



Shenandoah National Park Benthic Macroinvertebrate Monitoring Protocol

Version 3.0

Natural Resource Report NPS/SHEN/NRR—2018/1621



ON THE COVER

Portable invertebrate box sampler used by David Demarest on Twomile Run in Shenandoah National Park, 2010.
Photograph courtesy of NPS/Wendy Hochstedler.

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Protocol Revision History

All protocols, regardless of how sound they are originally, require modification as new and different information becomes available. Version numbers increase incrementally by hundredths (e.g. version 1.01, version 1.02...etc.) for minor changes. Major revisions should be designated with the next whole number (e.g., version 2.0, 3.0, 4.0 ...). Small changes or additions to existing methods will be reviewed by Shenandoah National Park or Mid-Atlantic Network staff. However, if a complete change in methods is sought, an outside review is required.

Revision History Log.

Version #	Date	Revised By	Changes	Justification
1.0	1990	Original protocol	NA	NA
2.0	1997	Demarest, Atkinson, Voshell	Sampling changed to spring only, 17 additional sites added to annual effort	See sampling design
3.0	2018	NPS, Voshell	Protocol updated to meet IMD requirements	IMD requirement

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Executive Summary

This document culminates an attempt to update a legacy National Park Service (NPS) monitoring protocol to current monitoring protocol standards (Oakley 2003). A legacy protocol, as discussed here, is a protocol with a history predating the National Park Service Natural Resource Inventory and Monitoring Program (I&M) which was established by Congressional mandate in 1998. Legacy protocols may have objectives, sampling designs, and other procedures that may be dated and may not follow current I&M guidance. Updating the protocol generally entailed providing more thorough written documentation on protocol procedures, goals, and objectives. No consideration was given to altering historic methods, procedures, or sampling designs. The primary purpose of this document is to clearly articulate how the protocol has been implemented for the past thirty years. Much of this document was based upon the Shenandoah National Park Long-term Ecological Monitoring Plan (LTEM) which was developed in the late 1980's (Voshell and Hiner 1990). The original protocol was updated to this current form by cooperators (R. Voshell) who were involved in the development of the original LTEM program and NPS staff (J. Wofford and E.D. Demarest). E.D. Demarest has participated with implementation of this monitoring protocol since 1991.

During the mid-1980's Shenandoah National Park (SHEN) identified benthic macroinvertebrates as an important component of ecosystem monitoring for park management. A more recent assessment confirmed this determination when the National Park Service's Mid-Atlantic Inventory and Monitoring Network (MIDN), also identified benthic macroinvertebrate communities as an important ecosystem "vital sign" for SHEN (Comiskey and Callahan 2008).

A benthic macroinvertebrate monitoring protocol was initiated at Shenandoah National Park in 1987. Conceptually, the purpose of the protocol is to determine the status and trends of stream health in SHEN in order to aid in park management decision making. The protocol entails annually monitoring macroinvertebrates at 28 "primary" sites distributed across the park and loosely stratified by park district, underlying geologic formations, and elevation. Each year, an additional six sites are selected from a list of 40 "secondary" sites that are monitored on a three to nine year rotation. Monitoring occurs during spring seasons. At all sites, three quantitative samples of the benthic community are collected from riffles with a Portable Invertebrate Box Sampler (PIBS) or Surber sampler. One qualitative sample is also collected from non-riffle stream habitats (inclusive) with a D-net. In the laboratory, all the captured macroinvertebrates are identified to the lowest practical taxonomic level. From the resulting taxonomic data, a number of metrics are calculated to assess stream health. In addition to collecting macroinvertebrates, measures of water chemistry, stream discharge, and stream habitat are also collected during every site visit.

Background and Objectives

Background and history of the resource issue being addressed

This document culminates an attempt to update a legacy National Park Service (NPS) monitoring protocol to current monitoring protocol standards (Oakley 2003). A legacy protocol, as discussed here, is a protocol with a history predating the National Park Service Natural Resource Inventory and Monitoring Program (I&M) which was established by Congressional mandate in 1998. Legacy protocols may have objectives, sampling designs, and other procedures that may be dated and may not follow current I&M guidance. Updating the protocol generally entailed providing more thorough written documentation on protocol procedures, goals, and objectives. No consideration was given to altering historic methods, procedures, or sampling designs. The primary purpose of this document is to clearly articulate how the protocol has been implemented for the past thirty years. Much of this document was based upon the Shenandoah National Park Long-term Ecological Monitoring Plan (LTEM) which was developed in the late 1980's (Voshell and Hiner 1990). The original protocol was updated to this current form by cooperators (R. Voshell) who were involved in the development of the original LTEM program and NPS staff (J. Wofford and E.D. Demarest). E.D. Demarest has participated with implementation of this monitoring protocol since 1991.

Shenandoah National Park (SHEN) was authorized by Congress in 1926 and established in 1935, with a formal dedication by Franklin D. Roosevelt in 1936. Shenandoah National Park “preserves and protects nationally significant natural and cultural resources, scenic beauty, and congressionally designated wilderness within Virginia’s northern Blue Ridge Mountains, and provides a broad range of opportunities for public enjoyment, recreation, inspiration, and stewardship” (NPS 2015). The park is significant for its unique natural and cultural resources, including mountain peaks and high-elevation ecosystems, cascading streams, historic lodges, Skyline drive, a presidential retreat (Rapidan camp), and an abundance of native and globally rare animal and plant populations.

Shenandoah National Park encompasses 199,016 acres (805 square kilometers) of forested terrain on the crest of the Blue Ridge Mountains in northern Virginia. The Park has a long linear shape with a highly uneven boundary and variable width. The Park is divided east to west by the ridgetop, which Skyline Drive and the Appalachian Trail follow, and into three districts north to south by U.S. Highways 33 and 211. Shenandoah is entirely contained within the Blue Ridge physiographic region and can be characterized by steep slopes, narrow ridges, and high relief (Bailey 1999). Elevations within the park range from 192 to 1231 meters (530–4050 feet), with about 60 peaks above 914 meters (3000 feet) (Conners 1988). Low elevations in the park generally experience mild winters and hot, humid summers—average monthly temperatures range from 41.9°F (5.5°C) in January to 85.5°F (29.7°C) in July at low elevations. At higher elevations, winters are moderate and summers are relatively cool—average monthly temperatures range from 24.8°F (-4.0°C) in January to 65.5°F (18.6°C) in July on higher ridge tops (Vana Miller and Weeks 2004). Hydrographs are generally characterized with peak stream flows in the spring and low flows occurring in late summer or fall (Lynch 1987). SHEN contains three major bedrock units of different age and composition which can be loosely characterized as granitic, basaltic, and siliciclastic (Gathright 1976). Each of the three main bedrock formations associated with these rock types have distinct chemical and physical

properties that directly influence the surrounding landscape, including terrestrial and aquatic communities (Bulger et al. 1995, Butler 2006, Southworth et al. 2009, Young et al. 2009, Jastram et al. 2013).

Shenandoah's Mid-Atlantic location straddles conditions of both the northern and southern Appalachian Mountains allowing over 1,300 species of vascular plants to occur in the park. Ninety-five percent of SHEN is forested with eastern deciduous woodland. In 2005 a vegetation map was completed that divides the park into 34 vegetation communities (Young et al. 2009). These include 22 upland forests and woodland community types, one alluvial/riparian forest, six non-alluvial wetlands, and five rock outcrop communities.

The park contains portions of the headwaters of three large river basins within Virginia: the James, the Shenandoah, and the Rappahannock watersheds. Streams within SHEN are generally 1st to 3rd order, with about 90 watersheds that contain perennial stream flow. Though inherently variable, streams tend to be of relatively high gradient, with step-pool or cascade morphology (Montgomery and Buffington 1997). Most streams are well shaded by vegetation and maintain cold or cool water temperatures during summer months. Springs and seeps provide the primary source of water for the streams that are further augmented by rain. Water quality within Shenandoah's streams is relatively good, though acid deposition and associated stream acidification are well documented (see Vana Miller and Weeks 2004 for review, Jastram et al. 2013). Additional baseline information on the environmental setting of Shenandoah National Park can be found in Costanzo (2016).

In 1984, SHEN was designated as one of the prototype monitoring parks (i.e. a park designated for pilot monitoring efforts) for the relatively new Inventory and Monitoring Initiative. Realizing that this program would take several years to establish, development was divided into segments or phases. The first phase of this effort was to design and implement a core monitoring program. This program, later named the Long-Term Ecological Monitoring System, was designed to monitor a basic set of ecological parameters that would characterize the broad types and rates of ecological changes likely to take place in SHEN. At the time, there was a particular concern with ecosystem impacts of the invasive gypsy moth (*Lymantria dispar*). Therefore, much of the original monitoring design was focused on those ecosystem components most likely to be affected by widespread defoliation, such as forest composition in the terrestrial environment and benthic macroinvertebrate composition in the aquatic environment. The effect of acid deposition, which was known to be occurring, was also emphasized in conjunction with gypsy moth effects. Other possible perturbations, such as wildfire, other forms of air pollution, and visitor use, were also considered so that the basic monitoring design would reflect the widest possible range of change (Voshell and Hiner 1992).

