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Ambient Biological Monitoring in Rivers and Streams: Benthic Macroinvertebrates and Periphyton

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Quality Assurance Monitoring Plan

Ambient Biological Monitoring in Rivers and Streams: Benthic Macroinvertebrates and Periphyton

August 2010

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EAP - Environmental Assessment Program.
EIM - Environmental Information Management system.

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Abstract

Each study conducted by the Washington State Department of Ecology (Ecology) must have an approved Quality Assurance Project Plan. The plan describes the objectives of the study and the procedures to be followed to achieve those objectives.

This document describes Ecology's Freshwater Ambient Biological Monitoring program.

Objectives of this program are to:

- Provide baseline information from “reference condition” streams across the Washington landscape.
- Develop and periodically calibrate biological assessment models for all regions in Washington State.
- Monitor long-term trends of stream health in the absence of human disturbance.
- Examine where and how biological information can be applied in water resource management.
- Guide management decisions that would restore biological integrity of the stream.

These objectives will be accomplished by collecting annual samples from the biological communities (aquatic macroinvertebrates and periphyton) in wadeable rivers and streams across the state.

Outlined within the document are:

- Sampling design.
- Site selection process.
- Field implementation.
- Laboratory processing of data.
- Analysis and interpretation of data.

The first appendix is a glossary that describes acronyms and terms. Field operations methods are found in the subsequent appendices and are consistent with previous work (Merritt, 2009; Plotnikoff, 1992; 1994; 1998; 1999; Plotnikoff and Ehinger, 1997; Plotnikoff and Wiseman, 2001).

Background

The Federal Clean Water Act (Section 101(a)) mandates that states develop programs to evaluate, restore, and maintain the chemical, physical, and biological integrity of the nation's waters (33 USC §1251 Sec 101(a)). Measurements of chemical and physical components alone do not provide enough information to fully address surface water problems. Biological assessments enhance chemical and physical evaluation by:

- Capturing impacts of pollutants for which there are currently no criteria and no regulation by Washington's Water Quality Program.
- Directly measuring the most sensitive resources at risk.
- Measuring stream components that reflect natural variation over time.
- Providing a diagnostic tool that synthesizes chemical, physical, and biological perturbations (Hayslip 1993).

Ecology has collected biological data from rivers and streams throughout the state since 1993. This data allows exploration of spatial patterns and temporal trends in benthic macroinvertebrate communities. The current dataset represents a range of biological conditions from reference (minimally or least impacted) to degraded conditions.

The Ambient Freshwater Biological Monitoring program focuses on annual index period monitoring of biological and habitat conditions among relatively natural streams (Stoddard et al., 2006) in Washington State. This provides a baseline of information for understanding the added impact of human activity. The program also monitors streams that have been selected for short term biological investigations. These are often disturbed streams.

Study Area and Surroundings

During 1993 to 2006, Ecology staff collected annual biological samples from riffles (fast-flowing water) of 12 wadeable reference streams among five of the level III ecoregions in Washington: Coast Range, Puget Lowlands, Cascades, Columbia Plateau, and Northern Rockies. See Figure 1, (Omernick and Gallant, 1986).

Ecoregion Representation

Ecoregions are geographical regions of relative homogeneity either in ecological systems or involving relationships between organisms and their environment (Omernik and Gallant, 1986; Pater et al., 1998). Map makers use surficial, climactic, and hydrological characteristics to define these regions of relative homogeneity. These characteristics include land surface form, potential natural vegetation, land use, soils, mean annual precipitation, and mean temperature.

Staff can extrapolate reference site information to other similar streams within each ecoregion. Regional biological description is defined by including information from a variety of reference sites within the ecoregion.

Washington State overlaps eight ecoregions: Coast Range, Puget Lowland, Cascades, Columbia Plateau, Northern Rockies, Willamette Valley, Blue Mountains, and Eastern Cascade Slopes and Foothills (Figure 1).

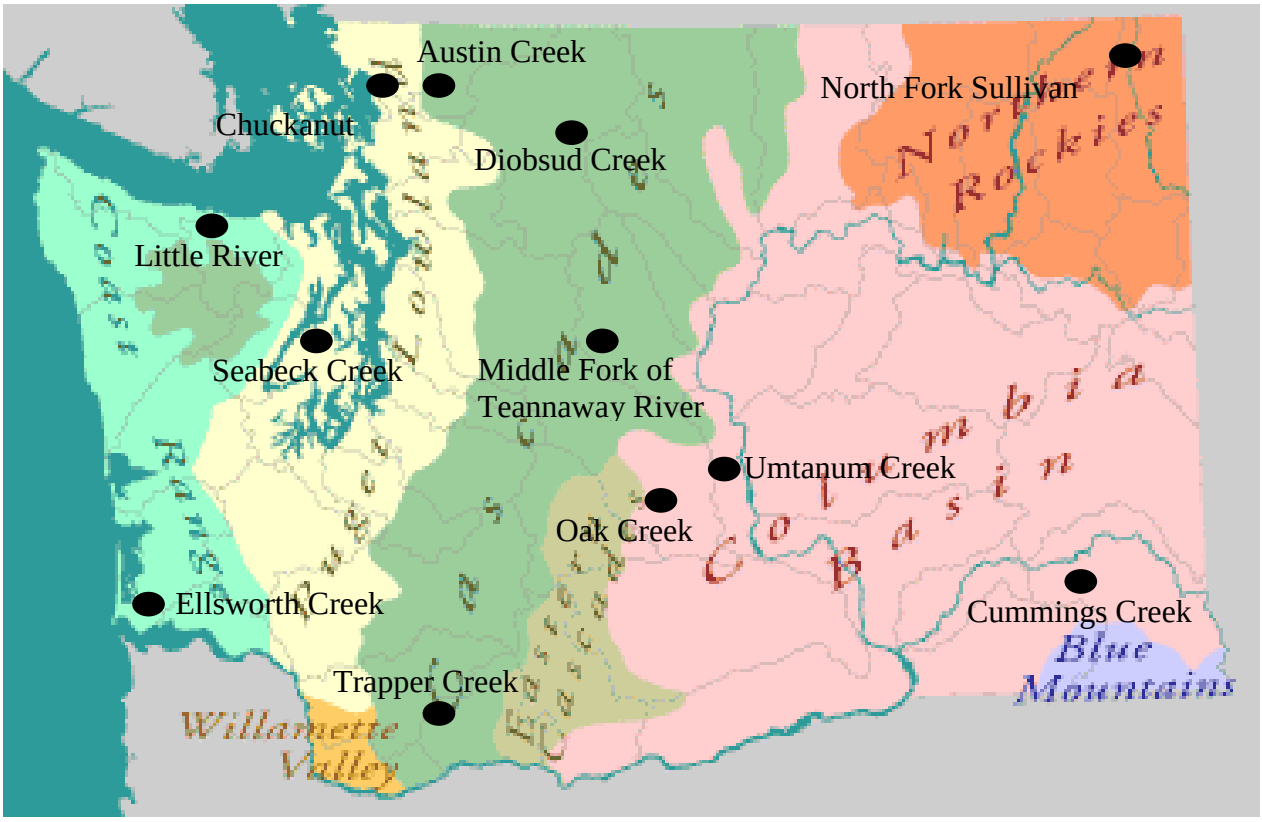

Figure 1. Washington State Ambient Biological Field Sites (1993-2006) and Ecoregions as defined by Omernik and Gallant (1986).

Staff describe annual biological conditions within regions over five-year periods to quantify natural variability. Using the regions helps to describe the range of conditions. Cyclic environmental phenomena are described using the inter-annual measurements. Staff use long-term biological monitoring sites to calibrate inter-annual variability and currently have 12 of these long-term reference sites located among five regions across the state. The plan is to identify new reference sites in each ecoregion. These sites will expand the current data set, and data will help test and refine regional reference condition definitions. Ideally, staff will incorporate up to 10 reference sites in each of five ecoregions: Coast Range, Puget Lowlands, the Cascades, the Columbia Basin, and the Northern Rockies.

Evaluation of regional patterns and variability is most effective in the absence of any human degradation. Degraded sites may introduce error into observed regional patterns, unless there are intrinsic biological attributes within a stream class that persist over a degradation gradient. If all streams in a region have been disturbed to a certain degree, a least disturbed condition (LDC)

should be identified and used for that region. This situation may be the case in the Columbia Basin and in the Puget Lowlands.

The Ambient Freshwater Biological Monitoring Program currently uses ecoregions as an a priori stream classification approach for the distribution of biological monitoring sites. When staff have identified and sampled an adequate number of reference sites from each ecoregion, the data will be used to build and periodically recalibrate multimetric and multivariate bioassessment models. These models may or may not be based on ecoregion delineation, depending on the actual invertebrate distribution in reference conditions across the state.

Reference Conditions

This program focuses on the ambient biological conditions of reference streams. The "reference condition" is the physical, chemical, and biological condition of a class of streams with little or no human-induced degradation. High road densities and the presence of other human activities in Washington State require the definition of minimally disturbed conditions (MDC) or least disturbed (LDC) for reference. MDCs reflect sites that have experienced very little human activity. LDCs are based on measurements from best available streams given today's state of the landscape (Stoddard et al., 2006).

Reference sites highlight biological variability due to natural disturbances, e.g., precipitation or drought. Non-reference sites reflect a range of human impacts that can be analyzed independently from changes due to natural disturbance once the natural baseline is established. This knowledge helps staff implement realistic and relevant restoration efforts in disturbed streams. It can also inform the regulatory process, allowing a more accurate determination of human- induced degradation. Remedies can then be addressed through the Total Maximum Daily Load (TMDL) process.

Reference site information is also used as a measure of biological potential for particular stream settings. Responses in the biological community to environmental degradation are described by comparing a test site to a paired reference site. The difference in physical, chemical, and biological conditions between the two sites represents a change away from reference conditions at the test site. Conditions at the paired reference site can serve as a benchmark for restoration success.

For consistency in a monitoring program, establishment of reference sites should follow these guidelines:

- Map potential areas where reference sites are expected.
- Eliminate areas with relatively high human modifications, past and present.
- For long-term monitoring, spread locations geographically and across a range of habitat types in each ecoregion.
- For targeted studies, choose reference sites that approximate the stream type and setting of test sites.
- Visit the site to verify condition.

Logistical Problems

The fieldwork for this monitoring program requires one full workday (6-8 hours) per site to complete. If the combined travel time and work time requires more than 12 hours, staff should plan to travel to a nearby town to stay overnight.

Sites should be selected and visited before the sampling season each year. This will allow assessing reference condition of the site as well as working out travel timing and issues before sampling.

Permission to access property should be obtained before sampling takes place. If access to a stream is denied by one land owner, access may be possible through a neighboring property.

History of Ambient Biological Monitoring in Washington State

A broad range of climatic and geographic conditions exist across Washington State, creating distinct regional patterns among the benthic macroinvertebrate and periphyton communities.

Ecology has conducted biological monitoring since 1993. Throughout the history of Ecology's sampling effort, protocols have varied. From 1993 to 1996, Ecology's Freshwater Ambient Biological Assessment Program sampled both riffle and pool habitat. They kept the composited riffle sample separate from the pools sample at each reach. Then, in 1997, Plotnikoff and Ehinger found that even though degradation may selectively occur in pool habitat and not in riffles, no consistent biological differences (due to degradation) between riffles and pools were detected. As a result, sampling protocols changed and sampling was conducted exclusively in riffles from 1997 to 2006, increasing efficiency by not having two samples at each site.

The 13- year sampling effort has incorporated a variety of site conditions. Besides high-quality reference conditions in ambient monitoring, sites with various levels of physical and chemical disturbance have also been surveyed during targeted monitoring efforts. Results show the importance of scale in describing and predicting a reference community.

For example, Plotnikoff (1992) found that communities differ as a function of region and season among similar-sized streams in three ecoregions: Puget Lowland, Cascades, and Columbia Plateau. Surveys in the Yakima River Basin identified segment-level variables (valley type and watershed characteristics) as the best correlates with biological community expressions over basins and regions of the landscape (Carter et al., 1996). Plotnikoff and Ehinger (1997) stressed the importance of reach-level variables (temperature, pH, conductivity, wetted width/ bankfull width ratio, and elevation) in shaping the macroinvertebrate communities.

The past monitoring data set represents a gradient of biological conditions reflecting the range of existing stream conditions in Washington. With this data set, the biological monitoring program has been able to develop empirical models for bioassessment of streams including:

- a multimetric model similar to the Benthic Index of Biotic Integrity (BIBI) (Karr, 1991)
- a multivariate model similar to the Riverine Invertebrate Prediction and Classification System (RIVPACS) (Wright, 1995)

RIVPACS type models are called O/E (observed/expected) models (Ostermiller and Hawkins, 2004) A multimetric model was developed by Ecology for the Puget Lowlands and the Cascades (Wiseman, 2003) and multivariate O/E models are currently being developed for the Cascades by Von Prause and Adams. The ultimate goal for the biological monitoring program is to provide assessment models that address all regions of the state. While ecoregions are used as the conventional classification scale for creating and applying multimetric models, there is no such conventional scale for building multivariate O/E models. The Ambient Freshwater Biological Monitoring program will increase the number of reference sites to refine the accuracy of current models and to create both BIBI-type and O/E models that address conditions across the state.

Empirical Modeling of Stream Conditions

Stream condition using aquatic invertebrate and periphyton species distribution can be determined using empirical models (models based on real data) such as those mentioned above. Empirical measurements from minimally through highly disturbed ranges of conditions enable objective statistical evaluation of stream health. The best scale of model assessment and applicability is not clearly defined for Washington State. Many studies promote localized assessment, suggesting that large-scale regionalizations, such as ecoregions, do not account for an acceptable amount of natural variability for a synthesis (See Hawkins et al., 2000). One alternative is to use large-scale regionalizations as an initial stratification variable, followed by smaller scale variables to account for more of the natural variability within the region.

Multimetric models use an a priori classification of macroinvertebrate communities by ecoregion, assuming that the distribution of species is primarily determined by the physical environment. These models are built by correlating taxonomic composition and characteristics, such as life history traits and pollution tolerance, with the range of conditions found in each ecoregion from near pristine to highly disturbed sites. This creates a defined scale of community characteristics one would expect to find in reference and in disturbed stream conditions. Once this model is built, the characteristics of the taxa at a site are used to indicate the condition of the stream as good, fair, or poor.

Similarly, multivariate O/E models assess stream health by comparing the taxa that are found at a site with the taxa that would be expected at that site if it were in reference condition. To establish the “expected” condition, environmental and taxonomic data are collected from reference streams across the region of interest. However, the sites are not classified a priori by ecoregion. Sites are classified by stream types defined by environmental predictor variables not influenced by human activity, e.g., elevation, precipitation, and latitude. The “expected” taxonomic composition is defined by statistically modeling the natural grouping of invertebrate communities that live in similar stream types. When assessing stream condition, the stream’s

type is determined based on the data from the predictor variables. The site's stream type defines the taxonomic composition that is expected. The similarity of the community under investigation with that expected in the same stream type indicates the level of impairment of the stream.

Utility of Bioassessment Models

Current Washington State Water Quality Program Policy 1-11 states that biological data will be accepted for listing water quality impairment purposes for Section 303(d) of the Clean Water Act. Ecology currently accepts RIVPACS-type model results as the primary criterion, such that streams with a score of 0.73 or lower during their most recent year of data collection are listed as impaired (Table 1). Biological data from other models is accepted when at least 3 of the most recent 5 years of data demonstrate impairment, receiving a "poor" score using the BIBI-type models. These criteria may be refined as the program develops.

Future refinements of old RIVPACS-type and BIBI-type models and creation of new models built for Washington State will provide up-to-date and objective means to document impacts on resources. They will also provide a statistically defensible determination of impairment for listing streams on the 303(d) list.

Table 1. Biological criteria accepted for 303(d) listing purposes in Washington State according to Policy 1-11 (2006).
Models referred to here are Ecology-developed models.

Category	Status	Description
1	Meets criteria	RIVPACS-type model score of >0.86, from the most recent year of invertebrate data or 3 of the most recent 5 years of data from other models show no impact (BIBI-type model score – "Good").
2	Waters of concern	RIVPACS type model score of <0.86 and >0.73 from the most recent year of data or 3 of the most recent 5 years of data from other models show that the water body is starting to be degraded (BIBI-type model score – "Fair").
3	Lack of sufficient data	Data are insufficient to make a determination. Data will be stored in the EIM database until future assessment is possible.
4	Impaired, no TMDL required	4a when a TMDL is approved by EPA, 4b when a pollution control project is approved, and 4c when impairment is not due to a pollutant that can be addressed by a TMDL.
5	Impaired, TMDL or DIP required	RIVPACS-type model score is <0.73 from the most recent year of data or 3 of the most recent 5 years of data from other models show impairment (BIBI-type model score – "Poor").

Project Description

Goals and Objectives

The goal of this program is to conduct long- term biological monitoring and assessment of representative stream conditions outside of human influence in Washington State. Ambient biological monitoring refers to monitoring of aquatic macroinvertebrate and periphyton communities in healthy streams. The health of these communities reflects the physical and chemical stream environment. Data collection and evaluation occurs on an annual basis to meet the monitoring program objectives to:

- Provide baseline information from "reference condition" streams across the Washington landscape.
- Develop and periodically calibrate biological assessment models for all regions in Washington State.
- Monitor long-term trends of stream health in the absence of human disturbance.
- Examine where and how biological information can be applied in water resource management.
- Guide management decisions that would restore biological integrity of the stream.

Upon request, short term studies of non-reference streams are conducted to investigate specific concerns in a stream. These studies adhere to the same quality standards and protocols outlined in this document.

General Design

Benthic macroinvertebrate and periphyton communities are used to assess stream condition. Macroinvertebrates and periphyton provide information about environmental conditions based on the range of tolerance individual taxa have to environmental conditions. The members present in these communities indicate habitat conditions based on unique tolerance levels of those present or missing taxa. Fish community evaluations are not used because relatively few taxa in western North America exist and harvests are restricted for several threatened or endangered species including salmon (Moyle et al., 1986).

Physical habitat, water chemistry, and soil chemistry are measured and biological samples are collected. These measurements and samples describe the environment at the time of sampling.

The primary tasks conducted by the biological monitoring staff include:

- Site reconnaissance and selection
- Collection of
  - Water chemistry samples
  - Soil chemistry samples
  - Benthic macroinvertebrate samples
  - Periphyton samples
  - Habitat data
- Data analysis
- Result summarization on Ecology's Biological Monitoring webpage www.ecy.wa.gov/programs/eap/fw_benth/index.htm.

Index Period

Sampling will occur between July 1 and October 15 of each year. This timeframe was chosen for the following reasons:

- Adequate time has passed for the instream environment to stabilize following natural disturbances, e.g., spring floods.
- Many macroinvertebrates reach body sizes that can be readily identified.
- Representation of benthic macroinvertebrate species reaches a maximum, particularly during periods of pre-emergence, typically from mid-spring to late-summer.

Biological results are affected by the index period chosen. This is because natural seasonal disturbances and physical stream conditions strongly affect the rate of development, diversity, and abundance of both insects and periphyton (Hynes, 1970; Vannote et al., 1980). It is recommended that collection begin during the earlier part of the index period for drier ecoregions. Data should be gathered from all sites within an ecoregion over a condensed time within the index period.

Stream Size

We sample streams that are perennial and wadeable. We avoid ephemeral streams because of the drought's effect on the community. Seasonal drought disturbance selects for distinct specialist communities (Resh et al., 1988; Clifford, 1966) and does not support representative aquatic communities. Monitoring these communities would be contrary to our goal of describing representative stream conditions.

Habitat Type

Stream habitat is classified into (1) riffles (fast- moving surface water), (2) glides or runs (fast-moving with less surface turbulence), and (3) pools (slow-moving or eddying water). Ecology has conducted biological assessment in the past in riffle habitats only. However, when conducting long-term monitoring of general stream and habitat condition, we need to understand the condition of all habitats. In addition, many researchers have found that consistently

identifying habitat types is difficult. Targeting riffles can introduce sampling error (Poole et al., 1997).

For these reasons, investigators use a reach- wide sampling scheme (Hayslip, 2007), as opposed to a targeted "riffle only" sampling scheme. In a reach- wide scheme, equidistant transects are established along the stream reach (defined as 20 times bankfull width at the sample site) and sampling is conducted systematically without consideration for habitat type. For example, the sampler might start collecting on the left bank at transect one, move to midstream at transect two, and the right bank at transect three, and back again until 8 square feet have been sampled from randomly chosen transects. Sampling locations are denoted by dots in Figure 2. A sample is collected at each of these locations regardless of what type of stream habitat the site falls on.

While this is a useful scheme for long-term monitoring projects, special requests for monitoring may require targeted sampling. Noise in signals of impairment is introduced when habitat- wide data are included in a targeted study (Parsons and Norris, 1996). Data collected from habitat-specific samples help reduce noise and allow clear and consistent detection of response to pollutants at an impacted site. For example, the use of riffle habitats for targeted studies like TMDLs captures the composition of more pollution- sensitive communities.

Recently, studies have shown that there is no statistical difference between samples collected from riffle only and those collected from reach- wide designs (Kerans et al., 1992; Rehn et al., 2007). Therefore, historical Washington State data collected in riffles only are useful for describing past and current stream health in streams currently monitored using habitat- wide methods.

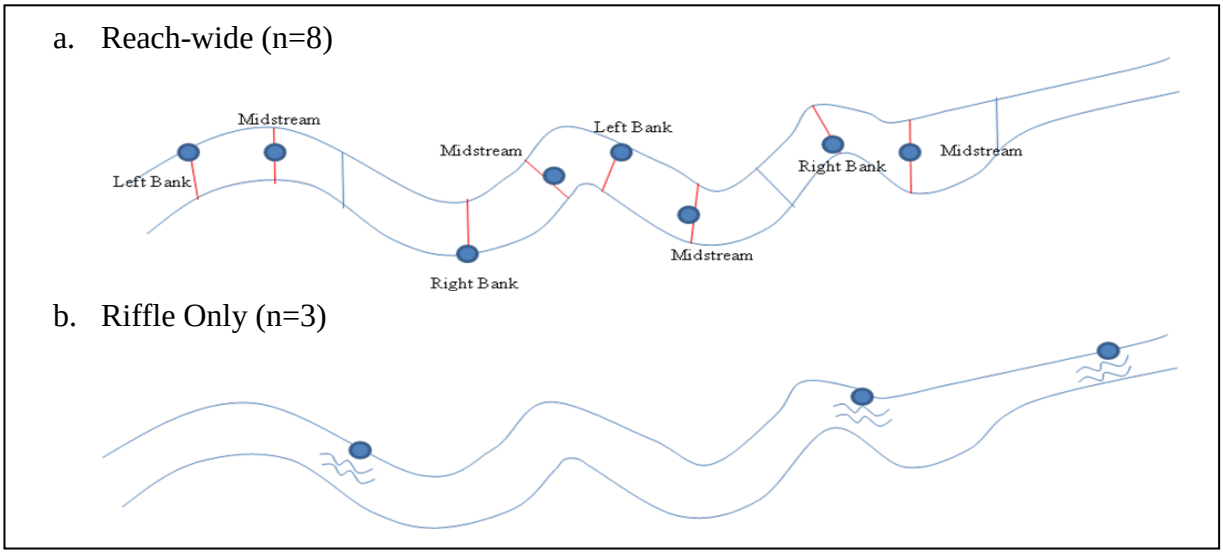

Figure 2. Schematic sampling design for (a) reach-wide sampling protocols compared with (b) riffle only protocols.

Organization and Schedule

The following people are involved in this project (Table 2). All are employees of the Washington State Department of Ecology. Table 3 outlines the schedule for completing field work, lab analysis, and data entry.

Table 2. Organization of project staff and responsibilities.

Staff (all are EAP except client)	Title	Responsibilities
Karen Adams Directed Studies Unit Western Operations Section Phone: (360) 407-6530	Project Manager/ Principal Investigator	Writes the QAMP. Oversees field sampling and transportation of samples to the laboratory. Conducts QA review of data, analyzes and interprets data, and enters data into EIM. Writes the draft report and final report.
George Onwumere Directed Studies Unit Western Operations Section Phone: (360) 407-6730	Unit Supervisor for the Project Manager	Provides internal review of the QAMP, tracks progress, approves the budget, and approves the final QAMP.
Robert F. Cusimano Western Operations Section Phone: (360) 407-6596	Section Manager for the Project Manager	Reviews the project scope, reviews the draft QAMP, and approves the final QAMP.
Will Kendra Statewide Coordination Section Phone: (360)-6698	Section Manager for the Project Area	Reviews the project scope, reviews the draft QAMP, and approves the final QAMP.
Jim Ross Eastern Regional Office Phone: (509) 329-3425	Field Investigator	Collects samples and records field information for the Eastern Region.
Dan Dugger Central Regional Office Phone: (509)454-4183	Field Investigator	Collects samples and records field information for the Central Region.
Gary Arnold Eastern Operations Section Phone: (360) 407-6730	Section Manager for the Field Investigators	Provides internal review of the QAMP and approves the final QAMP.
Stuart Magoon Manchester Environmental Laboratory Phone: (360) 871-8801	Director	Provides internal review of the QAMP and approves the final QAMP.
William R. Kammin Phone: (360) 407-6964	Ecology Quality Assurance Officer	Reviews the draft QAMP and approves the final QAMP.

EAP – Environmental Assessment Program.
EIM – Environmental Information Management system.
QAMP – Quality Assurance Monitoring Plan.

Table 3. Proposed schedule for completing field and laboratory work, data entry into EIM.

Field and laboratory work	Due date	Lead staff
Field work completed	July 1 to October 15 annually	Karen Adams
Biological laboratory analyses completed	November of the same year to March of the following year	
Chemical laboratory analyses completed	July 1 to October 15 of the same year	
Environmental Information System (EIM) database		
EIM user study ID	ID number BioMonitoring Program	
Product	Due date	Lead staff
EIM data loaded	April following data collection	Karen Adams
EIM Quality Assurance	April following data collection	May vary by year
EIM complete	May following data collection	Karen Adams
Data Reporting on Web		
Author lead	Karen Adams	
Schedule		
Calculations and results submitted to Gayla Lord	May following data collection	
Short Report		
Draft due to supervisor	May following data collection	
Draft due to client/peer reviewer	June following data collection	
Final (all reviews done) due to publications coordinator (Joan)	November 15, following year	
Final report due on web	December 31, following year	

Budget

Table 4 outlines the expected lab costs each year for the Ambient Biological Monitoring Program based on MEL prices posted for FY 2011. We expect to sample between 10 and 15 sites per year, for a total estimated annual cost of $9000 to $13,500. Because the location of the sites will differ each year, personnel and travel costs cannot be predicted.

Table 4. Lab budget per site for the Ambient Biological Monitoring Program.
Personnel and travel costs are dependent on number and distance of sites each year.

Parameter	Matrix	Cost Estimate Per Sample
Total Phosphorus	Water	$19
Total Perchlorate Nitrogen		$18
Chloride		$14
Turbidity		$12
Total Suspended Solids	Sediment	$12
Metals		$103
Polyaromatic Hydrocarbons		$33
Total Organic Carbon	Water	$44
Chlorophyll a		$45
Invertebrates		$300
Periphyton		$300
Total Per Sampling Event:		$900

Quality Objectives

Field Quality Assurance

The integrity of the data collected by this project is upheld by maintaining a high level of quality addressing the five objectives below. The quality of the sampling protocol is checked by analyzing the degree of sampling and visit precision, attempting to maintain less than 20% variation among reference stream data for taxa richness in benthos and periphyton samples. The aim is to collect samples that are representative of community and ecological conditions for each stream. Data are collected with common protocols used by other regional biological monitoring programs. This improves data comparability and usefulness among colleagues in biomonitoring.

Sampling and Visit Precision

Sampling precision measures the extent of random variability among replicate measurements of the same property and is a data quality indicator (USGS, 1998). Sampling precision is estimated by keeping the eight replicate samples separate at 10% of the reaches sampled annually. Sampling precision is calculated using the relative standard deviation (RSD) among the replicate samples and should be < 20% in reference streams when using the taxa richness metric (Plotnikoff, 1992). Staff expects collections of macroinvertebrates and periphyton from multiple sample locations to have similar community structure in reference streams.

Visit precision measures variability in the sampling method and is related to the variability of collecting a composite sample in a reach. Visit precision is estimated by collecting duplicate composite samples of the invertebrate and periphyton communities within the same reach during the same day at 10% of the reaches sampled annually. Visit precision is calculated using the relative standard deviation (RSD) from two replicate composite samples and should be < 20% in reference streams when using the taxa richness metric.

Bias

Sampling bias is the difference between the population mean and the true value. Bias usually describes a systematic difference reproducible over time and is characteristic of both the measurement system, and the analyte(s) being measured (Kammin, 2010; Ecology, 2004). Bias may be caused by the same field investigator conducting the same task at each site. It may also occur due to consistent misinterpretation of protocols by a group of field investigators.

Representativeness

Representativeness measures the degree to which a sample reflects the population from which it came - a data quality indicator. (USGS, 1998). For ambient monitoring, sites should be representative of minimally or least disturbed conditions in the sampled stream. For targeted monitoring, the sites should be representative of the range of conditions in the sample area. The sampling protocols in the appendices are designed to produce consistent and repeatable results in

each stream reach. Physical variability within reaches is accounted for through reach-wide sampling of the various depths, substrates, and flow conditions throughout the stream.

Completeness

Completeness is defined as the amount of valid data obtained from a data collection project compared to the planned amount and is usually expressed as a percentage (USEPA, 1997). Our target for completeness of data is 100%. Sample loss is minimized with sturdy sample storage vessels and adequate labeling of each vessel. Sample vessel type and labeling information are described under "Sampling Stream Macroinvertebrates", and “Sampling Periphyton” in the Appendix C. Sample contamination occurs when containers are improperly sealed or stored. Loss of material or desiccation diminishes the integrity of the sample. If the validity of the information from the sample is in question, the sample will be flagged and excluded from analysis.

Completeness is determined by four criteria:

- Number of samples collected compared to the sampling plan.
- Number of samples shipped and received in good condition by Manchester Environmental Laboratory (MEL) and the taxonomy contractor.
- Laboratory’s ability to produce usable results for each sampling event.
- Sample results accepted by the project manager.

Comparability

Comparability describes the degree to which different methods, data sets, and decisions agree or can be represented as similar (USEPA, 1997). Comparable data sets make sharing data with other organizations that adhere to the same protocols, such as field sampling and analytical methods, possible.

Once a year, the project manager will provide a list of relevant SOPs and protocols to all support staff. The project manager will conduct sampling with each support staff team once each year. At this time, the staff will verify, by signature, that applicable SOPs and protocols were reviewed. This will improve the comparability of data collected within the program.

Biological monitoring efforts within Ecology use the applicable protocols followed by Washington’s Status and Trends Monitoring Program. These protocols are similar to those of others in the region, including the Oregon Department of Environmental Quality's bioassessment program, and the U.S. Environmental Protection Agency's "Regional Environmental Monitoring and Assessment Program" (R-EMAP). Following these commonly accepted protocols will result in data that is comparable to other regional programs.

Sampling Process Design (Experimental Design)

Field Operations

At least one day is required to sample each site. Depending upon travel distances, the workday could last longer than eight hours. At least one day each week should be allowed for planning, cleaning, repair, and administrative duties. The relative timing of monitoring activities is variable and should be performed considering efficiency of effort and site-specific conditions. However, the crew must meet these requirements when organizing their day:

- Measure in-situ water chemistry (set 1) before in-stream activities upstream.
- Measure water and sediment chemistry before sampling biological and habitat.
- Sample benthos and periphyton immediately after site layout.
- Measure habitat after collecting benthos and periphyton. This will maintain biological data integrity by avoiding damage to the specimens due to substrate disturbance during work activities.
- Measure in-situ chemistry (set 2) just before departure.

Table 5 provides an example of how a two-person crew could typically collect data.

Table 5. Idealized daily work flow.

		Time Since Arrival On-site (Hrs)					
Activity	Persons	1	2	3	4	5	6
Verification& Layout	Lead						
Water/In-Situ	Assistant						
Water and Sediment Chemistry	Assistant						
Benthos/Periphyton	Lead+Assistant						
Habitat	Lead+Assistant						

Table 6 lists locations within the site where each of these activities is performed. Spatial descriptions of these transects are in Appendix A.

Table 6. Field procedures by station within a site.

Major Transect	Minor Transect	Thalweg Transect	Near the Index station (Transect F) [e]
Slope & Bearing (10)[a] Wetted width Bankfull width Bar width Substrate sizes Substrate depths Fish cover by class Shade Human Influence Riparian Vegetation Benthos [b] Periphyton [b] Large Woody Debris [d] GPS coordinates [d]	Slope & Bearing (10)[a] Wetted width Bankfull width Bar width Substrate sizes	Slope & Bearing (10)[a] Thalweg Depth Habitat Unit presence Side Channel presence Edge Pool presence Bar presence	In-situ measures Water Sampling Sediment Sampling Discharge GPS coordinates [d]

[a] Slope & Bearing: normally one measurement at each major transect and one at each minor transect. Supplemental measurements sometimes are needed from intermediate thalweg transects.
[b] The benthos and periphyton samples are composite samples from eight randomly selected major transects.
[c] Large woody debris is tallied across the full length of the site, but records are kept for counts between major transects, on the *Thalweg Data Form*.
[d] GPS is required at site coordinates (index station) and at two major transects for wadeable streams (top and bottom of site).
[e] Except for GPS coordinates, these measurements can be done anywhere within the site, but near the index station (mid-reach, transect F) is preferred.

Table 7 outlines the type of analysis, methods, and quality standards for all measurements.

Table 7. Lab sample information.

