

Table Mountain, and Virginia pine on northeastern aspects. Combinations which cannot be located can be disregarded without compromising the LTEMs objectives. Secondly, elevational differences throughout the SNP will make it difficult to find all ELUs in each District. The 3500 ft elevation classes may be especially difficult to locate in each District. While it is important that each ELU be represented by three sites within the SNP, it is not imperative that they be evenly divided among the three Districts. In these cases it is acceptable to select a second (or third) site within a single District.

Site Selection

The SNP has instituted a geographic information system that is linked to the LTEMs databases and provides immediate access to geographically referenced data to aid in making resource management decisions. The geographic information system/database provides the information used in the initial phase of LTEMs site selection. The term *site* in this case refers to the specific ELU location where the replicated research plots will be installed. Forest cover type, elevation, aspect, and administrative district are all data variables available from the SNP geographic information system database. The LTEMs site selection process is composed of two phases or levels. The first phase is based on the geographic information system/database and associated aerial imagery, and is accomplished in the office. The second phase is field verification in which the designated field party leader, using the geographic information system, travels to the specific site location to verify that the forest cover type, elevation, and aspect all meet the specified characteristics for an LTEMs site. The field party leader, after on-site verification, monuments the site location for the first plot, or rejects the site as not meeting the LTEMs requirements. In the case of site rejection, the field team leader would move to the next LTEMs site that was randomly selected as meeting the specific characteristics. This selection and verification process would continue until the required number of valid sites are found, or until it is determined that they do not exist within the boundaries of the SNP.

Permanent plot locations must be determined so that 1) they are representative of the forest conditions for which inferences will be made, 2) they can be relocated easily for remeasurement, and 3) their physical integrity can be maintained for the duration of the LTEMs program.

Aquatic Component

Site Selection

In order to select the best design for the aquatic component of the LTEMs, an extensive reconnaissance of SNP streams was conducted from January to April 1986. Thirty-three locations on thirty streams were visited. The reconnaissance consisted of making cursory examinations of the physical environment and macroinvertebrates at each location. Written notes and tape recordings of the investigators' observations were made for later review. In addition, information on geology (Gathright 1976), water chemistry (Dise 1984), and fishes (unpublished reports) were reviewed. From this information, the streams were classified in the following hierarchical system, and then representative study sites were selected from the different classifications. The rationale behind this design was to have all of the naturally occurring environmental conditions included in the LTEMs and to have sites spread throughout the area of the SNP. For the aquatic component, the term site refers to a designated length of stream along with the riparian area.

Administrative District

- North
- Central
- South

Geological Formation/Alkalinity

- Catoctin (100-200 ueq/L)
- Pedlar or Old Rag (20-100 ueq/L)
- Hampton or Erwin (<20 ueq/L)

Elevation

- Upper (>548.6 m [1800 ft])
- Lower (<548.6 m [1800 ft])

Other Factors

- Accessibility
- SNP trout monitoring sites
- Current research interests

Using the three administrative districts as the first level of classification was a convenient means of distributing the stream sites throughout the Park. In most cases, it will be impossible to predict where an adverse impact might occur. Therefore, spreading the sites throughout the Park increases the probability of having data from where they might be needed.

Geological formations were selected as the second level of classification because the SNP has a uniquely diverse geology and resulting water chemistry, particularly alkalinity. There are great differences among watersheds, and often between adjacent watersheds. Alkalinity and related water chemistry are probably significant factors affecting the distribution of the aquatic biota. Information on geological formations and alkalinity can be obtained from Gathright (1976) and Dise (1984), respectively. Streams in the SNP seldom have their watersheds entirely within a single geological formation; therefore, stream sites were established in watersheds that have at least 75% of their area within one of the geological formation categories.

Elevation affects the distribution of aquatic biota indirectly because of factors such as stream width, depth, current velocity, valley shading, and temperature. The SNP is located entirely within the northern section of the Blue Ridge physiographic province, which is the narrow (about 8 km) mountainous area between the low-lying Piedmont province to the east and the Ridge and Valley province to the west (Hoffman 1969, Gathright 1976). Drainage basins in Blue Ridge province typically have a steep gradient. On the west slope basins follow a trellis pattern, in which the small streams flowing down the mountains are parallel and join the main stream in the valley (Shenandoah River) at right angles (Reid and Wood 1976). Those on the east slope follow a dendritic pattern, so-called because of the resemblance to tree roots (Reid and Wood 1976). The criterion of 548.6 m (1800 ft) for distinguishing upper and lower sites was selected because field observations indicated that streams in the SNP tended to become very narrow and shallow (several m wide and less than knee deep) approximately above that elevation. This delimitation was somewhat arbitrary and was varied within reason when it was desirable to have both an upper and lower site on the same stream. Elevation was determined from the Potomac Appalachian Trail Club maps (scale 1:62,500). The USGS 7.5 min topographic maps were not as useful because they are not as accurate regarding trails, fire roads, and stream permanency.

Accessibility was given serious consideration when choosing sites. If a site cannot be reached with reasonable effort at the desired time, then the data are likely to be sporadic and would not be useful for monitoring. Trout populations in the SNP have been monitored for some time; therefore, it was logical to establish LTEMs sites at many of the same locations so that there would be ecological data to explain the results of fish sampling. Gypsy moth infestation and acid deposition are environmental concerns at the present time in the SNP. Preference was given to stream sites that are likely to be affected by either of these two perturbations

or sites that are being studied as part of other research projects.

One of the most important criteria that was used for designing the LTEMs was feasibility. For the purposes of monitoring, data that are not obtained consistently are of no value. Therefore, it was essential to design a system that would not be overly ambitious for the financial resources of the SNP. It was decided that it would be feasible to monitor about one-half of the sites that had been visited during the reconnaissance. Seventeen stream sites were selected for the aquatic LTEMs. These are listed below, according to the hierarchical classification system. Additional information on the sites is provided in Section III of this series (Aquatic Component User Manual).

South District

- Hampton or Erwin
- Paine Run (Upper)
- Paine Run (Lower)
- Twomile Run (Upper)
- Twomile Run (Lower)

Central District

- Pedlar or Old Rag
- Hazel River (Upper)
- Hazel River (Lower)
- Staunton River (Upper)
- Staunton River (Lower)
- North Fork Dry Run (Upper)

Catoctin

- White Oak Canyon Run (Upper)
- White Oak Canyon Run (Lower)
- Hog Camp Branch (Upper)
- Rose River (Lower)

North District

- Catoctin and Pedlar
- Piney River (Upper)
- Piney River (Lower)
- Catoctin, Hampton, and Weverton
- Jeremys Run (Upper)
- Pedlar
- Lands Run (Upper)

The streams sites are 100-m longitudinal reaches, which are marked inconspicuously with PVC pipes at the upstream and downstream boundaries. The 100-m sections were selected so that they included riffles and pools and as many microhabitats as possible (different sizes of rocks, logs, different current velocities, etc.). The rationale was that a well chosen 100-m reach of a stream would be large enough for all of the biota to be represented, including the fishes.

Parameters

Scientific defensibility was a second criterion that was given equal consideration along with feasibility in designing the aquatic component of the LTEMs. It was important to choose parameters that would be likely to show measurable changes in response to perturbations and to have information that could be used to explain any observed changes. Benthic macroinvertebrates were chosen to be the focus of the biological monitoring. Voshell et al. (1989) explained several reasons why benthic macroinvertebrates are commonly used for ecological monitoring. They are abundant in almost all freshwater environments. Sedentary habits and comparatively large size, in combination with abundance, make them relatively easy to collect. Most can now be identified reliably to genus with comprehensive taxonomic works that have become available (e.g., Pennak 1978, Brigham et al. 1982, Merritt and Cummins 1984). The benthic macroinvertebrates that exist in a given area are indicative of the environmental conditions that have occurred for at least several months previously because their life cycles last from several months to a year or more. Taken as a group, benthic macroinvertebrates have diverse habitat and food preferences, but many individual taxa have narrowly defined niches. Therefore, it is likely that some taxa will demonstrate a change in response to any perturbation that might take place. Activities of benthic macroinvertebrates affect major ecological processes of freshwater ecosystems. These significant activities include grazing on primary producers, decomposing organic matter, preying on smaller invertebrates, temporarily storing materials (spiralling), and providing food for fish.

Fish, especially brook trout, are one of the most important natural resources in the SNP. Protection of fish is one of the main reasons for conducting long-term aquatic monitoring in the SNP. Fish were not included as a parameter in the LTEMs that was developed by VPI & SU because fish populations were already being monitored by a joint effort between the SNP and the Virginia Commission of Game and Inland Fisheries. Whenever possible, the existing sites for fish sampling were also used as LTEMs sites.

Although the LTEMs emphasized biological monitoring of benthic macroinvertebrates and assumed that fish would also be monitored, it was also necessary to include a variety of physical and chemical parameters that could be used to explain any changes that might occur in the biota. The parameters that were chosen are listed below, according to broad categories of water quality and habitat. The rationale for choosing these parameters was that they could be measured

reliably and that they were related to the success of the biota. Detailed explanations of these parameters can be found in Section III of this series (Aquatic Component User Manual).

