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Benthic Macroinvertebrate Biological Monitoring Protocols for Rivers and Streams

2001 Revision

August 2001

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Benthic Macroinvertebrate Biological Monitoring Protocols for Rivers and Streams

2001 Revision

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Abstract

This document describes the Washington State Department of Ecology's Freshwater Ambient Biological Assessment Program. Outlined within the document are: 1) the sampling design, 2) the site selection process, 3) field implementation, 4) laboratory processing of data, and 5) analysis and interpretation of data. The document also includes all of the elements necessary to serve as a Quality Assurance Project Plan (QAPP) for biological monitoring. Field operations remain consistent with previous work (Plotnikoff 1992; 1994; 1998; 1999; Plotnikoff and Ehinger 1997). Relative to the original protocols document (Plotnikoff 1994), this revision provides additional detail for field operations, sub-sampling procedures, and data analysis procedures.

Introduction

Purpose of this Document

This document describes the Washington State Department of Ecology (Ecology) Freshwater Ambient Biological Assessment Program. Outlined here are:

- ◆ Sampling design.
- ◆ Site selection process.
- ◆ Field implementation.
- ◆ Laboratory processing of data.
- ◆ Analysis and interpretation of data collected by the program.

This document also includes all of the elements necessary to serve as a Quality Assurance Project Plan (QAPP). Field operations remain consistent with previous protocols (Plotnikoff 1992; 1994; 1998; 1999; Plotnikoff and Ehinger 1997). Relative to the original protocols document (Plotnikoff 1994), this revision provides additional detail for field operations, sub-sampling procedures, and data analysis procedures.

Background

The Federal Clean Water Act (Section 101) mandates the development of water management programs that evaluate, restore, and maintain the chemical, physical, and biological integrity of the nation's waters (U.S. EPA 1990). Traditional measurements of chemical and physical components for rivers and streams do not provide sufficient information to detect or resolve all surface water problems. Biological evaluation of surface waters provides a broader approach because degradation of sensitive ecosystem processes is more frequently identified. Biological assessments supplement chemical evaluation by:

- ◆ Directly measuring the most sensitive resources at risk.
- ◆ Measuring a stream component that integrates and reflects human influence over time.
- ◆ Providing a diagnostic tool that synthesizes chemical, physical, and biological perturbations (Hayslip 1993).

Ecology collects biological information from rivers and streams throughout the state. The long-term monitoring program was established in 1993 to explore spatial patterns and identify temporal trends in benthic macroinvertebrate communities. Gradually, the program has developed a large base of information that describes biological characteristics of reference and degraded conditions. Reference conditions are found in streams with little or no human impact.

This program is focused on determining methods for reliably interpreting biological and associated habitat data. This is accomplished by delineating regions of relatively homogenous natural biological communities in the state and comparing streams within each region along a gradient of impairment. Application of biological expectations are also applied to broad geographic areas defined by existing regional descriptors and by landscape variables such as elevation, climate zones, or topography. Identification of factors in streams that are directly correlated with biological attributes can be useful in tracing the sources of impacts. Tools for interpreting data are developed by using known stream conditions (naturally and highly degraded) and calibrating the responses of each biometric or community diversity measure to the known condition.

Biological information is used to evaluate stream impacts from point- and non-point sources of pollution. Integrating this information with physical and chemical characterization of a stream segment provides an effective way for diagnosing sources of degradation. The following discussion outlines the program under development for effectively using biological information in environmental management.

Objectives

Long-term biological monitoring of rivers and streams in Washington attempts to incorporate biological and associated habitat information into the regulation and conservation of environmental resources. Past and current development of biological criteria to detect human-induced impacts in streams is an essential step in this process. Evaluation of biological criteria follows the acquisition of new information, which is collected on an annual basis. Therefore, the objectives and applications of information from this monitoring program are as follows:

- ◆ Verify regional stream classification scheme previously defined by physico-chemical and species data.
 - ◆ Test existing physico-chemical dichotomous key stream classification
 - ◆ Test existing regional stream class indicator species lists and develop new regional lists.
- ◆ Develop criteria to evaluate human-induced disturbance in biological communities on a regional basis.
- ◆ Examine where and how biological information should be applied in water resource management.

Program Organization

Personnel

Field operations are completed with three personnel who gather samples and measure environmental variables at each site. The senior scientist or project leader designs and directs the components of the biological assessment program. A junior scientist or environmental technician collects biological and environmental data from rivers and streams, performs laboratory sample sorting and taxonomic identifications, and records data in a database.

Experience

The senior scientist must be able to: 1) independently design a project and direct field work, 2) identify benthic macroinvertebrate taxa to the lowest practical taxon (e.g. Plotnikoff and White (1996)) with available taxonomic literature, 3) understand and apply current stream ecology theory for interpretation of the biological data, 4) operate a variety of computer software including word processors, spreadsheets, statistical programs, and databases, and 5) supervise junior personnel.

The junior scientist/environmental technician must be able to: 1) understand project design and implement the components, 2) identify most taxa to family efficiently, 3) have a general knowledge of computer software operation, and 4) operate stream sampling equipment for measuring biological communities and physical variables.

Study Design

General Design

This program uses representative riffle-habitat (broken surface water) sampling of benthic macroinvertebrates, physical habitat, and water quality to describe biological community condition as a result of natural and human-induced disturbance. Normally, samples are collected from riffles to characterize the benthic macroinvertebrate community unless degradation is suspected in pool habitat (slow moving or eddying water). To distinguish natural versus human influence, data must be collected at reference sites and at degraded sites over a period of time to address spatial and temporal variability.

Reference sites are intended to represent relatively unimpacted or least impacted conditions. Minimally disturbed conditions reflect sites that have experienced very little historical activity that alters stream integrity. Least disturbed sites have been degraded historically, but exhibit some level of recovery. Reference sites are used to describe biological variability due to natural disturbances (*e.g.* precipitation, drought).

Degraded sites are surveyed to describe a continuum of human influence on natural stream communities. Identification of what a degraded macroinvertebrate community is and the factor(s) that caused the resulting condition defines severity of impact. This gradient of biological condition is used to determine the levels of human-induced disturbance that are excessive in a waterbody.

Long-term biological monitoring in this program has been conducted since 1993 and incorporated a variety of site conditions. Besides high-quality reference conditions, sites with high levels of physical and chemical modifications have been surveyed. The result is a data set that represents a gradient of biological conditions as a response to the existing stream condition.

The biological community in rivers and streams represents an important source of information when evaluating ecological integrity. We use a single biological component, the benthic macroinvertebrates, to evaluate stream condition. Evaluation of the fish community is not used as a sole source of information because of species paucity in western North America (Moyle et al. 1986) and continuing harvest restrictions on several salmon species.

Searching for a Regional Framework

Defining the distribution of aquatic invertebrate species is an important exercise in knowing how to use biological information as a guide for resource management. The search for spatial pattern in biological communities serves to describe minimum expectations in stream types across the state especially when a subset of streams is surveyed. A large base of biological information is usually required before community patterns emerge from analysis of the data, especially when the landscape has variable climatic topographic features.

In Washington State streams, distinct regional patterns exist among the benthic macroinvertebrate communities. Variables at different spatial scales are often required to explain regional patterns in benthic macroinvertebrate communities (Hawkins et al. 2000). Plotnikoff (1992) found that communities differ as a function of region and season among similar-sized streams in three ecoregions (Puget Lowland, Cascade Mountains, and Columbia Basin). Surveys in the Yakima River Basin, Washington 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, elevation) in shaping the macroinvertebrate communities. The large-scale expressions such as ecoregions (Omernik and Gallant 1986; Pater et al. 1998) or components of ecoregion descriptions like topography, elevation, or climate can be helpful in parsing streams into groups. These divisions are important for describing biological expectations in stream classes and are consistent with how criteria are applied in most regulatory agencies. Smaller-scale variables, however, are needed in order to explain additional natural variability and increase the likelihood of identifying human-induced changes in stream communities.

Reference Conditions

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 necessitate the use of a minimally disturbed or least disturbed definition for reference. Minimally disturbed conditions reflect sites that have experienced very little historical activity that alters stream integrity. Least disturbed sites have been degraded historically, but exhibit some level of recovery.

Reference site information is used as a measure of biological potential for particular stream settings. Identifying a response in the biological community to environmental degradation is determined by comparison to a reference site. For consistency in a monitoring program, identification of reference sites should follow these guidelines:

- ◆ Map potential areas where reference sites are expected.

- ◆ Evaluate whether candidate reference areas are concentrated in one part of a watershed or are in a variety of locations (candidate sites may not be physically comparable to degraded sites if they are unique to a small portion of a watershed).
- ◆ Eliminate areas with relatively high human modifications (past and present).
- ◆ Field visits; verify current condition of each site.
- ◆ Choose reference sites that approximate stream type and setting as those that will be surveyed for suspected degradation.

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 the region have been disturbed to a certain degree, however, a least disturbed condition must be identified and used for that region. We suspect this situation to arise in the Columbia Basin.