Analysis	Equipment Type and Method	Accuracy (deviation or % deviation from true value	Method Reporting Limits and/or Resolution	Number of Samples/ Measurements per site
Field Analysis				
Periphyton	Barbour et al. (1999)	90% RPD	NA	1 composite from 8 transects
Macroinvertebrate	Targeted = Plotnikoff and Wiseman (2001) Monitoring = Merritt (2009)	90% RPD	NA	1 composite from 8 transects
Dissolved Oxygen	EAP033 (Swanson, 2007)	+/- 0.5 mg/L	0.1 mg/L	2
Specific Conductivity	EAP033 (Swanson, 2007)	+/- 10 us/cm	0.1 µS/cm 0.2 @ 25° C	2

Analysis	Equipment Type and Method	Accuracy (deviation or % deviation from true value	Method Reporting Limits and/or Resolution	Number of Samples/ Measurements per site
pH	EAP033 (Swanson, 2007)	0.075 SU (pH<5.75) +/- 0.15 (pH>5.75)	1 to 14 SU	2
Temperature	EAP033 (Swanson, 2007)	+/- 1° C of thermometer reading	1 - 26° C	2
Slope	EAP062 (Werner, 2009a)	10% RPD	0.5 cm	101
Bearing	Merritt (2009)	10% RPD	0-360°	101
Thalweg Depth	EAP062 (Werner, 2009a)	10% RPD	0 – 1.2 meters	11
Habitat Unit Presence	Merritt (2009)	10% RPD	NA	11
Side Channel Presence	Merritt (2009)	10% RPD	NA	11
Edge Pool Presence	Merritt (2009)	10% RPD	NA	11
Bar Presence	Merritt (2009)	10% RPD	NA	11
Wetted Width	EAP062 (Werner, 2009a)	10% RPD	0.1 meters	11
Bankfull Width	EAP062 (Werner, 2009a)	10% RPD	0.1 meters	11
Bar Width	EAP062 (Werner, 2009a)	10% RPD	0.1 meters	11
Substrate Sizes	Merritt (2009)	10% RPD	NA	11
Substrate Depths	Merritt (2009)	10% RPD	0 – 1 meter	11
Shade	EAP064 (Werner, 2009b)	10% RPD	0-100%	11
Human Influence	Merritt (2009)	10% RPD	NA	11
Riparian Vegetation	Merritt (2009)	10% RPD	NA	11
Large Woody Debris	Merritt (2009)	10% RPD	NA	11
Laboratory Analysis				
Chlorophyll *a*	SM 10200H(3)	20% RPD	0.1 ppb	1
Ammonia	SM 4500-NH3-H	20% RPD	0.01 mg/L	1
Nitrate-Nitrite	SM 4500-NO3-I	20% RPD	0.01 mg/L	1

Analysis	Equipment Type and Method	Accuracy (deviation or % deviation from true value	Method Reporting Limits and/or Resolution	Number of Samples/ Measurements per site
Total Persulphate Nitrogen	SM 600/4-79-020 4500-NO3-B	20% RPD	0.025 mg/L	1
Total Phosphorus	SM4500PF	20% RPD	0.005 mg/L	1
Turbidity	SM 2130 B	20% RPD	0.5 NTU	1
Total Suspended Solids	SM 2540D	20% RPD	1.0 mg/L	1
Alkalinity	SM 2320 B	20% RPD	5 mg/L	1
Hardness	SM 2430B	20% RPD	NA	1
Chloride	EPA 300.0	20% RPD	0.1 mg/L	1
Total Organic Carbon in Sediment	PSEP (1986, with 1997 Update) MEL (2008)	20% RPD	0.1%	1
Arsenic	ICP Method 200.8 (EPA 1983) MEL (2008) Pg 134	20% RPD	0.1 mg/Kg	1
Copper	ICP Method 200.8 (EPA 1983) MEL (2008) Pg 134	20% RPD	0.1 mg/Kg	1
Lead	ICP Method 200.8 (EPA 1983) MEL (2008) Pg 134	20% RPD	0.1 mg/Kg	1
Zinc	ICP Method 200.8 (EPA 1983) MEL (2008) Pg 134	20% RPD	5 mg/Kg	1
Polynuclear Aromatic Hydrocarbons (Appendix B-16)	GC/MS Method 8270 (EPA 1996) MEL (2008) Page 164	+/- 50% RPD	40 µg/Kg	1

SM – Standard Method.
NA – Not applicable.
RPD – Relative Percent Difference.

Site Verification

Sampling crews will arrive at the sample site and verify that the following conditions for sampling are met:

1. They are at the correct location (Appendix B, B-1).
2. It is safe to enter.

Protocols listed in these appendices apply to waded streams and are restricted to sites that are less than 25 meters wide at the coordinates (sites less than 500 m long). Larger sites can be waded if shallow but will be sampled using a different protocol, for raft-based work (Merritt 2010).

In-Situ Measurements

Initial (time 1) in-situ measurements should be performed before staff enter upstream of the index station (transect F). After site verification, the crew can start preparing the in-situ instruments. Calibration and use of a Hydrolab instrument to make these measurements will be conducted before the site visit according to the Standard Operating Procedures for Hydrolab® DataSonde® and MiniSonde® Multiprobes (EAP033) (Swanson, 2007). Final (time 2) in-situ measurements are made just before leaving the site. Discharge can be measured at a convenient time (Appendix B, B-3).

Water and Sediment Chemistry

While one crew member is verifying the site, another crew member should collect water samples and sediment samples (Appendix B, B-4, and B-5). The crew member collecting this data should not use sunscreen and mosquito repellent until finished collecting the samples. The crew member must not contaminate the cap or the lip of the jar while collecting samples. Each sample is placed in a pre-labeled bottle ordered from MEL. All samples are stored on ice to maintain a temperature of below 6°C. Samples are sent to MEL within 7 days of collection and must include a chain of custody (COC) form (Appendix J). If the samples are sent to MEL two days or later after collection, a note must be made on the COC to alert the lab that some samples are closer to expiring than others.

Periphyton and Benthos

Depending upon the site-specific conditions, the same person might be assigned to sample benthos and periphyton. Each sample should be collected before staff enter upstream from points of collection. The benthos sampling method is found in Appendix C, C-1. The periphyton sampling method is found in Appendix C, C-3. Samples are sent to a taxonomist certified by the North American Benthological Society for identification.

Habitat

Habitat measurements are made last. At any station within the site, staff measuring habitat should follow later after staff collecting benthos and periphyton. The best allocation of staff and order of tasks depends on site-specific conditions. Methods for measuring physical habitat will include those outlined in SOPs EAP062-EAP067 (Clinton, 2009; Kennedy, 2009; Werner, 2009a, b, c, d), and are listed in Appendices B-6 through B-17.

Assumptions Underlying the Design

This monitoring program design is based on several assumptions. It is assumed that the sites are of least or minimally disturbed condition and that the land use is fairly stable over the long term. The site layout is designed for a long-term monitoring program rather than for a targeted study. While the data collection can proceed using the same protocols for either type of study, a targeted study may choose to use a habitat- specific approach, rather than reach-wide approach, thereby reducing the noise and amplifying the signal created by the conditions under investigation. This study design assumes that general trends in watershed and ecosystem health can be described with the parameters outlined here.

Safety – Field and Laboratory Preservatives

Biological samples collected from streams must be preserved immediately following storage in containers. Inadequate preservation often results in (1) loss of prey organisms through consumption by predators, (2) eventual deterioration of the macroinvertebrate specimens, and (3) deformation of macroinvertebrate tissue and body structures, making taxonomic identification difficult or impossible.

The field preservative used for biological samples is 85% denatured ethanol. The preservative is prepared from a stock standard of 95% denatured ethanol. Flammability, health risks, and containment information are listed on warning labels supplied with the preservative container. Detailed information can be found with the Materials Safety Data Sheets (MSDS) maintained by Ecology's Environmental Assessment Program. Minimal contact with the 95% denatured ethanol solution is recommended.

For the water samples, 0.25 ml of 1:1 hydrochloric acid (HCL) is used as a field preservative for the total phosphorus sample. For total persulphate nitrogen, 0.25 ml of 1:1 Sulfuric acid (H_2SO_4) is used as a field preservative. These jars must be handled carefully.

The preservative used in handling sorted laboratory samples is 95% ethanol (non-denatured). Seventy percent non-denatured ethanol is used for preserving voucher specimens in two dram vials (8 mL). Hazard Communication Training is required for all personnel who come into contact with hazardous materials while conducting program duties.

Safety – Miscellaneous

Field activities should be conducted by at least two persons, especially when in remote streams. A contact person should be designated at Ecology's headquarters office to which field personnel report at the end of each day at pre-designated times (Ecology, 2009).

Staff must carefully plan field activities and obtain permission to access private land. Staff can get land owner's written agreement before or at sampling time.

Decontamination/Prevention of Spread of Aquatic Invasive Species (AIS)

The Environmental Assessment Program of the Washington State Department of Ecology requires that all field work contacting surface water follow procedures outlined in the SOPs (EAP 1-15): *Minimize the Spread of Invasive Species from Areas of Moderate Concern.* These procedures will be followed at the end of each work day or upon leaving a water body before entering another. Some areas are designated to be of "Extreme Concern"; these areas are shown in maps at the following link: http://aww.ecology.ecy.wa.gov/programs/eap/index.html. These are areas where established AIS populations are a particular environmental or economic threat. Here, all equipment will be thoroughly cleaned and inspected to prevent introductions to other waterbodies. Acceptable methods of decontamination include hot water treatment (≥60 ºC) and hydrogen peroxide soak (≥15 min) for contaminated garments and tools.

Chain of Custody (COC) for Biological Samples

A standard chain of custody form is in Appendix J. This form is used for both macroinvertebrate and periphyton samples collected and transferred to the taxonomy lab. The COC for water and sediment chemistry samples is also found in Appendix J. This form must be completed before transferring samples to the lab.

Data Form Review

Staff will use the Status and Trends field teleforms to facilitate field data entry to the database. Examples of the field data forms are found in Appendix E. Before leaving the site, staff should review for completeness and accuracy. Staff should also scan completed forms for missing data and check for errors at the end of each week. The forms will then be filed for subsequent loading to the Status and Trends database.

Lab data for benthic macroinvertebrates will be reviewed and entered into the EIM database by the project lead upon notification from the lab that the data is available on the King County Stream Benthos Database (www.pugetsoundstreambenthos.org/). Lab data for periphyton will be reviewed and entered into an Access database until such time as it can be entered into the EIM database.

Data Management and Common Applications

Data Storage

Habitat data collected by Ecology's Freshwater Ambient Biological Assessment Program are stored in the Status and Trends Monitoring Program Database. Habitat data are loaded through the teleform process. Water and sediment chemistry data are loaded into the EIM database upon receipt of the MEL result summary reports.

The project lead will give a user name and password to the taxonomic labs to allow them to upload macroinvertebrate data to the King County Benthos Database (www.pugetsoundstreambenthos.org/). Once macroinvertebrate data are uploaded, the lab will notify the project lead. The project lead will download the data into the EIM database, under the project ID "BioMonitoringProgram". Voucher specimens for each year will be sent to the Orma J. Smith Museum of Natural History at the College of Idaho in Caldwell. Periphyton data will be sent back to the project lead in an electronic format and stored in an Access database with the Biological Monitoring Program at Ecology Headquarters until the EIM database has the capacity to store it.

Data Analysis

The purpose of data analysis in bioassessment is to describe trends in the biological health of sites over space and time. Two types of mathematical models will be developed based on the data collected in this program. These are: (1) multimetric models (similar to the BIBI) and (2) multivariate models, referred to here as O/E models. O/E models are so named based on the ratio that is computed by this model of the number of taxa observed over the number of taxa expected at a site. Both of these model types will be developed to describe the biological condition of streams in all of Washington's ecoregions. Confidence in these assessments is defined by the natural variability about the reference condition. The model design will include the quantification of spatial, temporal, and procedural variability in reference sites.

Data Preparation

Species Consolidation

A standard list of taxa is constructed for each site. Two rules are used to address common taxonomic issues. (1) The merge rule is used to combine related specimens to their most abundant taxonomic level. Unidentifiable specimens that are damaged or immature are assumed to be representatives of the next highest taxonomic level. (2) The drop rule is applied when the abundance of family level identifications is greater than the abundance of related genera. The generic categories are dropped and combined into the family taxonomic level. For example, identification to the generic level is difficult and sometimes unreliable for the Simuliidae and the Chironomidae. Therefore, taxonomic identifications below the family level are "dropped" for these groups and density estimates for each group are combined.

Data Reduction

Reduction of the taxa list prevents redundancy in taxonomic information, which often leads to complications while running the models. The taxa list may be reduced by eliminating all “rare” taxa, or those that are less than one percent of the total abundance in a sample. Additionally, a secondary reduction of taxa can be made using a rare taxa elimination rule of four percent. Rare taxa are difficult to collect and are not collected evenly across a region, making spatial patterns hard to interpret. Rare taxa elimination helps to clarify spatial patterns in biological communities (Clifford and Stephenson, 1975).

Audits and Reports

The taxonomic contractor and MEL will submit laboratory reports and QA information to the project lead according to the timeline (Table 3). Taxonomic reports will be delivered within 6 months from the date they were submitted and should include taxa lists, taxa counts, and standard and requested metrics for macroinvertebrates and periphyton. MEL will report all laboratory results for water chemistry, soil chemistry, and chlorophyll a to the project manager within 30 days of sample delivery. The reports will include narratives, numerical results, data qualifiers, and costs.

The laboratory will report any problems and associated corrective actions to the project manager who will flag data. These data may be dropped from analysis if the problem can't be addressed.

The project manager is responsible for periodic audit updates to the sampling team as well as for any reports upon request.

Data Verification

Data Verification

Data verification involves examining the data for errors, omissions, and compliance with quality control (QC) acceptance criteria.

Field staff will verify field results after measuring and before leaving site. They will keep field notes to meet the requirements for documentation of field measurements. The field lead will ensure that field data entries are complete and error -free. The field lead will check for consistency within an expected range of values, verify measurements, ensure measurements are made within the acceptable instrumentation error limits, and record anomalous observations. The project manager will verify field data to ensure that:

- Data are consistent, correct, and complete, with no errors or omissions.
- Results of QC samples accompany the sample results.
- Established criteria for QC results were met.
- Data qualifiers are properly assigned where necessary.
- Data specified in the Sampling Process Design were obtained.
- Methods and protocols specified in this QA Monitoring Plan were followed.

The project manager at the taxonomic lab will verify all taxonomic results. The taxonomic lab will:

- Review and report QC checks on instrument performance.
- Review and report case narratives. This includes comparing QC results with method acceptance criteria such as precision data, and laboratory control sample analysis.
- Explain flags or qualifiers assigned to sample results.
- Report the above information to the project manager or lead.

MEL will verify all analytical results. Reports of results and case summaries provide adequate documentation of the verification process. MEL analytical data will be reviewed and verified by comparison with acceptance criteria according to the data review procedures outlined in the *Lab Users Manual* (MEL, 2008). Appropriate qualifiers will be used to label results that do not meet QA requirements.
MEL will:

- Review and report QC checks on instrument performance such as initial and continuing calibrations.
- Review and report case narratives. This includes comparing QC results with method acceptance criteria such as precision data, laboratory control sample analysis, and procedural blanks.
- Explain flags or qualifiers assigned to sample results.
- Report the above information to the project manager or lead.

Data Usability Assessment

Data usability assessment follows verification. This involves a detailed examination of the data package using professional judgment to determine whether the quality objectives have been met. The project manager examines the complete data package to determine compliance with procedures outlined in the QA Monitoring Plan and Standard Operating Procedures. The project manager also ensures that the quality objectives for precision, bias, and sensitivity are met.

Part of this process is an evaluation of precision. Precision will be assessed by calculating relative standard deviations (RSDs) for field and laboratory duplicates. Laboratory duplicates will yield estimates of precision performance at the laboratory only. Field replicates will indicate overall variability (environmental + sampling + laboratory). Acceptable precision performance is outlined in the quality objectives (Table 5).

The project manager will assess completeness by examining the (1) number of samples collected compared to the sampling plan; (2) number of samples shipped and received at the taxonomic contractor in good condition; (3) lab's ability to produce usable results for each sample; and (4) sample results accepted by the project manager.

To analyze data for its usability, the project lead will consider precision, completeness, and documentation of adherence to protocols. Data will also be examined for extremes (i.e., against historical records and against the distributions of these project data). Extreme values will require logical explanations. Identified sources of bias will be described in the annual Short Report.

Acceptance Criteria for Existing Data

Data from past efforts within Ecology may be used to meet the objectives of this program if they are collected using the same protocols outlined in the appendices of this document or if the methods result in comparable data.

Data collected during this program will be used to meet the objectives of this program if they meet the requirements outlined above for data precision, completeness, representativeness, and comparability.

Data collected from outside Ecology will be used to meet the objectives of this program if they meet the requirements of the agency's credible data policy (www.ecy.wa.gov/programs/wq/qa/wqp01-11-ch2_final090506.pdf). This requirement does not apply to non-quality data such as flow or meteorological data. These data must have been collected using the protocols outlined in the appendices of this document or using methods that result in comparable data.

Any data that does not meet these criteria should be flagged as such. Those data will not be used to meet the objectives of this program.

Corrective Actions for Inadequate Data

If discrepancies in the data are found, there are two options for correction depending on when the problem is identified.

1. If the problem is identified before the end of the index period (July 1 to October 15), a review of the protocols and SOPs outlined in the appendices of this document is required. After this review, a repeat site visit may be made to re-collect the sample. This may occur if the data set is incomplete or incorrectly collected. Due to the inter-related nature of chemical and biological conditions, problems identified in the chemical or biological data should be addressed by again collecting the entire suite of chemical and biological analyses parameters. Because the habitat is mostly constant within an index period, if the data in question is related to habitat, only the missing habitat information needs to be collected. Before the second sampling, the investigator must review the SOPs and the appendices of this document to understand the protocols. Equipment should be cleaned and recalibrated and checked for proper function.
2. If the problem is identified after the index period, the data should be flagged and the problem explained in a comment in the database. This will allow the Ecology investigator, as well as external users of this data, to know how this data may be used in projects. If the data is incomplete, or if some data standard was not met, the data will not be used to meet the objectives of the Ambient Biological Monitoring Program.

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Appendix A. Glossary, Acronyms, and Abbreviations

Glossary

A priori: An a priori study is one that is designed independent of knowledge of the study site. This is opposite of a posteriori studies in which design and analysis of data is determined based on what one knows about the site. A priori is more random, whereas a posteriori is not random.

Alluvial Reach: Where the form of the streambed is composed of appreciable quantities of sediments that are transported and deposited in concert with streamflow (Armantrout, 1998).

Ambient: Background or away from point sources of contamination.

Artificial Structures: For this study, potential cover for aquatic vertebrates provided by human-introduced objects.

Bankfull Depth: This is the sum of thalweg wetted depth and bankfull height.

Bankfull Height: Vertical distance between surface of water and bankfull stage. For Status and Trends, this is measured in centimeters.

Bankfull Stage: This stage is delineated by the elevation point of incipient flooding, indicated by deposits of sand or silt at the active scour mark, break in stream bank slope, perennial vegetation limit, rock discoloration, and root hair exposure (Endreny, 2009).

Bankfull Width: Horizontal distance between the bankfull stage on the left bank and the bankfull stage on the right bank. This is measured in tenth of meters.

Bar: Bars are dry or exposed portions of the streambed. For this method, we are only counting bars that are surrounded by water (e.g. mid-channel bars or diamond bars). Bars are lower in elevation than the bankfull stage (islands are higher).

Bedrock Reach: Where the streambed lacks fill material except for temporary storage spots. Bedrock channels generally are confined by valley walls (Montgomery and Buffington, 1998).

Benthic Index of Biotic Integrity (BIBI): An index of the health of the benthic macroinvertebrate community in the streams of Western Washington's Puget Lowland ecoregion (Omernick and Gallant, 1986) by incorporating scores from multiple metrics into a single index number. These "multi-metric" style models were first used for bioassessment of streams by Karr et al. (1991) but have since been created for other locations to address the unique biological and environmental conditions on a level III ecoregional basis (Omernick and Gallant, 1986).

Biological Integrity: "The ability to support and maintain a balanced, integrated, and adaptive community of organisms having a species composition, diversity and functional organization comparable to those of natural habitats within a region" (Karr and Dudley, 1981).

Boulders: For this study, potential cover for aquatic vertebrates provided by rocks over basketball-size.

Braided Reach: Braided reaches are characterized by wide channels containing series of bars. They have a high supply of sediment. They have mobile bed forms. They lack valley confinement and are characterized erodible banks (Montgomery and Buffington, 1993).

Broadleaf Evergreen: Non-coniferous trees that maintain foliage through the seasons. A native example for Washington is the madrona (*Arbutus menziesii*).

Brush (dead): Potential cover for aquatic vertebrates provided by dead pieces of wood that are < 10 cm diameter *or* < 2 m long.

Bryos: For this study, potential cover for aquatic vertebrates provided by non-vascular plants such as mosses that reproduce using spores.

Canopy: The functional definition for this method: Vegetation above 5 meters high within a 10 meter x 10 meter riparian plot.

Cascade Reach: Cascade reaches occur on steep slopes where energy is high. They are characterized by disorganized cobbles and boulders and by confined valley walls (Montgomery and Buffington, 1998).

Clean Water Act: A federal act passed in 1972 that contains provisions to restore and maintain the quality of the nation's waters. Section 303(d) of the Clean Water Act establishes the TMDL program.

Colluvial Reach: Portion of the stream network that is typically in headwaters and typically consists of intermittent or ephemeral flow. In colluvial valleys, expect long-term accumulation of sediment, punctuated by periodic catastrophic erosion (Montgomery and Buffington, 1998).

Colluvial Material: Substrate of mixed sizes. It is recently eroded and transported locally through sheet flow such as avalanche or landslide (Armantrout, 1998).

Conductivity: A measure of water's ability to conduct an electrical current. Conductivity is related to the concentration and charge of dissolved ions in water.

Coniferous: Any of various mostly needle-leaved or scale-leaved, chiefly evergreen, cone-bearing gymnospermous trees or shrubs such as pines, spruces, and firs. This includes larch.

Cover: This can be thought of as the amount of shadow cast by a particular layer alone when the sun is directly overhead. Conceptually remove vegetation from higher layers before estimating.

Dammed Pool: A pool formed by impounded water from complete or nearly complete channel blockage (Armantrout, 1998).

DCE Data Collection Event: Data are indexed using this code which includes the SITE_ID, the date, and the time that the event began. It uses this format:

WAM06600-NNNNNN-dce-20YY-MMDD-HHMM

- **NNNNNN** = the number portion of the SITE_ID.
- **YY** = the last two numeric digits of the year that the event occurred.
- **MM** = the two numeric digits for the month that the event occurred.
- **DD** = the two numeric digits for the day within the month that the event occurred.
- **HHMM** = the military time when the event began.

Deciduous: Non-coniferous trees that shed their leaves annually. Examples include alder, oak, maple, and cottonwood.

Dissolved Oxygen (DO): A measure of the amount of oxygen dissolved in water.

Dry Channel: A habitat unit is designated as dry channel (DC) where flow is subsurface.

Duff: Organic matter in various stages of decomposition on the floor of the forest.

Ecological Quality: The ratio of observed to expected natural taxa (Wright et al., 2000). The observed number of native taxa collected relative to the number of taxa predicted based on a model of reference condition.

Edge Pool: Slow water at the edge of the wetted channel (but connected) where velocity is slow and often in a separate direction from the main flow. Imagine a line along the wetted edge of the wetted channel that is conceptually drawn across the mouth of the edge pool. The depth somewhere behind that line must be at least 30 cm. Edge pools must be at least 0.5 m long behind that imaginary line. Examples of edge pools include backwater pools, secondary channel pools, or alcoves that meet the size criteria described above.

Empirical Model: An empirical model is one that is based on actual data. O/E models are empirical models built on data that comes from the region surrounding the test site. The alternative is a mathematical model that is built based on assumed relative relationships between the parameters.

Eutrophic: Nutrient- rich and high in productivity resulting from human-caused conditions such as fertilizer runoff and leaky septic systems.

Fast Non-turbulent: Habitat unit with smooth, laminar flow that is less deep than in pools. Examples include a sheet or run.

Fast Turbulent: Habitat unit with supercritical flow, with hydraulic jumps sufficient to entrain air bubbles and create whitewater (Armantrout, 1998). Examples include waterfalls, cascades, rapids, and riffles.

Filamentous Algae: Potential cover for aquatic vertebrates provided by long, streaming filaments of microscopic algal cells that often occur in eutrophic water. Not to be confused with macrophytes and flowering aquatic plants.

Floodplain: The part of the valley floor over which a river spreads during seasonal or short-term floods (Small and Witherick, 1986).

Forbs: Broad-leaved herbs other than grass, such as those that grow in a field, prairie, or meadow.

Ground Cover: Vegetation or bare ground below 0.5 m high within a 10 m x 10 m riparian plot.

Habitat Unit: Habitat units are "quasi-discrete areas of relatively homogeneous depth and flow that are bounded by sharp physical gradient. Different types of units are usually in close enough proximity to one another that mobile stream organisms can select the type of unit that provides the most suitable habitat" (Hawkins et al., 1993). For Status and Trends, any unit (with two exceptions) must be at least as long as half their wetted width and they must include the thalweg. Plunge pools and dry channels are the exceptions. Plunge pools can be shorter than half their width. Dry channels have no wetted width and only need to extend 20% of a site's bankfull width (1/100th of the entire stream site's length).

Index Station: This is sometimes called "X". It is the location of the coordinates that represent the site. Normally "X" is located in the middle of the site length (i.e. at major transect F), but sometimes the site position can be adjusted to avoid changes in Strahler stream order or to avoid property where access has been denied.

Intermediate Axis: The diameter of a particle that is neither the longest nor the shortest of mutually perpendicular axes (Bain, 1999; Harrelson et al., 1994).

Island: A dry area between channels. It extends vertically at least as high as the bankfull stage.

Kick: One of the 8 components to a site's composite benthos sample. One kick is collected at each of 8 transects within the site. The area of a kick is 1 ft^2 (0.743 m^2) of stream bottom.

LWD: Large woody debris. This is dead wood that is at least 10 cm diameter and more than 2 meters long.

Least Disturbed Condition (LDC): A term used to describe near reference stream conditions. Least disturbed sites have experienced very little human impact. This is different from minimally disturbed conditions where a site has experienced recent human disturbance, which may be minimal or the site may be recovering from the disturbance.

Left Bank: The side of the stream that is to the left of a person facing downstream.

Live Trees/Roots: Potential cover for aquatic vertebrates provided living woody vegetation that is within the water.

Macrophytes: Potential cover for aquatic vertebrates provided by floating, submerged, or emergent water loving plants and wetland grasses that could provide cover for fish or macroinvertebrates. This category excludes mosses.

Main Channel: Channels in a stream are divided by islands (dry ground that rises above bankfull stage). Main channels contain the greatest proportion of flow. For this method it is called channel number 0.

Major Transect: One of 11 equidistant transects across the length of a site. These are labeled as follows: A (lowest), B, C,….K (highest)

Minimally Disturbed Conditions (MDC): Minimally disturbed conditions refer to near reference conditions in a stream that has experienced recent human disturbance which had little impact on the stream, or in streams that are recovering from past disturbance of varying strength.

Minor Transect: A subset of the thalweg transects. Each of 10 equidistant transects across the length of a site. These are situated mid-way between major transects and are labeled as follows: A5, B5, C5….K5.

Mixed Vegetation: More than 10% of the cover is made up of an alternate type.

Nonpoint source: Pollution that enters any waters of the state from any dispersed land-based or water-based activities. This includes, but is not limited to, atmospheric deposition, surface water runoff from agricultural lands, urban areas, or forest lands, subsurface or underground sources, or discharges from boats or marine vessels not otherwise regulated under the NPDES program. Generally, any unconfined and diffuse source of contamination. Legally, any source of water pollution that does not meet the legal definition of "point source" in section 502(14) of the Clean Water Act.

Nutrient: Substance such as carbon, nitrogen, and phosphorus used by organisms to live and grow. Too many nutrients in the water can promote algal blooms and rob the water of oxygen vital to aquatic organisms.

O/E Models: O/E models (Hawkins et al., 2000) were developed after the multivariate Riverine Invertebrate Prediction and Classification System (RIVPACS) models created in the United Kingdom (Wright, 1995) to describe the biological health of streams. O/E models are so called because the score that results from these models describes the ratio of the number of "Observed" taxa collected at a site to the taxa "Expected" to be encountered at that site. The model is built to describe expected biological conditions of a site based on the habitat characteristics of that site.

Overhanging Vegetation: Potential cover for aquatic vertebrates provided by vegetation that hangs to within 1 m of the water surface. Higher vegetation, e.g., perches for kingfishers or other predators does not count.

Plane-bed Reach: Plane-bed reaches are characterized by a relatively featureless gravel/cobble bed. There is an absence of tumbling flow, but may include glides, riffles or rapids. They lack lateral flow. Bed surfaces are often armored.

Plunge Pool: A pool created by water that passes over an obstruction and drops steeply to scour a basin in the streambed below (Armantrout, 1998). This plunge type of scour pool is coded separately because its length criteria are different. Plunge pools can be shorter than half the wetted width.

pH: A measure of the acidity or alkalinity of water. A low pH value (0 to 7) indicates that an acidic condition is present, while a high pH (7 to 14) indicates a basic or alkaline condition. A pH of 7 is considered to be neutral. Since the pH scale is logarithmic, a water sample with a pH of 8 is ten times more basic than one with a pH of 7.

Point Source: Sources of pollution that discharge at a specific location from pipes, outfalls, and conveyance channels to a surface water. Examples of point source discharges include municipal wastewater treatment plants, municipal stormwater systems, industrial waste treatment facilities, and construction sites that clear more than 5 acres of land.

Pollution: Such contamination, or other alteration of the physical, chemical, or biological properties, of any waters of the state. This includes change in temperature, taste, color, turbidity, or odor of the waters. It also includes discharge of any liquid, gaseous, solid, radioactive, or other substance into any waters of the state. This definition assumes that these changes will, or are likely to, create a nuisance or render such waters harmful, detrimental, or injurious to (1) public health, safety, or welfare, or (2) domestic, commercial, industrial, agricultural, recreational, or other legitimate beneficial uses, or (3) livestock, wild animals, birds, fish, or other aquatic life.

Pool: A habitat unit that has a maximum depth at least 1.5 times its crest depth.

Pool Crest Depth (scour pools): Thalweg depth at the shallowest tail-out (downstream) end of the pool.

Pool Crest Depth (dammed pools): Thalweg depth at the shallowest upstream end of the pool.

Pool Maximum Depth: Deepest thalweg depth in a pool habitat unit.

Pool- Riffle Reach: Pool riffle reaches are typically unconfined, with a laterally oscillating sequence of bars, pools, and riffles. There is local sediment accumulation in discrete bars. (Montgomery and Buffington, 1998).

QC Quality control: A quality control check is a measurement of a standard value to estimate the accuracy of an instrument.

QCCS: A quality control standard suitable for assessing errors of pH and conductivity of dilute neutral pH waters (Metcalf and Peck 1993). A dilute phosphate standard is prepared as a 100-fold dilution KH_2PO_4 and Na_2HPO_4 standard buffer solution (NIST pH buffer 6.865). It has a theoretical pH value of pH 6.98 and calculated conductivity value of 75.3 *µS cm-1 at 25 ˚C.* The stock solution should be kept refrigerated, but the 1:100 dilution should have no detectable change in pH or conductivity for at least 15 months when stored in polyethylene containers between 10-40 ^{o}C.

Reach: A specific portion or segment of a stream.

Regime Reach: Mobile bed forms provide the primary flow resistance. Regime channels are typically low-gradient sand bedded channels. Low slope, frequency and presence of ripples or

dunes throughout the channel bed distinguish regime channels from pool-riffle channels (Montgomery and Buffington, 1993).

Right Bank: The side of the stream that is to the right of a person facing upstream.

Riparian: Relating to the banks along a natural course of water.

RIVPACS (Riverine Invertebrate Prediction and Classification System): RIVPACS is the name of a model developed in the United Kingdom (Wright, 1995) to describe the biological health of streams. These models compare the taxa observed at a site to the taxa that were expected to exist at sites with similar environmental conditions.

Scour Pool: Pool created by the scouring action of current flowing against an obstruction (Armantrout, 1998). Examples include eddy pools, trench pools, mid-channel pools, convergence pools, and lateral scour pools.

Segments: The portions of the stream length over which incremental slope and bearing observations are made. There are at least 20 segments in each site, normally equal in size. Sometimes more segments or unequal segments are inserted to account for obscured lines-of-sight or sharp changes in channel direction.

Side Channels: Channels that contain less flow than the main channels. These are identified and enumerated (1, 2, 3, etc.) as encountered (see the method for thalweg measurements) during the DCE.

Similarity of communities: Communities are considered similar in this context if they are composed of similar taxa and those taxa are present in similar concentrations.

Site: A site is defined by the coordinates provided to a sampling crew and the boundaries established by the site layout method. Typically, the site extends 10 bankfull widths downstream from the coordinates and 10 bankfull widths upstream. The site also includes all riparian plots examined during the *Data Collection Event*. The site consists of many stations at which measurements or samples are collected.

Sonde: The cylindrical portion of the Hydrolab. It contains the sensors.

Station: Any location within the site where an observation is made or part of a sample is collected. For discharge measurements, these are the multiple (up to 20) data collection points along a transect that crosses the stream channel from left wetted margin to the right wetted margin. Margins are included.

Step-Pool Reach: Step-pool reaches consist of coarse materials that are organized into discrete series of steps separating pools containing finer materials. They consist of alternating turbulent flow over steps and tranquil flow in pools. (Montgomery and Buffington 1998).

Stormwater: The portion of precipitation that does not naturally percolate into the ground or evaporate but instead runs off roads, pavement, and roofs during rainfall or snow melt.

Stormwater can also come from hard or saturated grass surfaces such as lawns, pastures, playfields, and from gravel roads and parking lots.

Streamflow: Discharge of water in a surface stream (river or creek).

Surface Waters of the State: Lakes, rivers, ponds, streams, inland waters, salt waters, wetlands and all other surface waters and watercourses within the jurisdiction of Washington State.

Surveyor: The control and display portion of the Hydrolab.

Thalweg: Path of a stream that follows the deepest part of the channel (Armantrout, 1998).

Thalweg Depth: Water depth along the path of the thalweg.

Thalweg Transect: One of 101 equidistant transects across the length of a site. Labeling includes the name of the major transect. For example, the thalweg transects between (and including) major transects A and B would be labeled as follows:
A0, A1, A2, A3, A4, A5, A6, A7, A8, A9, B0 (i.e.., thalweg transect A0 is identical to major transect A).

