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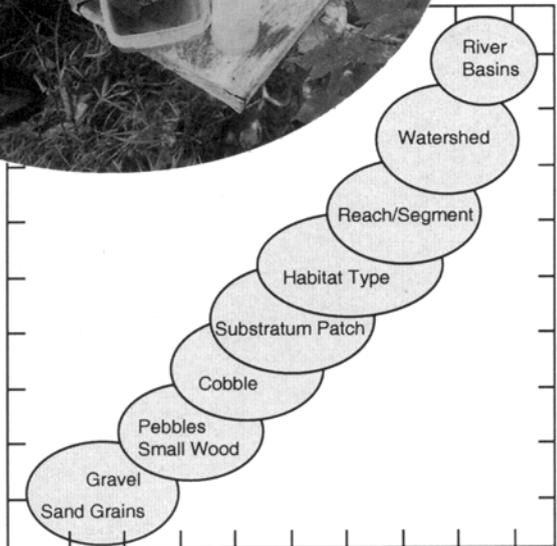
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# Monitoring Wilderness Stream Ecosystems

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## Abstract

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A protocol and methods for monitoring the major physical, chemical, and biological components of stream ecosystems are presented. The monitoring protocol is organized into four stages. At stage 1 information is obtained on a basic set of parameters that describe stream ecosystems. Each following stage builds upon stage 1 by increasing the number of parameters and the detail and frequency of the measurements. Stage 4 supplements analyses of stream biotic structure with measurements of stream function: carbon and nutrient processes. Standard methods are presented that were selected or modified through extensive field application for use in remote settings.

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# **Monitoring Wilderness Stream Ecosystems**

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# Introduction

Wilderness streams are a unique and valued resource, offering many of the “enduring benefits” envisioned by passage of the Wilderness Act of 1964. These benefits include fresh water and places to fish, relax, and enjoy nature; unique habitats for plants and animals; reference sites to judge direct and indirect impacts to our natural environment; and perhaps a place where we can learn how to be stewards of the land and water. Wilderness streams, because they are relatively unaffected by people compared to most other streams, present one of the best opportunities for learning about stream ecosystems and how they function. The value of wilderness streams as a place to learn and as an ecological benchmark to judge impacts is growing daily.

Myriad impacts threaten wilderness streams. Because of human and physical nature, most threats inexorably move toward streams. People who visit wilderness concentrate around streams and lakes, causing many types of problems, including:

- Removal of surrounding vegetation in turn causing increased erosion, sediment deposition, and turbidity;
- Introduction of human and other animal wastes, and chemicals such as fuel, soaps, and skin lotions;
- Trampling of bed material within streams and on stream margins thereby disrupting fish spawning and rearing areas, amphibian reproduction, and macroinvertebrates.

Other impacts include leachate from abandoned or active mines and atmospheric deposition of acids and other pollutants that eventually wash into streams and lakes. Cattle and other livestock spend much of their time close to water, especially in the drier wilderness areas of the western United States. Furthermore, compared to the total land area of most wildernesses, streams are rare and therefore impacts to them are of greater relative importance and significance.

Despite important social and biological values of wilderness streams and recognition of the many threats to them, our understanding of these relatively pristine aquatic ecosystems is meager. There are several reasons for this lack of knowledge. First, there are no roads in wilderness and roads have become the primary means of access for most scientists. The logistical and practical hurdles of hauling sampling gear on foot or horse deters most scientists. Second, there are no electrical outlets in the backcountry and

scientific equipment is increasingly dependent on electricity. And third, ecosystem-level understanding often requires manipulating the environment, and wilderness is the one place where such manipulation usually is not allowed. Also, understanding the functional parameters of ecosystems typically requires large amounts of expensive and bulky equipment that is costly and difficult to transport.

This manual provides information to overcome most or all of these challenges by demonstrating how to monitor streams in the backcountry wilderness using equipment that is lightweight, portable, and rugged. Our overall goal and purpose in developing this manual is to provide guidance to biologists and wilderness managers who are interested in developing baseline information and in evaluating known or likely impacts to wilderness streams.

## **Scope and Organization**

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This manual provides detailed guidance on how to acquire data on wilderness streams. We offer instruction on monitoring the entire range of structural and functional stream parameters in a staged monitoring system that provides increasing detail and rigor at each successive stage. This staged system offers maximum flexibility allowing modification for particular situations, goals, and needs. It is organized in a manner that, while ensuring the analysis of key factors, allows for modification to address particular objectives.

We begin, through the remainder of this introduction, by addressing the basic questions that occur when initiating a monitoring program. What stream components or factors should be measured? From where should samples be taken? How often should samples be collected? How are differences between or among locations and streams detected? Following the introduction, detailed discussions are presented of the methods that have been proven effective in evaluating the physical and biotic components in wilderness streams. The knowledge gained by the users of this manual will help to fill the information gap on wilderness streams.

## **Goals and Objectives**

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Clearly outlining the goals and objectives of a monitoring program will focus effort in the proper direction and thereby eliminate the needless costs associated with collecting irrelevant data. Monitoring goals generally fall into two main categories: obtaining baseline information or evaluating potential impacts. Wilderness areas often contain the only unimpacted streams within a region. Obtaining baseline data from within a wilderness area can provide important information on the structure and function of unimpacted stream ecosystems. These data then can be used to determine the extent of impact in streams subjected to various degrees or types of influence. Obtaining baseline data within wilderness areas also is beneficial for the evaluation of potential, unforeseen impacts. The monitoring

goal of obtaining baseline data can be further refined to a specific objective. For example, the effects of livestock grazing on small upland Forest Service streams may be a regional concern. The objective of the monitoring program would then be refined to obtain data on similar small upland wilderness streams. By defining goals and objectives, we have reduced potential sampling sites from all wilderness streams to small upland wilderness streams. More detailed stream classification (discussed below) can further reduce the number of potential sampling sites.

The same logic applies for the goal of evaluating impacts. For example, camp sites generally are concentrated within the stream/riparian corridor, particularly where trails approach or cross streams. This concentrated use could result in the compaction of soil, removal of riparian vegetation, increased streambank erosion, and clearing of downed timber for firewood. All these factors could negatively impact stream systems. Therefore, the monitoring objective may be to determine whether these camp sites are impacting the stream. Initial observations and stream classification could confirm such negative impact or demonstrate that most of the problem sites are on streams that have a low slope, are not confined, and have a relatively large floodplain. This information could help to further refine monitoring objectives and sampling locations.

## **Selecting Appropriate Measurements \_\_\_\_\_**

The stream factors measured at each sampling location are outlined in table 1 (Minshall 1994). The physical and biotic factors in table 1 are organized into four different stages. Each increase in stage increases the level of analysis and the number of factors measured. Stage 1 is considered the minimum level of analysis required. Each subsequent stage incorporates the measures of the previous stage. The procedures consist of a nested series of measurements grouped in units or “subsets” and arranged to progressively increase the information available for management decisions, and permit adjustments for specific types of problems. A nested arrangement assures that a basic set of comparable measurements will be made in all cases but also permits further tailoring of the program for specific needs and available resources. That is, the monitoring plan ensures measurements of basic ecosystem factors at stage 1, and provides flexibility through incorporation of additional levels (stages) of analysis for certain factors, or through higher levels of analysis.

The monitoring objective, type of problem (for example, nutrients versus toxic metals), and use of information (for example, a local management question versus legal litigation) will determine the necessary stage of analysis. However, selecting the appropriate stage of analysis will require a management decision based on monitoring objectives and a basic understanding of stream ecosystems. For example, if the monitoring objective is to obtain baseline information for comparison with potential future impacts to small upland streams, then stage 1 analysis could be conducted at most sites, with stage 3 or 4 analysis conducted at 1 or 2 long-term reference locations. If the monitoring objective is to evaluate potential changes in

**Table 1**—Hierarchical sequencing of measurements of stream environmental conditions suitable for application at the stream segment level and lower (excluding habitat features addressed in table 2), arranged in order of increasing detail, with each subsequent stage intended to be cumulative.

<b>Stage 1</b>	<b>Measurement/feature</b>	<b>Purpose</b>
Environmental factors: Temperature	24-hour maximum and minimum during warmest month of the year	Estimate of annual maximum and diel change
Solar radiation	Yearly estimates using Solar Pathfinder	Relative shading by vegetation and topographic features
Substratum	Mean and coefficient of variability (CV) of b-axis for $\geq 100$ randomly selected particles	Mean particle size distribution and heterogeneity
Alkalinity	Basic water chemistry analyzed using standard methods	General water quality
Hardness		
pH		
Turbidity		
Biotic factors: Large woody debris Macroinvertebrates	Total count within reach Rapid bioassessment protocol III	Abundance of structural component Biotic condition indicators and community structure indices
Fish (If specifically desired)	Appropriate metrics, density and biomass estimates	Biotic condition indicators and community structure indices
<b>Stage 2</b>	<b>Measurement/feature</b>	<b>Purpose</b>
Environmental factors: Solar radiation	Point incoming solar radiation reaching stream surface at 9, 12, 3, and 6 on a clear day in summer	Measurement of daily solar energy input
Temperature	Seasonal 30-day thermograph records	Improved characterization of thermal regime and heat budget

(con.)

**Table 1 (Con.)**

<b>Stage 2</b>	<b>Measurement/feature</b>	<b>Purpose</b>
Discharge	Summer baseflow	Characterize stream size; permit calculation of fluxes
Substratum	Embeddedness and stability	Estimate of suitability of streambed for fish (egg) and invertebrate survival
Calcium	Filtered sample	Delineation of main cations and principal plant nutrients
Magnesium	Colorimetric field procedure	
Nitrate-N		
Phosphorus (ortho)		
Sulfate		
Biotic factors:		
Large woody debris	Abundance and ranked score based on importance	Quantification of an important component of streams
Algae	Periphyton chlorophyll- <i>a</i> and biomass	Quantification of an important food source and biotic indicator
Benthic organic matter	Total	Quantification of an important food source
Invertebrates	Total density, biomass, and analysis by functional feeding group	Estimates of 2° consumer production
<b>Stage 3</b>	<b>Measurement/feature</b>	<b>Purpose</b>
Environmental Factors:		
Solar radiation	Stream surface, standard, depth and bottom PAR seasonally on clear days	Estimate of solar input
Temperature	Annual thermograph records	Improved information content
Discharge	Placement of stream stage height gauges; 5 seasonal instantaneous measurements	Improved characterization of flow regime
Current velocity and depth	Measured at random locations throughout study area. Determine mean current velocity	Characterization of stream habitat suitability; determination of hydraulic stress

(con.)

**Table 1 (Con.)**

<b>Stage 3</b>	<b>Measurement/feature</b>	<b>Purpose</b>
Ammonia-N Nutrient flux	Laboratory analysis of filtered samples Concentration X discharge (with concentration determinations upgraded to laboratory quality)	Further detail regarding nitrogen dynamics Measure of resource availability (Fisher 1990)
<b>Biotic factors:</b>		
Algae	Diatom community metrics	Biotic condition indicator
Benthic organic matter	Partitioned into coarse and fine sizes and main sources	Refined food resource analysis
Transported organic matter/invertebrate drift	Same as for benthic organic matter	Estimate of exported organic matter and food available for filter feeders and fish
Ecosystem production/ respiration	Total-system metabolism using open- system methods	Measure of ecosystem function, productivity, and trophic state
Nutrient spiraling/limitation	Open system nutrient spiraling parameters/ response to standard nutrient additions	Measure of ecosystem behavior and utilization/retention efficiencies/plant nutrient-growth status
<b>Stage 4</b>	<b>Measurement/feature</b>	<b>Purpose</b>
<b>Environmental factors:</b>		
Solar radiation	Annual solar radiation	Determine solar radiation regime and energy input
Discharge	Annual hydrograph records	Improved information content
<b>Biotic factors:</b>		
Organic matter decomposition	Leaf pack decay rates	Estimate of decomposition by microbial and invertebrate detritivores
Ecosystem production/ respiration	Activity rates of colonized trays of native substrata measured in recirculating chambers	Measure of ecosystem function, productivity, and trophic state for each component
Nutrient spiraling	Uptake rate of components measured in recirculating chambers	Uptake efficiencies of each component
Secondary production	Monthly measurements of invertebrate standing crops	Measure of impacts on fish-food producing capability of streams

water chemistry near camp sites, then stage 1 analysis should be enhanced by incorporating stage 4 analysis of water chemistry and stage 3 analysis of nutrient limitation.

Included are measurements of physical and chemical factors that address the known key stream ecosystem parameters (Minshall 1994). Established (standard) procedures are used, where possible, in order to permit rapid deployment and to assure comparability among studies and technical personnel. The recommended procedures are sufficiently robust to be applicable over a wide variety of situations.

## Stage 1

Stage 1 procedures are based on the Environmental Protection Agency's Rapid Bioassessment Protocols (RBP) (Plafkin and others 1989) for both habitat and biotic (macroinvertebrates, fish) components (MacDonald and others 1991). The combination of RBP III and V are used in the ecosystem assessments addressed in this study. We have modified the original RBP III protocol to involve the analysis of 300 or more specimens, and use of 250  $\mu\text{m}$ -mesh Surber net or comparable quantitative sampling device. Included in this stage is a basic evaluation of physical habitat (temperature, discharge, substratum) and diagnostic water quality conditions (alkalinity, hardness, pH, specific conductance, turbidity).

Stage 1 protocols assume that all of the data needed at this level of analysis will be obtained at the time the stream is visited and that may be only once a year or less. Consequently, this stage provides only the minimal information required to broadly characterize conditions. Maximum and minimum temperature measurements over 24 hours provides a measure of the range of values (both absolute and range) to which the organisms are exposed during any particular time of the year. Measurements during the warmest month provide information for one of the most stressful periods and, when combined with an estimate of the annual minimum temperature (often near 0 °C), can be used to estimate the annual range. Measurement of the intermediate axis of 100 or more randomly selected pieces of substratum (popularly known as the pebble count procedure) provides a good characterization of inorganic materials covering the streambed, and facilitates determination of a bed-stability index. Collectively, the suggested chemical measures can provide a good general characterization of water quality conditions (see Water Quality section).

In addition to the factors specified in table 1, a habitat characterization, as described by Plafkin and others (1989), and detailed site classification (table 2, 3) should be conducted as a means of adequately describing and classifying the study site and providing additional measures of physical conditions. Photographs supplement the site characterization and, along with global positioning systems, can be used to identify sampling locations in subsequent years.

**Table 2**—Spatial hierarchical classification of three Big Creek wilderness streams (from Monaghan and Minshall 1996).

<b>Stream habitat (linear spatial scale)</b>	<b>Defining measures</b>	<b>Stream characteristics</b>
Biogeoclimatic region (10 <sup>5</sup> m)	Regional climate  Regional geology Regional topography Regional terrestrial vegetation Flow regime	Northern Rocky Mountains Ecoregion, semi-arid steppe; hot dry summers, cold snowy winters (Bailey 1989; Robinson and Minshall 1995) Central Idaho northern Rocky Mountains (Alt and Hyndman 1989) Narrow steep-sided canyons; forested mountain tops Semi-arid steppe forest and grassland High snowmelt discharge, constant summer baseflow, rare summer spates
Stream system (10 <sup>3</sup> -10 <sup>4</sup> m)	Local climate  Local geology	74 cm precipitation annually, 54 percent between November and March Precambrian metamorphic schists and gneisses with Cretaceous and Eocene granitic intrusions of the Atlanta (Idaho) batholith (Alt and Hyndman 1989)
Segment system (10 <sup>2</sup> -10 <sup>3</sup> m)	Local topography  Local terrestrial vegetation  Thermal regime  Tributary junctions	Cliff Creek—southern aspect; Pioneer—northern aspect; Rush—northern aspect Douglas-fir and ponderosa pine; extensive areas of bare rock; open areas of sagebrush and grass Summer min/max of 9/20 °C Rush—between Lewis Creek tributary and confluence with Big Creek
Reach system (10 <sup>1</sup> - 10 <sup>2</sup> m)	Major geologic discontinuities  Channel slope Valley form  Bed material Riparian vegetation	Cliff—change from granite to schist/gneiss bedrock occurs above study reach; Pioneer—none noted; Rush—none noted Cliff—0.18; Pioneer—0.25; Rush—0.01 Cliff—narrow type A2 Rosgen (1994) classification; Pioneer—narrow type A3; Rush—less confined type B3 Eroded cobble and gravel Birch, alder, mountain maple, serviceberry

## Stage 2

Stage 2 provides a more complete measure of environmental conditions and an analysis of the food resources available to the heterotrophs. Thermograph records are used for identifying and quantifying important aspects of the thermal regime (Vannote and Sweeney 1980). They are equally important for quantifying thermal budgets (for example, cumulative degree-days) that are important in explaining aquatic invertebrate and litter-processing responses (Cummins and others 1989). The benthic invertebrate analysis is expanded beyond stage 1 to include total density (abundance per unit area), biomass (which require accounting for all organisms in a sample), and partitioning of the results by functional feeding group (Cummins 1973; Merritt and Cummins 1996). For this stage, habitat features are quantified using procedures such as those described by MacDonald and others (1991) and Platts and others (1983, 1987). However, a standard quantified protocol for habitat analysis comparable to the subjective protocols presented by Plafkin and others (1989) and Petersen (1992) has yet to be developed.

## Stages 3 and 4

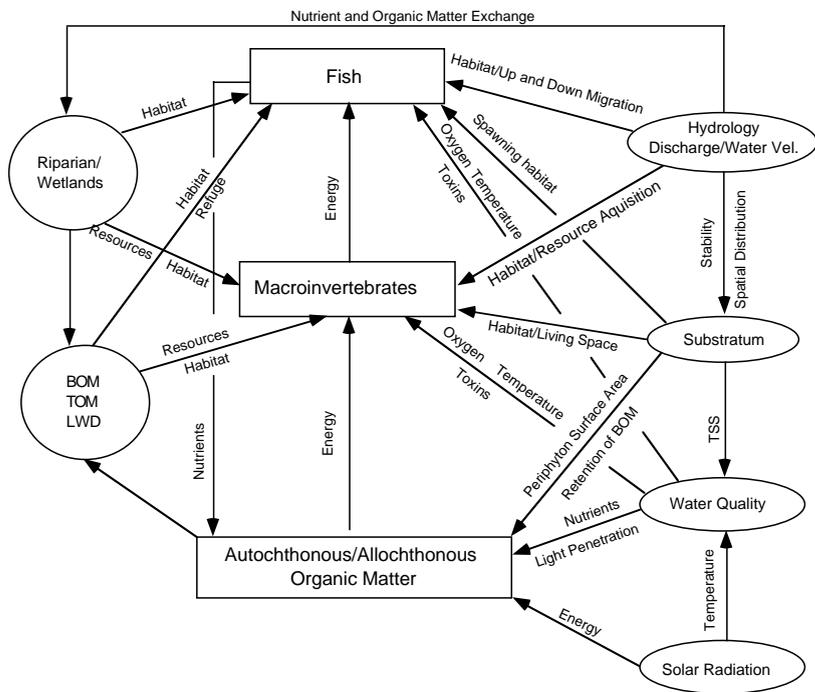
These two stages differ primarily in the level of detail involved and the incorporation of measurements of ecosystem function. Stages 3 and 4 supply additional environmental details and address some of the most important aspects of stream ecosystem function: decomposition rates, energy metabolism, and nutrient cycling. An ecosystem is at least a dual entity: structural and functional (MacMahon and others 1978; O'Neill and others 1986). Figure 1 is a simple model of a stream ecosystem. Quantification of each box, in other words, macroinvertebrate, fish, and algal community composition and biomass, would be a description of biotic structure. Biotic function is depicted in figure 1 by the arrows. Quantification of the flux of energy and elements among biotic and abiotic components would contribute to a description of stream ecosystem function. For example, primary production, the transfer rate of energy (solar radiation) and an element (carbon) to primary producers is a functional process. The structural (population-community) dimension is organized according to constraints involving organism interaction, natural selection (for example, competition) and the physical habitat. The functional dimension is established according to constraints that involve mass balance and thermodynamics. Only in unusual circumstances can one be considered in isolation from the other. That is, complete understanding (and monitoring) of stream ecosystems requires a quantification of biotic components (boxes) and the flux of energy and elements (arrows) among the different components. The description of the state (status) of an ecosystem or determination of changes in state must consider both biotic structural and functional attributes. Therefore, a sound bioassessment program must incorporate both structural and functional attributes of ecosystems. However, virtually

**Table 3**—Hierarchical classification of stream/riparian habitats (after Frissell and others 1986).

Stream habitat (linear spatial scale)	Defining measures		Boundaries		Application	Source of information	Procedure/guidelines references
	Longitudinal	Lateral	Longitudinal	Lateral			
Biogeoclimatic region (10 <sup>6</sup> m)	Regional climate Regional geology Regional topography Regional terrestrial vegetation Flow regime				Region; State; Forest District	Topographic maps (15') Geologic maps (15') Landsat photos Annual discharge records	Omernik 1987  Poff and Ward 1989
Stream system (10 <sup>2</sup> -10 <sup>4</sup> m)	Local climate Local geology Local topography Local terrestrial vegetation Thermal regime	Drainage divides, and seacoast, or catchment area	Drainage divides bedrock faults, joints controlling ridge valley development		Basin-wide surveys; Cumulative impacts; Integration of sites within watersheds	Topographic maps (7.5') Geologic maps Vegetation maps Aerial photos Annual temperature records	Omernik and Gallant 1986  Chorley and others 1984; Gregory and Walling 1973; Vannote and Sweeney 1980
Segment system (10 <sup>2</sup> -10 <sup>3</sup> m)	Tributary junctions Major geologic discontinuities	Tributary junctions major falls; bedrock lithologic or structural discontinuities	Valley sideslopes or bedrock out- crops controlling lateral migration		Paired watersheds Segment classes (for example uplands versus lowlands)	Topographic maps (7.5') Ground reconnaissance Low level aerial photos	(con.)

**Table 3 (Con.)**

Stream habitat (linear spatial scale)	Defining measures	Boundaries		Application	Source of information	Procedure/guidelines references
		Longitudinal	Lateral			
Reach system (10 <sup>1</sup> -10 <sup>2</sup> m)	Channel slope Valley form Bed material Riparian vegetation	Slope breaks: structures capable of withstanding <50-year flood	Local sideslopes or erosion-resistant banks; 50-year floodplain margins	Local effects: grazing allotments; dredging	Ground survey/mapping	Frissell and others 1986; MacDonald and others 1991; Minshall 1984; Minshall and others 1989; Petersen 1992; Plafkin and others 1983, 1987; Platts and others 1989; Rosgen 1994
Pool/riffle system (10 <sup>0</sup> -10 <sup>1</sup> m)	Bed form and material Origin Persistence Mean depth and velocity	Water surface and bed profile slope breaks; location of genetic structures	Mean annual flood channel; midchannel bars; other flow- splitting obstructions	Aquatic habitat inventories; fisheries censuses	Ground survey/mapping	Bisson and others 1981; Frissell and others 1986; McCain and others 1990
Microhabitat system (10 <sup>-1</sup> -10 <sup>0</sup> m)	Surface particle size; underlying particle size; water depth; velocity; overhead cover (type)	Zones differing substratum type; size arrangement	Same as longitudinal	Characterization of local spatial heterogeneity and effects (for example wading by fisherman)	Direct measurement	



**Figure 1**—Model of stream ecosystem identifying major biotic and abiotic components. The acronym BOM, refers to benthic organic matter, TOM, transported organic matter, TSS, total suspended solids, and LWD, large woody debris. The biotic components have both physical (circles) and biotic (rectangles) characteristics. That is, LWD provides both cover for fish (physical) and food for macroinvertebrates (biotic). Ecosystem structure is described by a quantification of the physical and biotic components (ovals, rectangles, and circles). Ecosystem function is described by the relationship between components (arrows). For example, the transfer of energy from the sun to fish is one description of ecosystem function.

all schemes to date have focused almost exclusively on structural features in spite of early admonitions by some aquatic ecologists (Cairns 1977) to include functional aspects as well.

## Selecting Sampling Locations \_\_\_\_\_

Selecting sampling locations involves two different processes. First, sampling reaches must be selected. This involves choosing reaches that will

be representative of the spatial scale of inference and that conform to the statistical design. Second, the exact location within the reach where measurements will be taken or samples obtained must be determined. These locations will depend both on the statistical design and the particular factor being measured, but usually are established in a random or stratified-random fashion.

## Selecting Sampling Reaches

As noted previously, monitoring of stream ecosystems usually is conducted to provide baseline data or to determine if some impact has significantly altered the integrity of the stream or site in question. For either of these monitoring goals, the scale of inference will influence the selection of appropriate sites. For example, if the objective is to describe the physical and biotic components within the ecoregion, then sample sites should represent the types of streams occurring within that spatial scale. Sites could be selected randomly among any sized stream (1st to 4th order) and any segment of these streams (confined high slope to unconfined shallow slope). In this case the variability in the data will be high and, while providing a means to distinguish differences among ecoregions, differences among locations within the ecoregion cannot be evaluated. On the other hand, sampling only sites located on steep sloped 1st order streams cannot provide data that is representative of all streams within the ecoregion. Stream classification provides a means of stratifying streams and identifying sampling locations that addresses the spatial scale of inference and objectives of the monitoring program.

A spatially nested hierarchical framework for classifying stream systems (table 2), allows managers to identify the spatial scale of inference (Frissell and others 1986; Hawkins and others 1993; Maxwell and others 1994). In a hierarchical system, lower levels are modified and constrained by factors operating at higher levels. Therefore, in an attempt to focus on factors influencing stream ecosystems on a small scale one must be aware of factors operating at larger scales. That is, one cannot evaluate and manage to alleviate the effects of intense recreational use at a stream crossing when similar or other impacts are occurring throughout the watershed. In addition, comparisons between stream reaches cannot be made if they are contained within different kinds of stream segments, systems, or ecoregions. In other words, one would not compare physical and biotic data obtained from a large river with similar data from a small headwater stream. Therefore, effective management of local ecosystems (for example, stream reaches or watersheds) requires attention to the landscape in which they are embedded (Agee and Johnson 1988; Jensen and Bourgeron 1993).

In this approach, the ecoregion is set at the upper level of the hierarchy (Minshall 1994). Stream systems, at successively lower levels of watersheds, consist of stream segments, reaches, pool/riffle complexes, and microhabitat subsystems. The pool/riffle complex (in other words, channel form) level can be further refined for more precise classification (Hawkins and others 1993). Initial classification according to ecoregion is based on Omernik (1987) and Gallant and others (1989). Inclusion of flow regime,

using the procedure of Poff and Ward (1989), further refines the biogeoclimatic aspects and makes the classification more directly related to flow: a major environmental driver of stream/riparian ecosystems. Classification of watersheds within an ecoregion is accomplished operationally by distinguishing between “regional” versus “local” climate, geology, and terrestrial vegetation. Proper classification at the watershed level requires the availability of long-term records of atmospheric temperature, precipitation, and stream discharge. Environmental data will, in many cases, be available from regional weather and stream-gauging stations (Finklin 1988; Mosko and others 1990). Snow cover and duration should be included when describing the local climate. Terrestrial plant records can be obtained from published sources such as Franklin and Dyrness (1973), Hall (1973), and Steele and others (1981). Incorporation of thermal regime, as recommended by Vannote and Sweeney (1980), permits stratification by catchment-level differences. Catchments may be similar in external or regional biogeoclimatic controls but differ in their thermal environments because of different make-up combinations of ground and surface water or different aspect of orientation to the sun.

Classification of stream segments is accomplished by conventional geomorphology practices which employ stream orders (Strahler 1957) or links (Shreve 1966), based on either tributary junctions, or major geologic discontinuities or both. Frissell and others (1986) and Rosgen (1994) provide criteria for distinguishing stream reach classes. Important driving factors at the stream reach level include substratum particle size and heterogeneity (Minshall 1984; Poff and Ward 1990) and woody debris accumulations (Cushing and others 1995; Elwood and others 1983; Marston 1982; Platts and others 1987; Sedell and others 1988; Trotter 1990). Several valley and channel features (Rosgen 1994) serve to further characterize the physical environment, and are obtained through the classification of the sampling sites. Channel slope (gradient), measured as the energy slope of the water surface, exerts a major control on current velocity, turbulence, and substratum composition. Valley form is expressed as the degree of entrenchment: the ratio of flood prone width divided by bankfull width. Bed form indicates whether the channel is straight, braided, or meandering. Sinuosity, the ratio of channel length to valley length, indicates the extent of meandering by the stream. Width/depth ratio, width at bankfull stage divided by bankfull depth, measures the distribution of energy within channels. The use of valley form (Minshall and others 1989; Rosgen 1994) in place of side-slope gradient is better for characterizing features likely to be important to riparian as well as stream dynamics at this classification level. Classification of pool/riffle systems is an important description of the templet on which patterns of biological diversity and production appear.

When monitoring to provide baseline data, maps should be used to classify the streams by habitat type within the ecoregion. From the maps, basin area, stream order, and estimates of stream slope and confinement can be determined. Site selection can then be stratified (see below for discussion of stratified sampling) or refined based on objectives. For example, if the focus is on the stream system scale (table 3), one 3rd order, three 2nd order, and seven 1st order streams reaches could be randomly

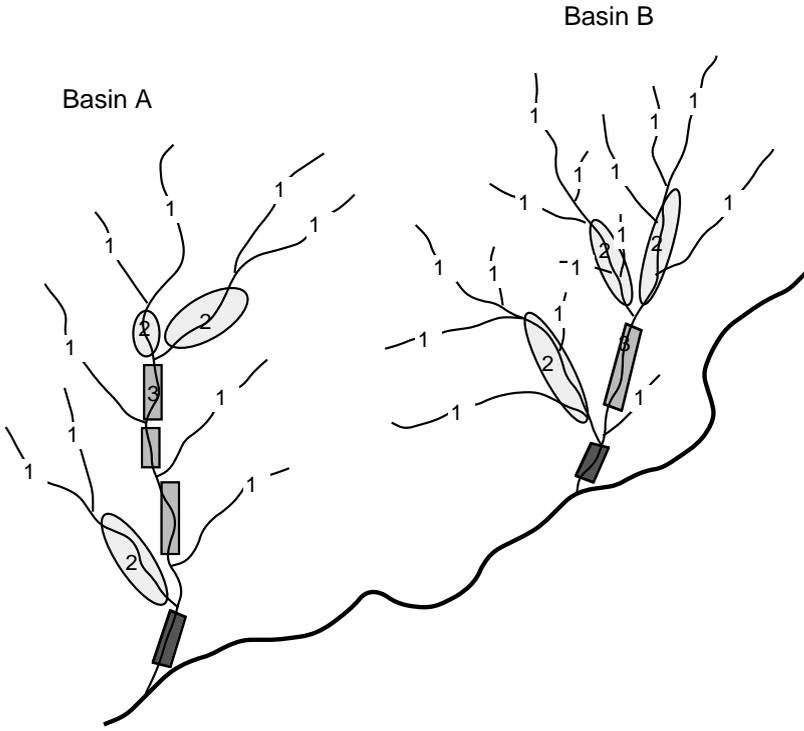
selected and monitored (stratification should be proportional to the frequency of a stream type). This manner of site selection will increase the level of resolution down to stream order, while still providing information relating to the ecoregion. Further classification and stratification can be extended to lower hierarchical levels (table 2) or by the reach classification of Rosgen (1994) but obviously will increase the cost and effort required to obtain data. Management objectives also can refine the spatial scale of monitoring efforts. For example, it may be that a large portion of wilderness use occurs at high elevations surrounding small 1st order streams. In this case, monitoring could include only streams in this category.

When monitoring to obtain baseline data, it is important to provide detailed classification of the sites monitored and to provide complete descriptions of the sampling methods and results. This allows for confident comparisons of the data with other sites or future studies.

Monitoring to determine possible impacts involves comparing impacted sites with reference sites. Reference sites are the field ecologist's equivalent of the experimentalist's more rigorously defined "control" condition. Reference sites can be of three types: a similar location upstream of the disturbance (for small scale impacts), the same location prior to disturbance, or a similar site(s) located on a different stream or streams (either historic or contemporary data). The selection of impacted and control sites will vary with the spatial scale of the disturbance. If the disturbance affects an entire basin, comparisons must be made with historic data (same location or different location within the ecoregion) or data from other streams in similar basins. Under ideal conditions, streams within the basin (impacted and reference) are classified, and sampling sites are stratified and selected randomly within each strata. Alternately, one representative impacted sampling reach is selected and compared to a reference site. If one sampling reach is used, it should be upstream of the mouth of the highest order stream in the basin. This allows for the integration of multiple impacts throughout the basin (fig. 2).

If impacts are confined to a stream segment, then multiple sampling reaches or a representative sampling reach should be monitored. These reaches can be selected randomly or by the investigator's judgment. A stream sampling reach is an arbitrary unit and is often defined as 20 times bankfull width. For small streams, however, a minimum reach length of 50 to 100 m is established. Stream reaches also can be based on regular patterns of morphology (Gordon and others 1992). For example, a reach could be a section of stream containing two pools and two riffles. If a representative reach is selected by the investigator, obvious biases should be avoided. A reach should not be selected based on access if it is not representative of the stream segment under investigation. Sampling locations should avoid modified sites, such as trail crossings, bridges, or campsites, unless assessing their effects. Sampling reaches also should avoid tributary inputs and be at least one reach upstream from a stream confluence or mouth.

No matter what spatial scale the disturbance is impacting, reference sites should have as similar a classification to impacted sites as possible. In many cases, the best reference sites will not be those which are immediately adjacent (or even in close proximity) to impacted sites. Proper and similar classification of impact and reference reaches ensures viable comparisons.



**Figure 2**—Streams within two basins are classified by stream order. For basin wide comparisons, sampling can be stratified based on the classification. Potential reaches are determined within the 1st order, 2nd order (shaded ovals), and 3rd order (shaded rectangles) segments. Dark rectangles represent potential sampling segments when only one site in each basin can be monitored.

For this reason, obtaining prior baseline data, particularly when future impacts are expected, is preferred.

## Selecting Sampling Locations Within a Reach

Once the sampling reaches are determined, the exact locations within the reach where data will be collected must be identified. These decisions will depend on the study design and whether statistical comparisons will be made. Detailed explanation of research design can be obtained by referring to statistics texts (Green 1979; Sokal and Rohlf 1969; Zar 1974) and will be outlined only briefly here. The type of statistical or comparative analysis for each of the physical and biotic components is outlined in table 4 and described in more detail in their respective chapters. For comparative data, the sampling location is selected to provide the best measurement of

**Table 4**—Outline of site selection, sampling frequency, and type of data analysis for each monitoring component.

<b>Factor/component</b>	<b>Site selection/ sampling locations</b>	<b>Sampling frequency</b>	<b>Data analysis</b>
Temperature	One representative location. Avoid slack water: sloughs or side channels.	Varies with stage of analysis: seasonal, monthly, continuous.	Comparative or statistical
Discharge	One location where flows are concentrated and channel uniform.	Varies with stage of analysis and objectives.	Comparative or statistical
Solar radiation	In small (1st and 2nd order) streams, mid-channel at 5 randomly selected transects. In larger streams stratified into margins and mid-channel.	Stages 2 and 3, seasonally and 4 times daily. Stage 4, continuous at representative location (location determined during early stage analysis).	Comparative or statistical
Water chemistry	One transect within sampling reach.	Varies with stage of analysis. In stage 4, stratified with flows.	Comparative and statistical
Morphology/substratum	Morphology: 5 randomly selected transects within reach. Substratum: systematic sampling.	Annual or greater unless bankfull-flows occur more often.	Statistical: contingency table
Macroinvertebrates	Random, stratified random, or systematic sampling.	Varies with stage of analysis: annual, seasonal, monthly.	Comparative (metrics) or statistical (con.)

**Table 4 (Con.)**

<b>Factor/component</b>	<b>Site selection/ sampling locations</b>	<b>Sampling frequency</b>	<b>Data analysis</b>
Algae/periphyton	Five or more stones selected haphazardly within reach.	Varies with stage of analysis, annual, seasonal, monthly.	Statistical but with caution due to potential bias
Large woody debris (LWD)	Total population within reach.	Annual	Comparative (metrics)
Benthic organic matter (BOM)	Random, stratified random, or systematic sampling.	Varies with stage of analysis: annual, seasonal, monthly.	Statistical
Transported organic matter (TOM)	Three or more replicates at one representative location. Drift should be stratified by time of day.	Varies with stage of analysis. In stage 4, stratified with flows.	Statistical
Organic matter decomposition	Three or more randomly selected locations. Can be stratified.	Annual	Statistical
Primary production	Three or more replicates selected randomly within reach.	Annual or seasonal	Statistical or comparative
Nutrient dynamics	Nutrient limitation, one representative location or open and shaded sites.	Annual or seasonal	Statistical

the parameter. For statistical comparisons all suitable locations within the reach should have an equal probability for being selected as sampling sites.

Four types of sampling are used in this monitoring manual: random, systematic, stratified random, and haphazard sampling. For random sampling, each location within the reach has an equal chance of being sampled. This is accomplished by dividing the stream reach into discrete sections (the area of each section equals the area of the sampler in use), each section is then numbered, and numbered sections are chosen by referring to a random numbers table. For example, the area of a Surber sampler (most common invert sampler) is 0.12 m<sup>2</sup>. For a stream that is 2 m wide, reach length might be 40 m, and total area 80 m<sup>2</sup>. Therefore, there are over 600 potential sampling locations. Five randomly selected sampling locations are selected from the 600 potential sampling sites. Random sampling is designed for homogeneous environments. Potentially all or most of the samples could end up being collected in one area rather than throughout the study reach. One way to spread out the potential sampling locations is to divide the stream reach into transects. For the 2 m wide stream, potential transects are spaced at 2 m intervals. There are 21 potential transects in the reach. Five of these transects are selected randomly. Each transect is then divided into 10 equal sections, one of which is randomly selected as a sampling location. In heterogeneous environments (most streams), more representative sampling may be obtained by the systematic or stratified random approaches.

There often is a large degree of variation in biotic characteristics among the different stream macrohabitats. Invertebrate community composition of pools may be very different from those residing in riffles. This large variability reduces the probability of determining differences between impacted and reference sites. Stratified random sampling divides the stream reach based on these distinct habitats or strata. A random sample is then drawn from each strata. The number of samples taken within each strata should be proportional to the area of each strata. That is, if 20 percent of the stream reach is classified as pools, then 20 percent of the samples should be taken within this habitat type. Further stratification might involve selecting a single strata, for example, riffles.

In systematic sampling, the initial sampling location is selected randomly and subsequent sampling locations or transects are selected at fixed intervals from this point. This method of sampling is used for determining substratum size distribution.

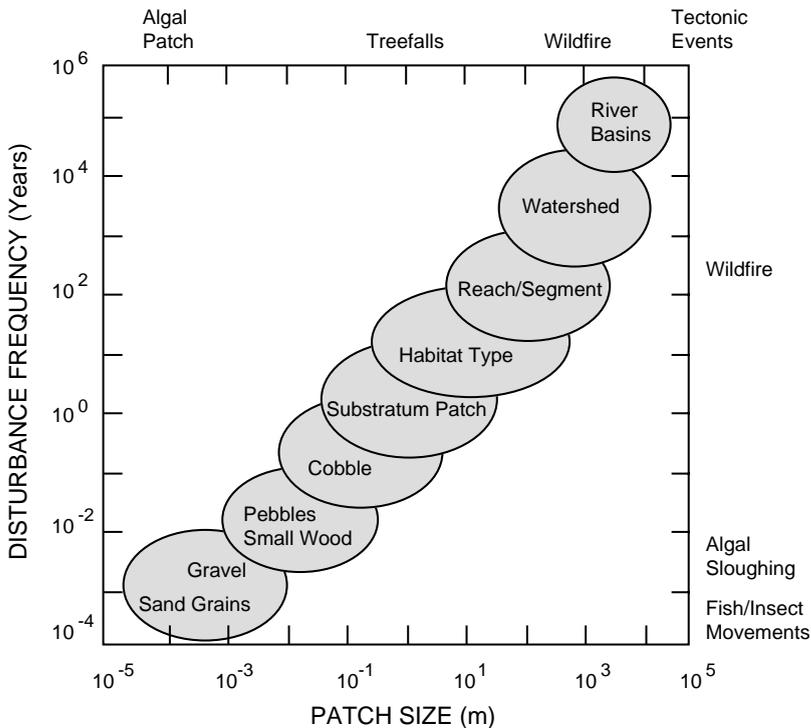
Haphazard sampling is occasionally used when completely random sampling is not practical. Haphazard sampling depends on the investigator obtaining random samples based on his/her judgment. Sampling locations are chosen by the investigator. For example, the area of the periphyton sampler described in this manual is  $3.54 \times 10^{-4}$  m<sup>2</sup>. For the 80 m<sup>2</sup> sampling reach there would be over 225,000 potential sampling locations. Dividing a stream reach into this many sections would be impractical, and so rocks sampled are best selected haphazardly or in association with established transects. Similarly, limitations imposed by the sampling gear may preclude strict random sampling.

# Sampling Frequency

Sampling frequency can be broken or subdivided into two different temporal scales. The larger temporal view addresses scale of inference and is determined by the sampling objectives and the spatial level of disturbance or interest. The smaller temporal scale addresses how often samples must be taken to adequately characterize the factor being measured. This depends on the factor and stage of analysis.

## Spatial Scale and Sampling Frequency

Natural landscape disturbances of a given frequency often are associated with a particular spatial scale (O'Neill and others 1986; Urban and others 1987). In general, the longer the recurrence interval of a disturbance, the larger the spatial scale and the higher the organizational level of the system that must be considered (O'Neill and others 1986). For example, small forest fires occur frequently but over small areas, and fires that occur over larger areas have much longer recurrence intervals (fig. 3). The



**Figure 3**—Relationship between time and spatial scales of natural disturbances in reference to stream ecosystems (Minshall 1994).

relationship between natural spatial and temporal scales of disturbance can help in determining sampling frequency. If the objective is to obtain background or reference data, then the scale of inference (spatial scale) can be used to establish sampling frequency. For example, if the scale of inference is the ecoregion and sites are stratified by stream order, then one may want to sample annually at the first-order sites, every other year at third-order sites, and every 5 years at sites greater than fifth order. Small-order sites drain a smaller area than large-order sites. Therefore, stream conditions likely will vary on a shorter temporal scale and should be sampled more frequently to document natural variability.

The relationship between spatial and temporal scales also can be used for evaluating impacts. For example, atmospheric deposition of toxins or nutrients likely will operate at the spatial scale of a watershed or ecoregion. Impacts at this spatial scale (depending on intensity) will influence stream systems at a temporal scale from 10 to 100 years. In this case, monitoring every few years would be more appropriate than a monthly monitoring frequency. However, in the case of intense recreational use of streamside locations, an annual monitoring regime would be warranted with monthly sampling during the summer months to evaluate the influence of altered riparian cover on factors such as water temperature, algal abundance, and macroinvertebrate community composition.

Selection of the appropriate temporal scale of operation will facilitate the selection of the optimal sampling frequency to identify deviations in stream structure and function. However, long-term monitoring will be required to determine if deviations are outside the normal variability seen in stream ecosystems. That is, when monitoring to determine the potential effects of concentrated recreational use, differences observed between impact and control sites may confirm suspected problems. However, annual sampling for multiple years or comparison to long-term sampling locations may be required to determine if differences are outside the range of natural variability.

## **Sampling Frequency and Investigated Parameters**

How often must samples be taken to adequately describe the investigated parameter? As shown in table 4, this depends on the parameter and the stage of analysis. Some parameters are adequately described through annual sampling. For example, both large woody debris and substratum size distribution largely are influenced by bankfull flows. For streams in the western United States, bankfull flows generally occur during annual snowmelt. Therefore, more frequent measurements of these parameters is not warranted. Most of the parameters measured vary throughout the year and sampling frequency increases with the stage of analysis to better characterize these changes. Stage 1 and stage 2 sampling can be completed in a single day. Stage 3 requires several visits a year. Stage 4 was designed for extensive analysis and will require frequent sampling. At stage 1, midsummer daily temperature range is determined. This gives some information toward the physical characteristics of the stream. At stage 2,

this information is increased to obtain monthly energy budgets, furthering an understanding of this parameter. At stage 3, annual temperature data are obtained thus completing the analysis of the variable on an annual basis. Therefore, for most variables, selection of the stage of analysis will determine sampling frequency.

## Evaluating Differences ---

As stated previously, the objective of the monitoring program often is to determine whether impacted sites are different from reference sites. How does one assess whether conditions are different at an impacted site in comparison to a reference site? This will depend on the impact under investigation and often will require statistical comparisons. When monitoring the biotic and physical characteristics of stream ecosystems, the entire group of elements, or the total population, rarely are collected. Sampling is a way to obtain a portion of the total population from which inferences about the total population can be made. The characteristics of the total populations are called parameters. An estimate of the population parameter is called a statistic and is obtained from the sample. That is, the arithmetic mean obtained from the samples is a statistic and is used to estimate the population mean. The more samples obtained, the closer the sample statistics are to the population parameters.

If the total population were sampled, differences could be determined by comparing parameters. However, because samples of the population are being compared, statistical analyses are used to determine the probability that the samples from the reference and impacted sites are from the same population. This question is stated formally as a null hypothesis: there is no difference between impacted and reference sites. There are two possible errors associated with answering this question. First, one could conclude that the samples are from different populations when in fact they are not. This is a type I error. Second, one could conclude that the samples are from the same population when they are not. This is a type II error. Since increasing the number of samples causes sample statistics to approach population parameters, increasing sample size can reduce the probability of committing type II errors.

Increasing the number of samples increases sampling and processing time and associated costs. Therefore, in selecting the number of samples taken, one attempts to increase confidence in statistical analysis while reducing time and costs. We recommend that at least 5 samples be taken when statistical analysis are to be performed. There is a proportionally larger increase in statistical confidence (statistical confidence per sample size) when increasing the sample size from 3 to 5 than can be obtained by increasing the sample size from 5 to 60 (Platts and others 1983, p. 37). The exact number of samples required to obtain a certain level of confidence in the statistical analysis can be calculated based on the magnitude of difference in populations to be determined and the variability among samples (refer to statistical texts).

Performed statistical analyses can be either parametric or nonparametric. Parametric tests require that certain assumptions be met. These assumptions are that samples are selected randomly, that samples come from a normal population, and that variances are equal. There are a number of different ways to transform the data if the assumptions of a normal distribution and equality of variance are not met (Zar 1974). If these assumptions cannot be met, nonparametric alternatives should be considered.

When comparing reference and impacted sites there are only two populations: factors at reference sites and those at impacted sites. Therefore, statistical tests generally are t-tests or some other nonparametric alternative for continuous data, and chi-square tests for discrete data. An exception is testing for nutrient limitation when the investigator instigates four different treatments (dependent variables for each factor and stage are presented in their respective chapters). When only one reference and one impact site are compared, some of the factors outlined in this document can only be used comparatively. Data variation when only two sites are sampled from within each reach and sample size is the number of replicate samples obtained.

When multiple reference and treatment sites are compared there are still only two populations: impacted and reference. However, variance in this case is from a number of different replicate streams. Because the variance is from a number of different streams, it is important to make sure that both reference and impact sites are of similar classification. Many of the factors measured vary considerably among differently classified stream reaches. For example, substratum particle size will be larger in small upland confined streams than in larger floodplain streams. This inherent variability will mask impact effects, increasing the chance of committing type II errors. If impacts occur at discrete locations, then a paired t-test can be used as the statistical design. For example, multiple sites may be potentially impacted by trail crossings. Impacted sites are selected below the crossing and reference sites above. These two sites are paired and the sampling statistic is the difference in factors between these two sites at multiple locations. This reduces the among stream variability and reduces the probability of committing a type II error.

Analogous to multiple reference and impacted sites is the situation where multiple years of data are available at both locations. In this case data variance is from the same stream over time. If each site were sampled over the same time interval, then each year could be compared individually. This may be beneficial when the impact is of short duration or management has altered the conditions. For example, if significant differences were determined between sites above and below a particular stream crossing, a bridge could be constructed. If sampling continued for a number of years after constructing the bridge, one may want to compare each year of data independently.

Multivariate analyses also are applicable in some situations. Impacted or treatment sites may vary in intensity. Treatment intensity may vary directly or over time. Using the previous example, ANOVA (or a

nonparametric alternative) could be used to determine the effectiveness of bridge construction with each year representing a separate factor. Likewise, correlation between stream condition and years since bridge construction could be used to evaluate management actions. In this case, treatment intensity changes with time. If one were evaluating the effect of stream crossings on stream ecosystems, multiple reference and treatment sites may be selected. However, some stream crossings may be used more often than others. Treatment sites, could be subdivided into low and high impact sites and significant differences determined with multivariate statistics.

There are many different statistical designs depending on the monitoring objectives and impact under consideration. Therefore each situation must be evaluated independently. Once the sampling objectives are determined, it is beneficial to consult with a biometrician to determine the appropriate sampling and statistical design.

# Temperature

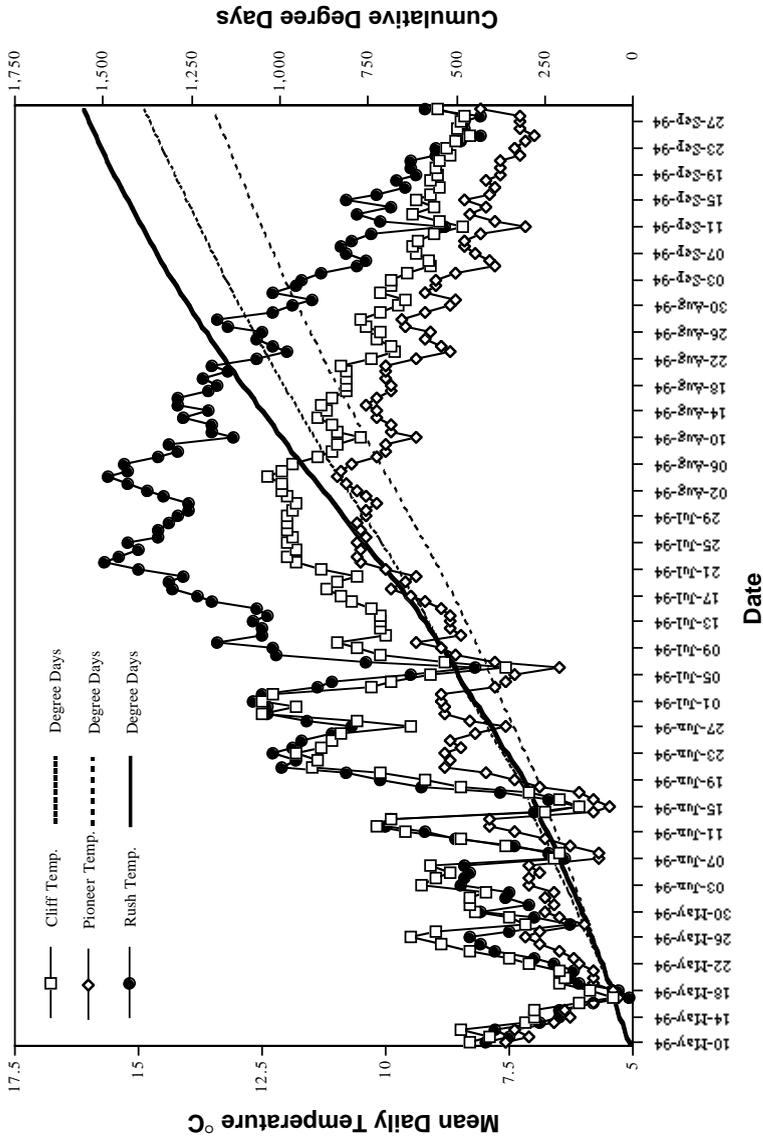
Stream water temperature is an important environmental factor because it affects many biotic processes. Stream temperature results from a combination of factors: source of water (snowmelt, groundwater, rain), air temperature, solar energy input, and surface to volume ratio. In turn, water temperature influences decomposition processes, primary production, invertebrate larval development, fish embryo development, and salmonid survival.

Snow- and rain-derived stream water is generally colder or warmer than groundwater sources and exhibits greater diel ranges. These relationships are demonstrated in seasonal graphs of stream water temperatures obtained at three different Idaho wilderness streams in 1994 using a continuous recording device. The graph of mean daily values in Cliff Creek (fig. 4), shows a decrease in temperature consistent with a loss of surface-fed discharge. At this time, diel temperature range dropped from 6° to 2-3 °C per day. The effect of solar input is demonstrated by the increase in mean temperatures in all three streams through the season, and the difference in mean temperature among the three streams during midsummer. The temperature variation among the three streams represents differences in solar energy input caused by drainage aspect and light attenuation by the riparian canopy.

## Methods: Stage 1, Stage 2, Stage 3 \_\_\_\_\_

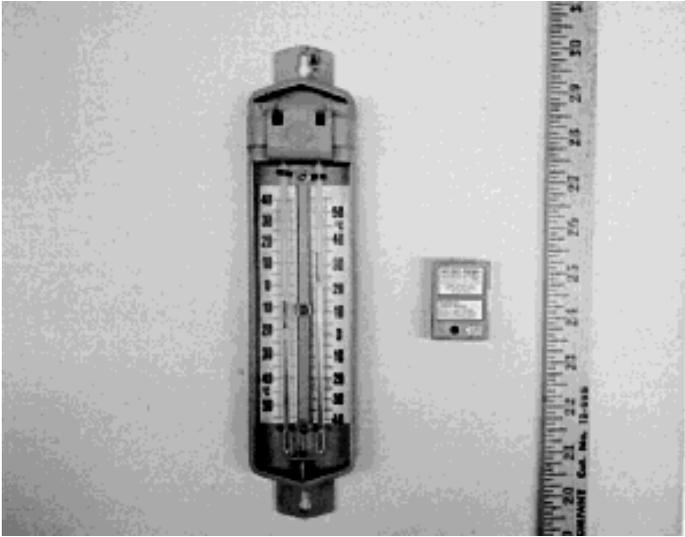
Stream water temperature is measured at one representative location. Water temperature should not be measured in backwater areas or sloughs unless these habitats comprise a significant portion of the total habitats; water mixing in these areas is reduced and temperatures can exceed those in flowing water. Daily maximum and minimum temperature during the warmest month of the year is obtained at stage 1. Sampling frequency increases to obtain 30-day thermograph and annual thermograph records at stages 2 and 3, respectively. The following tabulation outlines this process:

	<u>Dependent variables</u>	<u>Analyses</u>
<b>Stage 1</b>	Maximum daily temperature, minimum daily temperature, daily temperature range	Comparative or statistical if multiple years or multiple sites are sampled
<b>Stage 2</b>	Maximum seasonal temperature, minimum seasonal temperature, seasonal temperature range	Comparative or statistical if multiple years or multiple sites are sampled
<b>Stage 3</b>	Annual (or seasonal) cumulative degree days	Comparative or statistical if multiple years or multiple sites are sampled



10-May-94

Figure 4—Mean daily temperature and cumulative degree days for three streams in the Frank Church Wilderness Area.



**Photograph 1**—Maximum/minimum thermometer and HOBO temperature data logger. HOBO loggers available from Onset Instruments Corporation, Pocasset, MA.

Minimum and maximum stream temperatures demonstrate the variability of stream water with solar input and air temperature. Maximum temperatures indicate the suitability of the system for cold water fish. Maximum/minimum-recording thermometers (photograph 1), are relatively inexpensive, and can be placed within the stream during summer baseflow and retrieved at a later date. The thermometer should be protected from physical damage by PVC casing. The thermometer casing should be firmly attached to a stationary object, such as a large root, with plastic-coated steel cable to keep it from being swept away during high flow. Placement of the thermometer should be in an inconspicuous location buried in the streambed and should ensure coverage of the thermometer by water at baseflow. Before final placement within the stream, the thermometer should be equilibrated with the stream water temperature and indicators shaken down to rest on top of the mercury column.

Temperature-data loggers are capable of recording daily, seasonal, and annual temperature information. Though more expensive than maximum/minimum thermometers, the continuous data obtained often warrants their use. Temperature loggers, such as those manufactured by the Onset Corporation (HOBO Temp and Stowaway models) are small (3 x 4 cm) and light (2.06 g); and therefore particularly suited for wilderness use (photograph 1). These loggers are capable of recording temperatures every 4.8 hours for 360 days. Waterproof cases are needed to prevent water and physical damage. Placement within the stream is the same as described for maximum/minimum recorders. Alternatively, temperature data loggers may be fastened to a stationary object, such as a metal rod, using stainless steel hose clamps. Figure 4 displays data obtained from HOBO temperature loggers through the summer of 1994.

# Discharge

Discharge, at summer base flow, is a measure of minimum stream size and an indicator of potential habitat for fish and aquatic invertebrates. Discharge (Q) or flow is the product of mean water velocity (v) and cross sectional area (width (w) x depth (d)) ( $Q = wd\bar{v}$ ). Water velocity varies with slope, stream depth, hydraulic head, bed roughness, and viscosity. Water velocity is important biologically by transporting food to filter feeders, and by influencing the ability of organisms to obtain nutrients, meet respiratory and photosynthetic requirements, avoid competitors and predators, and leave unfavorable locations. Some of the methods described in this chapter differ from standard methods used by stream physical scientists. The primary purpose of this book is to understand biological systems in streams, and the methods we describe for monitoring stream discharge are sufficiently accurate for this purpose. If more comprehensive hydrogeomorphological methods are desired, the reader should consult the *National Handbook of Recommended Methods for Water Data Acquisition* (U.S. Geological Survey 1977) and *Stream Channel Reference Sites: An Illustrated Guide to Field Techniques* (Harrelson and others 1994) for clear and detailed directions.

## Methods: Stage 2

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A summer baseflow discharge measurement is obtained at this stage. A crude measurement of stream discharge in a wilderness setting may be obtained by determining mean velocity using the average time it takes five water-filled fishing bubbles to float a given distance and determining area as the product of stream width times mean depth. More accurate measurements of discharge require dividing the stream into segments, calculating discharge for each segment, and summing all segments to obtain total discharge.

Total flow, as the sum of individual component flows, can be calculated through the following equation (Platts and others 1983; Rantz and others 1982) (fig. 5):

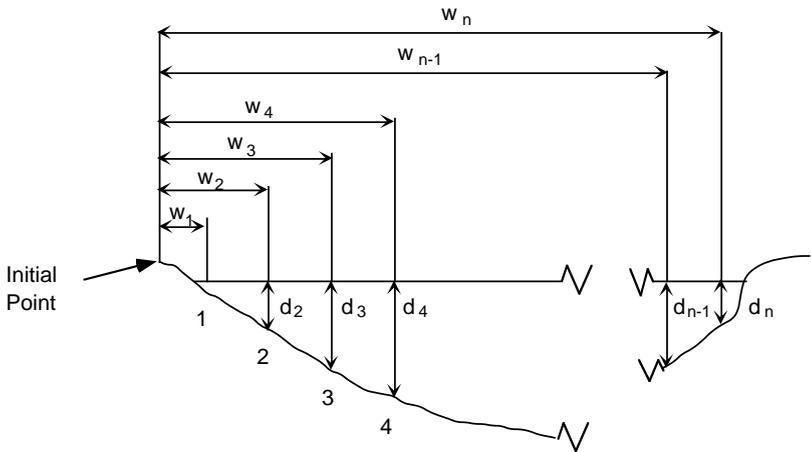
$$Q = \sum_{i=1}^k v_i d_i \left( \frac{w_{(i+1)} - w_{(i-1)}}{2} \right) \quad (1)$$

where

$w_i$  = horizontal distance from the initial point,

$d_i$  = water depth for each section,

$v_i$  = measured velocity for each section.



**Figure 5**—Schematic diagram of measurements taken for stream discharge calculations (Platts and others 1983).

The number of sections measured varies with stream size, but no more than 10 percent of total stream flow should pass through each section. Water velocity is measured at 0.6 times depth (0.6d) from the surface at most locations. However, if water depth is below 0.1 m then velocity is measured at 0.5d, and if depth is greater than 0.76 m, velocity should be measured at 0.2d and 0.8d and averaged.

Single, uniform stream channels should be used for discharge transect locations. Confined channels with underlying bedrock direct most of the flow into the open channel and allow for better discharge measurements. Stream width is measured with a fiberglass tape stretched from bank to bank and secured at or above the high water mark. Depth is measured with a meter stick. Many different water-velocity meters are available including propeller (Ott meters) and electronic- (Marsh-McBirney) based equipment. The USGS recommends Price Type AA meters for use in large streams and Price Pygmy meters in small streams. All velocity meters should be calibrated prior to use. Top-set rods are desirable but cumbersome in backcountry conditions.

## Methods: Stage 3, Stage 4 \_\_\_\_\_

Annual discharge can be monitored by obtaining a relationship between discharge and water depth (stage). Water depth in remote areas generally is evaluated by placement of an enamel-coated steel staff gauge. However, staff gauges must be observed directly each time a measurement is desired, thereby severely restricting the frequency and timing of measurements. Continuous records can be obtained from clock or battery driven stage height recorders or battery operated pressure transducers.

The staff gauge is firmly held within the stream by attachment to a stationary object. For temporary placement, attachment can be made to a

post driven into the streambed or a tree, rock face, or bridge abutment on the stream edge. The gauge is placed out of the main channel to avoid obstruction of floating debris. The lower edge of the gauge must remain under water during low flow and the upper edge must be above the high water mark. Water depth must be read off the gauge and recorded each time a measurement is obtained.

A high flow gauge, for determining the maximum height of flows for a given period, can be made by drilling a series of downward angled holes along a board or pole and inserting plastic test tubes in the holes. The height of the highest tube containing water is determined and measured. Then the tubes are emptied and reset for the next period.

In some cases the use of staff and/or high-flow gauges may be aesthetically inappropriate. Some alternatives may be employed under these circumstances. In wilderness streams, wooden gauges could be constructed from natural materials and placed under bridges at stream crossings. Another way of obtaining consistent water depth may be to drive a large spike, or scribe a mark into the base of bridges at stream crossings or permanent trees on the stream edge. Location of the spike or mark must be carefully documented. A measuring tape could then be packed in and gauge height monitored from this fixed location. Other methods include marks scribed onto rock faces or large boulders. Additionally, one could use a pair of bearing trees, one on each side of the stream, or other off-stream markers, and a tightly stretched line. One would then measure the distance from the line to the water surface.

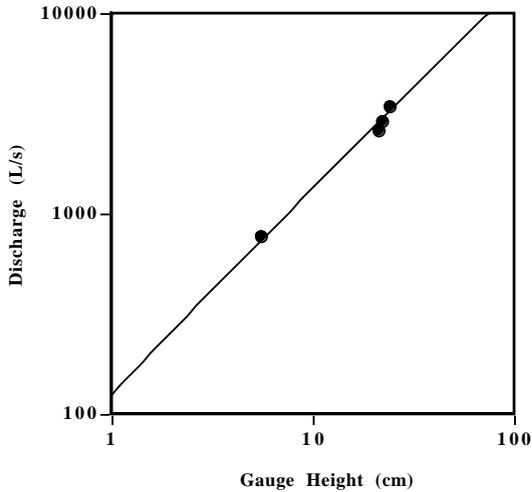
Stage height also can be determined from changes in pressure. Pressure transducers are available from a number of vendors including the Water Log from H,OFX and the Accustage Level Recorder from Yellow Springs Instrument (see appendix B). These transducers can be programmed to obtain readings at desired intervals and the data transmitted by telemetry from remote locations.

The gauge height/discharge relationship or rating curve is established through multiple measurements of both variables (minimum 3). Discharge (see above) is measured along with staff height, and both are plotted on a log-to-log scale (fig. 6). A best fit, or regression line is then drawn through the data points. Discharge can be determined directly from the graph or calculated with the regression equation (example 1).

Estimates of annual peak flows can be obtained using the slope-area method and Manning's equation. Manning's equation is:

$$Q = \frac{1}{n} AR^{2/3} S^{1/2} \quad (2)$$

where  $Q$  = discharge ( $m^3/s$ ),  $n$  = Manning's  $n$ ,  $A$  = cross-sectional area ( $m^2$ ),  $R$  = hydraulic radius (m),  $S$  = slope. Manning's  $n$  is an indication of streambed roughness. As bed roughness increases, turbulence and friction cause a decrease in water velocity. Therefore, as Manning's  $n$  increases, discharge decreases. Manning's  $n$  can be calculated from previous discharge measurements by solving the equation above for  $n$ . This value will remain valid if the streambed composition remains similar with increasing flows. In many cases high flows inundate the riparian vegetation, greatly decreasing water velocity. In this case, a different  $n$  value is determined for this portion of the channel and total discharge is obtained from the sum of



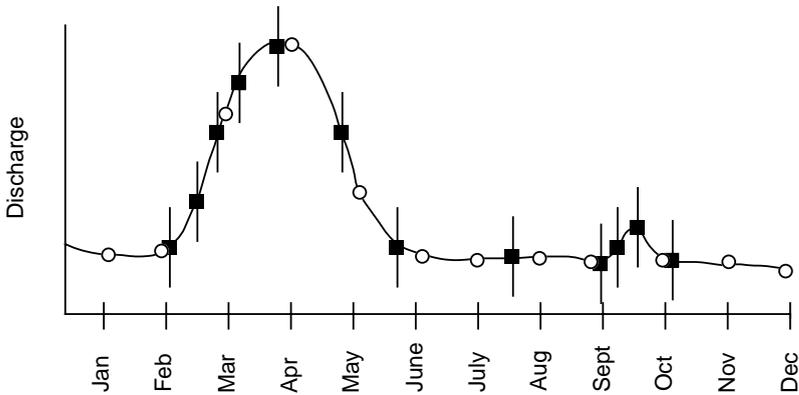
**Figure 6**—Plot of discharge as a function of gauge height on a log-log scale.  $R^2 = 0.990$ . Discharge (L/s) =  $10^{(2.166+0.966 \cdot \text{Log}_{10}(\text{Gauge height cm}))}$ . Data from Rush Creek (1994) in the Frank Church Wilderness Area, Idaho. Many more data points than those presented here should be obtained before applying this procedure.

each separate estimate (Gordon and others 1992). For peak flows, cross-sectional area is measured from the seasonal high flow line. This line usually is marked by the deposition of organic matter (twigs and leaves) along the stream margin. Hydraulic radius and stream slope are described further in the chapter titled “Stream and Substratum Morphology.”

**Example 1**—Regression relationship between the log of discharge in L/s, and the log of gauge height in cm for a straight line. From:  $y = b + m(x)$ ; Log discharge =  $2.166 + 0.966(\text{log gauge height})$ . Discharge values can easily be converted to other units: (L/s) =  $0.001(\text{m}^3/\text{s})$  and 0.0353 (cfs).

$y = \text{Log}$ (flow L/s)	$x = \text{Log}$ (gauge cm)	$y = y_i -$ mean(y)	$x = x_i -$ mean(x)	$x^2$	$xy$
3.454	1.342	0.133	0.146	0.021	0.019
3.409	1.322	0.088	0.126	0.016	0.011
3.537	1.380	0.216	0.184	0.034	0.040
2.884	0.740	-0.437	-0.456	0.208	0.199
Mean	Mean			Sum	Sum
3.321	1.196			0.279	0.270

Slope (m) =  $\Sigma xy / \Sigma x^2 = 0.270 / 0.279 = 0.966$   
Y intercept = Mean y - m(Mean x) = 2.166



**Figure 7**—Increased sampling during the changing hydrograph or event sampling (filled squares) is demonstrated in relationship to monthly or fixed sampling (open circles). An identical number of samples is shown in both cases but event sampling provides more information during times when dissolved and suspended matter are likely to vary markedly.

Estimates of long-term discharge data can be obtained through comparisons with local U.S. Geological Survey gauging stations. The regression relationship between measured discharge data and data from the gauging station is determined. Historic data from the gauging station then can be used to estimate discharge at the sampling location.

In stage 4 of the monitoring design, sampling frequency will vary with objectives. Important discharge characteristics include, maximum and minimum flows, timing of peak discharge, total yield, and the change in hydrograph with storm events. With multiple years of data, these characteristics can be used to determine important physical flow variables that modify the biotic community: flood frequency, flood predictability, and flow variability (Poff and Ward 1989). All of these characteristics can be obtained with continuous stage height monitoring. If stage height is recorded manually, then sampling frequency should increase when there are rapid changes in flow such as during spring runoff and storm events. More intensive sampling during high flows or storm events will provide a better measure of the annual hydrograph (fig. 7). The following tabulation outlines this process:

	<b>Dependent variables</b>	<b>Analyses</b>
<b>Stage 2</b>	Seasonal base flow	Comparative or statistical if multiple years or multiple sites are sampled
<b>Stage 3</b>	Seasonal or 30-day range Seasonal or 30-day yield	Comparative or statistical if multiple years or multiple sites are sampled
<b>Stage 4</b>	Annual yield Annual range Flood frequency Flow duration analysis	Comparative or statistical if multiple years or multiple sites are sampled

# Solar Radiation

Measuring solar radiation is important because of its primary and secondary effects on instream processes. Solar radiation can directly control rates of instream photosynthesis, and has secondary effects on stream temperature and flow regime. The amount of solar radiation reaching a stream surface each day is influenced primarily by stream aspect, latitude, time of year, and degree of shading. The first three factors affect the amount of radiation contacting a given surface area. For example, as latitude increases, the portion of incoming light energy is spread over a greater surface area. By similar means, a southern aspect (in the northern hemisphere) concentrates solar energy on a reduced surface area. Time of year also affects solar angle. Secondarily, the amount of solar radiation reaching a stream surface is influenced by land forms, (for example, canyon or open), clouds, and vegetative cover that intercept part of the available solar radiation.

Measurements of solar radiation are reported in distinct units based on two theories of light properties: wave and photon. Radiant energy is reported in the SI energy unit of Joules. Radiant flux is the energy per unit time (J/s) and is recorded as Watts. Pyranometers measure radiant flux over a unit of area and therefore the results are recorded as  $W/m^2$ . Photosynthetically active radiation (PAR) is the light energy between the wavelength of 400 and 700 nanometers (nm) that is used for photosynthesis. PAR is measured with a quantum meter and is reported in terms of photons. The units used in PAR measurements are moles or Einsteins (E), and flux per unit area is related as  $\mu\text{moles}/m^2/s$  or  $\mu\text{E}/m^2/s$ . PAR values can be converted to energy units by multiplying by 0.2174; however, this still represents only energy within the 400 to 700 nm wavelength and is not comparable with pyranometer measurements.

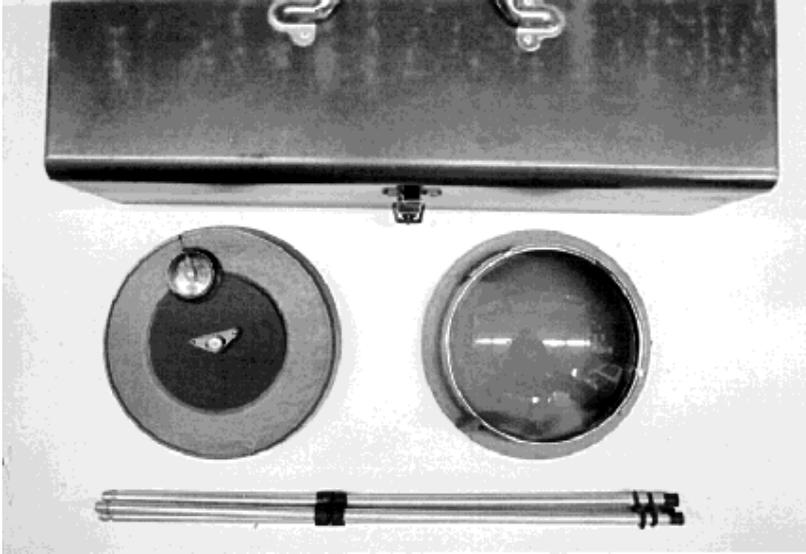
In small streams (2 to 3 m width), solar radiation is measured at mid channel at five randomly selected transects in addition to one representative open site. In larger streams solar radiation sample sites, at each transect, should be stratified with right and left stream margin measurements taken at half the distance from mid channel to bank. Measurements taken continuously or at least hourly from sunrise to sunset are desirable; however, sampling every 4 hours can be adequate. The following tabulation outlines this process:

	<u>Dependent variables</u>	<u>Analyses</u>
<b>Stage 1</b>	Annual solar input	Comparative
<b>Stage 2</b>	Mean daily solar radiation Mean daily percent of total	Statistical
<b>Stage 3</b>	Mean seasonal solar radiation Mean seasonal percent of total Mean extinction coefficient for each season	Statistical
<b>Stage 4</b>	Mean annual solar radiation	Statistical

## Methods: Stage 1

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A rough estimate of actual solar energy reaching the surface of a stream can be determined with a Solar Pathfinder instrument (appendix B). This instrument was designed to estimate the PH2 energy available for photo-voltaic panels (photo 2) but also has found application to ecological topics (Tait and others 1994).



**Photograph 2**—Solar Pathfinder (above) showing, reflecting dome, tripod and carrying case. A lighter carrying case can be constructed for wilderness use. PAR sensor and LI-1000 data recorder below.

The Solar Pathfinder estimates energy input based on location and the portion of total available energy reaching the site (Platts and others 1987), that is, total energy minus that intercepted by trees, mountains, or other obstructions. The Solar Pathfinder is set up in the middle of the test stream, leveled, and oriented to face south. Obstructions that would block solar input are reflected on the domed surface. The reflection is then outlined on a solar chart. Values representing the percent of total daily input for each month are calculated to get independent percent per day values for each month. These monthly values are then multiplied by a published energy value for the closest permanent climatological site to obtain energy units per day for each month. Energy per day for each month is then multiplied by the number of days in the month, to get a total monthly value (energy per day x days per month = energy per month), and total monthly values are summed to obtain an annual value in BTU per ft<sup>2</sup>. This value can be multiplied by 0.01136 to convert units to Megajoules per m<sup>2</sup>. Estimations are fairly accurate when few obstructions are reflected on the domed surface; however, outlining the dense riparian canopy of a small stream is difficult and the measurements are correspondingly rough.

## Methods: Stage 2 ---

Stage 2 measurements of solar radiation give an estimate of the portion of total solar radiation reaching the stream surface. Solar radiation, with a quantum probe and meter, is measured hourly (at least 0900, 1200, 1500, and 1800 hour) throughout the day at selected stream transects and at a location that receives direct sunlight. Solar radiation for both sites is plotted as a function of time; both curves are then integrated to give daily values. Percent PAR is then calculated as the ratio of these two integrated values times 100.

## Methods: Stage 3 ---

Solar radiation varies seasonally due to the changing angle of the sun and the presence of deciduous leaves. In addition, the amount of radiation reaching the stream bottom is attenuated by the water column. Absorption of light by the water column will vary seasonally with turbidity and depth of the water. Therefore, a more comprehensive measurement of available light energy is obtained by seasonal measurements of surface and depth-integrated PAR.

Seasonal measurements of surface PAR are obtained by the methods outlined in stage 2, repeated in the spring, summer, and autumn. Depth-integrated PAR is obtained by taking instantaneous light measurements at multiple depths. This requires a submersible PAR probe. For example, PAR is measured at the surface of the water, and at depths of 10 cm, 20 cm, 30 cm, and so forth until the stream bottom is reached. Estimation at depth, and comparisons between seasons and streams can then be made by

calculation and comparison of extinction coefficients. Extinction coefficients are calculated by solving the equation:

$$I_z = I_0 e^{-kz}$$

where  $I_z$  = light at depth  $z$ ,  $I_0$  = light at the surface,  $k$  = the extinction coefficient, and  $z$  = depth. The extinction coefficient is calculated by plotting the natural log of  $I_z/I_0$  as a function of depth. The negative slope of this line is  $k$ .

## Methods: Stage 4 \_\_\_\_\_

Stage 4 solar radiation measurements expand upon those outlined in stage 3 by obtaining continuous measurements. Solar radiation is continuously monitored by using a PAR probe and data logger. The probe is fixed in a location characteristic of local riparian cover. Solar radiation is measured throughout the year. The actual amount of radiation reaching the stream surface, and at various water depths, can be calculated from monthly estimates of percent of total radiation at the stream surface and extinction coefficients (as explained under stage 3).

# Stream and Substratum Morphology

The stream substratum is the site of most biotic activity, particularly in stream sizes most often found in wilderness areas. The composition and diversity of aquatic insects is often the result of the substratum present (Minshall 1984). The substratum is the site of algal growth, insect growth and development, and fish egg incubation. Substratum is determined by parent geology, but is modified by catchment-level and local processes. That is, the substratum is affected by inputs from terrestrial sources, and the forces of water flow. A stable channel has reached an equilibrium point, balancing inputs with outputs. Monitoring substratum provides a means of determining stream stability and evaluation of catchment level activities. As in the chapter on Discharge, some of the methods described in this chapter differ from standard methods used by stream physical scientists. The primary purpose of this book is to understand biological systems in streams, and the methods we describe for monitoring stream and substratum morphology are sufficiently accurate for this purpose. If more comprehensive hydro-geomorphological methods are desired, the reader should consult the *National Handbook of Recommended Methods for Water Data Acquisition* (U.S. Geological Survey 1977) and *Stream Channel Reference Sites: An Illustrated Guide to Field Techniques* (Harrelson and others 1994) for clear and detailed directions.

Quantification of surface substrata size distribution is accomplished by conducting pebble counts (Wolman 1954). The intermediate (b) axis of 100 randomly selected stones is measured. The substratum size distribution is plotted as cumulative percent finer as a function of particle size class. This distribution is then used for within and among stream comparisons and estimates of bed stability. Streambed stability is determined by relating substratum particle size distribution to the kinetic energy of water at bankfull discharge.

Measurements of channel morphology and substratum size distribution usually are taken once a year. More frequent measurements are required only if high flows occur more frequently. The following tabulation outlines this process:

	<b>Dependent variables</b>	<b>Analyses</b>
<b>Stage 1</b>	Mean stream width Mean stream depth Mean width/depth ratio Mean and CV of particle size	Statistical  Comparative or statistical if multiple sites or years are available
<b>Stage 2</b>	Size distribution Mean percent embeddedness	Chi-square Statistical
<b>Stage 3</b>	Mean and CV of water velocity Mean and CV of shear stress	Statistical Statistical

## Methods: Stage 1

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The mean and coefficient of variation (CV) in streambed particle sizes and five measurements of stream cross-sectional morphology are obtained in stage 1. Beginning at the downstream end of the sampling reach, the intermediate axis of rocks is measured at roughly one meter intervals as the investigator moves upstream, continually moving at an angle from bank to bank (see Bevenger and King 1995). A meter stick (the multipurpose backcountry measuring tool) may be used to measure these rocks. For greater measuring accuracy and consistency, a light-weight aluminum measuring template may be used. The *Hand Held Size Analyzer* (US SAH-97) is available from the Federal Interagency Sedimentation Project at <http://fisp.wes.army.mil>, and the *Gravel Sizing Template* is available from Hydro Scientific Ltd at <http://members.aol.com/HydroSci>. Mean particle size (or the more commonly used 50 percent median particle diameter size class) and the coefficient of variation are used to derive a general impression of the stream particles and should not be used to statistically compare different sampling reaches or streams. Substratum particle size is inherently variable, a condition that reduces the power of statistical comparisons at this stage. The CV is a measure of habitat variability, and is used as a dependent variable in statistical comparisons.

Cross-sectional morphology is measured for at least five systematically selected transects, and may be combined with discharge measurements. A tape is fixed to the right bank above high water mark, stretched level across the stream, and secured to the left bank. The distance from the right, vertical distance to the streambed, and vertical distance to the water surface is measured at a minimum of 10 points covering the stream channel. The frequency of measurements should increase with rapid changes in the channel cross-sectional profile, and measurements should be taken at all points of significant change in channel form. It is important to make sure the tape is level; this can be accomplished by ensuring equal distance from the tape to stream surface at both stream margins. Be certain to record the point of bankfull width. Data analysis consists of calculating the mean width, depth, and width/depth ratio for the sampling reach.

In streams that are outside of wilderness, cross-section locations may be permanently marked with rebar stakes allowing long-term monitoring of streambed morphology. Inside wilderness, however, there are significant ethical concerns about permanently marking these locations, as well as logistical concerns about transporting rebar or wooden stakes. The manager of each wilderness needs to be consulted for allowable practices. Where long-term monitoring is deemed necessary, some managers may allow rebar stakes to be driven all the way into the ground so they can be relocated with a metal detector. Use of a survey-grade Global Positioning System would allow relocating cross-sections without the use of stakes.

## Methods: Stage 2

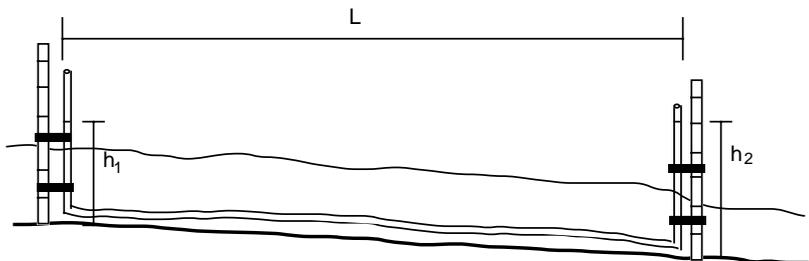
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Stage 2 analysis includes two additional field measurements: embeddedness and slope, and estimation of streambed stability. Embeddedness is

the filling-in of the interstitial spaces surrounding rocks on the streambed by silt or fine sand. This is different than armoring, which is the protection or covering of fine material by a layer of larger cobbles and boulders. Embeddedness can reduce streambed surface area and living space, the flow of oxygen and nutrients to developing fish eggs and aquatic invertebrates, storage of organic carbon, and entrance to and movement within the streambed by invertebrates.

Embeddedness is a qualitative estimate of the percent of the substratum particles covered by fine materials. For each stone-intermediate axis measurement, the percent of the particle embedded, in 25 percent increments, is recorded. Values are reported with simple statistics: mean, standard deviation, and coefficient of variation.

Stream slope can be calculated by hydrostatic leveling, hand level and rod, or with a clinometer. For hydrostatic leveling, two meter sticks and a 20-m length of 10-mm ( $\frac{3}{8}$  inch) inside diameter tubing are required (fig. 8). The hose is filled with water and extended along the streambed. When the water within the tubing stabilizes, the change in height is determined by the difference in water column height between the upstream and downstream end. The slope is the height (m) difference divided by length (m). The resulting ratio is unitless but often is multiplied by 100 and reported as a percentage. To use a hand level and rod, the level is supported on a stick cut in the field to a known length. A second person supports a rod 25- to 50-m downstream (folding or pocket rods are suitable for use in remote locations, available through forestry suppliers, appendix B). By sighting through the hand level, the height is determined from the rod. The difference between the level support length and the sighted height on the rod over the distance between level and rod is the slope. To determine slope with a clinometer, one person supports a rod marking eye-level while a second person walks upstream. Looking back downstream through the clinometer the cross-hairs are lined up with eye-level on the rod. Slope in degrees, or as a percentage, is read off of the meter. Slope, as a ratio, is equal to the tangent of slope in degrees. Clinometers are convenient for wilderness use but estimating slope by this method is difficult when visibility is limited and hydrostatic leveling provides a more accurate measurement. With all these methods every attempt should be made to measure slope between consistent stream features, such as from the top of one riffle to the top of the next riffle, or from the bottom of one pool to the bottom of the next pool. If this is not possible, take several measurements, for example with the 20 m hydrostatic level, and average them.



**Figure 8**—Diagram showing the calculation of slope by hydrostatic leveling.

## Methods: Stage 3

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Stage 3 measurements of water velocity and shear stress further characterize the physical habitat. The hydraulic habitat variability as indicated by the CV of water velocity and shear stress could influence invertebrate species diversity up to some maximum value above which species diversity declines (Monaghan and Minshall 1996).

Water velocity and stream water depth are measured at 20 random locations within the sampling reach. Water velocity is measured at 0.6 to 0.8 times stream depth to characterize the streambed invertebrate habitat. Shear stress ( $\tau$ ) is determined using the equation:

$$\tau = gSd\rho \quad (3)$$

(Statzner and others 1988), where  $g$  is acceleration due to gravity ( $980 \text{ cm/s}^2$ ),  $S$  is the slope of the water surface,  $d$  is water depth (cm), and  $\rho$  is the density of water ( $1 \text{ g/cm}^3$ ).

# Water Quality

Analysis of stream water chemistry provides an understanding of the environment to which biota are subject and the availability of macronutrients required for growth and reproduction. Stage 1 analysis provides an initial evaluation of the general chemical environment. Turbidity is a measure of light absorbance by water. Turbidity is altered by the amount of particulate matter in the water column and is an important measurement when waters are subject to potential sediment inputs. Stream water pH is a measure of hydrogen ion activity which affects many cellular and biogeochemical processes. The pH of water is affected by the dissolution of carbonate rocks and biological processes. In addition, the pH of natural waters can be affected by dissolved nitrogen, phosphorus, and sulfur compounds in precipitation. Alkalinity is the ability of stream water to accept hydrogen ions, thereby buffering changes in pH. In natural waters, alkalinity is due to carbonate and bicarbonate salts. Hardness is a measure of calcium and magnesium ions that usually are the principal cations in solution and are required for biotic growth. Specific conductance is the reciprocal of electrical resistance; in other words, the ability of water to conduct an electrical current. The conductance of water is affected by temperature, therefore, specific conductance is standardized by temperature, usually 20 or 25 °C. In addition to temperature, specific conductance is controlled by the concentration of dissolved salts in water. Specific conductance can therefore be used to estimate the total dissolved solids in water. Together, total dissolved solids, hardness, and alkalinity can provide valuable insights concerning the main components dissolved in the water (Methods: Stage 1).

Stage 2 analysis of water chemistry is a direct measurement of the major constituents dissolved in water which have biological significance: calcium, magnesium, sulfate, nitrate-nitrogen, and dissolved orthophosphorus. Stage 1 analysis gives an indirect estimate of calcium- and magnesium- and a direct measure of carbonate-concentrations. Stage 2 analysis partitions hardness into its two main components, calcium and magnesium. Sulfate is the most common anion dissolved in water after carbonate. Sulfate can contribute to total dissolved solids and high levels may have adverse effects on stream fauna (for example, Winget and Magnum 1979). Nitrogen and phosphorus are the main nutrients regulating production and decomposition in streams. At stage 2, chemical analysis is conducted either in the field or in the laboratory, using prepackaged reagents (Hach Chemical Co., see appendix B) and spectrophotometry. Field analysis requires a battery-powered spectrophotometer produced by the Hach Chemical Company

(appendix B). The DR 700 model weighs 487 g (add 1.7 kg for rechargeable battery) and is 10 x 22 x 7 cm in size. These methods provide only a rough estimate for chemicals occurring in low concentrations, particularly nitrogen and phosphorus. Therefore, more accurate (stage 3) evaluation of these important elements is obtained by field preservation of water samples and laboratory analysis. The American Public Health Association (APHA) describes the water sampling, preservation, and analysis methods outlined in stage 3 (see APHA [1995] for invertebrate and algal preservation and analysis).

Water samples are taken at one representative location within the sampling reach where the stream water is well mixed. Always take water samples upstream from where you are standing and avoid touching the inside of the sample container or lid. All water samples should be collected in clean polyethylene bottles. Bottles should be filled and rinsed three times before the sample is retained. Water samples should be depth integrated. Depth integrated samples can be taken by inverting the sample bottle, trapping air within, and then submerging to the bottom of the stream. The bottle is then allowed to fill as it is slowly moved to the surface. The following tabulation outlines this process:

	<b>Dependent variables</b>	<b>Analyses</b>
<b>Stage 1</b>	Water chemistry values (WCV)	Comparative or statistical if multiple sites or years are available
<b>Stage 2</b>	WCV for each season independently Mean seasonal WCV Maximum season WCV Season range of WCV	Comparative or statistical if multiple sites or years are available Statistical Comparative or statistical if multiple sites or years are available
<b>Stage 3</b>	Mean annual WCV nutrient flux	Statistical

## Methods: Stage 1

Stage 1 water samples are taken once a year usually at baseflow. The following methods are based on materials in Lind (1985) and APHA (1995).

### Specific Conductance/Total Dissolved Solids

Specific conductance is determined using a conductivity probe and meter. Specific conductance meters usually standardize for a particular temperature, generally 20 or 25 °C, or manual compensation can be made (see instruction manual for particular instrument). If temperature compensation is unavailable, temperature adjustments can be estimated as conductivity increases from 2 to 3 percent for each degree Celsius. Specific conductivity (sc) is based on the distance between electrodes, which is usually 1 centimeter, however, check the probe being used to determine the cell constant. The cell constant is multiplied by specific conductance reading to give the final value. Specific conductance is reported in mhos (reciprocal of ohms) or Sems (1 Sem = 1 mho) per centimeter. Total dissolved solids (TDS) can be estimated by multiplying specific conductance by

0.65 (Rainwater and Thatcher 1960). For more accurate work, the conversion factor ( $k$ ) for TDS should be determined directly for each stream, region, or geologic type by measuring specific conductance, evaporating the sample until dry, and determining the weight of the precipitate. The conversion factor is proportional to the ratio of TDS (mg/L) to specific conductance ( $\mu\text{S}/\text{cm}$ ):

$$k \propto \frac{\text{TDS}}{\text{SC}}. \quad (4)$$

## pH

The pH of water is measured using a hydrogen ion probe and meter. Most modern pH probes have internal temperature compensation for variable temperatures (see specific manual). The pH meter should be calibrated prior to use. Calibration buffer solutions should bracket the expected pH. In addition, buffer temperatures should be within 10 °C of the stream water. Laboratory quality pH measurements can be obtained using some portable field meters that are suitable for wilderness use and available from a number of suppliers (appendix B).

## Turbidity

Turbidity is measured with a nephelometer. This instrument measures the light reflected at a 90° angle. Nephelometers are available from Hach Chemical Company. Methods require following the manufacturer's instructions. Turbidity is recorded in nephelometric turbidity units (NTU).

## Alkalinity

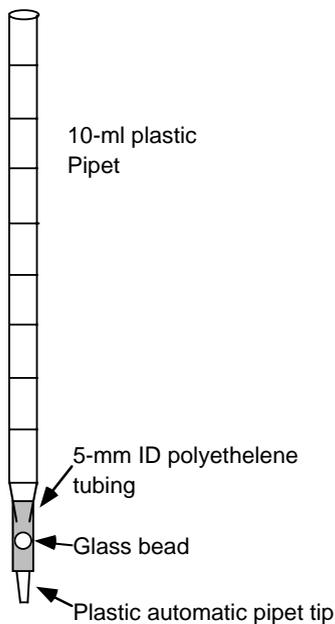
The alkalinity of water is subject to change with time, so measurements should be done in the field when possible. However, high alkalinity (>50 mg/L  $\text{CaCO}_3$ ) samples may be stable for a week or so if not exposed to harsh conditions.

### Equipment and Materials—

1. pH meter with buffer solutions (same as for pH described above)
2. 60-ml plastic syringe or 100-ml graduated plastic cylinder
3. 0.02-N sulfuric acid solution. Dilute 200 ml of 0.1 N sulfuric acid into 1 liter of carbon dioxide free water (need about 5 ml of solution for each water sample at alkalinities of 50 mg/L  $\text{CaCO}_3$ )
4. Calibrated dispenser (fig. 9)
5. 250-ml Erlenmeyer flask

### Reagents—

1. Use 60-ml syringe or plastic graduated cylinder to dispense 100 ml of stream water into the flask.
2. Stir gently with calibrated pH probe.
3. Fill dispenser with 0.02-N sulfuric acid solution.
4. Titrate water to pH of 8.3, and record ml of titrant. Titrate water to pH of 4.5 and record ml of titrant.



**Figure 9**—Portable calibrated solution dispenser. The glass bead is forced inside the polyethylene tubing which is fitted over a 10-ml disposable-plastic pipette. The tip of a plastic automatic pipette tip is fit into the other end. The pipette can then be filled with solution. Drops of the solution then can be released by pinching the tubing at the glass ball.

5. Calculate carbonate and total alkalinity by the following formulas:

Carbonate alkalinity as mg  $\text{CaCO}_3$  per liter =  $A \times N \times 50,000/\text{ml sample}$   
 and total alkalinity as mg  $\text{CaCO}_3$  per liter =  $B \times N \times 50,000/\text{ml sample}$ ,  
 where  $A$  = ml titration to pH 8.3,  $B$  = ml total titration from start to pH 4.5,  
 and  $N$  = Normality of acid.

Under conditions of low pH, the Gran titration method should be used for alkalinity analysis. This method is based on the rate of pH change with the addition of acid and provides more precise measurements.

**Procedure—**

1. Using 60-ml syringe or graduated cylinder, fill titration flask with 100 ml of sample. More precise estimates of sample volume can be obtained by weighing the titration vessel and the vessel with sample. Sample volume is computed from sample mass and the density of water.

2. While maintaining continuous stirring, the initial pH is measured with a rinsed and calibrated pH probe and meter. If the pH drifts, read after 60 seconds.

3. Using a Gilmont Syringe burette, or other calibrated dispenser, add 0.1 N (or 0.02 N) HCl to sample until pH is less than 4.3. Allow 30-60 seconds for the pH to stabilize before recording. Record pH and volume of added titrant.

4. Make two further acid additions between pH of 4.3 and 3.7. Record volume of titrant added and pH after stabilization for the second and third additions.

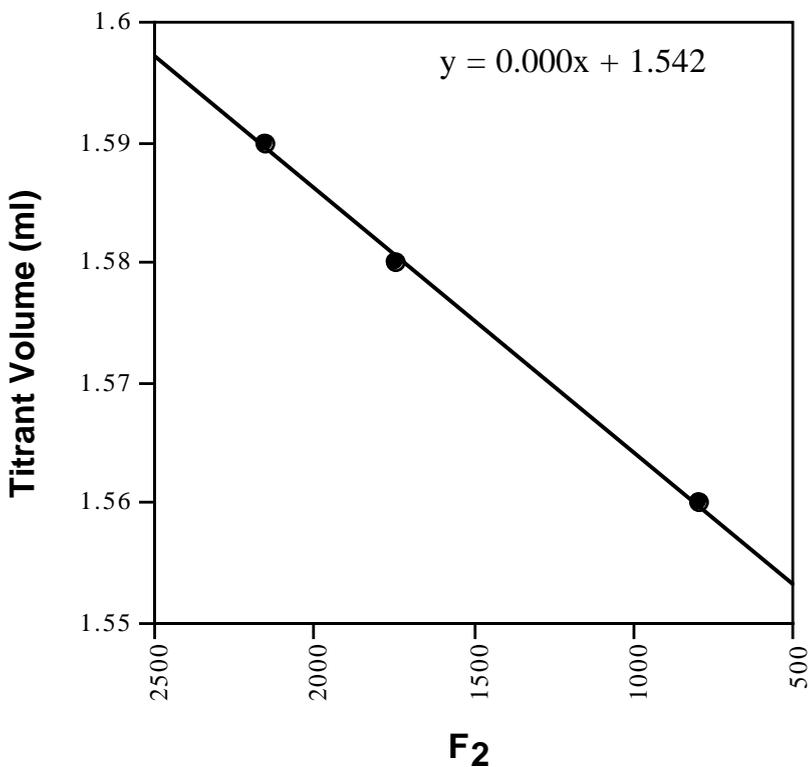
5. Total alkalinity milliequivalents (*meq/L*) is calculated from the equation:

$$\text{CaCO}_3(\text{meq/L}) = V_2 N \frac{1000}{V_s}, \quad (5)$$

where  $V_s$  is the sample volume (ml),  $N$  is the acid normality, and  $V_2$  is the Y-axis intercept of the regression relationship between  $V_t$  (titrant volume) as a function of  $F_2$  (fig. 10).  $F_2$  is calculated for each titration as follows:

$$F_2 = 10^{(5-pH)} (V_s + V_t) \quad (6)$$

$$\text{CaCO}_3 (\text{meq/L}) = 50.04 \text{ CaCO}_3 (\text{mg/L})$$



**Figure 10**—Calculation of  $V_2$  from the regression relationship between  $F_2$  and  $V_t$ . From the regression equation the  $V_2$  (y-axis) intercept is 1.542. Sample volume was 119.0 ml, and acid normality was 0.10; therefore, total alkalinity as  $\text{CaCO}_3 = 1.30$  (meq/L) or 64.8 (mg/L).

## Hardness

Unpreserved water samples can be returned to the laboratory or analyzed in the field.

### Equipment and Materials—

1. 60-ml plastic syringe or 100-ml graduated plastic cylinder
2. Calibrated dispenser
3. 250-ml Erlenmeyer flask
3. Stirring rod
4. White paper (we used reverse side of photocopied methods)
5. Distilled water (25 ml for each sample)

**Reagents**—These may be made up in the laboratory as indicated below or purchased in prepared form from major chemical supply houses (appendix B).

1. Buffer solution. Dissolve 16.9 g ammonium chloride ( $\text{NH}_4\text{Cl}$ ) in 143 ml concentrated ammonium hydroxide ( $\text{NH}_4\text{OH}$ ). Add 1.25 g magnesium salt of ethylenediaminetetraacetic acid (EDTA) and dilute to 250 ml with distilled water.

2. Indicator. Mix 0.8 g Eriochrome Black T dye and 100 g NaCl to prepare a dry powder mix.

3. Standard EDTA titrant (0.01 M). Dissolve 0.3723 g  $\text{Na}_2\text{EDTA}$ -dihydrate in distilled water and dilute to 100 ml. Check by titrating against a standard calcium solution: 1.00 ml = 1.00 mg  $\text{CaCO}_3$  = 0.4008 mg Ca.

4. Standard calcium solution. Weigh 1.000 g anhydrous calcium carbonate powder, primary standard grade, into a 500 ml Erlenmeyer flask. Add slowly one volume HCl diluted with an equal volume of distilled water until all the  $\text{CaCO}_3$  has dissolved. Add 200 ml distilled water and boil for a few minutes to expel  $\text{CO}_2$ . Cool and adjust to pH 5.0 with either  $\text{NH}_4\text{OH}$  or 1 + 1 HCl. Transfer to a 1-liter volumetric flask, washing out the Erlenmeyer flask several times with distilled water and adding to volumetric flask. Then dilute to mark with distilled water.

### Procedure—

1. Dilute 25 ml of sample to about 50 ml with distilled water in titration flask.

2. Add 1 to 2 ml of buffer solution to bring pH to 10.0 or 10.1.

3. Add approximately 0.1 g indicator powder.

4. Titrate with EDTA over a white surface with daylight or white light. Stir continuously until the last red tinge disappears. Add the last drops slowly, allowing about 5 seconds between drops. The entire duration of titration should not exceed 5 minutes and should not require more than 15 ml of titrant. If more titrant than this is used, take a smaller aliquot and repeat titration. An indistinct end point suggests interference and calls for an inhibitor after step 2. Old indicator powder also produces an indistinct end point.

$$\text{Hardness as mg CaCO}_3/\text{L} = A \times B \times 1,000/\text{ml of Sample} \quad (7)$$

where  $A$  = ml titration, and  $B$  = mg  $\text{CaCO}_3$  equivalent to 1.00 ml EDTA titrant.

## Estimation of Major Ions

Estimates of the major cations and anions in water are possible using measurements of total dissolved solids, alkalinity, and hardness. The major cations in water are  $\text{Ca}^+$ ,  $\text{Mg}^{2+}$ , and  $\text{Na}^+$ . The major anions in water are  $\text{HCO}_3^-$ ,  $\text{SO}_4^{2-}$ , and  $\text{Cl}^-$ . For example, if total dissolved solids are 200 mg/L, hardness is 150 mg/L, and alkalinity is 100 mg/L, then calcium and magnesium carbonates constitute 100 mg/L. Therefore 50 mg/L are calcium or magnesium sulfates or chlorides (difference between hardness and alkalinity). The remainder of the total dissolved solids, 50 mg/L (difference between hardness and total dissolved solids) are sodium sulfates or chlorides.

## Methods: Stage 2

---

### Calcium

#### Equipment and Materials—

1. 60-ml plastic syringe or 100-ml graduated plastic cylinder
2. Calibrated dispenser
3. 250-ml Erlenmeyer flask
3. Stirring rod
4. White paper (plastic laminated 3 x 5 card)

#### Reagents—

1. Sodium hydroxide, 1 N. Dissolve 4 g NaOH in distilled water and, when cool, dilute to 100 ml.
2. Murexide indicator. Grind together in a mortar 0.2 g powdered dye and 100 g NaCl. Store in tightly stoppered bottle.
3. Standard EDTA titrant, 0.01 M. Same as in hardness determination. (1.00 ml = 0.4008 mg Ca).

#### Procedure—

1. Take a sample that contains less than 10 mg calcium. Usually a 50 ml water sample is correct but, if total alkalinity is greater than 250 mg/L, it probably will be better to take a smaller aliquot and dilute to 50 ml with distilled water.
2. Add 1 to 2 ml NaOH solution to produce a pH of 13 to 14. Stir.
3. Add about 0.2 g indicator powder. The color change is from pink to purple on titration.
4. With continuous stirring, titrate slowly over a white surface with the EDTA titrant. Since this is a gradual color change, the end point recognition is facilitated by preparing a reference end point by adding NaOH, indicator, and 1 or 2 ml EDTA to 50 ml distilled water.

$$\text{mg Ca/L} = A \times B \times 400.8/\text{ml of sample,}$$

where A = ml titration for sample and B = mg  $\text{CaCO}_3$  equivalent to 1.00 ml EDTA titrant.

**Magnesium**—If both calcium concentration and hardness are known, magnesium concentration can be calculated by difference (Rainwater and

Thatcher 1960). Milliequivalents of hardness per liter are calculated from milligrams of hardness per liter. The milliequivalents of calcium per liter are subtracted from this, and the difference is multiplied by the equivalent weight of magnesium to express magnesium in milligrams per liter.

$$\text{milliequivalent hardness/L} = \text{mg hardness/L} \times 0.01998$$

$$\text{milliequivalent Ca}^{+2}/\text{L} = \text{mg Ca}^{+2}/\text{L} \times 0.0499$$

$$\text{mg Mg}^{+2}/\text{L} = 12.16 \times (\text{meq hardness/L} - \text{milliequivalent Ca}^{+2}/\text{L})$$

Alternatively, Standard Methods (APHA 1995) states: hardness, mg equivalent  $\text{CaCO}_3/\text{L} = 2.497 (\text{Ca, mg/L}) + 4.118 (\text{Mg, mg/L})$ .

**Sulfate**—The following methods are from Hach Chemical Company

#### **Equipment and Materials—**

1. Portable spectrophotometer
2. 2.54-cm test tubes or cuvettes
3. 125-ml Erlenmeyer flask
4. 60-ml syringe

#### **Reagents—**

1. Standard sulfate solution (1.00 ml = 0.10 mg  $\text{SO}_4$ ). Using a microburette, measure 10.41 ml standard 0.02-N  $\text{H}_2\text{SO}_4$  titrant (from alkalinity procedure) into a 100 ml volumetric flask and dilute to mark with distilled water.

2. SulfaVer powder. From Hach Chemical Company

#### **Procedure—**

1. To a 25 ml sample in a 125 ml flask add 1.0 g SulfaVer powder, and swirl evenly for 1 minute. A suspension of barium sulfate forms.

2. Pour entire sample into one of a pair of 2.54-cm test tubes that are matched for spectral qualities, and let stand for 3 minutes.

3. Read absorbance produced by this suspended turbidity at a wavelength of 420 nm on a spectrophotometer. Estimate milligrams of sulfate by comparing with a standard curve prepared by applying the same procedure to a series of known standard concentrations. The highest standard should not exceed 40 mg/L (1 mg/25 ml sample), since this method fails above that concentration.

## **Nitrate Nitrogen**

#### **Equipment and Materials—**

1. Portable spectrophotometer
2. 2.54-cm test tubes or cuvettes
3. 125-ml Erlenmeyer flask
4. 60-ml syringe

#### **Reagents—**

1. Hach NitraVer VI powder pillows (Hach Chemical Company)
2. Hach NitriVer III powder pillows (Hach Chemical Company)

3. Stock nitrate solution (1 ml = 100  $\mu\text{g NO}_3\text{-N}$ ). Dissolve 0.7218 g anhydrous potassium nitrate ( $\text{KNO}_3$ ) and dilute to 1,000 ml with demineralized water.

4. Standard nitrate solution (1.00 ml = 2.5  $\mu\text{g NO}_3\text{-N}$ ). Dilute 25 ml stock solution to 1000 ml with demineralized water. Prepare fresh weekly.