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). Surficial, climactic, and hydrological characteristics are used 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. The Freshwater Ambient Biological Assessment Program currently uses ecoregions as an *a priori* stream classification approach.

Information from sample sites is extrapolated to other similar streams within an ecoregion framework. It is, therefore, important to represent the variety of stream conditions within ecoregions to interpret results generated by this program. Regional biological description is defined by including information from a variety of reference sites within the ecoregion.

Washington State is comprised of eight ecoregions: Coast Range, Puget Lowland, Cascades, Columbia Basin, Northern Rockies, Willamette Valley, Blue Mountains, and Eastern Cascade Slopes and Foothills (Figure 1). Biological condition is described within-regions and inter-annually over five year periods in order to quantify natural variability. Analysis of data within regions describes the range of biological community conditions expected spatially. Measuring inter-annual variability identifies the influence of cyclic environmental phenomena (e.g. annual precipitation patterns, ambient air temperature) on biological communities. A few long-term benthic monitoring sites are used as a calibration tool that measures inter-annual variability. New reference sites are sampled periodically in each ecoregion in order to test and refine regional reference condition.



Figure 1. Washington State Ecoregions as defined by Omernik and Gallant (1986).

Classification by Empirical Modeling

Many studies have suggested that large-scale regionalizations, such as ecoregions, do not account for an acceptable amount of natural variability (See Hawkins et al. (2000) for a synthesis). One alternative is to use large-scale regionalizations as an initial stratification variable, and then use smaller scale variables to account for more of the natural variability. Modeling reference streams along environmental gradients at large and small spatial scales can accomplish this. The first step is to construct biological classes from the biological similarity of reference streams throughout the state. Stream communities can be clustered into biological classes with multivariate classification tools.

Multivariate classification clusters sites in such a way that within-class variability is minimized relative to between-class similarity (i.e. those that are most similar to each other). Reference conditions defined by *a posteriori* clustering differs from *a priori* regionalizations because the biology is used to delineate natural breaks in biological reference condition. When biological classes are identified, regional and reach-scale environmental variables from the same reference sites are used to construct models for each biological class. The same environmental variables from the independent test sites can then be applied to the empirical models to ascertain biological class membership. Biological class membership is defined in terms of probabilities, so a test site may belong, in part, to many biological classes (Moss et al. 1987).

Land Use Representation

Stream sample sites that have a gradient of land-use influences are chosen annually for monitoring in at least two ecoregions. The type of land use within an ecoregion influences biological communities and these relationships are described with independent stream surveys. Dominant land use within priority basins and ecoregions is initially determined. A visual estimate of the severity of land use is made to ensure that sites are chosen to represent a gradient of human influence. Visual estimates are based on maps and ortho-photos, followed by ground truthing in the watershed. This hypothetical impact gradient is further validated when field information is analyzed as described in a subsequent section of this document. Sampling and analysis of degraded stream reaches has a two-fold purpose:

- ◆ Validate acceptable reference condition delineation.
- ◆ To determine the sensitivity of biological information in detecting impacts.

The land use coverage that has been used was Anderson *et al.* (1976). Current land use coverages, such as those found at <http://www.epa.gov/mrlc/nlcd.html> and <http://www.epa.gov/OST/BASINS/> are being used in the program. The following list details the land uses represented in past analyses:

Residential	Sandy Areas Other Than Beaches
Commercial and Services	Bare Exposed Rock
Industrial	Strip Mines, Quarries, and Gravel Pits
Transportation, Communications, Utilities	Transitional Areas
Mixed Urban or Built-Up Land	Mixed Barren Land
Other Urban Land	Shrub and Brush Tundra
Agricultural	Herbaceous Tundra
Cropland and Pasture	Bare Ground Tundra
Orchards, Groves, Vineyards, and Nurseries	Wet Tundra
Confined Feeding Operations	Mixed Tundra
Other Agricultural Land	Perennial Snowfields
Herbaceous Rangeland	Glacier
Shrub and Brush Rangeland	
Mixed Rangeland	
Deciduous Forest Land	
Evergreen Forest Land	
Mixed Forest Land	
Lakes	
Reservoirs	
Bays and Estuaries	
Forested Wetland	
Nonforested Wetland	
Beaches	

Index Period

The index period is a time span during the year in which samples are collected. The index period used in this program (July 1st -October 15th) was chosen for the following reasons:

- ◆ Adequate time is available 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 mid-spring to late-summer).

Biological assessments can yield different interpretations depending on the index period chosen. This is because natural seasonal disturbances and physical stream conditions strongly affect the diversity, abundance, and life stage progression of aquatic insects (Hynes, 1970; Vannote *et al.* 1980). It is recommended that collecting begins earlier in the index period for drier ecoregions and that all sites within an ecoregion are visited during an abbreviated period of time.

Stream Size

We sample streams that are perennial and wadeable. Seasonal drought disturbance selects for distinct specialist communities (Resh *et al.* 1988; Clifford 1966). Stochastic drought is a catastrophic natural disturbance that eliminates many taxa (Resh 1982; Resh *et al.* 1988). These natural disturbances introduce natural variability into the data set, making community pattern identification difficult. Unwadeable streams present logistical problems and require different macroinvertebrate sampling techniques that are beyond the scope of this protocol.

Habitat Type

Stream reaches contain two easily identified and contrasting habitats: riffles (broken surface water) and pools (slow-moving or eddy water). The primary reason for surveying these two habitats is to measure habitat-specific signals from stressed invertebrate communities. Degradation may selectively occur in pool habitat and not in riffles. Comparison of pool invertebrate assemblages to riffle invertebrate assemblages may reveal the effect of natural hydrologic disturbance, as well as the biological response resulting from physical disturbance (Minshall and Minshall 1977; Brown and Brussock 1991). From 1993- 1996, Ecology's Freshwater Ambient Biological Assessment Program sampled both riffle and pool habitat. 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 in this survey (Plotnikoff and Ehinger 1997). As a result, we have placed exclusive regular monitoring effort in sampling riffles following work completed in 1997. Pools are sampled when degradation is suspected in that habitat.

Field Quality Assurance

Sampling and Visit Precision

Sampling precision is related to the variability of the four riffle samples that are composited. Sampling precision is estimated by keeping the four replicate riffle samples separate at 10% of the reaches sampled annually. Pool habitat is not examined for sampling precision estimates. Sampling precision is calculated using the coefficient of variation (CV) from four replicate riffle samples and should be $\leq 20\%$ in reference streams when using the taxa richness metric (Plotnikoff, 1992). We expect collections of macroinvertebrates from multiple sample locations to have similar community structure in reference stream riffles.

Visit precision is related to the variability of collecting a composite sample in a reach. Visit precision is estimated by collecting three composite riffle samples within the same reach during the same day at 10% of the reaches sampled annually. Pool habitat is not examined for visit precision estimates. Visit precision is calculated using the coefficient of variation (CV) from three replicate composite samples and should be $\leq 20\%$ in reference streams when using the taxa richness metric.

Representativeness

Representativeness of benthic community conditions is determined by the sample program design (Lazorchak and Klemm, undated). The sampling protocol is designed to produce consistent and repeatable results in each stream reach. Physical variability within riffles is accounted for through stratified sampling based on depth, substrate distribution, and location within the sample reach.

Completeness

Completeness is defined as the proportion of useable data gathered (Kirchmer and Lombard 2001). 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." Sample contamination occurs when containers are improperly sealed or stored. Loss of benthic material or desiccation diminishes the integrity of the sample. If the validity of the information from the sample is in question, the sample is excluded from analysis. The goal for completeness of benthic macroinvertebrate data sets is 95% of the total samples collected. Completeness is defined as the total number of samples that we are confident in using for further data analysis following field collection.

Sampler and operator efficiency both influence completeness. One measure of sampler/operator efficiency is the number of taxa collected or "total taxa richness." The discrepancy between transects in the total number of taxa collected is attributed to sampler/operator efficiency (*i.e.* the ease with which various species can be collected)

and the distributional characteristics of benthic dwelling organisms. Some species are considered rare and may be difficult to collect due to low abundance or are difficult to sample in certain habitats.

Comparability

Comparability describes the confidence in comparing one data set to another. Many private, academic, and governmental entities are currently generating biological information for rivers and streams that could potentially be incorporated into a larger data set. Comparability of data sets is primarily achieved through adherence to commonly accepted protocols (*e.g.* field sampling, analytical methods and objectives). Our multihabitat collection approach using a D-frame kicknet was chosen largely to provide necessary comparability with other programs. These programs include the Oregon Department of Environmental Quality's bioassessment program, the Environmental Protection Agency's "Regional Environmental Monitoring and Assessment Program" (R-EMAP), and a reference site monitoring program that developed probability models based on biodiversity in Washington streams (Hawkins and Ostermiller, Personal Communication).