TMDL Process: Total Maximum Daily Loads (TMDLs) are used to address water pollution issues and place a limit on the amount of each pollutant that enters a water body and has a negative impact on the designated uses of that water body. This process begins with identifying priority areas to implement a water cleanup plan. It then studies the ability of a stream to carry pollutants without diminishing its designated uses. It then implements remedial measures for the pollutants and monitors and adapts management to ensure effectiveness of the remediation.

Transect: A line of study that crosses the direction of flow, divided into intervals where observations are collected.

Turbidity: A measure of water clarity. High levels of turbidity can have a negative impact on aquatic life.

Undercut Banks: For this study, potential cover for aquatic vertebrates provided by banks (at the wetted margin) that extend over deeper water. Fish cover assessment is by area, rather than by length. Therefore undercut banks rarely provide more than 10% cover for a plot.

Understory: For this study, vegetation below 5 meters high but above 0.5 meters high within a 10 meter x 10 meter riparian plot.

Watershed: A drainage area or basin in which all land and water areas drain or flow toward a central collector such as a stream, river, or lake at a lower elevation.

Wetted Width: Farthest horizontal distance between water edge on the left and right sides of a channel.

Woody Debris (dead): Potential cover for aquatic vertebrates provided dead pieces of wood that are ≥ 10 cm diameter and ≥ 2 meters long.

Acronyms and Abbreviations

Following are acronyms and abbreviations used frequently in this report.

Ecology	Washington State Department of Ecology
EIM	Environmental Information Management database
EPA	U.S. Environmental Protection Agency
GPS	Global Positioning System
MEL	Manchester Environmental Laboratory
NIST	National Institute of Standards and Technology
QA	Quality assurance
RSD	Relative standard deviation
SOP	Standard operating procedure
USGS	U.S. Geological Survey
WDFW	Washington Department of Fish and Wildlife

Units of Measurement

cfs	cubic feet per second
ft	feet
km	kilometer, a unit of length equal to 1,000 meters.
m	meter
mL	milliliters

Appendix B. Measurement Procedures

B-1. Site Verification and Layout for Wadeable Streams

Personnel Responsibilities

This method is performed by 2 or more trained staff.

Equipment, Reagents, Supplies

- GPS
- *GPS Positions Form*
- Measuring rod
- 50-m tape
- Flagging
- Permanent marker
- Laser rangefinder
- Soft-lead pencil
- *Site Verification Form*
- Wading gear
- No. 2 pencil
- Maps

Summary of Procedure

The crew first navigates to the site using the coordinates provided by the Master Sample. They then verify that they are at the correct location and determine if the site is suitable for sampling. Next, they define the upper and lower boundaries and they define the transects within the site.

Establish the Data Collection Event

Prior to leaving the office, refer to the *GPS Positions Form* (Figure B-1.1). Enter the SITE_ID portion of the DCE using a number 2 pencil. Enter the Master Latitude and Master Longitude as listed on the Master Sample file. Navigate to the site using the GPS receiver. Upon arrival, record the date (MMDD) and time (military) portion of the DCE. Record the GPS-measured coordinates for the Index Station. Identify the bank at which these coordinates were measured (left and right are interpreted when facing downstream). Also note the precision of the GPS measurement. Other notes on location can also be recorded. Record the turn-by-turn directions taken to reach the site's access point.

Reviewed by (Initials):

Status and Trends Program - GPS Positions Form

Site Number — YY — MMDD — HH — MM

DCE: WAM0600-000018-DCE-2009-0815-09:00

Station	Bank (circle one)	Master Lat dec deg e.g. 47.123456	Master Lon dec deg e.g. 120.123456	GPSLatDD e.g. 47.123456	GPSLonDD e.g. 120.123456	Accuracy	Accuracy Units (ft, EPE, etc.)	Flag
INDEX STATION	L (R)	46.62843992	-122.04131986	46.62844	-122.04132	3	meters	
A0	L (R)			46.62770	-122.04160	3	meters	
B0	L R							
C0	L R							
D0	L R							
E0	L R							
F0	L (R)			46.62844	-122.04132	3	meters	
G0	L R							
H0	L R							
I0	L R							
J0	L R							
K0	(L) R			46.6290	-122.0410	3	meters	
PUTIN	L R							
TAKEOUT	L R							

ALL COORDINATES TO BE RECORDED IN NAD83

Position comments including accuracy: Index station is at transect F0

Directions to access point

From Hwy 706 in Ashford, drive south on FR26 for 10 miles. Hike west about 0.5 miles (no trail).

Draft

Figure B-1.1. The *GPS Positions Form* with example data. Note: Sometimes streams have re-routed after production of the map from which the Master coordinates were generated. In these cases navigate to the closest (most representative) point on the stream.

Determine Site Suitability

After arrival and recording the DCE, determine whether the site is suitable for sampling. Refer to the *Site Verification Form* (Figures B-1.2, and B-1.3).

Reviewed by (Initials):

Status and Trends Program - Site Verification Form 2009

	Site Number	YY	MMDD	HH	MM
DCE:	WAM0600-000018-DCE-	2009-	0701-	09:	00

DCE Start Date	07/01/2009	DCE End Date	07/01/2009

Water Name: Johnson Creek at Johnson Road

Waterbody Type: Saltwater/Brackish ☐ River/Stream ☒ Canal/Ditch ☐ Wetland ☐ Reservoir ☐ Lake ☐ Other ☐

Safe to Sample?: (Y) N

Permission?: (Y) N

Sampled?: (Y) N

Wade or Raft?: (W) R

If not sampled, why not?

Crew	1 (Leader)		Crew Member 2		Crew Member 3		Crew Member 4	
First Name / Last Name	Roberto	Clemente	Joni	Mitchell	David	Jordan	Manon	Rheaume
Organization	Acme Sampling, Inc.		Acme Sampling, Inc		Acme Sampling, Inc		Acme Sampling, Inc.	
Habitat	☒		☒		☒		☒	
Water	☐		☐		☐		☒	
Sediment	☐		☐		☐		☒	
Invertebrates	☐		☐		☒		☐	
Fishing	☒		☒		☒		☐	

Other People?

Montgomery & Buffington Reach Type	Bankfull Width Estimate near Index Station (avg. of 5) (m)	Site Length 20 x BFW but between 150-2000 (m)
	12	240
	Downstream Thalweg Distance (X to A) (m.x)	**Upstream Thalweg Distance (X to A) (m.x)**
	120	120

Colluvial ☐
Alluvial:Braided ☐
Alluvial:Regime ☐
Alluvial:Pool-Riffle ☒
Alluvial:Plane Bed ☐
Alluvial:Step Pool ☐
Alluvial:Cascade ☐
Bedrock ☐

General Notes

The index station was located at transect F0.

Draft

Figure B-1.2. The front side of the Site Verification Form with example data.

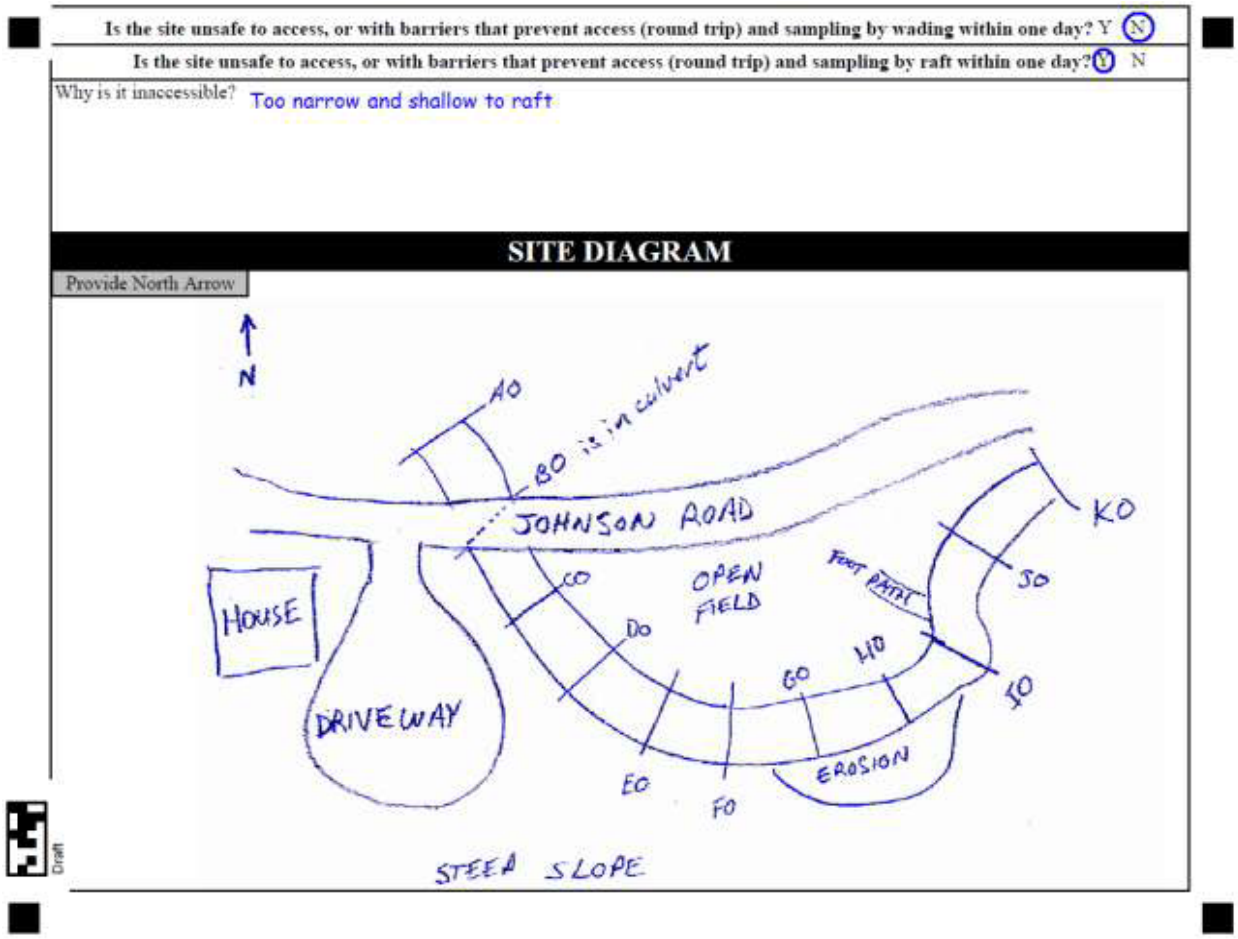


Figure B-1.3. The back side of the Site Verification Form, with example data.

Desktop evaluation of the site was performed earlier according to the method described elsewhere in this protocol. Verify that conditions at the site are truly suitable for sampling during the day of arrival. Complete the appropriate fields in the top third of the front side of the *Site Verification Form*, indicating whether the site is being sampled and, if so, whether this is by wading or by rafting. The site should not be sampled if it is deemed:

- Unsafe to enter.
- To have permission denied by land owners.
- Not a stream or river (e.g., a wetland, lake).
- Not freshwater.
- Within an artificial channel (e.g., canal or ditch).
- Not perennial.
- Not with surface flow for more than 50% of the length.

Record Event Information

Next, on the *Site Verification Form* (Figure B-1.2), record the information below about the data collection event

Crew

Record the names of those who are in the crew. Also note the organization that each staff represents. The crew lead will be recorded in column 1. Staff sampling roles can be recorded later, after the day is done, by using the check boxes provided on the form.

Site

Bankfull Stage

Near the Index Station (X), visually estimate the bankfull stage. This is best done after considerable training. There are at least three good on-line sources of training materials for identifying bankfull stage:

1. http://preview.tinyurl.com/8aabbm (Buffington, 2007)
2. www.dnr.wa.gov/Publications/fp_bfw_video_pt1.wmv

www.dnr.wa.gov/Publications/fp_bfw_video_pt2.wmv (Grizzel, 2008)

3. www.stream.fs.fed.us/publications/bankfull_west.html (Leopold et al, 1995)

Bankfull stage height is *not* a value that gets recorded on the *Site Verification Form*. The crew merely uses their visual estimate to help understand where to measure bankfull width.

Bankfull Width

Using the estimated bankfull level, measure the channel width at each of 5 transects near the Index Station:

1. The Index Station (X)
2. 1 bankfull width upstream from X
3. 2 bankfull widths upstream from X
4. 1 bankfull width downstream from X
5. 2 bankfull widths downstream from X

Record the average (nearest meter) of these 5 bankfull width measurements on the *Site Verification Form* (Figure B-1.2). Width measurements can be made using either a 50-m tape, a measuring rod, or (if the channel is wide) with a laser rangefinder.

Site Length

Sites must be no shorter than 150 m and no longer than 2000 m. Multiply the average bankfull width times 20. This value (whole meters) is the site length for a path that follows the main flow of the river. However, for any site with bankfull width less than 8 meters, the site length will be extended to 150 m; for any site with bankfull width over 100 m, reduce the length to 2000 m. Record the site length on the *Site Verification Form* (Figure B-1.2). Sampling methods for waded streams are restricted to sites that are less than 25 meters wide (less than 500 m long). Larger sites can be waded if shallow, but will be sampled using raft protocols. This rule will allow sampling on large streams to be accomplished within a single work day.

Relative position of the Index Station (X) within the site

The index station (X) is normally located at the middle of the site (i.e. at major transect F). On the *Site Verification Form* (Figure B-1.2), record the distance (tenths of meters) from X to the bottom of the site (i.e., to major transect A) and the distance from X to the top of the site (i.e., to major transect K). This distance is measured along the thalweg channel. Unless there is a reason to adjust the position of X, the distance will be equal to half the site length, in each direction. The relative position of X can be adjusted for reasons such as to keep the top or bottom of the site in lands where permission has not been denied, or to keep from changing Strahler (1957) stream order (at the 1:100,000 scale), or to account for barriers such as lakes. The location of the Index Station's coordinates can never be changed. These are pre-defined by the survey design. Although the site position can change relative to X (called "sliding" the site), the site must always contain X.

Bed Form

Assess the site for its predominant reach type according to Montgomery and Buffington (1993, 1997). Review the source materials hot-linked in the references to help understand the differences between bed forms. These references discuss details and provide images of examples. First decide whether the site is predominated by a reach that is colluvial, alluvial, or bedrock. Colluvial streams have a low chance of being sampled by this Status and Trends program, because we are limiting our sample to perennial streams. Bedrock streams are confined locations with little depositional material present. Most streams sampled will be alluvial. Next, if the site is predominantly alluvial, decide which one of the following sub-classifications can be used to describe the site.

- cascade
- step-pool
- plane-bed
- pool-riffle
- regime
- braided

Place an X in the appropriate box of the *Site Verification Form* (Figure B-1.2) to describe the predominant bed form within the site. Refer to the references (Montgomery and Buffington, 1993, 1997, 1998) for help. Figures B-1.4 and B-1.5 might help.

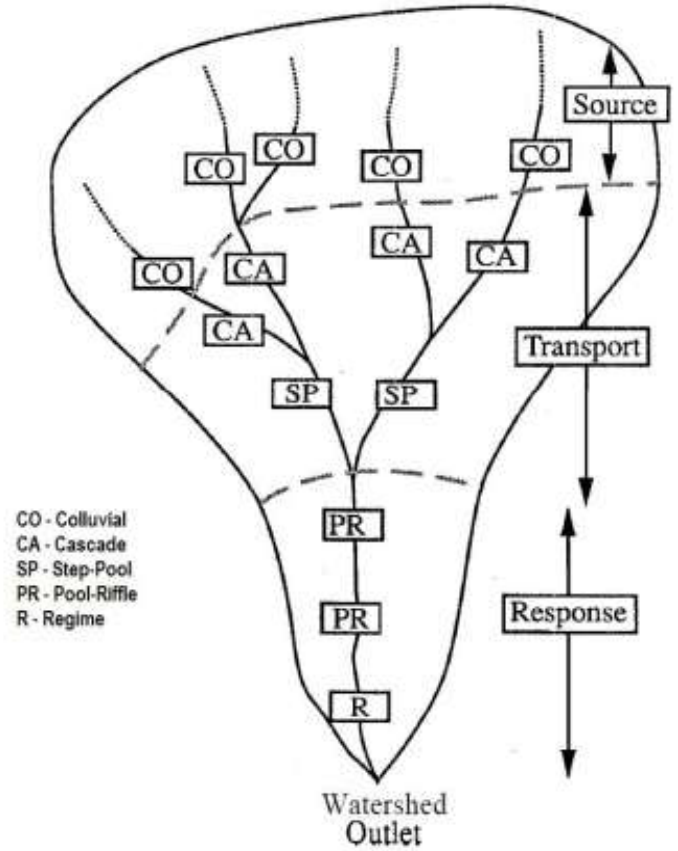


Figure B-1.4. Idealized positions (aerial view) of bed form types within a watershed. Modified from figure 22 of Montgomery and Buffington (1993).

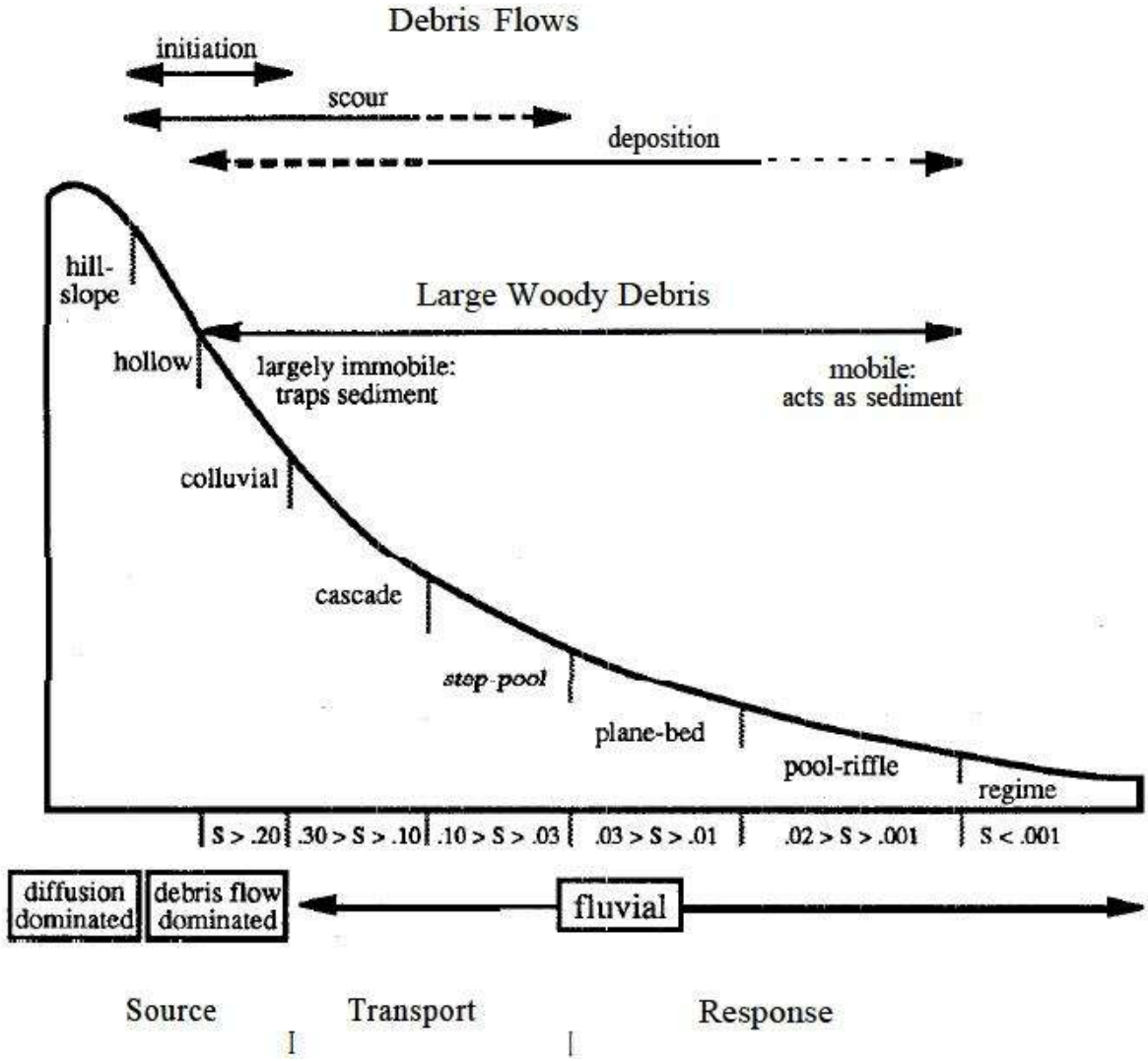


Figure B-1.5. Idealized positions (plan view) of bed form types within a watershed (from figure 16 of Montgomery and Buffington (1993)).

Layout the Reach

There are 3 types of transects that define the stream site (Table B-1.1): thalweg transects, major transects, and minor transects.

Thalweg Transects

Conceptually divide the stream site length using 101 transects which are perpendicular to the thalweg. These are called Thalweg Transects. They occur at regular intervals (0.2 bankfull widths). Thalweg transects, except for those that are also major transects (see below), do not need to be marked. Thalweg transects are useful in concept for describing relative positions within the site.

Major transects

Use orange flagging and a permanent marker to mark each of the 11 equidistant major transects. The lowest is *transect A0*, the highest is *transect K0*. Measure the distance between transects using either a 50-m tape or a measuring rod, by following the thalweg of the stream. The distance between flags should be 1/10th of the site length or (or 2 times the estimated bankfull width at the index station).

Minor Transects

Ten minor transects occur mid-way between the 11 major transects (Table B-1.1). The distance between major and minor transects is 1/5th the site length (or 1 bankfull width). Minor transects don't need to be marked.

Table B-1.1. The relative position of all transects on a stream site.

Station	Thalweg Transect	Major Transect	Minor Transect	Distance from Bottom (Bankfull Widths*)
A0	Yes	Yes		0
A1	Yes			0.2
A2	Yes			0.4
A3	Yes			0.6
A4	Yes			0.8
A5	Yes		Yes	1
A6	Yes			1.2
A7	Yes			1.4
A8	Yes			1.6
A9	Yes			1.8
B0	Yes	Yes		2
B1	Yes			2.2
B2	Yes			2.4
B3	Yes			2.6
B4	Yes			2.8
B5	Yes		Yes	3
B6	Yes			3.2
B7	Yes			3.4
B8	Yes			3.6
B9	Yes			3.8
C0	Yes	Yes		4
C1	Yes			4.2
C2	Yes			4.4
C3	Yes			4.6
C4	Yes			4.8
C5	Yes		Yes	5
C6	Yes			5.2
C7	Yes			5.4
C8	Yes			5.6
C9	Yes			5.8

Station	Thalweg Transect	Major Transect	Minor Transect	Distance from Bottom (Bankfull Widths*)
D0	Yes	Yes		6
D1	Yes			6.2
D2	Yes			6.4
D3	Yes			6.6
D4	Yes			6.8
D5	Yes		Yes	7
D6	Yes			7.2
D7	Yes			7.4
D8	Yes			7.6
D9	Yes			7.8
E0	Yes	Yes		8
E1	Yes			8.2
E2	Yes			8.4
E3	Yes			8.6
E4	Yes			8.8
E5	Yes		Yes	9
E6	Yes			9.2
E7	Yes			9.4
E8	Yes			9.6
E9	Yes			9.8
F0	Yes	Yes		10
F1	Yes			10.2
F2	Yes			10.4
F3	Yes			10.6
F4	Yes			10.8
F5	Yes		Yes	11
F6	Yes			11.2
F7	Yes			11.4
F8	Yes			11.6
F9	Yes			11.8
G0	Yes	Yes		12
G1	Yes			12.2
G2	Yes			12.4
G3	Yes			12.6
G4	Yes			12.8
G5	Yes		Yes	13
G6	Yes			13.2
G7	Yes			13.4
G8	Yes			13.6
G9	Yes			13.8
H0	Yes	Yes		14
H1	Yes			14.2
H2	Yes			14.4
H3	Yes			14.6
H4	Yes			14.8

Station	Thalweg Transect	Major Transect	Minor Transect	Distance from Bottom (Bankfull Widths*)
H5	Yes		Yes	15
H6	Yes			15.2
H7	Yes			15.4
H8	Yes			15.6
H9	Yes			15.8
I0	Yes	Yes		16
I1	Yes			16.2
I2	Yes			16.4
I3	Yes			16.6
I4	Yes			16.8
I5	Yes		Yes	17
I6	Yes			17.2
I7	Yes			17.4
I8	Yes			17.6
I9	Yes			17.8
J0	Yes	Yes		18
J1	Yes			18.2
J2	Yes			18.4
J3	Yes			18.6
J4	Yes			18.8
J5	Yes		Yes	19
J6	Yes			19.2
J7	Yes			19.4
J8	Yes			19.6
J9	Yes			19.8
K0	Yes	Yes		20

* For very small or very large sites (with length of 150 m or 2000 m), the transect spacing is 1/100th of the site length, and might not be 0.2 bankfull widths.

Record Coordinates

Refer to *GPS Positions Form* (Figure B-1.1). Record the GPS-measured coordinates at the bottom of the site (transect A0), and at the top of the site (transect K0). Note the bank at which the GPS was used and the accuracy of the measurements. You might also record coordinates for other major transects too, but this is not required for the waded streams.

B-2. In-Situ Measurements in Wadeable Streams

Purpose and Scope

This method explains how to collect in-situ measures of temperature, dissolved oxygen, pH, and conductivity at wadeable streams for the Status and Trends Program using a multi-probe (e.g., Hydrolab Minisonde). It requires adherence to calibration techniques discussed elsewhere in this procedure.

Personnel Responsibilities

This method is performed by 1 or more persons. This method is applied at every DCE, at the start *and* end of the sampling event. Staff performing this method must have been trained.

Equipment, Reagents, Supplies

- No. 2 pencil.
- Chemistry and Sampling Form.
- Completed Calibration Form.
- Hydrolab, components, maintenance kit (Swanson, 2007).
- Hydrolab Manuals (Hach 1999; 2006a; 2006b).

Summary of Procedure

Calibrate the instrument before sampling according to calibration methods discussed elsewhere in this protocol. Check the instrument after calibration, but before *and* after sampling, according to those same methods. Measure the stream twice.

Verify Quality Control

Prior to Sampling

Ensure that the calibrations and that QC checks have been performed according to methods described elsewhere in this protocol. Circle "Yes" on the top section of the Chemistry and Sampling Form (Figure B-2.1) for each sensor that checked out. Proceed with measurements using sensors that are within criteria.

After Sampling

Post-sampling calibration checks can be performed during the following day. Be sure to qualify data that were collected preceding calibration checks that failed to meet criteria.

Measure

Measure pH, water temperature, dissolved oxygen, oxygen percent saturation, and specific conductivity twice during a DCE - once at the start and once at the end. Record time (military) and location (thalweg transect). Both sets of in-situ measurements should usually be made near the middle elevation of the site, on the main channel. Measurements should *always* be taken within the boundaries of the site (between transects A0 and K0).

Place the probes into the stream and let them thermally equilibrate to the stream temperature. This might take 3-5 minutes. Then hold the sensors so that they are just below the surface of the water, and completely immersed. Avoid any turbulence. Make sure that readings are stable. On the Chemistry and Sampling Form (figure B-2.1), record temperature (˚ C, nearest tenth), pH (pH unit, nearest hundredth), specific conductivity (µS/cm at 25˚ C, nearest tenth), dissolved oxygen (mg/L, nearest tenth), and oxygen percent saturation (nearest tenth).

Reviewed by (Initials): JS

Status and Trends - Chemistry and Sampling Form

DCE: WAM0600 - 000001 - DCE - 2009 - 0701 - 13:25
(Site Number; YY; MMDD; HH : MM)

IN SITU WATER QUALITY CALIBRATION

Operator: Kurt Gowdy Unit #: 1

				Flag
T	Temp probe was checked vs NIST	(Yes)	No	F1
DO	Sensor Calibrated	(Yes)	No	F2
pH	Sensor Calibrated and Checked	(Yes)	No	F3
Cond	Sensor Calibrated and Checked	(Yes)	No	F3

Notes (in situ)
F1 - T - Checked pre-season
F3 - pH, Cond, calibrated and checked this morning at the lab.
F2 - DO calibrated streamside - Winkler comparison collected for July

Sed:%Gravel 0 %Sand 50 %Fines 50

In Situ Chemistry

Start Location (e.g. F0): F 0

Time1 13:45 hrs
Temp1 6.3 deg C
pH1 6.94 pH Units
DO1 10.9 mg/L %Sat1 102.5
Cond 27.8 uS/cm @ 25C

End Location (e.g. K0): F 0

Time2 19:50 hrs
Temp2 7.0 deg C
pH2 7.00 pH Units
DO2 10.4 mg/L %Sat2 99.5
Cond 27.9 uS/cm @ 25C

Sample	Primary Sample: No. of Jars	Duplicate Sample: No. of Jars (or ITIS for Fish Spp)	Destination	Tracking No. (if shipped)	Flag
TPN	1	0	MEL		
Tot P	1	0	MEL		
Cl	1	0	MEL		
Turb	1	0	MEL		
Sed PAH	1	0	MEL		
Sed Metals*	1	0	MEL		
Benthos	2	0	ETOH Shed at office		F4
Fish Spp1	1	ITIS: 159700	University Lab	FedEx: 835651465756	F5
Fish Spp2	1	ITIS: 167234	office lab for review under microscope		F6
Fish Spp3					

Water Sample Location (e.g. A5): F 0

Sample Notes (explain flags):
F4 - Invertebrate sample could not fit into a single jar. Two jars are taped together.
F5 - Lamprey ammocoetes in zipped bag on ice — Vert Collection Form "Jar" #1
F6 - Riffle sculpin in jar of ETOH - verify it is not a reticulate sculpin. — Vert Col. Frm Jar #2

Draft

*Sediment Metals jar includes sample material to be analyzed for TOC
Note: Use standard Manchester Environmental Lab forms for tracking water and sediment samples.

Figure B-2.1. The Chemistry and Sampling Form, with examples of in-situ data records.

B-3. Estimating Discharge in Wadeable Streams

Purpose and Scope

This method describes how to collect field data necessary for estimating instantaneous discharge (in cubic feet per second) during each data collection event for Washington's Status and Trends Program. Data will be used to categorize streams according characteristics of the stream at the time of sampling (approximate base-flow conditions). It applies to waded streams.

Personnel Responsibilities

One person performs this activity. Staff performing this method must have been trained.

Equipment, Reagents, Supplies

- Soft-lead pencil.
- *Chemistry and Sampling Form.*
- Distance measuring device (50-m tape or measuring rod).
- Flow Meter.
- Wading rod (top setting).
- Orange or other neutrally buoyant object.
- 5-gallon bucket.
- Stop watch.
- Field notebook.
- Calculator.

Discharge is normally measured near the index station ("X") where there is uniform (non-turbulent) flow. It can be done at any time during the data collection event, except prior to sampling for water, sediment, or invertebrates. This method references Sullivan (2007) and Kaufmann (2006). For operation of the flow meter, refer to the manufacturer's manual (e.g., Marsh-McBirney, 1990).

For calculation of discharge (from meter data), refer to USBR (2001). The QWIN program created by Larsen (2005) applies the USBR Midsection Method. Example data and on-line access to QWIN are provided by PSNS&IMF (2006). Conditions may not always allow for use of flow meters. In these cases, discharge can be estimated through alternate methods. Examples of alternate methods are:

- Velocity of floats (e.g., oranges).
- Time to fill a bucket of known volume (e.g., discharge off of a hanging culvert).
- Retrieval of data from co-located gages or models.

Velocity-Area Method

Use the Discharge Worksheet (Figure B-3.1), located on the back of the Chemistry and Sampling Form. Discharge can be calculated by converting widths to units of feet (nearest tenth) and applying the QWIN program (Larsen, 2005) as provided by PSNS&IMF (2006).

Flow Location - Thalweg Station (e.g. A5) : F 0 Flow Meter (Model / Unit #) Marsh McBirney Flo-Mate 2000 / 2

Discharge Worksheet

Flow Meter Zeroed Out? (Y) N Wetted Width: 1026 cm

Cell	Tape Distance Left to Right (cm)	Wetted Depth (ft.x)	Velocity (ft.xx/s)	Notes
01	10	0	0	Left edge of water
02	64	0.5	0.65	
03	118	1.0	0.49	
04	172	1.0	0.76	
05	226	1.3	2.29	
06	280	1.2	0.51	
07	334	1.8	1.66	
08	388	2.0	3.77	
09	442	2.1	3.61	
10	496	2.3	3.26	
11	550	2.2	3.21	
12	604	2.3	2.37	
13	658	1.7	1.74	
14	712	1.5	1.10	
15	766	1.0	0.95	
16	820	0.7	0.66	
17	874	0.5	0.54	
18	928	0.3	0.32	
19	982	0	0.15	
20	1036	0	0	Right wetted margin

Describe Alternate Method: N/A

Draft

Discharge: 8 8 . 2 cfs

Figure B-3.1. The Discharge Worksheet with example field data in blue.

Establish the cross-section

The velocity-area method is used at a transect location within the site that has the most of these conditions (based on Rantz and others, 1982).

- The stream is straight.
- Depths are mostly greater than 0.5 ft (15 cm).
- Velocities are mostly greater than 50 ft/s (0.15 ms/s).
- Local habitat is not a pool.
- The channel is “U-shaped”.
- The streambed is uniform and free of objects that cause turbulence.

Preference should be given to locations that are close to "X". Record the name of the nearest *Thalweg Transect*. Pull a measuring tape taught, perpendicular to the stream, and parallel to the stream surface (a measuring rod can be used for narrow streams). Record the tape value (cm) at the left wetted margin and at the right wetted margin. Subtract the left value from right value to determine the transect's wetted width. Record wetted width (cm) on the worksheet (Figure B-3.1).

Measure Distance, Depth, and Velocity

Define about 15-20 equally-spaced stations across the stream (possibly fewer for very small streams). To determine spacing between stations, divide the width by 20 and round up to a convenient number. Stations should not be closer than 10 cm to each other, even if this results in less than 15 stations. The first station is located at the left wetted margin, and the last station is located at the right wetted margin.