Water Quality

- Discharge
- Temperature
- Dissolved oxygen
- pH
- Alkalinity
- Conductivity
- Sulfate
- Nitrate
- Chloride
- Calcium
- Magnesium
- Potassium and sodium
- Silica
- Seston
- Bacteria

Habitat

- Depth
- Width
- Substratum
- Riparian vegetation cover
- Riparian vegetation type
- Debris retention
- Fish cover
- Pool-riffle ratio

Sampling Frequency

The feasibility criterion was given serious consideration in the decision about how often to collect samples. Because the emphasis of the LTEMs was on biological monitoring of benthic macroinvertebrates, which are comparatively long lived (about 1 yr), it was decided that sampling in the spring and summer quarters would be sufficient. The spring fauna is the most abundant and diverse in mountain streams such as those in the SNP, because many species are "cool adapted" and go into various resting stages during the warm months. Highest flows usually occur in spring, thereby increasing concentrations of suspended matter for food and the amount of usable habitat. The low flows of summer offer the greatest likelihood of degraded water quality, especially lower concentrations of dissolved oxygen caused by higher water temperatures. Lower flows during summer can also cause significant reductions in the amount of habitat that is usable by the biota. Therefore, it was thought that spring and summer sampling would reflect "best case" and "worst case" conditions, respectively.

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The purpose of this manual is to describe the field and laboratory techniques that should be used to acquire data for the aquatic component of the Long-Term Ecological Monitoring System (LTEMS) that has been developed for the Shenandoah National Park (SNP or park). The major aquatic resources in the SNP are the numerous small streams. The primary tasks that the manual addresses are: choosing and establishing stream sites, finding existing sites, taking measurements and samples in the field, analyzing samples in the laboratory, calculating final results, and keeping records of data prior to entry in the database management system. The user manual is intended to provide sufficient information for persons with some background in field biology (e.g., fisheries, wildlife, entomology, ecology, forestry, natural resource management, recreation). The most likely users will be National Park Service employees, but the manual will also enable other researchers in the various aquatic sciences to contribute to the LTEMS.

DESIGN

Site Selection

In order for the aquatic LTEMS to be successful, the stream sites must be representative of all the different environments in the SNP. For the aquatic component, the term site refers to a designated length of stream along with the riparian area. The stream sites are classified in the following hierarchical system:

Administrative District

South
Central
North

Geological Formation/Alkalinity

Catoctin (100-200 $\mu\text{eq/L}$)
Pedlar or Old Rag (20-100 $\mu\text{eq/L}$)
Hampton or Erwin (<20 $\mu\text{eq/L}$)

Elevation

Upper (>548.6 m [1800 ft])
Lower (<548.6 m [1800 ft])

Other Factors

Accessibility
SNP trout monitoring sites
Current research interests

Using the three administrative districts as the first level of classification is a convenient means of distributing the stream sites throughout the park. In most cases, it is impossible to predict where an adverse impact might occur. Therefore, spreading the sites throughout the park increases the probability of having data from where they might be needed.

Geological formations were selected as the second level of classification because the SNP has a uniquely diverse geology and resulting water chemistry, particularly alkalinity. There are great differences among watersheds, and often between adjacent watersheds. Alkalinity and related water chemistry are probably significant factors affecting the distribution of the aquatic biota. Information on geological formations and alkalinity can be obtained from Gathright (1976) and Dise (1984), respectively. Streams in the SNP seldom have their watersheds entirely within a single geological formation. An effort should be made to establish stream sites in watersheds which have at least 75% of their area within one of the geological formation categories.

Elevation affects the distribution of aquatic biota indirectly because of factors such as stream width, depth, current velocity, valley shading, and temperature. The SNP is located entirely within the northern section of the Blue Ridge physiographic province, which is the narrow (about 8 km) mountainous area between the low-lying Piedmont province to the east and the Ridge and Valley province to the west (Hoffman 1969, Gathright 1976). Drainage basins in the Blue Ridge province typically have a steep gradient. Drainage basins on the west slope follow a trellis pattern, in which the small streams flowing down the mountains are parallel and join the main stream in the valley (Shenandoah River) at right angles (Reid and Wood 1976). Those on the east slope follow a dendritic pattern, so-called because of the resemblance to tree roots (Reid and Wood 1976). The criterion of 548.6 m (1800 ft) for distinguishing upper and lower sites was selected because field observations indicated that streams in the SNP tended to become very narrow and shallow (several m wide and less than knee deep) approximately above that elevation. This delimitation is somewhat arbitrary and can be varied within reason when it is desirable to have both an upper and lower site on the same stream. Elevation is best determined from the Potomac Appalachian Trail Club maps (scale 1:62,500). The USGS 7.5 min topographic maps are not as useful because they are not as accurate regarding trails, fire roads, and stream permanency.

Accessibility should be given serious consideration when choosing sites. If a site cannot be reached with reasonable effort at the desired time, then the data

are likely to be sporadic and would not be useful for monitoring. Trout populations in the SNP have been monitored for some time; therefore, it is logical to establish stream plots at many of the same sites so that there will be ecological data to explain the results of fish sampling. Gypsy moth infestation and acid deposition are environmental concerns at the present time in the SNP. Some preference should be given to stream plots that are likely to be affected or are being studied as part of other research projects.

Site Locations

Based upon the hierarchical classification system described above, 17 stream sites were selected for the aquatic LTEMs. These are listed below, with exceptions to the classification system marked with an asterisk and explained in the following paragraph. Detailed descriptions of the site locations, including maps and travel directions, are given in Appendix III.A.

South District

- Hampton or Erwin
 - Paine Run (Upper)
 - Paine Run (Lower)
 - Twomile Run (Upper)*
 - Twomile Run (Lower)

Central District

- Pedlar or Old Rag
 - Hazel River (Upper)
 - Hazel River (Lower)
 - Staunton River (Upper)
 - Staunton River (Lower)
 - North Fork Dry Run (Upper)*

Catoctin

- White Oak Canyon Run (Upper)
- White Oak Canyon Run (Lower)
- Hog Camp Branch (Upper)
- Rose River (Lower)

North District

- Catoctin and Pedlar*
 - Piney River (Upper)*
 - Piney River (Lower)
- Catoctin, Hampton, and Weverton*
 - Jeremys Run (Upper)*
- Pedlar
 - Lands Run (Upper)*

The "upper" sites on Twomile Run, North Fork Dry Run, and Lands Run lie at an elevation below 548.6 m (1800 ft), but their physical characteristics more resemble the other upper elevation sites. North Fork Dry Run was selected for a stream site because of the comprehensive studies on acid deposition being conducted in this watershed. The upper reaches of Piney River, Jeremys Run, and Lands Run do not have 75% of their watershed contained within a single geological formation category. Upper elevation sites were established on these three streams because of the likelihood of gypsy moth defoliation.

Site Layout

The stream sites consist of 100-m longitudinal reaches, which are marked by 91-cm (36-inch) lengths of 5-cm (2-inch) (i.d.) PVC pipe, either painted entirely black or with black end caps. The PVC pipe is secured by driving a 102-cm (40-inch) length of sharpened 2-cm (3/4-inch) steel rebar into the ground, attaching a chain link to the rebar with a hose clamp, and then passing a 1 x 9-cm (1/4 x 3 1/2-inch) hexhead bolt through the pipe and chain link (Appendix III.B.1). A 1-cm (5/16-inch) hole is drilled 30 cm (12-inches) from the bottom of the PVC pipe prior to going into the field for placement. There are two markers for each site, one at the upstream boundary and one at the downstream boundary. Both markers are on the same side of the stream, with the side and distance from the stream varying among sites in order to provide the most protection from floods. Specific locations of the markers at each of the sites are included with the detailed descriptions of the site locations in Appendix III.A.

Parameters

This section lists all of the parameters that are included in the LTEMs. Also included are brief explanations of the parameters and their utility for ecological monitoring in streams.

Water Quality

Discharge. Discharge (or streamflow) is the volume of water, including dissolved and mixed sediments or solids, moving past a cross section of stream per unit time (Buchanan and Somers 1969). Discharge and the associated stream morphology affect the distribution of both fish and macroinvertebrates. Fish and macroinvertebrates, because of their diverse modes of existence and food habits, prefer a variety of habitats with different streamflows. During extreme conditions of flood and drought, discharge can change the stream habitat and drastically alter the entire stream morphology. Streamflow also affects water quality by diluting or

concentrating certain parameters. Extensive loss of vegetation in a watershed, such as might be caused by fire or insect damage, will lead to changes in discharge.

Temperature. Temperature is the degree of hotness or coldness. Temperature can be both a controlling and limiting factor in the aquatic environment (Warren 1971). The growth and life cycles of fish and invertebrates are significantly affected by temperature because they are poikilothermic (body temperature follows closely the temperature of the water). Many of the water quality parameters are also affected by temperature, especially dissolved oxygen. In small mountain streams, the temperature is usually below 20 °C, except perhaps for brief periods in summer. Like discharge, water temperature can be affected by watershed disturbances. Defoliation of the canopy over a stream by gypsy moths could affect the water temperature by allowing more sunlight to reach the stream.