Safety Procedures

Field and Laboratory Preservatives

Biological samples are preserved immediately following collection and consolidation into 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 in this program is 85% non-denatured ethanol. The preservative is prepared from a stock standard of 95% non-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 the Environmental Assessment Program Manager's Secretary. Minimal contact with the 95% non-denatured ethanol solution is recommended.

The preservative used in handling sorted laboratory samples is 95% ethanol (non-denatured). Seventy-percent non-denatured ethanol is used for preservation of voucher specimens in two dram vials (8 mL). Voucher specimens are stored in a flammables storage case in the Ecology Benthic laboratory. Bulk ethanol is stored in a separate flammables storage building. Hazard Communication Training is provided to all personnel that come into contact with hazardous materials while conducting program duties.

Miscellaneous

Field activities are conducted by at least two people. A contact person is designated at the headquarters office to which field personnel report daily at pre-designated times.

Careful planning of field activities is essential and permission to access private land must be obtained. Access to private land is usually obtained through verbal agreement with the landowner while at the proposed sample site.

Special safety equipment includes:

- ◆ Felt Soles or Cleats (for waders)
- ◆ Rain Gear
- ◆ Insulated Rubber or Neoprene Gloves
- ◆ First Aid Kit (stored in the vehicle)
- ◆ Department of Ecology Photographic Identification Card
- ◆ Certification in CPR/First Aid
- ◆ Defensive Driving Training

Field Operations

The sequence and spatial arrangement of field operations are outlined in two figures toward the end of this section. Companion pictures to the following field operations (page 18) can be found at

http://www.ecy.wa.gov/programs/eap/fw_benth/fwb_photos.html.

Landscape Attributes

Landscape attributes are often important correlates with macroinvertebrate community composition. The ecoregion, Strahler stream order (1:100,000 scale map), and basin are noted for each site. These attributes are often recorded from maps prior to sampling. Latitude, longitude, and elevation are recorded in the field with a GPS unit. Accuracy of these measurements is verified in the office by comparing against digital maps of stream locations in the state.

Reach Location

At each site, the stream reach location is determined by identifying the lower end of the study unit and estimating an upstream distance of 40 times the average wetted stream width. The lower end of a study unit is located to represent a given land use. The stream reach length should measure approximately 150 meters if stream width is narrow (< 3 meters). This reach length ensures that characteristic riffle/pool sequences are represented and potentially sampled.

Macroinvertebrate Sample Locations

Four biological samples are collected from riffle habitat in a reach. One sample is collected from each of four riffle habitats. A variety of riffle habitats are chosen within the reach to ensure representativeness of the biological community. The locations within a reach are determined by finding representative combinations of the following variables:

- ◆ Depth of riffle.
- ◆ Substrate size.
- ◆ Location within a riffle area of the stream (forward, middle, back).

Sampling among several riffles in a stream increases representation of physical differences in this habitat. Also, this sampling design maximizes the chance of collecting a larger number of benthic macroinvertebrate taxa from a reach than from fewer riffles. Variations in physical condition of the riffle habitat provide an opportunity to collect both common and rare taxa.

When sampling pool habitat, benthic macroinvertebrates are collected at four locations. Each sample is collected from its own respective pool. The locations within a reach are determined by finding representative combinations of the following variables:

- ◆ Depth of pool.
- ◆ Location within the channel (side, middle, behind a boulder/woody debris).

Absence of flowing water in pool habitat can result in low sampler efficiency. Most stream bottom samplers rely on flowing water to direct macroinvertebrates into a collection net. In the absence of flowing water, loss of individual organisms increases. Benthic organisms collected from pools provide reliable synoptic lists of taxa, but not community characterizations calculated with density estimates.

Sampling Stream Macroinvertebrates

Macroinvertebrate samples are collected from riffle and pool habitats with a D-Frame kicknet (500-micrometer net mesh). A device fastened to the base of the D-Frame kicknet encloses a one-foot by two-foot area in front of the sampler (sampling area= 0.19 m²). In riffle samples, coarse substrate in the enclosed area is removed and scrubbed with a brush to dislodge clinging invertebrates into the collection net. After scrubbing coarse substrate, all remaining substrate in the enclosed area is agitated to a depth of 15 cm for two minutes. Samples are stored in ethanol-filled containers.

Pool samples are more difficult to collect. Benthic-dwelling animals may escape in the absence of a steady current. The D-frame kicknet is placed on the stream bottom and a 1 foot x 2 foot area upstream of the collection net is disturbed by kicking. Stream bottom material will be suspended in the water column, particularly the organic material, and is actively “scooped” up with the collection net. Scooping requires the removal of the net from the stream bottom and collecting as much as possible from the water column. The net should follow a path of 1 x 2 feet through the water column when collecting the suspended material. Disturbance of the substrate and scooping with the net is done several times to ensure collection of most material in the pool collection area. Collection at each pool location within a reach is continued for a period of two minutes.

Macroinvertebrate samples from most sites are composited into single riffle and pool samples. As part of our data quality objectives, approximately 10 percent of total sites monitored in a year are included as part of an evaluation of community variability within a stream reach. Riffle samples are stored in separate containers as replicates at each of these streams. In projects that require a small-scale quantitative approach (e.g. upstream-downstream), the 0.19 m² samples are always kept separate as replicates.

The macroinvertebrate field samples are preserved in 85% ethanol. Storage containers can be either heavy-duty freezer bags or one-liter polycarbonate containers. A double bag system is used when storing samples in freezer bags. Sample labels are placed in the dry space between the inner- and outer freezer bags. Label information should contain: name of stream (including reach identification), date of collection, County and State, project name (if applicable), type of habitat (e.g. riffle 1, riffle 2, ..., riffle composite), and collector's name. Sample containers are assigned an identification number when stored in the laboratory. Additional physical and chemical stream information is associated with the numbered biological collections in the database.

Habitat Survey Rationale

The environmental characteristics of instream and riparian areas of streams have a substantial influence on the structure and function of benthic macroinvertebrate communities. Environmental characterization is used concomitantly with biological assessment surveys to: 1) understand the natural physical and chemical constraints imposed on macroinvertebrate communities, and 2) detect physical and chemical changes within sensitive stream areas and adjacent riparian zones.

Environmental variables used in this monitoring program are listed below:

Surface Water Quality

- ◆ temperature
- ◆ pH
- ◆ conductivity
- ◆ dissolved oxygen

Stream Flow

- ◆ discharge at base of reach
- ◆ average current velocity
- ◆ bottom current velocity

Stream Reach Profile

- ◆ maximum depth
- ◆ wetted width
- ◆ residual pool depth
- ◆ bankfull width
- ◆ stream gradient

Canopy Cover

- ◆ center-of-stream readings
- ◆ left bank/right bank readings

Substrate Characterization

- ◆ substrate composition (general description)

Human Influence

- ◆ type of activity
- ◆ proximity to the stream

Macroinvertebrate communities are affected by environmental variables on a number of spatial scales. In addition to landscape features, environmental variables in the sample reach are measured at the “reach” and “sample” scale. Figure 3 illustrates where each variable is sampled in the sampling reach. The field forms used to record measurements at each stream are in Appendix A.

Surface Water Quality

Surface water analysis is limited to temperature, pH, dissolved oxygen, and conductivity. These variables are routinely measured in most of Ecology's projects. Additional observations include water clarity, water/sediment odors, and surface films. Measurements of all surface water variables are made before biological samples are collected.

Water samples are collected directly from the lowest portion of the sample reach and transported back to the vehicle for measurement as quickly as possible. The following instruments and methods are used to measure surface water values:

Parameter	Method	Sensitivity
Temperature	YSI Thermistor	± 0.1°Centigrade
pH	Orion, Model 250A	± 0.1 pH Units
Conductivity	YSI Conductivity Meter, Null Indicator	± 2.5 µmhos/cm @ 25°C
Dissolved Oxygen	YSI Membrane Electrode, Model 57 or Winkler Titration	± 0.2 mg/L ± 0.1 mg/L

Quality Assurance

Replicate water quality measurements are made for one of five sample sites visited. Bias is determined by comparing instrument readings with solutions of known concentration (e.g. buffers for pH, conductivity standard, and calibration of the thermometer). Comparability is assured by using standard procedures.

Stream Flow

Stream discharge is measured with a Marsh-McBirney flow meter and a top-set wading rod. A stream cross-section at the bottom of the sampling reach is constructed perpendicular to the direction of flow. Velocities and depths are measured in cells across the distance of the cross-section. The cross-section is divided into at least 20 cells, following the U.S. Geological Survey (USGS) Mid-section method for instream flow measurements (United States Bureau of Reclamation, 1997). Total discharge is the summation of discharge in each cell. Discharge measured in each cell should not exceed ten percent of the overall discharge estimate.

Average water column current velocity (0.6 x depth from water surface) and bottom current velocity is measured at each macroinvertebrate collection site. These measurements provide information about the instream hydraulic conditions that biota experience.