Use a calibrated flow meter equipped with a top-setting wading rod that has depth increments in tenths of feet. At each station, record the tape distance (cm) from left to right. Record the water depth (nearest 0.1 ft). Place the sensor 60% of the distance down from the surface (Figure B-3.2). Measure and record water velocity (nearest 0.01 f/s).

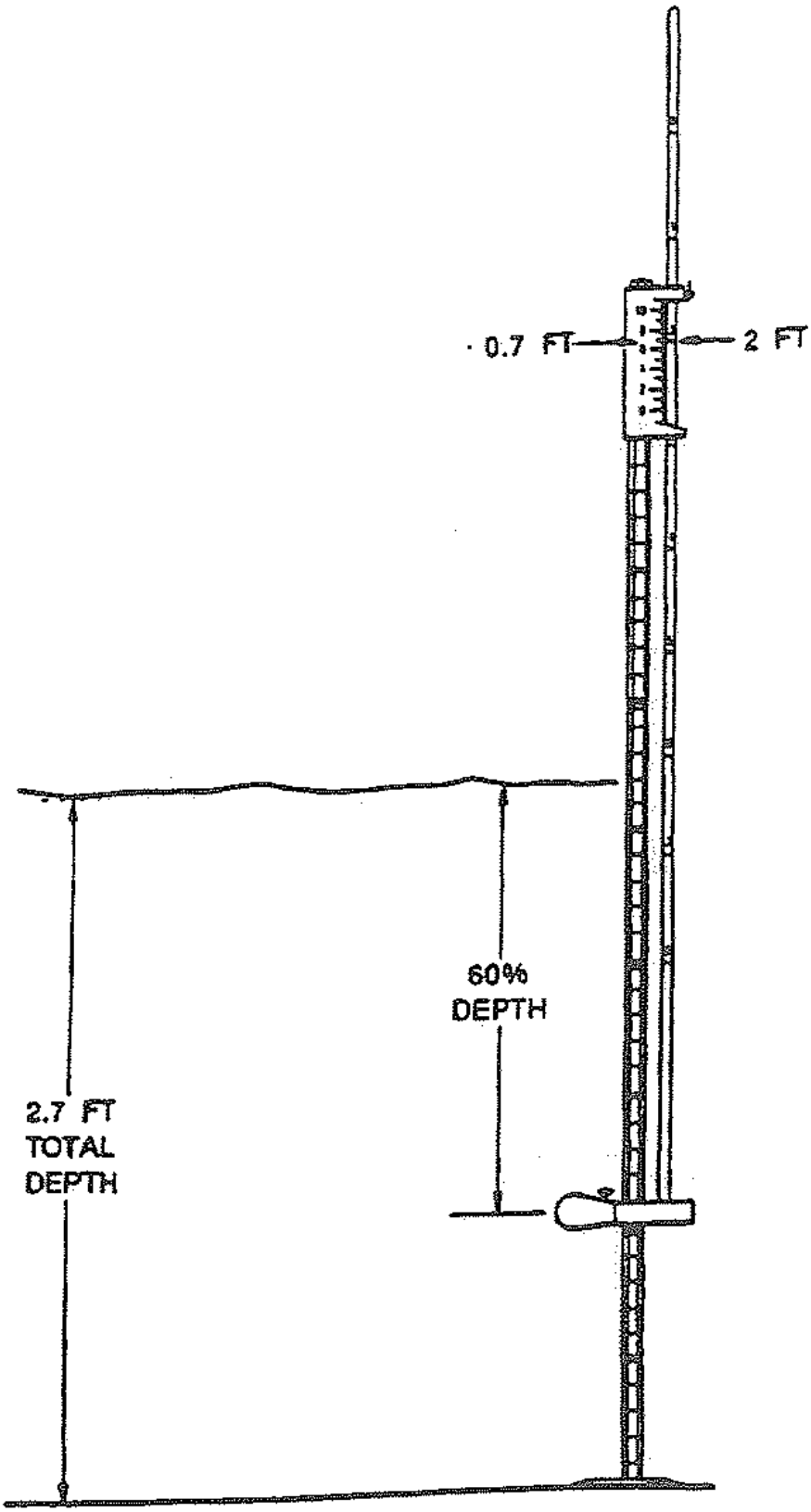


Figure B-3.2. Setting the wading rod at 60% depth when at a station that is 2.7 meters deep.

Alternate Methods

Conditions may not always allow for use of flow meters. In these cases, discharge can be estimated through alternate methods such as those listed below. At the bottom of the *Discharge Worksheet* (Figure B-3.1), record which alternate method was used. If an alternate method other than the timed float was used, record the estimated discharge value (cfs).

Examples of alternate methods are:

- Velocity of timed floats (e.g., oranges, plastic golf balls, sticks).
- Time to fill a bucket of known volume (e.g., discharge off of a hanging culvert).
- Obtaining collocated gage data or model data.

Timed Float

In the absence of a current meter, you can time the transport of a neutrally-buoyant object to estimate velocity. This method is similar to the Velocity-Area method because discharge is calculated as the product of water velocity and the stream cross-sectional area. Requirements are:

- The object must float, but very low in the water.
- The object must be small enough to *not* drag bottom.
- The segment must be somewhat strait, uniform, and non-turbulent.
- The segment must be long enough that it takes 10 to 30 seconds for the float to pass.

Velocity

Compute water column velocity in a field notebook. Determine the average time (seconds) for the float to travel the segment. Repeat twice more, each time releasing at a different position across the width of the stream. Compute an average for the three times. Measure the length of the segment (ft). Divide the segment length by the average time of travel (s) to estimate surface velocity (ft/s). Multiply this surface velocity by 0.85 to estimate water column velocity.

Cross-Sectional Area

Compute cross-sectional area (ft^2) in a field notebook. This can be done by summing the area for at least two trapezoids to approximate the cross section of the stream (Figure B-3.3). These should be centered on the thalweg.

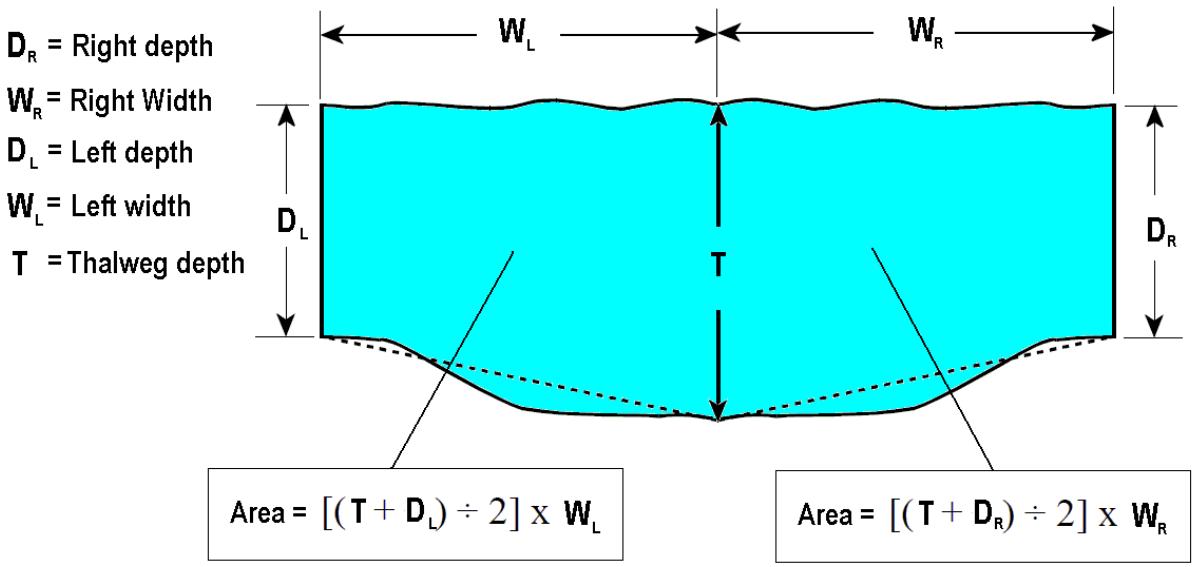


Figure B-3.3. The cross-sectional area of a stream segment as estimated by calculating the area of component trapezoids, centered on the thalweg.

Measure area for one or more cross-sections and average them. Only one cross-section is adequate if the channel is relatively uniform through the segment. Otherwise measure at these cross-sections:

- Near the top of the segment.
- Near the middle of the segment.
- Near the bottom of the segment.

If there is little change in channel width or depth, obtain measurements from a single "typical" cross-section within the segment.

Discharge

Convert cross-sectional area calculations to square feet (1 m^2 = 10.76391 ft^2). Then multiply water column velocity (ft/s) times the cross-sectional area (ft^2) to determine stream discharge for the site. Record this discharge (cfs) on the bottom of the discharge worksheet (Figure B-3.1). Also record "timed float" next to "Describe alternate method".

Timed Bucket-Filling

Place a bucket or other container with known volume below the discharge. Time how long it takes to fill the container. Repeat at least three times. Calculate discharge as the volume of the container divided by the average time to fill it. Use Table B-3.1 to translate from gallons or milliliters to cubic feet. Record discharge (cfs) at the bottom of the Discharge Worksheet (Figure B-3.1). Also record that the alternate method was by use of a timed bucket-filling.

Table B-3.1. Conversions for gallons or milliliters to cubic feet.

Gallons	Milliliters	Cubic Feet
0.1321	500	0.0176573
0.2642	1,000	0.0353147
1	3,785	0.1336806
5	18,927	0.6684028
7.480519	28,317	1

Existing Data

Record discharge (cfs) at the bottom of the Discharge Worksheet (Figure B-3.1) and note the data source, next to “Describe alternate method”.

B-4. Water Sampling in Wadeable Streams

Purpose and Scope

This method explains how to collect water samples at wadeable streams for the Status and Trends Program. Grab-water samples are collected for these 5 parameters:

1. Total Phosphorus (by colorimetric analysis)
2. Total Nitrogen (by persulfate method).
3. Chloride.
4. Turbidity.
5. Total Suspended Solids.

Personnel Responsibilities

This method is performed by 1 or more persons. This method is applied at every DCE, at the start of the sampling event. Staff performing this method must have been trained. Staff performing this method should refrain from the use of sun screen or insect repellent.

Equipment, Reagents, Supplies

- No. 2 pencil
- *Chemistry and Sampling Form*
- *Laboratory Analyses Required Form*
- Gloves - Non-powdered nitrile
- Garbage bag
- Cooler, Ice
- Sample Tags (with laboratory-assigned sample numbers)
- Jar#26 for TP (Figure B-4.1)
- Jar#19 for TPN (Figure B-4.2)
- Jar#22 for Cl (Figure B-4.3)
- Jar#22 for TURB (Figure B-4.4)
- Jar#23 for TSS (Figure B-4.3)

Figure B-4.1. The 60-mL jar for total phosphorus water samples.
(Manchester Laboratory Index # 26).

Figure B-4.2. The 125-mL jar for total persulfate nitrogen water samples.
(Manchester Laboratory Index # 19).

Figure B-4.3. The 500-mL jar. (Manchester Laboratory Index # 22).
There is one each for chloride and turbidity water samples (if not using a Turbidimeter).

Figure B-4.4. The 1000-mL jar for total suspended solids water samples.
(Manchester Laboratory Index # 23).

Summary of Procedure

This method is based on Joy (2006). Collect water samples first, before other in-stream activities. Fill stream water into each of 5 polypropylene jars (Figure B-4.5). Immediately chill the samples in the dark. Deliver them to the MEL or to its courier within 7 days of collection (Table B-4.1).

Table B-4.1. Handling requirements for water samples.

Parameter	Jar Size	Holding Time Before Analysis [A]
TP [b]	60 mL	28 days
TPN [c]	125 mL	28 days
CL	500 mL	28 days
TURB	500 mL	48 hours
TSS	1000 mL	7 Days

a All water samples need to be chilled (0-6 ˚C).
b The jar for total phosphorus is pre-acidified with 0.25 mL 1:1 HCl.
c The jar for total persulfate nitrogen is pre-acidified with 0.25 mL 1:1 H2SO4.

Pre-Sampling Preparation

Sample Numbers, Jars, and Tags

Prior to the field season, the Department of Ecology's Environmental Assessment Program (EAP) will help to prepare by performing two tasks. (1) EAP will obtain sample numbers from the MEL by submitting a *Pre-sampling Notification Form* (MEL, 2008). (2) EAP will order the sample jars and labels from the Manchester Environmental Laboratory by submitting the *Sample Container Request Form* (MEL, 2008).

Collecting Samples

Water samples are collected near the index station (near transect F0). Samples can be collected elsewhere within the site if necessary, but only if they can be collected from below transect K0 and above transect A0.

For each jar, remove the lid just before sampling. Be careful not to contaminate the cap, neck, or the inside of the bottle.

Chloride (CL), Turbidity (TURB, and Total Suspended Solids (TSS)

Stand in relatively deep, relatively non-turbulent water. Face upstream. Hold the container near its base, reach out in front of yourself as far a possible, and plunge it (mouth down) below the surface to about elbow depth. Make sure not to disturb sediments. Leave enough headspace so that the laboratory staff can mix the sample.

Total Phosphorus (TP) and Total Persulfate Nitrogen (TPN)

Stand in relatively deep, relatively non-turbulent water. Face upstream. Hold the container upright and place the lid over the mouth so that only a small area forms an opening (Figure B-4.5). Immerse the jar 15 cm (6 in) while holding the cap in position with your fingers as far away from the opening as possible. Carefully monitor the filling rate to avoid overfilling.

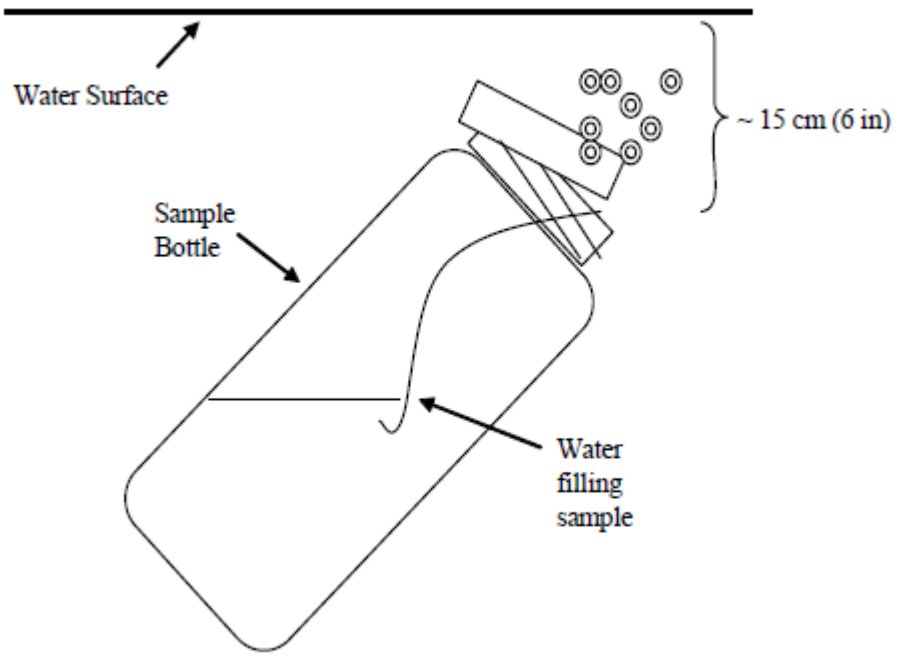


Figure B-4.5. Cap position during sample collection, when using jars that have been pre-filled with acid preservative (TPN and TP).

Field Processing

Labeling

For each jar, loop the string of the sample tag over the lid until it is secure. Use at least three loops for 250 mL jars and at least two loops for 500 mL jars or larger. Check the tag to ensure that the Master Sample SITE_ID number is recorded (this is the 6-digit number that follows "WAM06600-" on the SITE_ID. Also record the data and time that appears in the DCE. Use waterproof ink or pencil. An example tag is provided in Figure B-4.6.

PROJECT = STATUS & TRENDS in Puget STR
SITE_ID: WAM06600 - 0 0 0 0 0 1
MONTH 0 7 DAY 0 1 2009
dce TIME 1 3 : 2 5
PERSON WHO SAMPLED Kurt Gowdy
MEL SAMPLE # 0 0 0 0 0 0 0 - 9 6
PARAMETER Total P

PROJECT = STATUS & TRENDS in Puget STR
SITE_ID: WAM06600 - 0 0 0 0 0 1
MONTH 0 7 DAY 0 1 2009
dce TIME 1 3 : 2 5
PERSON WHO SAMPLED Kurt Gowdy
MEL SAMPLE # 0 0 0 0 0 0 0 - 9 6
PARAMETER TPN

PROJECT = STATUS & TRENDS in Puget STR
SITE_ID: WAM06600 - 0 0 0 0 0 1
MONTH 0 7 DAY 0 1 2009
dce TIME 1 3 : 2 5
PERSON WHO SAMPLED Kurt Gowdy
MEL SAMPLE # 0 0 0 0 0 0 0 - 9 6
PARAMETER TSS

PROJECT = STATUS & TRENDS in Puget STR
SITE_ID: WAM06600 - 0 0 0 0 0 1
MONTH 0 7 DAY 0 1 2009
dce TIME 1 3 : 2 5
PERSON WHO SAMPLED Kurt Gowdy
MEL SAMPLE # 0 0 0 0 0 0 0 - 9 6
PARAMETER TURB

PROJECT = STATUS & TRENDS in Puget STR
SITE_ID: WAM06600 - 0 0 0 0 0 1
MONTH 0 7 DAY 0 1 2009
dce TIME 1 3 : 2 5
PERSON WHO SAMPLED Kurt Gowdy
MEL SAMPLE # 0 0 0 0 0 0 0 - 9 6
PARAMETER Chloride

Figure B-4.6. Example tags for water chemistry jars.

Storage

If you are sampling close to your vehicle, immediately place samples in a cooler of ice. If you are sampling remotely, maintain samples in a sealed black garbage bag that is immersed in the stream and in the shade until you are ready to leave the site (Peck and others 2006). Place samples into a cooler of ice as soon as possible.

Chemistry and Sampling Form

Complete the relevant portions of the *Chemistry and Sampling Form* (Figure B-4.7) including how many jars were collected for each parameter. Also make sure that the header information is complete and that the sample location is specified according to the code for the closest Thalweg Transect. If the samples are delivered through a commercial courier, be sure to record the courier's tracking number for the shipment.

Reviewed by (Initials): JS

Status and Trends - Chemistry and Sampling Form

DCE: WAM0600 - 000001 - DCE - 2009 - 0701 - 13:25
(Site Number; YY; MMDD; HH : MM)

IN SITU WATER QUALITY CALIBRATION

Operator: Kurt Gowdy Unit #: 1

				Flag
T	Temp probe was checked vs NIST	(Yes)	No	F1
DO	Sensor Calibrated	(Yes)	No	F2
pH	Sensor Calibrated and Checked	(Yes)	No	F3
Cond	Sensor Calibrated and Checked	(Yes)	No	F3

Notes (in situ)
F1 - T - Checked pre-season
F3 - pH, Cond, calibrated and checked this morning at the lab.
F2 - DO calibrated streamside - Winkler comparison collected for July

Sed:%Gravel 0 %Sand 50 %Fines 50

In Situ Chemistry

Time1 13:45 hrs Start Location (e.g. F0): F0
Temp1 6.3 deg C
pH1 6.94 pH Units
DO1 10.9 mg/L %Sat1 102.5
Cond 27.8 uS/cm @ 25C

Time2 19:50 hrs End Location (e.g. K0): F0
Temp2 7.0 deg C
pH2 7.00 pH Units
DO2 10.4 mg/L %Sat2 99.5
Cond 27.9 uS/cm @ 25C

Sample	Primary Sample: No. of Jars	Duplicate Sample: No. of Jars (or ITIS for Fish Spp)	Destination	Tracking No. (if shipped)	Flag
TPN	1	0	MEL		
Tot P	1	0	MEL		
Cl	1	0	MEL		
Turb	1	0	MEL		
Sed PAH	1	0	MEL		
Sed Metals*	1	0	MEL		
Benthos	2	0	ETOH Shed at office		F4
Fish Spp1	1	ITIS: 159700	University Lab	FedEx: 835651465756	F5
Fish Spp2	1	ITIS: 167234	office lab for review under microscope		F6
Fish Spp3					

Water Sample Location (e.g. A5): F0

Sample Notes (explain flags):
F4 - Invertebrate sample could not fit into a single jar. Two jars are taped together.
F5 - Lamprey ammocoetes in zipped bag on ice — Vert Collection Form "Jar" #1
F6 - Riffle sculpin in jar of ETOH - verify it is not a reticulate sculpin. — Vert Col. Frm Jar #2

Draft

*Sediment Metals jar includes sample material to be analyzed for TOC
Note: Use standard Manchester Environmental Lab forms for tracking water and sediment samples.

Figure B-4.7. The Chemistry and Sampling Form, with example data for this method highlighted in yellow.

B-5. Sediment Chemistry Sampling

Purpose and Scope

This method explains how to collect a site-composite sediment sample in the Status and Trends Program. Stream sites are sampled within day-long data collection events (DCEs). Each composite sample will be composed of scoops taken from 3 separately-located shallow-water stations in the site. To detect the presence of select contaminants at the site, the sample will be analyzed for metals (copper, lead, zinc, arsenic) and a standard list of polynuclear aromatic hydrocarbon (PAH) compounds. To help interpret the results, the sample will also be analyzed for total organic carbon and grain size composition.

Personnel Responsibilities

This sampling method is performed by 1 person in the field. Pre-sampling pre-cleaning activities should be performed by staff familiar with MSDS and safety procedures. This method is applied at every DCE. Staff performing this method must have been trained. Staff performing this method should refrain from the use of sun screen or insect repellent.

Equipment, Reagents, Supplies

- Stainless steel bowl with sealed stainless steel cover (about 6)
- Stainless steel spoon (about 6)
- Turkey baster (3)
- No. 2 pencil
- *Chemistry and Sampling Form*
- *Laboratory Analyses Required Form*
- Gloves - Non-powdered nitrile
- Cooler, Ice
- Garbage Bag
- Sample Tags (with laboratory-assigned sample numbers)
- Jars (provided by the laboratory)
- Aluminum foil
- Wash bottle (labeled) with Liquinox or Alconox
- Wash bottle (labeled) with acetone (pesticide grade)
- Wash bottle (labeled) with 10% nitric acid
- MSDS
- Personal protective gear as specified by the MSDS
- Fume hood

Summary of Procedure

These procedures are derived from methods described in Johnson (1997), Blakley (2008a), and Manchester Environmental Laboratory (2008). Surface sediment samples are collected for laboratory analyses (Table B-5.1). The crew will analyze grain sizes of the sample while in the field. Samples are chilled immediately and delivered to the laboratory or a courier within 14 days of collection, but normally within 24 hours.

Table B-5.1. Laboratory methods for sediment chemistry samples.

Analysis	Analytical Method	Reporting Limits[a]
TOC	PSEP (1986, with 1997 update) MEL (2008) page 120	0.1%
As	ICP Method 200.7 (EPA 1983) [b] MEL (2008) page 134	0.1 mg/Kg, dry
Cu	ICP Method 200.7 (EPA 1983) [b] MEL (2008) page 134	0.1 mg/Kg, dry
Pb	ICP Method 200.7 (EPA 1983) [b] MEL (2008) page 134	0.1 mg/Kg, dry
Zn	ICP Method 200.7 (EPA 1983) [b] MEL (2008) page 134	5 mg/Kg, dry
PAHs	GC/MS Method 8270 (EPA 1996) [b] MEL (2008) page 164	40 µg/Kg, dry

a Find method quality objectives in Blakley (2008b), Table 8.
b Find method quality objectives in Meredith and Furl (2008), Table 2.

Pre-sampling Preparation

Sample Numbers, Jars, and Tags

Prior to the field season, the Department of Ecology's Environmental Assessment Program (EAP) will help to prepare by performing two tasks: (1) EAP will obtain sample numbers from the MEL by submitting a *Pre-sampling Notification Form* (MEL, 2008), and (2) EAP will order the sample jars and labels from the Manchester Environmental Laboratory by submitting the *Sample Container Request Form* (MEL, 2008).

Pre-Cleaning

On a weekly basis, field crews will pre-clean enough sampling tools to last a week (including spares). These are the pre-washing steps for each bowl, spoon, and turkey baster:

1. Wash in Liquinox detergent.
2. Rinse (three times) with tap water.
3. Wash with 10% nitric acid.
4. Rinse with deionized water.
5. In fume hood, rinse with acetone.

6. In fume hood, rinse with hexane.
7. In fume hood, air dry.
8. Wrap with aluminum foil (shiny side of foil facing out).
9. Properly dispose of hazardous wastes.

Sampling

Use pre-cleaned equipment that has been wrapped in foil. Collect the sample by compositing from each of three suitable locations near the point of arrival. A suitable location will have these characteristics:

- Surface sediment is dominated by particles < 2 mm diameter,
- Water depth above the sediment is < than 30 cm,
- The station is always under water throughout the day.
- Anywhere within 10 bankfull widths (upstream or downstream) of the index station.
- Upstream from where staff have entered the stream channel.

Using a stainless steel spoon, sample the top 2 cm of sediment and place it into a stainless steel mixing bowl. Let the sample settle, then use the turkey baster to remove overlying water. Homogenize the sample by stirring with the spoon until a uniform color and texture is achieved.

PAH Sample

Transfer sediment with the spoon into an 8-oz glass, wide-mouth jar (described as #6 by MEL, 2008). Screw the lid closed, label it, and place it into a cooler of ice. Record sample information on the *Chemistry and Sampling Form* (Figure B-5.1). Use the appropriate column depending upon whether documenting the primary sample or a duplicate for the date. For each sample: record “MEL” for the “Destination,” and record “1” for “No. Jars”.

Metals and TOC Sample

Transfer sediment with the spoon into an 8-oz glass, wide-mouth jar (described as #6 by MEL, 2008). Screw the lid closed, label it, and place it into a cooler of ice. Record sample information on the *Chemistry and Sampling Form* (Figure B-5.1). Use the appropriate column depending upon whether documenting the primary sample or a duplicate for the date. For each sample: record “MEL” for the “Destination,” and record “1” for “No. Jars”.

Grain Size Analysis: gravel (>2 mm), sand (2-16 mm), and fines (silt/clay/muck)

Visually estimate the composition of the sediment in the composite sample. Record percent gravel, percent sand, and percent fines on the *Chemistry and Sampling Form* (Figure B-5.1). Gravel should never be a dominant component of the sample. Sand is gritty to the touch whereas fines are not. You can check the feel of residue in the bowl for the presence of sand or fines only after sample jars have been filled and placed on ice.

Reviewed by (Initials): JS

Status and Trends - Chemistry and Sampling Form

DCE: WAM0600 - 000001 - DCE - 2009 - 0701 - 13:25 (Site Number; YY; MMDD; HH : MM)

IN SITU WATER QUALITY CALIBRATION

Operator: Kurt Gowdy Unit #: 1

				Flag
T	Temp probe was checked vs NIST	Yes	No	F1
DO	Sensor Calibrated	Yes	No	F2
pH	Sensor Calibrated and Checked	Yes	No	F3
Cond	Sensor Calibrated and Checked	Yes	No	F3

Notes (in situ)
F1 - T - Checked pre-season
F3 - pH, Cond, calibrated and checked this morning at the lab.
F2 - DO calibrated streamside - Winkler comparison collected for July

Sed:%Gravel 0 %Sand 50 %Fines 50

In Situ Chemistry

Start Location (e.g. F0): F0

Time1 13:45 hrs
Temp1 6.3 deg C
pH1 6.94 pH Units
DO1 10.9 mg/L %Sat1 102.5
Cond 27.8 uS/cm @ 25C

End Location (e.g. K0): F0

Time2 19:50 hrs
Temp2 7.0 deg C
pH2 7.00 pH Units
DO2 10.4 mg/L %Sat2 99.5
Cond 27.9 uS/cm @ 25C

Sample	Primary Sample: No. of Jars	Duplicate Sample: No. of Jars (or ITIS for Fish Spp)	Destination	Tracking No. (if shipped)	Flag
TPN	1	0	MEL		
Tot P	1	0	MEL		
Cl	1	0	MEL		
Turb	1	0	MEL		
Sed PAH	1	0	MEL		
Sed Metals*	1	0	MEL		
Benthos	2	0	ETOH Shed at office		F4
Fish Spp1	1	ITIS: 159700	University Lab	FedEx: 835651465756	F5
Fish Spp2	1	ITIS: 167234	office lab for review under microscope		F6
Fish Spp3					

Water Sample Location (e.g. A5): F0

Sample Notes (explain flags):
F4 - Invertebrate sample could not fit into a single jar. Two jars are taped together.
F5 - Lamprey ammocoetes in zipped bag on ice — Vert Collection Form "Jar" #1
F6 - Riffle sculpin in jar of ETOH - verify it is not a reticulate sculpin. — Vert Col. Frm Jar #2

Draft

*Sediment Metals jar includes sample material to be analyzed for TOC
Note: Use standard Manchester Environmental Lab forms for tracking water and sediment samples.

Figure B-5.1. The Chemistry and Sampling Form, with fields for sediment chemistry data highlighted.

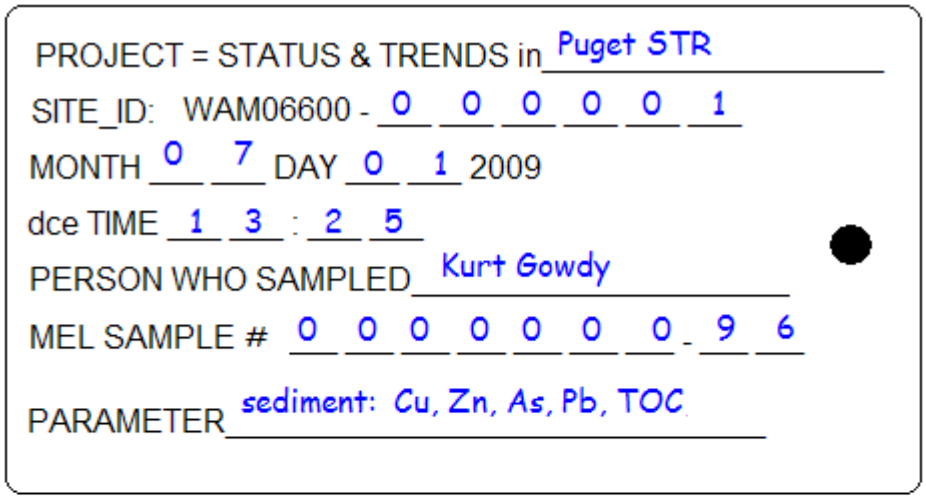
Field Processing

Storage

If you are sampling close to your vehicle, immediately place samples in a cooler of ice. If you are sampling remotely, maintain samples in a sealed black garbage bag that is immersed in the stream and in the shade until you are ready to leave the site. Place samples into a cooler of ice as soon as possible.

Labeling

For each jar, loop the string of the sample tag over the lid until it is secure. Use at least three loops for 250 mL jars and at least two loops for 500 mL jars or larger. Check the tag to ensure that the Master Sample SITE_ID number is recorded (this is the 6-digit number that follows "WAM06600-" on the SITE_ID. Also record the data and time that appears in the DCE. Use waterproof ink or pencil. An example set of tags is provided in Figure B-5.2.



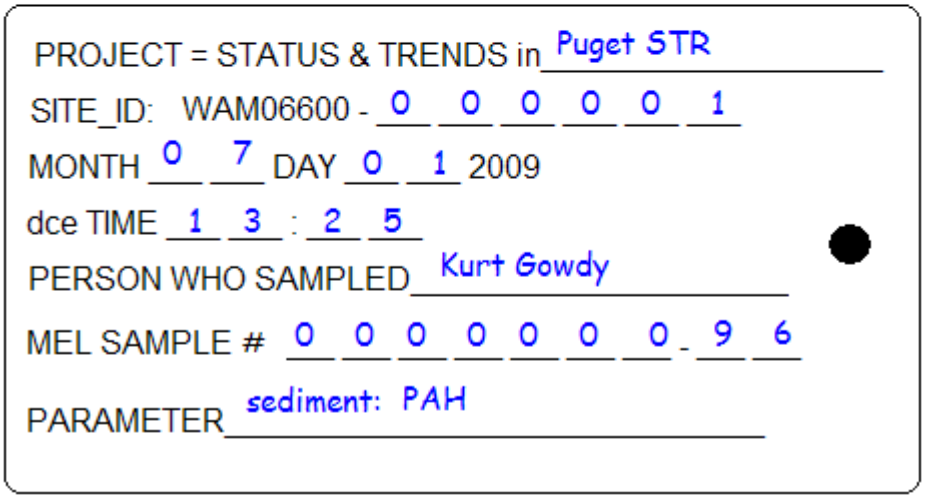


Figure B-5.2. Example tags for sediment chemistry sample jars.

Sample Delivery

Sample Crews will complete a *Laboratory Analyses required Form* (MEL, 2008) and submit it with samples at a drop-off location designated by the MEL.
The Laboratory Analyses Required (LAR) form will serve as a chain-of-custody form. Sediment chemistry samples have a 14-day field holding time (while at 0-6 ˚C). Normally though, they can be submitted with water samples which need to be submitted within 24 hours of collection.
Figure B-5.2. Example tags for sediment chemistry sample jars.

B-6. Bank Measurements at Major Transects in Waded Streams

Purpose and Scope

This method explains how to collect measurements for the Status and Trends Program at each of 11 equidistant transects at each site. Measurements in this procedure will be restricted to one main channel. Instruments included on the procedure include distance measuring devices (e.g., measuring rod, laser rangefinder, 50-m measuring tape), and hand-levels.

Personnel Responsibilities

This method is performed by 2 persons. This method is applied at every DCE, at each major transect. Staff performing this method must have been trained.

Equipment, Reagents, Supplies

- No. 2 pencil.
- Measuring rod.
- 50-m tape.
- Laser rangefinder.
- Hand level.
- Clinometers.
- Calculator.