Dissolved Oxygen. The amount of oxygen dissolved in water, which is supplied by the atmosphere and photosynthetic plants, is one of the most fundamental parameters of the aquatic environment because oxygen is essential for all aerobic aquatic organisms. Oxygen levels are affected by temperature; as the water temperature increases, the solubility of oxygen decreases. Cool mountain streams with abundant riffles usually have oxygen concentrations at saturation throughout the year (9 - 14 mg/L). Other influences on dissolved oxygen are altitude and aerobic bacteria of decay (Cole 1979).

Hydrogen Ion Activity. Hydrogen ion activity is the concentration of hydrogen ions and is expressed as pH (potentia hydrogenii), with a range of 1-14 (Cole 1979). At pH 7 the molar concentration of hydrogen is neutral. An increase in hydrogen results in a lowered pH; a decrease in hydrogen indicates an alkaline reaction and an increase in pH. Hydrogen ion activity is dependent on alkalinity, the capacity of the aquatic environment to neutralize acid (Feldman and Conner 1985). The composition and abundance of most stream biota are influenced by pH.

Alkalinity. Alkalinity, or buffering capacity, is an index of the ability of a solution to neutralize acid. Underlying geological formations and their degree of weathering influence alkalinity (Cole 1979). Alkalinity is responsible for much of the overall chemical environment because it is a major variable controlling the form and concentration of many ions as well as the hydrogen ion activity (Feldman and Connor 1985). The community structure of macroinvertebrates and fish are influenced by alkalinity, with the usual trend being greater abundance and diversity in streams with high alkalinity. The

water quality criterion for alkalinity is 20 mg/L or more for the protection of freshwater aquatic life, except where natural concentrations are less (U. S. Environmental Protection Agency 1976).

Conductivity. This parameter is a measure of the resistance of a solution to electrical flow (Wetzel 1983). Conductivity is indicative of the total ions in solution; therefore, it is sometimes considered a shortcut for analyzing total dissolved solids. Conductivity is a good indicator of a variety of impacts on the aquatic environment.

Sulfate. Sulfate is the predominant form of sulfur in water and is apparently necessary for plant growth. Sulfur is important in protein metabolism but is supplied to organisms originally as sulfate. The mean composition of sulfate in North American inland waters is 15.31% of total ions, with concentrations ranging from 1 to > 6000 mg/L (Reid and Wood 1976).

Nitrate. This oxide of nitrogen occurs in fresh waters through the sources of precipitation, surface and groundwater drainage, and fixation in both water and soil (Wetzel 1983). It is the most available form of nitrogen and is therefore very important for plant growth (Hynes 1970). Pollution from sewage and agricultural fertilizers can lead to high concentrations of nitrogen compounds that disrupt the balance of aquatic ecosystems. Nitrate averages 1.77% of the total ions found in North American inland waters; concentrations range from near zero to 9 mg/L (Reid and Wood 1976).

Chloride. Chloride is the major halide of chlorine stored in most freshwater algal cells (Cole 1979). The atmosphere and geological formations are the natural sources of chloride. Elevated levels usually indicate a source of pollution such as sewage. On the average, chloride accounts for 7.44% of total ions in North American inland waters, with concentrations ranging from near zero to > 5400 mg/L (Reid and Wood 1976).

Calcium. Implicated in numerous ways in the growth and population dynamics of freshwater flora and fauna, calcium is considered a micronutrient and varies with temperature, pH, and substrate composition (Wetzel 1983, Reid and Wood 1976). Limestone streams normally have high concentrations of calcium, which usually mean well buffered systems with abundant and diverse fauna. In soft water streams, which exist on igneous rock, calcium levels are low and the fauna is usually much sparser. The mean composition of calcium in North American inland waters is 19.36% of total ions, with concentrations ranging from near zero to 80 mg/L (Reid and Wood 1976).

Magnesium. Magnesium and calcium react similarly in streams although magnesium is normally found at lower concentrations (Reid and Wood 1976). Its source is the mineral crust of the earth, and the metabolic demand for magnesium is generally minor compared to its availability in freshwater (Wetzel 1983); however, it is still considered an important cation to the freshwater flora and fauna. Magnesium averages 4.87% of total ions in North American inland waters; concentrations range from near zero to 10 mg/L (Reid and Wood 1976).

Potassium and Sodium. These two closely related cations occur from the weathering of rock (Cole 1979). Any alteration of their concentrations in natural waters is not common (Wetzel 1983). Both potassium and sodium are important in algal metabolism. The mean compositions of sodium and potassium in North American inland waters are 7.46% and 1.77% of total ions, respectively; their combined concentrations range from near zero to 13,000 mg/L (Reid and Wood 1976).

Silica. Silica is one of the major forms of silicon that occurs in freshwater. It occurs from the weathering of rocks and is an essential nutrient for diatoms, which use it in construction of their frustules (Cole 1979). The mean composition of silica in North American inland waters is 8.60% of total ions (Reid and Wood 1976).

Seston. Seston is the particulate matter that is suspended in water, including both the organic and inorganic material. Organic seston is utilized for food by many macroinvertebrates. Collector-filterers, or suspension feeders, filter seston from the water column, while seston that has settled to the substrate is utilized by the collector-gatherers (Merritt and Cummins 1984). Although seston has a positive influence as food, excessive levels can be detrimental because the material settles on the firm substrates making them unsuitable for attachment by many macroinvertebrates. Seston is affected by discharge, stream morphology, type and amount of riparian vegetation, and debris retention structures. A review of studies in small streams indicated that the annual mean for organic seston is about 3 mg/L; however, during the year concentrations of organic seston may range from 0.04 to 15.0 mg/L (Webster et al. 1979).

Bacteria. Fecal streptococcus, total coliform, and fecal coliform are indications of the levels of animal intestinal organisms present in the water, but also include nonenteric (soil born) organisms as well. Ratios of these bacteria are used to indicate and distinguish between human and animal pollution (American Public Health Association et al. 1981).

Habitat

Depth. Stream depth is an important factor in determining the amount of usable habitat for macroinvertebrates and fish. It is defined as the height of the water column from the existing surface level to the channel bottom (Platts et al. 1983).

Width. The different communities of macroinvertebrates and fish that occur within watersheds appear to be related to stream width. The relationship is probably an indirect one, with wider streams having a greater diversity of microhabitats and warmer temperature regimes. Width is defined as the horizontal distance along a transect line from shore to shore at the existing water level, including only the distance that is covered with water (Platts et al. 1983).

Substratum. The substratum can be any organic or inorganic materials that are sufficiently stable for organisms to live on or in. In SNP streams the substratum consists almost entirely of mineral particles, with size of the particles being the most important ecological factor. Benthic macroinvertebrates are dependent upon a suitable substratum for attachment sites, hiding places, and in some cases food (periphyton). Coarse substrate (boulders, cobbles, pebbles) that lies loosely on the stream bottom provides the best habitat for macroinvertebrates because of the diversity of currents and pore spaces created. Gravel is important for the spawning of some stream fish.

Riparian Vegetation Cover. This parameter refers to the amount of shading provided by the tree canopy (overstory) and also the amount of litterfall that can be expected to be deposited in the stream. Small mountain streams typically have a dense canopy that shades the channel for much of the year. The light and temperature conditions that result from the shading are major factors in determining the communities of periphyton, macroinvertebrates, and fish, and the leaf input during autumn is the major source of energy for the ecosystem.

Riparian Vegetation Type. This parameter reflects the type of vegetation that would be expected to be predominant in the litterfall as well as the amount. Macroinvertebrate communities in small mountain streams are adapted for an energy input from leaves, rather than grasses and forbs. Trees contribute more usable litterfall than shrubs, which contribute more than grasses and forbs. The riparian vegetation type also indicates the stability of the banks. The heavy root systems of trees stabilize banks more than the lighter root systems of shrubs or grasses and forbs. Having no riparian vegetation along small streams would be indicative of perturbation.

Debris Retention. This parameter is an indication of a stream's capacity to retain litterfall and prevent its downstream transport. Debris retention structures include rock outcrops, boulders, trees rooted in the channel, and fallen logs. Higher productivity of macroinvertebrates and fish would be expected with more debris retention structures. In addition, debris retention structures are usually associated with fish cover and amount of pools (see below).

Fish Cover. Fish cover is considered to be the places where 15 to 20-cm (6 to 8-inch) brook trout could hide without being visible from above. Structures that provide cover are large rocks, logs, undercut banks, and terrestrial vegetation that bends over into the water. Cover is an important factor in evaluating the quality of habitat for fish, with greater survival and growth being positively correlated with more cover.

Pool-Riffle. This parameter indicates the relative amount of pool habitat (deep, still water) in comparison to the amount of riffle habitat (shallow, flowing water). In order to be considered a pool, the depth should be sufficient for 15 to 20-cm (6 to 8-inch) brook trout to reside there. Pool-riffle is another assessment of the quality of the habitat for fish, because some important species (e.g., brook trout) are more productive when there are ample pool areas. However, benthic macroinvertebrates, which are a major source of food, are more productive in riffles, and brook trout require riffle areas for spawning. The optimum pool-riffle ratio may be 1 to 1 (50% pool), or slightly higher.