Stream Reach Profile

A series of channel morphology features is measured at each macroinvertebrate sample site. A transect perpendicular to stream flow at each macroinvertebrate sample site is identified. Wetted width, bankfull width, and maximum depth is measured along each transect. The residual pool depth is measured in pool locations. The residual pool depth is the maximum pool depth minus the depth of the pool at the crest or “tailout”. Stream gradient is measured with a clinometer and reflects the local gradient of riffles where macroinvertebrates are collected.

Canopy Cover

Percent canopy cover is estimated with multiple densiometer readings along each macroinvertebrate sample transect. Four readings are taken at the sample point (facing upstream, facing downstream, facing the right bank, and facing the left bank). In addition, one reading is taken facing the bank at the wetted right bank and left bank, respectively. Each measurement is taken one foot above the water surface. The composite value is the sum of the four readings taken from the macroinvertebrate sample location.

Substrate Characterization

First, substrate is characterized at each macroinvertebrate sampling location. A metal grid with 50 equidistant points is placed on each macroinvertebrate sampling location. The substrate grid is an octagon with 21-inch dimensions from end to end. There are 50 points arranged 3 inches apart from each other on the grid. The substrate size at each point is categorized with a viewing tube. The viewing tube is a PVC tube with a Plexiglas bottom. The tube is 9 inches in diameter and twelve inches deep. Substrate classes are located on the field form in appendix A. This field exercise normally requires two personnel. The first person: 1) keeps the grid stationary, 2) moves the viewing tube from point to point, and 3) calls off the substrate class at each point. The second person simply records the information on the field sheet as tick marks. Four measurements (50 values per measurement) are taken during each visit at each macroinvertebrate sampling location. Representation of each substrate class is calculated by multiplying a substrate class tally by 2 and expressing as a percent. The measurements are taken before macroinvertebrate sampling occurs. Care is taken to avoid disturbing the substrate. In the deeper locations, disturbance is avoided by suspending the grid up above the substrate. In exceedingly shallow locations, adjacent substrate that is similar to the sampling location is used for substrate characterization.

An optional pebble count may be employed along each riffle cross-section contiguous with each macroinvertebrate sampling location to characterize substrate at the reach scale (Wolman 1954; Harrelson et al. 1994; Schuett-Hames et al. 1999). One Hundred substrate particles are collected and measured along each cross-section. Data collection starts at a randomly selected point at one of the bankfull elevations along the cross-section. With an averted gaze, the sampler picks up the first particle touched by the tip of the index finger at the toe of the wader (Harrelson et al. 1994). The particle is measured along its b-axis. A substrate particle is 3-dimensional, with a long side, a short side, and an intermediate side. The b-axis is the intermediate dimension that determines if the particle would pass through a sieve of that size. The sampler then steps in the direction of the opposite bank, picking up and measuring another substrate particle. The procedure is repeated until 100 measurements are made. The measurements are assigned to the proper size class and tallied. Size fractions are located on the field form in appendix A

The Wolman pebble-count has been used to characterize substrate composition along a “reach”. This method has been adopted for “site-specific” evaluations in this protocol.

Human Influence

Reach-scale human influence observations can be important for understanding biological and environmental information. Evidence of different types of human influence is noted in each sample reach. In addition, the proximity of each influence to the stream bank is visually estimated.

Sequence for Conducting Field Operations

Field procedures follow a sequence of measurements that ensure quality information is collected and a reasonable amount of time is spent at each site. The sequence and spatial arrangement of field operations is outlined in figures 2 and 3, respectively. The senior scientist selects sampling reaches prior to field work. The field crew consists of a lead and two assistants. Every person in the crew is qualified as at least a junior scientist, or under direct supervision of a junior scientist. One field crew lead and two assistants conduct biological monitoring. First, the two assistants collect surface water and discharge information at the furthest downstream portion of the sample reach. After surface water is collected for water quality measurements, the field crew lead selects macroinvertebrate sampling locations in different riffles. Sediment characterization and the macroinvertebrate collection ensues. The field crew lead identifies the macroinvertebrate sampling locations with numbered flags. The two assistants follow behind the lead and collect current velocity, stream reach profile measurements, and canopy cover at the sampling locations. One assistant alternates between the lead and the other assistant to help with different collection procedures. After macroinvertebrates are collected from all four sampling locations, they are deposited into a container and preserved with 85% ethanol. Evaluating human influence is the last component of a site visit. With this sampling sequence, stream disturbance is minimized before surface water and biological information is collected.

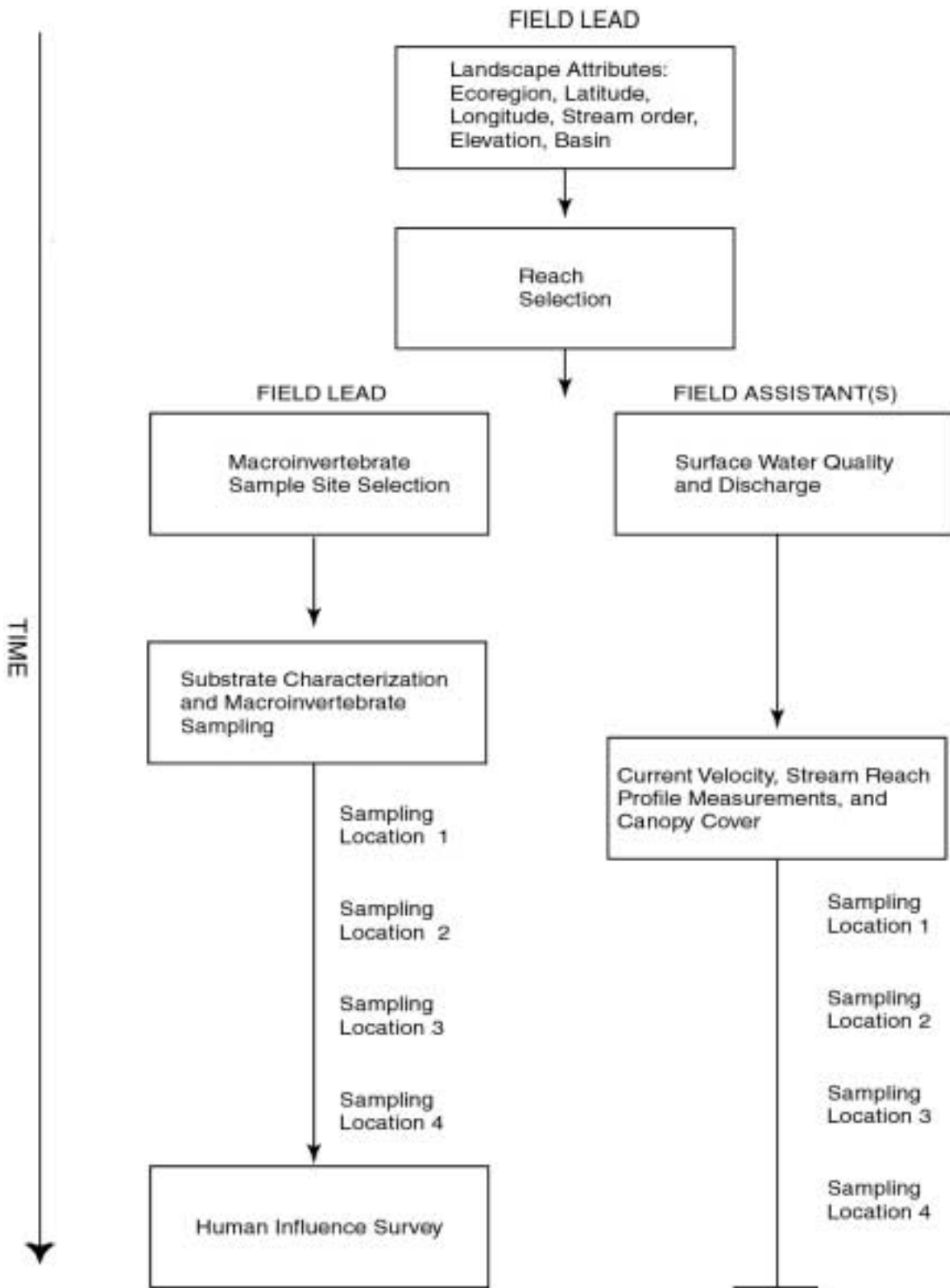


Figure 2. Sequence of field operations.