Summary of Procedure

Refer to the *Major Transect Form* (Figures B-6.1 and B-6.2). At each of the major Transects (A0- K0), assess the main channel. Measure these channel characters: bankfull width, wetted width, bar width, bankfull height, and bank instability. Describe flags.

BANK		
		Flag
Wetted Width XXX.X m	3.2	
Bar Width XX.X m	0	
Bankfull Width XXX.X m	5.4	
R Bankfull Height cm	35	
L BankfullHeight cm	32	
LB Instability %	50	F1
RB Instability %	0	

Figure B-6.1. A portion of the Major Transect Form, with example data for this method.

Flag	Comments
F1	slumping bank with cow prints

Figure B-6.2. A portion of the Major Transect Form, with an example flag qualifier.

Channel Dimensions

Bankfull Stage

At the transect, visually estimate the bankfull stage. This is best done after considerable training. There are at least four good on-line sources of training materials for identifying bankfull stage:

1. http://preview.tinyurl.com/8aabbm (Buffington, 2007).
2. www.dnr.wa.gov/Publications/fp_bfw_video_pt1.wmv. www.dnr.wa.gov/Publications/fp_bfw_video_pt2.wmv (Grizzel, 2008).
3. www.stream.fs.fed.us/publications/bankfull_west.html (Leopold et al., 1995)
4. www.fgmorph.com/fg_3_5.php (Endreny, 2009)

Use this visual estimate to help understand where to measure bankfull width and bankfull height.

Bankfull Width

After locating the bankfull stage at each bank, measure the bankfull width (Figure B-6.3) to the nearest tenth of a meter. Record this value on the *Major Transect Data Form* (Figure B-6.1). Width measurements can be made using either a 50-m tape, a measuring rod, or (if the channel is wide) with a laser rangefinder.

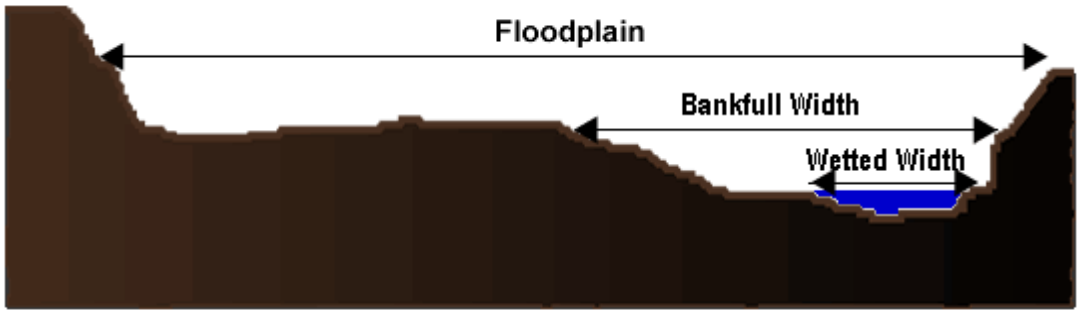


Figure B-6.3. Diagram of widths at the transect (Modified from Endreny 2009).

Wetted Width

Observe the wetted margins of the channel. On the *Major Transect Data Form* (Figure B-6.1), record the wetted width (or horizontal distance between these margins) to the nearest tenth of a meter. Do *not* subtract for bars.

Bar Width

Using the measuring rod, measure the width of each bar within the wetted channel. Record the sum (nearest tenth of a meter) for bar width.

Bankfull Height

Bankfull height is measured using a surveyor's rod with hand level or clinometer. On the *Major Transect Form* (Figure B-6.1), record bankfull height data in whole centimeters. Record the right bankfull height and left bankfull height (Figure B-6.4).

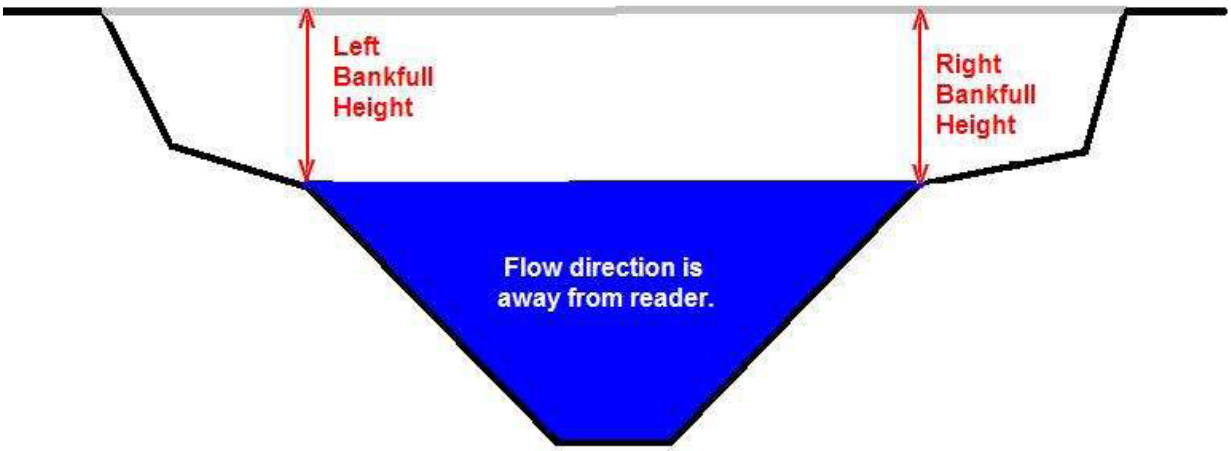


Figure B-6.4. Diagram of the left and right bankfull height measurements.

Bank Instability

For waded streams, evaluate how much of a 10-m length of each bank (centered on the primary transect) is unstable. Limit your observations of bank stability to the portion of the bank at and below the bankfull stage. A bank is unstable if it has eroding or collapsing banks. It may have the following characteristics:

- Sparse vegetation on a steep surface.
- Tension cracks.
- Sloughing.

On the *Major Transect Form* (Figure B-6.1), record right bank instability (%) and left bank instability (%).

B-7. Substrate and Depth Measurements at Major Transects in Waded Streams

Purpose and Scope

This method explains how to measure substrate characteristics for the Status and Trends Program at each of 11 equidistant transects at each site. Measurements in this procedure will be restricted to one main channel. This method must be preceded by the Major Transects Method. Instruments included on the procedure include distance measuring devices (e.g., measuring rod, or 50-m measuring tape, caliper), leveling device (hand level or clinometer) and a10-cm PVC ring.

Personnel Responsibilities

This method is performed by 2 persons. This method is applied at every DCE, at each major transect. Staff performing this method must have been trained.

Equipment, Reagents, Supplies

- No. 2 pencil.
- measuring rod.
- 50-m tape.
- PVC ring.
- hand-level.
- Clinometers.
- Calculator.

Summary of Procedure

Refer to the *Major Transect Data Form* (Figure B-7.1). At each of the major Transects (A0-K0), assess the main channel (channel number 0). Record these characters at each of 11 equidistant stations across the bankfull width:

- Wetted depth.
- Bankfull depth.
- Substrate type code.
- Embeddedness.

Station Location

Identify the position along the transect. Example stations along a transect would be:

1. **left bank** – at the left bankfull stage.
2. **.1** – 10% distance across the channel.

3. **.2** – 20% distance across the channel.
4. **.3** – 30% distance across the channel.
5. **.4** – 40% distance across the channel.
6. **.5** – half way across the channel.
7. **.6** – 60% distance across the channel.
8. **.7** – 70% distance across the channel.
9. **.8** – 80% distance across the channel.
10. **.9** – 90% distance across the channel.
11. **right bank** – at the right bankfull stage.

On the Major Transect Form (Figure B-7.1), insert data for depths, substrate type and embeddedness next to each station code. Describe flags (Figure B-7.2). Examples of data can be found in Figures B-7.1, B-7.2, and B-7.3.

SUBSTRATE					
	Wet Depth	BF Depth XXX CM	Size Class	Embd. 0-100%	Flag
left bank	-13	0	SA	100	
.1	-2	11	GF	90	
.2	0	13	GC	50	
.3	9	22	CB	25	
.4	17	30	SB	5	
.5	20	33	CB	25	
.6	17	30	CB	10	
.7	9	22	GC	10	
.8	0	13	WD	90	F1
.9	-1	12	FN	100	
right bank	-13	0	SA	100	

Figure B-7.1. Part of the Major Transect Form with example data for this method.

Flag	Comments
F1	WD = partially buried Douglas fir log, about 60 cm diameter

Figure B-7.2. Part of the Major Transect Form with example flag descriptions.

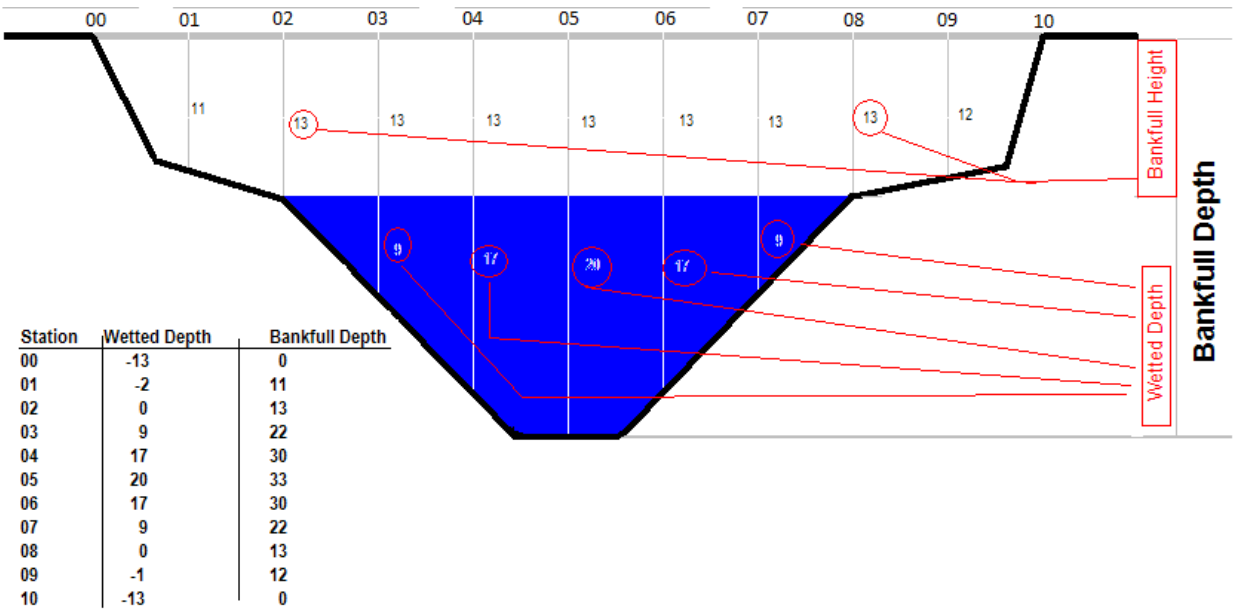


Figure B-7.3. Transect diagram showing example data for wetted depth, bankfull depth, and bankfull height. The bankfull depth equals the wetted depth plus average bankfull height.

Station Depth

For each station, record depth in whole centimeters. This should be the easiest to measure of either wetted depth or bankfull depth. The bankfull depth equals the wetted depth plus average bankfull height. Therefore, if you know one type of depth and the mean bankfull height, you also know the other type of depth.

Substrate Type

After recording depth, estimate the substrate particle type at the front of the measuring rod, where it rests on the surface of the streambed. Estimate the size class of that particle based on the intermediate axis length. Record the substrate type code. The choices are listed in Table B-

7.1. For fine gravel, coarse gravel and cobble use calipers to measure the intermediate axis length of the particle and confirm your estimate of size. For larger sizes, use the measuring rod to confirm your estimate.

Particles smaller than 100 mm are evaluated using a 10 cm ring surrounding the sample point. All particles within the ring are evaluated for size and embeddedness, not just the point. Record the estimated average for surface substrate within the ring.

Table B-7.1. Substrate codes, types, and sizes.

Code	Type	Range Size	Size Gauge
RS	Bedrock (smooth)	>4 m	larger than a car
RR	Bedrock (rough)	>4 m	larger than a car
RC	Concrete/Asphalt	>4 m	larger than a car
XB	Large Boulder	1-4 m	meter stick to car
SB	Small boulder	>250 mm–1 m	basketball to meter stick
CB	Cobble	>64 mm–250 mm	tennis ball to basketball
GC	Gravel, coarse	>16 mm to64 mm	marble to tennis ball
GF	Gravel, fine	>2 mm to16 mm	ladybug to marble
SA	Sand(2-16 mm)	>0.06 mm to2 mm	gritty to ladybug
FN	Fines(silt/clay/muck)	<0.06 mm	non gritty
HP	Hardpan- hardened fines	any size	
WD	Wood	any size	
OT	Other (doesn't fit choices above)	any size	

Embeddedness

At each station, touch the nearest particle to foot of the measuring rod then look at it. Estimate embeddedness (%). This is the fraction of a particle's surface that is surrounded by (embedded in) sand or finer sediments (≤ 2 mm). By default, sand or fines are 100% embedded. By default, bedrock is 0% embedded.

Particles smaller than 100 mm are evaluated using a 10 cm ring surrounding the sample point. All particles within the ring are evaluated for size and embeddedness, not just the point. Record the estimated average for surface substrate within the ring.

B-8. Shade Measurements at Major Transects in Waded Streams

Purpose and Scope

This method explains how to measure shade for the Status and Trends Program at each of 11 equidistant transects at each site. Measurements in this procedure will be restricted to one main channel. This method must be preceded by the Major Transects Method. Instruments included on the procedure include a distance measuring device (e.g., measuring rod), and a convex densiometer (modified according to Mulvey et al. (1992)).

Personnel Responsibilities

This method is performed by 1 person. This method is applied at every DCE, at each major transect. Staff performing this method must have been trained.

Equipment, Reagents, Supplies

- No. 2 pencil.
- *Major Transect Form.*
- Measuring rod or 50-m tape.
- Modified convex densitometer.

Summary of Procedure

Refer to the *Major Transect Form* (Figure B-8.1). At each of the major Transects (A0-K0), assess the main channel (channel number 0). Use a convex densiometer (Lemmon, 1957) that has been modified according to Mulvey et al. (1992; Figure B-8.2); it has 17 intersections.

DENSIOMETER MEASUREMENTS

(0-17Max)

		Flag			Flag
CenUp	5		CenR	9	
CenL	0		Left	0	
CenDwn	4		Right	17	

Figure B-8.1. Densiometer portion of The Major Transects Form, with example data.

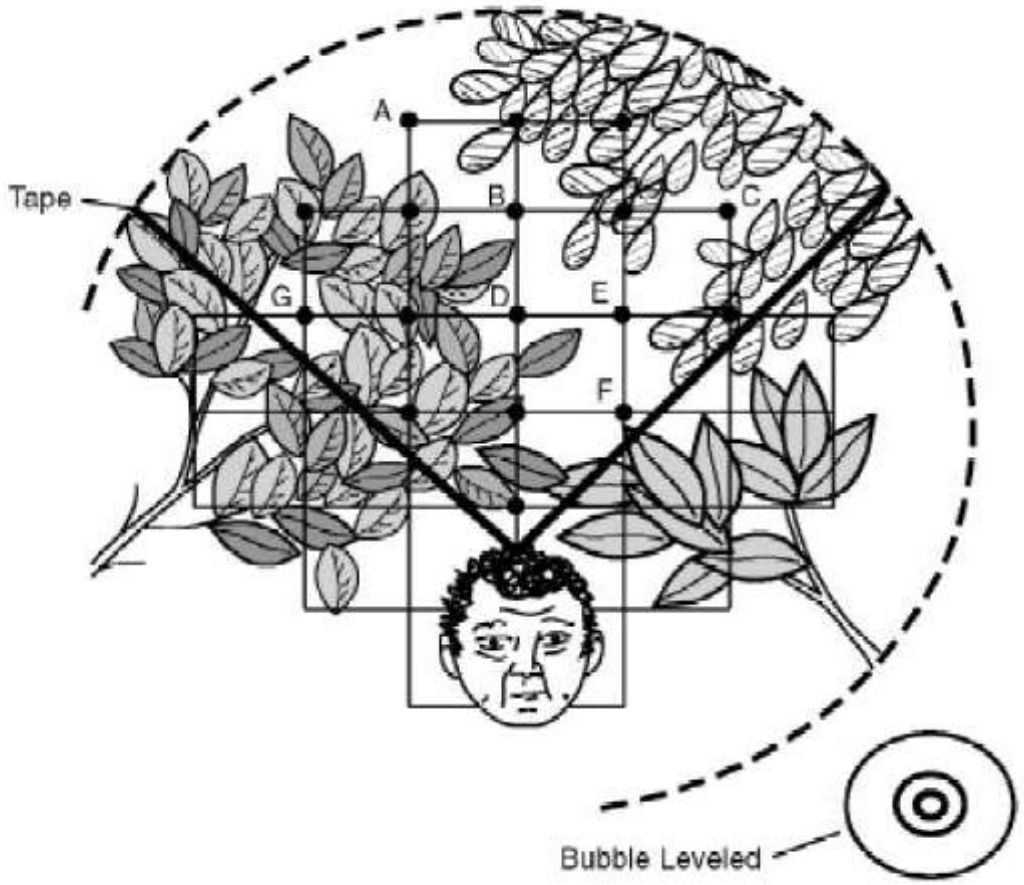


Figure B-8.2. An example reading from a modified convex densiometer. It shows 10 of 17 intersections with shade (a score of "10"). Note the proper positions of the bubble and head reflection (From Mulvey et al. 1992).

Record how many of the 17 cross-hairs have shade over them. Do this for each of six directions on the major transect (Figure B-8.3):

- Facing the left bankfull stage.
- Facing the right bankfull stage.
- Bankfull channel center, facing upstream.
- Bankfull channel center, facing right.
- Bankfull channel center, facing downstream.
- Bankfull channel center, facing left.

At each wetted station, hold the densiometer 30 cm above the water. At each dry station, hold the densiometer 30 cm above the ground. Bank readings should be able to detect shade from riparian understory vegetation such as ferns.

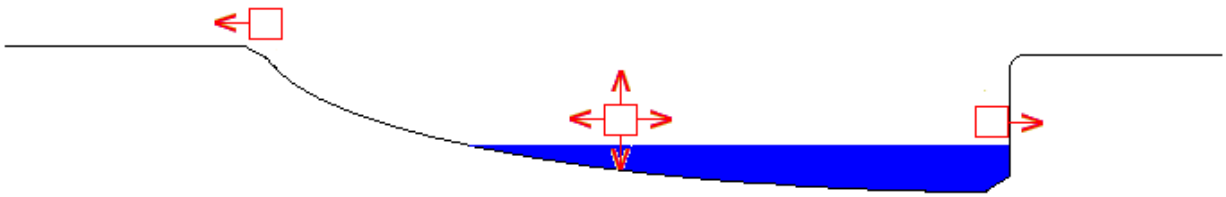


Figure B-8.3. Stations for densiometer measurement on each major transect. The densiometer is held level, and 30 cm above water for wet stations and 30 cm above ground for dry stations.

B-9. Estimating Fish Cover at Major Transects in Waded Streams

Purpose and Scope

This method explains how to estimate fish cover for the Status and Trends Program at each of 11 equidistant transects at each site. Measurements in this procedure will be restricted to one main channel. This method must be preceded by the Major Transects Method. Instruments included on the procedure include a distance measuring device (e.g., measuring rod).

Personnel Responsibilities

This method is performed by 1 person. This method is applied at every DCE, at each major transect. Staff performing this method must have been trained.

Equipment, Reagents, Supplies

- No. 2 pencil
- *Major Transect Form*
- measuring rod or 50-m tape

Summary of Procedure

This method is derived from that of Peck et al. (2006). Within the main channel, evaluate 11 plots (Figure B-9.1) with these characteristics:

- Centered at each major transect.
- Extends 5 meters upstream of each transect.
- Extends 5 meters downstream of each transect.
- Beneath the wetted surface.
- Visually assess the percentage of the water surface that has fish cover provided by each of 10.
- Cover types.

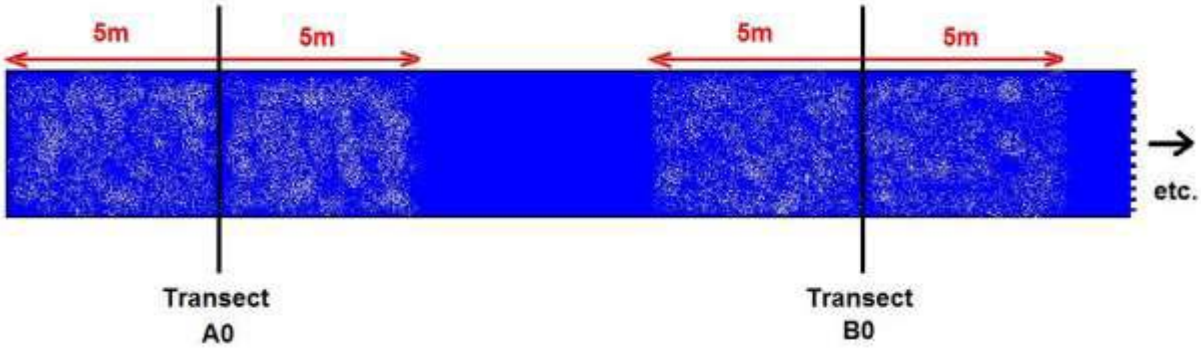


Figure B-9.1. Diagram of fish cover plots at each major transect of the main channel.

Refer to the *Major Transect Form* (Figure B-9.2). Circle the cover code that best characterizes each cover type.

FISH COVER	0 = Absent (0%) 1 = Sparse (<10%) 2 = Moderate (10-40%) 3 = Heavy (40-75%) 4 = Very Heavy (>75%) (circle one)	
	Cover in Channel	Flag
Filamentous Algae	(0) 1 2 3 4	
Macrophytes	(0) 1 2 3 4	
Woody Debris	0 (1) 2 3 4	
Brush	0 1 (2) 3 4	
Live Trees or Roots	(0) 1 2 3 4	
Overhanging Veg. =<1 m of Surface	0 1 2 (3) 4	
Undercut Banks	0 (1) 2 3 4	
Boulders	(0) 1 2 3 4	
Artificial Structures	(0) 1 2 3 4	
Bryophytes	(0) 1 2 3 4	

Figure B-9.2. Fish Cover portion of The Major Transects Form, with example records.

B-10. Human Influence at Major Transects in Waded Streams

Purpose and Scope

This method explains how to collect measurements for the Status and Trends Program at each of 11 equidistant transects at each site. Measurements in this procedure will be restricted to one main channel. This method must follow the method for establishing major transects.

Personnel Responsibilities

This method is performed by 1 person. This method is applied at every DCE, at each major transect. Observations are made at each bank of the main channel. Staff performing this method must have been trained.

Equipment, Reagents, Supplies

- No. 2 pencil.
- *Major Transect Data Form.*
- Measuring device (rod, tape, rangefinder).

Summary of Procedure

This procedure is derived from Peck et al. (2006) and Moberg (2007). Refer to the *Major Transect Data Form* (Figures B-10.1 and B-10.2). At each of the major Transects (A0-K0), assess the main channel. Record the appropriate *influence proximity code* for each of 13 human *influence types* (Figure B-10.1) relative to riparian plots (Figure B-10.3) on each bank of the transect. Influence proximity codes are:

- 0 = absent.
- 1 = beyond the plot, but within 30 meters of the bankfull margin.
- 2 = within the 10 meter by 10 m riparian plot.
- 3 = at least partially within the bankfull channel.

HUMAN INFLUENCE	0=not present, 1= 10-30m, 2= 0-10m, 3= on bank		
	Left Bank	Right Bank	Flag
Wall/Dike/Revetment/ Riprap/Dam	0 1 2 3	0 1 2 3	
Buildings	0 1 2 3	0 1 2 3	F1
Unpaved Motor Trail	0 1 2 3	0 1 2 3	
Clearing or Lot	0 1 2 3	0 1 2 3	
Human Foot Path	0 1 2 3	0 1 2 3	
PavedRoad/Railroad	0 1 2 3	0 1 2 3	
Pipes (Inlet/Outlet)	0 1 2 3	0 1 2 3	F2
Landfill/Trash	0 1 2 3	0 1 2 3	F3
Park/Lawn	0 1 2 3	0 1 2 3	
Row Crops	0 1 2 3	0 1 2 3	
Pasture/Range/Hay Field	0 1 2 3	0 1 2 3	
Logging Operations	0 1 2 3	0 1 2 3	
Mining Activity	0 1 2 3	0 1 2 3	

Figure B-8.1. A portion of the Major Transect Form, with example data.

Flag	Comments
F1	single-family home
F2	possible irrigation source
F3	beer cans

Figure B-10.2. A portion of the Major Transect Form with example comments for data flags.

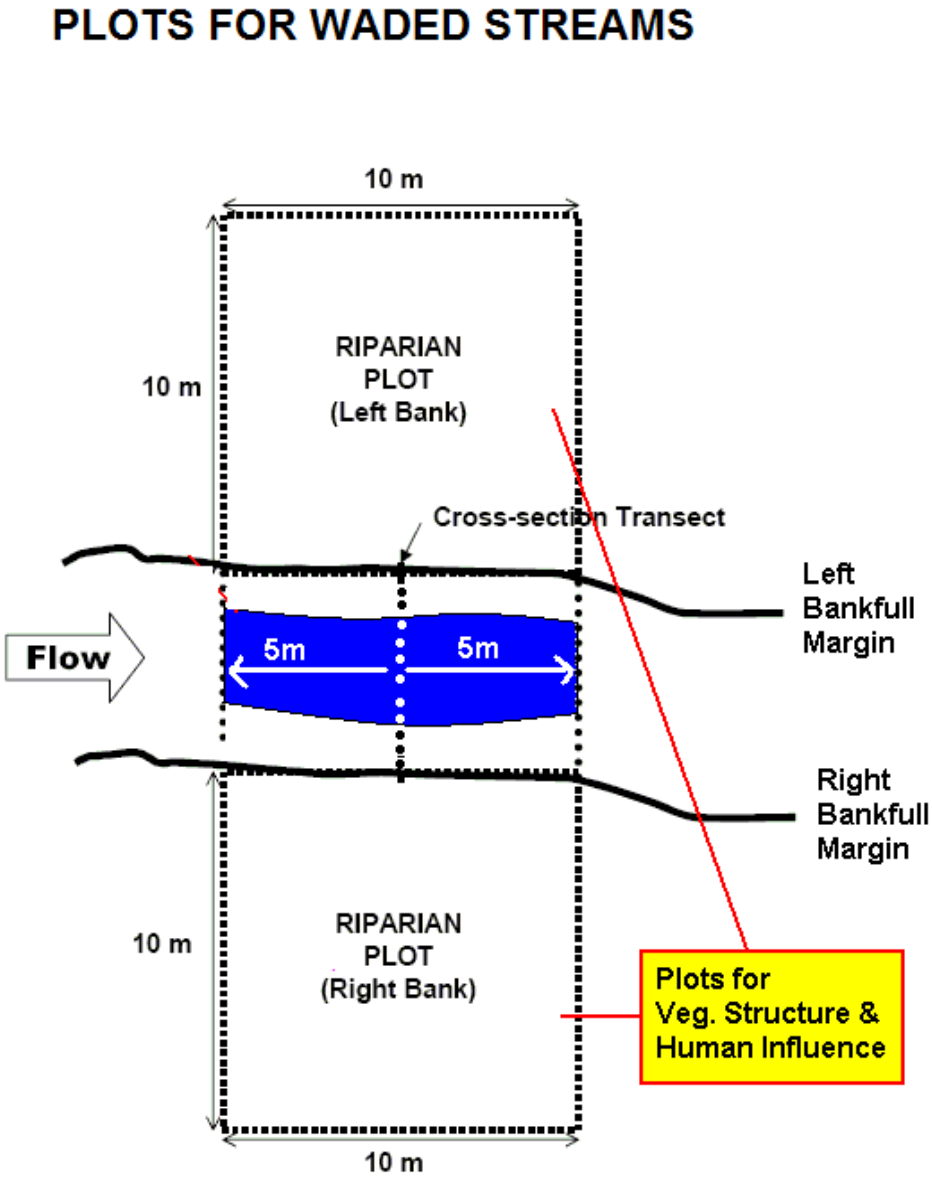


Figure B-10.3. Riparian plots.

B-11. Riparian Vegetation Structure at Major Transects in Waded Streams

Purpose and Scope

This method explains how to collect measurements for the Status and Trends Program at each of 11 equidistant transects at each site. Observations in this procedure will be restricted to one main channel. This method must follow the method for establishing major transects.

Personnel Responsibilities

This method is performed by 1 person. This method is applied at every DCE, at each major transect. Observations are made at each bank of the main channel. Staff performing this method must have been trained.

Equipment, Reagents, Supplies

- No. 2 pencil.
- *Major Transect Data Form.*

Summary of Procedure

This procedure is derived from Peck et al. (2006) and Moberg (2007). Refer to the *Major Transect Data Form* (Figure B-11.1).

RIPARIAN	0 = Absent (0%) 1 = Sparse (<10%) 2 = Moderate (10-40%) 3 = Heavy (40-75%) 4 = Very Heavy (>75%)	D = Deciduous C = Coniferous E = Broadleaf Evergreen M = Mixed N = None	
RIPARIAN VEGETATION COVER	Left Bank	Right Bank	Flag
	Canopy (>5 m high)		
Woody Vegetation Type	D C E (M) N	D (C) E M N	
BIG Trees (Trunk >0.3 m DBH)	0 (1) 2 3 4	0 1 2 (3) 4	
SMALL Trees (Trunk <0.3 m DBH)	0 1 2 (3) 4	0 (1) 2 3 4	
	Understory (0.5 to 5 m high)		
Woody Vegetation Type	D C E (M) N	D C E (M) N	
Woody Shrubs & Saplings	0 1 2 (3) 4	0 1 (2) 3 4	
Non-Woody Herbs, Grasses, & Forbs	0 (1) 2 3 4	0 (1) 2 3 4	
	Ground Cover (<0.5 m high)		
Woody Shrubs & Saplings	0 1 2 (3) 4	0 1 (2) 3 4	
Non-Woody Herbs, Grasses and Forbs	0 1 (2) 3 4	0 1 (2) 3 4	
Barren, Bare Dirt or Duff	0 (1) 2 3 4	0 1 (2) 3 4	

Figure B-11.1. A portion of the Major Transect Data Form, with example data.

On each major transect of the main channel, assess a plot on each bank. Each plot extends 5 meters downstream, 5 meters upstream, and 10 meters back from the bankfull margin (Figure B-11.2). The riparian plot dimensions can be estimated rather than measured. On steeply sloping channel margins, plot boundaries are defined as if they were projected down from an aerial view.

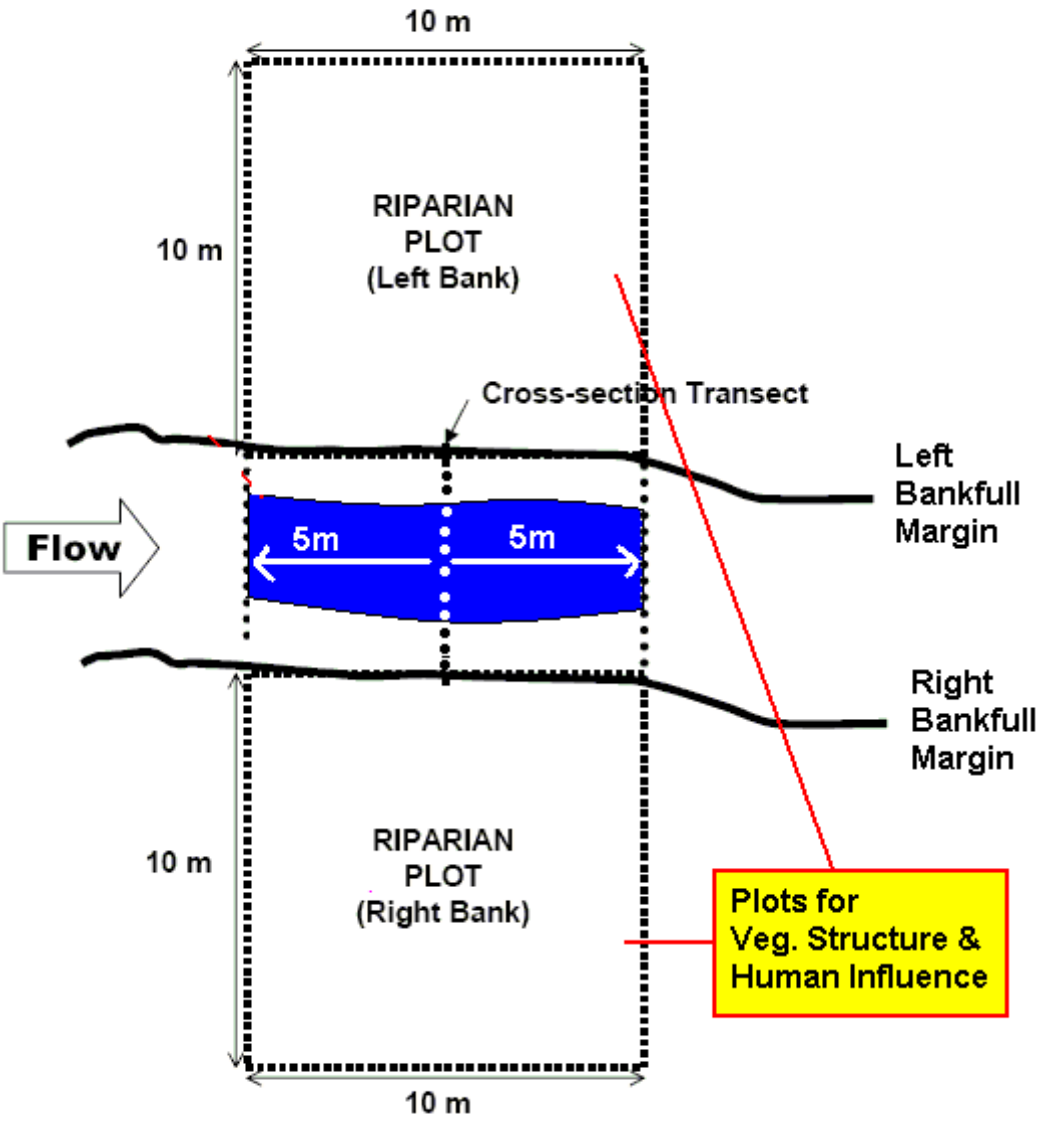


Figure B-11.2. Riparian plots.