Biological

Periphyton. The community of algae that lives attached to rocks and other firm substrates accounts for almost all of the primary production in small mountain streams. Periphyton comprises an important portion of the diet of many grazing macroinvertebrates. Periphyton may also provide detritus for collector macroinvertebrates when it dies. The amount of chlorophyll *a* in the periphyton is indicative of the biomass of live algae. The autotrophic index can be used to determine the relative amounts of live algae as compared to nonliving detritus attached to firm substrates. Any perturbation that changes the amount of light reaching the stream (e.g., gypsy moth defoliation) would cause a corresponding change in periphyton biomass.

Macroinvertebrates. The prefix "macro" has no exact definition. Macroinvertebrates are generally considered to be those worms, molluscs, and arthropods that are large enough to be seen with the unaided eye. It should be kept in mind that this delimitation refers to the mature stages of those organisms; the early stages

of some macroinvertebrates can only be seen with the aid of a stereomicroscope or magnifier. The term "benthic" refers to organisms living on the bottom of aquatic environments or on firm substrates protruding above the bottom. The benthic community contains a variety of organisms; however, in small streams like those in the SNP, most of the community members are the immature stages (nymphs and larvae) of insects. All of the so-called "aquatic" insects actually leave the water for a terrestrial adult stage, during which reproduction takes place.

Benthic macroinvertebrates are commonly used for ecological monitoring for several reasons (Voshell et al. 1989). They are abundant in almost all freshwater environments. Sedentary habits and comparatively large size, in combination with abundance, make them relatively easy to collect. Most can now be identified reliably to genus with comprehensive taxonomic works that have become available (e.g., Pennak 1978, Brigham et al. 1982, Merritt and Cummins 1984). The benthic macroinvertebrates that exist in a given area are indicative of the environmental conditions that have occurred for at least several months previously because their life cycles last from several months to a year or more. Taken as a group, benthic macroinvertebrates have diverse habitat and food preferences, but many individual taxa have narrowly defined niches. Therefore, it is likely that some taxa will demonstrate a change in response to any perturbation that might take place. Activities of benthic macroinvertebrates affect major ecological processes of freshwater ecosystems. These significant activities include grazing on primary producers, decomposing organic matter, preying on smaller invertebrates, temporarily storing materials (spiralling), and providing food for fish.

Three types of macroinvertebrate samples are taken at each stream site: quantitative benthic, qualitative benthic, and aerial. The quantitative benthic samples are the mainstay of the macroinvertebrate monitoring program. The data from those samples can be used to make statistical comparisons among streams and over time. Benthic macroinvertebrates are microhabitat specialists, and quantitative sampling devices cannot be placed in some of the microhabitats that occur in streams. Therefore, qualitative benthic samples are also taken to determine at least the presence or absence of taxa in those locations so that the taxa lists for each stream site will be comprehensive. The aerial samples, which are also qualitative, are taken to capture the adult stages of the insects that are aquatic as immatures. Aerial sampling of adults is done for two reasons: (1) adult specimens can usually be identified to the species level, whereas immature specimens can be identified usually only to genus; and (2) the taxa lists

for the sites will be more comprehensive because the immature aquatic stages might be missed or be absent as a result of a recent emergence.

Sampling Frequency

The LTEMs was designed to store and analyze data according to quarters of years, defined as spring (April, May, June), summer (July, August, September), fall (October, November, December), and winter (January, February, March). Because of the effort required, it is likely that it will be possible only to sample in one or two quarters consistently. If this is the case then the spring and summer quarters should be emphasized, in that order. The spring fauna is the most abundant and diverse in mountain streams such as those in the SNP, and this is the time of year when most taxa can be collected in both immature and adult stages to facilitate taxonomic identifications. Early stages of some of the summer fauna are also present in the spring. Highest flows usually occur in spring, diluting dissolved substances and increasing concentrations of suspended matter. The low flows of summer offer the greatest likelihood of degraded water quality, especially lower concentrations of dissolved oxygen caused by higher water temperatures. Lower flows during summer can also cause significant reductions in the amount of habitat that is usable by the biota. Therefore, summer sampling will probably reflect "worst case" conditions.

Within any quarter, all stream sites should be sampled as close in time as possible in order to reduce variability caused by the rapidly changing life cycles of the aquatic insect fauna. It would be ideal if all sites could be sampled within 2 wk, but the time for sampling all sites should not exceed 4 wk. Sampling for the spring and summer quarters should be concentrated in May and August, respectively.

FIELD SAMPLES AND MEASUREMENTS

General Information

Effort

This section describes the methods for the parameters that can be measured in the field and for taking samples of those parameters that require final analysis in the laboratory. It takes two persons approximately 4 hr to complete these procedures at each stream site, excluding hiking time.

Preliminary Tasks

Before leaving the vehicle the stream site code, date, and names of the persons doing the field work

should be entered on the Field Records, Discharge Measurement, and Habitat Measurements sheets (Appendices III.C.1, III.C.2, and III.C.3, respectively). The sample bottles for water quality, bacteria, and periphyton should be numbered or otherwise labeled externally and entered on the Field Records sheet. The labels for all macroinvertebrate samples should be made ready to drop in the containers immediately after collection. The composition of these labels and the writing upon them should be sufficient to withstand the effects of the preservatives in which they will be placed (alcohol or formaldehyde). A particularly effective labeling technique is to use one of the devices that embosses self-sticking plastic tape, and then drop the strips of plastic tape into the samples without exposing the adhesive backing. Using bright colors of plastic tape makes the labels easier to find in the samples. A less effective alternative is to use paper with at least 50% rag content and to write with #2 pencil or India ink. If electronic instruments (pH, conductivity, dissolved oxygen) are going to be used later at the vehicle rather than *in situ*, those instruments should be prepared for use when the samples are returned. Two backpacks (one bare frame and one with bag) are required to transport the apparatus to the stream. A checklist of the equipment that must be carried is found in Appendix III.C.16.

Sampling Sequence

Upon reaching the stream sites, it is important to follow a protocol designed so that the disturbance caused by some activities does not affect any samples or measurements that follow. All bottles for water quality and bacteria samples should be filled and instrument readings taken before any disturbance of the stream bottom. The temperature reading should be taken at this time also. The 100-m tape for marking transects at 10-m intervals can be placed along the center of the stream channel at any time after the water quality and bacteria samples are taken, but care should still be taken not to disturb the bottom any more than necessary while traversing the length of the site. Next, the discharge is measured below the site, then the periphyton and macroinvertebrates are sampled, always moving progressively upstream. The discharge measurement and the quantitative benthic macroinvertebrate samples require two people, but an effective division of labor after that is for one person to take the periphyton and qualitative benthic macroinvertebrates samples while the other person takes the aerial samples of adult insects. The final measurements involve the habitat parameters, and these require both persons. Habitat measurements can be made by proceeding in either direction in the stream, and there is no concern about disturbing the bottom because there is no further sampling at the site.

Upon return to the vehicle, water quality and periphyton samples that require later analysis in the laboratory should be packed on ice, water quality measurements with electronic instruments should be completed (pH, conductivity, dissolved oxygen) if they were not done at the stream, and macroinvertebrate samples should be preserved.

Equipment

A list of all equipment needed for the field and laboratory procedures is provided in Appendix III.B.2.

Water Quality

Physical/Chemical and Bacteria

Samples should be collected before disturbing the stream in any manner. Try to fill bottles without entering the stream. Collect samples in moving water where there is no accumulation of debris on the surface and no indication of stagnation, but do not sample in an area where the current is so swift that splashing occurs. If it is necessary to enter the stream to find a suitable area, then stand still and reach as far upstream as possible when filling the bottles. Special care should be taken when collecting the water samples to be used for measurements of bacteria. When filling the bottle, it is necessary to avoid contamination from the person taking the sample. This is best accomplished by holding the bottle near its bottom and slanting it slightly in the upstream direction when filling. When measuring temperature, avoid standing water and sunny spots because these might be atypical areas with slightly elevated temperatures. These guidelines also apply to the use of electronic instruments, which may be used for *in situ* measurements of temperature, pH, conductivity, or dissolved oxygen. After all bottles have been filled, leave them in shallow water in a shaded area of the stream while the remaining samples and measurements are being taken.

A summary of how measurements should be made or samples collected is given below. There are many alternatives for water sample containers. Because the SNP will be contracting some of the water quality analyses, other laboratories may have individual preferences that would also be acceptable.

Temperature, pH,
conductivity,
dissolved oxygen

Electronic
instruments
or kits

Alkalinity, pH, conductivity,
nitrate, sulfate,
calcium, magnesium,
chloride, sodium,
potassium, silica

1-L dark plastic
bottle

Seston

1-L dark plastic
bottle

Bacteria

1-L plastic bottle,
autoclaved

Temperature
(Optional)

Long-stem
thermometer

When taking field measurements with electronic instruments or kits, it is sometimes difficult to know if the equipment is functioning properly. Therefore, it may be useful to be familiar with the ranges that have been recorded in the SNP for the water quality parameters that will be measured in the field:

Temperature (°C)	0.0 - 22.0
pH	4.95 - 7.39
Conductivity (umhos)	10 - 93
Dissolved oxygen (mg/L)	5.6 - 10.9

Immediately upon returning to the vehicle, all of the water quality samples should be packed on ice in a cooler.

Discharge

Discharge should be measured by the six-tenths-depth method as recommended by the U. S. Geological Survey (Buchanan and Somers 1969) and the U. S. Forest Service (Platts et al. 1983). Methods are described in detail in the preceding publications. The principle is that depth and current velocity are measured at frequent intervals along a transect, the discharge is calculated for each individual section along the transect, then the total discharge of the stream is calculated by summing the discharges from all of the individual sections. Discharge should be measured below the site at the closest suitable location. Either a mechanical or electronic current meter (e.g., Scientific Instruments Model 1205 or Marsh-McBirney Model 201, respectively) can be used to make the velocity measurements (see equipment list in Appendix III.B.2). A separate data sheet is used to record the measurements in the field and to facilitate the discharge calculations (Appendix III.C.2). After selecting a suitable location, consisting of a stable, flat streambed with the threads of velocity parallel to each other, the transect is established by stretching a tag line across the stream at right angles to the direction of flow. "Tag line" is the term used by hydrologists for a small-diameter cord marked

with appropriate intervals to indicate distances when measuring discharge. The tag line can be secured by small metal rods or surveyor's pins driven into the streambank. Because discharge is measured outside of the stream site, rocks or debris can be moved to improve flow and streambed profile.

Using the tag line, determine the stream width to the nearest 0.1 m and record on the data sheet. The depth and current velocity must now be measured at frequent intervals along the transect that is demarcated by the tag line. This can be done from either side of the stream. The initial reference point for distance along the transect is where the tag line is secured to the pin. Beginning at one edge of the stream and proceeding at intervals of 0.1 - 0.3 m, measure and record the distance from the initial point (nearest 0.1 m), depth (nearest 0.01 m), and current velocity (direct reading from an electronic instrument or revolutions and seconds from a mechanical instrument). At both stream edges the depth and velocity will normally be zero. The number of recordings across the tag line varies with stream morphology, but as a general rule the narrower the stream (typically the upper sites) the smaller the recording intervals (0.1 m), and the wider the stream (typically the lower sites) the larger the intervals (0.3 m). When the bottom and current are irregular, recordings should not necessarily be made at regular intervals. Where current is visibly different over a short distance, recordings should deliberately be taken in the fastest and slowest water. Where rocks protrude sharply from the channel bottom, recordings should be made at the apex and close to the base. According to the six-tenths-depth method, the meter should be placed at six-tenths of the depth below the surface or four-tenths of the depth above the stream bottom.

SNP personnel use a Marsh-McBirney Model 201 electronic flow meter with a top-setting wading rod for measuring velocity. The following instructions apply to the use of that particular instrument. Attach the sensor to the wading rod, being careful not to overtighten the thumbscrew. Immerse the sensor in the water and turn the selector switch to "CAL" to check for electronic failure or low batteries. The readout should fall between 9.8 and 10.2 if everything is functioning properly. To set the sensor at six-tenths depth, the sliding decimeter scale should be lined up with the centimeter scale on the top-setting wading rod. For example, if the measured depth was 52 cm, line up the 5 on the sliding scale with the 2 on the centimeter scale. This will set the sensor at six-tenths depth. To get a velocity reading, set the selector switch on "M/sec." To help stabilize the readings, use the time constant switch. A time delay amounting to the switch setting multiplied by 5 sec occurs before the first full scale reading is reached. For example, if the time constant is set on 6, the first reading is reached at

30 sec. It is recommended by the manufacturer that readings be attempted at the smallest time constant first. If the readings do not stabilize adequately after the required time delay, then turn the time constant to the next highest number and try again.

Biological

Periphyton

A quantitative measurement can be made by scraping the periphyton from a known area of substrate. Four samples should be taken from each stream site by a stratified random design. (See the next section, Macroinvertebrates - Quantitative Benthic, for an explanation of stratified random design.) One sample should be taken from each of four cobble-size rocks selected from different segments of the site. The rocks must be of suitable shape and texture for attaching the bar-clamp sampler (Appendix III.B.3). The thickness of the rock cannot exceed the maximum opening of the bar-clamp sampler (11 cm), and the surface must be reasonably flat and smooth in order for the rubber gasket to seal properly. Also, do not choose rocks that are covered with obvious sedimentary materials; this can usually be avoided by selecting rocks from shallow riffle areas.

Attach the bar-clamp sampler securely to the rock, making sure of the seal by visual examination. Clean the entire area of the rock contained within the sampler with an acid etching brush. Move the brush firmly but slowly in all directions, being careful not to let any material "spring" off the brush. Rinse the inside of the sampler and the brush with distilled water from a squeeze bottle, making sure that no water leaves the sampler. The rinse water containing the periphyton in the sampler is then removed with an eye dropper and placed in a 60-ml dark plastic bottle. Repeat the procedure as necessary. The rock surface should appear clean (different color) when the periphyton has been satisfactorily removed.

Keep the periphyton sample cool at the stream by placing the bottle in shallow water at a shady spot, and pack the sample on ice in a cooler upon returning to the vehicle.

An alternative to sampling periphyton on natural substrates such as rocks is to use artificial substrates, which are usually standard 25 x 75-mm glass microscope slides. A floating rack constructed of clear vinyl plastic and styrofoam is commonly used to hold the slides (American Public Health Association et al. 1981). The periphyton from measured areas of each of several slides is scraped into dark plastic bottles. A major disadvantage of using this method is that it requires two

visits: one to place the samplers and one to retrieve them several weeks later. In addition, the glass slides do not collect the same community of algae as the rock substratum. Sampling natural substrates may be preferable for the LTEMs because the primary interest is how much periphyton biomass, either alive or as detritus, is available to consumers.

Macroinvertebrates

The LTEMs includes three types of macroinvertebrate sampling: quantitative benthic, qualitative benthic, and aerial. It is essential to collect both types of benthic samples consistently. Aerial sampling of adult aquatic insects is optional. The main purpose of collecting adult specimens is to obtain species level identifications, but the identifications can be done only by specialists in systematics.

Quantitative Benthic. The sampling devices that are recommended for the LTEMs are the Portable Invertebrate Box Sampler (PIBS) or the Surber Sampler (Appendix III.B.4). Although there are many different types of quantitative samplers, the PIBS is the best device for macroinvertebrates in rocky-bottomed streams (Voshell et al. 1989). The foam-lined bottom establishes a thorough seal by taking the shape of the bottom; therefore, the PIBS is effective on irregular cobble substratum as well as pebbles and gravels. In addition, the PIBS is completely enclosed, with wire mesh on the front side and solid side walls, so organisms cannot escape while sampling is in progress. The square shape and the edges at the bottom make it easy to attach to a backpack frame for transporting to remote areas. If the PIBS is attached to the backpack frame so that the catch net is up, the net can be inverted toward the wire mesh and used to carry gear. The Surber Sampler is not as effective at preventing organisms from escaping during sampling because it is not completely enclosed and does not fit closely to irregular substratum. The Surber Sampler should only be used when the water is not deep enough to use the PIBS. Do not use two different devices at a stream site on the same occasion. Both sampling devices should be outfitted with 350- μ m mesh catch nets. This mesh size has been shown to be fine enough to retain most organisms, yet not so fine that stream currents will cause a backwash out of the net. Coarser mesh nets (> 500 μ m) do not retain many of the small macroinvertebrates, most notably the abundant Chironomidae (Insecta: Diptera) (Voshell et al. 1989).

Quantitative benthic sampling cannot be completely random in rocky-bottom streams, such as those in the SNP. The physical placement of the sampling device is restricted by factors such as size and shape of the substratum, current velocity, depth of water, and in

some cases, even the width of the channels containing water. For these reasons, sampling in rocky-bottomed streams is usually designed as "stratified random," meaning that samples are always taken in the same habitat or "stratum." For the LTEMs the sampling stratum should be shallow riffles where the substratum consists predominantly of cobble and pebble/gravel. The sampler should be placed in areas where the depth and current are sufficient to wash dislodged organisms into the catch net but not so deep that the sampler is completely submersed. The substratum where the sampler is placed should not be so irregular that the sampler will not seal against the stream bottom. The area of stream bottom enclosed by the sampler should contain a mixture of substrate, consisting mostly of cobbles, some pebble/gravel, and a little underlying sand. (See the section on Habitat and Appendix III.C.7 for an explanation of substratum categories.) Homogeneous areas of small gravel and sand should be avoided because the diversity and abundance of macroinvertebrates will be very low.

Three quantitative samples should be taken at each stream site. Take the samples in different segments of the site, always beginning downstream and walking upstream to find the next suitable sampling area. Benthic macroinvertebrates are motile, and measurements of their abundance will be affected by walking through areas to be sampled. Spread the samples out as much as possible within the site, but when suitable benthic habitats are scarce take the samples wherever depth, flow, and substratum are optimum. After placing the sampler on the bottom, check to make sure that there is a good seal. The best position for the person taking the sample is to kneel behind the sampler with the catch net passing between the legs and the knees on the edges of the device; alternatively, the person can sit on the box (Appendix III.B.4). With either position the weight of the person taking the sample pushes the PIBS tightly against the substratum. If it is difficult to establish a good seal, the second person can assist by standing on the PIBS at the appropriate place. The instruments that will be needed for sampling are a vegetable brush, a small hand rake, and forceps. Brush each individual rock on all sides, so that the organisms will be dislodged and swept into the catch net. This is best done by holding the rocks underwater to make sure that no organisms are thrown out of the sampler. Each rock should also be visually examined at close range, because many aquatic insects have special means of attaching themselves very tightly to rock surfaces. Use forceps to remove any organisms found clinging after brushing. It is more efficient if a second person examines the individual rocks and removes the firmly attached organisms. After all of the larger rocks have been brushed, examined, and removed, rake the remaining fine substrate to stir up the sediment inhabiting organisms. Try

to rake down to a depth of about 10 cm. After raking, remove the catch net from the PIBS, or pick up the sampler in the case of the Surber. The catch net should be washed several times to concentrate the contents into the end. This is best accomplished by taking the net to a nearby pool area. The mouth of the net is briefly submersed and then raised rapidly. The contents of the sample are placed into a storage container by inverting the net, pushing an arm into the net, and gently tapping the apex from the inside. It is usually necessary to reverse and rewash the net several times to get all of the contents into the container. The appropriate label should be placed in the container immediately. Completely invert the catch net and backwash it before proceeding to take the next sample.

Any container that is a practical size and leakproof will suffice for storing benthic macroinvertebrate samples. Wide-mouth plastic jars are good because they do not break, but glass canning jars are also commonly used. A technique that has been used successfully in the Aquatic Entomology Program at VPI&SU for over a decade is to place the samples in small plastic trash bags that measure 23 x 20 x 46 cm (9 x 8 x 18 inches) and hold a volume of 15 L (4 gal). These bags are available in grocery stores. They can be doubled if there is concern about the contents of a sample making punctures (e.g., sticks). When the samples are first collected the plastic bags are tied loosely to contain the material, then upon returning to the vehicle, they are reopened, preservative is added, and the bags tied tightly. All of the bags containing separate samples from one stream site are placed in a 2.5-L plastic bucket with a snap-top lid (such as the ones used for cottage cheese). The appropriate labeling code should be written on the outside of the plastic bucket with a wax pencil.

There are several choices of fluids that can be used to preserve macroinvertebrates; formaldehyde, ethanol, and isopropanol are most commonly used. Benthic samples, which contain much bacteria-laden detritus and bottom sediment, are more difficult to preserve than separate organisms. Formaldehyde is the most effective preservative for benthic samples, but it must be used with caution because of human-health risks. If formaldehyde is used, the concentration should be 5% of the standard stock solution that is called formalin. Formalin contains 37% formaldehyde, so the concentration of formaldehyde in the preservative is a little less than 2%. The material in the sample only needs to be covered with the 5% formalin solution; it is not necessary to fill the sample container. Both ethanol and isopropanol are adequate substitutes for formaldehyde, and there is little difference between the two alcohols. Macroinvertebrates are best preserved with

a 70% solution of alcohol. More concentrated solutions make the specimens brittle and difficult to identify, while lower concentrations will not adequately retard decomposition by bacteria in the detritus and bottom sediment. Because benthic samples will retain some water and the live organisms contained in the sample have a great deal of water in their bodies, special attention must be given to dilution when preserving with alcohol. A 70% effective concentration can be approximated in the field by generously covering the material in samples with undiluted alcohol.

Qualitative Benthic. These samples are taken with a D-frame dip (or kick) net. The major objective is to obtain specimens from the many microhabitats that cannot be sampled with the quantitative device. Examples of these other microhabitats include leaf packs, surfaces of large rocks or logs, underneath large rocks or logs, exposed roots at the margins, and vegetation (either aquatic or terrestrial) hanging into the water (Appendix III.B.5). The investigators should use their powers of observation (and imagination) to find as many places as possible where macroinvertebrates might reside. It is usually best to dislodge the organisms with the hands and let them wash into the net. It is sometimes necessary to move objects with the feet, but this should be avoided usually because the specimens are often damaged. The net should be emptied at frequent intervals (at least after each microhabitat), combining all material into a single container. It is not necessary to retain all material in the net since this is a qualitative sample. It is difficult to state how much qualitative sampling is "enough" to achieve the goal of obtaining a complete taxa list. Try to sample each noticeable microhabitat, and try to expend equal effort among stream sites. The qualitative benthic samples are preserved in the same manner as the quantitative ones.

Aerial. The qualitative sample of emergent adult insects is obtained with an aerial net (Appendix III.B.6). One investigator should walk the entire site one time in one direction, while alternately sweeping both banks of the stream. Sweep the riparian vegetation with the net and periodically examine the contents. Sample different types of vegetation and different distances above the ground. The most effective use of the net is to sweep the tips of the vegetation or to bring the net up under branches and tap them. Sometimes flying insects will be observed and can be captured in flight. Captured insects can be retained while the investigator is walking by looping the net sideways over the rim. Periodically the contents of the net should be examined and the insects removed individually with forceps. The specimens are placed in a small jar (2 oz is convenient) containing 70% alcohol, along with the appropriate

label. It is desirable to retain only the aquatic insects, but this requires some entomological experience. The alternative is for the investigator to retain all specimens.

Habitat

Habitat measurements are made at 10-m intervals along each 100-m stream site. The 100-m measuring tape should be extended longitudinally between the upper and lower boundaries of the site. The tape should be placed along the middle of the stream, following the general shape of the channel as much as possible. Surveyor's pins or other type of stakes can be used to secure the ends of the tape, or a large rock can simply be placed on the tape. All habitat measurements are recorded on a separate field sheet (Appendix III.C.3). Measurements can be made by proceeding either upstream or downstream, but this should be recorded by circling the appropriate arrow for flow on the field sheet. The tag line used in the discharge procedures is also used to mark each transect in succession for the habitat measurements. The tag line can be secured on the streambanks with surveyor's pins. The overall scheme for habitat measurements is illustrated for a representative stream site in Appendix III.C.4. Note that transects T0 through T10 demarcate segments S1 through S10, respectively. Width, depth, riparian vegetation, and substratum are measured at each transect. Debris retention, fish cover, and pool-riffle ratio are measured throughout each segment.

Depth

Three depth measurements should be made along each transect, at distances of one-fourth, one-half, and three-fourths of the transect width. Depth is best measured with a wading rod, which is sturdy enough to withstand the current and the flat base will not slip into crevices. Other alternatives are a folding rule or meter stick. Depth measurements should be recorded to the nearest cm.

Width

Measure the width of the stream at each transect to the nearest 0.1 m. Measure the width between the two edges of water, not the banks of the channel. Islands, which are defined as rock or land structures greater than 30 cm (1 ft) high and 30 cm (1 ft) wide, are excluded from the measurement of stream width.

Riparian Vegetation Cover

This measurement should only be made when the leaves of deciduous trees would be expected to be present. At each transect, look directly up and rank the amount of cover over the stream channel into one of

three categories: 3 = 67-100% covered; 2 = 34-66% covered; 1 = 0-33% covered. This qualitative estimate is recorded on the habitat field sheet in the column "r. cover." Photographs of conditions representing each category are provided in Appendix III.C.5. In Category 1 the stream is exposed to the sun almost the entire day. Category 1 is not likely to occur in the SNP unless there has been extensive perturbation of the surrounding forest. Category 1 is the situation that would normally be expected for a small stream located in a forest during the early stages of succession, or for a river which is too large for the trees to shade. Most of the streams in the SNP, particularly the smaller ones at higher elevations, are likely to have riparian cover that fits Category 3. This is the condition when the limbs of most of the trees on both banks meet over the stream channel, thereby shading most of the water. In Category 2 the limbs of the trees do not meet over the water, but the trees are tall enough and the stream narrow enough that the water is shaded except during the middle of the day.

Riparian Vegetation Type

At each transect examine the vegetation on both banks that would fall or blow into the stream and decide whether the majority of the material would come from trees, shrubs, or grasses and forbs. Be sure to include the overstory that arises from the streambanks when making this qualitative evaluation. The categorization is recorded on the habitat field sheet in the column "r. type," as either T = trees, S = shrubs, or GF = grasses and forbs. No riparian vegetation is recorded as N. Photographs of flora representing each category are provided in Appendix III.C.6.

Substratum

The composition of the stream bottom is visually analyzed at 0.3-m intervals along the width of each transect. The tag line from the discharge measurements is useful for this purpose because it has convenient markings. In SNP streams the substratum consists almost entirely of mineral particles. Look directly under each 0.3-m segment of the tag line, and categorize the dominant substrate material according to the following sizes and terms:

Large, immovable	Bedrock (BR)
>25 cm, movable	Boulder (B)
6-25 cm	Cobble (C)
.2-6 cm	Pebble/Gravel (PG)
<.2 cm	Sand (S)

Photographs of each substrate category are provided in Appendix III.C.7 for reference. A length of PVC pipe with the substrate sizes delineated ("cobblestick") is very useful for making rapid and accurate decisions.

Record the abbreviation of the dominant type for each 0.3 m in the spaces on the habitat field sheet under "Predominant Substrate."

Debris Retention

Examine each segment between adjacent transects and categorize them according to the abundance of retention structures: 3 = abundant; 2 = intermediate; and 1 = none. Debris retention structures include rock outcrops, boulders, trees rooted in the channel, and fallen logs. The amount of debris accumulated on the structures does not enter into the decision. These qualitative estimates should be recorded on the habitat field sheet in the column "d. reten." Photographs of conditions representing each category are provided in Appendix III.C.8 for reference.

Fish Cover

Examine each segment between two adjacent transects and categorize them according to the abundance of fish cover: 3 = abundant (more than 50% of segment has cover); 2 = intermediate (25-50% of segment has cover); and 1 = exposed (less than 25% of segment has cover). Fish cover is considered to be places where 15 to 20-cm (6 to 8-inch) brook trout could hide without being visible from above. Structures that provide cover are large rocks, logs, undercut banks, and terrestrial vegetation that bends over into the water. In order to be usable cover, the water should be at least 15 cm deep and the current should be less than 15 cm/s. Depths and velocities must be estimated visually when categorizing fish cover. Photographs of conditions representing each category are provided in Appendix III.C.9 for reference. These qualitative estimates should be made according to the existing water level. Estimates are recorded on the habitat field sheet in the column "f. cover."

Pool-Riffle

Examine each segment between two adjacent transects and estimate the length of the segment that is pool (deep, still water) and the length that is riffle (shallow, flowing water). In order to be considered a pool, the depth and current should be appropriate for 15 to 20-cm (6 to 8-inch) brook trout to reside there (at least 15 cm deep with current less than 15 cm/s). Pocket water in the channel during low flow can be considered as a pool; glides and runs (smooth, slow-flowing water) are considered to be riffles. The 100-m tape that is stretched longitudinally along the channel is helpful, but pool-riffle is still largely a qualitative estimate. Depth

and current must be estimated visually. It should be kept in mind that pool-riffle refers only to major differences along the length of a segment and that minor differences across the width of a segment are disregarded. Photographs in Appendix III.C.10 provide some examples of pool-riffle estimates. The estimates are recorded on the habitat field sheet in the column "P:R", to the nearest 1 m and totaling 10 m.

LABORATORY ANALYSES AND CALCULATIONS

This section explains how the samples that have been collected in the field are analyzed to produce the final results. In addition, some measurements that were taken in the field require further calculations to reach appropriate units for reporting. Many of these analyses and calculations are standard methods that are described in readily available books and bulletins, which will be referenced without repeating the details. Several summary and worksheets are provided so that the laboratory analyses and calculations can be performed efficiently and accurately (Appendices III.C.11 - 15). On all of these sheets, the site code, date that the site was sampled, and name of the person who is compiling the data should be entered. Upon completing the procedures described in this section, all data are ready for entry into the database management system.

Water Quality

Discharge

Buchanan and Somers (1969) provide a thorough description of how to calculate discharge from the measurements that were taken. The Discharge Measurement sheet that was used in the field (Appendix III.C.2) has spaces to complete the calculations. The final result should be entered in the appropriate space on the Water Quality Summary sheet (Appendix III.C.11).

Chemical Parameters

Upon returning from field sampling, refrigerate all samples until they are analyzed. All chemical parameters should be analyzed according to methods recommended by either the American Public Health Association et al. (APHA, 1981, or later editions) or U.S. Environmental Protection Agency (USEPA, 1983). The following are specific recommendations within these two references for some of the parameters. Record all final results, including those measurements taken in the field (temperature, conductivity, pH, dissolved oxygen), on the Water Quality Summary sheet (Appendix III.C.11).

Alkalinity: double endpoint titration; APHA et al. (1981), method 403.

Sulfate: turbidimetric; USEPA (1983), method 375.4.

Nitrate: spectrophotometric, cadmium reduction; USEPA (1983), method 353.3.

Chloride: colorimetric, automated ferricyanide AAI or AAI; USEPA (1983), methods 325.1 or 325.2.

Calcium: atomic absorption spectrophotometric; APHA et al. (1981), method 303 A.

Magnesium: atomic absorption spectrophotometric; APHA et al. (1981), method 303 A.

Potassium: atomic absorption spectrophotometric; APHA et al. (1981), method 303 A.

Sodium: atomic absorption spectrophotometric; APHA et al. (1981), method 303 A.

Silica: colorimetric; USEPA (1983) method 370.1.

Seston

The methods for seston analysis are almost the same as those that APHA et al. (1981) suggest for residue analysis (Section 209). Use the Seston Worksheet (Appendix III.C.12) to record the sample weights (pans and filters) at each step. Use the Water Quality Summary sheet (Appendix III.C.11) to record the final calculations (sample weights minus tare weights of clean filters and pans divided by volume of water filtered). Weights should be reported in mg and final concentrations in mg/L. Other references that may be useful are Gurtz et al. (1980) and Voshell and Parker (1985).

Total seston, or suspended particulate matter, is synonymous with total nonfilterable residue and can be measured by a slight modification of Method 209 D. Because of the low concentration of seston in SNP streams, at least 1 L of water should be filtered. The sample should be dried at 60 °C for 24 hr, instead of 103-105 °C for 1 hr, to make sure that no organic matter is volatilized. This weight is recorded on the worksheet in the column labeled "Dry Wt." The concentration of total seston is calculated by subtracting the tare weight from the dry weight of the sample and dividing by the volume of water filtered. The concentration should be recorded on the water quality summary sheet in the space labeled "Total".

Inorganic seston is synonymous with the ash of nonfilterable residue and can be measured by a slight

modification of Method 209 G. The sample should be ignited at 500 °C for 1 hr, instead of 550 °C for 15 min. After cooling, a few drops of distilled water should be placed on the filter to restore the water of hydration. Then the sample should be dried at 60 °C for 24 hr, weighed, and recorded on the worksheet in the column labeled "Ash Wt." The concentration of inorganic seston is calculated by subtracting the tare weight from the sample ash weight and dividing by the amount of water filtered. Record the concentration on the Water Quality Summary sheet in the space labeled "Inorganic."

Organic seston is synonymous with volatile nonfilterable residue and can also be measured by the same modification of Method 209 G. The amount of organic seston in sample is the difference between the dry weight of the sample containing total seston and the weight after ignition, wetting, and redrying. Record this sample weight on the worksheet in the column labeled "AFDW" (ash-free dry weight). Calculate the concentration of organic seston by subtracting the tare weight from the ash-free dry weight and dividing by the amount of water filtered. Record the concentration on the Water Quality Summary sheet in the space labeled "Organic." The organic seston/inorganic seston ratio should be calculated by division, rounded to the nearest 0.01, and entered in the space labeled "O/I."

Bacteria

Total Coliform: standard MPN test; APHA et al. (1981), Method 908 A.

Fecal Coliform: MPN procedure; APHA et al. (1981), Method 908 C.

Fecal Streptococcus: multiple-tube technic; APHA et al. (1981), Method 910 A.

Record all results on the Bacteria and Seston Worksheet (Appendix III.C.14). Results should be expressed as MPN (Most Probable Number)/100 ml.

Habitat

The Habitat Measurements sheet that was used in the field (Appendix III.C.3) should be used as a worksheet to make the calculations described below. Final results should be recorded on the summary sheet for habitat measurements (Appendix III.C.13).

Depth

Calculate the mean depth at each transect by adding the three measured depths and dividing by four to account for zero depths at the shore (Platts et al. 1983). The overall mean depth at the site is calculated

by averaging the 11 transect mean depths. Round to the nearest cm.

Width

The mean width of the site is calculated by averaging the 11 transect widths. Round to the nearest 0.1 m.

Riparian Vegetation Cover

Average the 11 rankings taken at each transect. Round to the nearest 0.1. Results can be interpreted as follows: 2.6 - 3 is mostly shaded; 1.6 - 2.5 is mostly intermediate conditions of shading; 1 - 1.5 is mostly exposed to sunlight.

Riparian Vegetation Type

Add the number of entries for each of the four vegetation types at the 11 transects. Calculate the proportions of each type for the entire site. Round to the nearest 1%.

Substratum

Add the number of entries for each of the five substrate types at all 11 transects. Calculate the proportions of each type for the entire site. Round to the nearest 1%.

Debris Retention

Average the rankings for the 10 segments. Round to the nearest 0.1. Results can be interpreted as follows: 2.6 - 3 is mostly abundant retention capacity; 1.6 - 2.5 is mostly intermediate retention capacity; 1 - 1.5 is mostly without the capacity to retain debris.

Fish Cover

Average the rankings for the 10 segments. Round to the nearest 0.1. Results can be interpreted as follows: 2.6 - 3 is mostly abundant cover; 1.6 - 2.5 is mostly intermediate cover; 1 - 1.5 is mostly exposed.

Pool-Riffle

Average the pool values for the 10 segments, then average the riffle values. Round both the pool and riffle averages to the nearest whole number. An important rule to remember when rounding off is that if the digit to be rounded is followed by a 5 standing alone or followed only by zeros, the digit is not changed if it is even but is increased by one if the digit is odd (e.g., 2.5 rounds to 3, 7.5 rounds to 8) (Sokal and Rohlf 1981). The two

averages should total 10. Report the average results as the pool:riffle ratio. This ratio can also be interpreted as the proportion of pool and riffle at the site (e.g., 2:8 ratio means that 20% of the site is pool and 80% is riffle).