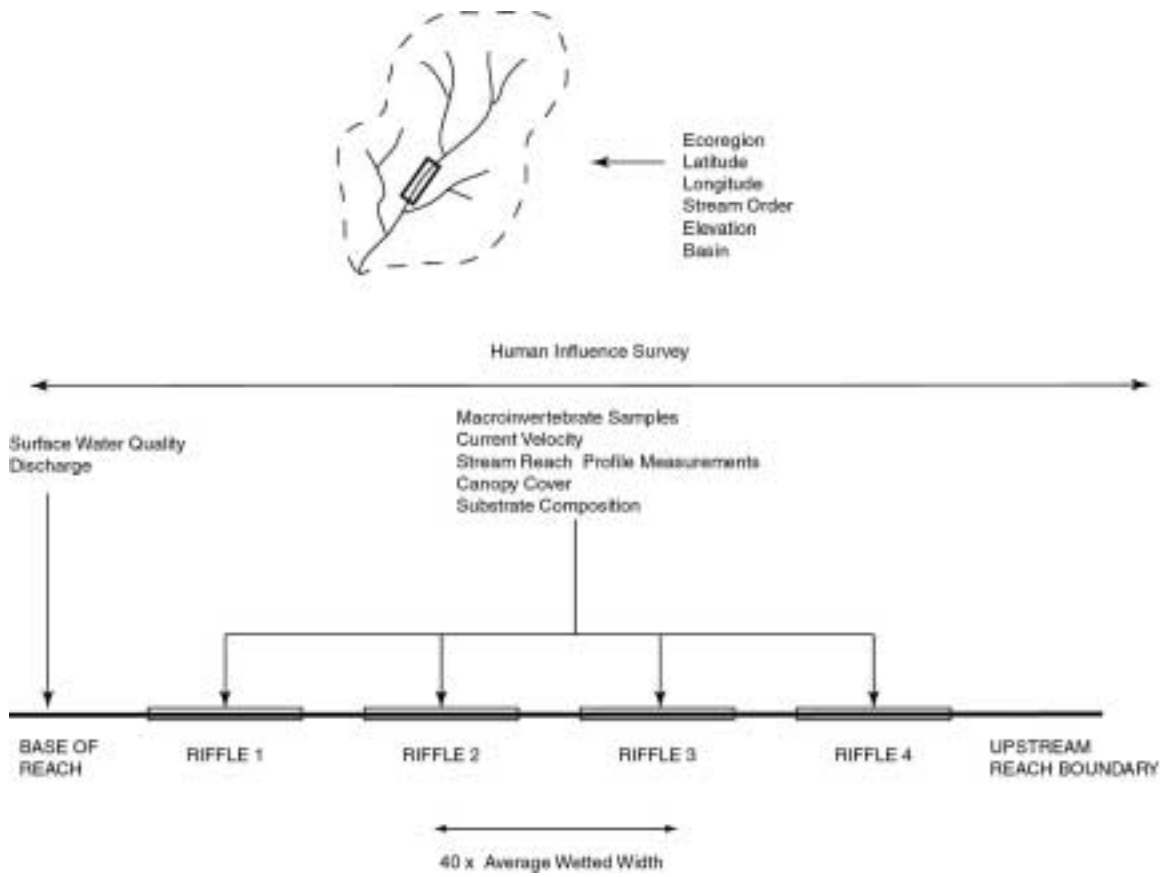


Figure 3. Spatial distribution of field operations.

Laboratory Sample Processing

Benthic Macroinvertebrate Samples

The riffle samples collected at each site are sub-sampled using a 500-organism count. Macroinvertebrates are removed from a minimum of two randomly chosen squares in a sub-sampling grid containing 30 squares. The dimension of each square is 6 cm x 6 cm and the tray has an overall dimension of 30 cm x 36 cm. The sample material from a field container is spread evenly on the base of the grid tray. We assume that the procedure is random and unbiased. All organisms are removed from randomly chosen squares until a minimum of 500 macroinvertebrates are picked and the process is continued to include all remaining organisms in the selected squares. Larger macroinvertebrates are removed from the sample square prior to use of a dissecting scope. In most cases, 500 macroinvertebrates or more are sub-sampled using this procedure. A 300-organism count was employed during 1993, 1994, 1995, and 1998. In 1996, 1997, 1999, 2000, 500 macroinvertebrates were sub-sampled from each sample. A 500-organism count will be employed consistently in future years.

Pool and riffle samples remain in separate containers following the sub-sampling procedure. In cases where the four riffle sample replicates from a site are in separate field containers, separate laboratory storage containers are used for organisms sub-sampled. All sub-sampled macroinvertebrates are placed in 70% ethanol that is prepared from a stock solution of 95% non-denatured ethanol.

Benthic Macroinvertebrate Identification

All major orders of freshwater macroinvertebrates are identified to at least the genus level, including the Chironomidae, and to species where existing taxonomic keys are available. Each taxon has an associated source key used for the identification so that future revision of macroinvertebrate taxonomy will be easily incorporated into the database. Taxa groups normally identified to coarser taxonomic levels include: Simuliidae, Lumbriculidae, Naididae, select families of Coleoptera, Planariidae, and Acari (water mites). The following list represents the major taxonomic keys used to complete taxonomic identification:

- ◆ (Merritt and Cummins 1996) An Introduction to the Aquatic Insects of North America
- ◆ (Pennak 1989) Freshwater Invertebrates of the United States
- ◆ (Usinger 1956) Aquatic Insects of California with keys to North American genera and California species
- ◆ (Edmondson 1959) Freshwater Biology
- ◆ (Needham *et al.* 1935) The Biology of Mayflies

- ◆ (Edmunds *et al.*, 1976) The Mayflies of North and Central America
- ◆ (Jensen, 1966) The Mayflies of Idaho (Ephemeroptera) **some material is outdated*
- ◆ (Baumann *et al.* 1977) The stoneflies (Plecoptera) of the Rocky Mountains
- ◆ (Stewart and Stark 1988) Nymphs of North American Stonefly genera (Plecoptera)
- ◆ (Wiggins, 1995) Larvae of the North American caddisfly genera (Trichoptera), 2nd ed.
- ◆ (McAlpine *et al.* 1981) Manual of Nearctic Diptera, Volume 1
- ◆ (Burch 1982) Freshwater Snails (Mollusca: Gastropoda) of North America

Additional literature is summarized in Plotnikoff and White (1996) and used to confirm distributions and variations in characteristics of individual taxa. Descriptions of biology are used to confirm likely distributions, particularly when larval or nymphal forms of macroinvertebrates are difficult to identify.

Laboratory Quality Assurance

Macroinvertebrate Sorting

Samples are either sorted whole or, in the case of large sediment volumes, sub-sampled so that only a fraction of the original is analyzed. Precision of the sub-sampling process is evaluated by re-sorting a new sub-sample of the original samples. Ten percent of the benthic macroinvertebrate samples (e.g. 1 of 10 samples) are re-sorted by a second laboratory technician. Sorting results that are less than 95% similar would indicate the need for:

- ◆ More thorough distribution of sample materials in the sub-sampling tray.
- ◆ Special attention given to easily missed taxa when sorting (*i.e.* increased magnification).

Taxonomic Accuracy and Precision

Correct identification of benthic organisms is important for accurate description of community structure and function. Taxonomic misidentification results in inadequate stream biology characterization. Errors in identification of benthic macroinvertebrate taxa should be $\leq 5\%$ of the total taxa in the sample. Re-identification of samples is done for 10% of the total number of samples collected in each year. Secondary identification is conducted by experienced taxonomists in order to maintain confidence in the data set. Difficult taxa are sent to museum curators whose specialty includes members of a particular order. A voucher collection is maintained by the Department of Ecology and is updated on an annual basis with macroinvertebrate specimens from each year's collection.

Data Analysis

The purpose of data analysis in bioassessment is to determine the biological health of sites in space and time. Biocriteria are typically defined by univariate biological endpoints. The biological health of a test site is determined by comparing the test site biological endpoint with the reference condition. Confidence in these assessments is defined by the natural variability about the reference condition. The program design should include the quantification of spatial, temporal, and procedural variability in reference sites. The linkage of biological health with environmental variables (i.e. water quality) is typically assessed with ordination and correlation techniques, as described in this section.

Data Preparation

Species Consolidation

A standard list of taxa is constructed for all collections and two rules are used to condense site species lists. 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.

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

Data reduction is employed before using multivariate analysis. Reduction of the species matrix accommodates: (1) redundancy or validity in taxonomic information, and (2) limited computational capacity of software. The taxa list may be reduced by eliminating all taxa that are less than one percent of the total abundance in a sample. The purpose for “rare” taxa elimination would be for clarity of spatial patterns in biological communities in ordination space. Additionally, a secondary reduction of taxa can be made using a rare taxa elimination rule of four percent. The reason for reduction of rare species is that they are more difficult to collect and may confound identification of consistent diagnostic features of streams in spatial analysis. (Clifford & Stephenson 1975).

Data Transformations (selecting the type of transformation)

Assumptions for distribution of data should be met before information is analyzed. There are a few commonly used transformations for biological data that precede analysis using univariate or multivariate statistical methods. Conformation of data to a normal distribution can be checked with statistical packages that run “probability-plots” or “Q-Q” plots. Systematic application of any available transformations can be applied that result in the best approximation of a normal distribution.

Standardization and Transformation of Abundance Data

The reduced taxa abundance data are initially standardized with a percent transformation. An additional $\log_{10}(x+1)$ transformation of the percent standardized data is used to downweight the contribution of very abundant taxa (Zar 1999). Rare taxa may become unique attributes of a site, in some instances, and should be retained for further testing in pattern analysis. Severe downweighting is chosen to lessen the effect of short-lived, abundant taxa on analytical results. This results in a more reliable estimate of community composition of a site. Effectiveness of different transformations in cluster analysis is evaluated by comparing the log transformed data with results from square root-, double square root-, and presence-absence transformations. Data that is expressed as a percentage of abundance estimates are non-normally distributed and should be transformed using the arcsine function.

Criteria Development

Biological criteria for stream health are generally based on a multimetric or multivariate approach. The current multimetric approach uses an aggregation of individual community metrics that comprise the Benthic Index of Biological Integrity (B-IBI). A current multivariate approach is the River Invertebrate Prediction and Classification System (RIVPACS). Development of biocriteria for these tools requires different steps (see Barbour et al. 1999 for an overview). Both methods, however, share the same conceptual framework. Reference condition and its variability need to be established before human-induced impacts can be assessed in test sites. First, reference streams with similar habitat and/or biological attributes are grouped into classes. Next, non-biological criteria are used to assign degraded test sites to classes in which reference condition has been defined. Class biological attributes are used to define numerical reference condition. Degraded test sites are then assessed based on their biological attributes relative to their class reference condition. Detailed methods for the multimetric B-IBI approach are outlined in Barbour et al. (1999), Karr (1987), and Karr and Chu (1999). Detailed methods for the RIVPACS approach are outlined in Wright et al. (1993), Wright (1995), Reynolds et al. (1995), and Norris and Georges (1993).

Pattern Analysis- Ordinations

Searching for patterns in physical, chemical, and biological attributes on a landscape scale is a prerequisite for developing biological criteria. The primary goal is to identify spatial continuity in stream attributes that are assumed to be important features in all streams of a given region. Statistical ordinations and classification techniques based on biological, physical, and chemical attributes can identify environmental gradients that are influential in shaping stream communities.

Principal Components Analysis

Surface water collection and characterization is a common exercise in environmental monitoring programs. Surface water information can be used separately from biological information to describe regional stream characteristics. Principal Components Analysis (PCA) is used to find associations among stream sites with similar water quality and then to graphically represent these associations. PCA assumes linear relationships between variables. Thus, PCA is inappropriate for species data, but acceptable for water quality data. In addition, PCA is appropriate when using variables that are not measured with units in the same order of magnitude (e.g. pH, temperature, conductivity).

Correspondence Analysis

Ordinations such as canonical correspondence analysis (Ter Braak 1986) are exploratory techniques that identify the relationships between biota and environmental variables. In Canonical Correspondence Analysis, biotic ordination axes are created with the restriction that axes are linear combinations of environmental variables. Thus, environmental variables can be directly related to the invertebrate assemblages. Detrended correspondence analysis can be applied to remove bell-shaped species response curves to environmental gradients (Ter Braak 1986).

Correlation between Environmental Variables with Biological Condition

Analytical results from the classification of sites can be associated with environmental variables (Clarke and Ainsworth 1993), allowing determination of those that have the highest correlations with the invertebrate matrix. The distribution for each environmental variable can be examined with a 'density' graphics function (Wilkinson 1990), using a $\log_{10}(x+1)$ transformation for those variables that do not approximate a normal distribution. Combinations of variables that show strong colinearity should be eliminated. Remaining environmental variables are correlated with the results of cluster analysis. The harmonic (weighted Spearman) rank correlation (r) is used to express the proportion of the variance explained for relationships between the biotic similarity matrix and the abiotic similarity matrix. Harmonic rank correlation (r) values derived from the environmental data matrix are not equivalent to the Spearman correlation coefficient.

The numeric values reported indicate the strength of the relationship between the biotic similarity matrix and a select group of environmental variables.

Pattern Analysis- Clustering

a priori classification

A common exercise in developing biometric (B-IBI) criteria is to construct *a priori* reference site classifications based on landscape features. Classification strength is tested and confirmed with macroinvertebrate reference community similarity. Test sites within the contiguous area are compared to the reference condition. Common landscape classification delineations are based on ecoregions. Ecology currently uses ecoregions (Omernik and Gallant 1986; Pater et al. 1998) to classify streams in Washington State. Recent work, however, demonstrated that invertebrate community similarity crossed some ecoregion boundaries in Washington State (Plotnikoff and Ehinger 1997).

a posteriori classification

Site classification in RIVPACS is based on invertebrate community composition. Sites are classified based on invertebrate community similarity, and relationships between the communities and environmental variables (non-human influenced). Invertebrate community similarity can be determined using a statistical technique called cluster analysis. Community similarity is measured by using either the Sorensen Dice (presence/ absence data) or the Bray-Curtis (abundance data) coefficients. These coefficients focus on taxa presence, rather than common taxa absences. Similarity values are clustered with the Beta- UPGMA algorithm (unweighted pair-group method using arithmetic averages). This cluster algorithm is described in Belbin and McDonald (1993).

Assessment of Classification Strength

Mean Similarity

Classification strength is defined by the degree to which classifications minimize within-class biotic similarity relative to between-class similarity (VanSickle 1997; VanSickle and Hughes 2000). The mean similarity dendrogram is an effective graphic that displays these similarity values between classification schemes.

Dichotomous Key, Indicator Groups and Species

Determining how well new test sites fit into a classification scheme further indicates classification strength. Ecology developed a dichotomous key constructed from the habitat variables associated with a previous biological reference site classification (Plotnikoff and Ehinger 1997). Test sites that fall into a particular class based on habitat variables should have an invertebrate assemblage that is commonly present.

Characteristic species of site clusters are described by examining the constancy and fidelity of species groups. The constancy of a species group is the proportion of sites in a cluster at which taxa from a distinct group appears. Fidelity of a species group is a measure of its 'uniqueness' to a site cluster from among all sites surveyed. Species are listed in Indicator groups when a species cluster had a constancy of $\geq 70\%$. Species are listed as indicator taxa when fidelity of a species cluster is high. The classification strength of the dichotomous key is tested each year by comparing the newly collected macroinvertebrates with the expected indicator groups and taxa. Thus, independent assessments of classification strength are periodically performed.

Data Management

Data collected in Ecology's Freshwater Ambient Biological Assessment Program as well as in the Washington R-EMAP program are stored in a Microsoft Access® Database. Data are partitioned into multiple tables. Several tables contain data specific to a site visit. Secondary tables contain related information, such as water-body segment information, taxonomic codes, species information, and taxonomic references. A simple illustration of the database structure is presented in Figure 4. The flexible nature of this database structure allows for detailed queries. Data may be requested from the authors of this document. Read-only site profiles are located at http://www.ecy.wa.gov/programs/eap/fw_benth/fwb_intr.html. We expect data from this program to be available on the Ecology EIM database in the future.

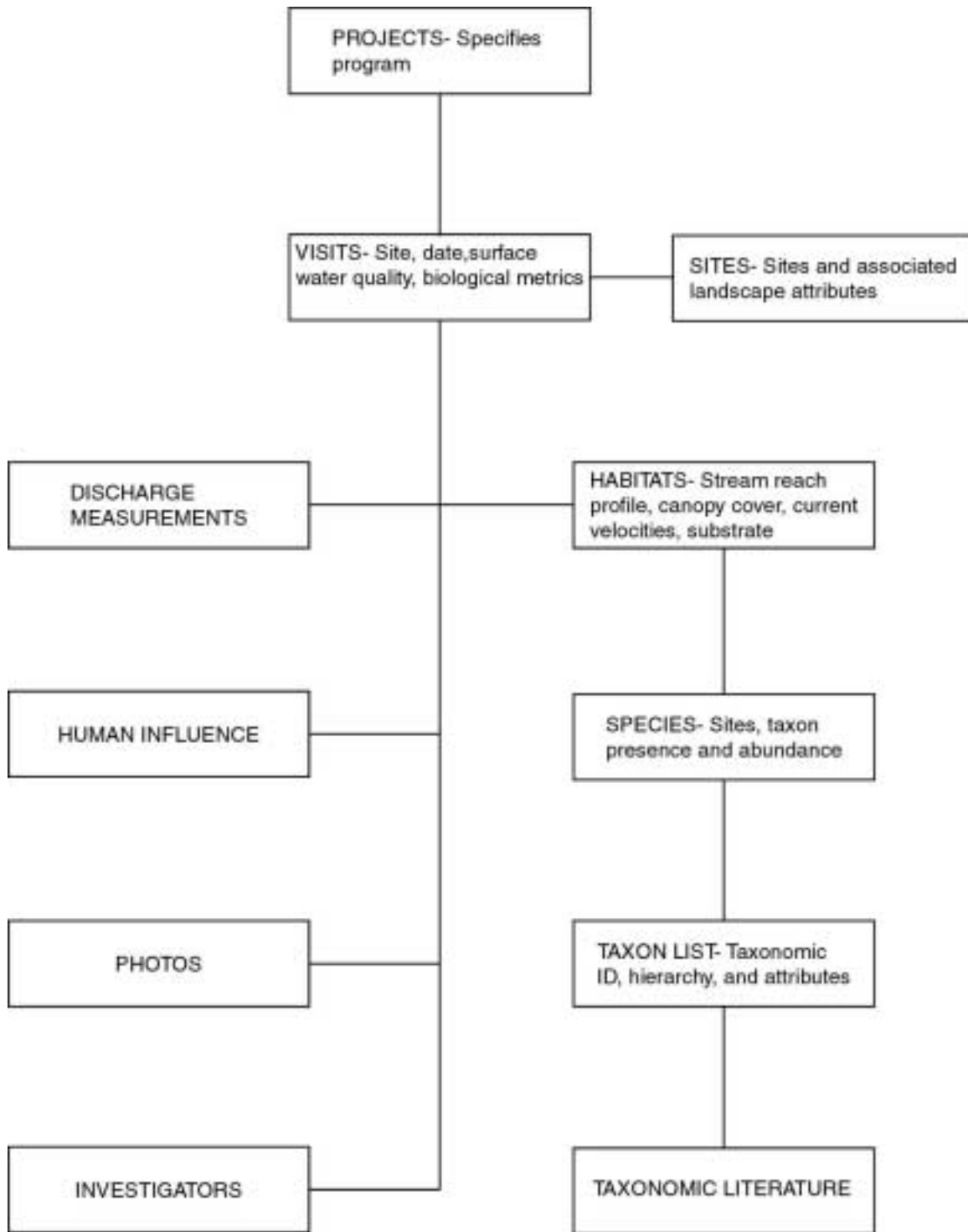


Figure 4. Database structure used by the Ecology Freshwater Ambient Biological Assessment Program.

Literature Cited

- Anderson, J.R., E.E. Hardy, J.T. Roach and R.E. Witmer. 1976. A land use and land cover classification system for use with remote sensor data. Geological Survey Professional Paper 964. United States Government Printing Office, Washington D.C., 28 pp.
- Barbour, M.T., J. Gerritsen, B.D. Snyder, and J.B. Stribling. 1999. Rapid bioassessment protocols for use in streams and wadeable rivers: periphyton, benthic macroinvertebrates and fish, 2nd ed. EPA 841-B-99-002. U.S. Environmental Protection Agency; Office of Water; Washington D.C.
- Baumann, R.W., A.R. Gaufin, and R.F. Surdick. 1977. The Stoneflies (Plecoptera) of the Rocky Mountains. *Memoirs of the Station Technical Bulletin*. 134: 1- 152.
- Belbin, L. and C. McDonald. 1993. Comparing three classification strategies for use in ecology. *Journal of Vegetation Science* 4: 341- 348.
- Brown, A.V. and P.P. Brussock. 1991. Comparisons of benthic invertebrates between riffles and pools. *Hydrobiologia*. 220: 99- 108.
- Burch, J.B. 1982. Freshwater snails (Mollusca: Gastropoda) of North America. EPA-600/382-026. United States E.P.A., Cincinnati, Ohio, 294 pp.
- Carter, J.L., S.V. Fend, and S.S. Kennelly. 1996. The relationships among three habitat scales and stream benthic invertebrate community structure. *Freshwater Biology* 35: 109-124.
- Clark, K.R. and M. Ainsworth. 1993. A method of linking multivariate community structure to environmental variables. *Marine Ecology Progress Series*. 92: 205-219.
- Clifford, H.F., 1966. The ecology of invertebrates in and intermittent stream. *Invest. Indiana lakes Streams*. 7: 57- 98.
- Clifford, H.T. and W. Stephenson. 1975. An introduction to numerical classification. Academic Press, New York.
- Edmondson, W.T. 1959. *Freshwater Biology*, 2nd ed. John Wiley & Sons, N.Y. 1248 pp.
- Edmunds, G.F., Jr., S.L. Jensen, and L. Berner. 1976. *The Mayflies of North and Central America*. University of Minnesota Press, Minneapolis, 330 pp.

- Harrelson, C.C., C.L. Rawlins, and J.P. Potyondy. 1994. Stream channel reference sites: an illustrated guide to field technique. USDA For. Serv. Gen. Tech. Rpt. RM-245. Rocky Mnt. For. Range Exp. Stat. Fort Collins.
- Hawkins, C.P., R.H. Norris, J. Gerritsen, R.M. Hughes, S.K. Jackson, R.K. Johnson, and R.J. Stevenson. 2000. Evaluation of the use of landscape classifications for the prediction of freshwater biota: synthesis and recommendations. *Journal of the North American Benthological Society*. 19 (3): 541- 556.
- Hawkins, C.P. and Ostermiller. Personal Communication. Department of Fisheries and Wildlife, Watershed Science Unit, and Ecology Center, Utah State University, Logan, Utah.
- Hayslip, G.A. (Ed.). 1993. EPA Region 10 In-Stream Biological Monitoring Handbook: for Wadable Streams in the Pacific Northwest. U.S. Environmental Protection Agency- Region 10, Environmental Services Division, Seattle, WA. 75 pp.
- Hynes, H.B.N. 1970. *The Ecology of Running Waters*. University of Toronto Press, Toronto. 555 pp.
- Jensen, S.L. 1966. *The Mayflies of Idaho*. Unpublished Master's Thesis, University of Utah. 367 pp.
- Karr, J.R. 1987. Biological monitoring and environmental assessment: a conceptual framework. *Environmental Management*. 11: 249- 256.
- Karr, J.R. and E.W. Chu. 1999. Biological monitoring: essential foundation for ecological risk assessment. *Human and Ecological Risk Assessment*. 3: 933-1004.
- Kirchmer, C.J. and S.M. Lombard. 2001. Guidelines for preparing quality assurance project plans for environmental studies. Washington State Department of Ecology, Environmental Assessment Program, Olympia, WA. Publication No. 01-03-003.
- Lazorchak, J.M. and D.J. Klemm (Eds.), undated. Generic quality assurance project plan guidance for bioassessment/Biomonitoring Programs. United States Environmental Protection Agency, Environmental Monitoring Systems Laboratory, Cincinnati, OH.
- McAlpine, J.F., B.B. Peterson, G.E. Shewell, H.J. Teskey, J. R. Vockeroth, and D.M. Wood (coords.). 1981. *Manual of Nearctic Diptera, Vol. 1*. Research Branch of Agriculture Canada, Monograph 27, 674 pp.
- Merritt R.W. and K.W. Cummins (ed.s). 1996. *An Introduction to the Aquatic Insects of North America*, 3rd ed. Kendall/Junt Publishing Company, Dubaque, IA, 862 pp.

- Minshall, G.W. and J.N. Minshall. 1977. Microdistribution of Benthic Invertebrates in a Rocky Mountain (USA) Stream. *Hydrobiologia*. 55: 231- 249.
- Moss, D., M.T. Furse, J.F. Wright, and P.D. Armitage. 1987. The prediction of the macro-invertebrate fauna of unpolluted running-water sites in Great Britain using environmental data. *Freshwater Biology*. 17. 41- 52.
- Moyle, P.B., L.R. Brown, and B. Herbold. 1986. Final report on development and preliminary tests of indices of biotic integrity for California. Final report to the U.S. Environmental Protection Agency, Environmental Research Laboratory, Corvallis, Oregon.
- Needham, J.G., J.R. Traver, and Y.C. Hsu. 1935. The biology of mayflies with a systematic account of North American species. Comstock, Ithaca. 759 pp.
- Norris, R.H. and A. Georges. 1993. Analysis and interpretation of benthic macroinvertebrate surveys. Pages 234- 286 in D.M. Rosenberg and V.H. Resh (editors). *Freshwater Biomonitoring and Benthic Macroinvertebrates*. Chapman and Hall, New York, New York.
- Omernik, J.M. and A.L. Gallant. 1986. Ecoregions of the Pacific Northwest. EPA/600/3-86/033. U.S. Environmental Protection Agency, Environmental Research Laboratory, Corvallis, OR, 39 pp.
- Pater, D.E., S.A. Bryce, T.D. Thorson, J. Kagan, C. Chappell, J.M. Omernik, S.H. Azevedo, and A.J. Woods. 1998. Ecoregions of Western Washington and Oregon. U.S. Geological Survey. ISBN 0607895713.
- Pennak, R.W. 1989. *Freshwater Invertebrates of the United States*, 3rd ed. J. Wiley & Sons, New York, 628 pp.
- Plotnikoff, R.W. 1992. Timber, Fish, and Wildlife Ecoregion Bioassessment Pilot Project. Washington Department of Natural Resources, Olympia, WA. TFW-WQ11-92-001. 57 p.
- Plotnikoff, R.W. 1994. Instream biological assessment monitoring protocols: benthic macroinvertebrates. Washington Department of Ecology, Olympia, WA. Ecology Publication no. 94-113. 27 p.
- Plotnikoff, R.W. 1998. Stream Biological Assessments (Benthic Macroinvertebrates) for Watershed Analysis: Mid-Sol Duc Watershed Case Study. Washington Department of Ecology, Olympia, WA. Ecology Publication no. 98-334. 37 p.