Conceptually divide the riparian vegetation into three layers:

- Canopy (> 5 m high).
- Understory (0.5 to 5 m high).
- Ground Cover layer (< 0.5 m high).

Within each layer, consider the type of vegetation present and the amount of cover provided. Do this independently of what is contained in higher layers. Cover quantity is coded on the field form (Figure B-11.1) as follows:

- 0 – absent.
- sparse (< 10% cover).
- 2 - moderate (10-40% cover).
- 3 - heavy (40-75% cover).
- 4 – very heavy (> 75% cover).

The maximum cover in each layer is 100%, so the sum of the cover for the combined three layers could add up to 300%.

Canopy

On the *Major Transect Form* (Figure B-11.1), circle the appropriate vegetation type code (D, C, E, M, or N). Type codes are defined on the form. Then circle the appropriate cover quantity code (0, 1, 2, 3, or 4) for each of 2 classes:

- Big trees – trees having trunks larger than 0.3 m diameter (at breast height).
- Small trees– trees having trunks smaller than 0.3 m diameter (at breast height).

Understory

On the *Major Transect Form* (Figure B-11.1), circle the appropriate vegetation type code (D, C, E, M, or N) for any *woody* vegetation that might be present. Then circle the appropriate cover quantity code (0, 1, 2, 3, or 4) for each of 2 classes:

- Woody vegetation - such as shrubs or saplings.
- Non-woody vegetation - such as herbs, grasses, or forbs.

Ground Cover

Circle the appropriate cover quantity code (0, 1, 2, 3, or 4) for each of 3 classes:

- Woody (living).
- Non-woody (living).
- Bare dirt (or decomposing debris).

The sum of cover quantity ranges for these 3 types of ground cover should include 100%.

B-12. Measuring Thalweg Depth in Waded Streams

Purpose and Scope

This method explains how to collect incremental depth measurements for the Status and Trends Program when traversing the length of the stream site. It also describes assessing the presence of bars and edge pools. Observations in this method will be restricted to the main channel.

Personnel Responsibilities

This method is performed by 2 persons: one measures and another records. This method is limited to the main channel. It must be preceded by the method for verification and site layout. Staff performing this method must have been trained.

Equipment, Reagents, Supplies

- No. 2 pencil.
- *Thalweg Data Form.*
- Measuring rod.

Summary of Procedure

This procedure is derived from Peck et al. (2006) and Moberg (2007). Refer to the *Thalweg Data Form* (Figure B-12.1).

Transect A	Thalweg Depth (cm)	Bar? (circle)	Edge Pool? (circle)
.0	69	Y N	Y N
.1	70	Y N	Y N
.2	75	Y N	Y N
.3	87	Y N	Y N
.4	70	Y N	Y N
.5	75	Y N	Y N
.6	33	Y N	Y N
.7	34	Y N	Y N
.8	32	Y N	Y N
.9	33	Y N	Y N

Figure B-12.1. A portion of the Thalweg Data Form, with example data.

While walking up the main channel, measure thalweg depth (cm) at each of 101 thalweg transects. To reference location:

- Record the letter code for the lowest major transect referenced (e.g., A).
- Record depth and occurrence data into the appropriate thalweg transect row (e.g., .0).

These thalweg stations are located 0.2 bankfull widths apart from each other; bankfull width is based on an estimate made during the site layout. While measuring thalweg depth, also evaluate whether each of these features is present at each thalweg transect:

- Bar.
- Edge pool.
- Circle “Y” for “yes” and “N” for “no”.

B-13. Large Woody Debris Tally for Waded Streams of Western Washington

Purpose and Scope

This method explains how to count pieces of large woody debris in waded streams for the Status and Trends Program when traversing the length of the stream site. Observations are limited to the main channel. This method applies to streams of Western Washington (west of the Cascade ridge), where natural conditions are expected to include larger sizes of wood.

Personnel Responsibilities

This method is performed by 1 person. This method is applied at every DCE. Observations are made while walking upstream in the main channel. Staff performing this method must have been trained.

Equipment, Reagents, Supplies

- No. 2 pencil.
- *Thalweg Data Form.*
- Measuring rod.
- Calipers.

Summary of Procedure

This procedure is derived from Peck et al. (2006) and Moberg (2007). One person, while walking upstream, counts the number of pieces of large woody debris (LWD), that are (at least partially) within the bankfull channel of each stream segment (e.g., A0 to B0) in the main channel. Pieces are tallied according to size classes (Table B-13.1), which differ by region (Table B-13.2).

Table B-13.1. Size classes for large woody debris.

Large Woody Debris measured in each thalweg			
Length (meters)			
West	>2-5 m	>5-15 m	>15 m
10-30 dia (cm)			
30-60 dia (cm)			
60-80 dia (cm)			
>80 dia (cm)			
Central & East	>1-3 m	>3-6 m	>6 m
10-15 dia (cm)			
15-30 dia (cm)			
30-60 dia (cm)			
>60 dia (cm)			

Table B-13.2. Washington state regions used to determine which size class of woody debris to use during bioassessment.

Region	LWD Size Class Used
Puget Sound	West
Coastal	West
Lower Columbia	West
Mid Columbia	East
Upper Columbia	East
Snake	East
Northeast Washington	East
Unlisted Washington	East

Considering taper

Wood pieces have a taper. Considerations for taper are illustrated in Figure B-13.1. The diameter of a log is based on the thickest end. The length of a log only counts the portion that has a diameter of more than 10 cm.

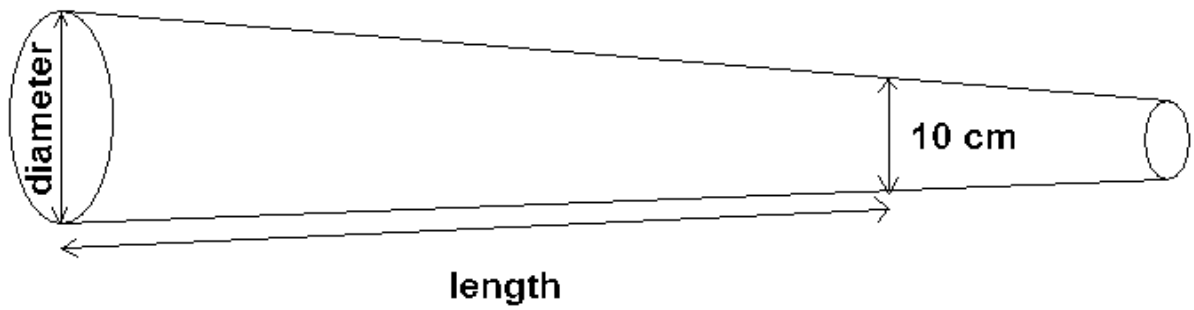


Figure B-13.1. Diagram of how to estimate the dimensions of a log.

Record

Refer to the *Thalweg Data Form* (Figure B-13.2). Identify and tally LWD pieces that lie in the bankfull channel. After tallying, sum the marks separately for each size class and enter the number into the corresponding box for each class.

LWD Count	Example: 6				Check box if all are zero ☐		
	2-5 m		5-15 m		>15 m		Flag
10-30 cm	3	3	5	5	2	2	
30-60 cm	1	1	3	3	1	1	
60-80 cm		0		0		0	
>80 cm		0		0		0	
LWD Notes:							

Figure B-13.2. A portion of the Thalweg Data Form, with example data.

B-14. Habitat Unit Descriptions Along the Main Channel Thalweg

Purpose and Scope

This method explains how to identify and count habitat units for the Status and Trends Program when traversing the length of the stream site. The habitat unit descriptions are based on the Hawkins et al. (1993) classification system (Figure B-14.1). Observations in this method will be restricted to the main channel.

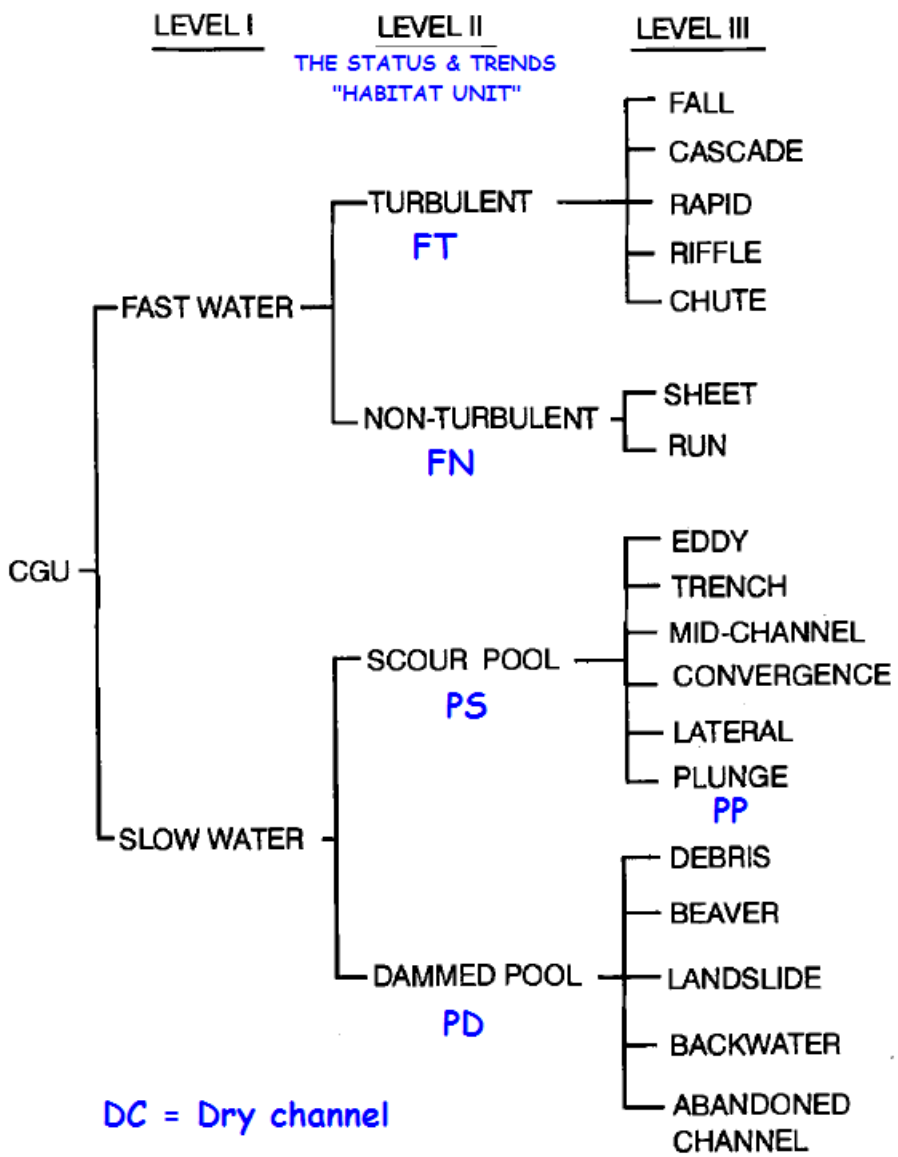


Figure B-14.1. Categories of channel geomorphic units (CGU) described by Hawkins et al. (1993) and their three levels of resolution.

This figure is modified from Hawkins et al. (1993), with Status and Trends habitat unit codes displayed in blue text.

Personnel Responsibilities

This method is performed by 1 person and who dictates data to a second person who records. This method is applied at every DCE. Observations are made while walking upstream in the thalweg of the main channel. Staff performing this method must have been trained.

Equipment, Reagents, Supplies

- No. 2 pencil.
- *Thalweg Data Form.*
- Measuring rod.
- 50-m tape or laser rangefinder.

Summary of Procedure

This procedure is derived from Moberg (2007). Refer to the *Thalweg Data Form* (Figures B-14.2 and B-14.3). Identify and code habitat units consecutively during the walk upstream. A separate Thalweg Data Form is recorded for sets of observations that span between major transects. Data will include:

- Type code.
- Unit identity (number).
- Pool forming code.
- Depths (for pools).

Habitat Unit Number	Habitat Unit Type FT, FN, PS, PD, PP,DC	Pool Forming Code (N, W, R, B, F)		HU Width (m.x)	Max Pool Depth (cm)	Crest Pool Depth (cm)	Channel Unit Notes:
		Code 1	Code 2				
1	PD	W	B	3.5	90	30	Pool formed by both boulder & wood
2	PP	W		4.2	75	15	
3	FN	N		4.8			

Figure B-14.2. A portion of the Thalweg Data Form, with example data for habitat unit type, pool forming code, habitat unit width, and pool depths.

Transect A	Thalweg Depth (cm)	Bar? (circle)	Edge Pool? (circle)	Habitat Unit Number
.0		Y N	Y N	1
.1		Y N	Y N	1
.2		Y N	Y N	1
.3		Y N	Y N	1
.4		Y N	Y N	2
.5		Y N	Y N	2
.6		Y N	Y N	3
.7		Y N	Y N	3
.8		Y N	Y N	3
.9		Y N	Y N	3

Figure B-14.3 A portion of the Thalweg Data Form, with example data for habitat unit locations relative to thalweg transects.

Type Code

With each step up the thalweg, evaluate the wetted channel for conformity to the Hawkins et al. (1993) classification system (Figure B-14.1). We are focusing on Level II designations. The main division is between slow water (pools) and fast water (e.g., cascades, riffles, or runs). All habitat units (except plunge pools or dry channels) must be at least as long as half the wetted width. All pools have specific depth criteria (Table 14-): the maximum depth must be at least 1.5 times the depth at the pool crest. Record the unit type code (Table B-14.1) on the *Thalweg Data Form*

Table B-14.1. Habitat unit type codes.

Unit	Type	Description
FT	Fast Turbulent	(riffle, cascade, waterfall)
FN	Fast Non-Turbulent	(sheet, run)
PS	Scour	pool
PD	Dammed	pool
PP	Plunge	pool
DC	Dry	channel

Unit Number

After you designate the habitat unit type (Table B-14.1), assign a habitat unit number. These are consecutive number counts for the whole stream site. For each form, record data for any new habitat units that appear since the last encountered major transect. For example, if habitat units

numbered 1, 2, and 3 were recorded between major transects A and B, then new units encountered between B and C would begin with habitat unit number 4.

Pool Forming Code

On the *Thalweg Data Form* (Figure B-14.2), record the pool forming code (Table B-14.2) to describe the obstruction that led to pool formation. Assign "N" for habitat units other than pools. If pool formation could be associated with two types (e.g., boulder *and* large wood), use both columns on the form, with one code per column.

Table B-14.2. Pool forming codes.

Pool Forming Code	Description
N	Not a pool
W	Large Woody Debris
R	Root wad
B	Boulder/Bedrock
F	Fluvial(non-specific stream process)

Habitat Unit Width

Estimate the average wetted width (nearest tenth of a meter) of the habitat unit for the full course of its length. Record this value on the *Thalweg Data Form* (Figure B-14.2) A measurement is not required. Just consider the relative width compared to the width measurements performed at nearby major transects and minor transects.

Pool Depths

With a measuring rod, measure water depth (cm) in each of two locations in the thalweg of pools:

- at the crest.
- at maximum depth.

Crest depth is measured differently, depending upon the pool type. For scour pools and plunge pools, the crest depth is measured where water exits the pool. For dammed pools, the crest depth is measured where water enters the pool. Record crest depth and maximum depth on the *Thalweg Data Form* (Figure B-14.2). No data need to be recorded for non-pool habitat units.

Position

After identifying and describing habitat units (Figure B-14.2), record the position of each habitat unit relative to thalweg stations (Figure B-14.3).

B-15. Side-Channel Descriptions

Purpose and Scope

This method explains how to identify and count side-channels of waded streams for the Status and Trends Program when traversing the length of the stream site. Observations are limited to portions of side channels that occur next to the sampled part of the main channel (above Transect A0 and below Transect K0).

Personnel Responsibilities

This method is performed by 1 person who dictates to another. This method is applied at every DCE. Observations are made while walking upstream to measure thalweg depths of the main channel. Staff performing this method must have been trained.

Equipment, Reagents, Supplies

- No. 2 pencil.
- *Thalweg Data Form.*
- Measuring rod.
- Field notebook.

Summary of Procedure

This procedure is derived from Moberg (2007). Refer to the *Thalweg Data Form* (Figures B-15.1 and B-15.2). Identify and count side channels occurring within the length of the sample site. Estimate their widths.

Identify and count

Identify and code side channels consecutively for the entire streams site. Number them as encountered while walking upstream. Note their presence for each of the 101 Thalweg Transects of the stream site. This will require 11 *Thalweg Data Forms* to complete (A-K).

Transect A	Thalweg Depth (cm)	Bar? (circle)	Edge Pool? (circle)	Habitat Unit Number	Side Channel Numbers				
.0		Y N	Y N						
.1		Y N	Y N		1				
.2		Y N	Y N		1				
.3		Y N	Y N		1				
.4		Y N	Y N		1	2			
.5		Y N	Y N		1	2			
.6		Y N	Y N		1	2			
.7		Y N	Y N		1	2			
.8		Y N	Y N		1	2	3		
.9		Y N	Y N		1	2	3		

Figure B-15.1. A portion of the Thalweg Data Form, with example data showing the presence or absence of side-channels at each Thalweg Transect.

Estimate Width

For each channel, estimate wetted width (nearest tenth of a meter). Make at least one representative measurement (in a notebook) between each major transect then visually estimate an average value for the length of the side-channel. Record this channel average on the *Thalweg Data Form* (Figure B-15.1). In your width estimate, do *not* include portions of the channel that occur below transect A0 or above transect K0.

Side Channel Number	Width (m.x)	Side Channel Notes:
1	1.0	left side of main channel
2	2.3	diverts from channel 1, not from main channel
3	3.7	Right side of main channel

Figure B-15.2. A portion of the Thalweg Data Form, with example data for channel width.

B-16. Width and Substrate Measurements at Minor Transects in Waded Streams

Purpose and Scope

This method explains how to measure width and substrate characteristics for the Status and Trends Program at each of 10 equidistant transects at each site. Measurements in this procedure will be restricted to one main channel. This method is performed in conjunction with the method for measuring thalweg depth. Instruments included on the procedure include distance measuring devices (e.g., measuring rod, or 50-m measuring tape, caliper), and a10-cm ring.

Personnel Responsibilities

This method is performed by 2 persons: an observer and a recorder. This method is applied at each minor transect. It is performed in conjunction with the method for measuring thalweg depth. Staff performing this method must have been trained.

Equipment, Reagents, Supplies

- No. 2 pencil.
- Measuring rod.
- 50-m tape.
- Calculator.
- 10-cm ring.

Summary of Procedure

Measure the channel width and then make observations about substrate size at 11 equidistant stations across the minor transect.

Widths

At each minor transect, measure distance (tenth of meters) for:

- Bankfull width.
- Wetted width.
- Total bar width (sum for all bars).

Record these widths on the Thalweg Data Form (Figure B-16.1).

Wet Width (m.x)	BF Width (m.x)	Bar Width (m.x)
4.2	5.3	0.3

Figure B-16.1. Part of the Thalweg Data Form, with example data for widths at the minor transect.

Station Location

Identify the ***Transect Station LeftRight***. Example stations for minor transect A5 would be:

12. **A500** – at the left bankfull stage.
13. **A501** – 10% distance across the channel.
14. **A502** – 20% distance across the channel.
15. **A503** – 30% distance across the channel.
16. **A504** – 40% distance across the channel.
17. **A505** – half way across the channel.
18. **A506** – 60% distance across the channel.
19. **A507** – 70% distance across the channel.
20. **A508** – 80% distance across the channel.
21. **A509** – 90% distance across the channel.
22. **A510** – at the right bankfull stage.

Substrate Type

Hold the measuring rod vertically and rest it on the substrate at each station. Estimate the substrate particle type at the front of the measuring rod, where it rests on the surface of the streambed. Estimate the size class of that particle based on the intermediate axis length. Record the *substrate type code* (Table B-16.1) on the Thalweg Data Form (Figure B-16.2) for each station. For coarse gravel and cobble, use calipers to measure the intermediate axis length of the particle and confirm your estimate of size. For larger sizes, use the measuring rod to confirm your estimate. Particles smaller than 100 mm are evaluated using a 10 cm ring surrounding the sample point. All particles within the ring are evaluated for size and embeddedness, not just the point. Record the estimated average for surface substrate within the ring.

Substrates at 5	LB	01	02	03	04	05	06	07	08	09	RB	Substrate Notes:
	SA	GF	GC	CB	CB	XB	XB	XB	XB	XB	FN	Stations 5-9 are one boulder

Figure B-16.2. Part of the Thalweg Data Form, with example data for substrate types along the minor transect.

Table B-16.1. Substrate codes, types, and sizes.

Code	Type	Range Size	Size Gauge
RS	Bedrock (smooth)	>4 m	larger than a car
RR	Bedrock (rough)	>4 m	larger than a car
RC	Concrete/Asphalt	>4 m	larger than a car
XB	Large Boulder	1-4 m	meter stick to car
SB	Small boulder	>250 mm–1 m	basketball to meter stick
CB	Cobble	>64 mm–250 mm	tennis ball to basketball
GC	Gravel, coarse	>16 mm to 64 mm	marble to tennis ball
GF	Gravel, fine	>2 mm to 16 mm	ladybug to marble
SA	Sand(2-16 mm)	>0.06 mm to 2 mm	gritty to ladybug
FN	Fines(silt/clay/muck)	<0.06 mm	non gritty
HP	Hardpan- hardened fines	any size	
WD	Wood	any size	
OT	Other (doesn't fit choices above)	any size	

B-17. Measuring Slope and Bearing in Wadeable Streams

Purpose and Scope

This method describes how to measure slope and bearing of the main channel at each site during a data collection event (DCE) for Washington's Status and Trends Program. It applies to waded streams. This method requires use of a hand level, measuring rod, and a compass to make incremental measurements across each of at least 20 segments of the stream site.

Personnel Responsibilities

Two persons perform this activity: one "rodder" who holds a measuring rod in a vertical position and a "sighter" who sights on the "rodder" with a hand level and compass to record data. Crew members must be trained prior to performing this method.

Equipment, Reagents, Supplies

- Hand level (5x magnification).
- Monopod for hand level.
- Measuring rod (telescoping).
- Compass (handheld, magnetic).
- Range finder.
- 50-meter tape.
- Slope and Bearing Form.
- Pencil.

Summary of Procedure

A two person-crew performs this procedure incrementally, once for each of at least 20 segments of the main channel for the entire site. Segments evaluated are normally between major and minor transects (e.g., A5-A0), but intermediate measurements may be used if necessary (e.g., due to thick vegetation or sharp bends in the channel). There should be no space between segments and no overlap of segments. The crew can either work moving up the stream or down, depending on efficiency of overall work flow. We will describe the technique for working from the top of the stream, downward. This method is based on modifications of Peck et al. (2006) and Moberg (2007).

Slope

The sighter stands at the water's edge of a transect at a higher elevation (Figure B-17.1). This person will sight downstream toward a measuring rod at a lower transect. Use a monopod to rest the hand level at a fixed eye height. The rodder holds the measuring rod vertically, with its base at the surface of the water. The rodder can assist by pointing to the numbers on rod and adjusting up or down as directed by the sighter. Record these things on the Slope and bearing Form (Figure B-17.2):

- Identity of transect where the sighter stands
- Identity of transect where the rodder stands
- Eye height (cm)
- Level Height (cm)

Note: Sometimes it is easier to sight in the wetted channel rather than the edge, to avoid vegetation. If the monopod or measuring rod rest below the surface of the water, subtract that depth from the eye height or level height.

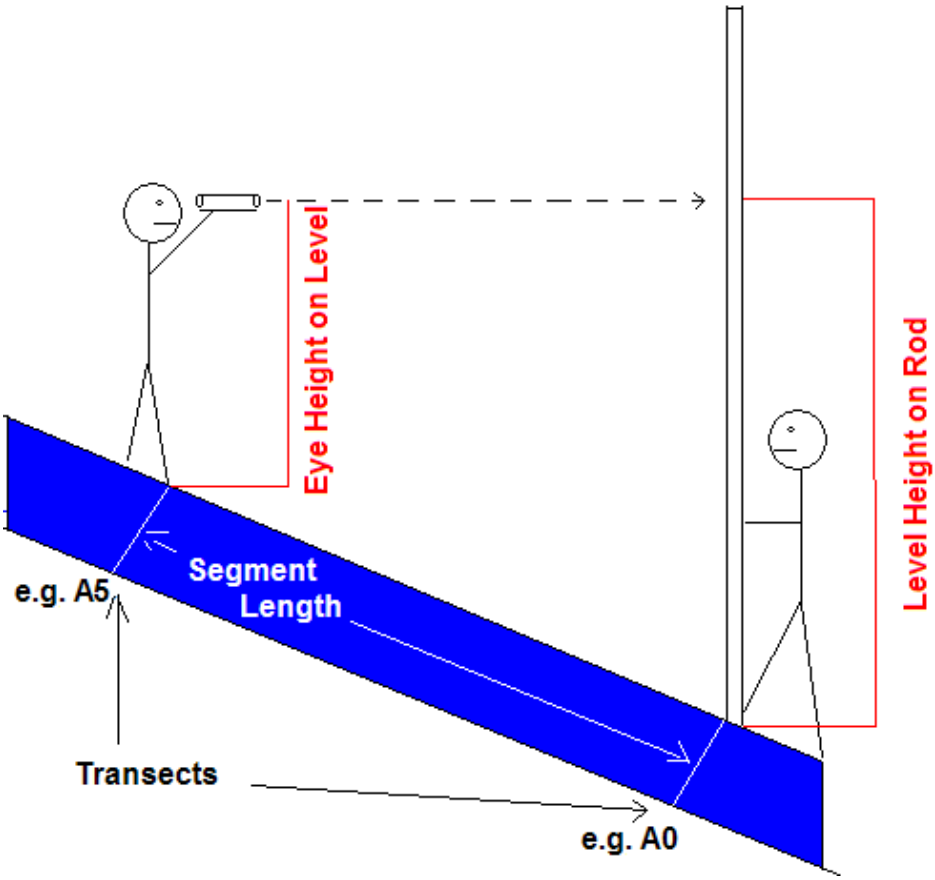


Figure B-17.1. Crew positions when measuring the slope and bearing

Status & Trends - Slope and Bearing Form

DCE: WAM0600 - 000152 - DCE - 2009 - 0715 - 10:00

Top Transect* LEVEL COMPASS	Bottom Transect* ROD	Segment Length (m.x)	Eye Height on Level (cm)	Level Height on Rod (cm)	Bearing (deg)	Flag	Comment
K0	J5	7.5	150	172	333		Started here
J5	J0	7.5	150	154	326		
J0	I5	7.5	150	154	293		
I5	I0	7.5	150	203	251		
I0	H5	7.5	150	285	262		
H5	H0	7.5	150	163	249		
H0	G5	7.5	150	225	227		
G5	G0	7.5	150	174	226		
G0	F5	7.5	150	173	237		
F5	F0	7.5	150	160	248		
F0	E5	7.5	150	171	259		
E5	E0	7.5	150	166	276		
E0	D5	7.5	150	230	310		
D5	D0	7.5	150	172	289		
D0	C5	7.5	150	188	301		
C5	C0	7.5	150	166	263		
C0	B5	7.5	150	172	237		
B5	B0	7.5	150	173	234		
B0	A5	7.5	150	166	262		
A5	A0	7.5	150	171	242		

Figure B-17.2. The Slope and Bearing Form, with example data.

Bearing

The sighter stands at a transect at a higher elevation (Figure B-17.1). This person will sight downstream toward the rodder at a lower transect. The sighter will then point the compass toward the rodder and parallel to the thalweg. On the Slope and Bearing Form, record the bearing (magnetic north) of the thalweg between the top and bottom of the segment.
Note: If sighting from bottom to top, record the bearing south.

Appendix C. Sampling Procedures

C-1. Field Sampling Benthos in Wadeable Streams

Purpose and Scope

This method describes how to collect benthic macroinvertebrate samples for conducting community level assessments in Washington's Status and Trends Program. Data will be used to describe biological integrity and ecological quality (or taxonomic loss). It applies to waded streams. This method requires measurement of the associated physical and chemical environmental variables described in other methods within this protocol.

Personnel Responsibilities

One person or more performs this activity. Staff performing this method must have been trained.

Equipment, Reagents, Supplies

- Wide-mouth polyethylene jar (128 oz or 3.8 L).
- D-Frame kick net with these characteristics.
  - Frame mouth that is 1 ft (30.5 cm) wide by 1 ft tall.
  - 500-µm mesh net.
- 95% Ethanol (add 3 parts by volume for each part sample).
- Label (waterproof) for jar exterior.
- Label (waterproof) for jar interior.
- Soft-lead pencil.
- Clear tape.
- Electrical tape.
- Pocket knife.
- Wading gear.

Summary of Procedure

Invertebrate sampling is one of the first methods to be performed on-site, after site verification and layout. It starts concurrently with water sampling, with initial components of the benthos sample collected downstream of the water sample. One kick sample is collected at each of 8 transects and added to the composite sample for the site. This method is taken from Hayslip (2007) with some details provided by Peck et al. (2006).

Choose transects

Randomly choose 8 transect stations out of these 11:

- A0
- B0
- C0
- D0
- E0
- F0
- G0
- H0
- I0
- J0
- K0

Identify kick stations

Start at the lowest transect and work upstream. At each transect, visually estimate the distance from left to right where the stream bottom will be sampled (Table C-1.1). Half the stations are in mid-channel. Half are in margins. If the water is too deep to sample at any station, collect the sample from the nearest feasible location. The kick net normally allows sampling up to about 50 cm depths.

Table C-1.1. Components of the macroinvertebrate composite sample.

Kick Station	Distance across wetted channel (left to right)
1st	25%
2nd	50%
3rd	75%
4th	50%
5th	25%
6th	50%
7th	75%
8th	50%

Collect each kick

A different procedure is needed depending upon whether the station sits within flowing water or slack water. Flowing water is where the stream current can sweep organisms into the net. Slack water is where water is so slow that active net movement is required to collect organisms.

Flowing water stations

Once the kick station is determined, place the net opening into the face of flow. Position the net quickly and securely on the stream bottom to eliminate gaps under the frame. Collect benthic

macroinvertebrates from a 1ft² (0.9 m²) quadrant located directly in front of the frame mouth. Work from the upstream edge of the quadrant 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, keeping the sampler securely in position, starting at the upstream end of the quadrant, kick the top 4 to 5 cm of the remaining finer substrate within the quadrant for 30 seconds. Pull the net up out of the water. Immerse the net in the stream several times or splash the outside of the net with stream water to remove fine sediments and to concentrate organisms at the end of the net. After completing the sample, hold the net vertically and rinse material to the bottom of the net.

After taking a sample, examine the contents of the net. Pick out coarse rocks and sticks. Closely examine them for clinging organisms; pick these animals off of the debris and place them into the sample jar. Discard the debris and empty the net's remaining contents into the sample jar. Add enough ethanol to the sample jar so that the resulting solution consists of 1/3 sample and 2/3 ethanol (by volume).

Slack water stations

Visually define a rectangular quadrant with an area of 1 ft² (0.09 m²). Inspect the stream bottom within the quadrant for any heavy organisms, such as mussels and snails. Remove these organisms by hand and place them into the sample jar. Pick up any loose rocks or other larger substrate particles within the quadrant and hold them in front of the net. Use your hands to rub any clinging organisms off of rocks or other pieces of larger substrate (especially those covered with algae or other debris) into the net. After scrubbing, place the larger substrate particles outside of the quadrant.

Vigorously kick the remaining finer substrate within the quadrant with your feet while dragging the net repeatedly through the disturbed area just above the bottom. Keep moving the net all the time so that the organisms trapped in the net will not escape. Continue kicking the substrate and moving the net for 30 seconds.

After 30 seconds, remove the net from the water with a quick upstream motion to wash the organisms to the bottom of the net. After taking a sample, examine the contents of the net. Pick out coarse rocks and sticks. Closely examine them for clinging organisms; pick these animals off of the debris and place them into the sample jar. Discard the debris and empty the net's remaining contents into the sample jar. Add enough ethanol to the sample jar so that the resulting solution consists of 1/3 sample and 2/3 ethanol (by volume).

Special circumstances

For samples located within dense beds of long, filamentous aquatic vegetation, kicking may not be effective. Use a knife to sample only the vegetation that lies within the quadrant. Don't include parts of the strands that extend beyond the quadrant.

Label and Seal the Composite sample

Using a number 2 pencil, complete two benthos jar labels (Figure C-1.1). Place one into the sample. Screw on the lid and seal it closed using electrical tape. Attach the other benthos label to the outside of the jar using clear tape. Record the DCE, which includes the Site ID, and site arrival time (year, month, day, hour, and minute). It should match the DCE recorded on the Site Verification Form. Be sure to note which transects were sampled, and which of these were sampled using the slack water technique.

500 µ D-frame kick	**Benthos Jar Label** Jar ___ of ___
Project	20__ Monitoring in the _____ STR
Stream	
Who collected? (full name)	
8 1-ft^2 Transects **(circle all sampled)**	A B C D E F G H I J K Transects sampled using slack-water technique: _____
Collectors Notes	
DCE	WAM06600-__ __ __ __ __ __-dce-2009__ __ __ __-__ __ __ __ m m d d h h m m

Figure C-1.1. The benthos jar label.

Enter Data to the Chemistry and Sampling Form

The sample jars will be stored by field crews and delivered *en mass* to the analytical laboratory at the end of the field season. The *Chemistry and Sampling Form* (Figure C-1.2) will be used to keep track of sample jar information. Note the Sample ID and number of jars per Sample ID. If there is more than one jar for a Sample ID, then ensure that the jars are located together. Taping the jars together with clear tape may be helpful. For destination, note the immediate place to where the sample will be stored, shipped, or delivered.