5. Standard curve for nitrate nitrogen concentration in the original water sample.

#### **Procedure—**

1. Add the contents on one NitriVer III powder pillow to a 25 ml sample in an Erlenmeyer flask. Shake for 30 seconds. If a pink color develops within 10 minutes, nitrite nitrogen is present. This may be quantified by starting with step 5 below.

2. Add the contents of one NitraVer VI powder pillow to a 30 ml water sample (or standard) in the glass bottle or flask. Stopper and shake vigorously for at least 3 minutes. Be sure standards and samples are shaken in exactly the same manner.

3. Wait 30 seconds to allow the cadmium metal to settle, then decant 25 ml into a clean flask.

4. Add the contents of one NitriVer III powder pillow and shake for 30 seconds.

5. If nitrate (or nitrite) nitrogen is present, a pink color will develop. Allow the color to develop. After 10 minutes, but before 20 minutes, measure the absorbance using the 2.54-cm test tubes and the spectrophotometer set at 500 nm. Determine the concentration of nitrogen from the standard curve. If nitrite was detected in step 1 but not quantified, report the results as combined nitrate and nitrite nitrogen.

## **Orthophosphorus**

#### **Equipment and Materials—**

1. Portable spectrophotometer
2. 2.54-cm test tubes or cuvettes
3. 125-ml Erlenmeyer flask
4. 60-ml syringe

#### **Reagents—**

1. PhosVer 3 (Hach Chemical Company)

2. Stock phosphorus solution. A stock solution in which 1.00 ml equals 0.05 mg phosphorus is prepared by dissolving 0.2197 g potassium dihydrogen phosphate in distilled water. Dilute this to 1.0 liter. Add 1 ml chloroform and store in the dark under refrigeration. The solution is stable for several months.

3. Standard solution. Dilute 10.0 ml of phosphorus solution to 1.0 liter with glass-distilled water. Should not be stored for more than a few days.

#### **Procedure—**

1. Fill an Erlenmeyer flask with 25 ml of sample water.

2. Add contents of PhosVer 3 powder pillow. Swirl and allow to react for 2 minutes.

3. Pour the solution into cuvette and read absorbance at 890 nm then calculate concentration from standard curve.

## Methods: Stage 3

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At stage 3, sampling frequency is adjusted to obtain an estimate of seasonal to annual changes in water chemistry and an estimate of nutrient flux. Therefore sampling frequency increases and should reflect changes in discharge, with more frequent sampling during the rising hydrograph (fig. 7). Stage 3 water analysis provides more accurate evaluation of element concentrations, particularly when concentrations are low. Stage 3 water analysis requires collecting and preserving samples in the field. Sample preservation per Standard Methods (APHA 1995) is as follows. However, preservation may vary with the laboratory conducting the analysis.

### Nitrogen: Ammonia

A 100-ml water sample is required and should be filtered immediately after collection, using a 0.45  $\mu\text{m}$  pore size filter. Filter holders that attach to leur-lock syringes are available from supply companies and are suitable for wilderness use. Preserve filtered samples with about 0.8 ml concentrated  $\text{H}_2\text{SO}_4/\text{L}$  to a pH between 1.5 and 2 and store at 4°C. The pH of the acid-preserved sample should be between 1.5 and 2.

### Nitrogen: Nitrate

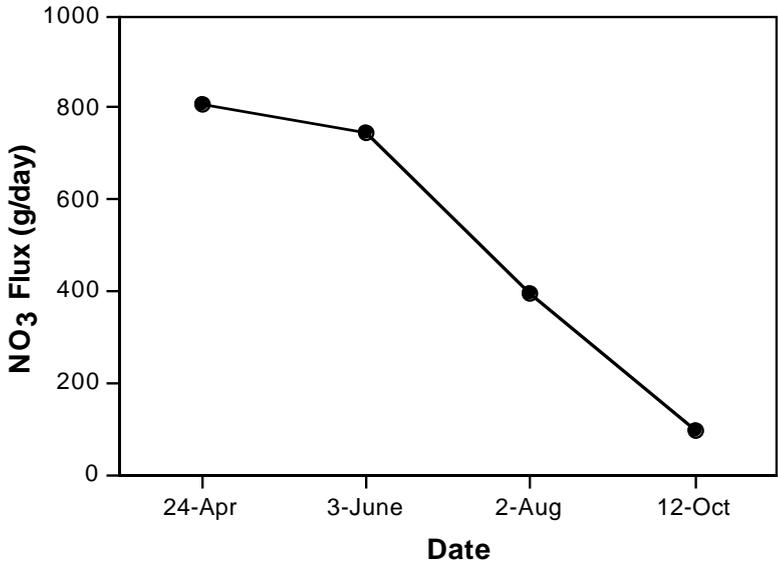
Samples (100 ml) should be filtered as above and frozen or stored at 4°C. If nitrite analysis is not required, samples can be acidified with 2 ml concentrated  $\text{H}_2\text{SO}_4/\text{L}$ .

### Dissolved Orthophosphorus

Filtered samples (100 ml) are stored acidified with 1 ml concentrated  $\text{HCl}/\text{L}$  and frozen. Do not store samples containing low concentrations of phosphorus in plastic bottles unless they are frozen, because phosphates can adsorb onto the walls of the bottles and be lost from solution. Rinse all glassware for storage and analysis in hot dilute (0.1 molar)  $\text{HCl}$ , then rinse several times in distilled water. Never use commercial detergents containing phosphate for cleaning glassware used in phosphate analysis.

### Nutrient Flux

Nutrient flux is a measure of the total quantity of an element passing a given point. Nutrient flux is a measure of nutrient availability (Fisher 1990) and has been used to evaluate changes in catchment level processes. Nutrient flux is the product of element concentration and discharge. Total yield for any given time interval can be determined by graphing nutrient flux over time and integrating the area under the curve (fig. 11). Standardization by basin area allows for comparable measurements.



**Figure 11**—Nitrate flux from April through October 1994 for Pioneer Creek within the Frank Church Wilderness. Total yield, calculated by determining the area under the curve, was 82.9 kg NO<sub>3</sub>-N. Standardized by basin area (17 km<sup>2</sup>) is 4.9 g/m<sup>2</sup>.

# Macroinvertebrates

Macroinvertebrates are important indicators of water quality and the primary food-base for fish in wilderness areas of the western U.S.A. Macroinvertebrates are good indicators of stream quality because of their relative lack of mobility and most have life spans of a few months to a few years (Plafkin and others 1989; Platts and others 1983). The limited mobility allows monitoring of local conditions, in addition to the integration of watershed-level disturbances. Their short life span makes them characteristic of conditions in the recent past (Platts and others 1983).

Multiple metrics are used because it is unlikely that any has sufficient sensitivity to be useful under all circumstances (Karr 1991). For the same reason, the values for each measure should be kept separate, in addition to summing them to produce a single index value. Separating the values gives additional information and avoids defeating the purpose for multiple analyses by preventing an inappropriate or insensitive index component from obscuring the “signal” from a component that is appropriate or sensitive or both (Steedman and Regier 1990). Graphing individual metric values from reference and impact sites and visually evaluating differences (Fore and others 1996) is the recommended method for determining the valuable metrics for a given impact. The following tabulation outlines this process:

	<u>Dependent variables</u>	<u>Analyses</u>
<b>Stage 1</b>	Mean total metric score Mean value for each metric	Statistical
<b>Stage 2</b>	Mean biomass	Statistical
<b>Stage 3</b>	Total production Production for each species and feeding group	Statistical

## Methods

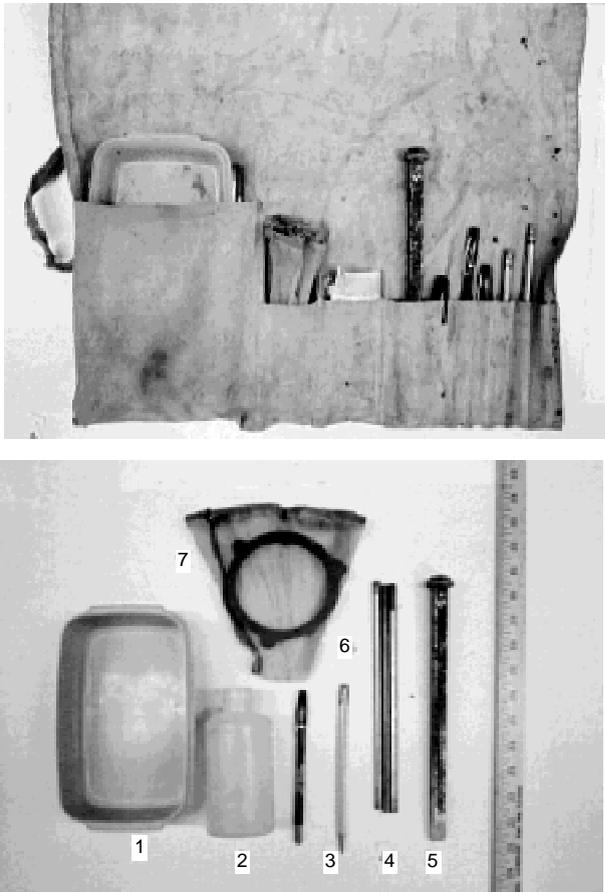
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The methods for invertebrate sampling are the same for the different stages of monitoring presented in this manual. A Surber sampler is recommended because of its portability and widespread use by Federal and State agencies. For wilderness use, it is recommended that repair equipment (for example, needle and thread, hot-glue stick) and a spare sampler be included as standard equipment. Progressing to a higher stage of analysis requires increased sampling frequency and level of data analysis.

1. The sampling location should be approached from downstream and the frame of the Surber sampler (250- $\mu$ m mesh net) placed into position as

quickly as possible to reduce the potential for escape by highly mobile macroinvertebrates. Try to keep the bottom square part of the frame flush with the substratum, and the bottom front edge of the net tight against the streambed.

2. The larger rocks within the perimeter of the open quadrant frame should be lifted by hand, rubbed and rinsed off at the mouth of the net opening, and removed from the sampler. The remaining substratum should be thoroughly disturbed to a depth of 10 cm by repeatedly digging and stirring with a probe (for example, a large nail or a railroad spike), included in the benthic monitoring kit (see photo 3). The invertebrates and lighter debris will be carried into the net by the force of the current.



**Photograph 3**—Benthic sampling kit in canvas carrying case (above) containing the following equipment (below): (1) plastic pan, (2) squirt bottle, (3) grease pencil and pencil, (4) legs of ring stand, (5) railroad spike, (6) ring stand, and (7) cone shaped net (100  $\mu$ m mesh).

3. When sampling is completed, the top of the net should be tipped downstream until a 45° angle is formed with the streambed and the sampler quickly removed from the water with a simultaneous forward and upward motion. The net should be dipped several times in the stream to wash the contents to the bottom, being careful not to submerge the net opening.

4. Grasp the net firmly with your thumb and forefinger just above the contents and invert the net into a shallow pan (a white enamel pan or plastic container approximately 40 cm long, 25 cm wide, and 5 cm deep is good) partially filled with water. It may be necessary to partition the contents into segments if it looks like they will fill the pan to overflowing. When the bulk of the contents have been removed from the net, re-invert the net, re-dip it in the stream, and again remove the contents. Carefully examine the interior of the net, especially the seams, for any adhering material and remove. A stream of water from a wash bottle is helpful at this stage.

5. Gently slosh the contents of the pan back and forth to suspend the invertebrates and other organic matter and quickly pour the suspended material into a cone-shaped net (a 3-legged ring stand makes a good holder for the net) (photo 4). Repeat the process until all organic matter is removed from the pan. As a final step, again partially fill the pan with water, spread the inorganic sediments in a thin layer evenly over the bottom of the pan, and examine the contents for any organisms remaining (photo 4) (for example, stone-cased caddisflies, planarians). Remove these with fingers or a forceps and place with the portion of the sample to be retained. When finished, discard the inorganic sediments.

6. Transfer the contents of the cone-shaped net into a sample container (for example, a whirl-pak bag) using a minimal amount of water (a wash bottle is helpful here), label with location and date, add sufficient water to cover the contents, and preserve to a final concentration of 5 percent



**Photograph 4**—Field processing benthic samples.

formalin (2 percent formaldehyde) (= 5 ml concentrated formalin (40 percent formaldehyde) per 100 ml water) or other preservative. For safety's sake all pouring and transfers should be done over the opening of the net or an empty pan. If a substantial amount of the sample is lost (an amount of material the size of a pea may contain a 1,000 or more organisms or several mg of organic matter on a per square meter basis), the entire sample should be discarded and a new sample taken. The properly packaged and preserved sample can then be transported to the laboratory for sorting and identification of macroinvertebrates.

7. Prior to identification, the sample should be coarse-sorted into major taxonomic groups. A small portion of the sample, no larger than a large teaspoon, should be placed into a clear petri dish containing a small amount of water. Invertebrates then are handpicked, using forceps, under a dissecting microscope, and placed in leakproof containers containing preservative (10-ml glass vials with plastic caps work well). All vials from a sample should be kept together and labeled appropriately. For large samples, subsampling facilitates the process and may be aided by the use of mechanical devices. If the sample is subsampled, a minimum of 300 individuals should be sorted (Plafkin and others 1989). The portion of the total sample examined must be recorded.

8. The invertebrates are then identified to the lowest taxonomic level feasible, given the goal of the particular study. Species is the preferred level of identification, because many species look alike but behave differently ecologically, however, in many cases genus is satisfactory for initial bioassessment purposes. The dipterans, Chironomidae, and Simuliidae commonly are identified only to subfamily due to the difficulty of more detailed identification. This usually can be accomplished with a dissecting microscope, but in some cases a compound microscope will be required. The number of individuals in a taxonomic group is recorded.

9. If biomass values are needed (see stage 2), organisms should be returned to storage vials after identification and counting. Each species group is placed in a separate vial. Each vial should be properly identified by sample location, date, and replicate.

## Methods: Stage 1

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Stage 1 data analysis and sampling frequency follows the procedures from Rapid Bioassessment Protocol III (RBP III) (Plafkin and others 1989), modified by the use of additional metrics. The calculation of some biotic metrics requires classification of aquatic insects by functional feeding group (Cummins 1973, 1974). Functional feeding groups provide information concerning resource utilization by invertebrates in streams. A shift in the relative abundance of the different functional feeding groups can therefore indicate a shift in the resource base. Initial placement of an aquatic insect into a particular functional feeding group can be accomplished by consulting "An Introduction to the Aquatic Insects of North America" (Merritt and Cummins 1996) However, direct analysis of gut contents is the preferred method for functional feeding group classification. A general outline of the functional feeding groups is given in table 5 (also see appendix C). Once organisms are identified to species, and classified by

**Table 5**—General functional feeding group divisions showing food resource, particle size and representative orders (after Merritt and Cummins 1996).

Functional group (based on feeding mechanism)	Subdivision of functional group		Feeding mechanism	Food particle size (micron)	Representative orders
	Dominant food				
Shredders	Living Vascular hydrophyte tissue		Herbivores-chewers and miners		Trichoptera Lepidoptera Coleoptera Diptera
	Decomposing Plant tissue (CPOM)		Detritivores-chewers and wood borers	>10 <sup>3</sup>	Plecoptera Trichoptera Coleoptera Diptera
			Detritivores-filterers or suspension feeders		Ephemeroptera Trichoptera Lepidoptera Diptera
Collectors	Decomposing fine particulate organic matter (FPOM)		Detritivores-gatherers or deposit feeders	<10 <sup>3</sup>	Collembola Ephemeroptera Hemiptera Trichoptera Coleoptera Diptera

(con.)

Table 5 (Con.)

Functional group (based on feeding mechanism)	Subdivision of functional group		Feeding mechanism	Food particle size (micron)	Representative orders
	Dominant food				
Collectors	Periphyton-attached algae and associated material		Herbivores-grazing scrapers of mineral and organic surfaces	<10 <sup>3</sup>	Ephemeroptera Hemiptera Trichoptera Lepidoptera Coleoptera Diptera
Scrapers	Living vascular hydrophyte cell and tissue fluid		Herbivores-pierce tissues or cells	>10 <sup>2</sup> -10 <sup>3</sup>	Neuroptera Megaloptera
Predators	Piercers-Living animal tissue		Carnivores-attack prey and pierce tissues and cells and suck fluids	>10 <sup>3</sup>	Plecoptera Odonata Hemiptera Neuroptera Trichoptera Coleoptera
	Engulfers-Living animal tissue		Carnivores-whole animals or parts	>10 <sup>3</sup>	Plecoptera Odonata Hemiptera Neuroptera Trichoptera Coleoptera

functional feeding group, the following metrics are calculated (after Robinson and Minshall 1995).

1. EPT/Chironomidae + Oligochaeta Ratio (EPT/C+O)—Based on the relative abundance of Ephemeroptera, Plecoptera, Trichoptera to Chironomidae and Oligochaeta to assess community health. A disproportionate number of the relatively pollution tolerant Chironomidae and Oligochaeta suggests degraded habitat conditions.

2. Species Richness (Sp. Rich)—This metric reflects health of the community through a measure of the number of distinct species (or taxa) present. Typically, a higher number of taxa suggests good habitat quality.

3. EPT Richness (EPT Rich.)—The total number of distinct taxa in the orders Ephemeroptera, Plecoptera, Trichoptera. These groups are generally sensitive to pollution, with a low EPT Richness indicating degraded habitat quality.

4. Hilsenhoff's Biotic Index (HBI) detects organic pollution stress in communities inhabiting stream riffles. HBI summarizes the pollution tolerance of each taxon in the community, based on the abundance of respective taxa, into a single value. Higher values typically indicate greater levels of organic pollution. HBI is calculated as:

$$HBI = \sum \frac{x_i t_i}{n} \quad (8)$$

where,  $x_i$  = number of individuals within a species,  $t_i$  = tolerance value of a species,  $n$  = total number of organisms in the sample. Tolerance values are available for the Western United States in Water Quality Monitoring Protocols Report No. 5 (Clark and Maret 1993) and are reprinted in appendix C.

5. EPT/Chironomidae Ratio—Uses the relative abundance of these indicator groups to assess community balance. A high number of Chironomidae indicates degraded habitat conditions.

6. Percent Dominance—A simple measure of a community's redundancy and evenness. The measure assumes that a highly redundant community is impaired. Percent dominance is the number of individuals in the dominant taxa (or 2 to 3 dominant taxa) to the total number of individuals times 100.

7. Simpson's Index (C)—A diversity index that reflects dominance or evenness of an assemblage. Simpson's index is:

$$C = \sum (p_i)^2 \quad (9)$$

where,  $p_i$  is the proportion of individuals in the  $i$ th species.

8. Percent Shredders—Measures the relative abundance of the shredding functional feeding group. A low number of shredders reflects poor or altered riparian conditions.

9. Density—The number of macroinvertebrates in a given area. Low benthic densities reflect degraded habitat conditions.

10. Percent Scrapers—A relative measure of the abundance of the scraping functional feeding group. A greater percentage of scrapers suggests good habitat quality.

11. Percent Filterers—A relative measure of the abundance of the filtering functional feeding group. A large percentage of filterers may indicate excessive sediment/organic load and consequently poor habitat quality for most of the community.

12. Percent EPT—The relative abundance of Ephemeroptera, Plecoptera, and Trichoptera in a stream. These groups are generally intolerant to pollution and used as indicator taxa.

13. Percent Chironomidae + Oligochaeta—Measure of the relative abundance of the generally pollution tolerant groups. A community with a high percentage of these organisms may indicate excessive erosion and/or sediment/organic load in the stream.

14. Percent Chironomidae—A measure of the relative abundance of the generally pollution tolerant group Chironomidae. A community with a high percentage of Chironomidae may indicate excessive erosion and sediment/organic load in the stream.

15. Percent Ephemeroptera, Percent Plecoptera, and Percent Trichoptera— Measure of relative abundance of these pollution intolerant groups.

In wilderness streams, the confidence interval for each metric obtained from multiple samples of similarly classified stream locations can be used to determine rank scores for each metric. Disturbance can then be evaluated by comparing metric scores from control and impacted sites. That is, how far does the stream in question vary from the confidence interval obtained from unimpacted sites. Alternatively, single stream trends can be monitored on an annual basis, or one control and impact site can be compared statistically. The first method however, is probably most consistent with the needs of wilderness stream managers and will be outlined in more detail.

The mean (5 replicates) metric values for each stream are recorded (example 2). The mean and 90 percent confidence interval for each column is calculated. Each metric is then given a rank score (SC): 5 if metric value better than upper confidence limit, 3 if within confidence limit, and 1 if below confidence limit. Each metric is then interpreted individually, along with the sum of all metric scores.

## Methods: Stage 2 ---

Stage 2 increases the level of analysis beyond the indices outlined in stage 1. In addition to the modified Rapid Bioassessment Protocol III, total invertebrate biomass is calculated. For biomass measurements, invertebrates are dried (60 °C for 24 hours). If the biomass for each individual taxa is required for secondary production calculations (see stage 4), each taxonomic group and each size class is dried and weighed separately. This requires a balance with the ability to measure to 10<sup>-5</sup> grams, that is, 0.1 to 0.01 mg. Alternatively, the entire invertebrate sample can be combined, dried, weighed, and standardized by surface area (cross sectional area of the sampler). For AFDM values, the sample is then ashed (550 °C for 2 hours) rewetted, dried, cooled to ambient temperature in a desiccator, and

**Example 2**—Raw data for selected metrics. Below each column is the mean, upper confidence interval, and lower confidence interval. Rank scores were based on the relationship to the raw score distribution. Total rank for each row in addition to individual metric scores are used to evaluate each stream.

Stream	EPT/C + O	Score	Sp. Rich	Score	EPT Rich	Score	HBI	Score
1	2.7	3	22	3	12	3	3.57	3
2	1.2	3	24	5	16	5	3.94	3
3	1.1	3	24	5	17	5	3.19	5
4	0.8	3	24	5	18	5	3.6	3
5	1.2	3	27	5	16	5	4.07	3
6	8.3	5	17	1	10	3	3.16	5
7	1.3	3	12	1	5	1	3.08	5
8	0.3	1	21	3	13	3	4.46	1
9	0.5	3	17	1	9	1	4.37	1
10	0.4	1	18	3	7	1	4.45	1
Mean	1.79		20.6		12.3		3.79	
St. Error	0.72		1.36		1.34		0.16	
Upper 90%CI	3.10		23.09		14.76		4.09	
Lower 90%CI	0.47		18.11		9.84		3.49	
Score	5 >3.1		>23		>14.8		<3.49	
	3 0.47-3.1		18-23		9.8-14.8		3.49-4.09	
	1 <0.47		<18		<9.8		>4.09	

reweighed and AFDM is obtained by difference. Invertebrate density, richness, and biomass values can then be used to compare different streams or to monitor streams over time. Additionally, AFDM values can be used to construct biomass pyramids and quantify food webs which can be partitioned by taxon or functional feeding group.

## Methods: Stage 3

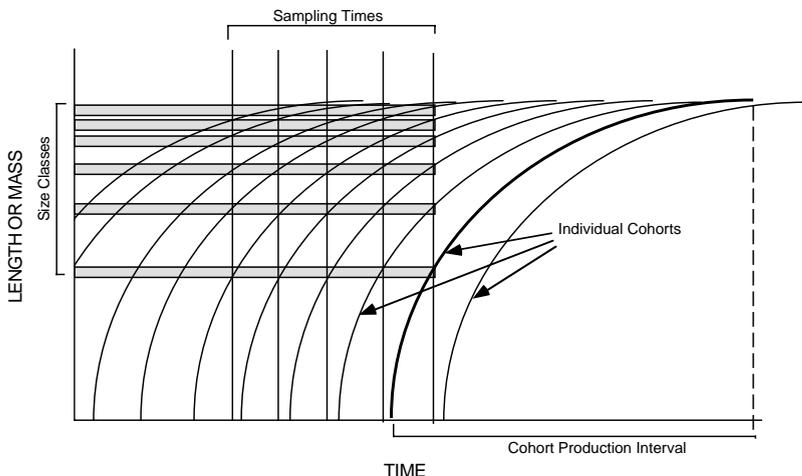
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At this stage of analysis, secondary production is calculated. Annual estimates are desirable but interval production for one or more seasons are valuable. Annual and interval estimates require at least monthly sampling. For annual estimates, sampling must continue throughout the year. Secondary production is a measure of the amount of energy transferred to primary consumers and predatory insects. Secondary production is important in quantifying the flow of energy through an ecosystem (Benke 1984). Secondary production also is an estimate of the energy available to fish, which are an important food and recreational resource. Evaluation of secondary production for functional feeding groups also gives a better understanding of the relative importance of various food resources.

Secondary production is calculated at the level of a population. Total community production, or production of functional feeding groups, can be obtained only by summing all population secondary production estimates. There are two general methods used to calculate secondary production (Benke 1993). The method used depends on whether or not individual cohorts can be followed. A cohort is a group of individuals of the same species that have similar hatching times and developmental rates; that is, a group of individuals that hatch on or near the same date and obtain similar sizes at similar times (fig. 12). If cohort production occurs, an invertebrate sample, on any given date, should contain individuals (within a population) of similar size. Non-cohort production occurs when hatching and development are distributed over time or when individuals from more than one life cycle are present at a time. Samples of a non-cohort population would produce individuals of many different sizes.

For cohort production, the instantaneous growth or increment-summation method can be used. Both of these methods are explained in Benke (1984). For non-cohort production, the size frequency method produces the best estimate. Because non-cohort production is common, and because the size-frequency method also can be used for cohort production, the size-frequency method is most generally applicable and will be described here.

The size-frequency method assumes that the size-frequency distribution, at any given time, will be similar. That is, that the density of individuals of a given size class should be similar across multiple sampling dates. However, this assumption does not have to be met. This method also assumes that the number of size classes present reflects the number of cohorts. That is, if species size distribution can be distributed into 11 different 1-mm size classes then 11 different cohorts are present at this time (fig. 12).

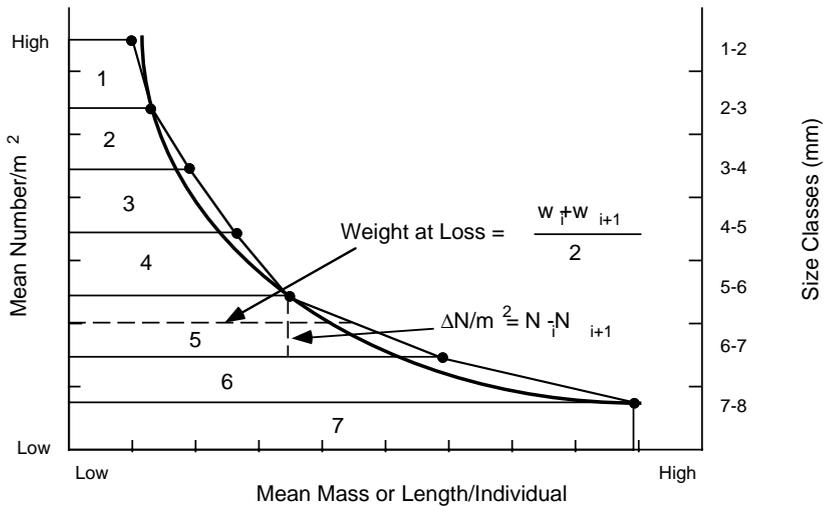


**Figure 12**—Representation of an invertebrate population over time. Each curved line represents growth of an individual cohort. The vertical lines indicate five different sampling periods. The horizontal rectangles represent six different size classes. Each sampling period transects six different cohorts but size class distribution is similar for each sampling period. The number of size classes equals the number of cohorts.

Secondary production is calculated, by integrating the area under the size-frequency distribution (Benke 1993), using means similar to removal summation methods for cohort production. However, the size frequency-distribution is constructed from the mean numbers of individuals from each size class throughout the sampling period (fig. 13) and the individual weights of the different size classes (table 6). This integrated value is multiplied by the number of size classes (representative of the number of cohorts) and corrected by cohort production interval (CPI). That is,

$$P = \frac{365}{CPI} i \sum \Delta N \bar{W}, \quad (10)$$

where  $i$  = the number of size classes,  $N$  = the mean number of individuals in that size class (individuals/  $m^2$ ), and  $W$  equals weight (mg/individual) (example 3). Integration of the area under the size-frequency distribution results in interval production (IP) (table 6) which is used to calculate the units used to describe secondary production.



**Figure 13**—Size-frequency distribution for macroinvertebrate secondary production estimates. The mean number of individuals over the sampling period are plotted against the mean mass for individuals in that size class. Interval production is calculated by integrating the area under the curve. Integration is accomplished by summing all numbered areas.

**Table 6**—Parameters used to describe secondary production (after Benke 1993).

Symbol	Definition	Units	Description
W	Individual weight	mg	Individual weight of animals
N	Density	No./m <sup>2</sup>	Density of individuals
B	Biomass	g/m <sup>2</sup>	Biomass of individuals
P	Annual production	g/m <sup>2</sup> /yr	Biomass produced over a year
IP	Interval production	g/m <sup>2</sup>	Biomass produced over an arbitrary time
IP <sub>c</sub>	Cohort production	g/m <sup>2</sup>	Biomass produced over the cohort production interval
IP <sub>c</sub> /B	Cohort P/B		Relationship between cohort production and biomass usually ranges from 2 to 8
P/B	Annual production	/yr	Relationship between annual production and biomass

**Example 3**—Calculation of secondary production for *Drunella doddsi* in Cliff Creek from Surber samples taken (five replicates) over a 7 month interval beginning in April 1994. The population is enumerated and each individual length is measured. Individuals are separated into 1-mm size classes, dried, and weighed. Column A is the length of size class. Column B is the total number of individuals in that size class collected over the entire sampling period. Column C is the mean number of individuals per sample, column B divided by 35 (7 months x 5 replicates). Column D is corrected by the area of the Surber sampler. Column E is the mean weight per individual in the size class and column F is weight per area (column D x C). The sum of column F is biomass (B). Column G is the difference in numbers (column D) between size classes, generally the number of individuals lost in moving to the next highest size class. Column H is the mean individual weight between adjacent size classes. Column I is the product of G and H. Interval production is the sum of I multiplied by the number of size classes.

	A Length (mm)	B Number	C Number/ Sample B/35	D Number/m <sup>2</sup>	E Weight (mg/Indiv.)	F Weight (mg/m <sup>2</sup> ) D x E	G $\Delta N/m^2$	H Weight at Loss (mg/Indiv.)	I Weight Loss (mg/m <sup>2</sup> ) G x H
1	0.8 - 2.0	148	4.229	45.517	0.036	1.635			
2	2.0 - 3.0	145	4.143	44.595	0.083	3.707	0.923	0.060	0.055
3	3.0 - 4.0	52	1.486	15.993	0.142	2.273	28.602	0.113	3.221
4	5.0 - 6.0	9	0.257	2.768	0.266	0.737	13.225	0.204	2.701
5	6.0 - 7.0	6	0.171	1.845	0.889	1.641	0.923	0.578	0.533
6	7.0 - 8.0	3	0.086	0.923	1.455	1.343	0.923	1.172	1.082
7	8.0 - 9.0	9	0.257	2.768	3.182	8.809	-1.845	2.319	-4.279
8	9.0 - 10.0	33	0.943	10.149	5.142	52.187	-7.381	4.162	-30.722
9	10.0 - 11.0	38	1.086	11.687	8.758	102.352	-1.538	6.950	-10.687
10	11.0 - 12.0	20	0.571	6.151	14.416	88.672	5.536	11.587	64.144
11	>12.0	5	0.143	1.538	27.749	42.670	1.538	21.082	97.258
						306.0		13.874	21.335
									144.6

IP = 11 x 144.6 mg/m<sup>2</sup> = 1,591.0 mg/m<sup>2</sup>  
or 1.59 g/m<sup>2</sup>  
B = 306.0 mg/m<sup>2</sup>  
or 0.306 g/m<sup>2</sup>  
IP/B = 5.2/7 months

# Fish

Sampling the fish community at stage 1 is optional but is included as a means to evaluate potential impacts and because fish are often of prime importance due their recreational and commercial value. Analysis of fish community data for the evaluation of impacts is limited due to planting of sport fish, fishing pressure, and in the West, low diversity. In anadromous fish streams, juvenile salmonids can vary with the number of returning adults and with different commercial and sport fish management plans. This variability makes statistical comparisons difficult.

## Methods: Stage 1

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Snorkeling is recommended as the preferred method for sampling fish in wilderness streams. This method requires little equipment, is cost effective, and fish are not handled, reducing potential mortality. This is particularly important in wilderness streams and in areas where protected species are present. The methods described in Thurow (1994) are briefly outlined below; however, this publication should be referenced for additional details.

In small streams, an individual snorkeler begins at the downstream end of the reach and moves slowly upstream. The snorkeler should move from side to side making sure that all habitat types, pools, eddies, and undercut banks are investigated. In larger streams, two observers move upstream with shoulders touching and count all fish passed between themselves and the bank. In some cases, stream depth is too great for upstream movement and the snorkeler must float downstream remaining as motionless as possible. All fish are identified, counted, and fish length is estimated in a single pass through the sampling reach. With training, the accuracy of species identification and estimates of fish length can be improved. Published relationships between fish length and fish weight can be used to estimate biomass.

The fish community is evaluated using the metrics from RBP V (Plafkin and others 1989). Additional metrics for Idaho coldwater streams have been developed by Chandler and others (1993) and Robinson and Minshall (1995). These metrics are as follows:

1. Number of native species
2. Number of sculpin species
3. Number of native minnow species
4. Number of sucker species

5. Number of intolerant species
6. Percent of common carp
7. Percent omnivores
8. Percent insectivores
9. Percent catchable salmonids
10. Number individuals per kilometer
11. Percent introduced species
12. Percent anomalies
13. Total biomass ( $g/m^2$ )
14. Salmonid biomass ( $g/m^2$ )
15. Percent young of the year
16. Salmonid density ( $m^{-1}$ ), and
17. Salmonid biomass ( $g/m^2$ )

Each metric can be used as a dependent variable for statistical comparisons between reference and impacted sites. Alternately, each metric is scored, based on the 90 or 95 percent confidence interval (example 3). Metric scores also can be determined based on visual evaluation of the range of data values (Fore and others 1996). The sum of all metric scores is then used for comparisons. The following tabulation outlines this process:

	<u>Dependent variables</u>	<u>Analyses</u>
<b>Stage 1</b>	Mean metric values Mean total metric score	Statistical

# Algae/Periphyton

Benthic algae, along with benthic organic matter and organic matter in transport, represent the primary energy source for herbivory and detrital food webs. The relative importance of these energy sources varies along a continuum from headwater streams to larger rivers (Vannote and others 1980). Three main groups comprise the majority of the benthic periphyton found in wilderness streams; Cyanophyta, Chlorophyta, and Chrysophyta. The Cyanophyta, or blue-greens, lack a nucleus and contain pigments within the cell membrane. The Chlorophyta are the green algae that are characterized by containing chloroplasts in which chlorophyll is the predominant pigment and energy is stored as starch. The diatoms (Bacillariophyceae) are the predominant class of organisms in the Chrysophyta division. Diatoms are generally unicellular, store food as oils, and are surrounded by a thick siliceous cell wall. Algae occur in association with, and are often embedded within the exudates of, heterotrophic bacteria and fungi; collectively, these constitute periphyton. For convenience, the algae and associated heterotrophic organisms and other organic matter are sampled and analyzed as a unit. Additional morphological (for example, counts of diatom frustules) or biochemical techniques (for example, chlorophyll or ATP analysis) may be employed to provide further information about the sample in general and the algae in particular. Algal production is directly affected by light, nutrients, water velocity, temperature, and indirectly by primary and secondary consumers. Therefore, alterations of these variables can result in different levels of algal biomass or changes in community composition. The following tabulation outlines this process:

	<u>Dependent variables</u>	<u>Analyses</u>
<b>Stage 2</b>	Mean AFDM Mean Chl-a Chl-a/ AFDM	Statistical
<b>Stage 3</b>	Diatom community metrics	Statistical

It is useful to divide incoming light into two components: light reaching the stream surface and light penetrating to the stream bottom. In many headwater streams in wilderness areas of the western U.S.A., benthic algae can be limited by the amount of light reaching the stream surface (Hill and Knight 1988; Shortreed and Stockner 1983). Alterations in the height and density of riparian plants can therefore be transmitted to changes in algal biomass. Community composition also can change with changes in light intensity, as diatoms are known to drift depending on light availability (Bothwell and others 1989). The amount of light penetrating to the bottom

of the stream can be altered by the turbidity of the water. Increasing instream sediment can alter light availability and reduce algal biomass (Davies-Colley and others 1992; Lloyd and others 1987; Quinn and others 1992).

The availability of limiting nutrients can alter algal biomass. Therefore, alterations in nutrient input to a stream can be monitored by changes in algal biomass. Algal community structure also can be affected by changes in nutrient concentrations. For example: the presence of the nitrogen-fixing cyanobacteria, *Nostoc*, is often an indication of low concentrations of nitrogen.

Water velocity can either enhance or degrade accumulation of algal biomass. Increasing stream velocities can facilitate the uptake of nutrients and the removal of metabolic waste products. As water velocity increases, the force of the turbulent water can remove dead or dying cells from the periphyton matrix or patches of living organisms.

Algal growth rates often are positively associated with increasing temperatures. Higher stream temperatures often are accompanied by enhanced algal biomass. Community structure also can change due to differential growth responses to different temperatures.

Algal biomass can be affected secondarily by herbivorous invertebrates (Hill and Knight 1987; Hill and others 1992; Lamberti and Resh 1983), which in turn can be altered by the presence of insectivorous fish or amphibians. For example, high levels of grazing insects can maintain a low level of algal biomass; however, if fish reduce the density of grazing insects, algal biomass can increase.

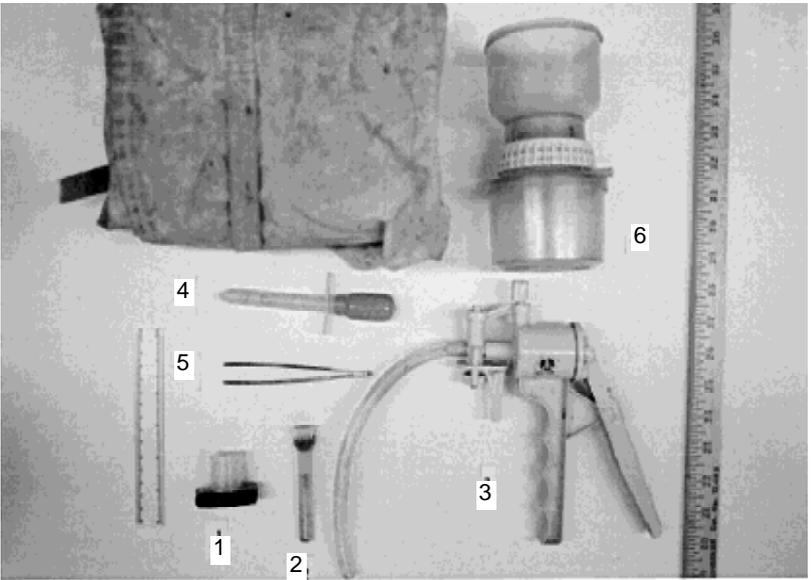
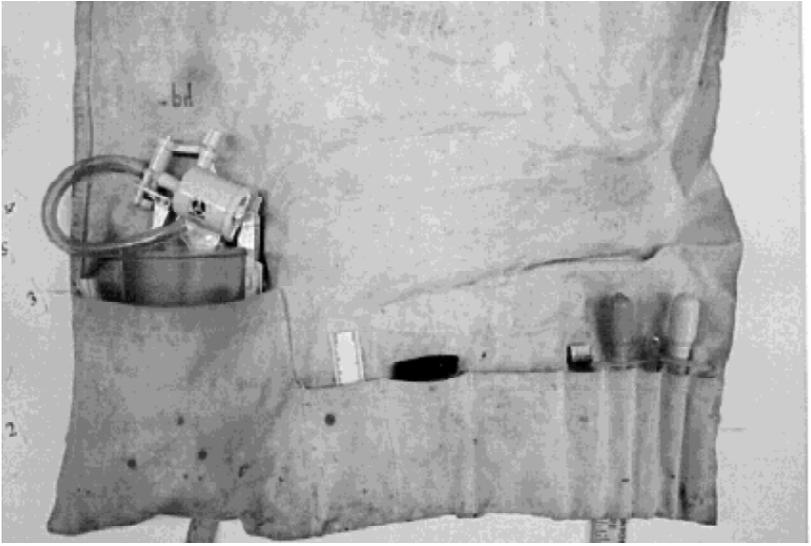
## Methods: Stage 2

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Within the sampling area, algae from a known surface area of five randomly chosen rocks is removed, filtered, and preserved in the field. Removal and filtration of attached algae requires the use of tools contained within the periphyton sampling kit (photo 5). Contents of the sampling kit are described below.

1. Periphyton sampler constructed from the barrel of a 30-cc plastic syringe which has been cut off 4 cm from the open end (the end that has protrusions for fingers). The bore of the syringe barrel is used to delineate the area for removal of periphyton. Neoprene foam (4-mm thick wetsuit material) is glued, using a combination of epoxy and RTV-silicone neoprene cement, to the flat surface of the syringe barrel to provide a water seal when the sampler is placed snugly against the rock surface.

A heated cork borer provides a convenient means for making a hole in the neoprene. The actual area circumscribed on a rock by the sampler can be determined by using the sampler as a "rubber stamp" to transfer an impression (using an ink pad) repeatedly onto paper. The area contained within the donut-shaped impression is determined. The area of a sampler fabricated as above is approximately 3.45 cm<sup>2</sup>.



**Photograph 5**—Periphyton sampling kit in canvas case (above) and containing. (1) sampler, (2) plastic brush, (3) hand suction, (4) medicine dropper, (5) forceps, and (6) filter holder.

2. A small brush constructed by gluing a 8 x 8 mm portion of a hard-bristled toothbrush onto the end of a handle such that the bristles are parallel with the handle.

3. Large plastic medicine dropper or plastic volumetric pipette with suction bulb for aspirating periphyton from sampler. The pipette tip should have an opening with a diameter of about 4 mm to facilitate suction of large particles (a 5-ml medicine dropper is ideal).

4. Forceps, for handling filters and for use in removal of filaments or invertebrates which otherwise would interfere with determination of periphyton biomass.

5. Portable 47-mm filter holder with base and trap for filtrate.

6. Hose and suction device for filter. Hand-operated pump can be used for suction.

7. Glass fiber filters (Whatman GF/F, 47 mm or equivalent with a nominal pore size of 0.7  $\mu\text{m}$ ) pre-combusted in a muffle furnace for 1 h at 475 °C. Filters should be pre-weighed and stored in individual dust-free containers if an estimate of periphyton dry weight or percent organic is desired. Storage containers for filters can take various forms including plastic scintillation or other vials, cryotubes, or 49 mm-diameter plastic petri dishes.

8. Labels for filter containers. PolyPaper labels (Nalgene No. 6309 or 6315, 19 x 38 cm) are good since they are waterproof and can be removed easily after laboratory analysis.

Algal or periphyton sampling follows the procedure outlined below.

1. The five rocks are brought to a central sampling location and placed under water maintaining the original orientation. A rock is removed from the water and a representative sampling location is identified on the upper surface.

2. The periphyton sampler is held onto the rock surface with adequate force to retain water within the plastic cylinder. With the medicine dropper or wash bottle, add sufficient particle-free water into the cylinder to fill it to a depth of 1-2 cm, brush vigorously for about 10 seconds to dislodge periphyton, aspirate the contents with the dropper or pipette, and expel the contents into filter head (photo 6). Repeat this process three times; more if necessary to remove particularly large accumulations. The final rinse should be particle-free. It may be necessary to repeat this procedure several times for each rock in order to fully load the filter with material. The objective is to collect sufficient material to minimize errors during gravimetric analysis (no less than 10 mg dry weight).

3. After filtration, the filter is placed within a labeled storage container, and placed in a cool, dark place. If analysis of chlorophyll-a is desired, the filtered sample must be stored in the dark at temperatures below 4 °C until analysis (APHA 1995). A liquid-nitrogen cooled 3DS Dry Shipper (Union Carbide Corporation: height 478 mm, diameter 194 mm, weight 6.8 kg) or packing in dry ice can be used to freeze samples. Small (1.8 ml) cryogenic vials work well for storage of filters in conjunction with Dry Shipper. If values of ash free dry mass only are desired, the filtered algae can be



**Photograph 6**—Field sampling of periphyton.

preserved in formalin. In the absence of a portable Dry Shipper, the difficulty of freezing samples in remote wilderness streams may limit analysis to AFDM.

4. Laboratory analysis of chlorophyll-a (corrected for pheophytin) and AFDM is done following methods in the current Standard Methods (APHA 1995). We have found that accurate chlorophyll-a and AFDM values can be obtained from each filtered sample.

- a. Place thawed sample into a tissue grinder and cover with 3 ml of 90 percent acetone (or methanol). Grind sample for 1 minute.
- b. Transfer sample to centrifuge tube and add an additional 7 ml of acetone. Be sure to rinse all residual material from the grinder.
- c. Place the centrifuge tube into a refrigerator (4 °C) for at least 2 hours.
- d. Clarify sample by centrifuging for 20 minutes at 500 g.
- e. Transfer 3 ml of the extract into a 1-cm cuvette and measure absorbance at 664 and 750 nm.
- f. Acidify with 2 drops of 0.1N HCl. After 90 seconds, measure absorbance at 665 and 750 nm.

$$\text{Chlorophyll} - a(\text{mg} / \text{m}^2) = \frac{26.7((664_b - 750_b) - (665_a - 750_a))V}{A \times L} \quad (11)$$

where  $V$  is the volume of extract (Liters),  $A$  is the area of the sampler ( $\text{m}^2$ ),  $L$  is the light path length (cm), and the subscripts  $b$  and  $a$  denote before and after acidification, respectively.

- g. Return extract to centrifuge tube and transfer contents to crucible.
- h. Place crucible under an exhaust fan until all the acetone has evaporated.
- i. Dry sample in drying oven (60 °C) for 24 hours, remove and cool to room temperature in a desiccator, and obtain dry weight.
- j. Ash samples in muffle furnace (550 °C) for 2 hours. Rewet samples with distilled water and return to drying oven for 24 hours.
- k. Place samples in desiccator and allow to cool to room temperature. Obtain final dry weight.

$$AFDMg/m^2 = \frac{W_1 - W_2}{A} \quad (12)$$

where  $W_1$  is initial dry weight (g) and  $W_2$  is final ash weight (g).

## Methods: Stage 3

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Stage 3 analysis is increased to include the preservation and identification of diatoms. These data are used to calculate diatom community metrics using Montana Water Quality Bureau Protocol II after Bahls (1993) or regionally refined metrics where available.

Diatom algae are collected from randomly selected rock substrates comprising a mix of habitats representative of a particular study site. Samples are brushed or scraped into a container, preserved in a 5 percent formalin solution, labeled, and returned to the laboratory. Samples may be processed and analyzed by the investigator or sent to a specialist. For the investigator, generic keys include Barber and Haworth (1981), and Prescott (1970). Patrick and Reimer (1966) provide a key to most North American species. A number of laboratories and/or individuals identify diatoms for biological monitoring projects, several of which are listed (appendix B). This list is not comprehensive, and is included here only to provide managers that require diatom identification with a starting point in their search.

For analysis, the composite sample is boiled in concentrated nitric acid, rinsed, mounted in Naphrax mountant, and examined under 1000X oil immersion. Analysis of the diatom community metrics requires identification of genera and, where possible, species. Counts of 600 to 1000 diatom valves are made from each slide to determine relative density. Diatoms are analyzed in terms of species richness, Simpson's Index, Shannon diversity, pollution tolerance index, siltation index, and a similarity index. These values are calculated using relative abundance data for each site (Bahls 1993; Minshall 1996; Robinson and others 1994).

# Large Woody Debris

Large woody debris (LWD) plays an important role in lotic ecosystems. It serves to stabilize the stream channel, retard the export of organic matter and nutrients, and provide protection and habitat for invertebrates and fish. Quantification of LWD often is ignored in ecological assessments because it is regarded as difficult and time consuming. Here we propose a relatively simple and straightforward technique for determining the amount of LWD in and immediately adjacent to the active stream channel, and evaluating several characteristics indicative of the contribution the material is likely to make in terms of channel/substratum stability, organic matter retention, and habitat for fish. The following tabulation outlines this process:

	<u>Dependent variables</u>	<u>Analyses</u>
<b>Stage 1</b>	Total piece count Total debris dam count	Comparative or statistical if multiple years or sites are available
<b>Stage 2</b>	LWDI Total piece by size class Total pieces in zones 1 and 2 Total piece volume	Comparative or statistical if multiple years or sites are available

## Methods: Stage 1

Large woody debris is described as the organic matter over 1 m in length and at least 10 cm in diameter at one end (sticks to logs). When multiple pieces of debris accumulate in the stream channel and retard water flow, a debris dam is formed. Stage 1 LWD analysis is an inventory of all LWD and debris dams over the entire sampling reach. All woody debris and debris dams within the bankfull channel are counted and recorded. Total counts are standardized by reach length or reach area. Large woody debris sampling is conducted once a year or longer.

## Methods: Stage 2

The functional influence of LWD on stream ecosystems varies with many factors, in addition to total counts of pieces and debris dams. The size relative to stream size, position in channel, and stability of LWD will determine its influence on streams. At stage 2 analysis, these factors are quantified to provide a score for each piece and debris dam, which will reflect their relative importance (table 7). The total score for LWD pieces and debris dams over the sampling reach is summed to provide a large woody debris index (LWDI) (example 4). The LWDI is standardized by reach length or area.

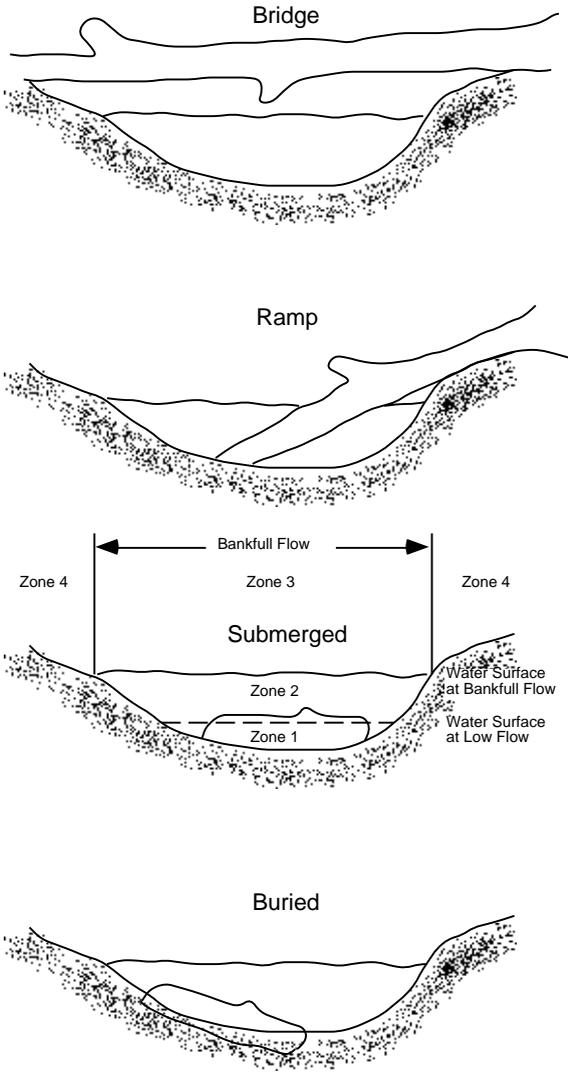
**Table 7**—Rank scores for pieces and dams of large woody debris (LWD) based on their potential to influence stream morphology, hydrology, and organic matter retention.

Pieces	Score				
	1	2	3	4	5
Length/bankfull width	0.2 to 0.4	0.4 to 0.6	0.6 to 0.8	0.8 to 1.0	>1.0
Diameter	10-20 cm	20-30 cm	30-40 cm	40-50 cm	≥50 cm
Location	Zone 4		Zone 3	Zone 2	Zone 1
Type	Bridge		Ramp	Submersed	Buried
Structure	Plain		Intermediate		Sticky
Stability	Moveable		Intermediate		Secured
Orientation	0-20°	20-40°	40-60°	60-80°	80-90°
<b>Debris dams</b>					
Length (% of bankfull width)	0 to 20	20 to 40	40 to 60	60 to 80	80 to 100
Height (% of bankfull depth)	0 to 20	20 to 40	40 to 60	60 to 80	80 to 100
Structure	Coarse		Intermediate		Fine
Location	Partially in high flow	In high flow	Partially in low flow	In mid low flow	In low flow
	Channel	Channel	Channel	Channel	Channel against bank
Stability	Moveable		Intermediate		Secured

**Example 4**—Data sheet for determining a large woody debris index (LWDI). Each piece of large woody debris (LWD) and one debris dam were ranked from the sampling reach. For example, 16 pieces were counted (number of marks in row). Eight of these pieces had a length/ bankfull width ratio of 0.2 to 0.4, 5 with a ratio of 0.4 to 0.6, and so forth. The far right column totals are the sum of the number of marks times the rank score. For example, length to bankfull width ratio,  $31 = (8)(1) + (5)(2) + (2)(4) + (1)(5)$ . Total piece score (PS) is 255 and total debris dam score (DDS) is 23.  $LWDI = \sum PS + 5\sum DDS = 255 + 5(23) = 370$ .

Pieces	Score					Total
	1	2	3	4	5	
Length/Bankfull Width						31
Diameter						37
Location						40
Type						35
Structure						36
Stability						38
Orientation						38
Total	54	10	66	20	105	255
<b>Debris Dams</b>						
Length						5
Height						5
Structure						5
Location						5
Stability						3
Total			3		20	23

The size of individual pieces is determined by measuring the length and diameter of the largest end. Longer, larger pieces should have a greater influence, are less likely to be moved, and are given a higher score. The location score is based on the portion of time a piece is likely to be in the active channel. Pieces that are in the active channel only at bankfull flows are given a lower score than pieces that will be in the channel at all times. Score is based on the predominant location in one of the four stream zones (Robison and Beschta 1990) (fig. 14). The different types of debris are shown



**Figure 14**—Different “types” of large woody debris (LWD) pieces and four stream zones.

in figure 16. Scores for piece type are based on stability and their relative influence on morphology, flow, and organic matter retention. Structure score is based on the potential to retain organic matter. LWD with a “sticky” structure has numerous branches or roots over its entire length. LWD orientation is determined by the angle between the piece and the stream bank. Pieces perpendicular to stream flow are more likely to create dam and plunge pools, increasing habitat complexity and organic matter retention. Pieces oriented 60 to 80° from the bank often divert flow and cause scour pools.

Debris dam scores rank the length (across the channel), height, structure, and stability of the object. Length is relative to bankfull width. A debris dam extending all the way across a stream will have a greater influence on morphology, hydrology, and organic matter retention than one that only partially disrupts flow. Debris dam height is relative to bankfull depth and reflects the portion of the stream influenced. Location scores reflect the position of the debris dam in relation to the active channel at low flows. Structure relates to the retention capacity of the debris dam. A debris dam with a fine structure will filter out more organic matter than a coarse structured dam and is given a higher score. Stability scores are based on the likelihood that the dam will be retained over variable flows.

# Benthic Organic Matter

Benthic organic matter (BOM) is the non-living organic matter deposited on stream bottoms and can provide an important energy source for heterotrophic bacteria, fungi, invertebrates, and fish. In heavily shaded streams, most of this material originates from the leaves, needles, and associated litter of terrestrial plant and can be a major organic energy source. Quantification of this resource is therefore important in determining the maximum biomass expected at upper trophic levels. Stage 2 analysis provides a measurement of this food base. Further subdivision of BOM in stage 3 provides a measure of annual variation and the size fractions of this resource. Size fractionation provides information in understanding invertebrate distribution, particularly with respect to functional feeding groups, in relation to the condition of the riparian habitat and the adjacent forest (Cummins and others 1989).

## Methods: Stage 2

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Benthic organic matter can be obtained from the sample collected for aquatic invertebrates. After all invertebrates are removed from the sample, the remaining organic matter is rinsed and placed within a large crucible or other suitable container that is stable at temperatures up to 600 °C. AFDM is determined by methods outlined previously for periphyton. The sample is placed in a drying oven (60 °C) for 24 hours or until weight stabilizes. The sample is cooled to room temperature in a desiccator, weighed, and placed within a muffle furnace (550 °C) for 2 hours or until all of the organic matter is reduced to ash. Upon removal, the sample is rewetted with distilled water, dried, cooled, and reweighed. The rewetting process rehydrates all inorganic clays within the sample. The difference between the initial and final dry weight is the AFDM. Resulting AFDM values are standardized by sampler area and expressed as g AFDM/m<sup>2</sup>. If the benthic sample was subsampled, the value should be multiplied by the inverse of the portion sampled to obtain a mass/sample value.

## Methods: Stage 3

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Annual measurements of benthic organic matter can be obtained by following the above procedure on a monthly or more frequent basis. At a minimum spring, summer, and autumn values should be obtained to represent the main periods of input and utilization.

Fractionation of benthic organic matter requires sieving of samples, which is most conveniently done in the laboratory. Three commonly used size fractions are: coarse particulate organic matter (CPOM) (1 mm to 16 mm), fine particulate organic matter (FPOM) (0.05 mm to 1 mm), and ultra fine particulate organic matter (UPOM) (0.45  $\mu$ m to 0.05 mm). The use of these size fractions will be based on the type of mesh used for invertebrate analyses. There is a trade-off in the size of mesh used for sampling. Smaller mesh size allows for the collection of smaller invertebrates, and the lower organic matter fractions, but reduces the flow of water which can cause the loss of sample integrity due to part of the sample being flushed out of the open end of the Surber net. We have found a 250  $\mu$ m mesh size to be the minimum size for use in conjunction with invertebrate collection that does not result in a loss of sample. If this mesh size is used, passing the sample through a sieve with a mesh size of 1 mm will provide coarse and fine fractions. The two fractions are then processed separately for AFDM as above.

Where more specific information is desired regarding specific size classes of BOM and smaller particles, further refinement can be obtained by sampling solely for BOM and adding a 52  $\mu$ m net to collect an additional FPOM fraction. Sampling of UPOM would require that the material passing through the 52  $\mu$ m-mesh net be subsampled and collected on a 0.45  $\mu$ m glass fiber filter (see Minshall and others 1983 for details).

In addition to size fractionation, identification of the types of plants and algae that contribute organic matter to the benthos is useful for characterizing food quality. Direct observation through a dissecting microscope is used to identify the organic matter. Identification only is possible for the larger size fractions (>1 mm). Genus or species identification is preferred; however, percent woody, autochthonus versus allochthonus, and deciduous versus evergreen are adequate distinctions.

# Transported Organic Matter

Transported organic matter (TOM) and invertebrate drift samples provide further quantification of the organic food base and a direct measurement of the food base for fish. Many aquatic insects are adapted to filtering organic matter from the water column. Measurements of transported organic matter allow a better understanding of the distribution of aquatic invertebrates based on functional feeding group analysis. Many fish, including salmonids, feed mainly on aquatic insects drifting in the water column; therefore, quantification of this resource is important in estimating the potential food base for these fishes. If the resource is to be evaluated for fish, the following sampling regime should be expanded to include dawn and dusk sampling, as aquatic invertebrate drift is usually greater at these times. The following tabulation outlines this process:

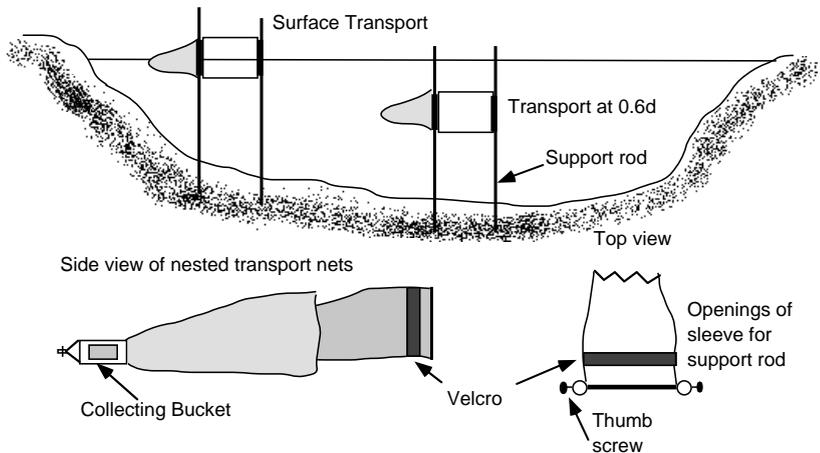
	<u>Dependent variables</u>	<u>Analyses</u>
<b>Stage 3</b>	Mean TOM, total and for each size class, and TOM flux.	Statistical

## Methods: Stage 3

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The sampling regime should coincide with benthic organic matter collection so that the relative importance of each resource can be determined. However, for more detailed measurements, TOM sampling should increase with changes in discharge as described for stage 3 water chemistry. Transport should be collected at 0.6 x depth, and at the surface, as a large portion of the coarse fraction is transported along the surface (fig. 15). The transport net frame should be constructed so that it can be supported at different depths within the water column. Nets of different mesh size can be nested so that multiple size fractions are collected simultaneously.

1. Rebar or steel spikes are passed through sleeves or collars on the side of the net frame and driven into the streambed. The net frame, with nested nets, is slid to the desired height and held in place by thumb screws passing through the sleeves.
2. Initial time is recorded. Water velocity into the net is measured and recorded by placing a velocity meter in front of the net opening.



**Figure 15**—Diagram of nested transport nets, frame, and stream placement.

3. Nets should be removed prior to sustained reduction of flow resulting from accumulation of materials in the net. Interruption of flow will result in underestimates of TOM, unless measured continuously. Time of removal is recorded.

4. Once the net is removed from the water column, the inner coarse net is slid up partially and all fine organic matter is rinsed into the apex of the fine net. This should be accomplished without submerging the opening of either net. The contents of the net are then emptied into a prelabelled whirl-pak bag and preserved with formalin (5 percent by volume). The content of the coarse net is treated similarly. Forceps may be useful in removing leaves and twigs from the net.

5. Upon returning to the laboratory, the sample is rinsed free of formalin, the aquatic invertebrates are removed and sorted into categories of similar appearance, identified to appropriate taxonomic level, counted, and weighed.

7. Each particulate organic matter size fraction is analyzed for AFDM as outlined previously for periphyton and BOM. The results are presented on a per-volume basis. Therefore, the AFDM value is standardized by the volume of water passing through the net in terms of  $\text{g}/\text{m}^3$ . Volume ( $\text{m}^3$ ) is calculated as the product of water velocity into the net ( $\text{m}/\text{s}$ ), area of net frame opening ( $\text{m}^2$ ), and total time the net was in place (s). Comparable units with benthic samples ( $\text{m}^2$ ) can be obtained by dividing the volume by mean stream depth (m).

# Organic Matter Decomposition

Decomposition rates are important indicators of the quality of benthic organic matter either as a food resource or microbial (bacterial and fungal) activity or both. Decomposition rates are influenced by many biotic and abiotic factors. These influences are summarized by Webster and Benfield (1986), from which the following discussion is derived. Decomposition rates are a function of temperature, invertebrate detritivores, the structural quality of the detritus, and the nutrient quality of the detritus and surrounding water. Decomposition generally is increased by elevated temperatures, as microbial enzymatic activity is enhanced. The structural quality of the litter also will influence breakdown rates, as fibrous cellular material is more resistant to decay. The nutrient quality of the litter also affects breakdown rates. In terrestrial systems, decay rates can be estimated from the C:N ratio of leaf litter. In aquatic systems, the nutrient content of litter can be augmented by dissolved elements. Generally, a higher nutrient content of the detritus and surrounding water results in faster breakdown rates of detritus. Invertebrate shredders act to mechanically fractionate the detritus and convert it into small fecal residue and food crumbs which are utilized directly by collectors or transported downstream. The pattern of detrital decomposition follows three stages. Initially, all soluble components of the cell are leached out. This results in a rapid weight loss in the first 24 hours. Next, decomposition is carried out by microbial and fungal breakdown. Finally, this conditioned detritus is fractionated by the combined effects of invertebrate shredders and physical processes.

Organic matter processing rates traditionally, as in this manual, have been determined by the mass loss of CPOM over time. These methods are an index of decomposition rates but provide little information concerning the breakdown of the smaller organic matter size fractions. The presence of extracellular enzymes has been used to estimate breakdown rates of FPOM and UPOM (Sinsabaugh and others 1994) and could be used to augment the methods outlined in this section.

## Methods: Stage 4

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The breakdown of CPOM is determined by containing leaves in a mesh bag or as a leaf pack, securing the leaves to the streambed, and measuring weight loss over time. Mesh bags may reduce the flow of dissolved nutrients and exclude invertebrates, thereby underestimating breakdown rates (Cummins and others 1980). However, the use of large mesh size alleviates

these problems (Benfield and Webster 1985). Leaf packs are constructed by binding leaves together with monofilament line and may be more representative of stream conditions by providing flow of nutrients and access to invertebrates. However, mechanical breakdown and loss of the smaller organic matter size fractions can increase decomposition estimates.

1. Leaf litter, that is representative of the riparian vegetation surrounding the stream in question, is collected from the forest floor. It is important to collect leaves after abscission because of the altered nutrient status of abscised leaves. Alternatively, a tarp may be spread out and trees or bushes shaken vigorously to dislodge dead leaves.

2. Leaves are dried at 60 °C until weight is stabilized; 5.0 to 10.0 g of dried leaves are placed within a mesh bag (mesh pore size of 2.5 cm<sup>2</sup>) or bound into a leaf pack.

3. The dry weight of individually labeled mesh litter bags is recorded. The number of mesh litter bags required is the product of replicates and sampling dates. That is, if three replicates are to be collected on six separate dates (day 1, 3, 10, 20, 30, and 60), then 18 litter bags are required.

4. Litter bags or packs are secured to the streambed at random locations within the dominant flow type (riffle, run, or pool) by securing the litter bag to a metal stake driven into the streambed or other stationary object such as a root.

5. Replicate-litter bags (three or more) are collected at predetermined sampling dates, emptied into whirl-pak bags, and preserved with formalin.

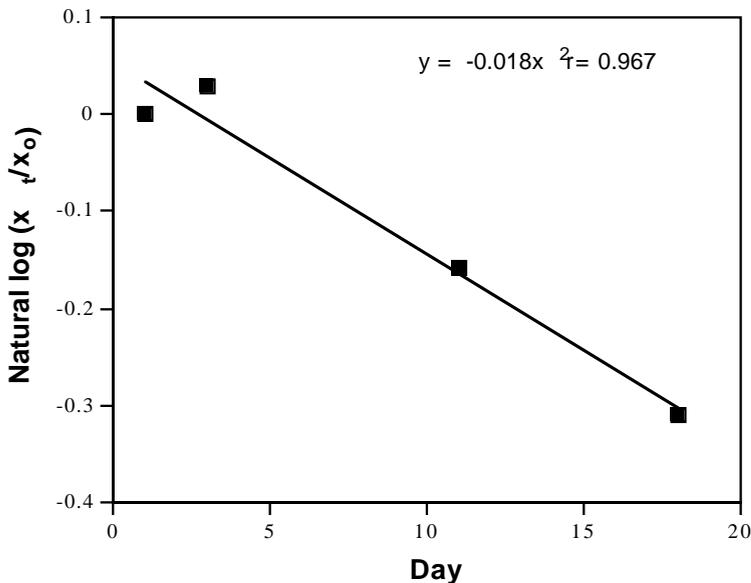
6. Upon returning to the laboratory, invertebrates are removed from the sample and identified. The remaining organic matter is rinsed thoroughly and dried to a stable weight at 60 °C. Dry weight and litter bag label information are recorded.

7. Where inorganic sedimentation may interfere with weight loss, initial and final AFDM values may be more representative of organic matter decomposition. In this case, the initial AFDM must be estimated from the relationship between dry weight and AFDM. At a minimum, thirty 5 g dry weight litter samples are ashed (550 °C for 2-3 hours), rewetted, dried and weighed. Regression analysis can be used to estimate AFDM as a function of dry weight.