The first three years of the parkwide program were focused on the establishment of the Park's overall I&M objectives, program design, selection of monitoring protocols, and the field testing of data collection methods. It was not possible for SHEN to monitor a large suite of environmental parameters. Therefore, the following factors, in order of priority, were used to determine what would be included in the initial SHEN I&M program (Ravlin et al. 1990).

Resources specifically mentioned in the Park's enabling legislation, listed as threatened or endangered, or otherwise given special status by law:

- 1) Key ecosystem processes that can be well defined and accurately accessed (high priority given to those likely to be impacted by gypsy moth);
- 2) Species or processes that may impact on public health or safety or are locally controversial (gypsy moth threat to safety from tree mortality in developed areas);
- 3) Events such as fire, flood, erosion, weather patterns, etc.;
- 4) Resources being threatened by visitor use;
- 5) Baseline monitoring of species, populations or compositions that enhance our understanding of particular phenomena, relationships or population dynamics;
- 6) Species or populations that are of interest but of no particular management or ecological concern.

An examination of these priorities in the mid-1980's resulted in the selection of benthic macroinvertebrates as one of the components of long term monitoring efforts in SHEN.

Rationale for monitoring benthic macroinvertebrates

Streams are one of the most important and conspicuous natural resources in Shenandoah National Park, and benthic macroinvertebrates are ideal for monitoring this resource. Benthic macroinvertebrates are aquatic invertebrate animals that are larger than microscopic size (i.e. they can be seen with the unaided eye). Because these animals are vital to the proper ecological function of stream ecosystems, they are often used as indicators of ecological integrity. Freshwater benthic macroinvertebrates are instrumental to nutrient and carbon dynamics and are an important link in stream food webs. Groups that are commonly used for biological monitoring include arthropods (insects, arachnids, and crustaceans), worms, clams, and snails. In addition to their importance in aquatic ecosystems, they are the most frequently used organisms in biomonitoring for many other reasons (Voshell et al. 1997, Barbour et al. 1999, Carter and Resh 2001), including:

- 1) Benthic macroinvertebrates are relatively easy to sample and sampling does not require complicated devices.
- 2) Most taxa can be identified easily to the family taxonomic level, even in the field. Identification is usually relatively easy to the genus level with a microscope and taxonomic keys.
- 3) Several life history traits make them uniquely advantageous for monitoring water quality. The duration of their life history is sufficiently long that they will likely be exposed to chronic or punctuated pollution and environmental stress, and the assemblage will not recover so quickly that the impairment will go undetected if monitoring is conducted annually. The most common life history is to produce one generation a year, and even among aquatic insects that become terrestrial adults, most spend about 10 months in the water.

- 4) They are ubiquitous and occur in almost all types of freshwater habitats. No habitat has features too small or large, temperature too cold or hot, current too slow or fast, substratum too soft or hard, nutrients too rich or poor, aquatic vegetation too lush or sparse, or detritus too abundant or scant to sustain a benthic macroinvertebrate assemblage.
- 5) There are many different taxa of benthic macroinvertebrates, and among these taxa there is a wide range of sensitivity to all types of pollution and environmental stress.
- 6) Benthic macroinvertebrates have mostly sedentary habits so they are likely to be exposed to pollution or environmental stress. They are seldom able to respond to pollution by moving away from the source.

In addition, an independent review by scientists and park managers indicated that, out of 43 potential vital signs that were assessed during vital sign development for SHEN, macroinvertebrate communities tied as #1 (in conjunction with water quality) based on combined ecological, management, and policy criteria (Comiskey and Callahan 2008).

Measurable objectives

Specific objectives for benthic macroinvertebrate monitoring in SHEN were not explicitly stated in the original protocol. Therefore, the objectives noted below were based upon an assessment of protocol design and methods, as well as upon more general text and interpretation of the original protocol.

- 1) Document status and trends of benthic macroinvertebrate communities at sites across the park representing geologic and elevational strata by means of metrics that reflect abundance, richness, evenness, environmental requirements, ecological roles, and responses to various potential stressors.
- 2) Relate measures of habitat condition (both instream and riparian) and water quality at the sampling sites to ecological health (i.e. status and trends) of benthic communities.
- 3) Relate the ecological health of benthic macroinvertebrate communities at individual sample sites to thresholds of ecological health.
- 4) Provide additional ecological information to supplement data from the fish monitoring protocol and to inform fish, especially brook trout, conservation and management.

Sampling Design

Sampling design rationale

The monitoring protocol for SHEN was originally developed in the mid-1980s. At that time, the U.S. Environmental Protection Agency had not issued the first rapid bioassessment protocols, and most states, including Virginia and surrounding mid-Atlantic region states, did not have standard monitoring protocols for benthic macroinvertebrates. Thus, the SHEN monitoring protocol was developed independently to address the primary stressors of concern in SHEN at that time: (1) defoliation from gypsy moth into the park from the north and (2) acidification of streams from atmospheric deposition. The resulting protocol was also designed to be broad enough to detect ecological changes over time that might be caused by other potential stressors, such as fire, floods, drought, visitor use, and sewage treatment plants.

Site selection

The target population for monitoring was restricted to permanently flowing waters within park boundaries. An extensive reconnaissance of SHEN streams was conducted from January to April 1986. Thirty-three locations on 30 streams were visited. The reconnaissance consisted of making cursory examinations of the physical environment and macroinvertebrates at each location. In addition, information on geology (Gathright 1976), water chemistry (Dise 1984), and fish (SHEN unpublished reports) were reviewed. From this information, the streams were stratified as follows and monitoring sites that were deemed representative of the streams were selected from the different strata.

1. Administrative District
 - a. North
 - b. Central
 - c. South
2. Geological Formation (Alkalinity)
 - a. Catoctin (i.e. Basaltic) (100-200 ueq/L)
 - b. Pedlar or Old Rag (i.e. Granitic) (20-100 ueq/L)
 - c. Hampton or Erwin (i.e. Siliciclastic) (<20 ueq/L)
3. Elevation
 - a. Upper (>548.6 m [1800 ft.])
 - b. Lower (<548.6 m [1800 ft.])
4. Other Factors
 - a. Accessibility
 - b. Fish monitoring sites
 - c. Research interests

Because park administration districts are spatially distributed latitudinally, using the three administrative districts as the first level of stratification facilitated assessing and managing the ecological effects of the spread of gypsy moth from north to south. In addition, this was an effective

means of spatially distributing stream sites throughout the park in order to provide data in a variety of watersheds where unanticipated environmental stressors might occur.

Geological formations were selected as the second level of stratification because SHEN has a uniquely diverse geology that strongly influences water chemistry, particularly alkalinity (Gathright 1976, Dise 1984). There are great differences in alkalinity among watersheds, and often between adjacent watersheds. Alkalinity and related water chemistry are significant factors affecting the distribution and abundance of aquatic biota in SHEN (see Vana Miller and Weeks 2004 for review, Jastram et al. 2013). Streams in SHEN seldom have their watersheds entirely within a single geological formation; therefore, most stream sites were established in watersheds that have at least 75% of their area within one of the geological formation categories. Nevertheless, in some cases other objectives (e.g. inform fish management decisions) resulted in the selection of some sites that do not fully meet this 75% geology goal.

The relationship between bedrock lithology and stream water quality, particularly as it pertains to acid-base status and associated acid-neutralizing capacity (ANC), is well documented in SHEN (Lynch and Dise 1985, Webb 1988, Cosby et al. 1991). In addition, numerous studies have noted the correlation between ANC and biological parameters (Smith and Voshell 1994, Moeykins and Voshell 2002, Bulger et al. 1999, Cosby et al. 2006, Jastram et al. 2013). These studies noted general declines in measures of ecosystem health (e.g. macroinvertebrate taxa diversity, brook trout condition factors, etc.) as ANC declined. Streams with the lowest ANC values have alkalinity ranges from 0 - 25 ueq/L (microequivalents per liter), are associated with siliciclastic formations in the watershed (e.g. Hampton and Erwin Formations), are all located along the southern half of the western slope in the park and have the fewest number of fish and macroinvertebrate taxa. Moderate ANC streams have alkalinity ranges from 50 - 100 ueq/L, are influenced by granitic formations (e.g. Old Rag and Pedlar Formations), are more evenly distributed parkwide and tend to have higher numbers of fish species and macroinvertebrate genera. Streams with higher ANC have alkalinity ranges from 150 - 200 ueq/L, are influenced by basaltic bedrock (e.g. Catoclin Formation), are also fairly well distributed throughout the park and also have relatively high numbers of fish species and macroinvertebrate taxa.

Elevation affects the distribution of aquatic biota indirectly because of factors such as stream width, depth, current velocity, valley shading, and temperature. SHEN is located entirely within the northern section of the Blue Ridge physiographic province, which is the narrow (about 8 km) mountainous area between the low-lying Piedmont province to the east and the Ridge and Valley province to the west (Gathright 1976). Drainage basins in the Blue Ridge province typically have a steep gradient. The criterion of 548.6 m (1800 ft.) for distinguishing upper and lower sites was selected because field observations indicated that streams in SHEN tended to become very narrow and shallow (less than several meters wide and less than knee deep) approximately above that elevation. This delimitation was varied within reason when it was desirable to have both an upper and lower site on the same stream, and the observed site characteristics generally fit the desired categorization.

Accessibility was also considered when choosing sites so that sampling could be accomplished safely with reasonable effort. Locations of existing fish monitoring sites were given priority in choosing

macroinvertebrate sampling sites so that there would be additional ecological data to explain the results of both monitoring programs.

The sampling unit for the SHEN macroinvertebrate monitoring program is a stream reach, which for this protocol, is defined as a length of stream chosen to represent a consistent set of physical, chemical, and biological conditions within the overall stream. SHEN streams sites are 100-m reaches, which were selected so that they included riffles and pools and as many microhabitats as possible (different substrate sizes, occurrence of woody debris, different current velocities, etc.). The rationale was that a well-chosen 100-m reach of a stream would be large enough for all of macroinvertebrate taxa likely to be present in the area to be represented. Some sites are less than 100 m in length. This occurred during site selection when certain portions of reaches were excluded from sampling due to extreme channel braiding.

Recommended number of sampling sites

The total number of samples taken each year was initially determined by the number of selected monitoring sites, available personnel, and budget. Over time, it was determined that approximately 34 sites could be sampled annually. Originally, 17 sites were sampled twice, once in spring and once in the fall. As noted below, in 1997 it was decided to sample 34 sites in spring only. Of the 34 sites sampled in a year, 17 sites are core sites from the original protocol. The 1997 revision resulted in the addition of seven more low ANC sites on six streams, and four sites on two streams affected by the 1995 floods. This resulted in 28 primary sites that are sampled annually. Forty additional sites (i.e. secondary) are also monitored, six of which are sampled each year over an approximate seven year panel (historically variable, with resampling occurring every three to nine years). Originally the program had an objective of including a secondary site on every fish-bearing stream but that objective was later abandoned as the number of documented fish-bearing streams increased. A map of primary and secondary sites in each park district and associated geological formations can be found in Figures 1, 2, and 3. Tables of primary and secondary sites and their corresponding elevation and geology can be found in Appendix A, Tables A-1 and A-2, respectively.

Macroinvertebrate Sampling Sites North District

National Park Service
U.S. Department of the Interior
Shenandoah National Park, VA

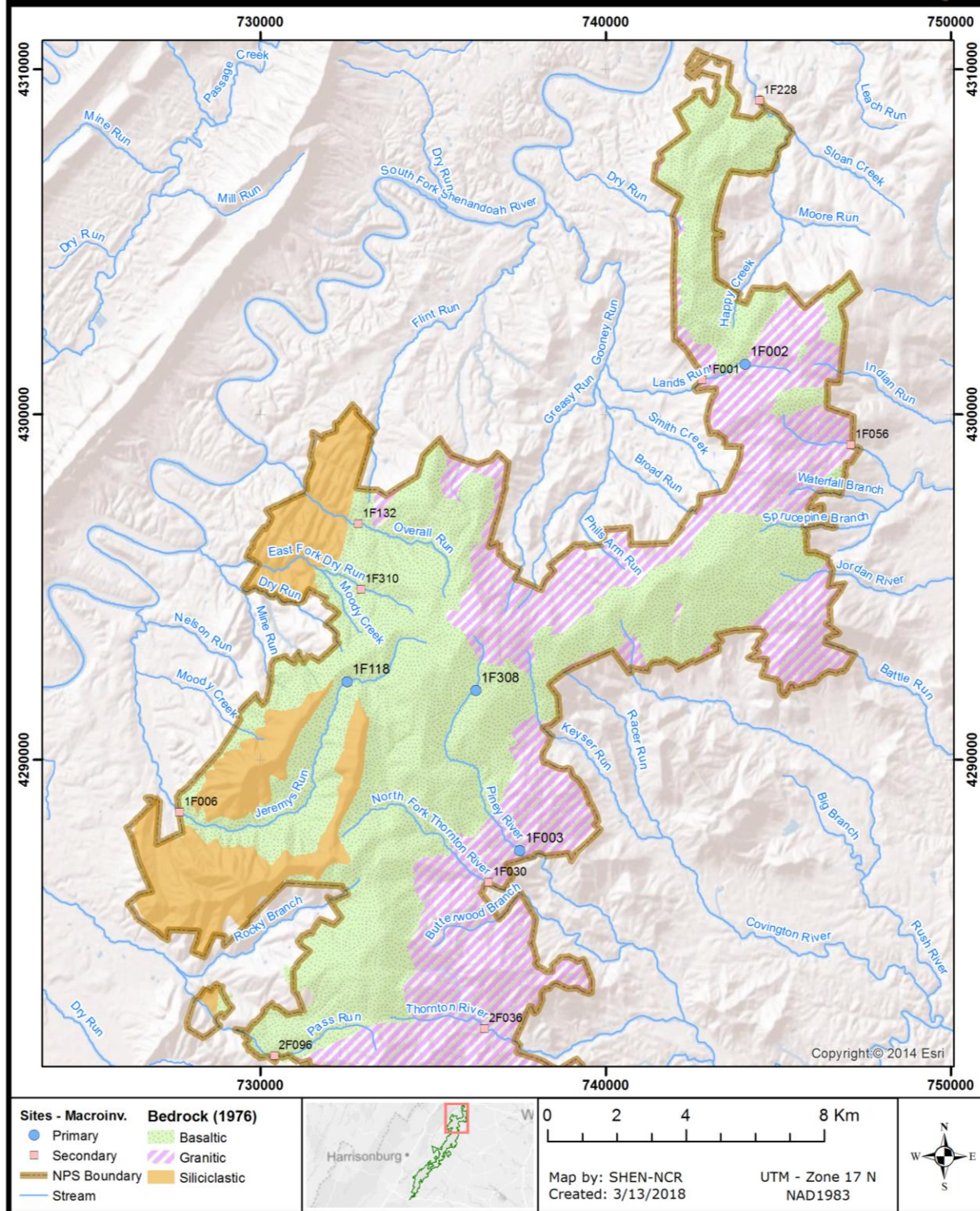


Figure 1. Map of primary and secondary macroinvertebrate sampling sites and underlying geology in the North District of Shenandoah National Park. Bedrock formations based on Gathright (1976).

Macroinvertebrate Sampling Sites Central District

National Park Service
U.S. Department of the Interior
Shenandoah National Park, VA

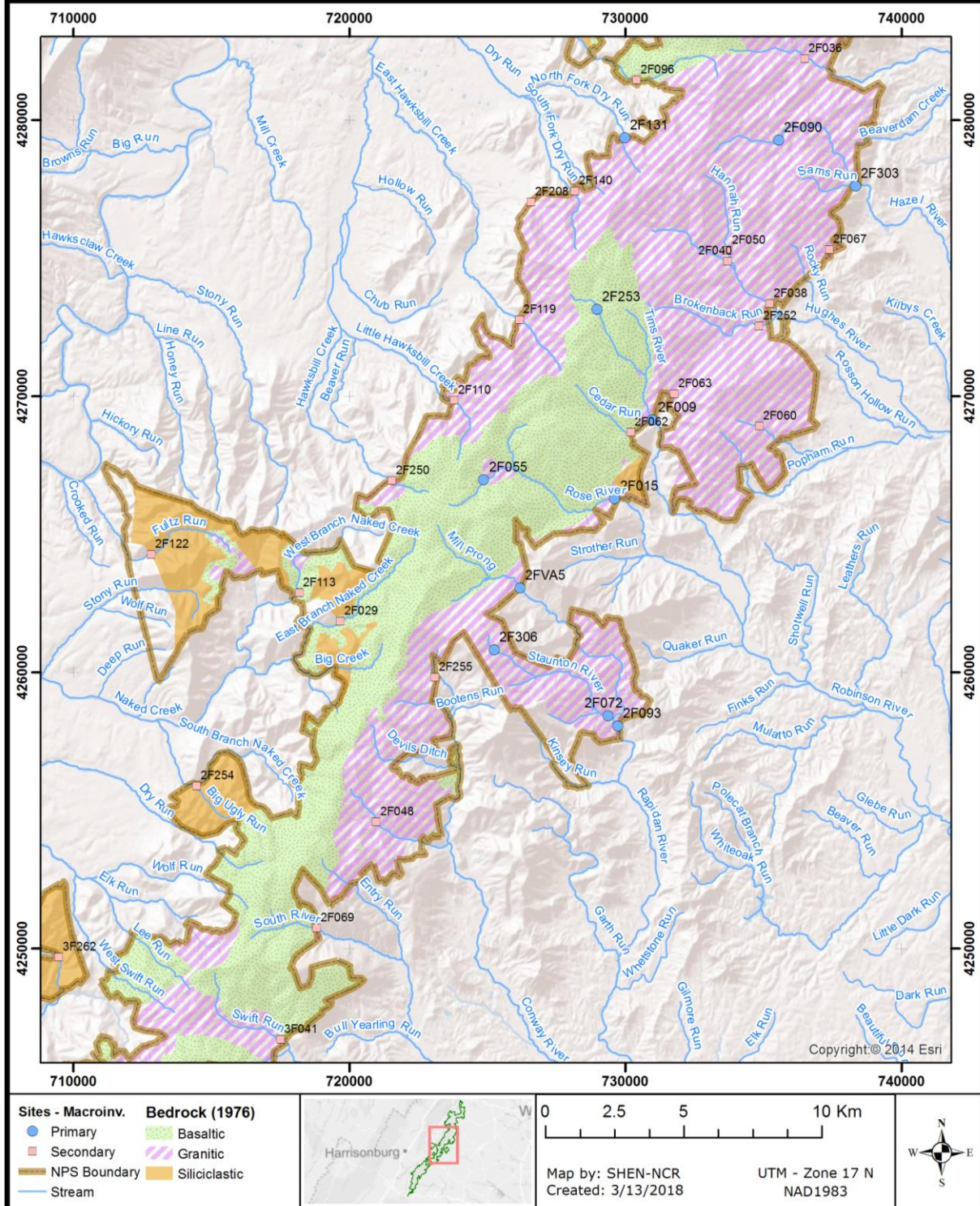


Figure 2. Map of primary and secondary macroinvertebrate sampling sites and underlying geology in the Central District of Shenandoah National Park. Bedrock formations based on Gathright (1976)

Macroinvertebrate Sampling Sites South District

National Park Service
U.S. Department of the Interior
Shenandoah National Park, VA

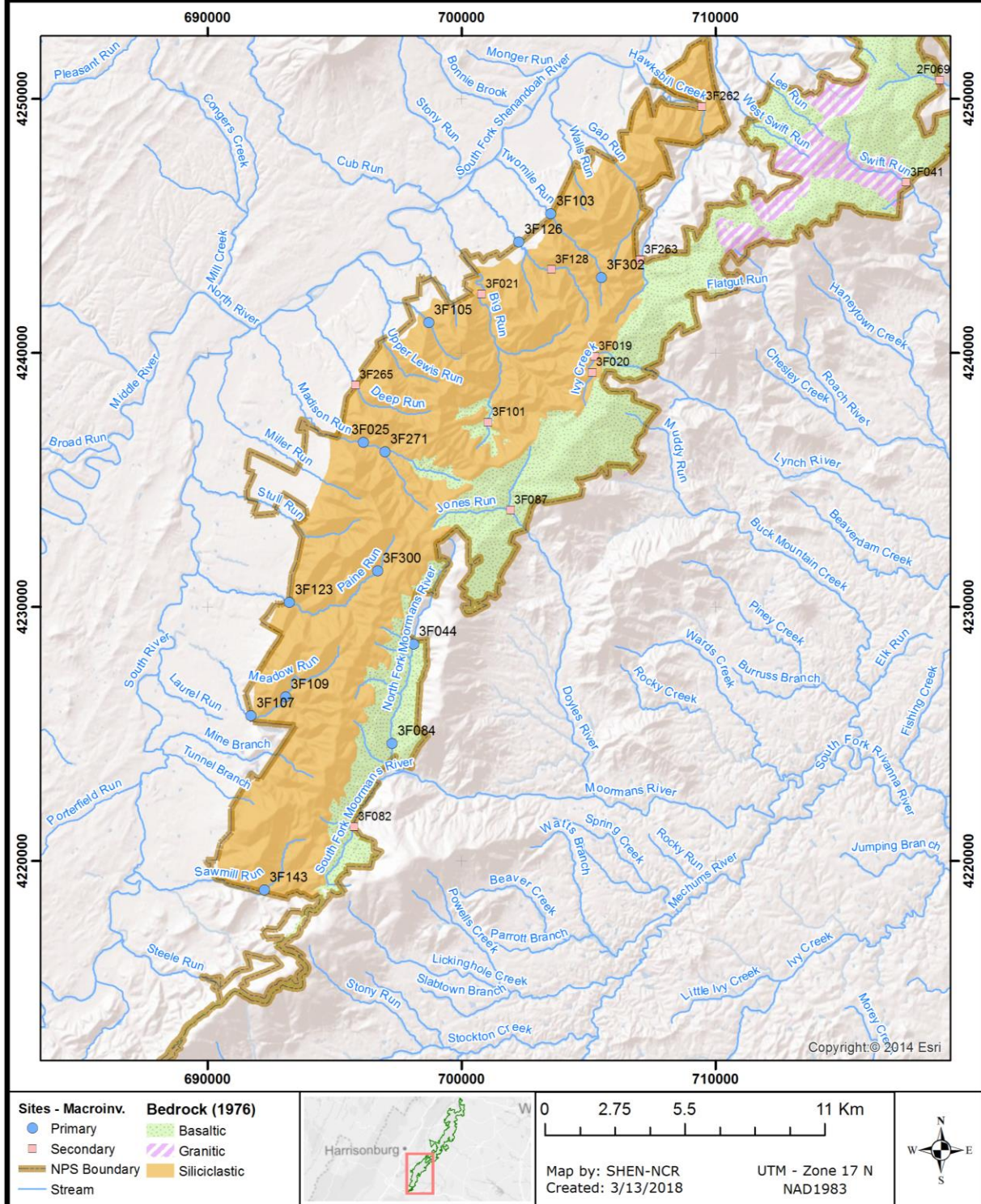


Figure 3. Map of primary and secondary macroinvertebrate sampling sites and underlying geology in the South District of Shenandoah National Park. Bedrock formations based on Gathright (1976).

Sampling frequency and replication

The original 1980's protocol recommended that macroinvertebrates should be sampled twice a year (i.e. in spring and in fall). Macroinvertebrate assemblages often exhibit their maximum richness in spring before the insect component begins to emerge as adults. Spring also often presents the optimum environmental conditions during the year because of cool water temperatures and reasonably high flows. Fall often presents the least favorable environmental conditions during the year because of elevated water temperatures and low flows. Although fewer taxa are present in fall because of their life history, some of the fall taxa are not present in spring because their life history is timed to coincide with annual leaf abscission. From 1988 to 1996, SHEN streams were sampled twice per year. At that point, analyses indicated that the fall samples were not contributing any additional inferences than the spring samples (Smith and Voshell 1994). Thereafter, the resources required for the fall samples were redirected toward taking samples at twice as many sites but only in the spring. Primary sites are sampled on an annual basis while secondary sites are sampled every three to nine years.

Inference

Primary sites were selected via a hierarchical stratification process. Professional opinion and analyses to date indicate that these sites are likely ecologically representative of aquatic systems within geologic strata, as geology and associated acid neutralizing capacity is a well-documented major driver of aquatic biological communities in SHEN (Bulger et al. 1999, Vana Miller and Weeks 2004, Jastram et al. 2013, Shenandoah National park unpublished data). Nevertheless because no randomization procedures were used in site selection, sites are not statistically representative of associated strata and extrapolation to larger scales should be done with caution.

Standard Operating Procedures

A series of standard operating procedures (SOPs) have been developed to ensure that the protocol is implemented in a consistent manner and high-quality data is captured. Unlike the protocol, these SOPs are intended to change more frequently over time as specific methods are refined and equipment is replaced. Therefore, the SOPs are published separately from the protocol to facilitate timely updates to procedures. The most updated versions of the SOPs will be maintained by SHEN. Updated SOPs will be published and made available on the [NPS Integrated Resource Management Application Data Store](#). Version numbers increase incrementally by hundredths (e.g. version 1.01, version 1.02...etc.) for minor changes. Major revisions should be designated with the next whole number (e.g., version 2.0, 3.0, 4.0 ...). Small changes or additions to existing methods will be reviewed by Shenandoah National Park or Mid-Atlantic Network staff. However, if a complete change in methods is sought, an outside review is required.

Field and Laboratory Methods

Detailed methods and procedures for field methods are outlined in the Standard Operating Procedures (SOPs) for the protocol (Table 1).

Table 1. List of Standard Operating Procedures associated with the field and laboratory methods for monitoring benthic macroinvertebrates in SHEN.

SOP Title	SOP #
Pre and post-season tasks	1
Training and safety	2
Sampling conditions and times	3
Locating sites in the field	4
Using the global positioning system	5
Water chemistry equipment calibration and cleaning	6
Water chemistry sampling	7
Quantitative sampling of macroinvertebrates	8
Qualitative sampling of macroinvertebrates	9
Site photography	10
Habitat sampling	11
Using a clinometer	12
Measuring discharge	13
Equipment cleaning and biosecurity	14
Laboratory processing of macroinvertebrate samples	15
Data forms	16

Field season preparations and equipment setup (SOP 1)

Preparations for the field season entails hiring seasonal technicians or interns, ensuring SHEN compliance documents are valid and up-to-date, and completing assessments or purchase of required equipment (SOP 1). The hiring process is generally initiated in October of the prior year. Barring major changes in protocol methods, compliance is renewed in conjunction with the fish monitoring program on a five-year basis. If compliance documents are expiring, the renewal process should begin in January of the monitoring year. Visual and mechanical assessments of field gear should begin in February of the monitoring year and are outlined in SOP 1.

Sequence of events during field season (SOPs 2-5)

The field component of the aquatic macroinvertebrate monitoring program typically begins in mid-April and ends in early June. In general, three technicians are required for efficient sampling of

macroinvertebrate communities under this protocol. Prior to conducting any field work, seasonal field technicians review a variety of job-hazard analyses (JHAs) to facilitate safe work practices (SOP 2). Training in the use of discharge meters, water quality equipment, and macroinvertebrate and habitat sampling procedures occurs on-the-job during initial monitoring efforts (SOP 2). Attempts are made to sample primary sites during the same week as in prior years depending on rain events. The secondary sites are sampled as feasible—often at the end of the sampling season.

Field work only occurs under certain environmental conditions (SOP 3). When these conditions are met, the general sequence of events during a day of field work is as follows:

- 1) crew arrives at park headquarters and water quality equipment is calibrated as necessary
- 2) field gear is loaded into vehicles
- 3) crew navigates to site
- 4) upstream and downstream reach boundaries are determined
- 5) water chemistry information is collected
- 6) quantitative macroinvertebrate samples are collected
- 7) qualitative macroinvertebrate samples are collected
- 8) digital photo of the site is taken
- 9) habitat and discharge information is collected
- 10) crew returns to park headquarters and gear is unloaded
- 11) equipment and gear is cleaned as appropriate
- 12) macroinvertebrates and data forms are appropriately stored

This daily sequence occurs until all sample sites have been visited. Normally more than one site will be visited in a day. Site locations and directions to sites are available in a variety of formats to assist in determining how many and which sites may be visited during a single day of field work (see SOPs 3, 4, and 5).

Details of taking measurements

Sample sites are generally 100 m long sections of stream, and macroinvertebrates are collected within the entire 100 m. Water chemistry, discharge, and photos are also collected during a site visit. Habitat measurements are collected at 10 m transects within the reach or within segments between transects (Figure 4), depending on the habitat metric. Details on data collection are documented below and in relevant SOPs. All data forms can be found in SOP 16.

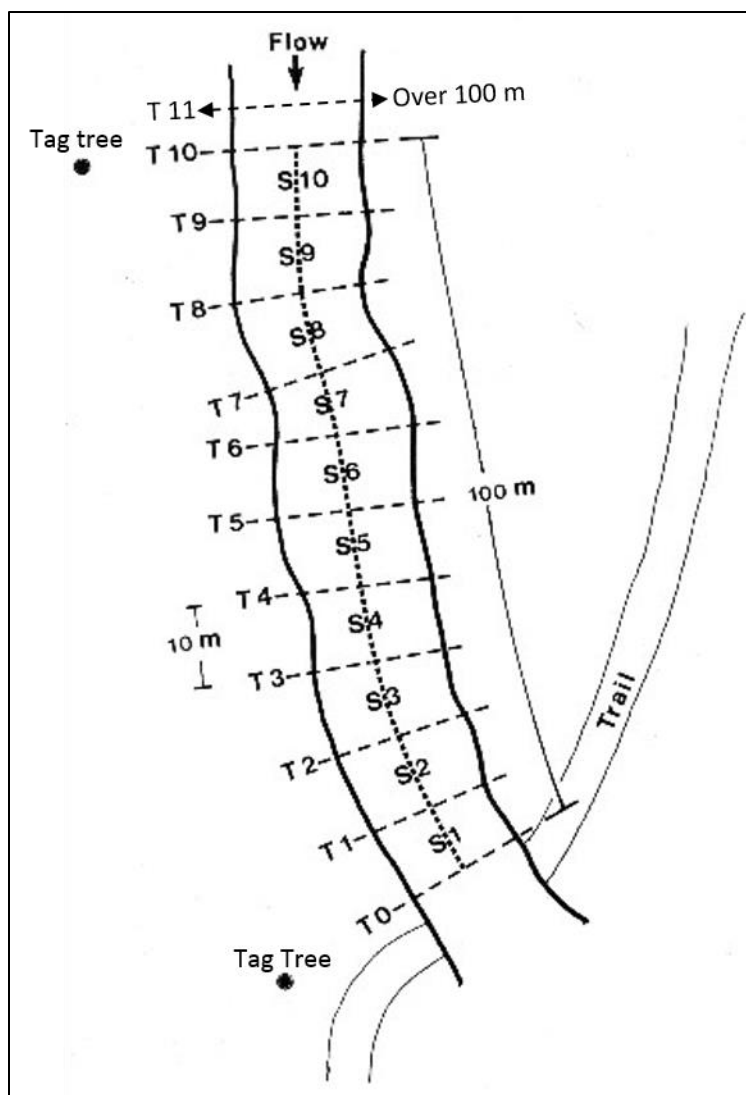


Figure 4. Diagram of a stream reach showing sample segments and transects. Depth, percent riparian vegetation, riparian vegetation type, and substratum are measured at each transect. Percent fish cover, Pool-riffle ratio, and % slope (gradient) are measured through each segment. T1 = transect 1, T2 = transect 2, etc. S1 = segment 1, S2 = segment 2, etc. See SOP 11 for more information.

Water chemistry sampling (SOPs 6 and 7)

Water chemistry sampling occurs prior to any in-stream disturbance associated with macroinvertebrate monitoring and is the first data collected during a site visit (SOPs 6 and 7). The “Core” water quality parameters are collected at each site (NPS 2002). Spot values of water temperature, pH, conductivity, dissolved oxygen, and total dissolved solids are collected during each sampling event. Relevant data forms can be found in SOP 16.

Macroinvertebrate collection

Following the collection of water quality information, both quantitative and qualitative macroinvertebrate samples are collected. Prior to all sampling, plastic bags with labels (includes site name, sample number, and date) are assembled. Quantitative samples provide estimates of abundance

and richness of taxa occurring in the most productive microhabitats in streams (i.e. riffles). They are collected with a Portable Invertebrate Box Sampler (PIBS) or a Surber sampler. Because quantitative samples only target riffle habitats, qualitative samples are also collected. Qualitative samples provide additional information about the diversity of taxa within all habitats in a sample reach. A D-frame dip net is used to collect macroinvertebrates from all available habitat types throughout the reach during the time the qualitative sampling is occurring. Relevant data forms can be found in SOP 16.

Quantitative samples (SOP 8)

Quantitative samples provide estimates of abundance and richness of taxa occurring in the most productive microhabitats in streams (i.e. riffles). These samples are collected with Portable Invertebrate Box Sampler (PIBS) or a Surber sampler, both outfitted with a 363- μ m mesh catch net. Three quantitative samples are taken in shallow, wadeable riffles (preferably three different riffles) at each sample site. The PIBS sampler is preferred, but in some cases, sampling conditions will require the use of the Surber sampler (see SOP 8). If it is necessary to use the Surber sampler at one sampling location at a site, then all three quantitative samples will be collected with the Surber—two different sampling devices will never be used for quantitative sampling in one site during the same sampling visit. That said, the PIBS and Surber only have minor differences in sample area and this difference can be accounted for in analyses if measures of macroinvertebrate density are calculated. After selecting a riffle for sampling, the sampling device will be placed on the substrate and each rock will be scrubbed, visually examined, and removed from the sample area after macroinvertebrates have been swept into the catch net. After all of the pebbles and cobbles (2mm – 25 cm) have been brushed, examined, and removed, the remaining substrate is disturbed in order to stir up sediment inhabiting organisms and allow them to wash into the catch net. Larger pieces of woody debris are cleaned and removed from the sample. In addition, fish and salamanders are also removed and the sample is placed with a label in a plastic bag. This procedure is repeated until three quantitative samples have been collected, ideally from separate riffles distributed along the reach.

Qualitative samples (SOP 9)

Because quantitative samples only target riffle habitats, a single qualitative sample is also collected to provide additional information about the diversity of taxa within all habitats in a sample reach. A D-frame dip net with 500- μ m mesh is used to collect macroinvertebrates from all available habitat types throughout the reach during qualitative sampling. The major objective of qualitative sampling is to obtain specimens from the many microhabitats that cannot be sampled with the quantitative device. Examples of these other microhabitats include leaf packs, surfaces of large rocks or logs, underneath large rocks or logs, exposed roots at the margins, and aquatic vegetation or terrestrial vegetation that is hanging into the water. The investigators should use their powers of observation (and imagination) to find as many places as possible where macroinvertebrates might reside. If possible, 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 may be damaged. The D-net is emptied at frequent intervals (at least after each microhabitat), combining all material into a single container. Large sticks or other debris that are captured in the D-net are examined for macroinvertebrates and then discarded. It is not necessary to retain all material in the net since this is a qualitative sample. Field technicians should try to sample

each noticeable microhabitat, and try to expend equal effort among stream sites and across years. Generally approximately the same amount of time is utilized to collect the single qualitative sample as is utilized to collect the three PIBS samples. Material from all microhabitats is placed with a label in one bag. As such, only one qualitative sample occurs per sample site, though that sample is composed of a collection from numerous habitat types. If the qualitative sample is being collected simultaneously as the quantitative samples, qualitative sampling should occur downstream of the quantitative sampling so that the quantitative sample isn't influenced by drifting insects disturbed during qualitative sampling.

Site photograph and habitat sampling (SOP 10 - 12)

Prior to collecting habitat information, a digital photo is taken from the lower end of the sample site. The picture is taken looking upstream, ensuring that the bottom transect (T0) of the reach is captured in the bottom of the picture frame (SOP 10). Relevant data forms can be found in SOP 16.

Habitat sampling includes a combination of actual measurements (i.e. stream width, site length, depth and gradient) and visual estimates (i.e. riparian cover, stream substrates and habitat features) (see SOP 11 and 12). These variables are used to provide a physical assessment of environmental attributes that may influence macroinvertebrate communities. Units for all measurements are in meters or percent slope.

Habitat measurements are collected at 10 m transects through the sample reach or within segments between transects depending on the habitat metric collected (see Figure 4). Transects and segments include only wetted portions of the stream channel. Wetted stream widths are recorded at each transect up to and including the end point at the upstream end of the monitoring site. This end point will be noted in the original site directions and will generally result in a reach that is near 100 m in length. Water depths are measured and stream substrates are estimated at every 0.3 m across each transect included the water's edge. At each 0.3 m point (0, 0.3 m, 0.6 m, etc.), the water depth is recorded to the nearest 0.01 m using the depth staff and the dominant substrate is recorded as organics, silt (<0.2 mm), sand (0.2 mm - 2.0 mm), pebble/gravel (2.0 mm - 6.0 cm), cobble (6.0 cm - 25.0 cm), boulder (>25 cm, mobile at flood stage or via debris flow), or bedrock (nearly fully embedded, immobile at all flows). From the center of each transect, an observer also records the riparian cover category that exists directly above the transect. This is a visual estimate of the degree of riparian canopy cover over the wetted channel. Riparian cover is classified as 0%, 1-33%, 34-66%, or 67-100%. In addition, observers note whether the canopy consists of trees, shrubs, forbs or grasses, or that no canopy is present.

Within each segment, observers also record gradient using a clinometer (SOP 12). An observer also conducts a visual estimate of the relative amount of pool and riffle habitat, debris retention potential, and fish cover. The ratio of pool to riffle habitat between transects is visually estimated with pool habitat defined as water with near-zero velocity and riffle habitat defined as locations where water velocity is noticeably greater than zero. Debris retention provides a visual estimate of the extent of structures such as rock outcrops or boulders that would hold stream debris such as leaf litter or small wood pieces. Areas between transects are rated as having debris retention structures covering 0-33%, 34-66%, or 67-100% of the wetted channel. Fish cover is also estimated between transects. Fish

cover is defined as habitat where a 15 to 20 cm (6 to 8 inches) brook trout could conceal itself from above (as under large wood or boulders). Fish cover can include turbulent water. Turbulent water must have a water depth of at least 15 cm and a water velocity of greater than 15 cm/s (as estimated) to be considered fish cover. In addition, depth and velocity alone can define fish cover, and any water greater than 25cm in depth and less than 15 cm/s in velocity is considered fish cover. Fish cover is characterized and recorded as covering 0-33%, 34-66%, or 67-100% of the wetted channel.

Specific procedure for measuring or estimating habitat parameters around islands can be found in SOP 11.

Discharge sampling (SOP 13)

Discharge is measured during each sampling event. Discharge is measured over cobble/gravel substrates with a uniform channel bed. Park staff utilize the 6/10ths flow measuring technique recommended by the USGS (Buchanan and Somers 1969) and the U.S. Forest Service (Platts et al. 1983). Measurements are taken from at least ten evenly spaced intervals at the selected cross section, perpendicular to stream flow. Relevant data forms can be found in SOP 16.

Cleaning equipment (SOP 14)

In order to limit the introduction and spread of invasive or exotic aquatic organisms in Shenandoah, the park implements specific cleaning procedures for equipment that contacts water during monitoring events. These cleaning procedures are outlined in SOP 14.

Laboratory processing of samples (SOP 15)

At the end of the monitoring season, collected insects are provided to a contractor for identification. Laboratory processing of macroinvertebrate samples involves washing the preserved samples, sorting the organisms from the debris, identifying each specimen, counting the number of individuals in each taxonomic unit, recording the data, and QA/QC. Each sample is maintained as a separate entity. The procedures for each of these steps are summarized below, and details are presented in SOP 15.

Samples are first washed with tap water in a 355-µm brass soil sieve (U.S. Series No. 45) in order to remove any remaining fine detritus and the original preservative. The purpose of this procedure is to make the sample clean enough for the organisms to be seen and removed during sorting.

The next step is sorting, which refers to removing all of the macroinvertebrates from the debris in the washed sample. Successive small amounts of the washed sample are placed in a shallow white enamel metal pan. This material is covered with approximately 1 cm of water, and the pan is examined systematically, proceeding along imaginary rows and columns. All macroinvertebrates are removed with forceps and placed into a single container containing 70% ethanol along with the original coded label. An illuminated 2X magnifier may be utilized during this process but is not essential.

All of the organisms that are sorted are then identified to the lowest practical taxonomic level and counted per taxonomic unit. The lowest taxonomic levels are: Insecta – genus (except Chironomidae – family), Crustacea – genus, Mollusca – family, Oligochaeta – class, Turbellaria – class. For the

quantitative benthic samples (PIBS and Surber), all specimens are enumerated. For the qualitative samples (D-frame dip net), taxa are only recorded as being present.

Macroinvertebrate data are entered into Shenandoah's database management system (see SOP 17). Quality Control (QC) checks are performed for sorting and identification on 5% of the samples in an annual set of samples (see SOP 15 for details). Following macroinvertebrate identification, data are entered into Shenandoah's database management system (see Data section below), and all samples are returned to the park.

Macroinvertebrate sample archiving

Since 1986, Shenandoah has retained all of the macroinvertebrate samples collected at the park. Once the samples are returned to Shenandoah from the contractor, they are unpacked and placed in cardboard herbarium trays, by year. These trays are stored in the "Gas House", in herbarium style cabinets. This building is heated but not air conditioned. Once every 12 to 18 months, the vials are checked and topped off with 70% ethanol as needed. More details on sample archiving can be found in SOP 1.

Data forms (SOP 16)

All of the SHEN macroinvertebrate monitoring data forms can be found in SOP 16.

Data Handling, Analysis, and Reporting

Detailed methods and procedures for data management are outlined in Standard Operating Procedures (SOPs) for the protocol (Table 2).

Table 2. List of Standard Operating Procedures associated with data management, analysis, and reporting for monitoring benthic macroinvertebrates in SHEN.

SOP Title	SOP #
Data forms	16
Data entry, editing, and verification	17
Data management and archiving	18
Data analysis	19
Reporting	20

Data entry, verification, and editing (SOP 17)

Accurate collection and stewardship of data are critical to the success of the benthic macroinvertebrate monitoring program. Ensuring data quality must be a priority during crew training, and throughout the data collection, entry, verification, and validation process.

Data entry occurs during days of inclement weather, and at the end of the field day, or at the end of the season. Data are first entered into a temporary database using a data-entry application. Then to help verify that the data entered matches what has been written on the data form, the data are entered a second time into a second temporary database by a different data-entry team. The two temporary databases are compared by the application and any discrepancies are reviewed and corrected. At the end of the season, the program lead or the lead biological technician reviews 1% of the entered data via a comparison of the database to data forms. Any errors found in this review will initiate review of additional data from the specific site where the error occurred and potential additional review of the data entry work of the technician associated with the error. Lastly, all data are visually screened for abnormal values for each quantitative parameter. Data forms are then electronically scanned.

Generally the contractor responsible for macroinvertebrate identification is responsible for entering macroinvertebrate taxa and enumeration information into an Access database provided by Shenandoah. Similar to above, taxa data are entered twice to ensure that entry errors have not occurred.

Data management (SOP 18)

The Shenandoah National Park aquatic macroinvertebrate data system consists of three main components, the data entry application, the temporary storage databases, and the master database. As noted above, field data are entered into the first temporary database using the data entry application. Then to help assure accurate data, the same data are entered a second time into a second temporary database. The two temporary databases are compared and discrepancies are reviewed and corrected.

At the end of the field season the data in the temporary databases are reviewed by the program staff and are then added to the master database. An ESRI shapefile is maintained to house the point locations of the sites. The shapefile can be joined to data exports in ArcGIS to display attributes of the plots spatially.

The current Shenandoah National Park aquatic macro-invertebrate monitoring database was developed in Microsoft Access 95 but has been converted and updated to the current version of MS Access. This database predates the NPS I&M Natural Resources Database Template (NRDT) but many of the design principles are present.

Data archival procedures (SOP 18)

Electronic data files including the databases, scanned data forms, and photo monitoring images are stored in a directory structure on a file server with appropriate access levels granted to users. Prior to a data entry session an automated backup of the back-end data file is made and stored on the server to allow “roll-back” if database corruption is encountered. A scheduled back-up utility makes copies of changed files to a server in a different building at the Shenandoah National Park Headquarters campus nightly. Annually, these back-up directories are evaluated and archived or replaced by a “fresh” copy of the directory.

Data compiled by Shenandoah National Park must be accompanied by Federal Geographic Data Committee (FGDC) compliant metadata. This includes both spatial and non-spatial datasets.

Data analysis (SOP 19)

Monitoring programs should produce data, analyses, and products that can be utilized to develop management actions that support park objectives. Data analysis of macroinvertebrate communities includes the assessment of a number of community metrics. Fourteen metrics have been identified that can be used to assess macroinvertebrate richness, balance, composition, tolerance, trophic structure, and habit. A multimetric index (VSCI: Virginia Stream Condition Index, Burton and Gerristen (2003)) has also been developed by VA DEQ and is used to assess status and trends and to compare results from the park to surrounding VA waters. The VSCI can also be utilized to compare the ecological condition of SHEN sites to established biological criteria or thresholds established by the Commonwealth of Virginia (i.e. impairment thresholds). The macroinvertebrate aggregate index score (MAIS) is an additional multimetric index that can be utilized, and ecological threshold values have been published for the MAIS (Smith and Voshell 1997, Moeykens 2002). Certain metrics may also be utilized to inform brook trout conservation and management. For example, the percent Leuctra metric may reflect the influence of stream temperature on the benthic community (Jastram et al. 2013), and stream temperature is a parameter of critical importance to brook trout.

Using a subset of metrics, control charts can be employed to visualize trends and changes in macroinvertebrate communities at the site, stream, and ANC group scale. Control charts allow for the visual display of trends and thresholds for management action or further data analyses (see Morrison 2008). Initially, the first ten years of data will serve as a baseline and thresholds will be defined as plus or minus two standard deviations around the ten-year mean (i.e. baseline) of the statistic (see SOP 19 for example). During any year where thresholds are crossed, more targeted data analyses will

be conducted to assess the driver responsible for exceeding the threshold and to help determine the potential need for management action. Baselines and thresholds may be altered in the future to potentially better reflect meaningful management or biological thresholds that might not be represented in the initial 10 year mean $\pm$ 2 standard deviations.

Most analyses are structured to address macroinvertebrate communities at multiple spatial scales or strata: sample site, stream (when more than one sample site occurs in a particular watershed), by bedrock geology type (i.e. ANC group ($<20\mu\text{eq/L}$, $20\text{--}100\mu\text{eq/L}$, $>100\mu\text{eq/L}$)), and by elevation ($<$ or > 549 m).

Reporting (SOP 20)

Annual data summaries

Annual data summaries can take the form of a report or resource brief. An example of a short report and a resource brief can be found in Wofford and Demarest (2012) and Wofford and Demarest (2014), respectively. These reports include brief summaries of annual data and generally don't include complex analyses. Recommended metrics for inclusion in annual reports are noted in SOP 19.

Comprehensive analyses and reports

Comprehensive analyses provide a more formal analysis of trend and occur on an approximate ten-year timeline. These analyses focus on trend detection and characterizing associations between habitat variables and benthic community metrics (see Jastram et al. 2013 for an example). Rather than requiring a detailed list of specific data analyses, the data analysis process should be flexible enough to allow the use of newly developed statistical and analytical techniques and tailoring of analyses for a variety of audiences with a variety of questions about the aquatic resources at Shenandoah. Univariate and multivariate analyses may occur at a variety of temporal and spatial scales.

Comprehensive analyses will likely include an assessment of all the metrics outlined in SOP 19.

Reporting format

Efficient reporting of monitoring results is critical in assisting park Resource Managers in management decisions. When writing reports, the most recent report template for regional natural resource technical reports is followed (<http://www.nature.nps.gov/publications/NRPM/index.cfm>). Natural resource reports are the designated medium for disseminating comprehensive information and analysis about natural resources and related topics concerning lands managed by the National Park Service. The natural resource data report series is used to disseminate basic data sets and data summaries. Ideally, for each report, a one or two page resource brief will also be produced that summarizes reported information.

Operational Requirements

Roles and responsibilities

The park fish and wildlife biologist (GS-11) coordinates all aspects of the program. Implementation of the protocol is led by a GS-07 crew leader and two technicians or interns. The park data manager (GS-11) provides leadership support and guidance on data entry, analysis, management, and archiving. An outside contractor is responsible for the laboratory analysis of the macroinvertebrate samples.

Fish and wildlife biologist

The park fish and wildlife Biologist operates as the project manager to oversee all aspects of the monitoring program, including:

- Permits, scheduling, hiring, purchasing, contracting, and budget
- Maintaining and updating the monitoring protocol
- Conducting or assisting in training and participating in field work as needed
- Recruiting and supervising the field crew, providing logistical, administrative, and technical support
- Analyzing data and preparing reports
- Ensuring QA/QC standards are met
- Coordinating with the park data manager
- Communicating results to the park I&M coordinator, other interested staff, cooperators, and the public

Lead biological technician

The lead biological technician is responsible for assisting the fish and wildlife biologist with program management obligations as well as taking the lead in the day-to-day operations of the field crew.

Tasks include:

- Coordinating and leading daily field activities and ensuring equipment is in working order
- Coordinating directly with biologist and communication center when conducting field work
- Conducting weekly safety briefings and offering safety reminders appropriate to current activities
- Ensuring that all field crew members are following proper data collection procedures
- Coordinating data entry into database, verification, corrections, and archiving of data from paper data forms
- Assisting the biologist with program obligations such as hiring, purchasing, contracting, budgeting, updating the protocol, analyzing data, and preparing reports
- Ensuring vehicles are properly maintained and cleaned
- Ensuring QA/QC standards are met

Monitoring crew technicians and interns

The crew technicians are responsible for data collection. Tasks include:

- Collecting field data accurately as described in the SOPs
- Recording data accurately on field forms and accurately entering data into the project database
- Ensuring all necessary equipment is available, well cared for, clean, and functional prior to each trip
- Performing work safely and efficiently

Data manager

The data manager in the Natural and Cultural Resources Division provides oversight for all data related aspects of the project including:

- Providing database training to the crew
- Maintaining the macroinvertebrate monitoring database
- Preparing maps of sample point locations
- Ensuring data management practices are met, including data backup, transfer, and archiving
- Ensuring QA/QC standards are met
- Running routine predetermined scripts/formulas for analysis and report writing

Outside contractor

- Wash and sort samples
- Identify macroinvertebrates to the genus taxonomic level (excluding Chironomids and Mollusca which are identified to family and Oligochaeta and Turbellaria which are identified to class)
- Count and record the number of individuals in each taxonomic unit
- As contract specifies, enter macroinvertebrate data into the Shenandoah National Park aquatic macroinvertebrate data system
- Return samples, identification data forms, and electronic data to Shenandoah

Annual workload and field schedule

Field work is conducted between mid-April and mid/late-May. Data entry, verification, and archiving is conducted between mid-April and the end of September and depends in part on when macroinvertebrate identification data is received from the contractor. Field planning, data analysis and report writing largely occur between October and April. Hiring of temporary employees commences in the late summer and continues throughout the spring. Budget and other administrative support activities occur year round.

Facility and equipment needs

The field crew is based at Shenandoah's Headquarters and operates out of the Natural and Cultural Resource Division's Lower office. All equipment needed to conduct the field work is listed in SOP 1, and is stored in the office or nearby storage buildings. The lead technician is responsible for ensuring that all equipment is in working order and coordinating with the Biologist to replenish supplies as needed.

Operating costs and budget considerations

Actual annual operating costs (2017) for implementation of this protocol, including personnel and supplies, are listed below (Table 3). Additional costs may be incurred to replace or repair equipment including computers, GPS units, cameras, and vehicles.

Table 3. Annual cost to implement Shenandoah National Park Macroinvertebrate Monitoring Protocol (2017). The permanent staff cost estimate includes 80 hours of a GS-11 data manager's time, 720 hours of a GS-11 biologist's time, and 680 hours of a GS-07 crew leader's time. Temporary staff costs include 640 hours of GS-05 technician's time. Macroinvertebrate ID costs include 34 sites with 3 PIBS samples per site ($34 \times 3 \times \$225 = \$23,000$) and 1 D-net sample per site ($34 \times 1 \times \$205 = \$7,000$).

Category	Expense (2017 dollars)
Permanent staff	\$ 61,900.00
Seasonal staff	\$ 12,600.00
Equipment	\$ 1,000.00
Supplies	\$ 2,000.00
Macroinvertebrate ID	\$ 30,000
Total	\$107,500.00

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Appendix A: Benthic macroinvertebrate monitoring sites.

Table A-1. Primary aquatic macroinvertebrate sampling sites in Shenandoah National Park. Primary sites are sampled annually. Table includes sample site elevation and the percent of the total watershed underlain by the three main geologic types.

STREAM	Site #	Sampling Scheme	District	Elevation (m)	Basaltic (%)	Granitic (%)	Siliciclastic (%)
Hazel River	2F303_1 ¹	NA	Central	321	0	100	0
Hazel River	2F303	Primary	Central	331	0	100	0
Hazel River	2F090	Primary	Central	667	0	100	0
Jeremy's Run	1F118	Primary	North	555	69	3	28
Land's Run	1F002	Primary	North	517	9	91	0
Lower Lewis Run	3F105	Primary	South	448	5	0	95
Madison Run	3F025	Primary	South	429	14	0	86
Meadow Run	3F107	Primary	South	449	0	0	100
Meadow Run	3F109	Primary	South	511	0	0	100
North Fork Dry Run	2F131	Primary	Central	493	0	100	0
North Fork Moormans River	3F084	Primary	South	338	66	1	33
North Fork Moormans River	3F044	Primary	South	498	66	1	33
Onemile Run	3F126	Primary	South	378	1	0	99
Paine Run	3F123	Primary	South	425	0	0	100
Paine Run	3F300	Primary	South	559	0	0	100
Piney River	1F003_1 ²	NA	North	346	58	42	0
Piney River	1F003_2 ²	NA	North	353	58	42	0
Piney River	1F003	Primary	North	379	58	42	0
Piney River	1F308	Primary	North	763	58	42	0
Rapidan River	2F093	Primary	Central	285	6	94	0
Rapidan River	2FVA5	Primary	Central	678	6	94	0
Rose River	2F015	Primary	Central	340	85	15	0
Rose River	2F055	Primary	Central	802	85	15	0

¹Original site moved to 2F303 in May 1994 due to extreme channel braiding.

²Original site moved twice (1F003_1 to 1F003_2 in June 1994, 1F003_2 to 1F003 in May 1996) due to incorrect boundary survey.

³Original site moved to 2F072 in September 1995 due to change in channel from a flood event.

⁴Original site was moved to 2F253 in May 1995 to incorporate hemlock headwaters.

Table A-1 (continued). Primary aquatic macroinvertebrate sampling sites in Shenandoah National Park. Primary sites are sampled annually. Table includes sample site elevation and the percent of the total watershed underlain by the three main geologic types.

STREAM	Site #	Sampling Scheme	District	Elevation (m)	Basaltic (%)	Granitic (%)	Siliciclastic (%)
Sawmill Run	3F143	Primary	South	463	20	0	100
Staunton River	2F072_1 ³	NA	Central	312	0	100	0
Staunton River	2F072	Primary	Central	316	0	100	0
Staunton River	2F306	Primary	Central	901	0	100	0
Twomile Run	3F103	Primary	South	371	0	0	100
Twomile Run	3F302	Primary	South	510	0	0	100
Whiteoak Canyon Run	2F009	Primary	Central	345	85	15	0
Whiteoak Canyon Run	2F300 ⁴	NA	Central	786	85	15	0
Whiteoak Canyon Run	2F253	Primary	Central	951	85	15	0
Whiteoak Run	3F271	Primary	South	444	14	0	86

¹Original site moved to 2F303 in May 1994 due to extreme channel braiding.

²Original site moved twice (1F003_1 to 1F003_2 in June 1994, 1F003_2 to 1F003 in May 1996) due to incorrect boundary survey.

³Original site moved to 2F072 in September 1995 due to change in channel from a flood event.

⁴Original site was moved to 2F253 in May 1995 to incorporate hemlock headwaters.

Table A-2. Secondary aquatic macroinvertebrate sampling sites in Shenandoah National Park. Secondary sites are sampled on a rotational basis. Table includes sample site elevation and the percent of the total watershed underlain by the three main geologic types.

STREAM	Site #	Sampling Scheme	District	Elevation (m)	Basaltic (%)	Granitic (%)	Siliciclastic (%)
Berry Hollow	2F063	Secondary	Central	437	0	100	0
Big Run	3F021	Secondary	South	377	19	0	81
Big Run	3F101	Secondary	South	500	19	0	81
Big Ugly Run	2F254	Secondary	Central	383	0	0	100
Bolton Branch	1F056	Secondary	North	375	7	93	0
Broad Hollow Run	2F067	Secondary	Central	359	0	100	0
Brokenback Run	2F252	Secondary	Central	345	6	94	0
Cedar Run	2F062	Secondary	Central	412	100	0	0
Conway River	2F255	Secondary	Central	663	13	87	0
Deep Run	3F265	Secondary	South	439	0	0	100
Doyles River	3F087	Secondary	South	435	92	0	8
East Branch Naked Creek	2F029	Secondary	Central	545	78	4	18
East Fork (Compton)	1F310	Secondary	North	401	70	1	29
East Hawksbill Creek	2F119	Secondary	Central	523	17	83	0
Fultz Run	2F122	Secondary	Central	398	11	9	80
Hannah Run	2F050	Secondary	Central	425	0	100	0
Happy Creek	1F228	Secondary	North	208	95	5	0
Hawksbill Creek (Beldor)	3F262	Secondary	South	333	64	16	20
Hawksbill Creek (Beldor)	3F263	Secondary	South	467	64	16	20
Hughes River	2F038	Secondary	Central	293	7	93	0
Hughes River	2F040	Secondary	Central	402	7	93	0
Ivy Creek	3F019	Secondary	South	627	73	1	26
Ivy Creek	3F020	Secondary	South	682	73	1	26
Jeremy's Run	1F006	Secondary	North	285	69	3	28
Kettle Canyon	2F208	Secondary	Central	450	3	97	0
Land's Run	1F001	Secondary	North	362	9	91	0
Lewis Spring Falls	2F250	Secondary	Central	582	83	17	0
Little Hawksbill Creek	2F110	Secondary	Central	506	48	52	0
North Fork Thornton River	1F030	Secondary	North	352	67	33	0

Table A-2 (continued). Secondary aquatic macroinvertebrate sampling sites in Shenandoah National Park. Secondary sites are sampled on a rotational basis. Table includes sample site elevation and the percent of the total watershed underlain by the three main geologic types.

STREAM	Site #	Sampling Scheme	District	Elevation (m)	Basaltic (%)	Granitic (%)	Siliciclastic (%)
Onemile Run	3F128	Secondary	South	451	1	0	99
Overall Run	1F132	Secondary	North	290	90	10	0
Pass Run	2F096	Secondary	Central	559	44	56	1
Pocosin Hollow Run	2F048	Secondary	Central	606	18	82	0
Ragged Run	2F060	Secondary	Central	484	0	100	0
South Fork Dry Run	2F140	Secondary	Central	509	21	79	0
South Fork Moormans River	3F082	Secondary	South	439	75	0	25
South Fork Thornton River	2F036	Secondary	Central	320	10	90	0
South River	2F069	Secondary	Central	542	100	0	0
Swift Run	3F041	Secondary	South	408	70	30	0
West Branch Naked Creek	2F113	Secondary	Central	488	70	3	27

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