Biological

Periphyton

Biomass. Spectrophotometric determination of chlorophyll *a* is used to estimate the biomass of live periphyton, according to Method 1002 G.3 in APHA et al. (1981). Three of the periphyton samples should be analyzed separately and averaged. This method includes a correction factor for the presence of pheophytin *a*, a common degradation product of chlorophyll *a* that interferes with determination of chlorophyll *a*. Use the Bacteria and Periphyton Worksheet (Appendix III.C.14) to record the various steps of this procedure. The final results should be expressed as $\mu\text{g}/\text{cm}^2$. Method 1002 G.3 is actually written for phytoplankton samples, which are reported according to volume. To report periphyton in terms of surface area, the area of the sampler in cm^2 should be substituted for V_2 in the equations. In addition the final result should be converted from mg to μg .

Autotrophic Index (AI). The AI is calculated by dividing the total biomass of periphyton by the amount of chlorophyll *a*, in accordance with APHA et al. (1981) Method 1002 G except that the units are $\mu\text{g}/\text{cm}^2$. Use the Bacteria and Periphyton Worksheet (Appendix III.C.14) to make the calculations. The amount of chlorophyll *a* is obtained from the procedures described above. The total biomass of periphyton, including both living and dead material, is measured by filtering the fourth periphyton sample and analyzing it by the same method as organic seston (Method 209 G, ash-free dry weight).

Macroinvertebrates

Quantitative and Qualitative Benthic. The analysis of each benthic sample is done in a series of steps that may take place over a period of several days; therefore, it is essential to establish a log that traces the analysis of each sample from its return to the laboratory to the final identification and enumeration of specimens. The log should have a separate entry for each benthic sample that was taken, including the code, location, type, replicate, and date collected. The steps that should be recorded in the log are: return to the laboratory, washing, sorting, and identification along with enumeration. The date that each step was completed and the initials of the person completing the step should be recorded. The log should be kept in a sturdy, permanently bound notebook, which cannot have pages removed.

The first step in analyzing benthic macroinvertebrate samples is to remove any remaining fine detritus and the original preservative, which will have become darkly stained from leaf leachate. This is accomplished by placing each sample in a 355- μ m brass soil sieve (U. S. Series No. 45) and washing thoroughly with tap water. A sieve of this particular mesh size is used in order to correspond with the mesh size of the nets on the samplers that were used to collect the samples. The sample should be gently stirred and shaken while it is being washed. The purpose of this procedure is to make the sample clean enough for the organisms to be seen and removed. Therefore, it is important to wash samples thoroughly; however, care should be taken not to damage the specimens because accurate identifications depend on having all external structures intact. All material should be removed from the sieve and placed in a suitable container for temporary storage. Round glass bowls, measuring 11.5 cm in diameter and 5.5 cm high, work very well for this purpose. The original coded label that was placed in the sample during field collection should be kept with the sample throughout all steps of analysis. If the sample is to be sorted immediately, water can be added to the container; if there will be a delay before sorting (e.g., overnight) then 70% ethanol should be added. Special efforts are required to make sure that all material is removed from the sieve. The material can be concentrated at the bottom of the sieve by holding the sieve at an angle under a faucet. A flexible hose attached to the faucet is particularly effective for this task. The sample is best transferred to the container by gently washing it out of the sieve with a squeeze bottle containing either water or alcohol. The bottom area of the sieve should be backwashed with the squeeze bottle to complete the removal of the sample.

The next step is to remove the organisms from the debris in the sample. This procedure can be referred to as "sorting," but it is commonly called "bug picking." The importance of this step cannot be over-emphasized. If all of the organisms are not consistently removed from the samples, significant variability will be introduced into the monitoring program, perhaps even making the careful field work for naught. Therefore, it is essential that a rigid protocol be established and followed. The sample should be placed in a shallow white enamel pan, covered with approximately 1 cm of water, and all organisms taken out with forceps. An efficient size of enamel pan is 19.5 cm wide x 31 cm long x 5.5 cm high. If the sample contains a great deal of debris, only a small portion should be placed in the sorting pan at one time. The pan should be examined in a systematic fashion, proceeding along imaginary rows and columns. The debris should be gently stirred with the forceps. There should be adequate lighting immediately above the pan, such as a desk lamp with a flexible arm. An

illuminated magnifier is helpful, but not essential for the size of organisms that will be retained by a 355- μ m mesh. An efficient approach is to carefully examine the entire pan with the unaided eye, then scan the entire contents a second time with the illuminated magnifier. As the organisms are removed they can be placed into a single container or they can be separated into taxonomic groupings, depending upon the expertise of the persons doing the sorting. A 59-ml (2-oz) bottle is a handy size for holding all organisms collectively; 7-g (4-dram) vials are best for holding individual taxonomic groups. After the second scan of the pan, dispose of the material and continue sorting portions of the sample until finished. Make sure that the containers are filled with 70% ethanol and are tightly sealed. Each jar or vial should contain a copy of the site code, and the original field label should be in one of the containers.

The last step involves identifying the specimens to the lowest possible taxonomic level and counting the number in each taxon. Each specimen must be carefully examined with a reasonably good quality stereomicroscope having magnification up to at least 40X. Identification is accomplished by using taxonomic publications that contain descriptions and keys. Brigham et al. (1982) and Merritt and Cummins (1984) are two excellent works that cover all of the aquatic insects that will be found in Virginia. Some more specialized publications that cover the most common individual orders of insects in more detail are: Edmunds et al. (1976), Ephemeroptera; Wiggins (1977), Trichoptera; and Stewart and Stark (1989), Plecoptera. Macroinvertebrates other than insects can be identified with Pennak (1978) or an older work that is still quite useful, Ward and Whipple (1959). Occasionally immature stages of terrestrial insects are found in aquatic samples because of accidental dislodgement into the water. These can be particularly troublesome because they may not key out with the references for aquatic fauna. A recent book (Stehr 1987), which covers the immature stages of all insects, contains a key to orders that can be used to resolve this problem. Benthic macroinvertebrates should be identified to at least the following taxonomic levels:

- Insecta - genus (except Diptera, family)
- Crustacea - genus
- Mollusca - family
- Oligochaeta - class
- Turbellaria - class

Sometimes it may not be possible to identify early stages to these levels. It should be kept in mind that good taxonomic references by themselves do not guarantee accurate identifications. Formal training in a course, such as aquatic entomology or invertebrate zoology, or experience obtained under the supervision

of a specialist is necessary. It is always a good idea to have representatives of each taxon verified by specialists at universities or museums.

As specimens are identified they should be sorted for separate storage. A series of vials lined up on the bench is handy for this purpose; special trays are available, or can be constructed, to keep the vials from tipping over. Watch glasses and porcelain spot test plates are also handy for temporarily holding individual taxa while a sample is being identified. Specimens should always be kept immersed in 70% alcohol. For the quantitative benthic samples all specimens should be enumerated. Individuals can either be tallied as they are identified or counted at one time after the entire sample is finished. It is probably more efficient to continuously tally the abundant taxa and count the sparse taxa at the end. Tally meters are useful for both continuous and final counting. For the qualitative benthic samples the taxa are only recorded as being present; there is no need to count the specimens. All data should be recorded on the Benthic Macroinvertebrate Worksheets (Appendix III.C.15). It is very important to fill in all of the information at the top of each worksheet (site code, date, sample type and replicate, person making the identifications). The column for comments should be used to record any special circumstances, such as questionable identifications that need more attention or specimens having been sent to a specialist for verification. Macroinvertebrate data are entered into the database management system by taxonomic codes, rather than scientific names. After the samples are identified and enumerated, the taxonomic codes should be written on the worksheets in the column labeled

"Taxa Codes" to make data entry faster and more accurate. The taxonomic codes can be found in the dictionary for the aquatic component of the database management system.

When a sample is completely identified and counted, all specimens should be stored individually in 4-dram vials containing 70% alcohol. Make sure the vials are filled and tightly sealed. Either patent-lip vials with neoprene stoppers or screw-cap vials with conical plastic liners will prevent evaporation of the alcohol for extended periods of time (several yrs). Each vial should contain a label bearing the site code, site name, date, taxonomic name, and name of the person making the identification. Vials should be stored in collective lots corresponding to site, date, sample type, and replicate. The original field label should be kept with the corresponding lot. Cardboard unit trays, which are available commercially, are efficient for storing samples in this manner. All samples should be kept at least until the data are entered into the database management system and successfully analyzed. Inevitably questions will arise, and certain taxa in some samples will have to be reexamined. Eventually storage space and costs of new vials will make it necessary to dispose of old samples. The SNP should decide how long samples will be kept; a minimum of 1 yr after entry into the database management system is recommended. It is imperative to keep several vials of each taxon collected each year as a voucher collection. The voucher collection serves as a tool for training employees or consultants, educating the public, and resolving any taxonomic questions that might arise in the future.

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