Decomposition rates are obtained by fitting the data to a mathematical model. Many different models are available. A review and comparison of these models is provided by Wieder and Lang (1982). Generally, the single exponential decay model is used to determine the decay rate constant  $k$ . This constant can then be compared with other studies. The exponential decay model is:

$$X_t = X_o e^{-kt} \quad (13)$$

where  $X_t$  is mass at time  $t$  (days),  $X_o$  is the initial mass, and  $t$  is time in days. The weight of the organic matter collected on day 1 is used as the initial weight to correct for material lost in transport and through leaching. The decay rate constant,  $k$ , is determined by graphing the natural log of  $X_t/X_o$  as a function of  $t$  (fig. 16). The negative slope of this line is  $k$ . The slope is calculated through least-squares regression (example 1). Due to the effect



**Figure 16**—Calculation of the decay rate constant,  $k$ , by plotting  $\ln X_t/X_0$  versus time. Slope of line is  $-0.018$ , so  $k = 0.018$ .

of temperature on decomposition rates, this value can be standardized by degree days (Minshall and others 1983; Paul and others 1983), which allows comparison of different streams or values obtained at one site at different seasons. This is accomplished by regressing  $\ln X_t/X_0$  as a function of degree days rather than days. For sites that are difficult to access, single 30-day removals from several streams may be a viable alternative. Single removals from several streams may result in less precise measurements but would at least allow for comparative 30-day organic matter losses. The typical values were as follows:

	<b>Method</b>	<b>k/day</b>	<b>Reference</b>
First order stream in Frank Church Wilderness Area, Idaho	Bags	0.018	Unpublished
Second order stream, Caribou National Forest, Idaho	Packs	0.0016	La Point (1980)
First order stream, Oregon ( <i>Carya tomentosa</i> )	Packs	0.0035	Minshall and others (1983)
First order stream, Idaho ( <i>Carya tomentosa</i> )	Packs	0.0037	Minshall and others (1983)
Third order stream, Michigan ( <i>Salix alaxensis</i> )	Packs	0.0105	Irons and others (1994)
Second order stream, Virginia ( <i>Cornus florida</i> )	Bags	0.0486	Benfield & Webster (1985)
Second order stream, Virginia ( <i>Acer rubrum</i> )	Bags	0.022	Benfield & Webster (1985)
Second order stream, Alaska ( <i>Alnus crispa</i> )	Packs	0.026	Irons and others (1994)
Second order stream, Alaska ( <i>Salix alaxensis</i> )	Packs	0.016	Irons and others (1994)

# Primary Production

Primary production is a measure of within-stream or autochthonous carbon fixation. This production may constitute a substantial carbon source for herbivory and detrital based food webs (Minshall 1978). The relative importance of primary production varies with stream size, increasing at mid-order streams as the filtration of light by the riparian canopy diminishes, and then decreasing in larger rivers as light attenuation through the water column increases (Bott and others 1985; Minshall and others 1983; Minshall and others 1992; Naiman and Sedell 1980). Primary production is described by three parameters: Gross Primary Production (GPP), Net Primary Production (NPP), and Respiration (R). These three parameters are related, because NPP is the total amount of carbon fixed (GPP) minus that respired (R). Primary production can be described in terms of the autotrophic component or at the community/ecosystem level. Attached algae reside in a matrix composed not only of algae but also associated bacteria and fungi. Therefore, autotrophic GPP consists of carbon fixed by algae, minus algal, bacterial, and fungal respiration. However, large portions of carbon are respired outside of this association. Therefore, ecosystem-level measurements, in addition to autotrophic processes, include respiration by animals, and that associated with the microbial breakdown of organic matter in transport, on the streambed, and beneath and lateral to the streambed.

Measurements of primary production provide information that is not available by evaluation of standing stocks of periphyton biomass or the change in biomass over time. Biomass measurements are the result of NPP minus the amount lost through herbivory and sloughing. Therefore, measurements of biomass underestimate the importance of autotrophic production as an energy source. Because of this underestimate, ratios of algal to benthic biomass do not reflect the relative importance of these two energy components. A more realistic evaluation is obtained by the ratio of Gross Community Production to Gross Community Respiration, or a P/R ratio (see Rosenfeld and Mackay 1987, and Meyer 1989 for a discussion of P/R ratios and their interpretation). The resulting typical values were as follows:

	<b>Method</b>	<b>GPP (mg/O<sub>2</sub>/m<sup>2</sup>/hr)</b>	<b>Reference</b>
First order, Tennessee	Open system	72.6	Marzolf and others (1994)
First order, Tennessee	Chamber	67.3	Marzolf and others (1994)
Second order, New York	Chamber	15.8 (NPP)	Fuller and Bucher (1991)
First order, Alaska,	Chamber	12.2-260.2	Duncan and Brusven(1985)
Second order, Idaho	Chamber	26.9-74.0	Davis (1995)
Second order, Idaho	Chamber	63	Minshall and others (1992)
First order, Oregon	Chamber	16	Naiman and Sedell (1980)

## Methods: Stage 3

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Ecosystem measures of metabolism using open-system methods is suggested for stage 3 level of analysis where flow conditions permit. The open system method (Odum 1956) involves measuring the change in oxygen or carbon dioxide from upstream to the downstream end of a stream segment. The changes in oxygen concentration must be corrected for oxygen accrual, through tributaries or groundwater, and atmospheric diffusion. Careful site selection can usually reduce non-photosynthetic oxygen accrual. Estimates of diffusion however, are difficult to obtain and the difficulty is accentuated in highly turbulent streams (Marzolf and others 1994). In rapid-headwater streams upstream-to-downstream changes in oxygen can be dominated by diffusion rather than biotic processes, and open system measurements are not recommended in these situations (Bott and others 1978). The use of streamside channels reduces diffusion and accrual problems and has been used as an alternative to true open system measurements (Guasch and others 1995; Triska and others 1983).

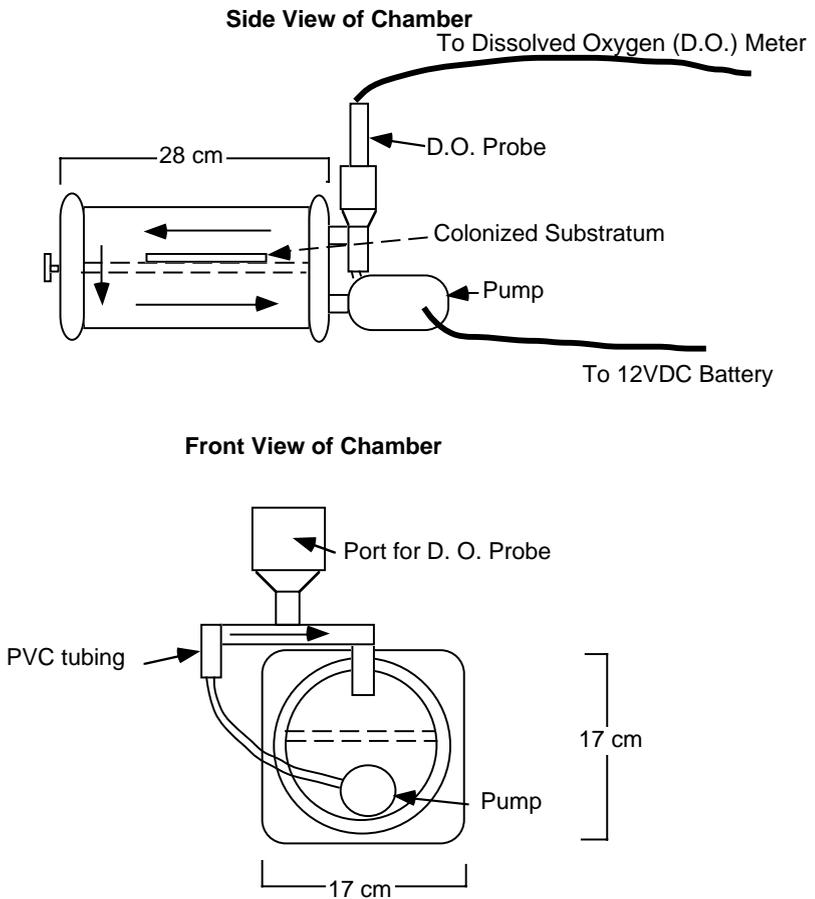
## Methods: Stage 4

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The second method used to measure primary production is through the isolation of a portion of the streambed within a closed microcosm. This method involves the use of recirculating chambers. Ecosystem-level measurements can be obtained by using microcosms that encompass most components of production, or by measuring each component separately and summing individual components. Periphyton productivity can be evaluated through the following chamber method using artificial or natural substrata. Artificial substrata such as unglazed ceramic tiles, have the advantage of easier determination of surface area, homogeneous colonization, and more simple algal biomass, but are disadvantageous because they may not reflect natural biomass and community composition (Cattaneo and Amireault 1992).

1. If artificial substrata are to be used, the material should be placed in the stream at least 1 month prior to production measurements. Algal-colonized tiles, or randomly selected rocks are placed within the chamber (fig. 17) (Bott and others 1978; Bowden and others 1992; Duff and others 1984). The chamber is sealed and placed in the stream to maintain ambient stream temperatures within the chamber. Placement location should reflect dominant light levels and light reaching chambers should be recorded during productivity measurements (see the Solar Radiation section).

2. Dissolved oxygen (D.O.), time, and water temperature are monitored at 15 to 30-minute intervals or recorded continuously with a data logger. Duration of incubations will depend on the productivity within the chamber and available power supply. Highly productive colonies will produce oxygen supersaturation within the chambers, leading to diffusion of oxygen out of the water. Therefore, chamber water should be renewed periodically to avoid supersaturation.



**Figure 17**—Diagram of photosynthesis chamber designed by Aliquot (Appendix B). Each chamber, exclusive of pump and probe, weighs 2.5 kg.

3. Night measurements or opaque coverings can be used to determine respiration rates.

4. After day and night measurements, the colonized substrata are removed from the chamber. Periphyton biomass and chlorophyll-a is evaluated by scrubbing all attached algae into a known volume of water. Subsamples of the algal slurry are removed and filtered, preserved, and returned to the laboratory for analysis (see the Algae/Periphyton section). Slurry and subsample volume must be recorded in order to calculate the total chamber chlorophyll-a and AFDM values.

5. Surface area of tiles can be determined by standard geometric formulas. The surface area of rocks can be determined by weight/area relationships. The area of the rock with attached algae is covered with

aluminum foil, being careful not to overlap the foil. The foil is then weighed. This weight is multiplied by the ratio of a known area of foil to the weight of that area.

6. Primary productivity parameters are calculated as follows:  $NPP \text{ (mg/h)} = \text{Final D.O.} - \text{Initial D.O. (mg/L)} \times \text{Chamber volume (L)} / \text{Time (h)}$ . A better estimate is obtained by regressing dissolved oxygen as a function of time. The slope of this regression line (mg-L/h) multiplied by chamber volume (L) equals productivity (mg/hr). Respiration is calculated in the same manner using dark chamber data.  $GPP = NPP + \text{Respiration}$ .

7. Total daily production values can be obtained by 24 hour incubations, summing all incubations over a 24 hour period, or by estimation from productivity rates.  $\text{Estimated } NPP \text{ (mg O}_2\text{)} = NPP \text{ (mg/h)} \times \text{photoperiod (h)}$ ;  $\text{Respiration (mg O}_2\text{)} = \text{Respiration (mg/hr)} \times 24 \text{ hours}$ ;  $GPP_{24} = NPP_{DL} + \text{Respiration}_{24}$ .

8. Productivity rates or production values are standardized by area, chlorophyll-a, or biomass.

Ecosystem-level measurements can be obtained by summing individual components, or by enclosing all components within the microcosm. To encompass all or most components of ecosystem productivity, trays containing native substrata can be submerged into the streambed. After at least 1 month colonization time, the tray can be removed and placed within a recirculating-photosynthesis chamber and productivity values can be determined as above (Bott and others 1985). Productivity or production is then standardized by the area of the colonization tray which is representative of streambed area. Total algal biomass and chlorophyll-a can be determined by scrubbing all rocks within the tray and washing all organic matter and periphyton through a 1 mm sieve into a calibrated bucket. Subsamples are then removed, and preserved for chlorophyll-a and AFDM analysis. Alternatively, frames can be placed directly over the tray which has been colonized in the stream. The frame is equipped with circulating pumps and opaque or translucent tops for light and dark incubations (Pennak and Lavelle 1979; Sumner and Fisher 1979). In this case, benthic organic matter, chlorophyll-a, and algal biomass can be estimated from instream values.

Summing individual components requires separate productivity measurements with chambers containing algae, benthic organic matter, and, in some cases, transported organic matter (Minshall and others 1983; Minshall and others 1992; Naiman 1983; Naiman and Sedell 1980). Algal metabolism is evaluated as above. Benthic organic matter respiration is determined by collecting BOM passively in trays placed within the streambed, or through collection of organic matter in depositional areas, and placing this organic matter in mesh bags. Values are expressed on a weight basis (for example, g O<sub>2</sub>/g AFDM) and then extrapolated to an areal measure based on the mean standing crop of BOM. TOM can be evaluated by collecting FPOM in transport. A slurry of TOM is made and a subsample removed and injected into the chamber. Light and dark metabolism measurements are made. Ecosystem-level metabolism is the sum of all individual components.

## Carbon Turnover Length

Using the BOM, TOM, and metabolism data, estimates of carbon turnover length can be calculated. Carbon turnover length is the average distance a fixed carbon atom travels before it is respired. Carbon spiraling length is a measure of the retention and utilization of available energy sources (Minshall and others 1992). Carbon turnover length,  $S$  (m), is calculated by the following equation (Elwood and others 1982; Newbold and others 1981, 1983):

$$S = v/k, \quad (14)$$

where,  $v$  (m/s), is the downstream velocity of carbon and is the product of TOM ( $\text{g}/\text{m}^3$ ) and discharge ( $\text{m}^3/\text{day}$ ) divided by BOM ( $\text{g}/\text{m}^2$ ) and stream width (m); and,  $k$  (m/s), is the portion of benthic organic carbon respired in a year and is the ratio of benthic respiration to BOM. Respiration values measured as the change in oxygen must be multiplied by 0.375 to convert values to carbon, and TOM and BOM values are multiplied by 0.454 to convert AFDM values to carbon.

# Nutrient Dynamics

Primary production in pristine streams often is limited by low levels of macronutrients required for algal growth and reproduction. Nutrient limitation in many situations is the result of low levels of nitrogen or phosphorus, or a combination of these two elements. Stream phosphorus concentrations are the result of the weathering of phosphorus-containing minerals and atmospheric deposition throughout the stream catchment, and their subsequent transformations through upland and riparian systems. Nitrogen in streams is the result of biological fixation and atmospheric deposition within the catchment. Organic nitrogen within the catchment is mineralized and nitrified to nitrates which are mobile within the groundwater. Nitrates are transformed by biogeochemical processes within the catchment and riparian areas before entering stream water. Once these macronutrients enter the stream, their concentrations and forms are modified further by in-stream processes. These processes include biological uptake and adsorption to organic and inorganic particles, and are affected by many variables, including water velocity, benthic organic matter, and retention in transient storage areas. Transient storage areas include the portion of the stream flowing within and below the bed (hyporheic zone) but distinct from the groundwater, slow water areas along the stream margins, and backwater areas behind debris dams and other obstructions.

Wilderness-stream nutrient concentrations and nutrient limitation can be altered by disrupting natural biogeochemical processes. Monitoring nutrient dynamics can provide historic data for undisturbed streams that can be used for future comparisons. Although wilderness areas are protected from many disturbances, they are not completely isolated. For example, alterations in global temperatures can change precipitation events and mineralization rates and their role in nutrient cycling. Atmospheric inputs of nitrogen and phosphorus compounds from industrial processes can increase inputs and alter stream water pH. On a smaller scale, recreation and grazing in riparian areas can influence nutrient concentrations directly (in other words, metabolic wastes or detergents) or indirectly by altering biological and microbial processes differentially affecting specific macronutrient inputs. Therefore, understanding and monitoring of nutrient dynamics in streams can alert management agencies to potential problems and provide insight to management alternatives.

Evaluating nutrient limitation can provide information that will assist in wilderness management. For example, managers may need to determine why excessive algal accumulations are occurring around popular camping

sites and how this problem should be addressed. If previous nutrient limitation experiments had demonstrated phosphorus limitation, then the changes in periphyton abundance hypothetically could be the result of phosphorus inputs from the use of detergents. This hypothesis could be tested and, if confirmed, appropriate action could be taken.

Nutrient uptake rates are influenced by many biotic and abiotic components including the amount, type, and retention of benthic organic matter, instream nutrient concentrations, and the hyporheic and lateral movement of water. Therefore, nutrient uptake rates and retention indices provide information concerning the interrelationships between biotic and abiotic processes. For example, excessive silting of the streambed could disrupt the connection between the stream and the hyporheic/groundwater zone. This could affect stream microbial processes and the survival of organisms dependent upon the movement of water through the streambed (macroinvertebrates and salmonid eggs) and could be demonstrated by ecosystem-level measurements of nutrient uptake rates. The process is as follows:

	<b>Uptake length (m)</b>	<b>Uptake rate (<math>\mu\text{g}/\text{m}^2/\text{min}</math>)</b>	<b>Mass transfer coeff. (<math>\times 10^{-5} \text{ m/s}</math>)</b>	<b>Reference</b>
<b>Phosphorus</b>				
Second order, Idaho	370	33.6	11.2	Davis (1995)
Second order, Idaho	370	84.0	11.3	Davis (1995)
First order, North Carolina	85	18.6	31.1	Munn and Meyer (1990)
First order, Oregon	697	1.54	0.51	Munn and Meyer (1990)
First order, Tennessee	22-97	1.3-15.5	2.2-5.2	Mulholland and others (1985)
<b>Nitrogen</b>				
Second order, Idaho	549	246	8.0	Davis (1995)
Second order, Idaho	1,839	449	2.27	Davis (1995)
First order, North Carolina	689	3.9	1.08	Munn and Meyer (1990)
First order, Oregon	42	11.9	9.88	Munn and Meyer (1990)

## Methods: Stage 3

Nutrient limitation can be estimated or evaluated by a number of different methods. Estimations can be made based on the relative amounts of elements in comparison to amounts required by biota. These estimations can then be confirmed through nutrient amendments and measurements of the resulting biotic effects. Nutrient amendments can be direct or indirect through nutrient diffusing substrata. The estimation of nutrient limitation based on nitrogen:phosphorus (N:P) ratios and enrichment through nutrient diffusers is described below.

### Nutrient Limitation: N:P Ratios

An initial method for evaluating nutrient limitation uses stream water nitrogen to phosphorus ratios. This concept is based on the "Law of the Limiting Factor" which states that at any given time only one resource can

limit production. The N:P ratio is the pivotal point at which either nitrogen or phosphorus becomes the limiting agent. A high N:P ratio denotes phosphorus limitation and a low N:P ratio is indicative of nitrogen limitation.

The N:P ratio is a molar ratio of species and therefore requires conversion of nutrient analysis results (often given in mg/L) to moles. Due to the many forms of nitrogen and phosphorus found in stream waters it is important to indicate which forms are used to construct N:P ratios. Nitrogen is found as nitrate, nitrite, ammonia, and organic nitrogen, and phosphorus as ortho-pyro- meta- and organic-phosphorus either in a dissolved or particulate form. N:P ratios will differ with the forms of nitrogen or phosphorus used. Most N:P ratios are in the form of total inorganic nitrogen (sum of nitrate, nitrite, and ammonia) to dissolved orthophosphorus, dissolved total, or total phosphorus.

N:P ratios are limited in their use as a predictor of nutrient limitation because optimal ratios are species specific. In a community of many different species therefore, there may be a large range of values that signify neither nitrogen nor phosphorus limitation. In addition, intraspecific optimal N:P ratios can shift with water velocity (Borchardt 1994), light (Wynne and Rhee 1986), and temperature (van Donk and Kilham 1990). Regardless of these problems, N:P ratios can provide insight toward potential nutrient limitation. Morris and Lewis (1988) concluded that the best indicators of nutrient limitation were total dissolved inorganic nitrogen (DIN) to total phosphorus (TP) or total dissolved phosphorus (TDP). In their study phosphorus was found limiting in lake waters at ratios above 12 and 20 for DIN:TP and DIN:TDP respectively. Nitrogen limitation occurred at ratios below 2, for both ratios (DIN:TP and DIN:TDP), and co-limitation or nonlimitation occurred at values within these ranges. In streams, nitrogen has been found to limit primary production at and below 18 while phosphorus has been found limiting at ratios at or above 18 (table 8).

## Testing Potential Nutrient Limitation

Evaluation of potential nutrient limitation can be tested through enrichment of stream water and monitoring the response of primary producers. Nutrient enrichment can be obtained through direct application of dissolved nutrients to stream water (Grimm and Fisher 1986; Hill and others 1992; Lohman and others 1991) or through nutrient diffusing substrata (Bushong and Bachmann 1989; Chessman and others 1992; Coleman and Dahm 1990; Fairchild and Everett 1988; Fairchild and Lowe 1984; Fairchild and others 1985; Gibeau and Miller 1989; Grimm and Fisher 1986; Hill and Knight 1988). The method used by Gibeau and Miller (1989), described below, is particularly suited for wilderness streams due to the small size and low weight of the diffusing substrata and the small amount of nutrients released.

1. Soak porous porcelain or fused silica crucible covers (2.6 cm diameter disc, Leco Corporation #528-042) in 10 percent HCl solution for 48 hours. Rinse copiously in deionized water.

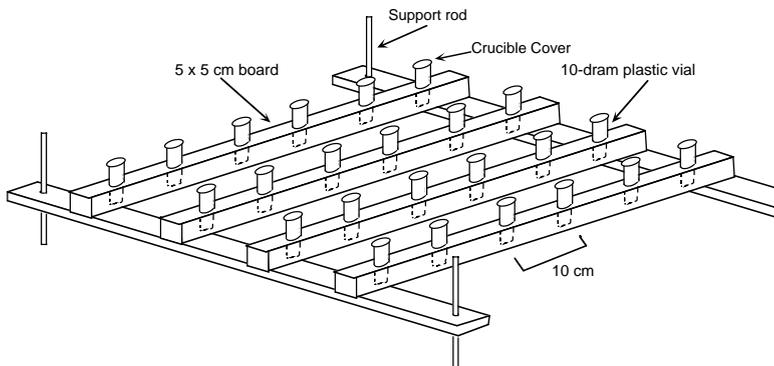
**Table 8**—Summary of stream nitrogen:phosphorus (N:P) ratios and nutrients determined limiting  
 TDN = total dissolved nitrogen; TDP = total dissolved phosphorus; TN = total nitrogen;  
 TIN = total inorganic nitrogen.

Location	N-limit	N:P	P-limit	N:P	Species	Reference
Rhine River	<10		>20		NO <sub>3</sub> -N:PO <sub>4</sub> -P	Schanz and Joun (1983)
Michigan			40		NO <sub>3</sub> -N:PO <sub>4</sub> -P	Pringle and Bowers (1984)
Alaska			60		TIN:TP	Peterson and others (1983)
Arizona	1.6-2.6				NO <sub>3</sub> -N:PO <sub>4</sub> -P	Grimm and Fisher (1986)
Missouri	<18		>19		TN:TP	Lohman and others (1991)
California	<2				NA	Hill and Knight (1988)
Australia	2				TIN:PO <sub>4</sub> -P	Chessman and others (1992)
Australia			>44		TIN:PO <sub>4</sub> -P	Chessman and others (1992)
Australia	6				TIN:PO <sub>4</sub> -P	Chessman and others (1992)
Australia			18		TIN:PO <sub>4</sub> -P	Chessman and others (1992)

2. Fill a 10-dram plastic vial (Dynalab Corporation #2636-0010) with 30 ml of 2 percent nutrient enriched or unenriched agar. Enriched agar is made by dissolving sodium nitrate (NaNO<sub>3</sub>) or potassium dibasic phosphate (KH<sub>2</sub>PO<sub>4</sub>) or both into a nutrient-free 2 percent agar solution. The agar is then heated to boiling and poured into the diffusers while still hot. The mass of chemicals added will vary with the enrichment concentrations required. The majority of studies have used 0.1 molar concentrations, which should be suitable for most wilderness streams. For 0.1 molar concentrations, 8.5 g of NaNO<sub>3</sub> and 13.6 g of KH<sub>2</sub>PO<sub>4</sub> per liter of agar are used. Treatments should include at least three replicates of control, phosphorus, nitrogen, and nitrogen plus phosphorus diffusers.

3. Once the vials are filled, heated crucible covers are melted into the top of the plastic vials, which are then turned upside down before the agar solidifies.

4. The vials are glued into 3-cm holes drilled into 5 x 5 cm (2 x 2 inch) lumber strips 70 to 100 cm long. Multiple strips can be combined to construct a rack which is then secured within the stream (fig. 18).



**Figure 18**—Nutrient diffuser frame showing vial placement within wooden crossmembers.

5. The nutrient-diffusing vials are left in the stream long enough for algal biomass to develop, but are removed before algal sloughing occurs. For most sites this will be from 10 to 30 days. After incubation, the vials are removed from the frame and the algal-colonized-crucible covers carefully lifted from the vial tops. The attached periphyton is scraped into a 250-ml graduated cylinder (or other suitable container) filled with 100 ml of water. Subsamples of this algal slurry can be removed for algal species identification prior to filtering. The filtered algae can then be analyzed for chlorophyll-a and AFDM (see *Algae/Periphyton* section). Surface area is calculated from the area of exposed crucible covers and area-specific chlorophyll-a, or AFDM values can be used to test for significant differences among treatments.

In some cases, neither nitrogen, phosphorus, or nitrogen and phosphorus enrichment results in any differential algal response. This implies that some other factor is limiting algal accumulation such as micronutrients (Pringle and others 1986), or light (Hill and Knight 1988; Triska and others 1983), or that differences are masked by grazing macroinvertebrates (Hill and others 1992). Evaluation of light limitation can be tested by placing sets of diffusers in locations within a stream that vary in light intensity. In this case greater care should be taken to insure that other factors are similar between sites, in particular current velocity. Testing for micronutrient limitation involves modification of elements dissolved within the agar matrix.

## **Ecosystem Uptake Parameters: Open System Methods**

Under conditions of nutrient limitation, the retention of elements is essential for the productivity of the system. Uptake parameters also are a measure of the “intactness” and proper functioning of stream ecosystems. The ability of a stream to retain nutrients is best described by the nutrient spiraling concept (Newbold and others 1981). Essentially, spiraling length is the distance a nutrient atom travels in dissolved form (uptake length) plus the distance traveled in particulate form (turnover length). Under base flow conditions, uptake length dominates total spiraling length, due to the rapid movement of nutrients in the water column. Uptake length is a function of uptake rate, streamwater nutrient concentrations, and water velocity. Therefore uptake length can be calculated by measuring these parameters.

The uptake of nutrients from the water column occurs through autotrophic and heterotrophic processes. Nutrients are removed from the water column by algae and incorporated into algal biomass, and by bacteria and fungi which remove nutrients from the water column to augment the breakdown of organic matter. The relative importance of these two uptake processes will vary with the stream in question. In many headwater streams, phosphorus uptake has been shown to be a function of the amount of benthic organic matter available (Mulholland and others 1985; Newbold and others 1983); however, in streams where autotrophic processes

dominate, algal uptake may dominate (Grimm 1987). The relative importance of these two processes is related to P/R ratios from productivity measurements.

The retention of nutrients is a measure of stream channel stability or the efficient use of available elements. Where organic carbon is the major site of nutrient uptake, the ability of the system to hold this organic matter will be important in nutrient retention. Undisturbed headwater streams, typical of wilderness areas, have been shown to be effective in organic matter retention (Minshall and others 1983). Retention of organic matter is the result of physical and biotic processes. Physical processes include debris dams, pools, and large woody debris in the stream channel. Biotic processes may include filtering of organic matter in transport by filter feeding invertebrates. Autotrophic uptake may be enhanced by the rapid regeneration of algal biomass as a result of invertebrate grazing. Loss of these biotic and abiotic processes, therefore, will lead to the inability of a stream to utilize process-limiting nutrients.

Nutrient uptake rate and uptake length, from whole stream nutrient releases, can be determined through two different methods. Both methods require the release of a conservative tracer in addition to the biologically active element under consideration. These two methods and their advantages and disadvantages were described by the Stream Solute Workshop (1990). The first method requires fitting the data obtained from the change in tracer and nutrient concentrations over distance to a mathematical model describing the dispersion of elements in the water column and uptake. The second method uses data obtained from the injection to directly estimate uptake rates and length (Munn and Meyer 1990). This second method will be described below, and entails injection of a  $\text{NO}_3\text{-N-PO}_4\text{-P}$ -chloride solution, and measurement of the resulting concentration at successive locations downstream. The solution is injected at a constant rate at an upstream location. The injection continues until constant elevated stream water nutrient concentrations (plateau concentrations) are obtained throughout the study reach. Replicate samples of the plateau concentrations are taken at multiple transects throughout the study reach. These water samples are then analyzed for  $\text{NO}_3\text{-N}$ ,  $\text{PO}_4\text{-P}$ , and chloride. The change in nutrient concentrations over distance, corrected by the change in chloride concentrations, is used to determine uptake.

1. The first step is the determination of the concentration of solutes in the injectate. This requires prior knowledge of stream water nitrogen, phosphorus, and chloride concentrations and stream discharge. Plateau concentrations should not exceed stream water concentrations by a large amount (usually 3 to 4 times background concentrations) and stream water N:P ratios should be maintained (Stream Solute Workshop 1990). Once plateau concentrations are determined, solution concentration and injection rate can be determined by the following formula (example 5):

$$Q = \frac{Q_i C_i}{C_p - C_b}, \quad (15)$$

where  $Q$  is discharge,  $C$  is concentration, and the subscript  $i$ , stands for injectate,  $p$ , for plateau, and  $b$ , for background. The limits of solute concentration are set by their saturation values, and the limits of the injection rate are determined by the metering pump or other means of nutrient injection being used. Saturation values are variable among sites and difficult to determine. However, as a general rule, stream water concentrations should be at or below 0.10 mg/L-N and 0.005 mg/L-P.

2. Once injectate concentrations are calculated, the total amount of nitrogen, phosphorus, and chloride salts needed should be determined, weighed out, and packaged in the laboratory in zip-lock bags or whirl-paks.

3. New water-sample bottles should be obtained with a separate bottle for each element, sample time, and transect. For example, if samples of the three elements are to be taken at seven transects, at eight different times (multiple samples of plateau concentrations) then 168 sample bottles are required. Sample bottles should be prelabelled.

4. The reach length and transect location should be determined before beginning the injection. Reach lengths should be long enough to ensure depletion of nutrient concentrations, but short enough to reduce the accrual of groundwater. In small streams (1-4 L/s discharge) 20-m reaches may be adequate whereas reaches of 300 m or longer will be required in larger streams (100-200 L/s). Five to seven transects are spaced evenly throughout the stream reach. The exact distance from the injection point to each transect is measured, and each transect identified with flagging or other marker.

5. The nutrient salts, 1-L graduated cylinder, 100-ml graduated cylinder, mixing bucket (4-6 L), metering pump, and 12-VDC battery are then carried to the upstream end of the reach. Stream water is used to dissolve the nutrients in the mixing bucket. The metering pump is used to drip the solution into the stream at the predetermined injection rate ( $Q_i$ ) and roughly 10 m above the first sampling transect. The injection rate should be determined manually prior to and after the injection, or

**Example 5**—Calculation of nutrient concentrations for uptake length experiments.

Stream water nutrient concentrations are 0.046 mg/L  $\text{NO}_3\text{-N}$ , 0.005 mg/L  $\text{PO}_4\text{-P}$ , and 0.22 mg/L Cl. Stream discharge is 170 L/s. Plateau concentrations desired are 0.1 mg/L  $\text{NO}_3\text{-N}$ , 0.011 mg/L  $\text{PO}_4\text{-P}$ , and 1.00 mg/L Cl. Injection rate will be 50 ml/min or  $8.3 \times 10^{-4}$  L/s.

Solving the formula for  $\text{NO}_3\text{-N}$ :

$$C_i = C_p - C_b)Q/Q_i = \\ (0.10 - 0.046)(170/8.3 \times 10^{-4}) = \\ 11,016 \text{ mg/L or } 11.02 \text{ g/L.}$$

For a two hour injection at 50 ml/min, the total volume required ( $50 \times 120$ ) will be 6.0 L. Therefore 66.12 g ( $6 \times 11.02$ ) of  $\text{NO}_3\text{-N}$  will be required. The total amount of nitrate salt as  $\text{NaNO}_3$  will be 66.12 g  $\text{NO}_3\text{-N}$  times the molecular weight of  $\text{NaNO}_3$  divided by the molecular weight of N.

$$\text{g NaNO}_3 = 66.12(85/14.01) = 401.2$$

The same computations are used for phosphorus and chloride.

continuously with an in-line meter. The solution is dispensed upstream of a turbulent area to allow complete mixing by the first sampling transect.

6. The nutrients are allowed to drip into the stream until plateau concentrations are reached. The time required to reach plateau will increase as transient storage areas increase. However, an hour generally is enough time to reach plateau. If based on stream morphology, an extensive hyporheic area is expected, initial injections of a NaCl solution could be used to determine the time required to reach plateau.

7. Once plateau concentrations are reached, water samples are taken roughly every 10 minutes at each transect. The total number of samples or duration of sampling is variable. Multiple samples provide a better measurement of plateau concentrations but require longer injection times. Measurements of conductivity can be used to replace chloride sampling and analysis.

8. After the sampling regime is completed, water samples are filtered and preserved for analysis (see section on water chemistry). The results from the water chemical analysis are then used to determine uptake rates and uptake length. Uptake lengths are calculated by solution of the following formula:

$$A_x = e^{-x/S_w}, \quad (16)$$

where  $A_x$  = the ratio of observed to predicted concentrations at distance 'x',  $x$  = distance downstream, and  $S_w$  = uptake length. Uptake length is then calculated by the same methods used to determine decay rate constants; that is, the  $\ln$  of  $A_x$  is plotted as a function of distance downstream. The slope of this line is  $1/S_w$ , so the inverse of the slope is uptake length (example 6 and fig. 19).

Predicted concentrations are based on the dilution of the conservative tracer and are calculated by the formula (Hart and others 1992):

$$C_p = C_o \frac{Cl_x}{Cl_o}, \quad (17)$$

Where  $C_p$  = the predicted concentration,  $C_o$  = concentration at transect 1,  $Cl_o$  = chloride concentration (or conductivity) at transect 1, and  $Cl_x$  = chloride concentration at transect  $x$ .

The uptake parameters, uptake rate and mass transfer coefficient ( $U/C$ ), can be calculated from their relationship to uptake length, water velocity, and mean depth (Stream Solute Workshop 1990). This relationship is shown in the following equation:

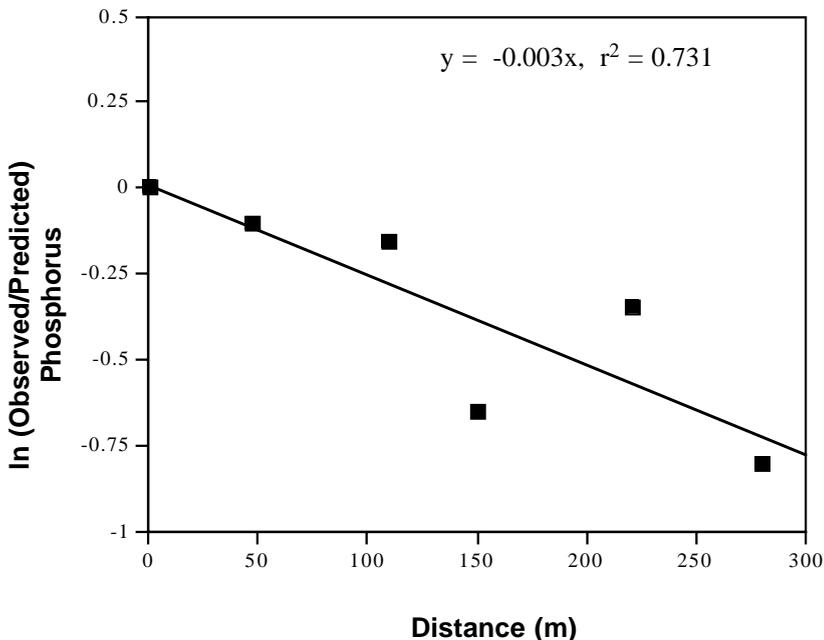
$$S_w = \frac{vh}{U/C}, \quad (18)$$

where  $U$  = uptake rate ( $\text{mg}/\text{m}^2/\text{s}$ ),  $C$  = concentration ( $\text{mg}/\text{m}^3$ )  $v$  and  $h$  are mean water velocity (m/s) and mean depth (m), respectively.

The uptake rate calculated above can be corrected for background streamwater concentrations. This correction is based on the assumption that at below limiting levels of nutrients, uptake increases proportionally with stream water concentrations. That is, the mass transfer coefficient

**Example 6**—Water samples were analyzed for chloride and phosphorus at 6 transects extending 280 m downstream. Expected phosphorus concentrations remained constant because plateau chloride concentrations did not change throughout the reach. The natural log of observed to expected concentrations is plotted against downstream distance (fig. 21). Uptake length was 333 m. Velocity was 0.28 m/s, mean depth 0.157 m, background  $\text{PO}_4\text{-P}$  concentrations were  $5 \text{ mg/m}^3$ , and plateau concentrations  $10 \text{ mg/m}^3$ . From equation 22, uptake rate, at plateau, was  $0.00132 \text{ mg/m}^2/\text{s}$  or  $79.2 \text{ } \mu\text{g/m}^2/\text{min}$ . Using equation 23, uptake rate was  $39.6 \text{ } \mu\text{g/m}^2/\text{min}$  at background stream water concentrations.

Transect	Distance <i>m</i>	Chloride <i>mg/L</i>	Observed $\text{PO}_4\text{-P}$ <i>mg/L</i>	Expected $\text{PO}_4\text{-P}$ <i>mg/L</i>	ln (observed/expected)
1	25.6	2.38	0.005	0.005	0.00
2	60.0	2.38	0.0055	0.005	-0.10
3	133.5	2.38	0.004	0.005	-0.16
4	175.5	2.38	0.0026	0.005	-0.65
5	220.3	2.38	0.0035	0.005	-0.35
6	282.9	2.38	0.00225	0.005	-0.88



**Figure 19**—The natural log of the ratio of observed to expected phosphorus concentrations is plotted against downstream distance. Uptake length,  $S_w$ , is the negative inverse of the slope of the regression relationship. Uptake Length =  $1/0.003$  or 333 m.

(uptake/concentration) is a constant under increasing concentrations (at a given time and location) below saturation. Therefore uptake at stream water concentrations is equal to:

$$U_c = C_b \frac{U_p}{C_p} \quad (19)$$

where  $U_c$  = corrected uptake rate,  $U_p$  = uptake at plateau concentrations,  $C_p$  = plateau concentration, and  $C_b$  = background concentration.

Uptake length is the average distance an element will travel before being taken up by the biota. Equation 17 demonstrates that uptake length is a combination of physical factors, such as water velocity and stream depth, and biotic factors, such as uptake rate per concentration or mass transfer coefficient. Uptake length should, therefore, increase with stream order and the associated increase in velocity and depth. In streams of similar size and slope, uptake length will increase as physical complexity of the channel decreases. The mass transfer coefficient will decrease as a result of factors influencing biotic activity and the total area available for biotic uptake. Siltation of the streambed will reduce the active area for periphyton

production and storage of allochthonous organic matter, decreasing nutrient uptake rates and causing uptake length to increase. Impacts including disruption of riparian nutrient dynamics, alterations of organic matter input and storage, alterations in litter quality, nutrient loading, channelization, and loss of retention devices, can potentially alter the functional integrity of streams and can be monitored through measurements of uptake parameters in stream ecosystems.

## Stage 4: Component Uptake Parameters \_\_\_\_\_

Measuring nutrient uptake in streams is analogous to measuring primary production. That is, individual components or intact systems can be evaluated. Chambers can be used for individual components or intact micro/mesocosm measurements, while nutrient injections (stage 3) can be used for whole-system measurements. Like productivity measurements, individual component measurements allow the separation and identification of active areas of uptake but are susceptible to the compounding minor errors during addition of components and extrapolation to whole stream values. The enclosure of intact systems within chambers reduces the magnification of errors but does not provide a means to identify active areas and still requires extrapolation to whole stream values. Both chamber methods likely exclude uptake within the hyporheic zone. Nutrient injections provide the most precise measurement of ecosystem level uptake parameters but must be combined with chamber studies to isolate and determine the relative importance of different components.

Measuring nutrient uptake rates in chambers (Duff and others 1984; Grimm 1987) can be accomplished simultaneously with chamber productivity measurements (see Primary Production section). Once the component in question, either algae, or detritus, or a tray containing both, is placed within the chamber, initial water samples are taken to determine nutrient concentrations (see Water Quality section). After each productivity run, or prior to flushing the chambers, a second water sample is taken. Water samples are analyzed for nitrate nitrogen, ammonia, and dissolved orthophosphorus.

Net uptake ( $U$ ) is calculated as the initial concentration ( $C_1$ ) minus the final concentration ( $C_2$ ), times chamber volume ( $V$ ), and divided by time ( $t$ ). That is:

$$U = \left( \frac{C_1 - C_2}{t_2 - t_1} \right) V. \quad (20)$$

This value is standardized by area, chlorophyll-a, or AFDM. These values can then be converted to values relative to the abundance of the particular component present in the test stream. For example, if uptake associated with BOM was 0.1 mg-P/g-AFDM/hr and stream BOM was 10 g-AFDM/m<sup>2</sup>, then instream uptake of BOM would be 0.1 x 10 or 1 mg-P/m<sup>2</sup>/hr. This same procedure is then used for each of the components measured. Total area uptake rates would be the sum of rates for each individual component.

Once total area uptake rates are known, uptake lengths can be calculated from the following equation (Stream Solute Workshop 1990).

$$S_w = \frac{vd}{U/C} \quad (21)$$

Where  $S_w$  = uptake length (m),  $v$  = mean stream water velocity (m/s),  $d$  = mean depth (m),  $U$  = uptake rate (mg/m<sup>2</sup>/s), and  $C$  = stream water element concentration (mg/m<sup>3</sup>).

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# Appendix A: Wilderness Monitoring Equipment List

## Stage 1

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### **Temperature**

- Maximum/Minimum thermometer or Hobo temperature data logger
- Protective PVC case
- Plastic coated steel cable
- U clamps
- Pliers

### **Substratum**

- Data sheet
- Meter sticks

### **Water Quality**

- pH meter and probe with buffer solutions (pH 10 and pH 4)  
(thermometer if not available with probe)
- Conductivity meter and probe
- Turbidity meter and probe
- Water analysis kit packed in Rubbermaid or other sealable container containing:
  - 60-ml plastic syringe or 100-ml plastic graduated cylinder
  - 0.02-N H<sub>2</sub>SO<sub>4</sub>, 5-ml per sample
  - Distilled water, 25-ml per sample
  - 250-ml Erlenmeyer flask
  - Calibrated dispenser
  - Stirring rod
  - Buffer solution
  - Indicator (hardness)
  - Standard 0.01 M EDTA titrant

### **Fish**

- Neoprene wetsuit
- hood
- gloves
- mask
- snorkel

### **Macroinvertebrates**

- Surber or Hess nets
- Whirl-pak bags, 5 for each site

500 ml bottle of formalin  
Shoulder length gauntlets (optional)  
Glue, needles, thread, glue stick (repair)  
Benthic sampling kit packed in canvas bag  
Labels  
Plastic pan  
Cone shaped bag  
Ring stand  
Forceps  
Spatula  
Pencils  
Marking pens  
RR spike  
250 ml Nalgene wash bottle

## Stage 2

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In addition to items contained under Stage 1 add:

### **Solar Radiation**

Pyranometer or PAR probe and meter

### **Discharge**

Data sheets  
Teflon tape 50-100 meter  
Meter stick

### **Substratum**

20-to-30 meters of polyethylene tubing or clinometer

### **Water Quality**

Portable Spectrophotometer with cuvettes  
Add to water analysis kit  
125-ml Erlenmeyer flask  
SulfaVer powder  
NitraVer VI  
NitriVer III  
PhosVer 3

### **Periphyton**

Pre-fired filters, 5 for each site  
Dewar's flask or suitable alternative  
Sampling kit packed in canvas bag  
Cushing samplers  
Plastic brush  
Filter manifold and funnel assembly  
25 ml Nalgene pipettes with bulb  
Forceps  
Pencils  
Marking pen  
Nunc tubes

## Stage 3

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In addition to items listed under Stage 1 and Stage 2

### **Discharge**

- Staff gauge, pressure transducer, or other alternatives
- Water velocity meter

### **Water Quality**

- Dropper with sulfuric acid
- 0.45 micron filters stored in distilled water
- 60 ml sterile syringes with filter caps
- Marking tape and permanent pen
- Cooler for storing water samples
- 250-ml plastic storage containers

### **Transported Organic Matter**

- Transport frames (20 x 35 mm)
- Transport nets (100 micrometer mesh)
- 9.5-mm diameter Rebar (50 cm length)
- Whirl-pak bags
- 500-ml bottle formalin
- 250-ml Nalgene wash bottle
- Forceps
- Stopwatch
- Digital flow meter

### **Whole System Nutrient Release**

- Preweighed nutrient salts
- Metering pump
- Sample vials-acid washed, but not with HCl.
- Marking tape
- Mixing bucket
- Stop watches, one for each transect
- Additional filters and sulfuric acid preservative

### **Nutrient Limitation**

- Nutrient diffusers
- Extra filters and nunc tubes

## Stage 4

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In addition to items contained in Stages 1 through 3

**Primary Production** (will vary with type of chamber and method used)

- Chambers
- Extra tubing and fittings
- Extra stopcocks
- Pumps and circuit box
- Extra fuses, 1A250V
- Extra pump
- 9 volt battery for volt meter
- Two rechargeable 12 VDC batteries

Battery charger (optional)  
Power source for charger, solar or water power (optional)  
D.O. probe and meter  
Data logger (optional)  
Substrate tiles or Trays

**Decomposition-Leaf Packs**

Pre-weighed (10 g dry weight each) marked packs. 20 for each site  
Additional whirl-pak bags  
An additional 500-ml bottle of formalin  
Metal stakes (16 cm nails), 20 for each site

**Miscellaneous**

Clinometer  
Topographic maps  
Camera- film, polarized filter.  
Data book  
Global Positioning System

# Appendix B: Vendor List

Addie Sewing  
531 S. Charles Street  
Salmon, ID 83467  
(208) 756-2291

Aldrich Chemical Company  
1001 W. Street Paul Avenue  
Milwaukee, WI 53201-9358  
(800) 558-9160

Aliquot  
P.O. Box 2616  
Boise, ID 83701  
(208) 322-8950

Alpkem Corporation  
P.O. Box 1260  
Clackamas, AZ 97015  
(800) 547-6275

Aquacare Environment Incorporated  
P.O. Box 4315  
Bellingham, WA 98227  
(368) 734-7964

Aquaculture Research Association  
P.O. Box 1303  
Homestead, FL 33090  
(305) 248-4205

Aquatic Ecosystem Incorporated  
2056 Apopka Blvd.  
Apopka, FL 32703  
(407) 886-3939

Bausch & Lomb  
635 St. Paul Street  
Rochester, NY 14602  
(716) 338-6000

Beckman Instruments Incorporated  
Diagnostic Division  
250 S. Krasmen Blvd.  
Le Brea, CA 92621  
(800) 526-5821

BelArt Products  
Pequanock, NJ 07440-1992  
(201) 694-0500

Ben Meadows Company  
3589 Broad Street  
Atlanta, GA 30341  
(800)241-6401

Benz Microscope Optics  
749 Airport Blvd. S1A  
Ann Arbor, MI 48107  
(313) 994-3880

Campbell Scientific Incorporated  
815 W. 1800 N.  
Logan, UT 84321-1784  
(801) 753-1342

Coffelt Manufacturing  
1311 E. Butter Avenue BDGB  
Flagstaff, AZ 86001  
(602) 774-8829

Cole Palmer  
625 E. Bunker Court  
Vernon Hills, IL 60061  
(800) 323-4340

Cryogenics Northwest  
4401 Airport Way South  
Seattle, WA 98108  
(206) 224-0430

Desert Research Institute  
7110 Dandini Blvd.  
Reno, NV 89512  
(702) 673-7300

Difco Laboratories  
P.O. Box 331058  
Detroit, MI 48232-7058  
(313) 462-8500

Duraframe Airport  
Route 2, Box 166  
Viola, WI 54664  
(608) 538-3140

Hach Chemical Co.  
P.O. Box 589  
Loveland, CO 80537  
(800) 227-4224

Dynalab Corporation  
Box 112  
Rochester, NY 14601  
(888)345-6040

H,OFX  
75 W. 100 S.  
Logan, UT 84321  
(801) 753-2212

Dynatech Laboratories  
14340 Sullyfield Circle  
Chantilly, VA 22021  
(800) 336-4543

Kahl Scientific Instruments  
P.O. Box 1166  
El Cajon, CA 92022-1166  
(619) 444-2158

Epic Incorporated  
654 Madison Avenue  
Suite 1706  
New York, NY 10021-8404

Lab-line Instruments Incorporated  
15th and Bloomindale Avenue  
Melrose Park, IL 60160-1491  
(800) 523-0257

Fisher Scientific  
2170 Martin Avenue  
Santa Clara, CA 95050-2780  
(603) 929-2650

Leco Corporation  
3000 Lakeview Avenue  
St. Joseph, MI 49085  
(800) 292-6141

Floy Tag & MFC, Incorporated  
4616 Union Bay Place, NE  
Seattle, WA 98105  
(206) 524-2700

Li-Cor Incorporated  
P.O. Box 4425  
Lincoln, NE 68504  
(800) 447-3576

Forest Densimeter  
5333 SE Cornel Drive  
Bartlerville, OK 74006

Markson Sciences Incorporated  
P.O. Box 1359  
Hillsboro, OR 97123  
(800) 528-5114

Freshwater Ecosystems  
2056 Apopha Boulevard  
Apopha, FL 32703-9950  
(800) 422-3939

Marsh McBirney  
4539 Metropolitan Center  
Fredrick, MD 21701  
(800) 368-2723

Forestry Suppliers Incorporated  
205 W. Rankor St.  
P.O. Box 8397  
Jackson, MS 39284-8397  
(800) 647-5368

Martek Instruments  
P.O.Box 97067  
Raleigh, NC 27624  
(800)628-8834

Frigid Units Incorporated  
3214 Sylvania Avenue  
Toledo, OH 43613  
(419) 474-6971

Onset Instruments Corporation  
P.O. Box 3450  
Pocasset, MA 02559  
(508) 563-9000

Gelman Sciences  
600 S. Wagner Rd.  
Ann Arbor, MI 48106-1448  
(313) 665-0651

Orion Research  
529 Main Street  
Boston, MA 02129  
(800) 225-1480

Philips Electronic Instruments  
P.O. Box 5370  
Arvada, CO 80005-5370  
(303) 467-9970

Real Goods  
966 Mazzoni Street  
Ukiah, CA 95482-3471  
(707) 468-9292

Royce Instruments Corporation  
13555 Gentilly Road  
New Orleans, LA 70129  
(800) 347-3505

S & M Microscopes Incorporated  
4815 List Drive, Suite 118  
Colorado Springs, CO 80919  
(719) 894-0123

Sargent Welch Scientific  
911 Commerce Court  
Buffalo Grove, IL 60089-2362  
(800) 727-4368

Sigma Chemical Company  
P.O. Box 14508  
St. Louis, MO 63178  
(800) 325-3010

So-Low Environment Equipment  
10310 Spartan Drive  
Cincinnati, OH 45215-1279  
(503) 772-9110

Solar Pathfinder  
25720 465th Avenue  
Hartford, SD 57033-6428  
(605) 528-6473

Tetho  
333 South Highland Avenue  
Briarcliff Manor, NY 10510  
(914) 941-7767

Thomas Scientific  
P.O. Box 99  
Swedesboro, NJ 08085-0099  
(800) 345-2100

Union Carbide Corporation  
Cryogenic Equipment  
4801 W. 16th St.  
Indianapolis, IN 46224  
(203)794-2000

USA Chemical Company  
South Highway  
Idaho Falls, ID 83401  
(208) 523-5816

Weathermeasue Corporation  
P.O. Box 41257  
Sacramento, CA 95841  
(209) 824-6577

Whatman Lab Sales  
P.O. Box 1359  
Hillsboro, OR 97123-9981  
(800) 942-8626

Wheaton Scientific  
1000 North 10th Street  
Millsville, NY 08332  
(609) 825-1100

Wildfire Materials Incorporated  
Route 1, Box 427A  
Carbondale, IL 62901  
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# Appendix C: Macroinvertebrate List

Taxon name	Taxon level	Order	Family	TV <sup>1</sup>	FFG <sup>2</sup>
ANNELLIDA	Phylum			5	CG
BRANCHIOBELLIDA	Class				
Branchiobdellidae	Family	Branchiobdellidae	Branchiobdellidae	6	CG
HIRUDINEA	Class			10	PR
OLIGOCHAETA	Class				CG
Tubificidae	Family	Tubificida	Tubificidae	10	CG
<i>Tubifex</i>	Genus	Tubificida	Tubificidae	10	CG
ARTHROPODA	Phylum				
ARACHNOIDEA	Class				
Acari	Order	Acari			PR
CRUSTACEA	Class			8	CG
Amphipoda	Order	Amphipoda		4	CG
Gammaridae	Family	Amphipoda	Gammaridae		
<i>Gammarus</i>	Genus	Amphipoda	Gammaridae	4	CG
<i>Anisogammarus</i>	Genus	Amphipoda	Gammaridae	4	CG
Talitridae	Family	Amphipoda	Talitridae	8	CG
<i>Hyallela azteca</i>	Species	Amphipoda	Talitridae	8	CG
Cladocera	Order	Cladocera		8	CF
Copepoda	Order	Copepoda		8	CG
Decapoda	Order	Decapoda		8	SH
Astacidae	Family	Decapoda	Astacidae	8	SC
<i>Pacifastacus connectens</i>	Species	Decapoda	Astacidae	6	OM
<i>Pacifastacus leniusculus</i>	Species	Decapoda	Astacidae	6	OM
<i>Pacifastacus gambelii</i>	Species	Decapoda	Astracidae	6	OM
Eubranchiopoda	Order	Eubranchiopoda		8	CF
Ostracoda	Order	Ostracoda		8	CG
INSECTA	Class				
Coleoptera	Order	Coleoptera			PR
Amphizoidae	Family				

(con.)

Appendix C (Con.)

Taxon name	Taxon level	Order	Family	TV <sup>1</sup>	FFG <sup>2</sup>
<i>Amphizoa</i>	Genus	Coleoptera	Amphizoidea	1	PR
Carabidae	Family	Coleoptera	Carabidae		PR
Dryopidae	Family	Coleoptera	Dryopidae	5	SH
<i>Helichus</i>	Genus	Coleoptera	Dryopidae	5	SH
<i>Helichus striatus foveatus</i>	Species	Coleoptera	Dryopidae	5	SH
Dytiscidae	Family	Coleoptera	Dytiscidae		PR
<i>Oreodytes</i>	Genus	Coleoptera	Dytiscidae	5	PR
Elmidae	Family	Coleoptera	Elmidae	4	CG
<i>Ampumixis dispar</i>	Species	Coleoptera	Elmidae	4	CG
<i>Atractelmis</i>	Genus	Coleoptera	Elmidae	4	CG
<i>Cleptelmis</i>	Genus	Coleoptera	Elmidae	4	CG
<i>Cleptelmis ornata</i>	Species	Coleoptera	Elmidae	4	CG
<i>Dubiraphia</i>	Genus	Coleoptera	Elmidae	4	CG
<i>Gonielmis</i>	Genus	Coleoptera	Elmidae	5	CG
<i>Heterilmnius</i>	Genus	Coleoptera	Elmidae	4	CG
<i>Heterilmnius corpulentus</i>	Species	Coleoptera	Elmidae	4	CG
<i>Lara avara</i>	Species	Coleoptera	Elmidae	4	CG
<i>Microcyloopus</i>	Species	Coleoptera	Elmidae	4	SH
<i>Microcyloopus similis</i>	Genus	Coleoptera	Elmidae	2	CG
<i>Narpus</i>	Genus	Coleoptera	Elmidae	4	CG
<i>Narpus concolor</i>	Species	Coleoptera	Elmidae	4	CG
<i>Obobrevia nubrifera</i>	Species	Coleoptera	Elmidae	4	CG
<i>Optoservus</i>	Genus	Coleoptera	Elmidae	4	SC
<i>Optoservus castanipennis</i>	Genus	Coleoptera	Elmidae	4	SC
<i>Optoservus divergens</i>	Species	Coleoptera	Elmidae	4	SC
<i>Optoservus quadrimaculatus</i>	Species	Coleoptera	Elmidae	4	SC
<i>Optoservus seriatus</i>	Species	Coleoptera	Elmidae	4	SC
<i>Rhizelmis</i>	Genus	Coleoptera	Elmidae	7	SC

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Taxon name	Taxon level	Order	Family	TV <sup>1</sup>	FFG <sup>2</sup>
<i>Stenelmis</i>	Genus	Coleoptera	Elmidae	7	SC
<i>Zaitzevia</i>	Genus	Coleoptera	Elmidae	4	CG
<i>Zaitzevia milleri</i>	Species	Coleoptera	Elmidae	4	CG
<i>Zaitzevia parvula</i>	Species	Coleoptera	Elmidae	4	CG
<i>Gyrinus</i>	Genus	Coleoptera	Gyrinidae	5	PR
Halipitidae	Family	Coleoptera	Halipitidae	7	MH
<i>Brychius</i>	Genus	Coleoptera	Halipitidae		SC
Hydrophilidae	Family	Coleoptera	Hydrophilidae	5	PR
<i>Crenitis</i>	Genus	Coleoptera	Hydrophilidae	5	PR
Psephenidae	Family	Coleoptera	Psephenidae	4	SC
<i>Eubrianix edwardsi</i>	Species	Coleoptera	Psephenidae	4	SC
<i>Psephenus falli</i>	Species	Coleoptera	Psephenidae	4	SC
Diptera	Order	Diptera		7	UN
<i>Atherix</i>	Genus	Diptera	Athericidae	2	PR
<i>Atherix variagata</i>	Species	Diptera	Athericidae	2	PR
Blephariceridae	Family	Diptera	Blephariceridae	0	SC
Ceratopogonidae	Family	Diptera	Ceratopogonidae	6	PR
Chironomidae	Family	Diptera	Chironomidae	6	OM
<i>Bezzia</i>	Genus	Diptera	Chironomidae	6	CG
<i>Boreochlus</i>	Genus	Diptera	Chironomidae	6	CG
<i>Boreocheptagya</i>	Genus	Diptera	Chironomidae	6	CG
<i>Brillia</i>	Genus	Diptera	Chironomidae	5	SH
<i>Brillia flavifrons</i>	Species	Diptera	Chironomidae	5	SH
<i>Brillia retifinis</i>	Species	Diptera	Chironomidae	5	SH
<i>Brundiniella</i>	Genus	Diptera	Chironomidae	6	PR
<i>Cardiocladius</i>	Genus	Diptera	Chironomidae	5	PR
<i>Gaetocloadius</i>	Genus	Diptera	Chironomidae	6	CG
<i>Chironomus</i>	Genus	Diptera	Chironomidae	10	CG
<i>Cladotanytarsus</i>	Genus	Diptera	Chironomidae	7	CG

(con.)

Appendix C (Con.)

Taxon name	Taxon level	Order	Family	TV <sup>1</sup>	FFG <sup>2</sup>
<i>Conchapelopia</i>	Genus	Diptera	Chironomidae	6	PR
<i>Constempellina</i>	Genus	Diptera	Chironomidae	6	CG
<i>Corynoneura</i>	Genus	Diptera	Chironomidae	7	CG
<i>Cricotopus</i>	Genus	Diptera	Chironomidae	7	OM
<i>Cricotopus bicinctus</i>	Species	Diptera	Chironomidae	7	OM
<i>Cricotopus festivellus</i>	Species	Diptera	Chironomidae	7	OM
<i>Cricotopus isocladus</i>	Species	Diptera	Chironomidae	7	OM
<i>Cricotopus nostocladius</i>	Species	Diptera	Chironomidae	7	OM
<i>Cricotopus tremulus</i>	Species	Diptera	Chironomidae	7	OM
<i>Cricotopus trifasciata</i>	Species	Diptera	Chironomidae	7	OM
<i>Cryptochironomus</i>	Genus	Diptera	Chironomidae	8	PR
<i>Diamesa</i>	Genus	Diptera	Chironomidae	5	CG
<i>Dicrotendipes</i>	Genus	Diptera	Chironomidae	8	CG
<i>Einfeldia</i>	Genus	Diptera	Chironomidae	9	CG
<i>Endochironomus</i>	Genus	Diptera	Chironomidae	10	OM
<i>Eukiefferiella</i>	Genus	Diptera	Chironomidae	8	OM
<i>Eukiefferiella brehmi</i>	Species	Diptera	Chironomidae	8	OM
<i>Eukiefferiella brevicar</i>	Species	Diptera	Chironomidae	8	OM
<i>Eukiefferiella claripennis</i>	Species	Diptera	Chironomidae	8	OM
<i>Eukiefferiella devonica</i>	Species	Diptera	Chironomidae	8	OM
<i>Eukiefferiella graeci</i>	Species	Diptera	Chironomidae	8	OM
<i>Eukiefferiella pseudomontana</i>	Species	Diptera	Chironomidae	8	OM
<i>Heleniella</i>	Genus	Diptera	Chironomidae	6	UN
<i>Heterotrissocladus subpilosus</i>	Species	Diptera	Chironomidae	0	CG
<i>Hydrobainus</i>	Genus	Diptera	Chironomidae	8	SC
<i>Larsia</i>	Genus	Diptera	Chironomidae	6	PR
<i>Limnophyes</i>	Genus	Diptera	Chironomidae	8	CG
<i>Lopescladius</i>	Genus	Diptera	Chironomidae	6	CG
<i>Macropelopia</i>	Genus	Diptera	Chironomidae	6	PR

(con.)

Appendix C (Con.)

Taxon name	Taxon level	Order	Family	TV <sup>1</sup>	FFG <sup>2</sup>
<i>Micropsectra</i>	Genus	Diptera	Chironomidae	7	CG
<i>Microtendipes</i>	Genus	Diptera	Chironomidae	6	CF
<i>Monodiamesa</i>	Genus	Diptera	Chironomidae	7	C
<i>Monopelopia</i>	Genus	Diptera	Chironomidae	6	PR
<i>Nanocladius</i>	Genus	Diptera	Chironomidae	3	C
<i>Nilotanytus</i>	Genus	Diptera	Chironomidae	6	PR
<i>Nimbocera</i>	Genus	Diptera	Chironomidae	6	C
<i>Odontomesa</i>	Genus	Diptera	Chironomidae	4	C
<i>Oliveridia</i>	Genus	Diptera	Chironomidae	6	C
<i>Orthocladius</i>	Genus	Diptera	Chironomidae	6	CG
<i>Orthocladius complex</i>	Species	Diptera	Chironomidae	6	CG
<i>Orthocladius eudactylocladius</i>	Species	Diptera	Chironomidae	6	CG
<i>Orthocladius euorthocladius</i>	Species	Diptera	Chironomidae	6	CG
<i>Orthocladius pogonocladius</i>	Species	Diptera	Chironomidae	6	CG
<i>Pagastia</i>	Genus	Diptera	Chironomidae	1	CG
<i>Parachaeotocladus</i>	Genus	Diptera	Chironomidae	6	CG
<i>Parakiefferiella</i>	Genus	Diptera	Chironomidae	6	CG
<i>Paramerina</i>	Genus	Diptera	Chironomidae	6	PR
<i>Parametrioconemus</i>	Genus	Diptera	Chironomidae	5	CG
<i>Paraphaenocladus</i>	Genus	Diptera	Chironomidae	5	CG
<i>Paratanytarsus</i>	Genus	Diptera	Chironomidae	6	CG
<i>Paratendipes</i>	Genus	Diptera	Chironomidae	8	CG
<i>Paratrichocladius</i>	Genus	Diptera	Chironomidae	6	CG
<i>Parorthocladius</i>	Genus	Diptera	Chironomidae	6	CG
<i>Pentaneura</i>	Genus	Diptera	Chironomidae	6	PR
<i>Phaenopsectra</i>	Genus	Diptera	Chironomidae	7	SC
<i>Polypedium</i>	Genus	Diptera	Chironomidae	6	OM
<i>Polypedium pentapedilum</i>	Species	Diptera	Chironomidae	6	OM
<i>Potthastia gaedii</i>	Species	Diptera	Chironomidae	6	OM (con.)

Appendix C (Con.)

Taxon name	Taxon level	Order	Family	TV <sup>1</sup>	FFG <sup>2</sup>
<i>Pothastia longimana</i>	Species	Diptera	Chironomidae	2	CG
<i>Procladius</i>	Genus	Diptera	Chironomidae	9	PR
<i>Prodiamesa</i>	Genus	Diptera	Chironomidae	3	CG
<i>Psectrocladius</i>	Genus	Diptera	Chironomidae	8	CG
<i>Psectrocladius allopsectroclad</i>	Species	Diptera	Chironomidae	8	CG
<i>Psectrocladius limbatellus</i>	Species	Diptera	Chironomidae	8	CG
<i>Psectrocladius sordidellus</i>	Species	Diptera	Chironomidae	8	CG
<i>Psectrotanypus</i>	Genus	Diptera	Chironomidae	10	PR
<i>Pseudochironomus</i>	Genus	Diptera	Chironomidae	5	CG
<i>Pseudodiamesa</i>	Genus	Diptera	Chironomidae	6	CG
<i>Pseudorthocladus</i>	Genus	Diptera	Chironomidae	0	CG
<i>Rheocricotopus</i>	Genus	Diptera	Chironomidae	6	CG
<i>Rheotanytarsus</i>	Genus	Diptera	Chironomidae	6	CF
<i>Stempellina</i>	Genus	Diptera	Chironomidae	2	CG
<i>Stempellinella</i>	Genus	Diptera	Chironomidae	4	CG
<i>Subletta</i>	Genus	Diptera	Chironomidae	6	UN
<i>Symbiocladius</i>	Genus	Diptera	Chironomidae	6	PA
<i>Sympothastia</i>	Genus	Diptera	Chironomidae	2	CG
<i>Synorthocladus</i>	Genus	Diptera	Chironomidae	2	CG
<i>Tanytarsini</i>	Sup-Genus	Diptera	Chironomidae	6	CF
<i>Tanytarsus</i>	Genus	Diptera	Chironomidae	6	CF
<i>Thienemannimyia</i>	Genus	Diptera	Chironomidae	6	PR
<i>Thienemanniola</i>	Genus	Diptera	Chironomidae	6	CG
<i>Tvetenia</i>	Genus	Diptera	Chironomidae	5	CG
<i>Tvetenia bavarica</i>	Species	Diptera	Chironomidae	5	CG
<i>Tvetenia discoloripes</i>	Species	Diptera	Chironomidae	5	CG
<i>Zavrella</i>	Genus	Diptera	Chironomidae	8	CG
<i>Zavrellimyia</i>	Genus	Diptera	Chironomidae	8	PR
Culicidae	Family	Diptera	Culicidae	8	CG

(con.)

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Taxon name	Taxon level	Order	Family	TV <sup>1</sup>	FFG <sup>2</sup>
<i>Deuterophlebia</i>	Genus	Diptera	Deuterophlebiidae	0	SC
Dixidae	Family	Diptera	Dixidae	1	CG
<i>Dixa</i>	Genus	Diptera	Dixidae	1	CG
Empididae	Family	Diptera	Empididae	6	PR
<i>Chelifera</i>	Genus	Diptera	Empididae	6	PR
<i>Clinocera</i>	Genus	Diptera	Empididae	6	PR
<i>Hemerodromia</i>	Genus	Diptera	Empididae	6	PR
<i>Oreothalia</i>	Genus	Diptera	Empididae	6	PR
<i>Wiedemannia</i>	Genus	Diptera	Empididae	6	PR
Ephydriidae	Family	Diptera	Ephydriidae	6	PR
Muscidae	Family	Diptera	Muscidae	6	CG
Pelecorhynchidae	Family	Diptera	Pelecorhynchidae	3	PR
<i>Glutops</i>	Genus	Diptera	Pelecorhynchidae	3	PR
Psychodidae	Family	Diptera	Psychodidae	10	CG
<i>Maruina</i>	Genus	Diptera	Psychodidae	10	CG
Ptychopteridae	Family	Diptera	Ptychopteridae	7	CG
Simuliidae	Family	Diptera	Simuliidae	6	CF
<i>Simulium bivittatum</i>	Species	Diptera	Simuliidae	6	FC
<i>Prosimulium</i>	Genus	Diptera	Simuliidae	3	CF
<i>Simulium</i>	Genus	Diptera	Simuliidae	6	CF
<i>Simulium vittatum</i>	Species	Diptera	Simuliidae	6	CF
<i>Twinnia</i>	Genus	Diptera	Simuliidae	6	CF
Stratiomyidae	Family	Diptera	Stratiomyidae	8	CG
<i>Euparyphus</i>	Genus	Diptera	Stratiomyidae	8	CG
Pericoma	Genus	Diptera	Sychochidae	4	CG
Tabanidae	Family	Diptera	Tabanidae	8	PR
Tipulidae	Family	Diptera	Tipulidae	3	OM
<i>Antocha</i>	Genus	Diptera	Tipulidae	3	CG
<i>Dicranota</i>	Genus	Diptera	Tipulidae	3	PR

(con.)

Appendix C (Con.)

Taxon name	Taxon level	Order	Family	TV <sup>1</sup>	FFG <sup>2</sup>
<i>Hesperoconopa</i>	Genus	Diptera	Tipulidae	1	OM
<i>Hexatoma</i>	Genus	Diptera	Tipulidae	2	PR
<i>Limnophila</i>	Genus	Diptera	Tipulidae	4	PR
<i>Limonia</i>	Genus	Diptera	Tipulidae	6	OM
<i>Pedicia</i>	Genus	Diptera	Tipulidae	6	PR
<i>Tipula</i>	Genus	Diptera	Tipulidae	4	OM
<i>Ephemeroptera</i>	Order	Ephemeroptera			
Baetidae	Family	Ephemeroptera	Baetidae	4	CG
<i>Baetis</i>	Genus	Ephemeroptera	Baetidae	5	OM
<i>Baetis bicaudatus</i>	Species	Ephemeroptera	Baetidae	2	OM
<i>Baetis insignificans</i>	Species	Ephemeroptera	Baetidae	6	CG
<i>Baetis intermedius</i>	Species	Ephemeroptera	Baetidae	6	CG
<i>Baetis tricaudatus</i>	Species	Ephemeroptera	Baetidae	5	OM
<i>Calibaetis</i>	Genus	Ephemeroptera	Baetidae	9	CG
<i>Centroptilum</i>	Genus	Ephemeroptera	Baetidae	2	CG
<i>Pseudocloeon</i>	Genus	Ephemeroptera	Baetidae	4	OM
Caenidae	Family	Ephemeroptera	Caenidae	7	CG
<i>Caenis</i>	Genus	Ephemeroptera	Caenidae	7	CG
Ephemerellidae	Family	Ephemeroptera	Ephemerellidae	1	CG
<i>Attenella</i>	Genus	Ephemeroptera	Ephemerellidae	3	CG
<i>Attenella delantala</i>	Species	Ephemeroptera	Ephemerellidae	3	CG
<i>Caudatella</i>	Genus	Ephemeroptera	Ephemerellidae	1	CG
<i>Caudatella edmundsi</i>	Species	Ephemeroptera	Ephemerellidae	1	CG
<i>Caudatella heterocaudata</i>	Species	Ephemeroptera	Ephemerellidae	1	CG
<i>Caudatella hystrix</i>	Species	Ephemeroptera	Ephemerellidae	1	CG
<i>Drunella</i>	Genus	Ephemeroptera	Ephemerellidae	0	SC
<i>Drunella coloradensis</i>	Species	Ephemeroptera	Ephemerellidae	0	PR
<i>Drunella doddsi</i>	Species	Ephemeroptera	Ephemerellidae	0	PR
<i>Drunella flavilinea</i>	Species	Ephemeroptera	Ephemerellidae	1	SC

(con.)

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Taxon name	Taxon level	Order	Family	TV <sup>1</sup>	FFG <sup>2</sup>
<i>Drunella pelosa</i>	Species	Ephemeroptera	Ephemerellidae	0	SC
<i>Drunella spinifera</i>	Species	Ephemeroptera	Ephemerellidae	0	SC
<i>Ephemerella</i>	Genus	Ephemeroptera	Ephemerellidae	1	CG
<i>Ephemerella aurivillii</i>	Species	Ephemeroptera	Ephemerellidae	0	CG
<i>Ephemerella grandis</i>	Species	Ephemeroptera	Ephemerellidae	1	CG
<i>Ephemerella inermis</i>	Species	Ephemeroptera	Ephemerellidae	1	SH
<i>Serratella</i>	Genus	Ephemeroptera	Ephemerellidae	2	CG
<i>Serratella tibialis</i>	Species	Ephemeroptera	Ephemerellidae	2	CG
<i>Timpanoga hecuba</i>	Species	Ephemeroptera	Ephemerellidae	2	CG
Heptageniidae	Family	Ephemeroptera	Heptageniidae	7	CG
<i>Cinygma</i>	Genus	Ephemeroptera	Heptageniidae	4	SC
<i>Cinygmula</i>	Genus	Ephemeroptera	Heptageniidae	4	SC
<i>Epeorus</i>	Genus	Ephemeroptera	Heptageniidae	4	SC
<i>Epeorus albertae</i>	Species	Ephemeroptera	Heptageniidae	0	SC
<i>Epeorus deceptivus</i>	Species	Ephemeroptera	Heptageniidae	0	SC
<i>Epeorus grandis</i>	Species	Ephemeroptera	Heptageniidae	0	SC
<i>Epeorus iron</i>	Species	Ephemeroptera	Heptageniidae	0	SC
<i>Epeorus longimanus</i>	Species	Ephemeroptera	Heptageniidae	0	SC
<i>Heptagenia</i>	Genus	Ephemeroptera	Heptageniidae	4	SC
<i>Heptagenia elegantula</i>	Species	Ephemeroptera	Heptageniidae	4	SC
<i>Ironodes</i>	Genus	Ephemeroptera	Heptageniidae	4	SC
<i>Nixe criddlei</i>	Species	Ephemeroptera	Heptageniidae	4	SC
<i>Nixe simpliciooides</i>	Species	Ephemeroptera	Heptageniidae	4	SC
<i>Rhithrogena</i>	Species	Ephemeroptera	Heptageniidae	2	SC
<i>Rhithrogena hageni</i>	Species	Ephemeroptera	Heptageniidae	0	SC
Leptophlebiidae	Family	Ephemeroptera	Leptophlebiidae	0	CG
<i>Leptophlebia</i>	Genus	Ephemeroptera	Leptophlebiidae	2	CG
<i>Paraleptophlebia</i>	Genus	Ephemeroptera	Leptophlebiidae	2	CG
<i>Paraleptophlebia bicornuta</i>	Species	Ephemeroptera	Leptophlebiidae	1	OM
				4	CG

(con.)

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Taxon name	Taxon level	Order	Family	TV <sup>1</sup>	FFG <sup>2</sup>
<i>Paraleptophlebia heteronea</i>	Species	Ephemeroptera	Leptophlebiidae	2	CG
Polymitarcyidae	Family	Ephemeroptera	Polymitarcyidae	2	CG
<i>Ephoron album</i>	Species	Ephemeroptera	Polymitarcyidae	2	CG
Siphonuridae	Family	Ephemeroptera	Siphonuridae	7	CG
<i>Ameletus</i>	Genus	Ephemeroptera	Siphonuridae	0	CG
<i>Ameletus velox</i>	Species	Ephemeroptera	Siphonuridae	0	CG
<i>Siphonurus</i>	Genus	Ephemeroptera	Siphonuridae	7	OM
Tricorythidae	Family	Ephemeroptera	Tricorythidae	4	CG
<i>Tricorythides</i>	Genus	Ephemeroptera	Tricorythidae	5	CG
<i>Tricorythodes minutus</i>	Species	Ephemeroptera	Tricorythidae	4	CG
Hirudinidae	Family	Gnathobdellida	Hirudinidae	7	PR
Naididae	Family	Haplotaaxida	Naididae		C
<i>Rhyacodrilus sodalis</i>	Species	Haplotaaxida	Tubificidae	10	CG
<u>Hemiptera</u>	Order	Hemiptera			
<i>Lethocerus</i>	Genus	Hemiptera	Belostomatidae		PR
Corixidae	Family	Hemiptera	Corixidae		OM
<i>Callicorixa</i>	Genus	Hemiptera	Corixidae		PR
<i>Cenocorixa</i>	Genus	Hemiptera	Corixidae		OM
<i>Cenocorixa bifida hungerfordi</i>	Species	Hemiptera	Corixidae		PR
<i>Corisella</i>	Genus	Hemiptera	Corixidae		PR
<i>Graptocorixa</i>	Genus	Hemiptera	Corixidae		PR
<i>perocorixa</i>	Genus	Hemiptera	Corixidae		PH
<i>Sigara</i>	Genus	Hemiptera	Corixidae		PH
<i>Sigara alternata</i>	Species	Hemiptera	Corixidae		PH
Gelastocoridae	Family	Hemiptera	Gelastocoridae		PR
<i>Gelastocoris</i>	Genus	Hemiptera	Gelastocoridae		PR
Gerridae	Family	Hemiptera	Gerridae	5	PR
<i>Gerris</i>	Genus	Hemiptera	Gerridae		PR

(con.)

Appendix C (Con.)

Taxon name	Taxon level	Order	Family	TV <sup>1</sup>	FFG <sup>2</sup>
<i>Gerris buenoi</i>	Species	Hemiptera	Gerridae	5	PR
<i>Gerris remigis</i>	Species	Hemiptera	Gerridae	5	PR
Naucoridae	Family	Hemiptera	Naucoridae	5	PR
<i>Microvelia</i>	Genus	Hemiptera	Velidae	PR	PR
<u>Hydracarina</u>	Order	Hydracarina	Hydracarina	8	PR
<u>Hygrobatidae</u>	Family	Hydracarina	Hygrobatidae	8	PR
<i>Hygrobates</i>	Species	Hydracarina	Hygrobatidae	8	PR
<u>Lebertiidae</u>	Family	Hydracarina	Lebertiidae	8	PR
<i>Lebertia</i>	Genus	Hydracarina	Lebertiidae	8	PR
<u>Piersigiidae</u>	Family	Hydracarina	Piersigiidae	8	PR
<i>Protzia californensis</i>	Species	Hydracarina	Piersigiidae	8	PR
Sperchonidae	Family	Hydracarina	Sperchonidae	8	PR
<i>Sperchon pseudoplumifer</i>	Species	Hydracarina	Sperchonidae	8	PR
<u>Hymenoptera</u>	Order	Hymenoptera	Sperchonidae	8	PR
<u>Isopoda</u>	Order	Isopoda	Sperchonidae	8	PA
<u>Asellidea</u>	Family	Isopoda	Asellidae	8	CG
<i>Asellus</i>	Genus	Isopoda	Asellidae	6	CG
<i>Asellus occidentalis</i>	Species	Isopoda	Asellidae	8	CG
<i>Caecidotea communis</i>	Species	Isopoda	Asellidae	8	CG
<u>Lepidoptera</u>	Order	Lepidoptera	Asellidae	6	CG
<u>Pyralidae</u>	Family	Lepidoptera	Pyralidae	5	SH
<i>Petrophila</i>	Genus	Lepidoptera	Pyralidae	5	SC
<u>Limnophila</u>	Order	Limnophila	Pyralidae	5	SC
<u>Lymnaeidae</u>	Family	Limnophila	Lymnaeidae	8	SC
<i>Fossaria</i>	Genus	Limnophila	Lymnaeidae	8	SC
<i>Lymnaea</i>	Genus	Limnophila	Lymnaeidae	8	SC
<i>Vorticifex</i>	Genus	Limnophila	Lymnaeidae	8	SC
<u>Megaloptera</u>	Order	Megaloptera	Planorbidae	8	SC
<u>Corydalidae</u>	Family	Megaloptera	Corydalidae	0	PR

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Appendix C (Con.)

Taxon name	Taxon level	Order	Family	TV <sup>1</sup>	FFG <sup>2</sup>
<i>Orohermes</i>	Genus	Megaloptera	Corydalidae	0	PR
<i>Sialis</i>	Genus	Megaloptera	Sialidae	4	PR
<u>Mesogastropoda</u>	Order	Mesogastropoda			
<i>Fluminicola</i>	Genus	Mesogastropoda	Bithyniidae	5	SC
<i>Fluminicola</i>	Genus	Mesogastropoda	Hydrobiidae	8	SC
<i>Hydrobiidae</i>	Family	Mesogastropoda	Hydrobiidae		SC
<i>Fontelicella</i>	Genus	Mesogastropoda	Hydrobiidae	8	SC
<u>Odonata</u>	Order	Odonata			
Aeshnidae	Family	Odonata	Aeshnidae	3	PR
<i>Anax</i>	Genus	Odonata	Aeshnidae	8	PR
Coenagrionidae	Family	Odonata	Coenagrionidae	9	PR
<i>Calopteryx</i>	Genus	Odonata	Calopterygidae	6	PR
<i>Argia</i>	Genus	Odonata	Coenagrionidae	7	PR
<i>Erallegma</i>	Genus	Odonata	Coenagrionidae	9	PR
<i>Ishnura</i>	Genus	Odonata	Coenagrionidae	9	PR
<i>Zoniagrion</i>	Genus	Odonata	Coenagrionidae	9	PR
Gomphidae	Family	Odonata	Gomphidae	1	PR
<i>Octogomphus</i>	Genus	Odonata	Gomphidae	1	PR
<i>Ohiogomphus</i>	Genus	Odonata	Gomphidae	1	PR
<i>Amphiagrion</i>	Genus	Odonata	Protoneuridae	5	PR
Eropobdellidae	Family	Pharyngodellida	Eropobdellidae	8	PR
<i>Dina parva</i>	Species	Pharyngodellida	Eropobdellidae	8	PR
<u>Plecoptera</u>	Order	Plecoptera			
Capniidae	Family	Plecoptera	Capniidae	1	UN
<i>Capnia</i>	Genus	Plecoptera	Capniidae	1	SH
<i>Eucapnopsis brevicauda</i>	Species	Plecoptera	Capniidae	1	SH
<i>Paracapnia</i>	Genus	Plecoptera	Capniidae	1	SH
Chloroperlidae	Family	Plecoptera	Chloroperlidae	1	PR
<i>Alloperla</i>	Genus	Plecoptera	Chloroperlidae	0	PR

(con.)

Appendix C (Con.)

Taxon name	Taxon level	Order	Family	TV <sup>1</sup>	FFG <sup>2</sup>
<i>Kathroperla perdita</i>	Species	Plecoptera	Chloroperlidae	1	OM
<i>Paraperla</i>	Genus	Plecoptera	Chloroperlidae	1	PR
<i>Swelta complex</i>	Species	Plecoptera	Chloroperlidae	1	PR
Leuctridae	Family	Plecoptera	Leuctridae	0	SH
<i>Despaxia augusta</i>	Species	Plecoptera	Leuctridae	0	SH
<i>Megaleuctra</i>	Genus	Plecoptera	Leuctridae	0	SH
<i>Mosellia infuscata</i>	Species	Plecoptera	Leuctridae	0	SH
<i>Paraleuctra</i>	Genus	Plecoptera	Leuctridae	0	SH
<i>Paraleuctra occidentalis</i>	Species	Plecoptera	Leuctridae	0	SH
<i>Perlomyia</i>	Genus	Plecoptera	Leuctridae	0	SH
Nemouridae	Family	Plecoptera	Leuctridae	0	SH
<i>Amphinemura</i>	Genus	Plecoptera	Nemouridae	2	SH
<i>Malenka</i>	Genus	Plecoptera	Nemouridae	2	SH
<i>Malenka</i>	Genus	Plecoptera	Nemouridae	2	SH
<i>Podmosta</i>	Genus	Plecoptera	Nemouridae	5	PR
<i>Prostola besametsa</i>	Species	Plecoptera	Nemouridae	2	SH
<i>Soyedina</i>	Genus	Plecoptera	Nemouridae	2	SH
<i>Visoka cataractae</i>	Species	Plecoptera	Nemouridae	2	SH
<i>Zapada</i>	Genus	Plecoptera	Nemouridae	1	SH
<i>Zapada cinctipes</i>	Species	Plecoptera	Nemouridae	2	SH
<i>Zapada columbiana</i>	Species	Plecoptera	Nemouridae	2	SH
<i>Zapada frigida</i>	Species	Plecoptera	Nemouridae	2	SH
<i>Zapada oregonensis</i>	Species	Plecoptera	Nemouridae	2	SH
Peltoperlidae	Family	Plecoptera	Peltoperlidae	2	SH
<i>Soliperla</i>	Genus	Plecoptera	Peltoperlidae	2	SH
<i>Yoraperla</i>	Genus	Plecoptera	Peltoperlidae	2	SH
<i>Yoraperla brevis</i>	Species	Plecoptera	Peltoperlidae	2	SH
<i>Yoraperla mariana</i>	Species	Plecoptera	Peltoperlidae	2	SH
Perlidae	Family	Plecoptera	Perlidae	2	SH
				1	PR

(con.)

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Taxon name	Taxon level	Order	Family	TV <sup>1</sup>	FFG <sup>2</sup>
<i>Beloneuria</i>	Genus	Plecoptera	Perlidae	3	PR
<i>Calineuria</i>	Genus	Plecoptera	Perlidae	3	PR
<i>Calineuria californica</i>	Species	Plecoptera	Perlidae	1	PR
<i>Claasenia</i>	Genus	Plecoptera	Perlidae	3	PR
<i>Claasenia sabulosa</i>	Species	Plecoptera	Perlidae	4	PR
<i>Doroneuria</i>	Genus	Plecoptera	Perlidae	1	PR
<i>Doroneuria baumanni</i>	Species	Plecoptera	Perlidae	1	PR
<i>Doroneuria theodora</i>	Species	Plecoptera	Perlidae	1	PR
<i>Hesperoperla pacifica</i>	Species	Plecoptera	Perlidae	1	PR
<i>Cascadoperta</i>	Genus	Plecoptera	Perlidae	2	PR
Perlodidae	Family	Plecoptera	Perlidae	2	PR
<i>Cultus</i>	Genus	Plecoptera	Perlidae	2	PR
<i>Diura knowltoni</i>	Species	Plecoptera	Perlidae	2	OM
<i>Frisonia picticeps</i>	Species	Plecoptera	Perlidae	2	PR
<i>Isogenus</i>	Genus	Plecoptera	Perlidae	2	PR
<i>Isoperla</i>	Genus	Plecoptera	Perlidae	2	PR
<i>Isoperla fluva</i>	Species	Plecoptera	Perlidae	2	PR
<i>Isoperla fusca</i>	Species	Plecoptera	Perlidae	2	PR
<i>Kogotus</i>	Genus	Plecoptera	Perlidae	2	PR
<i>Megarctys</i>	Genus	Plecoptera	Perlidae	2	PR
<i>Oroperla</i>	Genus	Plecoptera	Perlidae	2	PR
<i>Perlinoles aurea</i>	Species	Plecoptera	Perlidae	2	PR
<i>Pictetiella expansa</i>	Species	Plecoptera	Perlidae	2	PR
<i>Satvena bradleyi</i>	Species	Plecoptera	Perlidae	2	PR
<i>Skwala</i>	Genus	Plecoptera	Perlidae	2	PR
<i>Yugus</i>	Genus	Plecoptera	Perlidae	2	PR
Pteronarcyidae	Family	Plecoptera	Pteronarcyidae	0	OM
<i>Pteronarcella</i>	Genus	Plecoptera	Pteronarcyidae	0	OM
<i>Pteronarcella badia</i>	Species	Plecoptera	Pteronarcyidae	0	OM

(con.)

Appendix C (Con.)

Taxon name	Taxon level	Order	Family	TV <sup>1</sup>	FFG <sup>2</sup>
<i>Pteronarcys regularis</i>	Species	Plecoptera	Pteronarcyidae	0	OM
<i>Pteronarcys</i>	Genus	Plecoptera	Pteronarcyidae	0	OM
<i>Pteronarcys californica</i>	Species	Plecoptera	Pteronarcyidae	0	OM
<i>Pteronarcys princeps</i>	Species	Plecoptera	Pteronarcyidae	0	OM
Taeniopterygidae	Family	Plecoptera	Taeniopterygidae	2	UN
<i>Doddsia occidentalis</i>	Species	Plecoptera	Taeniopterygidae	2	UN
<i>Taenionema</i>	Genus	Plecoptera	Taeniopterygidae	2	SC
<i>Taenionema pallidum</i>	Species	Plecoptera	Taeniopterygidae	2	SC
Entocytheridae	Family	Podocopa	Entocytheridae	2	UN
Glossiphoniidae	Family	Rhynchobdellida	Glossiphoniidae	8	PR
<i>Glossiphonia complanata</i>	Species	Rhynchobdellida	Glossiphoniidae	8	PR
<i>Helobdella stagnalis</i>	Species	Rhynchobdellida	Glossiphoniidae	10	PR
<i>Piscicola salmositica</i>	Species	Rhynchobdellida	Piscicolidae	7	PR
**Corticacarus delicatus	Species	Rhynchobdellida	Hygrobatidae	8	PR
Trichoptera	Order	Trichoptera		8	UN
Brachycentridae	Family	Trichoptera	Brachycentridae	1	CF
<i>Amiocentrus</i>	Genus	Trichoptera	Brachycentridae	1	CG
<i>Amiocentrus aspilus</i>	Species	Trichoptera	Brachycentridae	2	CG
<i>Brachycentrus</i>	Genus	Trichoptera	Brachycentridae	1	OM
<i>Brachycentrus americanus</i>	Species	Trichoptera	Brachycentridae	1	OM
<i>Brachycentrus occidentalis</i>	Species	Trichoptera	Brachycentridae	1	OM
<i>Micrasema</i>	Genus	Trichoptera	Brachycentridae	1	MH
<i>Oligoptetrum</i>	Genus	Trichoptera	Brachycentridae	1	C
Calamocreatidae	Family	Trichoptera	Calamocreatidae	1	SH
<i>Heteropteron calliformicum</i>	Species	Trichoptera	Calamocreatidae	1	SH
Glossosomatidae	Family	Trichoptera	Glossosomatidae	0	SC
<i>Agapetus</i>	Genus	Trichoptera	Glossosomatidae	0	SC
<i>Anagapetus</i>	Genus	Trichoptera	Glossosomatidae	0	SC
<i>Culoptila cantha</i>	Species	Trichoptera	Glossosomatidae	0	SC

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Taxon name	Taxon level	Order	Family	TV <sup>1</sup>	FFG <sup>2</sup>
<i>Glossosoma</i>	Genus	Trichoptera	Glossosomatidae	0	SC
<i>Glossosoma alascense</i>	Species	Trichoptera	Glossosomatidae	0	SC
<i>Glossosoma intermedium</i>	Species	Trichoptera	Glossosomatidae	0	SC
<i>Glossosoma montana</i>	Species	Trichoptera	Glossosomatidae	0	SC
<i>Glossosoma oregonense</i>	Species	Trichoptera	Glossosomatidae	0	SC
<i>Glossosoma penitum</i>	Species	Trichoptera	Glossosomatidae	0	SC
<i>Glossosoma wenatchee</i>	Species	Trichoptera	Glossosomatidae	0	SC
<i>Protopitla</i>	Genus	Trichoptera	Glossosomatidae	1	SC
<i>Protopitla coloma</i>	Species	Trichoptera	Glossosomatidae	1	SC
<i>Protopitla tenebrosa</i>	Species	Trichoptera	Glossosomatidae	1	SC
Helicopsychidae	Family	Trichoptera	Helicopsychidae	3	SC
<i>Elicopsyche borealis</i>	Species	Trichoptera	Helicopsychidae	3	SC
<i>Helicopsyche</i>	Genus	Trichoptera	Helicopsychidae	3	SC
Hydropsyichidae	Family	Trichoptera	Hydropsyichidae	4	CF
Apataniinae	Sub-Family	Trichoptera	Hydropsyichidae	2	CF
<i>Cheumatopsyche</i>	Genus	Trichoptera	Hydropsyichidae	5	CF
<i>Cheumatopsyche campyla</i>	Species	Trichoptera	Hydropsyichidae	6	CF
<i>Cheumatopsyche enonis</i>	Species	Trichoptera	Hydropsyichidae	6	CF
<i>Cheumatopsyche pettiti</i>	Species	Trichoptera	Hydropsyichidae	6	CF
<i>Hydropsyche</i>	Genus	Trichoptera	Hydropsyichidae	4	CF
<i>Hydropsyche californica</i>	Species	Trichoptera	Hydropsyichidae	4	CF
<i>Hydropsyche occidentalis</i>	Species	Trichoptera	Hydropsyichidae	4	CF
<i>hydropsyche oslari</i>	Species	Trichoptera	Hydropsyichidae	4	CF
<i>Macronema</i>	Genus	Trichoptera	Hydropsyichidae	3	CF
<i>Parapsyche</i>	Genus	Trichoptera	Hydropsyichidae	1	PR
<i>Parapsyche almota</i>	Species	Trichoptera	Hydropsyichidae	3	PR
<i>Parapsyche elisis</i>	Species	Trichoptera	Hydropsyichidae	1	PR
Hydroptilidae	Family	Trichoptera	Hydroptilidae	4	PH

(con.)

Appendix C (Con.)

Taxon name	Taxon level	Order	Family	TV <sup>1</sup>	FFG <sup>2</sup>
<i>Agraylea</i>	Genus	Trichoptera	Hydroptilidae	8	PH
<i>Hydroptila ajax</i>	Species	Trichoptera	Hydroptilidae	6	SC
<i>Hydroptila</i>	Genus	Trichoptera	Hydroptilidae	6	PH
<i>Hydroptila arctica</i>	Species	Trichoptera	Hydroptilidae	6	SC
<i>Hydroptila argosa</i>	Species	Trichoptera	Hydroptilidae	6	SC
<i>Leucotrichia</i>	Genus	Trichoptera	Hydroptilidae	6	SC
<i>Neotrichia halia</i>	Species	Trichoptera	Hydroptilidae	4	S
<i>Ochrotrichia</i>	Genus	Trichoptera	Hydroptilidae	4	C
<i>Orthotrichia</i>	Genus	Trichoptera	Hydroptilidae	6	PR
<i>Stactobiella</i>	Genus	Trichoptera	Hydroptilidae	2	SH
Lepidostomatidae	Family	Trichoptera	Lepidostomatidae	1	SH
<i>Lepidostoma</i>	Genus	Trichoptera	Lepidostomatidae	1	SH
<i>Lepidostoma cinereum</i>	Species	Trichoptera	Lepidostomatidae	3	SH
Leptoceridae	Family	Trichoptera	Leptoceridae	4	CG
<i>Mystacides</i>	Genus	Trichoptera	Leptoceridae	4	C
<i>Nectopsyche gracilis</i>	Species	Trichoptera	Leptoceridae	3	S
<i>Nectopsyche halia</i>	Species	Trichoptera	Leptoceridae	3	S
<i>Nectopsyche lahontanensis</i>	Species	Trichoptera	Leptoceridae	3	S
<i>Nectopsyche stigmatica</i>	Species	Trichoptera	Leptoceridae	3	S
<i>Oecetis</i>	Genus	Trichoptera	Leptoceridae	8	PR
<i>Trietnodes</i>	Genus	Trichoptera	Leptoceridae	6	MH
Limnephilidae	Family	Trichoptera	Limnephilidae	4	OM
<i>Allocosmoecus partitus</i>	Species	Trichoptera	Limnephilidae	0	SC
<i>Apatania</i>	Genus	Trichoptera	Limnephilidae	1	SC
<i>Chyranda</i>	Genus	Trichoptera	Limnephilidae	1	SH
<i>Chyranda centralis</i>	Species	Trichoptera	Limnephilidae	1	SH
<i>Cryptochia</i>	Genus	Trichoptera	Limnephilidae	0	SH
Dicosmoecinae	Sub-Family	Trichoptera	Limnephilidae	1	OM
<i>Dicosmoecus</i>	Genus	Trichoptera	Limnephilidae	1	SH

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Taxon name	Taxon level	Order	Family	TV <sup>1</sup>	FFG <sup>2</sup>
<i>Dicosmoecus atripes</i>	Species	Trichoptera	Limnephilidae	1	PR
<i>Dicosmoecus gilvipes</i>	Species	Trichoptera	Limnephilidae	2	SC
<i>Ecclisocosmoecus scylla</i>	Species	Trichoptera	Limnephilidae	0	SH
<i>Ecclisomyia</i>	Genus	Trichoptera	Limnephilidae	2	OM
Goerinae	Sub-Family	Trichoptera	Limnephilidae	1	SC
<i>Goera archaon</i>	Species	Trichoptera	Limnephilidae	1	SC
<i>Grensia</i>	Genus	Trichoptera	Limnephilidae	6	SH
<i>Hesperophylax</i>	Genus	Trichoptera	Limnephilidae	5	OM
<i>Homophylax</i>	Genus	Trichoptera	Limnephilidae	0	SH
<i>Hydatophylax</i>	Genus	Trichoptera	Limnephilidae	1	SH
Limnephilinae	Sub-Family	Trichoptera	Limnephilidae	4	OM
<i>Limnephilus</i>	Genus	Trichoptera	Limnephilidae	5	OM
<i>Moselyana</i>	Genus	Trichoptera	Limnephilidae	4	C
<i>Neophylax</i>	Genus	Trichoptera	Limnephilidae	3	S
<i>Neophylax occidentalis</i>	Species	Trichoptera	Limnephilidae	3	S
<i>Neophylax rickeri</i>	Species	Trichoptera	Limnephilidae	3	S
<i>Neophylax splendens</i>	Species	Trichoptera	Limnephilidae	3	S
<i>Oligophlebodes</i>	Genus	Trichoptera	Limnephilidae	1	S
<i>Onocosmoecus</i>	Genus	Trichoptera	Limnephilidae	1	SH
<i>Onocosmoecus unicolor</i>	Species	Trichoptera	Limnephilidae	2	SH
<i>Pedomoecus sierra</i>	Species	Trichoptera	Limnephilidae	0	SC
<i>Psychoglypha</i>	Genus	Trichoptera	Limnephilidae	1	OM
<i>Psychoglypha bella</i>	Species	Trichoptera	Limnephilidae	2	OM
<i>Psychoglypha subborealis</i>	Species	Trichoptera	Limnephilidae	2	OM
Philopotamidae	Family	Trichoptera	Philopotamidae	3	CF
<i>Dolophilodes</i>	Genus	Trichoptera	Philopotamidae	3	CF
<i>Wormaldia</i>	Genus	Trichoptera	Philopotamidae	3	CF
Polycentropidae	Family	Trichoptera	Polycentropodidae	6	CF
<i>Polycentropus</i>	Genus	Trichoptera	Polycentropodidae	6	PR

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Taxon name	Taxon level	Order	Family	TV <sup>1</sup>	FFG <sup>2</sup>
Psychomyiidae	Family	Trichoptera	Psychomyiidae	6	CG
<i>Psychomyia lumina</i>	Species	Trichoptera	Psychomyiidae	2	SC
<i>Tinodes</i>	Genus	Trichoptera	Psychomyiidae	6	SC
Rhyacophilidae	Family	Trichoptera	Rhyacophilidae	0	PR
<i>Rhyacophila</i>	Genus	Trichoptera	Rhyacophilidae	0	PR
<i>Rhyacophila acropedes</i>	Species	Trichoptera	Rhyacophilidae	1	PR
<i>Rhyacophila alberta</i>	Species	Trichoptera	Rhyacophilidae	0	PR
<i>Rhyacophila angelita</i>	Species	Trichoptera	Rhyacophilidae	0	PR
<i>Rhyacophila arnaudi</i>	Species	Trichoptera	Rhyacophilidae	0	PR
<i>Rhyacophila betteni</i>	Species	Trichoptera	Rhyacophilidae	0	PR
<i>Rhyacophila blarina</i>	Species	Trichoptera	Rhyacophilidae	0	PR
<i>Rhyacophila brunnea</i>	Species	Trichoptera	Rhyacophilidae	0	PR
<i>Rhyacophila coloradensis</i>	Species	Trichoptera	Rhyacophilidae	0	PR
<i>Rhyacophila hyalinata</i>	Species	Trichoptera	Rhyacophilidae	0	PR
<i>Rhyacophila iranda</i>	Species	Trichoptera	Rhyacophilidae	0	PR
<i>Rhyacophila narvae</i>	Species	Trichoptera	Rhyacophilidae	0	PR
<i>Rhyacophila pellisa</i>	Species	Trichoptera	Rhyacophilidae	0	PR
<i>Rhyacophila rotunda</i>	Species	Trichoptera	Rhyacophilidae	0	PR
<i>Rhyacophila sibirica</i>	Species	Trichoptera	Rhyacophilidae	0	PR
<i>Rhyacophila vagrita</i>	Species	Trichoptera	Rhyacophilidae	0	PR
<i>Wormaldia verrula</i>	Species	Trichoptera	Rhyacophilidae	0	MH
<i>Wormaldia gabriella</i>	Species	Trichoptera	Rhyacophilidae	3	CF
Sericostomatidae	Family	Trichoptera	Sericostomatidae	3	SH
<i>Grumaga</i>	Genus	Trichoptera	Sericostomatidae	3	SH
Uenoidea	Family	Trichoptera	Uenoidea	0	S
<i>Neothremma alicia</i>	Species	Trichoptera	Uenoidea	0	S
<i>Neothremma</i>	Genus	Trichoptera	Uenoidea	0	S
MOLLUSKA	Phylum			7	SC
GASTROPODA	Class				(con.)

Appendix C (Con.)

Taxon name	Taxon level	Order	Family	TV <sup>1</sup>	FFG <sup>2</sup>
<u>Limnophila</u>	Order	Limnophila			
Ancylidae	Family	Limnophila	Ancylidae	6	SC
<i>Ferrisia</i>	Genus	Limnophila	Ancylidae	6	SC
Limnaeidae	Family	Limnophila	Limnaeidae	6	SC
Physidae	Family	Limnophila	Physidae	8	SC
<i>Physa</i>	Genus	Limnophila	Physidae	8	SC
<i>Physella</i>	Genus	Limnophila	Physidae	8	SC
Planorbidae	Family	Limnophila	Planorbidae	7	SC
<i>Gyraulus</i>	Genus	Limnophila	Planorbidae	8	SC
<i>Promentus</i>	Genus	Limnophila	Planorbidae	6	CG
<u>Mesogastropoda</u>	Order	Mesogastropoda			
<i>Juga</i>	Genus	Mesogastropoda	Thiaridae	7	OM
PELECYPODA	Class			8	CF
<i>Margaritifera</i>	Genus	Pelecypoda	Margaritiferidae	4	CF
<i>Margaritifera margaritifera falcata</i>	Species	Pelecypoda	Margaritiferidae	8	CF
Sphaeriidae	Family	Pelecypoda	Sphaeriidae	8	CF
<i>Pisidium</i>	Genus	Pelecypoda	Sphaeriidae	8	CF
<i>Pisidium casertanum</i>	Species	Pelecypoda	Sphaeriidae	8	SC
<i>Pisidium compressum</i>	Species	Pelecypoda	Sphaeriidae	8	CF
<i>Pisidium idahoenses</i>	Species	Pelecypoda	Sphaeriidae	8	CF
<i>Sphaerium patella</i>	Species	Pelecypoda	Sphaeriidae	8	CF
<i>Sphaerium striatum</i>	Species	Pelecypoda	Sphaeriidae	8	CF
Unionidae	Family	Pelecypoda	Unionidae	8	CF
<i>Gonidea</i>	Genus	Pelecypoda	Unionidae	4	CF

(con.)

**Appendix C (Con.)**

Taxon name	Taxon level	Order	Family	TV <sup>1</sup>	FFG <sup>2</sup>
<i>Anodonta nuttalliana idahoensis</i>	Species	Pelecypoda	Unionidae	8	CF
<i>Gonidea angulata</i>	Species	Pelecypoda	Unionidae	8	CF
NEMATODA	Phylum			5	F
PLATYHELMINTHES	Phylum				
TUBELLARIA	Class			4	PR
Tricladida	Order	Tricladida			UN
Planariidae	Family	Tricladida	Planariidae		OM

<sup>1</sup>Tolerance values (TV) range from 0 (low tolerance) to 10 (high tolerance) from Clark and Maret (1993)

<sup>2</sup>Functional Feeding Group (FFG) Designations: CF = Collector-Filterer; PH = Plercer-Herbivore; CG = Collector-Gatherer; PR = Predator; MH = Macrophyte Herbivore; SC = Scrapper; OM = Omnivore; SH = Shredder; PA = Parasite; UN = Unknown

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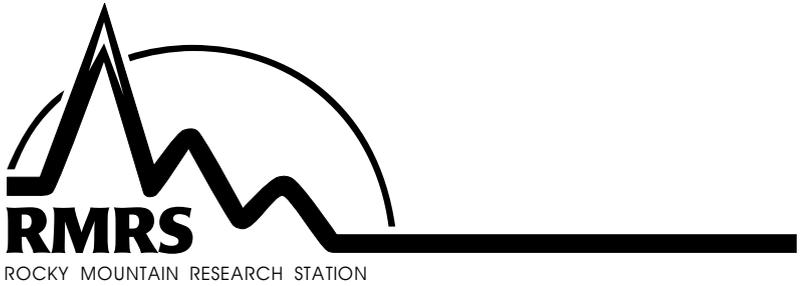
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