Reviewed by (Initials): PS

Status and Trends - Chemistry and Sampling Form

DCE: WAM0600 - 000001 - DCE - 2009 - 0701 - 13:25 (Site Number; YY; MMDD; HH:MM)

IN SITU WATER QUALITY CALIBRATION

Operator: Kurt Gowdy Unit #: 1

				Flag
T	Temp probe was checked vs NIST	(Yes)	No	F1
DO	Sensor Calibrated	(Yes)	No	F2
pH	Sensor Calibrated and Checked	(Yes)	No	F3
Cond	Sensor Calibrated and Checked	(Yes)	No	F3

Notes (in situ)
F1 - T - Checked pre-season
F3 - pH, Cond, calibrated and checked this morning at the lab.
F2 - DO calibrated streamside - Winkler comparison collected for July

Sed:%Gravel 0 %Sand 50 %Fines 50

In Situ Chemistry

Time1 13:45 hrs Start Location (e.g. F0): F0
Temp1 6.3 deg C
pH1 6.94 pH Units
DO1 10.9 mg/L %Sat1 102.5
Cond 27.8 uS/cm @ 25C

Time2 19:50 hrs End Location (e.g. K0): F0
Temp2 7.0 deg C
pH2 7.00 pH Units
DO2 10.4 mg/L %Sat2 99.5
Cond 27.9 uS/cm @ 25C

Sample	Primary Sample: No. of Jars	Duplicate Sample: No. of Jars (or ITIS for Fish Spp)	Destination	Tracking No. (if shipped)	Flag
TPN	1	0	MEL		
Tot P	1	0	MEL		
Cl	1	0	MEL		
Turb	1	0	MEL		
Sed PAH	1	0	MEL		
Sed Metals*	1	0	MEL		
Benthos	2	0	ETOH Shed at office		F4
Fish Spp1	1	ITIS: 159700	University Lab	FedEx: 835651465756	F5
Fish Spp2	1	ITIS: 167234	office lab for review under microscope		F6
Fish Spp3					

Water Sample Location (e.g. A5): F0

Sample Notes (explain flags):
F4 - Invertebrate sample could not fit into a single jar. Two jars are taped together.
F5 - Lamprey ammocoetes in zipped bag on ice — Vert Collection Form "Jar" #1
F6 - Riffle sculpin in jar of ETOH - verify it is not a reticulate sculpin. — Vert Col. Frm Jar #2

Draft

*Sediment Metals jar includes sample material to be analyzed for TOC
Note: Use standard Manchester Environmental Lab forms for tracking water and sediment samples.

Figure C-1.2. The *Chemistry and Sampling Form*, with fields that are relevant to benthos sampling highlighted.

C-2. Taxonomic Lab Sampling Benthos from Wadeable Streams

Purpose and Scope

Taxonomic identification is conducted by a lab that employs taxonomists certified by the North American Benthological Society. The taxonomist should have experience with the freshwater macroinvertebrates of the Pacific Northwest. All major orders of freshwater macroinvertebrates are identified to at least the genus level (see Appendix G), including the Chironomidae (See Appendix H) and Simuliidae, and to species where existing taxonomic keys are available. Taxon groups normally identified to coarser taxonomic levels include: Lumbriculidae, Naididae, Oligochaeta, select families of the Coleoptera, Planariidae, and Acari. If the taxonomist has a compelling reason (Appendix I) that a specimen cannot be identified to the genus level, they may decide to aggregate individuals in the next highest taxonomic level.

Personnel Responsibilities

One person or more performs this activity. Staff performing this method must have been trained.

Summary of Procedure

Sample Preparation

Samples are sub-sampled using a 500-organism count. According to Ecology protocols (Plotnikoff and Wiseman, 2001), macroinvertebrates are removed from a minimum of two randomly chosen squares from a 30 square sub-sampling grid. The dimension of each square is 6 cm x 6 cm and the grids overall dimensions are 30 cm x 36 cm. The sample material is thoroughly mixed and spread evenly across the grid. All organisms are removed from randomly chosen squares until a minimum of 500 macroinvertebrates are removed from the sample and placed in alcohol for subsequent identification under the dissecting scope. If a grid square is dominated by a single taxon, additional grids are selected and sorted, and notes are made in the report. In some cases, there may be less than 500 organisms in the whole sample. When the target count of organisms has been reached or the specified amount of material has been sorted, a special large and rare protocol may be followed, with these organisms placed in an additional labeled vial.

Large and Rare Specimen Identification

The remainder of the sample material in the tray will be searched for any large or rare taxa that may have been missed in the sub-sampled fraction. These specimens will be identified and placed together in a vial labeled “Large and Rare Taxa” for the voucher collection. This scan will include any adult aquatic invertebrates, which will be archived separately (not to be identified and included in dataset) for anyone interested in looking at the material in the future.

Reporting Requirements

The contractor will complete all analyses by the dates mentioned above and submit all final data and QA results via the Puget Sound Stream Benthos website, including:

- Sample ID.
- ITIS number.
- Taxon name.
- Taxon count.
- Density estimate per sample of each species in square meters.
- Percent Sample Sorted – Web site will ask for number or squares counted out of total number of squares.
- Number of individuals per species of organisms in a life stage other than Juvenile (i.e. eggs, adults, larval) in each sample
- Number of individuals that were damaged to the point that identification to the required resolution was not possible.

C-3. Field Sampling Periphyton in Wadeable Streams

Purpose and Scope

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 community structure, diversity, and density are useful in the assessment of biological condition for surface waters. Washington State's biological monitoring program follows Collyard (2010) for standard sampling procedure. For more information on periphyton and their use in bioassessments, refer to Barbour et al. (1999) and Stevenson et al. (1996).

Personnel Responsibilities

One person or more performs this activity. Staff performing this method must have been trained.

Equipment, Reagents, Supplies

- Wide-mouth polyethylene jar (1 L).
- Firm bristled toothbrush.
- Aluminum foil.
- Wash bottle.
- Plastic funnel.
- One gallon Ziplock bag.
- Large plastic bowl or tray to catch rinsate.
- 95% Ethanol (add 3 parts by volume for each part sample).
- Deionized or distilled water.
- Label (waterproof) for jar exterior.
- Soft-lead pencil.
- Clear tape.
- Electrical tape.
- Pocket knife.
- Wading gear.

Summary of Procedure

Sampling Method for Epilithic (Coarse Substrate) Habitats

Periphyton sampling can be performed on-site with macroinvertebrate sampling, after site verification and layout. One sample is collected at each of 8 major transects and are added to a single composite sample jar for the site.

1. Eight samples are randomly collected from each reach. A periphyton sample is taken at each major transect where macroinvertebrate samples are taken. Samples will be collected in close proximity to (but not within) the randomly selected macroinvertebrate sample locations. See *Benthos Sampling* above for description of selecting random sample locations.

2. Carefully remove a rock from each of the eight randomly selected sample locations, 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. 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 topsetting 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 α 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.

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):

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

Where,
n = number of discrete collections
p = 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 α analysis and taxonomic identification.

Sampling Method for Episammic (Pea gravel/Sand) and Epipelic (Silt) Habitats

Quantitative microalgal periphyton samples are collected from the upper 5 to 7 mm layer of episammic (pea gravel _ 5 mm/sand) and epipelic (silt) habitat in organically-rich depositional areas of the reach. Use the following method to sample episammic or epipelic 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® sample bottle. Use rinse water sparingly, but be thorough. Combine all five discrete sample collections in the 500 mL Nalgene® 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^2. 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 _ 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 epipelic) 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):

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.

7. 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.

8. 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.

9. 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.

10. 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):

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

    Where,
    n = number of discrete collections
    p = 3.1416
    di = mean diameter of each cylindrical stem section, in centimeters
    li = length of each cylindrical stem section, in centimeters

11. 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):

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

    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

12. 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.

13. Process the composite sample following steps described in Subsample Processing Procedures to extract subsamples for chlorophyll α 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, epipelic 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.

5. 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.

6. 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.

7. 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.

8. Fill a 1000 mL Nalgene® bottle with 300 mL of deionized or distilled water. Place sample segments in the Nalgene® bottle and cap the bottle. Shake the Nalgene® bottle vigorously for 30 seconds to dislodge attached periphyton. Open the Nalgene® 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® 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® bottle. Any dislodged periphyton remaining in the plastic tray should be inserted through a funnel into the 1000 mL Nalgene® 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.

9. 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[1]. Calculate the total sampled surface area by using the following formula (assumes a cylinder):

Total Sampled Area (cm2) = $\sum_{i=1}^{n} (\pi)(di)(li)$

Where,
n = number of discrete collections
p = 3.1416
di = mean diameter of each cylindrical stem section, in centimeters
li = length of each cylindrical stem section, in centimeters
[1]For samples with ≥ 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 ≥ 10 branches. In all cases, the stem must be measured in its entirety.

10. For non-cylindrical or broad-shaped leaf samples, place each cleaned leaf segment on a sheet of aluminum foil[2]. 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.

[2]For samples with ≥ 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 eaf segments and multiply by the total number of leaf segments in the sample. Record the total area on the datasheet.

11. Process the composite sample following steps described in Subsample Processing Procedures to extract subsamples for chlorophyll α analysis and taxonomic identification.

Subsample Processing Procedures

Each composite sample processed in the field is used to extract subsamples for chlorophyll α 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 α 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® 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 α subsample. Determine the number of aliquots filtered and record the chlorophyll α 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.

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/Epipelic 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 _ 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 _ 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.

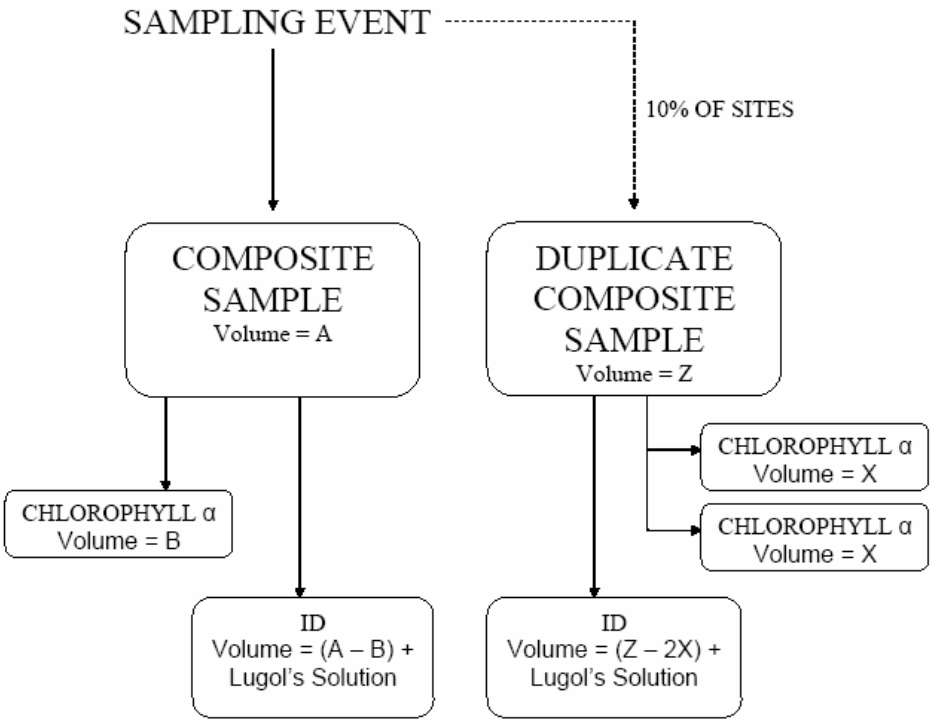


Figure C-3.1. Flow chart for the duplicate composite sample/subsample process.

Periphyton Jar Label Jar ___ of ___	
Project	20__ Monitoring in the _____ STR
Stream	
Who collected? (full name)	
8 samples/ 1 per transect (circle all sampled)	A B C D E F G H I J K
Collectors Notes	
DCE	WAM06600-__ __ __ __ __ __-dce-2009__ __ __ __-__ __ __ __ m m d d h h m m

Figure C-3.2. Periphyton jar label.

Reviewed by (Initials): PS

Status and Trends - Chemistry and Sampling Form

DCE: WAM0600 - 000001 - DCE - 2009 - 0701 - 13:25 (Site Number; YY; MMDD; HH:MM)

IN SITU WATER QUALITY CALIBRATION

Operator: Kurt Gowdy Unit #: 1

				Flag
T	Temp probe was checked vs NIST	Yes	No	F1
DO	Sensor Calibrated	Yes	No	F2
pH	Sensor Calibrated and Checked	Yes	No	F3
Cond	Sensor Calibrated and Checked	Yes	No	F3

Notes (in situ)
F1 - T - Checked pre-season
F3 - pH, Cond, calibrated and checked this morning at the lab.
F2 - DO calibrated streamside - Winkler comparison collected for July

Sed:%Gravel 0 %Sand 50 %Fines 50

In Situ Chemistry

Start Location (e.g. F0): F0

Time1 13:45 hrs
Temp1 6.3 deg C
pH1 6.94 pH Units
DO1 10.9 mg/L %Sat1 102.5
Cond 27.8 uS/cm @ 25C

End Location (e.g. K0): F0

Time2 19:50 hrs
Temp2 7.0 deg C
pH2 7.00 pH Units
DO2 10.4 mg/L %Sat2 99.5
Cond 27.9 uS/cm @ 25C

Sample	Primary Sample: No. of Jars	Duplicate Sample: No. of Jars (or ITIS for Fish Spp)	Destination	Tracking No. (if shipped)	Flag
TPN	1	0	MEL		
Tot P	1	0	MEL		
Cl	1	0	MEL		
Turb	1	0	MEL		
Sed PAH	1	0	MEL		
Sed Metals*	1	0	MEL		
Benthos	2	0	ETOH Shed at office		F4
Fish Spp1	1	ITIS: 159700	University Lab	FedEx: 835651465756	F5
Fish Spp2	1	ITIS: 167234	office lab for review under microscope		F6
Fish Spp3					

Water Sample Location (e.g. A5): F0

Sample Notes (explain flags):
F4 - Invertebrate sample could not fit into a single jar. Two jars are taped together.
F5 - Lamprey ammocoetes in zipped bag on ice — Vert Collection Form "Jar" #1
F6 - Riffle sculpin in jar of ETOH - verify it is not a reticulate sculpin. — Vert Col. Frm Jar #2

Draft

*Sediment Metals jar includes sample material to be analyzed for TOC
Note: Use standard Manchester Environmental Lab forms for tracking water and sediment samples.

Figure C-3.3. The Chemistry and Sampling Form, with fields that are relevant to periphyton sampling placed in the highlighted benthos fields. Use the flag to indicate volume in bottle.

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

C-4. Taxonomic Lab Sampling Periphyton from Wadeable Streams

Purpose and Scope

Taxonomic identification is conducted by a lab that employs taxonomists with degrees in a related field. The taxonomist should have experience with the freshwater periphyton of the Pacific Northwest. A standardized level of identification is required to allow for valid comparison of periphyton data sets between sampling locations. The standard level of identification required by Ecology is that diatoms be identified to species (or species variety, if possible), and non-diatoms (soft-bodied algae) be identified to genus.

Personnel Responsibilities

One person or more performs this activity. Staff performing this method must have been trained.

Summary of Procedure

Sample Processing and Subsampling

Samples are homogenized in a blender to break up large colonies and filaments and to evenly distribute individual cells. A sub-sample is transferred to a Palmer-Maloney counting chamber, and 300 live algae cells are counted at 400X. The sub-sample is diluted or concentrated to achieve an optimum concentration of 10-20 cells per field. For small filamentous cyanobacteria (e.g., *Phormidium*) and for coenocytic algae that lack cell walls (e.g., *Vaucheria*), the counting unit will be a 10-micron length of filament. Diatoms are identified only as diatoms; soft (non-diatom) algae are identified to genus, and to species if possible. The number of dead (empty) diatom frustules is also recorded on the bench sheet during the count of 300 live cells.

After the identification of soft algae, the homogenized sample is re-agitated and a portion of the raw sample is extracted to make duplicate diatom slides. The diatom sub-sample is cleaned of organic matter using sulfuric acid, potassium dichromate, and hydrogen peroxide. Following several dilutions with distilled water, two permanent diatom slides are prepared from cleaned material using Naphrax, a high refractive index mounting medium. At least 300 diatom cells (600 valves) are counted at random and identified to species, and variety if possible. Diatom naming conventions should follow those adopted by the Academy of Natural Sciences (Philadelphia) for USGS NAWQA samples (Morales and Potapova, 2000) as updated in 2005 (Morales and Charles, 2005). Separate bench sheets will be used for algae and diatom counts, and these will provided to Ecology when samples are completed each year.

Counts of soft algae and diatoms will be conducted with compound research microscopes using brightfield transmitted light or differential interference contrast. Proportional counts will be performed using 100X oil immersion objectives and 10X oculars.

Voucher slides will be provided to Ecology at the end of the project.

Remaining sample volume will be re-preserved and stored by the contract laboratory. Contractor storage of sample fractions may be as long as six months. Ecology may request that a subset of samples be returned for quality assurance purposes. Non-requested sample fractions will be appropriately discarded by the contractor.

Sample Submittal and Data Turn-Around

Ecology will collect samples between early June and late October. Ecology will ship samples to the contractor no later than December 1 of each year. Contracts between Labs and Ecology outline responsibility for costs of shipping samples and forms to the contract laboratory. The processing of samples and reporting of data will be completed within 90 days of the receipt of the samples by the contract laboratory. Data reported to Ecology will consist of:

1. The original (signed) chain-of-custody form.
2. A taxonomic matrix for each sample based on the required level of identification. (Excel or Access format).
3. Metric calculations (Excel or Access format).
4. Copies of bench sheets used by the laboratory.
5. Copies of all QA/QC documentation.

Reporting Requirements

Taxa lists for each sample should be reported in a taxa- density matrix (expressed as abundance per cm^2). Surface area sampled is provided by Ecology and included on the chain-of-custody form. The contract laboratory will analyze the taxonomy matrix for each sample and summarize with a suite of periphyton metrics. In most cases, diatoms and non-diatoms will be analyzed separately with a few exceptions (combined metrics) outlined below. This list represents the minimum metric reporting requirements. The contractor is encouraged to provide additional metrics if available and relevant.

Combined metrics

1. Total number of algal divisions.
2. Total number of algal genera.
3. Total number of algal species.

Diatom metrics

1. Total number of diatom genera.
2. Total number of diatom species.
3. Shannon Diversity Index.
4. Pollution Index (Lange-Bertalot, 1979; Bahls, 1993; Barbour et al., 1999).
5. Siltation Index (% *Navicula* + *Nitzschia* + *Surirella*) (Bahls, 1993; Barbour et al., 1999).
6. Percent live diatoms (Hill, 1997).
7. Valves counted.

8. Cells counted.
9. Percent dominant species.
10. Pollution tolerance (% by category).
11. Disturbance index (Bahls, 1993; Barbour et al., 1999).
12. Percent rhopalodiales.
13. Percent aerophiles.
14. Percent centrics.
15. Motility index.

Van Dam diatom metrics (% in each category)(Van Dam et al., 1994)

1. pH.
2. Salinity.
3. Nitrogen uptake.
4. Oxygen demand.
5. Saprobity.
6. Trophic state.
7. Moisture.

Non-diatom metrics

1. Dominant phylum.
2. Percent 5 dominant genera.
3. Percent 10 dominant genera.
4. Total number of genera (within each non-diatom algal division).
5. Total number of non-diatom genera.

Appendix D. Quality Control Procedures

D-1. Quality Control for In-Situ Meters

Purpose and Scope

This method explains how to verify that in-situ meters used for the Status and Trends Program are working properly. Instruments included on the procedure include probes for measuring temperature, pH, conductivity, and dissolved oxygen (Minisonde Multiprobes). It also includes the instrument used for measuring water velocity (Marsh-McBirney FloMate-2000).

Personnel Responsibilities

This method is performed by 1 or more persons. This method is applied at every DCE, before and after sampling, although some of the tasks are required less frequently. Staff performing this method must have been trained.

Equipment, Reagents, Supplies

- No. 2 pencil.
- Calibration Form.
- Flow Meter.
- Flow Meter batteries.
- Wading rod.
- Flow Meter Manual (Marsh-McBirney, 1990).
- Five-gallon bucket (for flow meter zero-adjust).
- Hydrolab, components, maintenance kit (Swanson, 2007).
- Hydrolab Manuals (Hach 1999, Hach 2006a, Hach 2006b).
- QCCS (Metcalf and Peck 1993).
- pH 7 buffer (7.00) – e.g., VWR - 23197-996.
- pH 4 buffer (4.01) – e.g., VWR - 23197-998.
- pH 10 buffer (10.01) – e.g., VWR - 23197-994.
- pH 7 standard (6.97) – e.g., Thermo 700702.
- pH 4 standard (4.10) – e.g., Thermo 700402.
- pH 9 standard (9.15) – e.g., Thermo 700902.
- Conductivity Standard (100 µS) – e.g., VWR 23226-589.
- Conductivity Standard (1,000 µS) – e.g., VWR 23226-603.
- Conductivity Standard (alternate as available).
- De-ionized water (DI).
- Tap Water.
- Lab tissues (e.g., KimWipes®).
- Barometer.
- Winkler sampling supplies (see Mathieu, 2007).

Summary of Procedure

Calibrate (Conductivity, pH, Dissolved Oxygen, and Velocity)

Calibration of the Hydrolab will be conducted based on EAP033 (Swanson, 2007). Use the *Calibration Form* (Figure D-1.1) to record calibrations and quality control checks. Each day calibrate conductivity (COND), pH, dissolved oxygen (DO) and velocity. The DO should be calibrated on-site or near the site, to match local barometric pressure of calibration and sampling. The pH and conductivity calibration standards should be chosen to bracket expected values. For example, most wadeable streams west of the Cascades or in moderate to high elevations will need to be calibrated with pH 7 and pH 4 standards, they will need to be calibrated with 0 and 100 µS conductivity standard. Many larger streams and rivers will need to be calibrated with pH 7 and ph 10 standards. Some rivers might need to be calibrated with 0 and > 100 µS conductivity standards. The order of calibration is normally:

1. COND (Hydrolab).
2. pH (Hydrolab).
3. DO (Hydrolab).
4. Velocity (zero-adjust the flow meter).

Before calibrating, make sure that a post-sampling QC check measurement has been made to verify the quality of sampling at the previously sampled site. QC checking is discussed in detail later in this document.

S&T Calibration Form prototype					Comments
Person Calibrating Hydrolab					
Date					
Hydrolab Sonde serial number					
Hydrolab Sonde Battery Voltage					
Hydrolab Surveyor serial number					
Hydrolab Surveyor Battery Voltage					
Flow Meter serial number					
Conductivity Calibration					
Standard Used Check one	100		1000		
Cond. (before adjusting)				µS/cm at 25˚C	
Cond. (after adjusting)				µS/cm at 25˚C	
Standard's Temperataure (˚C)				˚C	
QCCS Cond. (pre-field)				units	
QCCS temperature (pre-field)				˚C	
QCCS (pre-field) 65.3 to 85.3 µS/cm at 25˚C ?	Yes		No		No means "failed" - recalibrate
QCCS Cond. (post-field)				units	
QCCS temperature (post-field)				˚C	
QCCS date (post-field)				mm/dd/yyyy	
QCCS (post-field) 65.3 to 85.3 µS/cm at 25˚C ?	Yes		No		No means "failed" - qualify data
QCCS batch check				µS/cm at 25˚C	
QCCS batch check				˚C	
QCCS batch check				mm/dd/yyyy	
pH Calibration					
Standards Used: 6.95 and (Check one)	4.10		9.15	**OR**	
7 and (Check one)	4		10		
pH 6.95 or pH 7 temperature				˚C	
pH 6.95 or pH 7 value - before adjusting				units	
pH 6.95 or pH 7 value - after adjusting				units	
Temp. of 2nd pH standard after adjusting				˚C	
pH of 2nd standard pH - before adjusting				units	
pH of 2nd standard pH - after adjusting				units	
Calibration Slope				%	
Calibration Slope >90% (for dilute standards)	Yes		No	**4.10,6.95,9.15**	No means "failed" - recalibrate
Calibration Slope >___% (for buffer standards)	Yes		No	**4,7,10**	No means "failed" - recalibrate
QCCS pH (pre-field)				units	
QCCS temperature (pre-field)				˚C	
QCCS -(pre-field) 6.78 to 7.18 ?	Yes		No		No means "failed" - recalibrate
QCCS pH (post-field)				units	
QCCS temperature (post-field)				˚C	
QCCS date (post-field)				mm/dd/yyyy	
QCCS -post 6.78 to 7.18 ?	Yes		No		No means "failed" - qualify data
QCCS batch check				units	
QCCS batch check				˚C	
QCCS batch check				mm/dd/yyyy	
DO Calibration					
Atmospheric Pressure				mm HG	
Altitude				ft	
Calibration Temperataure				˚C	
Reading-DO				mg/L	
Reading-Percent Saturation				%	
QCC-Winkler reading				mg/L	
QCC-person titrating					
QCC-Winkler within 0.4 mg/L of Hydrolab	Yes		No		No means "failed" - qualify data
Temperature Calibration check					
Ice bath - Hydrolab				˚C	
Ice bath - NIST Thermometer				˚C	
Ice bath - measures within 1 ˚C	Yes		No		No means "failed" - qualify data
Warm bath - Hydrolab				˚C	
Warm bath - NIST Thermometer				˚C	
Warm bath - measures within 1 ˚C	Yes		No		No means "failed" - qualify data
Person checking					
Velocity Zero-adjust					
At zero in bucket?	Yes				
Adjustment necessary?	Yes		No		
Person checking flow meter					

Figure D-1.1. The Calibration Form.

Clean the Hydrolab Sonde and the Flow Meter Sensor

Refer to Swanson (2007) section 6.3 and to Marsh-McBirney 1990.

Rinse Hydrolab Sonde Between Each Operation

Rinse three times with tap water, three times with deionized water, then three times with the solution to be used for calibrating or testing.

Calibrate Conductivity to Bracket Expected Field Conductivity:

1. Dry the conductivity probe with a lab tissue (e.g., KimWipe®).
2. Using the Surveyor, enter 0 into SpCond, to dry calibrate to 0.
3. Fill the calibration cup to within a centimeter of the top of the calibration cup with dilute standard (either 100 µS or 1,000 µS) so that the probes are covered.
4. Make sure there are no bubbles in the cell, wait 2 minutes.
5. Using the Surveyor, enter the appropriate value for the standard into SpCond.

Table D-1.1. Theoretical pH values by temperature for each pH standard buffer.

Temp (˚C)	4a	7b	10c
4	4.00	7.09	10.26
5	4.00	7.08	10.25
6	4.00	7.08	10.23
7	4.00	7.07	10.22
8	4.00	7.07	10.21
9	4.00	7.06	10.2.0
10	4.00	7.06	10.18
11	4.00	7.05	10.17
12	4.00	7.05	10.16
13	4.00	7.04	10.14
14	4.00	7.04	10.13
15	4.00	7.03	10.12
16	4.00	7.03	10.11
17	4.00	7.02	10.10
18	4.00	7.02	10.09
19	4.00	7.02	10.08
20	4.00	7.01	10.06
21	4.01	7.01	10.05
22	4.01	7.01	10.04
23	4.01	7.00	10.03
24	4.01	7.00	10.02
25	4.01	7.00	10.01
26	4.01	6.99	10.00
27	4.01	6.99	9.99
28	4.01	6.99	9.98
29	4.01	6.99	9.98
30	4.02	6.98	9.97

Buffers: [a] Thermo 7.00, [b] Thermo 4.01, [c] Thermo 10.01. From:
www.thermo.com/com/cda/resources/resources_detail/1,2166,13217,00.html

Calibrate pH to Bracket Expected Field pH

1. Pour the pH 7 buffer into the calibration cup to cover the sensor and reference electrode. Enter the theoretical pH value units the Surveyor. Theoretical values are based on temperature of the standard and are listed in Table D-1.1.
2. Rinse and repeat step 1, using either the pH 10 buffer (when sampling in basic waters) or pH 4 buffer (when sampling in acidic waters).
3. On the calibration form, record the temperature and theoretical pH values (Table D-1.1) that were used to calibrate. Also record adjustments that were needed to calibrate to these theoretical values.
4. On the calibration form, record the percent slope of the calibration (displayed on the Surveyor). Be sure this percent slope matches the criteria described on the form. Otherwise, recalibrate.

Calibrate DO Using Water-Saturated Air

1. Fill the calibration cup with about 1/2 inch of DI; it should be below the sensor cap.
2. Use Kimwipes to dry any droplets from the sensor cap.
3. Invert calibration cup's cap and gently rest it on the cup.
4. Wait 5 minutes, making sure that temperature stabilizes.
5. Determine local barometric pressure (mm Hg) and enter this value into the Surveyor.
6. Click "Calibrate". A "Calibrate Successful" will be displayed.

DO calibration notes:

1. To retain calibration accuracy between measurements, store with the sensor immersed in water or within a water-saturated air environment such as a sealed storage cup with at least 10 ml of water.
2. It is important to have the water-saturated air and the sensor at the same temperature. Therefore, store a jar of DI in the same environment as the sonde, and calibrate in a similar air temperature as the water and sonde.
3. Stay out of direct sun or wind.
4. Refer to Table D-1.2 if necessary.

Table D-1.2. Unit conversions for pressure.

Atmospheres	Bars	Hg mm	inches Hg
1	1.01325	760	29.92126
0.9869233	1	750.0617	29.52999
0.001315789	0.001333224	1	0.03937008
0.03342105	0.03386388	25.4	1

Zero-Adjust the Flow Meter

Zero the flow meter prior to each use. Refer to Marsh-McBirney 1990 (pages 8-9).

Quality Control

Daily Checks

Check pH and conductivity at the start and end of each DCE by measuring the QCCS. Record the temperature of the QCCS too. *The pH should measure between 6.78 and 7.18 pH units.* Conductivity should measure between 65.3 and 85.3 µS/cm at 25 ˚C. Re-calibrate if the presampling check fails these criteria. Data from the DCE should be qualified if the post-sampling check fails these criteria. Record measurements on the *Calibration Form Monthly Checks* Once monthly, check the accuracy of the DO sensor on the Hydrolab. Collect a Winkler sample at the same location and time as an in-situ DO reading. Winkler samples are collected and analyzed according to Mathieu (2007).

Twice-Seasonal Checks

Before and after the season, check the regular pH calibrations against dilute pH standards:

- pH 7 standard (6.97) – e.g., Thermo 700702.
- pH 4 standard (4.10) – e.g., Thermo 700402.
- pH 9 standard (9.15) – e.g., Thermo 700902.

Calibrate first with the regular buffers as for the daily calibrations (e.g., first 7 and 4), then check using the QCCS. Re-calibrate, this time using the dilute standards (e.g., 6.97 and 4.10). Measure the QCCS and compare the difference in QCCS measures between calibrations. Repeat for the high-pH calibrations (7 and 10; 6.97 and 9.15). Theoretical values by temperature for the dilute pH standards are found in Table D-1.3.

Table D-1.3. Theoretical values by temperature for the dilute pH standards.

Temp (˚C)	4a	7b	9c
10	4.10	7.01	9.27
11	4.10	7.01	9.26
12	4.10	7.00	9.25
13	4.10	7.00	9.25
14	4.10	7.00	9.24
15	4.10	7.00	9.23
16	4.10	6.99	9.22
17	4.10	6.99	9.21
18	4.10	6.99	9.21
19	4.10	6.98	9.20
20	4.10	6.98	9.19
21	4.10	6.98	9.18
22	4.10	6.97	9.18
23	4.11	6.97	9.17
24	4.11	6.97	9.16
25	4.11	6.97	9.16
26	4.11	6.96	9.15
27	4.11	6.96	9.14
28	4.12	6.96	9.13
29	4.12	6.95	9.13
30	4.12	6.95	9.12

a Orion 700402, bOrion 700702, c Orion 700902

The Hydrolab's thermistor is factory calibrated. Check the settings before and after the field season by comparing with an NIST-traceable thermometer. Verify that it measures to within 1˚ C the thermometer. Do this in an ice water bath and in a warm water bath. Qualify the season's temperature data if the measures fall outside this range.

D-2. Quality Control for Laboratory Analysis – Benthos

Lab Quality Assurance Samples

Macroinvertebrate Sorting Efficiency
Quality control procedures for initial sample processing and subsampling involves checking sorting efficiency. These checks are conducted on 100% of the samples by independent observers who microscopically re-examine 20% of sorted substrate from each sample. All organisms that were missed are counted. Sorting efficiency is evaluated by applying the following calculation:

$SE = n_1/n_2 \times 100$

where SE is the sorting efficiency, expressed as a percentage, n 1 is the total number of specimens in the first sort, and n 2 is the total number of specimens in the first and second sorts combined. Sorting efficiency is recorded on each benchsheet, and this data is entered into the Rhithron database. If 95% sorting efficiency is not achieved for a given sample, a failure is recorded on the benchsheet and in the database. The sorted portion of that sample is then completely re-sorted before the sorting efficiency test is repeated for that sample. Sorting efficiency statistics for each technician and for the entire laboratory are reviewed monthly. Sorting efficiency for each sample in a project is reported to the client in the technical summary document. Technicians who do not maintain the target sorting efficiency are given remedial training, and larger portions of the samples they process are examined for the sorting efficiency test until they are able to maintain the target sorting efficiency.

A second evaluation of the sub-sampling process is applied to a small proportion of samples processed in each month; typically one sample per week is subjected to the following test of precision of the sub-sampling process. The procedure is only applied to samples where the target number of organisms was achieved in less than half of the Caton grids. A sample is randomly selected, and a second sub-sample is re-sorted from the unprocessed sample remnant. A second technician performs this sort. The resulting sub-sample is identified, and Bray-Curtis similarity index is calculated for the results of both sub-samples. Results that are less than 90% similar would indicate the need for more thorough distribution of sample materials in the subsampling tray or more special attention given to easily missed taxa when sorting (i.e. increased magnification).