- Plotnikoff, R.W. 1999. The Relationship Between Stream Macroinvertebrates and Salmon in the Quilceda/Allen Drainage. Washington Department of Ecology, Olympia, WA. Ecology Publication no. 99-311. 18 p.
- Plotnikoff, R.W. and S.I Ehinger. 1997. Using invertebrates to assess quality of Washington streams and to describe biological expectations. Washington Department of Ecology, Olympia, WA. Ecology Publication no. 97-332. 56 p.
- Plotnikoff, R.W. and J.S. White. 1996. Taxonomic Laboratory Protocol for Stream Macroinvertebrates Collected by the Washington State Department of Ecology. Washington State Department of Ecology. Publication No. 96-323.
- Resh, V. H. 1982. Age structure alteration in a caddisfly population after habitat loss and recovery. *Oikos*. 38: 280-284.
- Resh, V.H., A.V. Brown, A.P. Covich, M.E. Gurtz, H. W. Li, G. W. Minshall, S. R. Reice, A. L. Sheldon, J. B. Wallace, and R. C. Wissmar. 1988. The role of disturbance in stream ecology. *Journal of the North American Benthological Society*. 7 (4): 433- 455.
- Reynoldson, T.B., R.C. Bailey, K.E. Day, and R.H. Norris. 1995. Biological guidelines for freshwater sediment based on Benthic Assessment of Sediment (the BEAST) using a multivariate approach for predicting biological state. *Australian Journal of Ecology*. 20: 198- 219.
- Schuett-Hames, D., R. Conrad, A.E. Pleus, and K. Lautz. 1999. TFW Monitoring Program method manual for the salmonid spawning gravel scour survey. Prepared for the Washington State Dept. of Natural Resources under the Timber, Fish, and Wildlife Agreement. TFW-AM9-99-008. DNR #110. December.
- Stewart, K.W. and B.P. Stark. 1988. Nymphs of North American stonefly genera (Plecoptera). Thomas Say Foundation Series, Entomological Society of America. 12: 1- 460.
- Ter Braak, C.J.F. 1986. Canonical Correspondence Analysis: a new eigenvector technique for multivariate direct gradient analysis. *Ecology*. 67 (5): 1167- 1179.
- United State Bureau of Reclamation, 1997. Water Measurement Manual. U.S.G.P.O., Denver, Colorado.
- U.S. Environmental Protection Agency. 1990. Biological Criteria: National Program Guidance for Surface Waters. Office of Water Regulations and Standards. U.S. EPA, Washington D.C. EPA-440/5-90-004.
- Usinger. 1956. Aquatic Insects of California with keys to North American genera and California species. University of California Press, Berkeley, 508 pp.

- Vannote, R.L., G.W. Minshall, K.W. Cummins, J.R. Sedell, and C.E. Cushing. 1980. The river continuum concept. *Canadian Journal of Fisheries and Aquatic Sciences* 37: 130- 137.
- Van Sickle, J. 1997. Using mean similarity dendrograms to evaluate classifications. *Journal of Agricultural, Biological, and Environmental Statistics* 2: 370- 388.
- Van Sickle, J. and R.M. Hughes 2000. Classification strengths of ecoregions, catchments, and geographic clusters for aquatic vertebrates in Oregon. *Journal of the North American Benthological Society* 19 (3): 370- 384.
- Wiggins, G.B. 1995. Larvae of the North American caddisfly genera (Trichoptera), 2nd ed. University of Toronto Press, Toronto.
- Wilkinson, L. 1990. SYSTAT: The system for statistics. SYSTAT, Inc., Evanston, IL.
- Wolman, M.G. 1954. A method of sampling coarse bed material. *Trans. Amer. Geophysical Union*. 35: 951- 956.
- Wright, J.F., M.T. Furse, and P.D. Armitage. 1993. RIVPACS: A technique for evaluating the biological quality of rivers in the UK. *European Water Pollution Control* 3 (4): 15- 25.
- Wright, J.F. 1995. Development and use of a system for predicting the macroinvertebrate fauna in flowing waters. *Australian Journal of Ecology* 20: 181- 197.
- Zar, J.H. 1999. *Biostatistical Analysis*, 4th ed. Prentice Hall, Upper Saddle River, NJ. 663 pp.

Appendix A

Field Forms for Chemical and Physical Habitat Assessments

Site Name: _____
Stream Name: _____
Major Basin: _____
Date/Time: _____
Latitude/Longitude: *Latitude:* _____ *Longitude:* _____
Investigators: _____

SURFACE WATER INFORMATION		Thermometer, pH meter, Conductivity meter, D.O. Bottles	
Parameters	Measurement (Qualifiers)		
Temperature (°C)			
pH (standard units)	Calibration or Calibration Check:		
Conductivity (umhos/cm)	slope: _____ pH 1: _____ pH 2: _____		
Dissolved Oxygen (mg/L)	Bottle no.	(mL of titrant) or (mg/L)	Correction factor
Sample Time:			
Qualitative Observations			
Water Clarity (circle one)	Clear	Cloudy	Turbid
Water Odors (circle one)	Absent	Organic	Mucky
Sediment Odors (circle one)	Absent	Organic	Anoxic
Surface Films (circle one)	Absent	Foam	Sheen

Field Notes:	
Photograph:	
Photograph:	

Dominant Land Use (circle one): **Forest** **Agriculture** crops/pasture **Urban** industrial/residential **Other**

Weather (circle one): **Rain** **Drizzle** **Clear** **Overcast** **Other**

STREAM REACH PROFILE				
Equipment: Measuring tape, Clinometer, Wading Rod				
Channel Feature	Riffle 1 (riffles)	Riffle 2 (riffles)	Riffle 3 (riffles)	Riffle 4 (riffles)
Wetted Width (m)				
Bankfull Width (m)				
Maximum Depth (m)				
Gradient (%)				
RPD (m)				

Residual Pool : Dp=maximum depth of Dc=depth at pool crest (or RPD=residual pool depth (Dp-

CANOPY COVER MEASUREMENTS				
Equipment: Canopy Densiometer				
DENSIOMETER (count open intersections)				
Direction	Riffle 1	Riffle 2	Riffle 3	Riffle 4
Center (up)				
Center (down)				
Center (left)				
Center (right)				
Left Bank				
Right Bank				

SUBSTRATE MEASUREMENTS				
Equipment: Glass-bottom bucket, Gridded hoop				
Substrate Parameter	Riffle 1	Riffle 2	Riffle 3	Riffle 4
Depth (meters)				
Size Class (# intersections)				
Bedrock (smooth)				
Bedrock (rough)				
Boulder (250 to 4000 mm)				
Cobble (64 to 250 mm)				
Coarse Gravel (16 to 64 mm)				
Fine Gravel (2 to 16 mm)				
Sand (0.06 to 2 mm)				
Silt/Clay/Muck (not gritty)				
Wood (any size)				
Other (comment)				

Substrate measurements are made with a 60 cm diameter hoop and 50 equidistant observations

CURRENT VELOCITY				
Equipment: Wading rod and Flow meter				
Location in Water Column	Riffle 1	Riffle 2	Riffle 3	Riffle 4
Bottom of Stream				
0.6x Depth from Surface				

SUBSTRATE MEASUREMENTS- PEBBLE COUNTS				
Size Class (mm)	Riffle 1	Riffle 2	Riffle 3	Riffle 4
≥ 512				
≥ 256				
≥ 180				
≥ 128				
≥ 90				
≥ 64				
≥ 45				
≥ 32				
≥ 22				
≥ 16				
≥ 11				
≥ 8				
≥ 6				
≥ 4				
≥ 2				
< 2				

Equipment List

Biological Surveys

D-frame kick nets (2)
Scrub brush (2)
Cable ties (20)
Field Preservative (85% denatured ethanol)- 5 gallons
One liter Polycarbonate containers (40)
One gallon Heavy Duty freezer bags (30)
Hip waders
Chest waders
Wader repair kit
Labels (40)
Fine tip permanent markers (2)
Camera (1)
Slide film (2 rolls)

Habitat Surveys

Field forms for each site
150 ft tape measure (1)
Fiberglass stadia rod (1)
Clinometer (1)
Compass (1)
Flow meter (1)
Spare batteries for meter (2)
Flow rod (1)
Substrate grid (1)

Plexiglass substrate viewer (1)
Densimeter (1)
GPS unit (1)
"A Good Eye!"

Water Quality Surveys

250 ml water collection bottles (2)
Alcohol thermometers (2)
PH meter (1)
Conductivity meter (1)
Spare batteries for meters (2)
Winkler dissolved oxygen bottles/Tray (12 bottles)
Winkler reagents
De-Ionized water (2 ½ gallons)
Buffer Solutions (pH 4, pH 7, pH 10)
10% Hydrochloric Acid (250 ml)

Personal Gear

Elbow Length Insulated Gloves
Rain Gear
Leather gloves
Field vest
Eyewash kit
First aid kit
Drinking Water