

NEW RIVER GORGE NATIONAL RIVER  
LONG-TERM ECOLOGICAL MONITORING SYSTEM  
USER MANUAL  
FIELD AND LABORATORY METHODS

J. Reese Voshell, Jr.<sup>1</sup>, Donald J. Orth<sup>2</sup>,  
Stephen W. Hiner<sup>1</sup>, and Robert S. Easton<sup>2</sup>

<sup>1</sup>Department of Entomology

<sup>2</sup>Department of Fisheries and Wildlife Sciences  
Virginia Polytechnic Institute and State University  
Blacksburg, Virginia 24061

August 1996

In partial fulfillment of Cooperative Agreement CA4000-8-8008 between Virginia Polytechnic Institute and State University and USDI, National Park Service, New River Gorge National River, Oak Hill, West Virginia 25901

## CONTENTS

Introduction .....	5
Design .....	5
Study Sites .....	5
Bluestone Dam .....	6
Sandstone Falls .....	6
Prince .....	6
Thurmond .....	7
Fayette Station .....	7
Parameters .....	8
Water Quality .....	8
Discharge .....	8
Temperature .....	8
Dissolved Oxygen .....	8
Hydrogen Ion Activity .....	8
Alkalinity .....	9
Hardness .....	9
Conductivity .....	9
Seston .....	9
Biological .....	9
Periphyton .....	9
Phytoplankton .....	10
Macrophytes .....	10
Macroinvertebrates .....	10
Fish .....	11
Special Considerations for Macroinvertebrates .....	12
Heterogeneity of Communities Among Habitats .....	12
Optimum Study Design .....	13
Habitats .....	13
Quantitative Versus Qualitative Samples .....	14
Sampling Devices .....	14
Precision, Time Required, and Numbers of Replicate Samples .....	15
Levels of Effort .....	16
Sampling Frequency .....	21
Field Samples and Measurements .....	21
Water Quality, Algae, Macrophytes, Macroinvertebrates .....	21
General Information .....	21
Effort .....	22
Preliminary Tasks .....	22
Sampling Sequence .....	22
Equipment .....	23

Water Quality .....	23
Algae .....	24
Periphyton .....	24
Phytoplankton .....	25
Macrophytes .....	25
Submerged .....	25
Emergent .....	25
Macroinvertebrates .....	25
Quantitative Samples .....	25
Cobble/Pebble Riffles .....	25
Rock Outcrops .....	26
Emergent Macrophytes .....	27
Qualitative Samples .....	27
Cobble/Pebble Riffles .....	27
Rock Outcrops .....	27
Emergent Macrophytes .....	28
Pools .....	28
Preserving and Storing .....	28
Fish .....	29
General Information .....	29
Effort .....	29
Preliminary Tasks .....	29
Sampling Sequence .....	29
Equipment .....	30
Collection .....	30
Laboratory Analyses and Calculations .....	30
Water Quality, Algae, Macrophytes, Macroinvertebrates .....	30
Water Quality .....	31
Water Chemistry .....	31
Seston .....	31
Algae .....	32
Periphyton .....	32
Biomass .....	32
Autotrophic Index .....	32
Phytoplankton .....	32
Biomass .....	32
Macrophytes .....	32
Justicia .....	32
Biomass .....	32
Podostemum .....	33
Biomass .....	33
Macroinvertebrates .....	33
Fish .....	35
References .....	36

## Appendices ..... 39

## A. Maps

1. Five permanent study sites
2. Bluestone Dam study site
3. Sandstone Falls study site
4. Prince study site
5. Thurmond study site
6. Fayette Station study site

## B. Equipment

1. Periphyton sampler
2. Macroinvertebrate samplers
3. Electrofishing sampling gear

## C. Field and Laboratory Work

1. Field records sheet for water quality, algae, macrophytes, and macroinvertebrates
2. Equipment checklist for field sampling of water quality, algae, macrophytes, and macroinvertebrates
3. Sources of field and laboratory equipment and supplies for water quality, algae, macrophytes, and macroinvertebrates
4. Checklist of required field and laboratory equipment and supplies for fish sampling, identification, and measurements
5. Sources of field and laboratory supplies for fish sampling, identification, and measurements
6. Field and laboratory worksheet for fish
7. Label for fish samples
8. Laboratory worksheet and summary for water quality, algae, and macrophytes
9. Laboratory worksheet for seston
10. Laboratory worksheet for macroinvertebrates
11. Potential repositories for archiving fish specimens and persons to contact about fish identification

## INTRODUCTION

The purpose of this manual is to describe the field and laboratory methods that should be used to acquire data for the Long-Term Ecological Monitoring System (LTEMS) that has been developed for the aquatic resources in the New River Gorge National River (NRGNR). The primary tasks that the manual addresses are: 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 useful data to the NRGNR LTEMS.

## DESIGN

### Study Sites

The LTEMS was designed to monitor the section of the New River from Bluestone Dam to the National Park Service (NPS) boundary at Fayette Station. The New River is a 6th order stream throughout this 91-km reach. The Bluestone Lake impoundment exerts a strong influence on the New River tailwaters (VPI&SU 1985). At the normal summer pool the reservoir has a surface area of 816 ha. Water is released from 16 inversely gated sluices that measure 3.0 m high x 1.7 m wide. The bottoms of the gates are situated 6.3 m below the surface. Because of the small size of the reservoir in comparison to the inflow, retention time is short, and there is no permanent thermal stratification. When temporary stratification does occur in the summer, the metalimnion usually forms at 8-9 m; therefore, water is always released from the epilimnion and there are no problems with reductions in temperature or dissolved oxygen, which sometimes occur with hypolimnetic releases. Because Bluestone Lake is eutrophic, the water released from the epilimnion is somewhat warmer than ambient temperatures in the New River and contains moderately high concentrations of organic seston. The seston contains a high proportion of algae because of the abundance of phytoplankton in the eutrophic reservoir. The detritus in the seston also probably originates from the phytoplankton in the reservoir. The extremely high productivity of aquatic insects in the tailwaters has been attributed to the rich food resource provided by the seston released from Bluestone Lake (Voshell 1985). This positive influence on productivity does not appear to extend as far downstream as Sandstone Falls (16 river km).

The aquatic LTEMS is restricted to the New River and does not include any of the 47 tributary streams. In order to reflect the possible influences of the tributary streams, it was necessary to establish study sites at periodic longitudinal intervals. Five study sites were selected and assigned the following names: Bluestone Dam, Sandstone Falls, Prince, Thurmond, and Fayette Station (Appendix A.1). The term "site" refers to a designated length of the river where samples and measurements are taken. The number of study sites was a compromise between the amount of information that would be needed to determine the cause of an ecological change and the amount of resources that would be feasible for continuously maintaining the LTEMS. The longitudinal distribution of these five sites should make it possible to detect localized changes that might occur and to follow trends in the overall

ecosystem. If ecological changes are detected, it may be desirable to add more study sites for short-term, intensive studies.

### **Bluestone Dam**

This site is located immediately below Bluestone Dam (Appendix A.1) at the public park maintained by the U.S. Army Corps of Engineers (Town of Hinton, Summers County, West Virginia). The exact location is shown on the topographic map in Appendix A.2. Access is from the parking lot. Macroinvertebrate samples are taken randomly from specified habitat types (stratified random design) within the section that extends from 100 to 300 m downstream of the spillway. The exact locations where fish are collected are shown as shaded areas on the topographic map in Appendix A.2.

At normal summer flows (approximately 3500 cfs) most of this site is wadable, with an average depth of about 1 m. The width is approximately 300 m. At the Bluestone gauge, which measures discharge immediately below the dam, the 54-year average for mean daily discharge has been 5600 cfs. The substratum consists of rock outcrops, boulders, and cobble/pebble riffles. There are two major aquatic macrophytes: the submerged *Podostemum ceratophyllum* (river weed) that grows on the rock outcrops and boulders and the emergent *Justicia americana* (water willow) that grows along the banks and islands. The substratum often has dense growths of filamentous algae during the summer months.

### **Sandstone Falls**

This site is located 16 river km downstream from Bluestone Dam (Appendix A.1). The exact location is shown on the topographic map in Appendix A.3. Access is from Route 26, on the west side of the New River, just upstream from the falls. Macroinvertebrate samples are taken randomly from specified habitat types (stratified random design) within the section that is 50 to 250 m upstream from the falls, which are an abrupt 8-10 m drop. The exact locations where fish are collected are shown as shaded areas on the topographic map in Appendix A.3.

The depth is shallow enough to be wadable (0.1-1.0 m) during summer flow conditions. The width of this section is approximately 300 m. The substratum consists almost entirely of rock outcrops, with only a few small cobble/pebble riffles adjacent to both banks. *Podostemum* and *Justicia* are the two dominant aquatic macrophytes. The rock outcrops often have dense growths of filamentous algae during the summer months. There are 12 tributaries between Sandstone Falls and Bluestone Dam. Eleven of these are very small streams, but the Greenbrier River, which enters from the east 1 km below Bluestone Dam, contributes 29% of the annual discharge below the confluence. At the Hinton gauge, which includes the contribution of the Greenbrier, the 50-yr average (1936-86) mean daily discharge for the New River has been 7,889 cfs. During this 50-yr period the maximum mean daily discharge was 246,000 cfs on Aug 15, 1940, and the minimum was 620 cfs on Nov 3, 1981.

### **Prince**

The Prince study site is located approximately 41.5 river km below Bluestone Dam (Appendix A.1). The exact location is shown on the topographic map in Appendix A.4. Access to the site is achieved from a dirt road on the south

bank, which forks from Route 19 and 41 just prior to crossing the river. Macroinvertebrate samples are taken randomly from specified habitat types (stratified random design) within the 200-m section that is 1.75 km upstream of the Route 19 and 41 bridge. The sampling site is the first riffle above the bridge and includes the pool area immediately below the riffle. The exact locations where fish are collected are shown as shaded areas on the topographic map in Appendix A.4.

The New River is approximately 150 m wide at the Prince study site. Under summer flow conditions much of the river is wadable; however, the main channel and several backwaters are too deep or swift to wade. Cobbles and boulders are the dominant substratum; however, there are also several sandy areas. *Justicia* was the only macrophyte observed by VPI&SU personnel during the development of the LTEMs (1988-90), but *Podostemum* has been observed by NPS personnel in succeeding years. *Justicia* occurs in low abundance and is usually out of the water during normal summer flows. Twelve tributaries enter the New River between the Sandstone Falls and the Prince study sites; however, the discharge at Prince is only slightly greater than at Sandstone Falls.

### **Thurmond**

The Thurmond study site is located approximately 61.6 river km below Bluestone Dam (Appendix A.1). The exact location is shown on the topographic map in Appendix A.5. Access to the river is achieved from a dirt road which forks from the paved road just prior to the Stonecliff bridge. Macroinvertebrate samples are taken randomly from specified habitat types (stratified random design) within the section 3 km upstream of the town of Thurmond, approximately 50 m upstream of the Stonecliff bridge. The exact locations where fish are collected are shown as shaded areas on the topographic map in Appendix A.5.

At the Thurmond study site the New River is approximately 150 m wide. The site consists of a 50-m riffle between two long, deep pools. Under summer flow conditions most of the riffle is wadable except for the thalweg (main stream channel). Only the extreme river margins of the pools are wadable. Cobbles and boulders are the dominant substratum in the riffle. The substratum of the pool margins is mostly silt and sand. The dominant aquatic macrophytes include *Justicia* and *Podostemum*. Eight tributaries enter the New River between Prince and Thurmond. A gauge is located at Thurmond, 0.1 km upstream of Dunloup Creek on the right bank. The 6-yr average discharge at the Thurmond site between 1981 and 1987 was 9003 cfs.

### **Fayette Station**

This study site is located approximately 91.4 river km downstream from Bluestone Dam (Appendix A.1). The exact location is shown on the topographic map in Appendix A.6. Access is achieved from Route 82 and an NPS public access area. Macroinvertebrate samples are taken randomly from specified habitat types (stratified random design) within the section that extends from the riffle just below the confluence of Wolf Creek to 300 m upstream of the riffle. The exact locations where fish are collected are shown as shaded areas on the topographic map in Appendix A.6.

The river is approximately 100 m wide at the Fayette Station site. Sampling at this site is limited to the extreme river margins because of the high current velocity in the riffle and the depth of the pools. Boulders are the dominant

substratum in the riffles, and sand is dominant in the pools. No aquatic macrophytes are present at this site. Fifteen tributaries enter the New River between Thurmond and Fayette station, but the discharge at Fayette Station is only slightly greater than at Thurmond.

### 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 flowing waters.

#### 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.

**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 macroinvertebrates 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. Medium-sized streams, such as the New River, are classified as "warm-water", meaning that summer temperatures exceed 20 °C for considerable periods of time. Water temperatures in the NRGNR are affected appreciably by the Bluestone Lake impoundment and the free-flowing Greenbrier River.

**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 the metabolic activities of all aerobic aquatic organisms. Oxygen levels are affected by temperature; as the water temperature increases, the solubility of oxygen decreases. Medium-sized rivers that are mostly shallow with abundant riffles would be expected to have oxygen concentrations at saturation throughout the year (8 - 14 mg/L), except perhaps during periods of exceptionally low flow in summer. 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, with 6 - 9 being regarded as the range in which most organisms are successful.

**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).

**Hardness.** Water hardness is caused by the concentration of dissolved polyvalent metallic ions. These ions are principally calcium and magnesium, but other metals such as iron, strontium, manganese, and sodium often contribute appreciably to the hardness. The occurrence of algae, macrophytes, macroinvertebrates, and fish are often correlated with hardness, but the relationship has not been explained. The explanation probably lies with concentrations of individual ions rather than the overall effect of hardness (Hynes 1970, U. S. Environmental Protection Agency 1976). Hardness is a useful parameter for the LTEMs because it is related to the success of a wide variety of biota and it integrates the concentrations of several ions. Hardness is usually reported as an equivalent concentration of calcium carbonate. A commonly used classification is: 0-75, soft; 75-150, moderately hard; 150-300, hard; > 300, very hard (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.

**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).

## **Biological**

**Periphyton.** Periphyton is the community of algae that lives attached to rocks and other firm substrates. In medium-sized rivers, which are shallow and relatively open to sunlight, primary production by the periphyton may be a significant input of energy to the ecosystem. Periphyton comprises an important portion of the diet of many grazing macroinvertebrates, and dead periphyton may also provide detritus for collector macroinvertebrates. The amount of chlorophyll a in the periphyton can be used to estimate 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 increases the amount of nutrients (especially phosphorus) entering the stream (e.g., agricultural runoff, sewage discharge) can cause a dramatic increase in periphyton biomass. Such "blooms" of periphyton are usually detrimental to the fauna because the spaces between the particles of substratum become clogged and the surfaces become so thickly covered that organisms cannot attach themselves. In addition, an overabundance of periphyton can reduce dissolved oxygen concentrations when live cells respire at night or when cells die and decay.

**Phytoplankton.** The phytoplankton community consists of the algae that live suspended in the water. There is usually no permanent phytoplankton community in flowing waters (sometimes referred to as potamoplankton), except perhaps in very large, sluggish rivers. There will be a temporary phytoplankton community in medium-sized rivers that is derived from dislodged periphyton and organisms that develop in pools and backwaters. An impounded river, such as the New River, can transport appreciable amounts of phytoplankton that are released from the reservoir. When present in rivers, phytoplankton can be an important source of food for filter-feeding insects and the early life stages of some fish. Like periphyton, too much phytoplankton can be detrimental to the fauna of a river. This situation could occur below a eutrophic reservoir, such as Bluestone Lake on the New River. The same parameters are used to measure the phytoplankton as the periphyton.

**Macrophytes.** The various rooted, vascular plants that occur in surface waters are referred to collectively as macrophytes. In flowing waters macrophytes exhibit their greatest abundance in medium-sized rivers, which are shallow and relatively open to sunlight. There are submerged and emergent species of macrophytes. Macrophytes are important in nutrient cycles and as habitat for macroinvertebrates and fish. Although they are usually not consumed while alive, macrophytes provide a constant food supply of detritus during the growing season as fragments break off and a pulse of detritus for food when they die back at the end of the growing season. The presence of macrophytes greatly increases the surface area (effective habitat) available for macroinvertebrates to cling, climb, build silk retreats, or hide. Removing the macrophytes from a known area, then drying and weighing the material, provides a measurement of the 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, 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 (nutrient spiralling), and providing food for fish.

Two types of benthic macroinvertebrate samples are taken at each site: quantitative and qualitative. The quantitative benthic samples are the mainstay of the macroinvertebrate monitoring program. The data from those samples can be used to make statistical comparisons at individual sites over time and among sites. Benthic macroinvertebrates are microhabitat specialists, and quantitative sampling devices cannot be placed in some of the microhabitats that occur in streams. In addition, laboratory analyses of quantitative samples can be very time consuming. Therefore, qualitative benthic samples are also taken to determine the relative abundance of taxa as well as other indices that can be used to make similar, but less rigorous, comparisons.

**Fish.** Sampling effort for fish collections is standardized to allow for comparisons among collection sites and among collection years. At each collection site, 13 samples are collected. Each sample consists of a 10-min electrofishing period. All fish that are stunned during electrofishing collections are recovered for data collection.

Because species may react differently to environmental changes it is important to record the types of fish species collected, total number of species collected, and the numeric and percentage composition of species at a collection site. Changes in the total number of species or the species composition at a collection site may indicate changes in physical habitat quality, energy sources, and water quality.

Total length (TL) of each specimen is measured to allow for future analysis of species length and weight relationships or age analysis via length frequency distributions. Total length is defined as the length from the anterior-most part of the fish to the tip of the longest caudal fin rays when the fin lobes are compressed dorso-ventrally (Anderson and Gutreuter 1983).

Total weight of each specimen is measured to calculate mean biomass collected per 10-min sample and species diversity based on biomass. Total fish weight measurements can also be used to calculate the biomass of individual species or total biomass collected from a sample site. Mean weight and biomass measurements provide useful descriptions of fish populations for comparisons among collection sites and among collection years.

## Special Considerations For Macroinvertebrate Sampling

### Heterogeneity of Communities Among Habitats

Macroinvertebrates exhibit heterogenous (clumped) distribution patterns within a body of water. Individual taxa usually have very specific ecological requirements. Often, only a very slight difference in one ecological characteristic will make a habitat (the place where an organism lives) either favorable or unfavorable for a particular type of macroinvertebrate. Habitats usually differ in a number of ecological characteristics, and there is a continuum of differences for each characteristic. In addition, there are interactive effects between different ecological characteristics and the constituent organisms themselves. Finally, macroinvertebrates are small, sessile organisms, so they only require a small portion of the overall habitat (a microhabitat) for their success. The net effect is that very different assemblages of macroinvertebrates (communities) often exist in habitats that appear to be only slightly different and are located very close to each other.

For example, mineral substratum (rocks, sand, etc.) and vegetation (either live plants or tangles of plant debris) usually harbor different communities of macroinvertebrates. This may have to do with macroinvertebrates' abilities to hold on to the different surface textures of rocks versus plants. Within each of these broad habitat categories, the macroinvertebrates will also differ according to the size of the spaces available. Different sizes of space offer different current velocities, types and sizes of food, and opportunities for attachment and concealment from predators. Some macroinvertebrates depend upon water current to bring them food, and most need current to accomplish gas exchange. Many macroinvertebrates are adapted for a narrow range of current velocities. In regard to food, some macroinvertebrates (scrapers) feed exclusively on periphyton. Therefore, scrapers are only found on the surfaces of rocks or plants exposed to sunlight, where their food grows. Others, called collector-filterers, must remove fine particles from the water with silk nets or body hairs, so they must attach themselves where they are exposed to currents. Shredder macroinvertebrates feed only on coarse particles (> 1 mm diameter) of plant detritus, and will be found where this material accumulates (on the upstream side of rocks and logs in riffles and on the bottom of pools). Collector-gatherers feed on fine particles of detritus, so they are usually found in places such as under rocks and in plant roots in riffles and on the bottom of pools.

Medium-sized streams, such as the New River, contain the greatest diversity of habitats among flowing waters. They have shallow areas with current (riffles, runs) where the bottom is mostly rocky, as well as deep areas with no current (pools) where the bottom is mostly sand and silt. In addition, medium-sized streams are wide enough that much of the water surface is exposed to sunlight, so plants grow in shallow areas. Studies conducted as part of this project documented that there are different macroinvertebrate communities in several of the habitats found in the New River (Interim Report No. 3, 1990; Interim Report No. 6, 1991). In these reports, the macroinvertebrate communities at each of the five study sites were compared in the following habitats: cobble/pebble riffles, *Podostemum* on rock outcrops, outcrops without *Podostemum*, *Justicia* at the edges, and pools. The method of comparison was similarity, which was calculated as percent of taxa in common. The criterion that was used to determine if sampling two habitats would be redundant was 80% of taxa in common. The outcome of these analyses was that, although communities in some of the habitats were

essentially similar at some sites, the communities in none of the habitats were consistently similar at all sites.

### Optimum Study Design

We placed high priority on designing a monitoring system that is both scientifically accurate and feasible for the Park Service to continue on a permanent basis. The criteria that we used for selecting the types of samples to be taken were:

- 1) provide information on the greatest number of different taxa;
- 2) offer an acceptable level of precision (low variability among replicate samples);
- 3) require a feasible amount of time for laboratory analyses.

**Habitats.** The more taxa that are collected by standard repeatable methods, the greater the sensitivity of the NRGNR LTEMs will be for detecting changes in ecological integrity. In addition, two of the habitats that were evaluated, the aquatic macrophytes *Podostemum* and *Justicia*, are biological resources themselves, so measures of their success can also be effective for monitoring ecological integrity. Therefore, the recommendation was to conduct stratified sampling in all of the habitats mentioned above. The only change that was made was to drop *Podostemum* as a separate habitat in favor of outcrops, with or without *Podostemum*. The reason for this was that *Podostemum* does not occur at all five study sites, but outcrops do occur at all five study sites. The most likely comparison that will be made in the NRGNR LTEMs is over time at individual study sites. Therefore, it is likely that outcrops with *Podostemum* will always be compared to outcrops with *Podostemum* and outcrops without *Podostemum* will always be compared to outcrops without *Podostemum*. A brief description of the recommended sampling strata (habitats) follows.

**Cobble/pebble riffles** are those areas where there is moderate to swift current and the substratum consists primarily of rocks in the cobble size category (3.2 - 25.6 cm) overlying some smaller rocks in the pebble size category (1.6 - 3.2 cm). These substratum size categories can be recognized visually after some experience, but inexperienced investigators should probably take a measuring stick with them. The range of the cobble size category is approximately between that of an average fist and head.

**Rock outcrops** are delimited as the areas where there is moderate to swift current and the substratum consists entirely of bedrock or imbedded, immovable boulders. There are no overlying smaller rocks, only the impenetrable surface of the outcrop, which may have mats of *Podostemum* growing on it. Samples should be taken in *Podostemum*, if present, and on the bare rock surface if *Podostemum* is absent.

**Justicia** is an emergent macrophyte that occurs in dense stands at the river margins where there is moderate current with gravel and sand substratum. During periods of exceptionally low flow the stands of *Justicia* may have no surface water passing through them.

**Pools** are the areas where there is no perceptible current. The depth is usually greater than in riffles or on outcrops, and the substratum consists primarily of fine mineral particles (sand, silt) and particles of organic matter, which settle out of suspension because of the reduced current velocity.

**Quantitative Versus Qualitative Samples.** Macroinvertebrates can be sampled by quantitative or qualitative methods. Quantitative sampling methods for macroinvertebrates obtain an estimate of their absolute abundance, usually as numbers of organisms per unit of bottom area in the aquatic habitat. Quantitative sampling devices must isolate a known area of bottom and prevent macroinvertebrates from leaving or entering during sampling. Data from quantitative sampling can be used as replicates in statistical tests that infer whether two or more populations or communities are different or not, with a specified level of certainty that the observations are not the result of chance. Qualitative sampling methods for macroinvertebrates only obtain an estimate of the relative abundance of organisms (percentages of different taxa) and taxa richness (number of different taxa), because the sampling devices do not delimit a specific area of the stream bottom. Data from qualitative sampling can be analyzed by several statistical techniques to examine if two or more communities are similar or different, but the data are not amenable to the more rigorous statistical tests.

There are advantages and disadvantages to each sampling approach. Quantitative sampling offers the advantage of more rigorous statistical testing; however, replicate samples are necessary (at least 3-5), which require considerable time for laboratory analyses. While qualitative sampling does not lend itself to rigorous statistical testing, not as much time is required in the laboratory because there are no replicates. A distinct advantage to qualitative sampling is that more taxa are usually encountered because the most common sampling device (D-frame net) is used in several different places within a habitat type and can be used in places where the larger quantitative devices will not fit. Studies conducted as part of this project documented that more taxa were usually sampled by the qualitative use of the D-frame net than by quantitative techniques in the same habitats (Interim Report No. 3, 1990; Interim Report No. 6, 1991).

Therefore, our recommendation for the optimum study design of the NRGNR LTEMS was to conduct both quantitative and qualitative sampling, so that as many taxa as possible would be included in the community analyses. For the habitats included in the NRGNR LTEMS, quantitative and qualitative samples can be taken in cobble/pebble riffles, rock outcrops, and emergent macrophytes. Only qualitative samples can be taken in the pool habitat, because the sand and silt substratum also contains many cobble- and pebble-size rocks. All quantitative sampling devices for habitats with no current involve taking grabs or cores of soft sediment. These devices will not operate properly in the pools of the New River.

**Sampling Devices.** Three quantitative samplers and one qualitative sampler are used in the NRGNR LTEMS. These samplers are described briefly below, and their use is explained in detail in the section on Field Samples and Measurements - Macroinvertebrates.

The **Portable Invertebrate Box Sampler** (PIBS; Appendix B.2) is a completely enclosed 0.10-m<sup>2</sup> sampler, with wire mesh on the front side, solid side walls, and a 350- $\mu$ m mesh catch net on the back. It has a foam-lined bottom that establishes a thorough seal by taking the shape of irregular cobble and pebble substratum so organisms cannot escape while sampling is in progress. Because of the foam on the bottom, the PIBS can be used only when the water is > 0.10 m deep. 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** (Appendix B.2) is similar in size (0.09 m<sup>2</sup>) and function (350- $\mu$ m mesh catch net) as the PIBS, but the Surber has the front and most of the sides open and the bottom is a bare metal frame. It 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. However, the Surber can be used in very shallow water and in narrow channels because of its smaller size.

The **Rock Outcrop Community Sampler (ROCS)** (Appendix B.2) is a fully enclosed 0.01-m<sup>2</sup> sampler with no catch net in the water. It is 0.80 m high so that it can be used at depths up to 0.75 m, which are often encountered on the rock outcrops in the New River. A strip of foam around the bottom edge assures a tight seal to the substratum. A lightweight diaphragm pump, which is designed to handle slurries, is used to transport the sample into a 350- $\mu$ m mesh catch net that is suspended out of the water. The pump is permanently mounted on the ROCS, and the net is removable. The ROCS was designed specifically for studies of benthic macroinvertebrates in the New River (Voshell et al. 1992).

All qualitative samples are taken with a **D-Frame Dip Net** (Appendix B.2). The bottom of the net frame measures 0.30 m, and the mesh of the net is 350  $\mu$ m. Sampling is standardized as much as possible according to area and effort. By making the samples "semi-quantitative," comparisons among times and places are more accurate.

**Precision, Time Required, and Numbers of Replicate Samples.** Precision is a measure of the similarity of repeated (replicate) measurements. In statistical testing, replicates within a treatment must be reasonably similar in order to prove that any observed differences were caused by the treatment and not by inherent variability. A common measure of precision is the standard error. Levels of precision for macroinvertebrate samples are commonly in the range of 25 to 50% of the mean for the standard error, which is equal to a range of  $\pm 50$  to 100% for the 95% confidence limits of the mean.

Studies conducted as part of this project analyzed the level of precision obtained with different numbers of replicates of each type of quantitative sample and the amount of time required in the laboratory for each type of sample (Interim Report No. 3, 1990; Interim Report No. 6, 1991). Precision (D) was defined as the 95% confidence limits of the mean and was analyzed for total density, mean number of taxa, diversity index (Shannon), and density of the four most abundant taxa at each site. We analyzed the precision of the quantitative samples taken in the different habitats at the Bluestone and Thurmond study sites because Bluestone had the highest densities, and Thurmond was representative of the downstream sites with lower densities. We defined criteria for the acceptability of precision as follows:

Very good	D < 50%
Acceptable	D = 50 - 75%
Unacceptable	D > 75%

Higher values for D indicate replicates that are more variable and less precise.

Although it is desirable to have information on as many taxa as possible and have acceptable levels of precision, we realized that the LTEMs also must be feasible within the resources of NRGNR. The greatest cost for the macroinvertebrate portion of the LTEMs is the time required for analyzing the samples in the laboratory. The macroinvertebrate samples that can be collected during one day in the field may require several months in the laboratory to analyze. Therefore, we kept records of the amount of time required to analyze each type of sample in the laboratory.

The following times were the average required by VPI&SU personnel for each individual sample and include washing, sorting ("bug picking"), identifying, counting, and recording on bench sheets:

Quantitative		
	Cobble/pebble	22 h
	<i>Podostemum</i>	6 h
	<i>Justicia</i>	9 h
	Outcrops	2 h
Qualitative		
	All types	5 h

The most noticeable difference was the amount of time required for the cobble/pebble samples. Therefore, we analyzed the use of individual cobble rocks as a substitute for the cobble/pebble sample taken with a Portable Invertebrate Box Sample (PIBS). We speculated that using an individual cobble rock as a sampling unit might collect the same taxa with equivalent precision but would require much less time to process in the laboratory. Although the time required for analyzing the individual rock samples in the laboratory was very good (0.5 h), this type of sample was not a suitable substitute for the more labor intensive cobble/pebble samples. The individual rock samples collected far less taxa than the cobble/pebble samples (54-67%) and exhibited very low precision when densities were low.

Based on our analyses of precision and time required in the laboratory, our recommendations for numbers of replicates for each type of quantitative sample were:

Cobble/pebble	3
<i>Justicia</i>	3
Outcrops	5

### Levels of Effort

We were asked to develop a hierarchy of several different study designs for macroinvertebrates. The hierarchy was to include the most complex design that would be feasible (the optimum study design described above) and the least complex design that would provide some scientifically defensible data. Complexity meant the total amount of time required for all aspects of the design. It was not possible to make any type of quantitative sampling less time consuming than what has been recommended, because the numbers of replicate samples has been reduced to the lowest possible numbers with reasonable precision. We had hoped that the individual cobble rock samples would alleviate the problem with the most time consuming type of sample (cobble/pebble PIBS samples), but they omitted many taxa and had very poor precision. The only other alternative was to eliminate some types of quantitative samples in favor of qualitative samples in particular

habitats. Four levels of effort for monitoring macroinvertebrates are summarized below, proceeding from the most complex (Level 4) to the least complex (Level 1). Level 4 is the optimum study design described in the previous section.

**Level 4. Optimum study design. Quantitative samples in three habitats; qualitative samples in four habitats. Comprehensive supporting data on water quality and biological components related to food and habitat.**

Macroinvertebrates

Quantitative

3 habitats

cobble/pebble (3 replicates)

outcrop (5 replicates)

*Justicia* (3 replicates)

Qualitative

4 habitats

cobble/pebble (1 replicate)

outcrop (1 replicate)

*Justicia* (1 replicate)

pool (1 replicate)

Supporting Data

Water Quality

temperature

dissolved oxygen

pH

alkalinity

hardness

conductivity

seston

Biological

periphyton

phytoplankton

macrophytes (*Podostemum*, *Justicia*)

**Level 3. Quantitative samples in one habitat; qualitative samples in four habitats. Complete supporting data on water quality; partial supporting data on biological components related to food and habitat.**

Macroinvertebrates

Quantitative

1 habitat

outcrop (5 replicates)

Qualitative  
 4 habitats  
     cobble/pebble (1 replicate)  
     outcrop (1 replicate)  
     *Justicia* (1 replicate)  
     pool (1 replicate)

Supporting Data

Water Quality  
     temperature  
     dissolved oxygen  
     pH  
     alkalinity  
     hardness  
     conductivity  
     seston

Biological  
     periphyton  
     phytoplankton  
     macrophytes (*Podostemum*)

**Level 2. No quantitative samples; qualitative samples in four habitats. Complete supporting data on water quality; very limited supporting data on biological components related to food and habitat.**

Macroinvertebrates

Qualitative  
 4 habitats  
     cobble/pebble (1 replicate)  
     outcrop (1 replicate)  
     *Justicia* (1 replicate)  
     pool (1 replicate)

Supporting Data

Water Quality  
     temperature  
     dissolved oxygen  
     pH  
     alkalinity  
     hardness  
     conductivity  
     seston

Biological  
     periphyton  
     phytoplankton

**Level 1. No quantitative samples; qualitative samples in one habitat. Partial supporting data on water quality; no supporting data on biological components related to food and habitat.**

Macroinvertebrates

Qualitative  
1 habitat  
cobble/pebble (1 replicate)

Supporting Data

Water Quality  
temperature  
dissolved oxygen  
pH  
conductivity

The complete evaluation of the different levels of effort also requires careful consideration of the time required for each. A summary of the records that we kept for the Water Quality, Algae, Macrophytes, and Macroinvertebrates components of the NRGNR LTEMs is presented below. These estimates are based on the work being done by personnel with expertise in field and laboratory methods for aquatic ecology and macroinvertebrate taxonomy.

**Average Time Required  
(Person-Hours)**

	<b>Level 4</b>	<b>Level 3</b>	<b>Level 2</b>	<b>Level 1</b>
Field				
Collect samples	120	96	40	32
Laboratory				
Macroinvertebrates	705	240	140	20
Water Quality, Algae, Macrophytes	41	40	39	--
	—	—	—	—
Totals	866	376	219	52

While Levels 3, 2, and 1 offer obvious advantages in the amount of time required, there are corresponding losses in the amount of information about NRGNR and the ability to interpret and make conclusions about biological conditions. Eliminating sampling in some habitats has a major disadvantage in that some taxa will not be sampled and monitored. Eliminating quantitative sampling eliminates the ability to analyze the data with rigorous statistical tests.

**Level 4** (optimum study design) has been explained thoroughly in the preceding section.

**Level 3** retains quantitative sampling in one habitat (outcrops); therefore, inferential statistical testing would be possible. This habitat was chosen because the highest precision is obtained in relation to the amount of time that must be invested for processing the samples in the laboratory. Qualitative samples are taken in all habitats, so the maximum number of taxa should be encountered and can be reported at least in terms of relative abundance and presence or absence. The only biological supporting data that are omitted in Level 3 are samples of *Justicia*. However, the macrophyte *Podostemum* is included in Level 3, and it is probably more important as habitat for macroinvertebrates and is a better biological monitor of some types of water quality degradation (e.g., acidification, sedimentation) than *Justicia*.

**Level 2** has no quantitative sampling, so inferential statistical testing would not be possible. However, qualitative samples are taken in all habitats like Level 3, so the maximum number of taxa should be encountered and can be reported at least in terms of relative abundance and presence or absence. Each of the four types of standardized qualitative samples are very similar to the "rapid bioassessment" techniques that have been developed in recent years (see Plafkin et al. 1989, Barbour et al. 1992, Resh and Jackson 1993, Lenat and Barbour 1994, Barbour et al. 1995). Rapid bioassessment includes the calculation of a variety of descriptive statistics (usually called "metrics" in this approach), which can be used to make comparisons between different times or places. Although not as rigorous as statistical testing, these metrics do provide numerical comparisons that will provide increasing insight as long-term data are acquired. Level 2 does not include any information on either of the macrophytes that are abundant in much of NRGNR.

**Level 1** has no quantitative sampling, so inferential statistical testing would not be possible. Qualitative samples are taken in only the cobble/pebble riffle habitat, so there would be no information on some taxa. However, this habitat has the greatest taxa richness in NRGNR, so a great deal of information would be obtained and could be analyzed by the rapid bioassessment techniques discussed in the preceding paragraph on Level 2. There are no supporting biological data in Level 1.

NPS personnel should decide which level of effort for macroinvertebrates is appropriate, based upon the thoroughness of monitoring that is desired and the financial resources available. It is not necessary to work at the same level every year. The database that was developed for the NRGNR LTEMs stores, retrieves, and summarizes each component of Level 4 independent of any other components (see User Manual, Database Management System, VPI&SU 1994). Therefore, it is possible to shift back and forth among levels of effort in different years, then make comparisons of only the same samples. For example, if Level 4 was used in even years and Level 1 was used in odd years, inferential statistical tests could be conducted for three habitats among even years, but rapid bioassessment analyses could be conducted for the cobble/pebble habitat among all years.

## Sampling Frequency

The NRGNR LTEMS was designed to store and analyze data for one sampling period per year. Because of the high flows in the New River, it is usually only possible to take samples during the period from June through December. Even during this period, there are intervals immediately after heavy rainfall when it is not possible to wade or maneuver boats to collect samples. For the purposes of long-term monitoring it is essential that samples be collected consistently from year to year without any missing data. While data from other times of the year might be useful for making ecological interpretations, it is unlikely that such data could be obtained every year. Another complicating factor in the New River is that all biological samples require a great deal of time and effort for processing because of the abundance of the biota.

Therefore, it is recommended that samples be taken annually in August when flows are usually at their lowest and almost all of the biota would be expected to be present. 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 August all 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 1 wk, but the time for sampling all sites should not exceed 2 wk.

## FIELD SAMPLES AND MEASUREMENTS

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.

### WATER QUALITY, ALGAE, MACROPHYTES, MACROINVERTEBRATES

#### General Information

This section describes the sampling that is done for Level 4, which is the most comprehensive level of effort in the Water Quality, Algae, Macrophytes, and Macroinvertebrates components of the NRGNR LTEMS. The Water Quality component involves measuring seven parameters. The Algae component involves measuring the biomass of the two major communities of algae, periphyton and phytoplankton. The Macrophytes component consists of making biomass measurements of the two major species, *Podostemum* and *Justicia*. For the Macroinvertebrates component, three habitats are sampled quantitatively and four qualitatively. If lower levels of effort are desired, sampling can be reduced (Levels 1-3) as described previously in the Design section. Levels 1-3 involve measuring or sampling for fewer parameters, taking samples in fewer habitats, or doing qualitative rather than quantitative sampling. Levels 1-3 do not involve any type of sampling that is not part of Level 4.

## Effort

With a field crew of 3 people, it takes approximately 120 person-hours to complete these procedures at all five sites, including travel time. Investigators should allow the following amounts of time for travel between sites: Bluestone Dam to Sandstone Falls - 0.5 hr, Sandstone Falls to Prince - 2.0 hr, Prince to Thurmond - 1.0 hr, Thurmond to Fayette Station - 0.5 hr.

## Preliminary Tasks

Before leaving the vehicle the code, date, and initials of the persons doing the field work should be entered on the Field Records sheet (Appendix C.1). The sample bottles for water quality, 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 collecting samples. 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 river. A checklist of the equipment that must be carried is found in Appendix C.2.

## Sampling Sequence

Upon reaching the site, 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 phytoplankton samples should be filled and instrument readings taken before any disturbance of the river bottom. The temperature reading should be taken at this time also. After taking the water samples, an effective division of labor is for two persons to take the quantitative macroinvertebrate samples while the third person takes the periphyton samples. It is essential that the macroinvertebrate samples and periphyton samples not be taken in places where the investigators have walked. The investigators can avoid this by deciding upon a sampling itinerary before entering the river and observing each other while the samples are being collected. A general guideline is for sampling to proceed upstream. The different habitats where macroinvertebrate samples are collected can be visited in any sequence. The macrophyte samples are taken concurrently with the quantitative macroinvertebrate samples. The person taking the periphyton samples usually finishes first and then begins taking the qualitative macroinvertebrate samples. When the other two persons finish taking the quantitative macroinvertebrate samples, they help complete the qualitative macroinvertebrate sampling.

Upon return to the vehicle, water quality measurements that are made with electronic instruments (pH, conductivity, dissolved oxygen) should be completed if they were not done at the river. All other samples, which require later analysis in the laboratory, should be either preserved or stored on ice.

## Equipment

A list of all equipment needed for the field and laboratory procedures is provided in Appendix C.3.

### Water Quality

Water samples and instrument readings should be taken before the biological samples are collected. Take 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. Still water (pools, backwaters) may have higher temperature and lower dissolved oxygen concentration. Splashing water (riffles) may have a supersaturated concentration of dissolved oxygen and elevated concentration of seston. It will be necessary to enter the river to find a suitable spot. These guidelines also apply to taking a temperature measurement with a thermometer and using electronic instruments, which may be used for *in situ* measurements of temperature, pH, conductivity, or dissolved oxygen.

When filling water bottles, stand still and reach as far upstream as possible. It is a good practice to let the water flow slowly into the bottles, rather than submerging the bottles and letting the water "bubble" in. After all bottles have been filled, leave them at the river's edge in shallow water with moderate current while the remaining samples 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. The laboratory that conducts the water quality analyses may have individual preferences that would also be acceptable.

Temperature, pH, conductivity, dissolved oxygen.	Electronic instruments or kits
Alkalinity, pH, conductivity, hardness	1-L dark plastic bottle
Seston	Two 1-L dark plastic bottles
Temperature (Optional)	Long-stem thermometer
Dissolved oxygen (Optional)	0.3-L BOD bottle

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 know the ranges of measurements that have been recorded in the New River during August:

Temperature (°C)	23.5 - 29.5
Hydrogen ion (pH)	7.0 - 9.3
Conductivity ( $\mu$ mhos)	70 - 160
Hardness (mg CaCO <sub>3</sub> /L)	68 - 104
Dissolved oxygen (mg/L)	6.5 - 10.0

Immediately upon returning to the vehicle, any water quality samples that will be analyzed later should be packed on ice in a cooler. If the Winkler method is being used for dissolved oxygen, those samples should be fixed with the specified reagents (see American Public Health Association et al. 1989)

## Algae

### Periphyton

A quantitative measurement can be made by scraping the periphyton from a known area of substratum. Ten samples should be taken from each site by a stratified random design. (See the section, Macroinvertebrates - Quantitative, for an explanation of stratified random design.) One sample should be taken from each of 10 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 B.1). 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 samples cool by placing the bottles in shallow water (shaded, if possible), while the remaining work is done at the site. Pack the samples 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. 1989). The periphyton from measured areas of each of several slides is scraped into dark plastic bottles. There are several important disadvantages to using artificial substrates. Two visits to each site are required: one to place the samplers and one to retrieve them several weeks later. 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 is available to consumers, as either live or dead cells. In addition, there is a high probability of having missing data if artificial substrate

samplers are used in the New River because of losses from vandalism and flooding.

## **Phytoplankton**

Duplicate phytoplankton samples are collected by filling two 1-L dark plastic bottles with water. The samples are taken at the same time and in the same manner as the water quality samples discussed previously. These should be stored on ice in a cooler, like the periphyton samples, for transportation to the laboratory.

## **Macrophytes**

### **Submerged**

Samples of the dominant submerged macrophyte, *Podostemum ceratophyllum*, are collected as part of the quantitative macroinvertebrate samples that are taken on the rock outcrops. (See the section, Macroinvertebrates - Quantitative, for an explanation of how this macrophyte is removed and preserved.)

### **Emergent**

The dominant emergent macrophyte, *Justicia americana*, is sampled concurrently with the macroinvertebrates in that habitat. (See the section, Macroinvertebrates - Quantitative, for an explanation of how this macrophyte is removed and stored.)

## **Macroinvertebrates**

Take the samples within the designated habitat strata, but spread them out as much as possible in different areas of the site. However, if appropriate habitat strata are scarce take the samples wherever depth, flow, and substratum are optimum. Always be careful not to take samples where someone has already walked. Many macroinvertebrates swim or drift when disturbed, so measurements of their abundance will be affected by walking through areas to be sampled.

### **Quantitative Samples**

**Cobble/Pebble Riffles.** Three samples should be taken in this habitat stratum at each site with either the Portable Invertebrate Box Sampler (PIBS) or the Surber Sampler (Appendix B.2). The Surber Sampler must be used at Fayette Station because the cobble/pebble habitat is found only in narrow channels where the wider base of the PIBS will not fit. Otherwise, the PIBS is the best device for sampling macroinvertebrates (Voshell et al. 1989) and should be used at all other sites.

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 submerged. The substratum where the sampler is placed should not be so irregular that the sampler will not seal against the bottom. The area of bottom enclosed by the sampler should contain mostly cobble (6.4 - 25.6 cm) with some pebble (1.6 - 6.4 cm) and a little underlying gravel (0.2 - 1.6 cm) and sand. Homogeneous areas of gravel and sand should be avoided because the diversity and abundance of macroinvertebrates will be very low.

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 kneeling 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. 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. The second person holds the instruments, labels, and storage containers while the other person takes the sample.

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 the 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 substratum 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 submerged and then raised rapidly. Repeat the procedure for each side of the net. 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 immediately in the container. Completely invert the catch net and backwash it before proceeding to take the next sample. Samples are preserved and stored as described below.

**Rock Outcrops.** Five samples should be taken in this habitat stratum at each site. All samples should be taken in mats of *Podostemum*, if this submerged macrophyte is present at the site. If *Podostemum* is not present, then the samples should be taken from bare outcrops. Outcrop samples should never be taken from the mats of filamentous algae that are often conspicuously abundant, because macroinvertebrates are almost always very sparse.

Samples should be taken only with the Rock Outcrop Community Sampler (ROCS, Appendix B.2), which was designed specifically for New River studies (Voshell et al. 1992). Like the PIBS, sampling with the ROCS is most efficient with two persons, with one taking the sample and the other holding the instruments, labels, and storage containers. The investigators stand to use the ROCS. After placing the ROCS in the desired location on the outcrop, the person taking the sample holds the device in place with his or her feet. Because of the narrow shape of the ROCS, long-handled tools are used to dislodge the *Podostemum* and macroinvertebrates. If *Podostemum* is present, it is removed with a paint scraper. If *Podostemum* is not present, then a stiff 3.8-cm paint brush is used to dislodge the organisms. The sample is removed and transferred into the net by pumping all of the water out of the ROCS. After the ROCS has been pumped dry, approximately 2 L of river water is poured into the sampler, and then it is pumped dry again. This is

repeated one more time to make sure that all macroinvertebrates and vegetation are collected in the net. Transfer and label the rock outcrop samples the same as described above for the cobble/pebble samples. The macroinvertebrates and *Podostemum* are preserved and stored together by the procedures described below. The *Podostemum* will be removed later in the laboratory for a separate measurement of plant biomass.

**Emergent Macrophytes.** Three samples are taken from this habitat stratum by placing the PIBS in representative stands of *Justicia*. The depth and current should be the same as described previously for the cobble/pebble samples. *Justicia* usually grows only in gravel/sand substratum, so there is little concern about having to fit the PIBS on irregular bottom. The procedures for taking these samples are almost identical to those for the cobble/pebble samples, except that the units of substratum that have to be dealt with are stalks and leaves of *Justicia* instead of rocks.

All of the *Justicia* stalks contained in the PIBS are cut immediately above the river bottom with pruning shears. Vigorously wash each stalk in the water within the PIBS so that most of the organisms dislodge and wash into the catch net. Then the second person examines each stalk carefully and all organisms that remain attached are removed with forceps and added to the macroinvertebrate sample container. Place all pieces of *Justicia* that have been harvested and washed in a separate sample container so that the macrophyte biomass can be measured later in the laboratory. After removing all of the *Justicia*, rake the remaining fine substratum to stir up the sediment inhabiting organisms, but do not pull up the plant roots. Transfer the macroinvertebrate sample from the catch net and label it as described above for the cobble/pebble samples. Preserve and store the macroinvertebrate samples as described below, but the *Justicia* sample should only be placed on ice.

### Qualitative Samples

Qualitative samples are taken with a D-frame dip net. Sampling is standardized as much as possible according to area and effort. The same stratified random sampling design is used: cobble/pebble riffles, rock outcrops, emergent macrophytes, pools. 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. All qualitative samples, especially those from pools, should be washed as much as possible by moving the net vigorously through the water after taking the sample.

**Cobble/Pebble Riffles.** The D-frame net is placed in only one location where the conditions appear to be optimum for macroinvertebrates (sufficient current, heterogenous substratum). The loose rocks are moved and rubbed to dislodge organisms.

**Rock Outcrops.** The D-frame net is placed on a rock surface that is relatively flat and where there is sufficient current to carry away sediment. Qualitative samples should be taken in mats of *Podostemum*, if this submerged macrophyte is present at the site. If *Podostemum* is not present, then the samples should be taken from bare outcrops. Outcrop samples should never be taken from the mats of filamentous algae that are often conspicuously abundant, because macroinvertebrates are almost always very sparse. A square area, approximately equal to the width of the net frame (0.30 m), is disturbed with the hands or feet so

that the dislodged organisms are swept into the net. This is then repeated at one more place on another rock outcrop at the site.

**Emergent Macrophytes.** In the *Justicia* habitat, the D-frame net is pushed vigorously for a distance of 3.0 m, which is estimated as two lengths of the net handle. The frame of the net is moved just above the bottom, while intermittently prodding the roots of the *Justicia*. This sample is completed by cutting six stalks of *Justicia*, just above the substratum, and placing these in the same bag containing the contents of the D-frame net sample. The 3.0-m sweep with the net and the six stalks of *Justicia* may come from different places within the site.

**Pools.** In the pool habitat, the D-frame net is pushed along sand/silt substratum for a distance of 3.0 m. Like the *Justicia* habitat, this distance is estimated by two lengths of the net handle. The frame of the net is held about 1-2 cm under the soft substratum. The 3.0-m sampling distance may come from different areas within the site.

The qualitative macroinvertebrate samples are preserved and stored in the same manner as the quantitative ones, which is described below.

### **Preserving and Storing**

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 almost 20 yr 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 large cottage cheese containers used for sales to restaurants and institutions). 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 for field preservation. 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.

## **FISH**

### **General Information**

#### **Effort**

Fish sampling requires three persons for approximately 8 h, excluding travel time. Approximately 2 h are needed for unloading and loading the vehicle with equipment and 6 h for the actual fish collection. Each sample takes 10 min to collect and approximately 15-25 min to work up, depending on the make up of the sample. Thirteen samples are collected from each site. Each site is sampled once in early- to mid-August. This time of year is optimal because flows are usually low, and young-of-the-year fishes are large enough to be sampled effectively. All five sites should be sampled in 1 work-week if river flows and the weather permit.

#### **Preliminary Tasks**

To save time on the sampling day, equipment should be loaded into the vehicle prior to the sampling day. Before loading the generator onto the vehicle, check the oil level and add oil if necessary and start the generator to ensure it is operating properly. Before leaving for the collection site review the field portion of the equipment checklist (Appendix C.4) to ensure no equipment is forgotten.

At the collection site, fill the generator with gasoline and unload collection equipment onto the river bank. At this point the field crew should put on chest waders and place the boat or canoe into the river. Next, load the collection equipment into the boat. Place the generator so it can be easily started, stopped, and refilled with gasoline. Place the voltage regulator where it can be reached to adjust the power output and where the wattage output meter can be read. Make certain the voltage regulator is above the bottom of the boat to prevent it from getting wet if water gets into the boat. Set the cord on the bottom of the boat in an area free of other equipment to reduce entanglement. Leave space in the boat for storing the probes and nets when not in use.

When all the gear is loaded and properly arranged in the boat, plug the voltage regulator into the generator (Appendix B.3). Connect the cord to the voltage regulator and the probes. Hang the cathode (+) cable, which is attached to the cord, over the side of the boat. Before sampling begins the equipment should be tested to ensure everything is operating correctly and to adjust power output. Start the generator and switch the voltage regulator to DC pulse. Adjust the power output of the voltage regulator until the wattage meter reads 150-175 watts when the probe ends are submerged and the probe switches are depressed.

#### **Sampling Sequence**

There are three primary steps to the collection of each fish sample: (1) start the generator and electrofish for 10 min, (2) stop the generator and record length, weight, and species of all fish that can be identified and weigh more than 2 g, and (3) preserve and label all fish that cannot be positively identified or accurately weighed. This sequence is repeated thirteen times.

## **Equipment**

A list of equipment needed for field and laboratory procedures is provided in Appendix C.4. Sources for purchasing replacement parts and supplies is included in Appendix C.5.

## **Collection**

The exact locations where fish have been collected at each site are shown as shaded areas on the topographic maps in Appendices A.2-6. As stated previously, three persons are necessary for electrofishing; two persons to electrofish while the third person maintains the boat. The person controlling the boat is also responsible for running the stopwatch and preventing the cord from becoming tangled. Each person electrofishing holds a probe in one hand while netting fish with the other hand. Electrofishing should proceed upstream through any wadable area with the person maintaining the boat following behind (within 0-5 m). The best areas for sampling are along bank and island margins and in shallow riffles. The probes should be worked around structures and through open water. Sampling areas should be covered completely with little overlapping among sampling units. Every 0.5-2.0 min (depending on the numbers of fish being captured) the nets should be handed back to the person maintaining the boat and emptied into buckets of water. Probe safety switches must be depressed continuously throughout the 10-min sampling period otherwise unequal sampling effort will result among sampling periods.

After 10 min of sampling the person maintaining the boat calls out "stop" and the two persons electrofishing release the probe safety switches. The person maintaining the boat then turns off the generator. At this point the fish sample is worked up. One person identifies each fish and measures the total length. Another person weighs each fish. The third person completes the top portion of the data form (Appendix C.6; date, site, sample number, and field crew) and records the fish species and measurements. After the species, length, and weight have been recorded for a fish it should be released downstream of the area to be sampled next. A quick return to the river increases the survival of released fish and releasing the fish downstream of the next sampling area ensures the fish will not be captured twice. Any fish that cannot be positively identified or weighs less than 2 g is preserved immediately in 5-10% formalin. Each preserved sample is labelled (sample label Appendix C.7) by placing a piece of waterproof paper with the date, sample site, and sample number into the storage container.

## **LABORATORY ANALYSES AND CALCULATIONS**

### **WATER QUALITY, ALGAE, MACROPHYTES, MACROINVERTEBRATES**

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 worksheets and summary sheets are provided so that the laboratory analyses and calculations can be performed efficiently and accurately (Appendices C.8-10). On all of these sheets, the site, 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

### Water Chemistry

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. (1989) or U. S. Environmental Protection Agency (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 Worksheet and Summary (Appendix C.8).

Alkalinity: single endpoint titration; APHA et al. (1989), method 2320 B.

### Seston

The methods for seston analysis are almost the same as those that APHA et al. (1989) suggest for solid analysis (Section 2540). Use the Seston Worksheet (Appendix C.9) to record the sample weights (pans and filters) at each step. Use the Water Quality Worksheet and Summary (Appendix C.8) 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 suspended solids and can be measured by a slight modification of Method 2540 D. Filter two samples of 1 L each. 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 labelled "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 fixed solids and can be measured by a slight modification of Method 2540 E. 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 Worksheet in the space labeled "Inorganic."

Organic seston is synonymous with volatile solids and can also be measured by the same modification of Method 2540 E. 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 mg, and entered in the space labeled "O/I."

## ALGAE

### Periphyton

**Biomass.** Spectrophotometric determination of chlorophyll *a* is used to estimate the biomass of live periphyton, according to Method 10200 H.2 in APHA et al. (1989). Five 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 Water Quality Worksheet and Summary (Appendix C.8) to record the various steps of this procedure. The final results should be expressed as  $\mu\text{g}/\text{cm}^2$ . Method 10200 H.2 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  $\text{cm}^2$  should be substituted for  $V_2$  in the equations. In addition the final result should be converted from mg to  $\mu\text{g}$ .

**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. (1989) Method 10300 C.6, except that the units are  $\mu\text{g}/\text{cm}^2$ . Use the Water Quality Worksheet and Summary (Appendix C.8) 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 remaining five periphyton samples and analyzing them by the same method as organic seston (Method 2540 E, ash-free dry weight). These five samples should be analyzed separately and averaged.

### Phytoplankton

**Biomass.** Use method 10200 H.2 in APHA et al. (1989) to measure the biomass of living phytoplankton. Use the Water Quality Worksheet and Summary (Appendix C.8) to record the various steps in the spectrophotometric determination. Each phytoplankton sample should be analyzed separately and the results averaged. Final results must be converted from mg/L to  $\mu\text{g}/\text{L}$ .

## MACROPHYTES

### Justicia

**Biomass.** Soon after returning from the field, remove the samples of *Justicia* from the plastic bags and place each sample in a separate enamel pan with the original sample label. Dry the plant material at room temperature for approximately 1 wk. Weigh the samples on a triple-beam balance to the nearest 0.1 g. Record the weights on the Water Quality Worksheet and Summary (Appendix C.8).

## Podostemum

**Biomass.** This submerged macrophyte is preserved along with the macroinvertebrate samples from the rock outcrop habitat and must be removed during the sorting process (see Macroinvertebrates, Quantitative and Qualitative, in next section). As each rock outcrop macroinvertebrate sample is sorted, the *Podostemum* is removed and placed in a separate plastic dish with a sample label. Dry the samples in a drying oven at 60 °C for 24 hr, then weigh them on a triple-beam balance to the nearest 0.1 g. Record the results on the Water Quality Worksheet and Summary (Appendix C.8).

## MACROINVERTEBRATES

The analysis of each macroinvertebrate 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 macroinvertebrate samples is to remove any remaining fine detritus and the original preservative, which will have become darkly stained from the chlorophyll in the algae and macrophytes. This is accomplished by placing each sample in a 355- $\mu$ 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 overemphasized. 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- $\mu$ 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 West 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 book by Stehr (1987) covers the immature stages of all insects and 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. 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. All data should be recorded on the Benthic Macroinvertebrate Worksheet (Appendix C.10). 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 taxa 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 years). 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 NRGNR personnel 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.

## FISH

After a minimum of three days in formalin, preserved fish can be transferred to a preservative less hazardous to human health. Under a hood, formalin from each sample container should be drained off into a waste formalin carboy leaving the fish and the label in the sample container. Soak the fish in water to remove excess formalin by adding water to each sample container. After 24 h, drain off the water and refill the containers with fresh water. This sequence should be repeated for 3 d at which point the containers can be refilled with 70-75% ethanol.

Fish identification should be done for one sample container at a time to prevent the chance of mixing samples. Place the specimens and the sample label

into a shallow porcelain sorting pan and fill the pan until the fish are covered with water. At this point the fish can be sorted visually into groups of similar looking species either in the same pan or into several pans depending on the numbers and sizes of the fish. With experience and familiarity, many genera and some species can be identified without the aid of dichotomous keys; however, to ensure proper identification of all specimens the keys should be used whenever any uncertainty arises. We recommend Jenkins and Burkhead (1994) for fish identification; however, other books such as Eddy and Underhill (1978) and Page and Burr (1991) will provide additional aid in identification.

After all fish in a sample have been identified to species, measure each fish for total length and weight. Record these data directly on data forms, which were partially completed in the field and correspond to the sample being analyzed. Return the label and fish to the sample container after species, lengths, and weights have been recorded. All preserved specimens should be retained to resolve any taxonomic questions that might arise. One year retention is recommended before the samples are relinquished to a permanent museum. Appendix C.11 provides a list of potential repositories for samples as well as a list of individuals to contact about fish identification.

Information on data analysis and data entry into the computer are provided in the LTEMs database user manual.

## REFERENCES

- American Public Health Association, American Water Works Association, and Water Pollution Control Federation. 1989. Standard methods for the examination of water and wastewater, 17th edition. American Public Health Association, Washington, D.C.
- Anderson, R. O. and S. J. Gutreuter. 1983. Length, weight, and associated structural indices. Pages 283-300 *in* L. A. Nielsen and D. L. Johnson, eds. Fisheries techniques. American Fisheries Society. Bethesda, MD.
- Barbour, M. T., J. L. Plafkin, B. P. Bradley, C. G. Graves, and R. M. Wisseman. 1992. Evaluation of EPA's rapid bioassessment benthic metrics: metric redundancy and variability among reference stream sites. *Environmental Toxicology and Chemistry* 11: 437-449.
- Barbour, M. T., J. B. Stribling, and J. R. Karr. 1995. Multimetric approach for establishing biocriteria and measuring biological condition. Pp. 63-77 *in* Davis, W. S. and T. P. Simon, eds. Biological assessment and criteria. Tools for water resource planning and decision making. CRC Press, Boca Raton, Florida.
- Brigham, A. R., W. U. Brigham and A. Gnilka, eds. 1982. Aquatic insects and oligochaetes of North and South Carolina. Midwest Aquatic Enterprises, Mahomet, Illinois.
- Buchanan, T. J. and W. P. Somers. 1969. Discharge measurements at gaging stations, techniques of water-resources investigations of the United States Geological Survey. Book 3, Chapter A8, U. S. Government Printing Office, Washington, D.C.

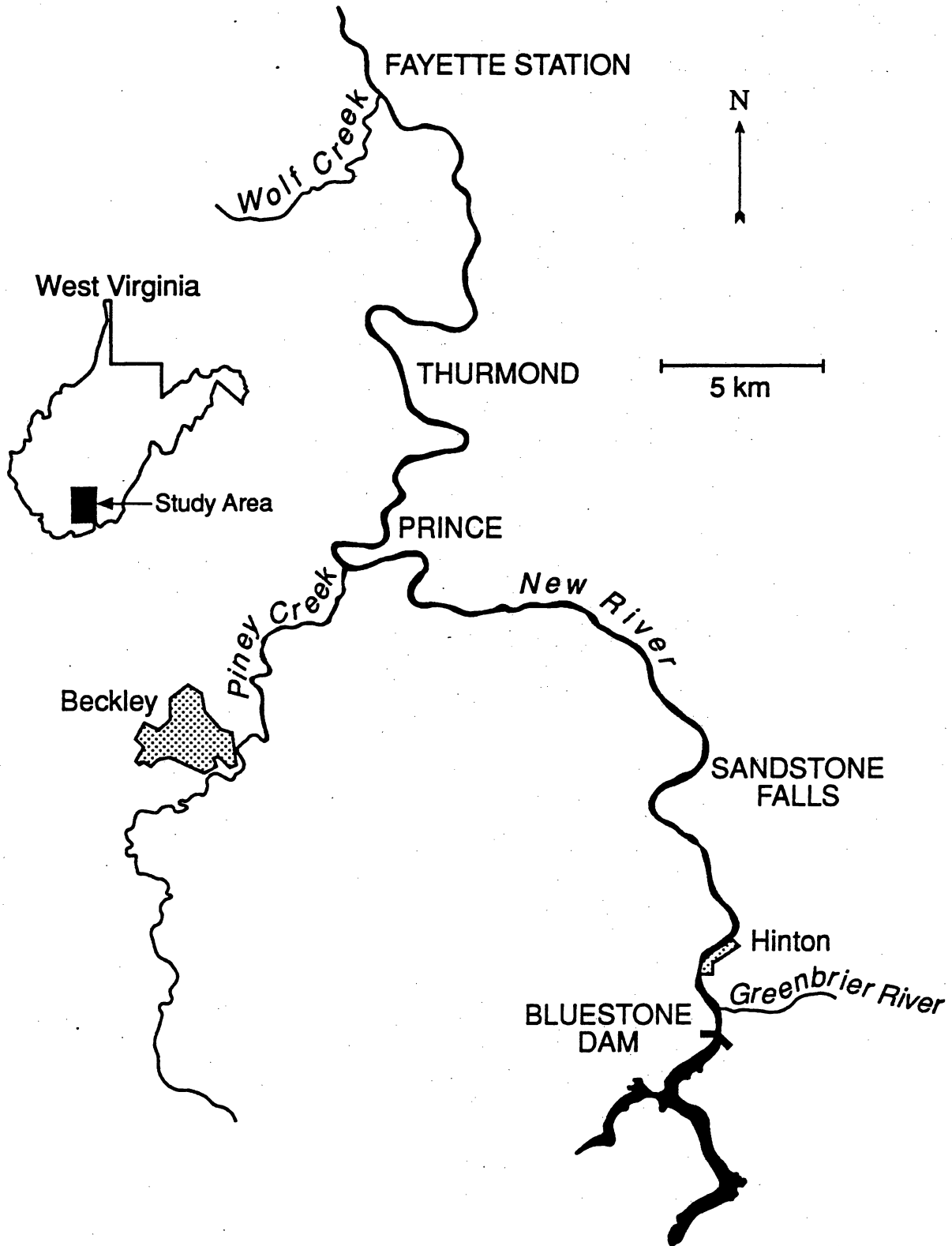
- Cole, G. A. 1979. Textbook of limnology, 2nd ed. The C. V. Mosby Company, St. Louis, Missouri.
- Eddy, S. and J. C. Underhill. 1978. How to know the freshwater fishes. William C. Brown Company, Publishers. Dubuque, IA. 215 pp.
- Edmunds, G. F., Jr., S. L. Jensen, and L. Berner. 1976. The mayflies of North and Central America. University of Minnesota Press, Minneapolis.
- Feldman, R. S. and E. F. Connor. 1985. Influences of low alkalinity and pH on invertebrate community structure: a controlled replicated stream design. Unpublished report, Department of Environmental Sciences, University of Virginia, Charlottesville.
- Gurtz, M. E., J. R. Webster and J. B. Wallace. 1980. Seston dynamics in southern Appalachian streams: effects of clear-cutting. *Can. J. Fish. Aquat. Sci.* 37:624-631.
- Hynes, H. B. N. 1970. The ecology of running waters. University of Toronto Press, Toronto, Ontario.
- Jenkins, R. E. and N. M. Burkhead. 1994. Freshwater Fishes of Virginia. Am. Fish. Soc., Bethesda, Maryland.
- Lenat, D. R. and M. T. Barbour. 1994. Using benthic macroinvertebrate community structure for rapid, cost-effective, water quality monitoring: rapid bioassessment. Pp. 187-215 in Loeb, S. L. and A. Spacie, eds. Biological monitoring of aquatic systems. CRC Press, Boca Raton, Florida.
- Merritt, R. W. and K. W. Cummins, eds. 1984. An introduction to the aquatic insects of North America, 2nd ed. Kendall/Hunt, Dubuque, Iowa.
- Page, L. M. and B. M. Burr. 1991. A field guide to freshwater fishes of North America north of Mexico. Houghton Mifflin Company. Boston, MA. 432 pp.
- Pennak, R. W. 1978. Fresh-water invertebrates of the United States, 2nd ed. John Wiley and Sons, New York.
- Plafkin, J. L., M. T. Barbour, K. D. Porter, S. K. Gross, and R. M. Hughes. 1989. Rapid bioassessment protocols for use in streams and rivers. Benthic macroinvertebrates and fish. EPA/444/4-89/001. Office of Water Regulations and Standards, U. S. Environmental Protection Agency, Washington, D. C.
- Resh, V. H. and J. K. Jackson. Rapid assessment approaches to biomonitoring using benthic macroinvertebrates. 1993. Pp. 195-223 in Rosenberg, D. M. and V. H. Resh, eds. Freshwater biomonitoring and benthic macroinvertebrates. Chapman and Hall, New York.
- Stehr, F. W., ed. 1987. Immature insects. Kendall/Hunt, Dubuque, Iowa.
- U. S. Environmental Protection Agency. 1976. Quality criteria for water. U. S. Government Printing Office, Washington, D.C.

- U. S. Environmental Protection Agency. 1983. Methods for chemical analysis of water and wastes. EPA-600/4-79-020, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio.
- Virginia Polytechnic Institute and State University. 1985. An ecological investigation of the New River and Bluestone Lake. Report. Huntington District, U. S. Army Corps of Engineers. 185 pp. text + 241 pp. data appendix.
- Voshell, J. R., Jr. 1985. Trophic basis of production for macroinvertebrates in the New River below Bluestone Dam. Report submitted to West Virginia Department of Natural Resources, Charleston, WV.
- Voshell, J. R., Jr., S. W. Hiner, and R. J. Layton. 1992. Evaluation of a benthic macroinvertebrate sampler for rock outcrops in rivers. *J. Freshwat. Ecol.* 7: 1-6.
- Voshell, J. R., Jr., R. J. Layton, and S. W. Hiner. 1989. Field techniques for determining the effects of toxic substances on benthic macroinvertebrates in rocky-bottomed streams. Pp. 134-155 in U. Cowgill and L.R. Williams, eds. *Aquatic toxicology and hazard assessment: 12th volume.* ASTM STP 1027, American Society for Testing and Materials, Philadelphia.
- Voshell, J. R., Jr. and C. R. Parker. 1985. Quantity and quality of seston in an impounded and a free-flowing river in Virginia, U.S.A.. *Hydrobiologia* 122: 271-280.
- Ward, H. B. and G. C. Whipple. 1959. *Fresh-water biology*, 2nd ed. John Wiley and Sons, New York.
- Warren, C. E. 1971. *Biology and water pollution control.* W. B. Saunders Company, Philadelphia.
- Webster, J. R., E. F. Benfield, and J. Cairns, Jr. 1979. Model predictions of effects of impoundment on particulate organic matter transport in a river system. Pp. 339 - 364 in J. V. Ward and J. A. Stanford, eds. *The ecology of regulated streams.* Plenum Press, New York.
- Wetzel, R. G. 1983. *Limnology*, 2nd ed. W. B. Saunders Company, Philadelphia.
- Wiggins, G. B. 1977. *Larvae of North American caddisfly genera (Trichoptera).* University of Toronto Press, Toronto, Ontario.

APPENDICES

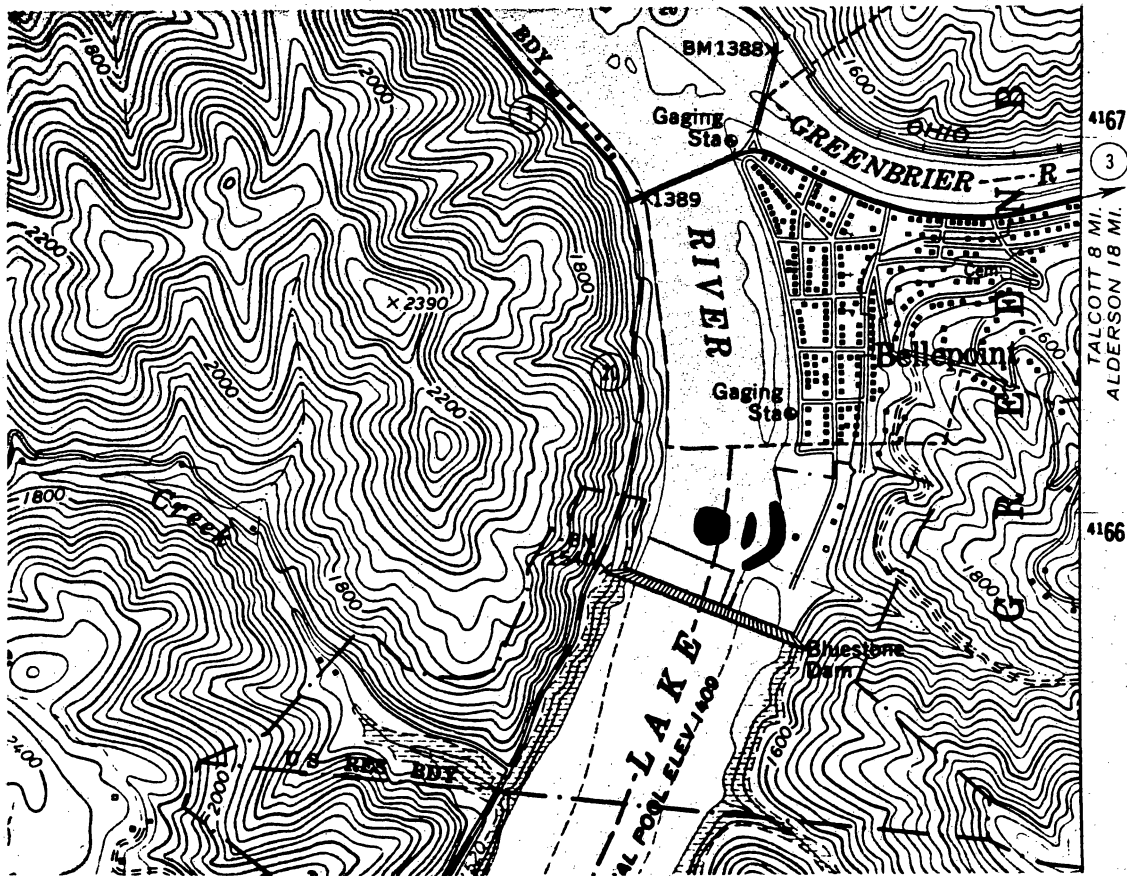
Appendix A.1.

Map of five permanent study sites that were established for the New River Gorge National River Long-Term Ecological Monitoring System (Bluestone Dam, Sandstone Falls, Prince, Thurmond, Fayette Station).



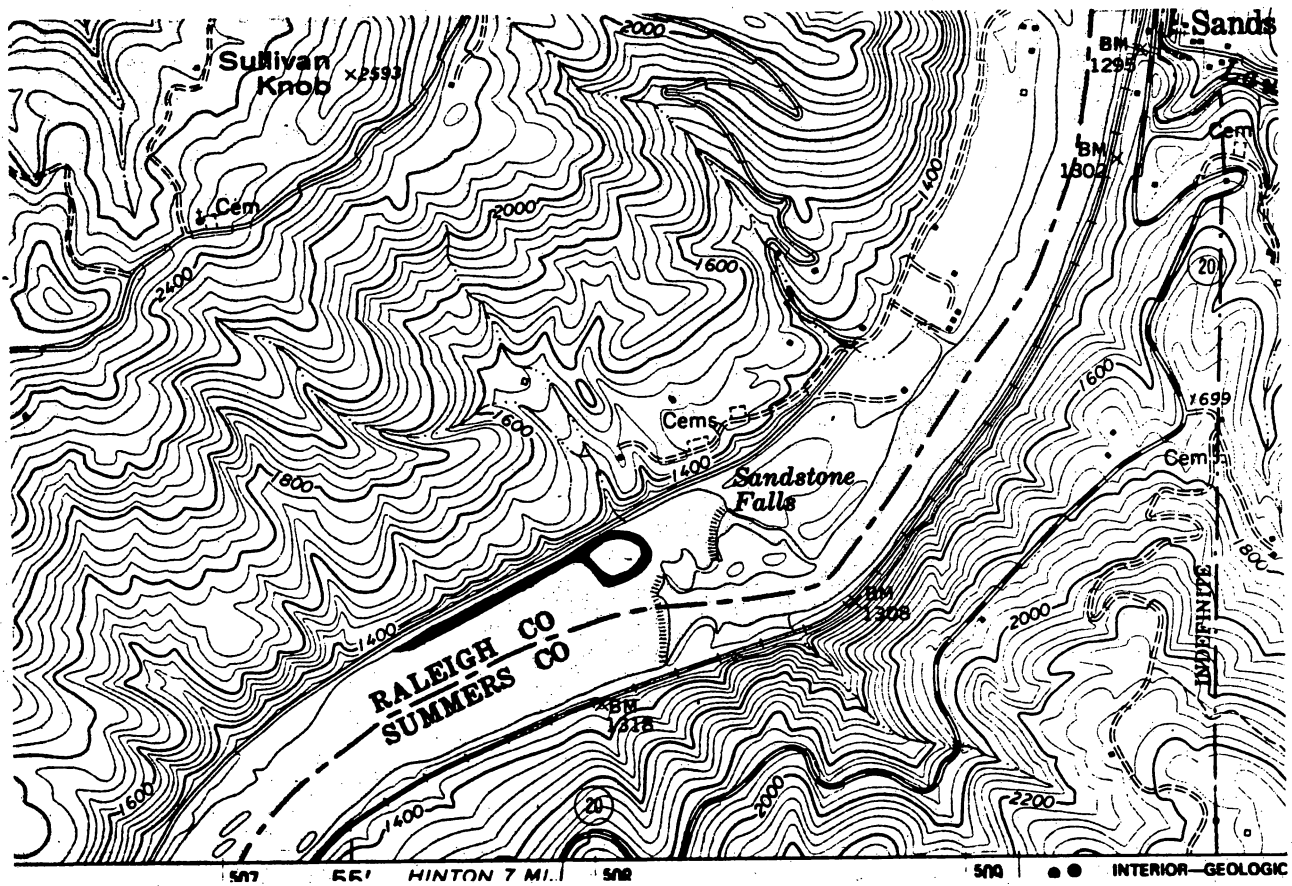
Appendix A.2. Bluestone Dam Study Site (USGS 7.5 Minute Topographic Map - Hinton Quadrangle).

This site is located immediately below Bluestone Dam at the public park maintained by the U.S. Army Corps of Engineers (Town of Hinton, Summers County, West Virginia). Access is from the parking lot. Macroinvertebrate samples are taken from specified habitats within the section from 100 to 300 m downstream of the spillway. The exact locations where fish are collected are shown as shaded areas.



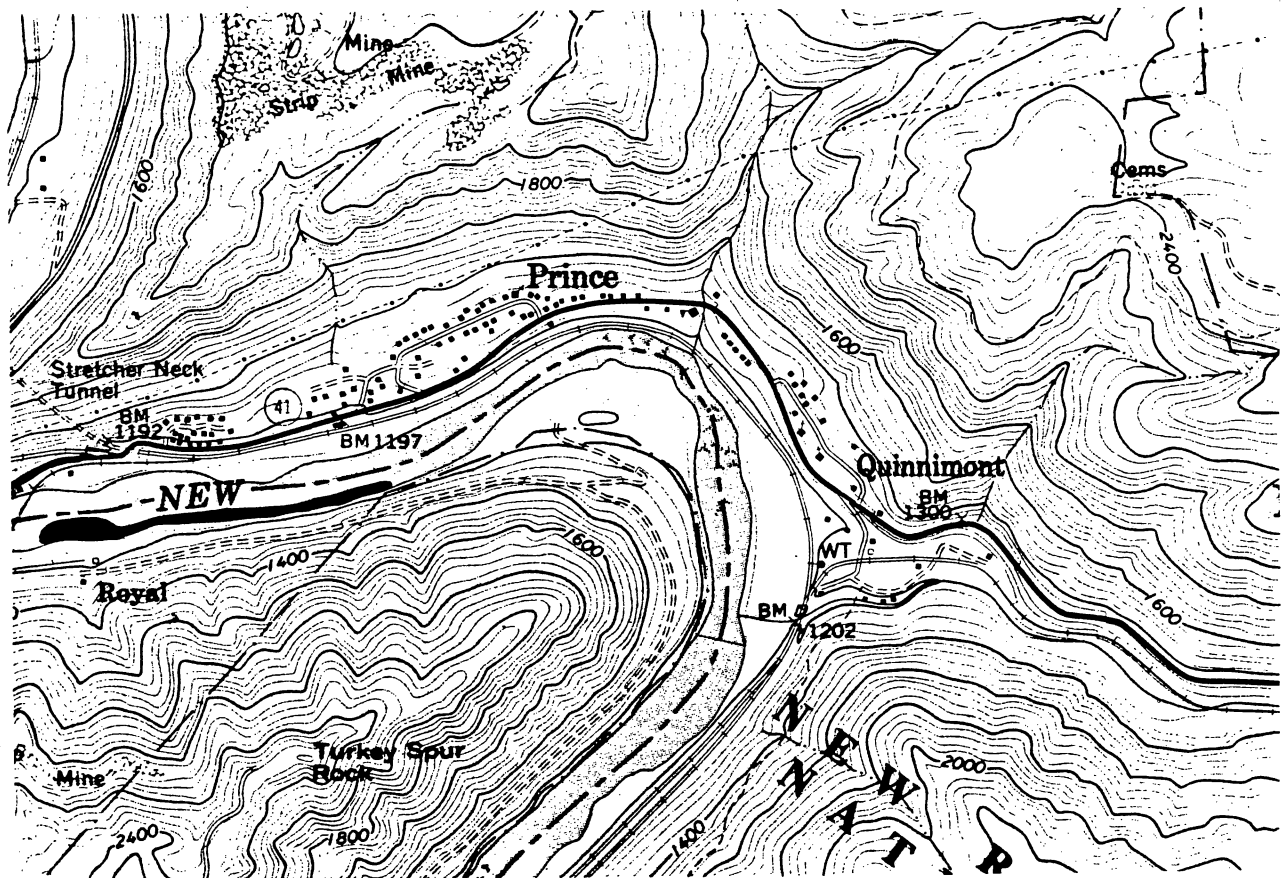
Appendix A.3. Sandstone Falls Study Site (USGS 7.5 Minute Topographic Map - Meadow Creek Quadrangle).

This site is located 16 river km downstream from Bluestone Dam. Access is from Route 26, on the west side of the New River, just upstream from the falls. Macroinvertebrate samples are taken from specified habitats within the section that is 50 to 250 m upstream from the falls, which are an abrupt 8-10 m drop. The exact locations where fish are collected are shown as shaded areas.



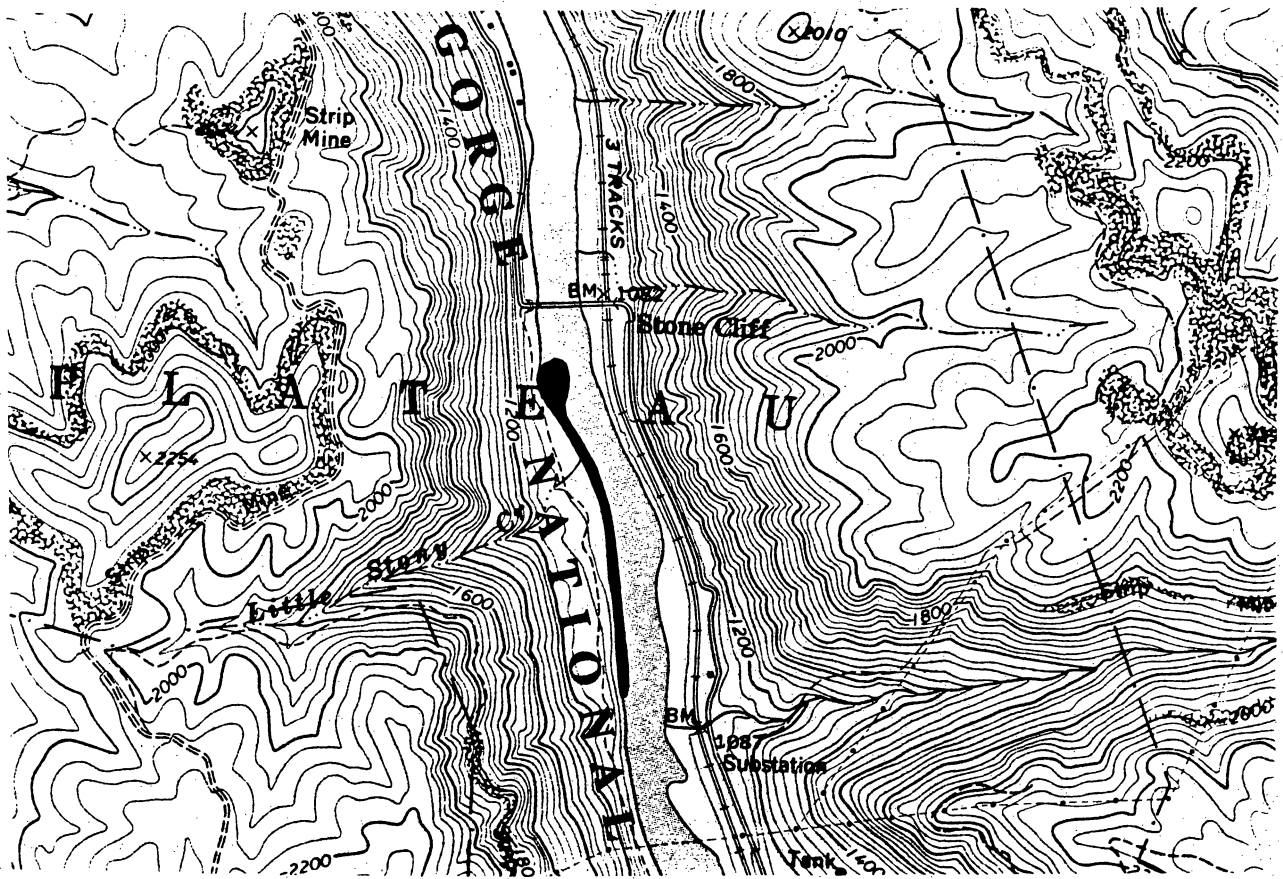
Appendix A.4. Prince Study Site (USGS 7.5 Minute Topographic Map - Prince Quadrangle).

This site is located approximately 41.5 river km below Bluestone Dam. Access to the site is achieved from a dirt road on the south bank, which forks from Route 19 and 41 just prior to crossing the river. Macroinvertebrate samples are taken from specified habitats within the 200-m section that is 1.75 km upstream of the Route 19 and 41 bridge. The sampling site is the first riffle above the bridge and includes the pool area immediately below the riffle. The exact locations where fish are collected are shown as shaded areas.



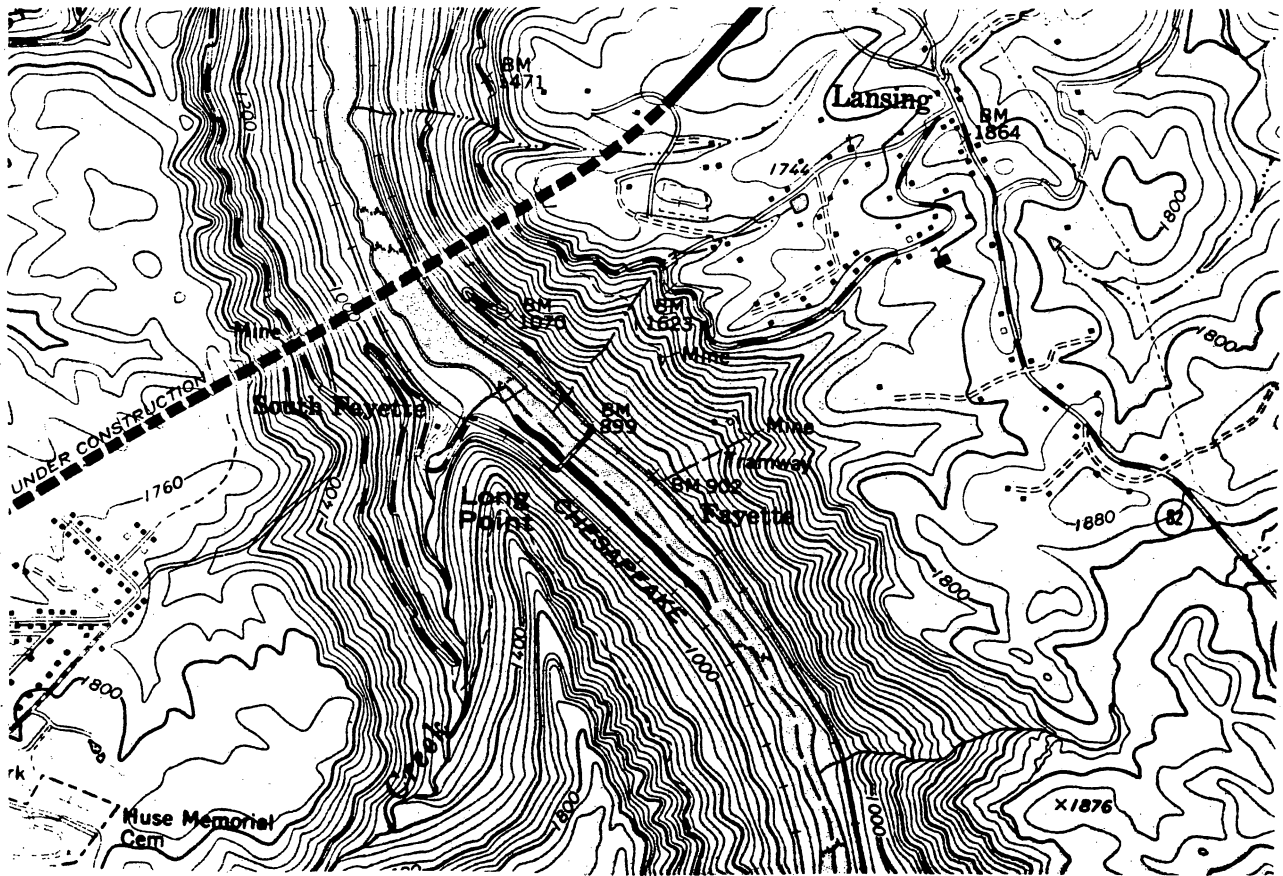
Appendix A.5. Thurmond Study Site (USGS 7.5 Minute Topographic Map - Thurmond Quadrangle).

This site is located approximately 61.6 river km below Bluestone Dam. Access to the river is achieved from a dirt road which forks from the paved road just prior to the Stonecliff bridge. Macroinvertebrate samples are taken from specified habitats within the section 3 km upstream of the town of Thurmond, approximately 50 m upstream of the Stonecliff bridge. The exact locations where fish are collected are shown as shaded areas.

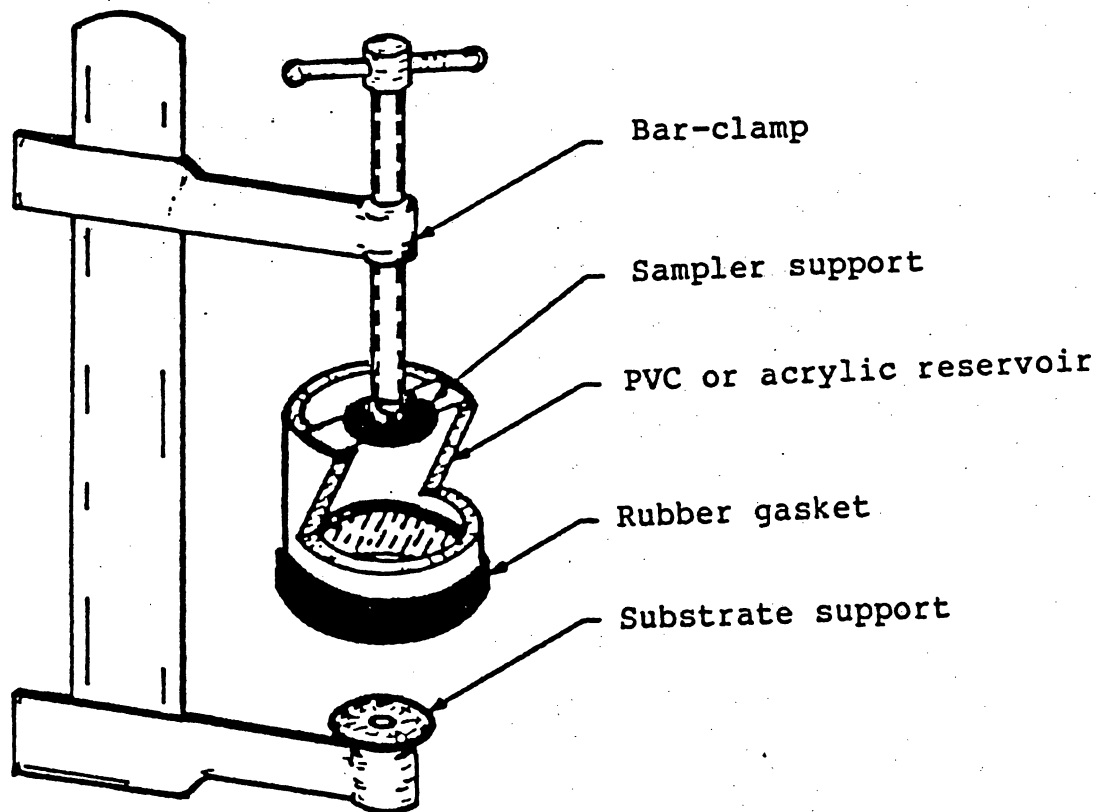


Appendix A.6. Fayette Station Study Site (USGS 7.5 Minute Topographic Map - Fayetteville Quadrangle).

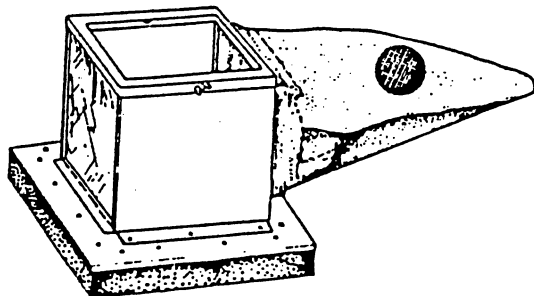
This site is located approximately 91.4 river km downstream from Bluestone Dam. Access is achieved from Route 82 and an NPS public access area. Macroinvertebrate samples are taken from specified habitats within the section that extends from the riffle just below the confluence of Wolf Creek to 300 m upstream of the riffle. The exact locations where fish are collected are shown as shaded areas.



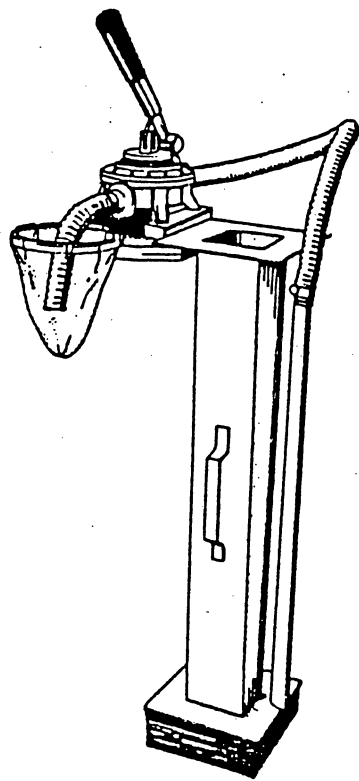
Appendix B.1. Periphyton Bar-Clamp Sampler.



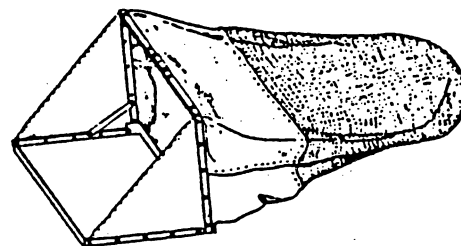
Appendix B.2. Macroinvertebrate quantitative samplers.



Portable Invertebrate Box Sampler (PIBS)

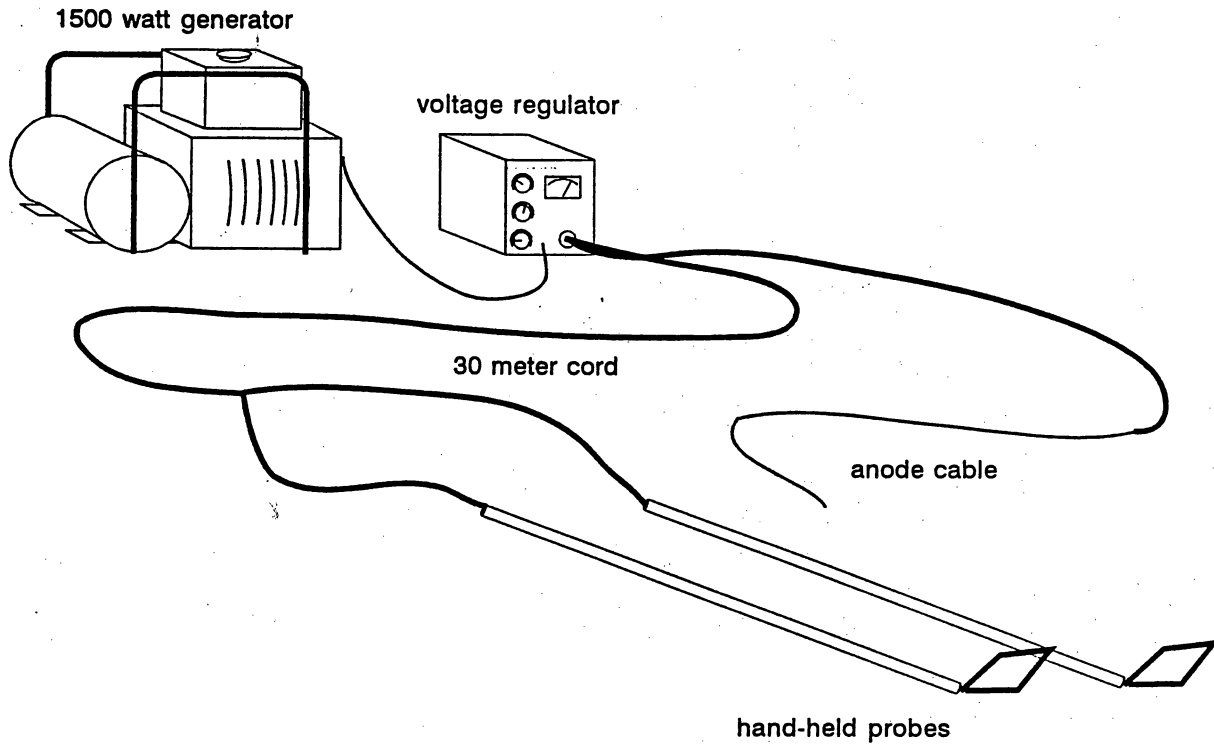


Rock Outcrop Community Sampler (ROCS)



Surber Sampler

Appendix B.3. Electrofishing sampling gear.



Appendix C.1. Field records sheet for water quality, algae, macrophytes, and macroinvertebrates.

**FIELD RECORDS**  
**WATER QUALITY, ALGAE, MACROPHYTES, MACROINVERTEBRATES**

Site \_\_\_\_\_ Date \_\_\_\_\_  
Taken By \_\_\_\_\_  
Start \_\_\_\_\_ Finish \_\_\_\_\_

Water Temperature \_\_\_\_\_ °C Discharge \_\_\_\_\_ cfs

**Bottle #'s**

Alkalinity	_____	Dissolved Oxygen	_____
Seston	_____	Phytoplankton	_____
Hardness	_____	pH	_____
Conductivity	_____		
Periphyton	_____		

**Other Samples Collected**

Macrophytes \_\_\_\_\_

Macroinvertebrates

**Quantitative**

**Qualitative**

Rock Outcrop	_____	_____
Cobble-Pebble	_____	_____
Justicia	_____	_____
Pool	_____	_____

**Comments**

\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

**EQUIPMENT CHECKLIST**  
**WATER QUALITY, ALGAE, MACROPHYTES, MACROINVERTEBRATES**

- \_\_\_\_\_ Data sheets/clipboard
- \_\_\_\_\_ Pencils
- \_\_\_\_\_ PIBS
- \_\_\_\_\_ Surber sampler
- \_\_\_\_\_ Vegetable brush
- \_\_\_\_\_ Hand rake
- \_\_\_\_\_ Forceps
- \_\_\_\_\_ ROCS
- \_\_\_\_\_ ROCS tools
- \_\_\_\_\_ D-frame dip net
- \_\_\_\_\_ Plastic bags
- \_\_\_\_\_ Labels for macroinvertebrate samples
- \_\_\_\_\_ Alcohol
- \_\_\_\_\_ Thermometer
- \_\_\_\_\_ Bar-clamp sampler
- \_\_\_\_\_ Periphyton tools
- \_\_\_\_\_ Plastic buckets
- \_\_\_\_\_ Wax pencils
- \_\_\_\_\_ Sample bottles
- \_\_\_\_\_ D.O. kit or meter
- \_\_\_\_\_ Conductivity meter
- \_\_\_\_\_ Hardness kit
- \_\_\_\_\_ pH Meter
- \_\_\_\_\_ Waders
- \_\_\_\_\_ Coolers with ice
- \_\_\_\_\_ Backpack and pack frame
- \_\_\_\_\_
- \_\_\_\_\_
- \_\_\_\_\_
- \_\_\_\_\_
- \_\_\_\_\_
- \_\_\_\_\_

Appendix C.3. Sources of field and laboratory equipment and supplies for water quality, algae, macrophytes, and macroinvertebrates.

Parameters	Equipment	Addresses and Phone Numbers of Suppliers
Water temperature	YSI Model 33 meter, long-stem thermometer	American Scientific 8855 McGaw Rd. Columbia, MD 21045
Dissolved oxygen	YSI Model 58 meter	Fisher Scientific P.O. Box 40339 Raleigh, NC 27629
	Hach kit	Hach Company P.O. Box 389 Loveland, CO 80539
Conductivity	YSI Model 33 meter	American Scientific Fisher Scientific
pH	Fisher Accumet 640 meter with calomel reference electrode and universal electrode (or equivalent), electrode storage solution	Fisher Scientific
Alkalinity, conductivity, pH	1-L dark plastic collection bottle	Fisher Scientific
Hardness	Hach kit	Hach Company
Seston	1-L collection bottle, vacuum pump, filter funnel (47mm), filter flask, vacuum hose, glass fiber filters (47mm), aluminum pans, filter forceps, filter flask (2L), graduated cylinder (1L), muffle/ashing furnace, convection drying oven, electronic analytical balance (0.00001g)	Fisher Scientific GELMAN filtering products

Appendix C.3. List of required field and laboratory equipment and supplies for water quality, algae, macrophytes, and macroinvertebrates. [CONTINUED]

Parameters	Equipment	Addresses and Phone Numbers of Suppliers
Periphyton (chlorophyll a)	Bar-clamp sampler (fabricated), acid etching brush, wash bottle, eye dropper, 60-ml dark plastic bottles, filtering equipment (see Seston), hydrochloric acid, aqueous acetone, magnesium carbonate, whirl-pak bags, tissue grinder, grinding chamber and bit, 12-ml graduated centrifuge tubes, centrifuge, pipets, cuvetts, spectrophotometer	hardware store
		Fisher Scientific
Phytoplankton (chlorophyll a)	1-L dark plastic collection bottle (same equipment as used for Periphyton)	Fisher Scientific
Macrophytes	Portable invertebrate box sampler (PIBS) (350 um net)	Ellis-Rutter P.O.Box 401 Punta Gorda, FL 33950
	Rock outcrop community sampler (ROCS) (350 um net) (fabricated)	machine shop
	pruning shears	hardware store
	plastic bags	grocery store
	Dymo labeler	office supply store
Macro-invertebrates	D-frame kick net,	BioQuip 1320 E. Franklin Ave. El Segundo, CA 90245

Appendix C.3.

List of required field and laboratory equipment and supplies for water quality, algae, macrophytes, and macroinvertebrates. [CONTINUED]

Parameters	Equipment	Addresses and Phone Numbers of Suppliers
	Surber sampler (350 nm net)	Wildco 301 Cass St. Saginaw, MI 48602
	Portable invertebrate box sampler (PIBS) (350 nm net)	Ellis-Rutter
	2.5-L plastic containers with lids	Genpak Corp. Box 727 Glens Falls, NY 12801
	Plastic bags	grocery store
	4-dram vials, 2-oz jars, formaldehyde(opt.) ethanol, forceps	Fisher Scientific
	Vegetable brush, hand rake	hardware store
	Dymo labeler	office supply store
Miscellaneous	Chest waders, backpacks, coolers	outdoor recreation supplier

Appendix C.4. Checklist of required field and laboratory equipment and supplies for fish sampling, identification, and measurements.

**EQUIPMENT CHECKLIST  
FISH**

*Field*

- \_\_\_\_\_ 1500-watt Homelite generator
- \_\_\_\_\_ Coffelt VVP-2C, voltage regulator
- \_\_\_\_\_ 30-m cord with anode cable and couplings for connection to probes and Coffelt VVP-2C
- \_\_\_\_\_ Coffelt electrofishing probes (anodes), approx. 1.75 m long with waterproof switches
- \_\_\_\_\_ John boat or canoe
- \_\_\_\_\_ Canoe straps and carrier
- \_\_\_\_\_ 2 dip nets, 1.75 m long with 0.5 cm (3/16 in) nylon mesh
- \_\_\_\_\_ 3-5 20-L plastic buckets
- \_\_\_\_\_ 20-L gasoline can, full
- \_\_\_\_\_ Funnel
- \_\_\_\_\_ 3 pair chest waders with felt soles or aluminum cleats
- \_\_\_\_\_ Rubber gloves
- \_\_\_\_\_ Stopwatch or timer
- \_\_\_\_\_ 15-20 250-ml plastic storage jars (for each sample day)
- \_\_\_\_\_ 4 L 5-10% formalin (for each sample day)
- \_\_\_\_\_ Measuring board (0-1000 mm)
- \_\_\_\_\_ 3 Pesola spring scales, (one each size, 0-10 g, 1-100 g, and 0-300 g)
- \_\_\_\_\_ Platform scale (0-10 kg)
- \_\_\_\_\_ Notepad
- \_\_\_\_\_ 15-20 copies of LTEMs fish worksheet (for each sample day)
- \_\_\_\_\_ Waterproof notepaper
- \_\_\_\_\_ Pencils
- \_\_\_\_\_ West Virginia Department of Natural Resources sampling permit
- \_\_\_\_\_ Polarized sun glasses (at least two pairs, one for each person netting fish)
- \_\_\_\_\_ The Freshwater Fishes of Virginia, (Jenkins and Burkhead 1994) and/or A Field Guide to Freshwater Fishes of North America North of Mexico, (Page and Burr 1991)
- \_\_\_\_\_
- \_\_\_\_\_

Appendix C.4.

Checklist of required field and laboratory equipment and supplies for fish sampling, identification, and measurements.  
[CONTINUED]

**EQUIPMENT CHECKLIST  
FISH**

*Laboratory*

- \_\_\_\_\_ Measuring board (0-500 mm)
- \_\_\_\_\_ Electronic scale (0-300 g)
- \_\_\_\_\_ Partially completed LTEMs fish worksheets
- \_\_\_\_\_ Pencils
- \_\_\_\_\_ The Freshwater Fishes of Virginia, (Jenkins and Burkhead 1994)
- \_\_\_\_\_ 4-5 porcelain sorting pans (45 cm x 30 cm x 10cm)
- \_\_\_\_\_ Forceps
- \_\_\_\_\_ Probes
- \_\_\_\_\_ Dissecting microscope
- \_\_\_\_\_ Dissecting lamp
- \_\_\_\_\_ 20-30 L 70% ethanol
- \_\_\_\_\_ List of fishes in New River and LTEMs species codes
- \_\_\_\_\_
- \_\_\_\_\_
- \_\_\_\_\_
- \_\_\_\_\_
- \_\_\_\_\_

Appendix C.5. Sources of field and laboratory equipment and supplies for fish sampling, identification, and measurements.

Equipment	Addresses and Phone Numbers of Suppliers
voltage regulator cord probes	Coffelt Manufacturing 1311 East Butler Avenue, Bldg. B Flagstaff, AZ 86001 (602) 774-8829
dip nets	Coffelt Manufacturing, see above
rubber gloves	Memphis Net and Twine Company, Inc. 2481 Matthews Avenue P. O. Box 8331 Memphis, TN 38108 (800) 238-6380
measuring board	Wildlife Supply Company 301 Cass Street Saginaw, MI 48602 (517) 799-8100
formaldehyde ethanol storage bottles dissecting tools	Fisher Scientific 711 Forbes Avenue Pittsburg, PA 15219 (800) 245-2230
scales	Fisher Scientific, see above
balance waterproof notepaper	Ben Meadows Company, Inc. P. O. Box 80549 Chamblee, GA 30366 (800)241-6401
	Forestry Suppliers, Inc. P. O. Box 8397 Jackson, MS 39284-8397 (800) 674-5368
waders cleats sunglasses	Cabela's, Inc. 812 13th Avenue Sidney, NE 69160 (800) 237-4444
	Gander Mountain, Inc. P. O. Box 248, Hwy W Wilmot, WI 53192 (800) 558-9410



Appendix C.7. Label for fish samples.

<p style="text-align: center;"><b>New River Gorge LTEMs Fish Sample</b></p> <p><b>Site</b> _____</p> <p><b>Date</b> _____</p> <p><b>Sample Number</b> _____</p>
---

Appendix C.8. Laboratory worksheet and summary for water quality, algae, and macrophytes.

**WORKSHEET AND SUMMARY  
WATER QUALITY, ALGAE, MACROPHYTES**

Site \_\_\_\_\_ Date \_\_\_\_\_  
 Analyzed By \_\_\_\_\_

Physical/Chemical Analyses:

Water Temperature \_\_\_\_\_ °C      pH \_\_\_\_\_      Conductivity \_\_\_\_\_ umhos  
 Dissolved Oxygen \_\_\_\_\_ mg/L      Hardness \_\_\_\_\_ mg/L  
 Alkalinity \_\_\_\_\_ mg/L =  $A \times N \times 50000$  / ml of sample  
 \_\_\_\_\_ ml sample A = \_\_\_\_\_ ml acid N = \_\_\_\_\_ acid normality

Seston Analysis:

Filter#	Total mg/L	Organic mg/L	Inorganic mg/L	O/I
_____	_____	_____	_____	_____
_____	_____	_____	_____	_____
Mean	_____	_____	_____	_____

Periphyton Analysis:

Chlorophyll a:

Correction Factors for Calibration      750 nm \_\_\_\_\_ 664 nm \_\_\_\_\_

Rep/tube #s	mL in extraction	w/o acid		w/ acid		ug/cm <sup>2</sup>
		750nm	664nm	750nm	665nm	
_____	_____	_____	_____	_____	_____	_____
_____	_____	_____	_____	_____	_____	_____
_____	_____	_____	_____	_____	_____	_____
_____	_____	_____	_____	_____	_____	_____
_____	_____	_____	_____	_____	_____	_____

Mean chlorophyll a \_\_\_\_\_ ug/cm<sup>2</sup>

Appendix C.8. Laboratory worksheet and summary for water quality, algae, and macrophytes. [CONTINUED]

Total Biomass:

Filt. #	Area Sampled (cm <sup>2</sup> )	Tare Wt. (ug)	Dry Wt. (ug)	Ash Wt. (ug)	AFDW (ug)
_____	_____	_____	_____	_____	_____
_____	_____	_____	_____	_____	_____
_____	_____	_____	_____	_____	_____
_____	_____	_____	_____	_____	_____
_____	_____	_____	_____	_____	_____

Mean AFDW \_\_\_\_\_ ug/cm<sup>2</sup>

Autotrophic Index:

A.I. = \_\_\_\_\_ mean AFDW ug/cm<sup>2</sup> / \_\_\_\_\_ mean chl a ug/cm<sup>2</sup> = \_\_\_\_\_

Phytoplankton Analysis:

Chlorophyll a

Rep/tube #s	mL in extraction	w/o acid		w/ acid		ug/L
		750nm	664nm	750nm	665nm	
_____	_____	_____	_____	_____	_____	_____
_____	_____	_____	_____	_____	_____	_____

Mean chlorophyll a \_\_\_\_\_ ug/L

Macrophyte Analyses:

*Podostemum* weights (gDW/sample)

1	2	3	4	5	mean	gDW/m <sup>2</sup>
_____	_____	_____	_____	_____	_____	_____

*Justicia* weights (gDW/sample)

1	2	3	mean	gDW/m <sup>2</sup>
_____	_____	_____	_____	_____





Appendix C.11. Potential repositories for archiving fish specimens and persons to contact about fish identification.

Dr. Brooks Burr  
Department of Zoology  
Southern Illinois University  
Carbondale, IL 62907  
(618) 453-4112

Dr. Dave Etnier  
Department of Zoology  
University of Tennessee  
Knoxville, TN 37916  
(615) 974-3107

Dr. Carter Gilbert  
Florida State Museum  
University of Florida  
Museum Road  
Gainesville, FL 32611  
(905) 392-1721

Dr. Richard L. Mayden  
Department of Biology  
P.O. Box 870344  
University of Alabama  
Tuscaloosa, AL 35487-0344

Dr. Larry Page  
Center for Biodiversity  
Illinois Natural History Survey  
Champaign, IL 61820  
(217) 244-2104

Local contact persons for verification of fish identifications:

Dr. Robert Jenkins  
Department of Biology  
Roanoke College  
Salem, VA 24153  
(703) 375-2463

Dr. Mel Warren  
Department of Fisheries and Wildlife Sciences  
Virginia Polytechnic Institute and State University  
Blacksburg, VA 24061-0321  
(703) 231-3402