Taxonomic Accuracy and Precision

Taxonomic misidentification results in inadequate biological characterization of a stream. Errors in identification should be less than 5% of the total taxa in the sample. Re-identification of samples is conducted for 10% of the total number of samples in each year. Secondary identification is conducted by experienced taxonomists in order to maintain confidence in the data set. Difficult taxa should be sent to museum curators whose specialty includes members of the order in question. A voucher collection has been maintained by Ecology and is being transferred to the Orma J. Smith Museum of Natural History in Caldwell, Idaho for curation. A

voucher collection should be prepared from the set of samples for the year and shipped to the address below:

The Orma J. Smith Museum of Natural History
College of Idaho
2112 Cleveland BLVD
Caldwell, ID 83605-4432

Documentation necessary for acceptance by the museum will be delivered to the successful bidder with the samples.

D-3. Quality Control for Laboratory Analysis – Periphyton

Taxonomic Accuracy and Precision: Taxonomic misidentification of diatom and algal taxa results in inadequate biological characterization of a stream. Errors in identification should be less than 5% of the total taxa in the sample. Re-identification of samples is conducted for 10% of the total number of samples in each year. Secondary identification is conducted by experienced taxonomists in order to maintain confidence in the data set. Difficult taxa should be sent to specialists whose area of expertise includes members of the order in question.

Chlorophyll *a* analyses will be performed in accordance with the *Manchester Environmental Laboratory Users Manual* (MEL, 2008). This manual indicates that the reporting limits listed in Table 5 can be achieved by using analytical methods listed. Laboratory staff will consult the project manager if there are any changes in procedures over time timeline, or if other difficulties arise.

After subsamples are withdrawn from the composite sample, the remaining sample volume will be re-preserved and stored by the contract laboratory. Contractor storage of sample fractions may be as long as six months. Ecology may request that a subset of samples be returned for quality assurance purposes. Non-requested sample fractions will be appropriately discarded by the contractor.

Appendix E. Field Forms for Ambient Biological Monitoring in Washington.

There are 8 data forms that will be scanned using the Teleform system to enter data into the Status and Trends system. These are:

- GPS Positions Form.
- Site Verification Form.
- Site Diagram.
- Chemistry and Sampling Form.
- Discharge Worksheet.
- Major Transect Form.
- Thalweg Data Form.
- Slope and Bearing Form.

Table E-1. The juxtaposition of field forms and number of copies needed per data collection

Front Side	Back side	Copies per DCE
Site Verification Form	Site Diagram	1
Chemistry and Sampling Form	Discharge Worksheet	1
Major Transect Form	Thalweg Data Form	11
Slope and Bearing Form	GPS Positions Form	1
Vertebrate Collection Form front	Vertebrate Collection Form back	1

See Figures E-1 to E-10 for each of the forms that will be scanned into the Status and Trends Database using the Teleform system.

Figure E-1. The Site Verification Form.

Is the site unsafe to access, or with barriers that prevent access (round trip) and sampling by wading within one day?	**Y N**
Is the site unsafe to access, or with barriers that prevent access (round trip) and sampling by raft within one day?	**Y N**

Why is it inaccessible?

SITE DIAGRAM

Provide North Arrow

Draft

Figure E-2. The Site Diagram Form.

Reviewed by (Initials): _____

Status and Trends - Chemistry and Sampling Form

Site Number — YY — MMDD — HH : MM

DCE: W A M 0 6 0 0 - _ _ _ - D C E - 2 0 _ _ - _ _ _ - _ _ : _ _

IN SITU WATER QUALITY CALIBRATION				
Operator _____		Unit # _____		Flag
T	Temp probe was checked vs NIST	Yes	No	
DO	Sensor Calibrated	Yes	No	
pH	Sensor Calibrated and Checked	Yes	No	
Cond	Sensor Calibrated and Checked	Yes	No	

Notes (in situ)

Sed:%Gravel _____ %Sand _____ %Fines _____

In Situ Chemistry

Start Location (e.g. F0) ☐☐

Time1 ___:___ hrs
Temp1 ___.___ deg C
pH1 ___.___ pH Units
DO1 ___.___ mg/L %Sat1 ___.___
Cond ___.___ uS/cm @ 25C

End Location (e.g. K0) ☐☐

Time2 ___:___ hrs
Temp2 ___.___ deg C
pH2 ___.___ pH Units
DO2 ___.___ mg/L %Sat2 ___.___
Cond ___.___ uS/cm @ 25C

Sample	Primary Sample: No. of Jars	Duplicate Sample: No. of Jars (or ITIS for Fish Spp)	Destination	Tracking No. (if shipped)	Flag
TPN					
Tot P					
Cl					
Turb					
Sed PAH					
Sed Metals*					
Benthos					
Fish Spp1					
Fish Spp2					
Fish Spp3					

Water Sample Location (e.g. A5) ☐☐

Sample Notes (explain flags):

Draft

*Sediment Metals jar includes sample material to be analyzed for TOC
Note: Use standard Manchester Environmental Lab forms for tracking water and sediment samples.

Figure E-3. The Chemistry and Sampling Form.

Flow Location - Thalweg Station (e.g. A5) : ____ Flow Meter (Model / Unit #) _____ / _____

Discharge Worksheet

Flow Meter Zeroed Out?	Y N	Wetted Width:	

Cell	Tape Distance Left to Right (cm)	Wetted Depth (ft.x)	Velocity (ft.xx/s)	Notes
01				Left edge of water
02				
03				
04				
05				
06				
07				
08				
09				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				Right wetted margin

Describe Alternate Method:	Flow Method: (circle one)	Flow Meter	Time of Travel
		Bucket Flow	Gage

Draft

Discharge: ___ , ___ . ___ cfs

Notes (discharge)

Figure E-4. The Discharge Worksheet.

Major Transect Form

Reviewed by (Initials):_____

Transect	Channel #	Site Number	YY	MMDD	HH : MM
		DCE: WAM0600 - - DCE - 20	-	-	:

SUBSTRATE

	Wet Depth	BF Depth XXX CM	Size Class	Embd. 0-100%	Flag
left bank					
.1					
.2					
.3					
.4					
.5					
.6					
.7					
.8					
.9					
right bank					

FISH COVER

0 = Absent (0%)
1 = Sparse (<10%)
2 = Moderate (10-40%)
3 = Heavy (40-75%)
4 = Very Heavy (>75%)
(circle one)

	Cover in Channel	Flag
Filamentous Algae	0 1 2 3 4	
Macrophytes	0 1 2 3 4	
Woody Debris >0.3 m(BIG)	0 1 2 3 4	
Brush/Woody Debris < 0.3 m (SMALL)	0 1 2 3 4	
Live Trees or Roots	0 1 2 3 4	
Overhanging Veg. =<1 m of Surface	0 1 2 3 4	
Undercut Banks	0 1 2 3 4	
Boulders	0 1 2 3 4	
Artificial Structures	0 1 2 3 4	
Bryophytes	0 1 2 3 4	

RIPARIAN

0 = Absent (0%)
1 = Sparse (<10%)
2 = Moderate (10-40%)
3 = Heavy (40-75%)
4 = Very Heavy (>75%)

D = Deciduous
C = Coniferous
E = Broadleaf Evergreen
M = Mixed
N = None

RIPARIAN VEGETATION COVER	Left Bank	Right Bank	Flag
Canopy (>5 m high)			
Woody Vegetation Type	D C E M N	D C E M N	
BIG Trees (Trunk >0.3 m DBH)	0 1 2 3 4	0 1 2 3 4	
SMALL Trees (Trunk <0.3 m DBH)	0 1 2 3 4	0 1 2 3 4	
Understory (0.5 to 5 m high)			
Woody Vegetation Type	D C E M N	D C E M N	
Woody Shrubs & Saplings	0 1 2 3 4	0 1 2 3 4	
Non-Woody Herbs, Grasses, & Forbs	0 1 2 3 4	0 1 2 3 4	
Ground Cover (<0.5 m high)			
Woody Shrubs & Saplings	0 1 2 3 4	0 1 2 3 4	
Non-Woody Herbs, Grasses and Forbs	0 1 2 3 4	0 1 2 3 4	
Barren, Bare Dirt or Duff	0 1 2 3 4	0 1 2 3 4	

HUMAN INFLUENCE	0=not present, 1= 10-30m, 2= 0-10m, 3= on bank		
	Left Bank	Right Bank	Flag
Wall/Dike/Revetment/ Riprap/Dam	0 1 2 3	0 1 2 3	
Buildings	0 1 2 3	0 1 2 3	
Unpaved Motor Trail	0 1 2 3	0 1 2 3	
Clearing or Lot	0 1 2 3	0 1 2 3	
Human Foot Path	0 1 2 3	0 1 2 3	
PavedRoad/Railroad	0 1 2 3	0 1 2 3	
Pipes (Inlet/Outlet)	0 1 2 3	0 1 2 3	
Landfill/Trash	0 1 2 3	0 1 2 3	
Park/Lawn	0 1 2 3	0 1 2 3	
Row Crops	0 1 2 3	0 1 2 3	
Pasture/Range/Hay Field	0 1 2 3	0 1 2 3	
Logging Operations	0 1 2 3	0 1 2 3	
Mining Activity	0 1 2 3	0 1 2 3	

BANK

		Flag
Wetted Width XXX.X m		
Bar Width XX.X m		
Bankfull Width XXX.X m		
R Bankfull Height XX.X		
L BankfullHeight XX.X		
B Height Average		
LB Instability %		
RB Instability %		

Densiometer

(0-17Max)

		Flag			Flag
CenUp			CenR		
CenL			Left		
CenDwn			Right		

Flag codes: K = Sample not collected; U = Suspect sample; F1, F2, etc. = flag assigned by field crew. Explain all flags in comment sections.

Flag	Comments

Draft

04/07/2009

Figure E-5. The Major Transect Form.

Thalweg Data Form

Reviewed by (Initials):_____

Site Number	YY	MMDD	HH : MM
DCE: WAM0600- -	DCE-20	-	- :

Transect	Thalweg Depth (cm)	Bar? (circle)	Edge Pool? (circle)	Habitat Unit Number	Side Channel Numbers					Wet Width (m.x)	BF Width (m.x)	Bar Width (m.x)	Thalweg Notes:
.0		Y N	Y N										
.1		Y N	Y N										
.2		Y N	Y N										
.3		Y N	Y N										
.4		Y N	Y N										
.5		Y N	Y N										
.6		Y N	Y N										
.7		Y N	Y N										
.8		Y N	Y N										
.9		Y N	Y N										

Substrates at 5	LB	01	02	03	04	05	06	07	08	09	RB	Substrate Notes:

Habitat Unit Number	Habitat Unit Type FT, FN, PS, PD, PP,DC	Pool Forming Code (N, W, R, B, F) Code 1	Code 2	HU Width (m.x)	Max Pool Depth (cm)	Crest Pool Depth (cm)	Channel Unit Notes:

Side Channel Number	Width (m.x)	Side Channel Notes:

LWD Count — Example: 6 — Check box if all are zero ☐

	2-5 m	5-15 m	>15 m	Flag
10-30 cm				
30-60 cm				
60-80 cm				
>80 cm				

LWD Notes:

Draft

Figure E-6. The Thalweg Data Form.

Reviewed by (Initials):_____

Status & Trends - Slope and Bearing Form

Site Number YY MMDD HH : MM

DCE: W A M 0 6 0 0 - - D C E - 2 0 - - : :

Top Transect* LEVEL, COMPASS	Bottom Transect* ROD	Segment Length (m.x)	Eye Height on Level (cm)	Level Height on Rod (cm)	Bearing (deg)	Flag	Comment

Reviewed by (Initials):

Status and Trends Program - GPS Positions Form

Site Number YY MMDD HH : MM

DCE: W A M 0 6 0 0 - - D C E - 2 0 - - :

Station	Bank (circle one)	Master Lat dec deg e.g. 47.123456	Master Lon dec deg e.g. 120.123456	GPSlatDD e.g. 47.123456	GPSLonDD e.g. 120.123456	Accuracy	Accuracy Units (ft, EPE, etc.)	Flag
INDEX STATION	L R							
A0	L R							
B0	L R							
C0	L R							
D0	L R							
E0	L R							
F0	L R							
G0	L R							
H0	L R							
I0	L R							
J0	L R							
K0	L R							
PUTIN	L R							
TAKEOUT	L R							

ALL COORDINATES TO BE RECORDED IN NAD83

Position comments including accuracy

Directions to access point

22965

Figure E-8. The GPS Positions Form.

Biomonitoring Program – Photo Log Form			
Photo Number	Date/time	Field Site Number	Photo Description

Figure E-9. The Photo Log Form.

Appendix F. Field Equipment Checklist

Table F-1. Checklist of equipment necessary to complete the procedures in the Ambient Biological Monitoring Program at Ecology.

Check Box (√)	Category	Item
	General	No. 2 Pencils
	General	Pencil sharpener
	General	Waders/Boots
	General	Backpacks
	General	This manual
	General	Clip Boards (3): Habitat, Chemistry, Biology
	General	Camera
	General	Calculator
	General	Field notebook
	General	Stopwatch
	General	Calipers
	Forms and labels	Site Verification Form x Site Diagram (1)
	Forms and labels	Chemistry and Sampling Form x Discharge Worksheet (1)
	Forms and labels	Major Transect Form x Thalweg Data Form (11)
	Forms and labels	Slope and Bearing Form x GPS Positions (1)
	Forms and labels	Calibration Form (1)
	Forms and labels	Laboratory Analyses Required Form
	Forms and labels	Benthos Label (waterproof) for jar exterior
	Forms and labels	Benthos Label (waterproof) for jar interior
	Forms and labels	Periphyton Label (waterproof) for jar exterior
	Forms and labels	Periphyton Label (waterproof) for jar interior
	Calibration	Hydrolab, components, maintenance kit
	Calibration	Hydrolab Manuals
	Calibration	QCCS
	Calibration	pH 7 buffer (7.00) - e.g., VWR - 23197-996
	Calibration	pH 4 buffer (4.01) - e.g., VWR - 23197-998
	Calibration	pH 10 buffer (10.01) - e.g., VWR - 23197-994
	Calibration	pH 7 standard (6.97) - e.g., Thermo 700702
	Calibration	pH 4 standard (4.10) - e.g., Thermo 700402
	Calibration	pH 9 standard (9.15) - e.g., Thermo 700902
	Calibration	Conductivity Standard (100 ?S) - e.g., VWR 23226-589
	Calibration	Conductivity Standard (1,000 ?S) - e.g., VWR 23226-603
	Calibration	Conductivity Standard (alternate as available)
	Calibration	De-ionized water (DI)
	Calibration	Tap Water
	Calibration	Lab tissues (e.g., KimWipes®)
	Calibration	Barometer
	Category	Item
	Calibration	Winkler sampling supplies
	In-situ	Hydrolab, components, maintenance kit
	In-situ	Hydrolab Manuals
	Discharge	Flow Meter

Check Box (√)	Category	Item
	Discharge	Flow Meter Manual
	Discharge	Batteries
	Discharge	Wading rod (top setting)
	Discharge	Orange or other neutrally buoyant object.
	Discharge	5-gallon bucket
	Benthos sampling	Wide-mouth polyethylene jar (128 oz or 3.8 L)
	Benthos sampling	D-Frame kick net with handle and frame
	Benthos sampling	95% Ethanol (add 3 parts by volume for each part sample)
	Benthos sampling	Clear tape
	Benthos sampling	Electrical tape
	Periphyton sampling	Firm bristle toothbrush
	Periphyton sampling	Plastic tray or bowl
	Periphyton sampling	Rinse bottle
	Periphyton sampling	Aluminum foil
	Periphyton sampling	Wide-mouth polyethylene jar (1 L)
	Periphyton sampling	95% Ethanol
	Periphyton sampling	Gallon Ziplock bags
	Periphyton sampling	Plastic funnel
	Periphyton sampling	Distilled or deionized water
	Periphyton sampling	Pocket knife
	Habitat	GPS
	Habitat	Maps
	Habitat	Measuring rod
	Habitat	50-m tape
	Habitat	Laser rangefinder
	Habitat	Hand level with monopod
	Habitat	Clinometer
	Habitat	PVC ring with 10-cm ID
	Habitat	Modified convex densiometer
	Habitat	Flagging
	Habitat	Permanent marker
	Habitat	Calipers
	Habitat	Field notebook
	Habitat	Compass

Appendix G. Standard Taxonomic Effort (except Chironomidae)

Ephemeroptera

- Genus, with exceptions noted:
- Baetidae
  - Acentrella—species.
  - Acerpenna—species.
  - Baetis—species.
  - Baetodes—species.
  - Cloeodes—species.
  - Diphetor hageni—monotypic.
  - Fallceon quilleri—distribution, genus for far SW US projects.
  - Paracloeodes minutus—distribution.
  - Psuedocloeon sp.—species.
- Caenidae
  - Amercaenis ridens—distribution.
  - Caenis—species.
- Ephemerellidae
  - Attenella—species.
  - Caudatella—species.
    - Note that C. cascadia has been synonymized with C. hystrix.
  - Caurinella idahoensis—monotypic.
  - Drunella—species.
    - Use D. coloradensis/flavinea for D. coloradensis and D. flavinea.
  - Ephemerella—species.
    - Use E. inermis/infrequens for E. inermis and E. infrequens.
  - Eurylophella—species.
  - Serratella—species.
  - Timpanoga hecuba—monotypic.
- Ephemeridae
  - Ephemera simulans—distribution.
- Heptageniidae
  - Epeorus—species for Rocky Mountain specimens, genus otherwise.
  - Stenacron—species.
  - McCaffertium—species.
- Leptophlebiidae
  - Neochoroterpes—species.
  - Thraulodes—species.
  - Traverella sp.—species.
- Leptohyphidae
  - Leptohyphes—species.
  - Vacupernius packeri—monotypic.

Odonata

Species for mature specimens of most taxa (exception below), genus otherwise:

Coenagrionidae—genus.

Plecoptera

Genus for most taxa (exceptions below):

Capniidae—family except for late instar larvae.

Leuctridae—family except for late instar larvae.

Despaxia augusta—monotypic.

Moselia infuscata—monotypic.

Nemouridae

Visoka cataractae—monotypic.

Zapada—species.

Use Z. oregonensis gr. for members of that species group.

Perlodidae

Frisonia picticeps—monotypic.

Kogotus/Rickera—for indeterminate specimens.

Osbenus yakimae—monotypic.

Perlinodes aurea—monotypic.

Pictetiella expansa—monotypic.

Rickera sorpta—monotypic.

Perlidae

Acroneuria—species.

Calineuria californica—monotypic.

Claassenia sabulosa—distribution.

Hesperoperla—species.

Pteronarcyidae

Pteronarcys—species for mature specimens.

Hemiptera

Genus for most taxa (exceptions below):

Gerridae—ignore.

Veliidae—ignore.

Coleoptera

Genus for most taxa (exceptions below):

Elmidae

Ampumixis dispar—monotypic.

Atractelmis wawona—monotypic.

C. Barr undescribed sp.—used for that genus soon to be described by C. Barr.

Cleptelmis addenda—monotypic.

Macronychus glabratus—monotypic.

Ordobrevia nubifera—monotypic.

Rhizelmis nigra—monotypic.

Psephenidae

Eubrianax edwardsi—monotypic.

Megaloptera

Genus except for:

Orohermes crepusculus—monotypic.

Diptera

Larvae to genus with a few exceptions noted below and the following to family:
Thaumaleidae, Dolichopodidae, Syrphidae, Tabanidae, Ephydridae, Muscidae, Sciomyzidae
pupae to family except cased Simuliidae to genus, Antocha to genus.

Tipulidae

Rhabdomastix—larvae to species group.

Ceratopogonidae

Bezzia/Palpomyia—use for those two genera inseparable as larvae.

Chaoboridae

Eucorethra underwoodi—monotypic.

Psychodidae

Pericoma/Telmatoscopus—use for those two genera inseparable as larvae.

Stratiomyidae

Hedriodiscus/Odontomyia—use for inseparable larval specimens.

Trichoptera

Larvae generally to genus except monotypic species, other exceptions noted below:
Pupae to family.

Rhyacophilidae

Rhyacophila—most larvae to species group using Smith designations, with the following exceptions:

R. betteni gr.

R. malkini—distinctive.

R. leiftincki gr.

R. arnaudi—only N.A. species in group.

R. sibirica gr.

R. narvae—usually distinctive, leave at species group if unsure.
R. blarina—distinctive.
R. pellisa/valuma—use this for all R. atrata subgroup.

Hydropsychidae

Arctopsyche—larvae to species.
Parapsyche—larvae to species.
Potamyia flava—distribution.
Smicridea—to subgenus.

Polycentropodidae

Cyrnellus fraternus—distribution.

Psychomyiidae

Psychomyia—larvae to species.

Apataniidae

Pedomoecus sierra—monotypic.

Brachycentridae

Amiocentrus aspilus—monotypic.
Brachycentrus—larvae to species.

Calamoceratidae

Heteroplectron californicum—distribution.

Leptoceridae

Mystacides—larvae to species.

Oecetis—larvae of O. avara, O. disjuncta to species, all others to genus.
Limnephilidae
Allocosmoecus partitus—monotypic.
Amphicosmoecus canax—monotypic.
Chyranda centralis—monotypic.
Clostoeca disjuncta—monotypic.
Dicosmoecus—larvae to species.
Ecclicosmoecus scylla—distribution.
Hydatophylax hesperus—distribution.
Uenoidae
Neophylax—larvae to species.
Sericostriata surdickae—monotypic.
Lepidoptera
Larvae—Petrophila and Paraponyx to genus, most others to family, order if uncertain
pupae—order.
Cnidaria
Genus.
Nemertea
Genus.
Turbellaria
Phylum, except Polycelis to genus.
Nematoda
Phylum.
Nematomorpha
Phylum.
Gastropoda
Genus in most cases, with exceptions noted:
Valvatidae
Species for mature specimens, if immature leave at genus.
Hydrobiidae—family.
Lymnaeidae
Radix auricularia—monotypic.
Bivalvia
Genus for mature specimens.
Branchiobdella
Order (Branchiobdellida).
Polychaeta
Manayunkia speciosa—distinctive.
Hirudinea
Genus, with exceptions noted:
Erpobdellidae—family.
Glossiphoniidae
Glossiphonia complanata—distinctive.
Helobdella stagnalis—distinctive.
Piscicolidae
Piscicola—species for mature specimens.

Crustacea

Genus, with exceptions noted:
Astacidae—species.
Cambaridae—species for mature males.
Ostracoda—class.
Branchiopoda—ignore.
Copepoda—ignore.

Acarina

Genus for adults, use ‘Acari’ for indeterminate specimens, leave Oribatei at suborder (Oribatei).

Appendix H. Taxonomic Effort for Chironomidae

To maintain data consistency, Chironomidae identifications should be to the genus level when practical except for the following taxa:

Cardiocladius albiplumus

Cricotopus (Isocladius) Type I.

(EcoAnalysts in-house designation) presence of two "racing stripes" on the dorsum of the light colored (yellow) head, body with gray mottling, dark mentum with 15 teeth, first lateral teeth closely pressed to the median teeth, and the last two pairs of lateral teeth appear to be reduced and slightly separated from the other lateral teeth.

Cricotopus (Nostococladius) nostocicola

(previously referred to as *Cricotopus (Nostocladius)* sp. by EcoAnalysts, changed in 2002.)

Cricotopus bicinctus gr.

Cricotopus trifascia gr.

Heterotrissocladius

(identify to species group following Wiederholm, 1983 and 1986.)

Hyporhygma quadripunctatum (monotypic).

Lauterborniella agrayloides (monotypic).

Microtendipes

(identify to species group following Wiederholm, 1983 and 1986.)

Orthocladius Complex

(encompasses *Orthocladius* sp.; and *Cricotopus* sp. that are inseparable from *Orthocladius* sp.; and *Paratrichocladius* sp.)

Orthocladius (Symposiocladius) lignicola

Paralauterborniella nigrohalteralis (monotypic)

Paramerina/Zavrelimyia sp.

(This includes *Reomyia* sp.)

Paraphaenocladius "n. sp."

(EcoAnalysts in-house designation) single median tooth, "long" antenna, six antennal segments (sixth hairlike), second antennal segment with a "break."

Platysmittia

(identify to species following Epler, 2001 and Jacobsen, 1998.)

Potthastia

(identify to species group following Wiederholm, 1983 and 1986.)

Robackia

(identify to species following Wiederholm, 1983 and Epler, 2001.)

Saetheria tylus

(larval diagrams in Epler, 2001 and Merritt and Cummins, 1996.)
Tempisquitoneura merrillorum
Thienemannimyia gr. sp.
(consists of the genera *Arctopelopia*, *Conchapelopia*, *Hayesomyia*, *Helopelopia*, *Meropelopia*, *Rheopelopia*, *Telopelopia*, and *Thienemannimyia*.)

Tribelos jucundum

Tvetenia

(identify to species group following Bode, 1983 ie. discoloripes grp. and bavarica grp.)
Unniella multivirga (monotypic).
Xenochironomus xenolabis (monotypic).
Xylotopus par (monotypic).

Appendix I. Aggregation of Taxa

1. When to aggregate.

Unless the taxonomist has a compelling reason (listed below) to believe the unidentifiable individuals are unique, those individuals shall be aggregated.

An unidentifiable individual will be considered unique (distinct) if and only if:

A. Morphological characteristics preclude the unidentified individual from being any of the other taxa identified (in other words, the specimens can not be the same thing). If they cannot be the same, then they are considered distinct.

B. Due to maturity an unidentifiable specimen is more than two major taxonomic levels removed from its nearest possible relative.

2. How to aggregate.

Unidentifiable individuals shall be aggregated with the nearest related lower taxon or taxa using all available information (e.g., ecological, life history, developmental, etc.). In cases where no other clues are available, individuals shall be apportioned according to the relative abundance of each related taxon.

3. Notation.

Each taxon that contains aggregated individuals will be marked using a simple note, e.g.. A7, where “A” represents aggregate and “7” being the number of individuals aggregated.

Appendix J. Standard Chain of Custody Form

PAGE ___ of ___ Biological Monitoring Project Chain of Custody Complete one form for each sample type	Project Lead:_____ Staff member :_____ submitting sample to lab Date samples transferred to Lab:_____ Date Project Lead downloads data:_____ Lab staff member receiving samples_____ Date samples received by Lab:_____ Date vouchers transferred to archive:_____ Date data available to Project Lead for download:_____ Archive staff member receiving vouchers_____ Date vouchers received by archive:_____

Sample ID	Sample Type P=Periphyton M= Macroinvertebrate	Sample Location Latitude DMS	Sample Location Longitude DMS	# Jars/ sample
Total Samples Transferred				

PAGE ___ of ___
Biological Monitoring Project
Chain of Custody
Date Samples Transferred to Lab:

Sample ID	Sample Type P=Periphyton M= Macroinvertebrate	Sample Location Latitude DMS	Sample Location Longitude DMS	# Jars/ sample
Total Samples Transferred				

Laboratory Analyses Required

Page ____ of ____

Project Name: _____

SIC: __ __ __ __ __ Program: _____

Send Results to: _____ Mail Stop: _____

☐ Preliminary Investigation ☐ For HW Designation

☐ Monitoring ☐ For NPDES

Date Results needed by: _____

Name/Reference # of QAPP for this project: _____

| Sampling | | | | | | General Chemistry | | | | | |

Matrix Codes

Code	Description
10	Water
11	Field Filtered Water
12	Filter from Water
13	Water to be filtered upon receipt at lab
40	Soil/Sediment
41	Frozen Soil/Sediment (PSEP)
45	Semi-Solid/Sludge
70	Tissue
80	Oil/Solvent
90	Waste
00	Other (Use only if no other apply)

Source Codes

Code	Description
00	Unspecified Source
01	Unknown Liquid Media (Drum/Tank)
02	Unknown Liquid Media (Spill Area)
03	Unknown Liquid Media (Waste Pond)
10	**Water (General)**
12	Ambient Stream/River
13	Lake Reservoir
14	Estuary/Ocean
15	Spring/Seepage
16	Rain
17	Surface Runoff/Pond (general)
18	Irrigation Canal/Return Flow
20	**Well (General)**
21	Well (Industrial/Agricultural)
22	Well (Drinking Water Supply)
23	Well (Test/Observation)
24	Drinking Water Intake
25	Drinking Water (At Tap)
30	**Effluent Wastewater (General)**
31	Municipal Effluent
32	Municipal Inplant Waters
33	Industrial Surface Runoff/Leachate
34	Industrial Effluent
35	Industrial Inplant Waters
36	Industrial Surface Runoff/Pond
37	Industrial Waste Pond
38	Landfill Runoff/Pond/Leachate
40	**Sediment (General)**
42	Bottom Sediment or Deposit
44	Sludge (General)
45	Sludge (WastePond)
46	Sludge (Drum/Tank)
48	Soil (General)
49	Soil (Spill/Contaminated Area)

Code	Description
50	Bore Hole Material
60	**Air (General)**
61	Ambient Air
62	Source or Effluent Air
63	Industrial or Workroom Air
70	**Tissue (General)**
71	Fish Tissue
72	Shellfish Tissue
73	Bird Tissue
74	Mammal Tissue
75	Macroinvertebrate
76	Algae
77	Periphyton
78	Plant/Vegetation
80	**Oil/Solvent (General)**
81	Oil (Transformer/Capacitor)
82	Oil/Solvent (Drum Tank)
83	Oil/Solvent (Spill Area)
84	Oil/Solvent (Waste/Pond)
90	**Commercial Product Formulation**
95	Well Drill Water
96	Well Drill Mud
97	Well Sealing Material
98	Gravel Pack Material

PRE-Sampling Notification

Fax to Manchester Laboratory: (360) 871-8850

Enforcement
Monitoring
Emergency
Class II
Preliminary Invest.
Special turnaround

Project Name: _____ **SIC:** _____

Requested by: _____ Sampling Date(s): _____

Program: _____ Date to Lab: _____

Phone No.: _____ Sample Pickup Location: _____

QAPP: ☐Yes ☐ No Date results needed by: _____

General Chemistry	W	S	O	Microbiology		W	S	O	Organic Chemistry	W	S	O
Alkalinity				Fecal Coliforms ☐ MF ☐ MPN					Base/Neutral/Acids (BNA)			
Conductivity				E. Coli MF ☐ "MUG" ☐ mTEC2					Polynuclear Aromatics (PAH)			
Hardness				E. Coli MPN								
pH				% Klebsiella					Volatile Organic Analysis (VOA)			
Turbidity									BTEX			
☐ Fluoride ☐ Chloride ☐ Sulfate									Pest/PCB's (Organochlorine)			
Cyanide ☐ Total ☐ Dissociable				**Metals**	W_T	W_D	S	O	Pesticides only (Organochlorine)			
Total Solids				Priority Pollutant Metals (13 elements)					PCB's only			
Total Nonvolatile Solids				TCLP metals					OP - Pests (Organophosphorous)			
Total Suspended Solids				Hardness					Herbicides (Chlorophenoxy)			
Total Nonvolatile Suspended									Nitrogen Pesticides			
Total Dissolved Solids				Mercury (Hg) ☐ Low Level ☐ Regular					PCL Pesticides (8085)			
Chlorophyll ☐ Filtered in field ☐ Filtered at lab				Other: List individual elements:					PBDEs			
% Solids									Hydrocarbon ID (match to source)			
% Volatile Solids (TVS)									TPH-ID (gas/diesel/oil)			
Total Organic Carbon									$TPH\text{-}G_x$			
Dissolved Organic Carbon									$TPH\text{-}D_x$			
Biochemical Oxygen Demand (BOD) 5 day												
BOD - Inhibited									TCLP-VOA			
BOD - Ultimate									TCLP-BNA			
Ammonia									TCLP-Herbicides			
Nitrate-Nitrite									TCLP-Pesticides			
Orthophosphate												
Total Phosphorous												
☐ TPN ☐ TKN				Asbestos								

Comments: Enter the number of samples in the appropriate box(es) above

W = water S = soil/sediment O = other (please specify)
W_{TR} = water total W_D = water dissolved

ECY 040-2-62 (Rev. 06/05) Ecology is an Equal Opportunity Employer.

Sample Container Request Form

Please FAX to Leon Weiks: (360) 871-8850
(Phone for Leon Weiks: (360) 871-8825)

Requestor: _____ Project Name: _____

Phone: _____ Today's Date: _____

Location for Delivery: _____ Date Needed by: _____

Index #	Description	Qty.
1	1 gallon jar *	
2	1/2 gallon jar *	
3	1 liter jar * (wide mouth) (special request only)	
4	1 liter jar (narrow mouth) (oil & grease) **	
5	8 oz short jar *	
6	8 oz short jar **	
8	4 oz short jar *	
9	4 oz short jar **	
11	40 mL vial w/septum *	
13	2 oz short jar w/septum * (Volatiles: solids only)	
14	125 mL amber glass bottle * (carbamate)	
15	1 liter amber bottle * (narrow mouth)	
16	500 mL HDPE bottle (metals)	
17	1 gallon cubitainer	
19	125 mL clear Nalgene (nutrients or COD; bottle contains 1:1 sulfuric acid)	
20	125 mL amber Nalgene (filters and syringe also required for orthophosphate)	
21	125 mL polypropylene bottle (hardness - bottle contains acid) **For Hexachrome, request bottle without acid**	

Index #	Description	Qty.
22	500 mL poly bottle	
23	1000 mL poly bottle	
24	1000 mL amber poly	
25	250 mL amber poly bottle (Cyanide) (contains Sodium Hydroxide)	
26	60 mL poly bottle (TOC/DOC or TP; bottle contains 1:1 hydrochloric acid)	
27	250 mL glass or poly bottle (fecal coli)	
28	500 mL glass bottle or poly (multiple micro tests)	
29	250 mL glass or poly bottle **with thiosulfate** (fecal coliform - chlorinated)	
30	500 mL glass or poly bottle **with thiosulfate** (multiple micro tests - chlorinated)	
31	8 oz plastic jar (grain size only)	
32	1 liter jar ** with **sulfuric acid** (wide mouth, clear; for phenolics)	
33	sterile specimen cup (micro)	
34	2 oz short jar (TOC - NO septum)**	
35	Soil VOA/BTEX Airtight Sampling Capsules (3 per sample)	
36	Soil VOA/BTEX Sampling Handle (1 per sampling event)	
	Other Supplies	

*organic free with Teflon lined lids, with Certificate of Analysis. **same as "*" but does not include Certificate of Analysis.

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Taken From MEL (2008).