8 - 30 | 4 - 8 | 1 - 4 | 0.1 - 2 | <0.1 | <3 |
| Typical confinement | Strongly confined | Strongly confined | Strongly confined | Moderately confined | Variable | Unconfined | Unconfined | Unconfined |
| Pool spacing (channel widths) | Variable | Variable | <1 | 1- 4 | None | 5 - 7 | 5 - 7 | Variable |

Site Data Form

| | |
|---|---|
| Site Name: | Latitude: |
| Site Code: | Longitude: |
| Date: Vehicle: | Elevation (m): Map = GPS = |
| State: Land Ownership: | Initial Time: Initial Temperature: |
| Crew: Collector's Name- Recorder's Name- | Final Time: Final Temperature: |
| Type of Site (circle one): Reference Test | Overall Site Condition (circle one): Is this a good reference site? Yes No |

| Site Evaluation | | |
|---|---|---|
| Vegetative cover | Score: | 4 = >95% 3 = 85 - 94% 2 = 75 - 84% 1 = <75% |
| Erosional deposition from surrounding slopes | Score: | 4 = None 3 = Some in specific, limited locales 2 = Obvious signs 1 = Mass wasting. |
| Consumption of trees & shrubs by livestock | Score: | 4 = 0 - 5% 3 = 5 - 25% 2 = 25 - 50% 1 = >50% |
| Stream incisement | Score: | 4 = No incisement. 3 = Old Incisement 2 = Deep incisement; new floodplain development 1 = Deep incisement; active downcutting |
| % bank with lateral cutting | Score: | 4 = < 5% 3 = 5 - 15% 2 = 15 - 35% 1 = >35% |
| % bank with deep, binding root masses | Score: | 4 = >85% 3 = 65 - 85% 2 = 35 - 64% 1 = < 35% |

| Management Activities (Rank and Describe) and Livestock Use Index | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Activity | Rank | Notes | | | | | | | |
| Logging | | | | | | | | | |
| Agriculture | | | | | | | | | |
| Mining Activities | | | | | | | | | |
| Recreation | | | | | | | | | |
| Roads | | | | | | | | | |
| Stream Diversions | | | | | | | | | |
| Urbanization | | | | | | | | | |
| Livestock Grazing | | | | | | | | | |
| Livestock Use Index: Number of fecal droppings | | Left Transect | | | | Right Transect | | | |
| | | Cow-Old | Cow-New | Sheep-Old | Sheep-New | Cow-Old | Cow-New | Sheep-Old | Sheep-New |
| | | | | | | | | | |

| Water Sample (check all collected) | Unfiltered with $H_2SO_4$ | Filtered and Frozen | Filtered with $H_2SO_4$ | Filtered with $HNO_3$ | Notes |
|---|---|---|---|---|---|
| Stream Water | | | | | |
| Field Blanks | | | | | |

| Site Measurements (also see back of form) | | | | | | |
|---|---|---|---|---|---|---|
| Conductivity (µS/cm) | P Alkalinity (ppm CaCO3) | Total Alkalinity (ppm CaCO3) | Stream Travel Time (s/50m) lead | trail | Stream Slope ( %) | Periphyton Sample Volume (mL) |
| | | | | | | |
| Channel Classification: Braided Regime Pool-Riffle Plane-Bed Step-Pool Cascade Bedrock Colluvial | | | | | | |
| Dominant Fast-Water Habitat Type: Rapid Riffle Run Steprun | | | | | | |
| Dominant Slow-Water Habitat Type: Lateral Scour Plunge Dammed | | | | | | |

| Photographs | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Looking Upstream | Roll#: | Exp#: | Looking Downstream | Roll#: | Exp#: | Stream Overview | Roll#: | Exp#: |

| | Stream Depth/Width Transects | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Transect | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| Width (m) | | | | | | | | | | |
| Depth (cm) at 0.25 width | | | | | | | | | | |
| Depth (cm) at 0.50 width | | | | | | | | | | |
| Depth (cm) at 0.75 width | | | | | | | | | | |

| | Densiometer Measurements | | | |
|---|---|---|---|---|
| Direction | Unit 1 | Unit 2 | Unit 3 | Unit 4 |
| Upstream | | | | |
| Left bank | | | | |
| Right bank | | | | |
| Downstream | | | | |

| Visual estimate of % of riffles comprised of 7 substrate types / sizes (mm) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Aquatic Plants | BR | Boulder | Cobble | Pebble | Gravel | Sand | Silt /Muck |
| Name | | BR | > 256 | 64-256 | 16-64 | 2- 16 | < 2 | |
| | | | | | | | | |
| | | | | | | | | |
| Notes: | | | | | | | | |

| Visual estimate of median substrate size (use 12 size classes below). | Name: | Size: | Name: | Size: |
|---|---|---|---|---|

| | Stream Bed Particle Size Counts | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Unit 1 | | Unit 2 | | Unit 3 | | Unit 4 | | Total Counts |
| Particle Size Class | Tallies | Count | Tallies | Count | Tallies | Count | Tallies | Count | |
| Bedrock | | | | | | | | | |
| 180 | | | | | | | | | |
| 128 | | | | | | | | | |
| 90 | | | | | | | | | |
| 64 | | | | | | | | | |
| 45 | | | | | | | | | |
| 32 | | | | | | | | | |
| 22.6 | | | | | | | | | |
| 16 | | | | | | | | | |
| 11 | | | | | | | | | |
| 8 | | | | | | | | | |
| < 8 | | | | | | | | | |
| | | | | | | | Total # of particles counted in all units: | | |

| | Moss Cover Index | | | |
|---|---|---|---|---|
| Category | 0 | 1 | 2 | 3 |
| Tally | | | | |
| Count | | | | |

| | Macro-Algae Cover Index | | | |
|---|---|---|---|---|
| Category | 0 | 1 | 2 | 3 |
| Tally | | | | |
| Count | | | | |

| | Micro-Algae Cover Index | | | | | | |
|---|---|---|---|---|---|---|---|
| Category | 0 | 0.5 | 1 | 2 | 3 | 4 | 5 |
| Tally | | | | | | | |
| Count | | | | | | | |

Comments: