National Park Service
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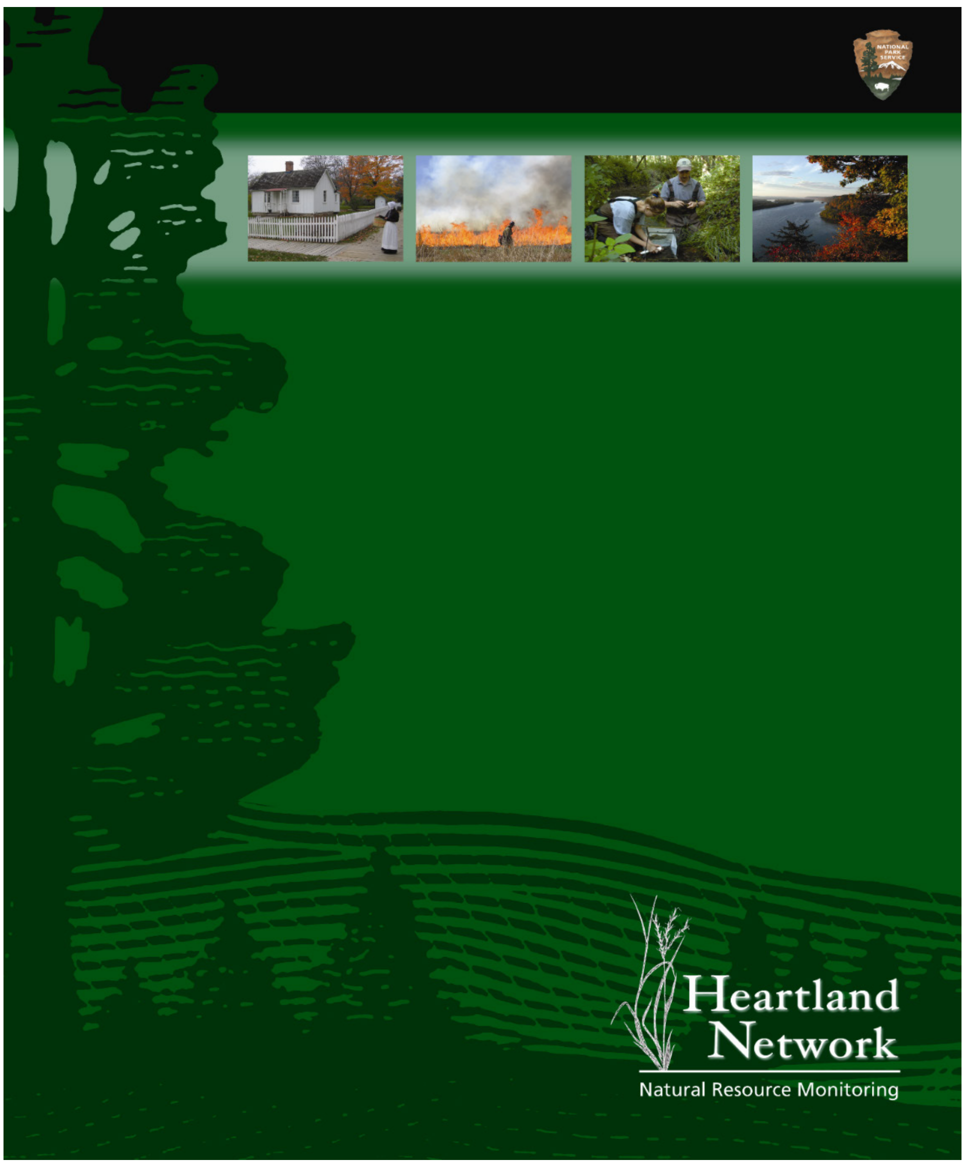
Natural Resource Stewardship and Science

Protocol for Monitoring Aquatic Invertebrates at Ozark National Scenic Riverways, Missouri, and Buffalo National River, Arkansas

Version 2.1

Natural Resource Report NPS/HTLN/NRR—2020/2206



ON THE COVER
Herbert Hoover birthplace cottage at Herbert Hoover National Historic Site, prescribed fire at Tallgrass Prairie National Preserve, aquatic invertebrate monitoring at George Washington Carver National Monument, and the Mississippi River at Effigy Mounds National Monument.
Photography by NPS

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The National Park Service, Natural Resource Stewardship and Science office in Fort Collins, Colorado, publishes a range of reports that address natural resource topics. These reports are of interest and applicability to a broad audience in the National Park Service and others in natural resource management, including scientists, conservation and environmental constituencies, and the public.

The Natural Resource Report Series is used to disseminate comprehensive information and analysis about natural resources and related topics concerning lands managed by the National Park Service. The series supports the advancement of science, informed decision-making, and the achievement of the National Park Service mission. The series also provides a forum for presenting more lengthy results that may not be accepted by publications with page limitations.

All manuscripts in the series receive the appropriate level of peer review to ensure that the information is scientifically credible, technically accurate, appropriately written for the intended audience, and designed and published in a professional manner.

This report received formal peer review by subject-matter experts who were not directly involved in the collection, analysis, or reporting of the data, and whose background and expertise put them on par technically and scientifically with the authors of the information.

This report is available from the Heartland Network Inventory and Monitoring website, and the Natural Resource Publications Management website. If you have difficulty accessing information in this publication, particularly if using assistive technology, please email irma@nps.gov.

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Contents

Page

Contents (continued)

Figures

Tables

Appendices

Protocol Revision History

The original protocol narrative version 1.0 was published in 2007 (Bowles et al. 2007). This document is protocol version 2.1. The previous protocol version 2.0 incorporated the recommended changes described in DeBacker et al. (2012). Those changes are not further detailed here. Changes made to the SOPs since the first versions were published are referenced in the individual SOPs.

Revision History Log

Prev. Version #	Revision Date	Author (s)	Changes made	Reason for Change	New Version #
1.0	12/2019	David E. Bowles, Lloyd W. Morrison, Hope R. Dodd, Gareth A. Rowell, Michael D. DeBacker, Janice A. Hinsey, J. Tyler Cribbs, Jennifer L. Haack-Gaynor, and Jeffrey M. Williams	Changes to habitat data collection, invertebrate data analysis, and a new spatial and temporal sampling design of sites	Recommenda-tions described in DeBacker et al. (2012)	2.0
2.0	07/2020	David E. Bowles, Lloyd W. Morrison, Hope R. Dodd, Gareth A. Rowell, Michael D. DeBacker, Janice A. Hinsey, J. Tyler Cribbs, Jennifer L. Haack-Gaynor, and Jeffrey M. Williams	Minor formatting updates and minor wording changes.	Discoverability	2.1

Standard Operating Procedures

The following Standard Operating Procedures (SOPs) are published as separate documents.

SOP 1: Preparation for Field Sampling and Laboratory Processing

SOP 2: Training for Field Sampling and Laboratory Processing

SOP 3: Sampling Invertebrates and Collecting Habitat Data

SOP 4: Laboratory Processing and Identification of Invertebrates

SOP 5: Measuring Stream Discharge

SOP 6: Documenting CORE 5 Water Quality Variables

SOP 7: Data Management

SOP 8: Data Analysis

SOP 9: Data Reporting

SOP 10: Procedures and Equipment Storage After the Field Season

SOP 11: Revising the Protocol

Executive Summary

Ozark National Scenic Riverways (OZAR) and Buffalo National River (BUFF) were established to preserve and interpret the free-flowing Buffalo, Jacks Fork, and Current rivers. Much of their respective watersheds lie outside of park boundaries placing the water quality of the rivers and tributaries at risk. Wadeable streams of the Ozarkian region, including those at BUFF and OZAR, are generally considered to be in good condition overall, but they are threatened by numerous stressors. Stream condition and ecosystem health are dependent on processes occurring in the entire watershed as well as riparian and floodplain areas; therefore, they cannot be manipulated independently of this interrelationship. Land use activities in the Ozarks Highlands, such as timber management, landfills, grazing, swine and poultry operations, urbanization, gravel mining, stream channelization, removal of riparian vegetation, and lead-zinc mining, threaten stream quality. Accordingly, the framework for aquatic monitoring at OZAR and BUFF is directed towards maintaining the ecological integrity of the rivers and tributaries in those parks.

Invertebrates are an important tool for understanding and detecting changes in ecosystem integrity, and they can be used to reflect cumulative impacts that cannot otherwise be detected through traditional water quality monitoring. The broad diversity of invertebrate species occurring in aquatic systems similarly demonstrates a broad range of responses to different environmental stressors. Furthermore, changes in the diversity and community structure of benthic invertebrates are relatively simple to communicate to resource managers and the public. Benthic invertebrate community structure can be quantified to reflect stream integrity in several ways, including the absence of pollution sensitive taxa, dominance by a particular taxon combined with low overall taxa richness, or appreciable shifts in community composition relative to reference condition. To assess the natural and anthropogenic processes influencing invertebrate communities, this protocol has been designed to incorporate the spatial relationship of benthic invertebrates with their local habitat, including substrate size and embeddedness and water quality parameters (temperature, dissolved oxygen, pH, specific conductance, and turbidity). Rigid quality control and quality assurance are used to ensure maximum data integrity. Detailed standard operating procedures (SOPs) and supporting information are associated with this protocol.

Acknowledgments

We thank the peer reviewers whose comments improved upon the original protocol. We also thank the many people who helped us in the field as we undertook the first few years of monitoring that led to this revised protocol.

Heartland Inventory and Monitoring Network

The National Park Service has organized its parks with significant natural resources into 32 networks linked by geography and shared natural resource characteristics. The Heartland Inventory and Monitoring (I&M) Network (Heartland Network) is composed of 15 NPS units in eight Midwestern states. These parks contain a wide variety of natural and cultural resources including sites focused on commemorating civil war battlefields, Native American heritage, westward expansion, and our U.S. Presidents. The Network is charged with creating inventories of its species and natural features as well as monitoring trends and issues in order to make sound management decisions. Critical inventories help park managers understand the natural resources in their care while monitoring programs help them understand meaningful change in natural systems and to respond accordingly. The Heartland Network helps to link natural and cultural resources by protecting the habitat of our history.

The I&M program bridges the gap between science and management with a third of its efforts aimed at making information accessible. Each network of parks, such as the Heartland Network, has its own multi-disciplinary team of scientists, support personnel, and seasonal field technicians whose system of online databases and reports make information and research results available to all. Greater efficiency is achieved through shared staff and funding as these core groups of professionals augment work done by individual park staff. Through this type of integration and partnership, network parks are able to accomplish more than a single park could on its own.

The mission of the Heartland Network is to collaboratively develop and conduct scientifically credible inventories and long-term monitoring of park *vital signs* and to distribute this information for use by park staff, partners, and the public, thus enhancing understanding which leads to sound decision making in the preservation of natural resources and cultural history held in trust by the National Park Service.

https://www.nps.gov/im/htln/index.htm

Heartland Network

Natural Resource Monitoring

I. Background and Objectives

Issues Being Addressed and Rationale for Monitoring Benthic Invertebrates

Ozark National Scenic Riverways (OZAR) in Missouri and Buffalo National River (BUFF) in Arkansas were established to preserve and interpret the free-flowing Buffalo, Jacks Fork, and Current rivers. The parks were designated for river corridor protection, but much of their respective watersheds outside of park boundaries were left unprotected, placing the water quality of the rivers and tributaries at risk (Panfil and Jacobson 2001). The NPS jurisdictional boundaries around the Buffalo, Current, and Jacks Fork rivers are generally narrow corridors that encompass only a small area within the respective watersheds of these rivers. The jurisdictional boundary of OZAR encompasses only about 5% of the watershed of the Current and Jacks Fork rivers, and the boundary of BUFF is only 11% of the Buffalo River's watershed. Over 50% of the watersheds in both parks are in private ownership.

Both parks are also located in an area of extensive karst topography making the rivers vulnerable to contaminated groundwater recharge and interbasin transfer of groundwater from adjacent watersheds. Because streams at OZAR primarily receive their baseflows from groundwater, contamination of groundwater is of special concern due to the rapid nature of recharge and transport of contaminants through the soluble bedrock system of caves, springs, and sinkholes. Wadeable streams of the Ozarkian region, including those at BUFF and OZAR, are generally considered to be in good condition overall although they are threatened by numerous stressors (United States Environmental Protection Agency 2006).

Stream condition and ecosystem health are dependent on processes occurring in the entire watershed as well as riparian and floodplain areas;. therefore, they cannot be manipulated independently of this interrelationship (Doppelt et al. 1993). Land use, particularly land clearing practices and associated increases in sediment load, nutrient concentrations, nutrient enrichment and other point and nonpoint sources, have been reported as the largest long-term threat to streams in the Ozark Highlands (Duchrow 1977; Mott 1997; Scott and Udouj 1999). Land use practices at the watershed level appear to overwhelm localized protection of stream corridors. For example, measures of land use and riparian vegetation at larger spatial scales (watershed level) were superior to local measures at predicting stream conditions within that watershed (Roth et al. 1996).

Since the establishment of Buffalo National River in 1972, more of the watershed has been deforested than is protected within the boundaries of the park (Mott 2000). Over a 27-year study period, the annual increase in pasture land in the BUFF watershed was almost equal to the annual decrease in forested land (Scott and Hofer 1995). Land use activities in the Ozarks Highlands include timber management, landfills, grazing, swine and poultry operations, urbanization, gravel mining, stream channelization, removal of riparian vegetation, and lead-zinc mining. Impacts to stream integrity from land use include disruptions in channel geomorphology, increased suspended and deposited sediments, bank erosion, increased light penetration and water temperature, higher periphyton biomass, and decreases in leaf litter and woody debris.

The framework for aquatic monitoring at OZAR and BUFF is directed towards maintaining the ecological integrity of the rivers and tributaries in those parks. Invertebrates are an important tool for understanding and detecting changes in ecosystem integrity, and they can be used to reflect cumulative impacts that cannot otherwise be detected through traditional water quality monitoring. The broad diversity of invertebrate species occurring in aquatic systems similarly demonstrates a broad range of responses to different environmental stressors. Benthic invertebrates are relatively easy to collect, and they can be analyzed at many different levels of precision. They are sensitive to a wide variety of impacts that occur in the Ozark Highlands, such as changes in chemical constituents (including metals), hydrological alterations, sedimentation and bank erosion, and land use and other changes in the watershed. Furthermore, changes in the diversity and community structure of benthic invertebrates are relatively simple to communicate to resource managers, administrators, and park visitors because the loss of biological communities is of interest and concern to these groups. Benthic community structure can be quantified to reflect stream integrity in several ways, including the absence of pollution sensitive taxa, dominance by a particular taxon combined with low overall taxon richness, or appreciable shifts in community composition relative to reference condition (Plafkin et al. 1989; Lazorchak et al. 1998; Barbour et al. 1999; United States Environmental Protection Agency 2006).

Aquatic communities can be impacted by land use practices in the watershed, particularly by the conversion of forestland to pasture (Sweeney 1995). For example, Quinn et al. (1997) found densities of mayflies, stoneflies, and caddisflies that were two to three times higher in forested streams versus streams dominated by pasture. Water quality and invertebrate community monitoring at BUFF (Bryant 1997; Mott 1997; Usrey 2001) have shown a strong negative correlation between agricultural nonpoint source chemical pollution (nitrates) and stream water quality and invertebrate community structure. Increased bank erosion rates and changes in channel morphology through time have been correlated with increased land clearing of steep uplands within a tributary basin (Stephenson and Mott 1992) and with historical riparian land clearing (Jacobson and Primm 1997).

To assess the natural and anthropogenic processes influencing invertebrate communities, this protocol has been designed to incorporate the spatial relationship of invertebrates with their habitat. Local variables, such as conductivity, water temperature, pH, dissolved oxygen, turbidity, current velocity, substrate size, and other habitat variables will be measured along with more extensive site surveys of stream geomorphology established through a separate protocol.

History of Invertebrate Monitoring

Ozark National Scenic Riverways (OZAR)

Prior to the implementation of Bowles et al. (2007), there had not been any long-term monitoring of invertebrate communities conducted at OZAR, but some short-term studies and special projects have been undertaken. These include invertebrate inventories (Blackwood 2001; Doisy et al. 1997; Poulton and Stewart 1991; Moulton and Stewart 1996; Trial 2000; Sarver and Kondrateiff 1997), distribution and community ecology studies (Doisy and Rabeni 2001; Rabeni et al. 2002; Whitledge and Rabeni 2000; Ball 2001, 2002), water quality and other impact studies (Duchrow 1977; Doisy et al. 2002), and preliminary work towards developing a long-term biomonitoring program for the riverways (Rabeni et al. 2002; Rabeni and Wang 2001; Rabeni et al. 1997, 1999; Doisy and Rabeni 1999).

Clifford (1966) conducted one of the earliest general aquatic resource studies about three years before the establishment of OZAR. This study looked at several chemical, physical, and biological parameters in Ozark streams, including invertebrates (Clifford 1966). However, no major trends or correlations were noted, but mayflies were listed as the chief component of the bottom fauna (Clifford 1966). Similarly, Duchrow (1977) published a study of invertebrates of the Current and Jacks Fork rivers, which evaluated the presence and absence of pollution sensitive taxa as well as invertebrate community structure and diversity as indicators of water quality using Missouri state standards. The results of that study indicated the Current and Jacks Fork rivers were not seriously degraded, and water quality criteria for unpolluted Missouri streams were equaled or exceeded by the riverways (Duchrow 1977).

Work at OZAR by Doisy et al. (1997) showed that high gradient riffles, coarse runs, and low gradient riffles had the highest diversity of invertebrate species. Additionally, Doisy and Rabeni (2001) showed high variability occurs within invertebrate communities, including within individual sites. Their results showed that variation in community structure within any single segment of stream was actually greater than variation throughout the total stream system, indicating that local habitat conditions have the greatest influence on community structure.

Doisy and Rabeni (2001) also identified current velocity as the best indicator of invertebrate community composition and found that diversity was positively correlated with current velocity. Indeed, Rabeni et al. (2002) showed that fast water channel units (high gradient riffles) have the most consistent community structure with higher densities and less variability compared to the other habitat units. This is an important finding for planning a long-term monitoring program because it provides justification for focused sampling on high gradient riffles rather than more time-consuming and expensive multiple habitat sampling protocols.

Finally, Doisy et al. (2002) described potential impacts to invertebrate communities from timber harvest and other deforestation activities in headwater tributary streams of the Current River system. Invertebrate communities from every stream that was sampled fell within the range of normally functioning communities. On the local level, the variables showing the greatest influence on invertebrate communities included substrate size and organic matter, and on the reach level, they included stream bank erosion and canopy cover. However, on the watershed level, road densities and unbuffered stream banks showed the greatest acute effects. Their study found that relative densities of stoneflies and caddisflies decreased with increased road density in the watershed because of increased fine sediment deposition and lower organic matter inputs (Doisy et al. 2002). Removal of riparian buffers resulted in decreased substrate size and less organic matter entering the system, resulting in acute negative effects on invertebrate community diversity.

Buffalo National River (BUFF)

Similar to OZAR, no long-term monitoring of aquatic invertebrates and stream habitat were conducted for the Buffalo River prior to the implementation of Bowles et al. (2007). However, some studies were conducted that assessed invertebrate community structure in the Buffalo River and its relationship to physical habitat and chemical degradation. In 1982, Geltz and Kenney, in an unpublished report, conducted one of the first known surveys of invertebrates along the river. The authors recommended development of a diversity index to reflect the invertebrate species composition in the river.

Mathis (1990) conducted an assessment of invertebrate community structure on selected sites in the Upper Buffalo River using Hilsenhoff's Biotic Index (HBI; Hilsenhoff 1982, 1988) based on pooled data collected from November to March. Sites were selected as pairs, one of each pair with higher water quality and one with lower. Main channel sites included the relatively pristine reach in the upper Boxley Valley just below the boundary of the Upper Buffalo Wilderness Area and two more disturbed sites at the downstream end of the valley near Ponca. Tributary sites included a pristine site at Cecil Creek near Erbie and a more disturbed site at Mill Creek near Pruitt. This study showed no distinct seasonal patterns among the invertebrate community structure during this index period, and there were no consistent differences between Upper Boxley and Ponca, suggesting these sites had a high level of ecological integrity. However, Mill Creek showed a consistently lower diversity when compared to the other three sites, suggesting it may be impacted by anthropogenic disturbances. Mathis (1990) also reported a greater abundance of pollution intolerant taxa at these sites compared to Mill Creek and the Buffalo River at Ponca (Mathis 1990). Additional studies of Mill Creek water quality by Manner and Mott (1991) found that 96% of the nitrogen load being carried by the Buffalo River below the confluence was supplied by Mill Creek, and likely came from the interbasin transfer of groundwater with a nearby watershed.

Although the Buffalo River at Ponca may be classified as relatively high quality, some anthropogenic impacts have occurred there. According to Mathis (1990), results obtained from this site always were poorer than those obtained at his Upper Boxley site. Numerous published reports and the River Continuum Concept [see discussion below] suggest that natural increases in species richness and diversity should have occurred on the stream between Upper Boxley and Ponca. The Mathis study showed the opposite, and he contended the river is being slightly impacted by the disturbances associated with agricultural practices as the stream flows through Boxley Valley. Subsequent to the Mathis study, water quality data collected from 1991 to 1998 indicated the Ponca site had significantly higher fecal coliform concentrations compared to other sites on the Buffalo River (Mott and Luraas 2004).

Bryant (1997) tested the River Continuum Concept (RCC) along fourth and fifth order reaches of the Buffalo River. The RCC predicts biotic transformations along a stream's gradient (Vannote et al. 1980). The RCC predicts species richness and diversity should be lower in headwaters reaches, increase in mid-reaches, and then return to decreased richness and diversity in lower reaches (Vannote et al. 1980). Bryant found that discharge, conductivity, pH, and temperature increased in the downstream direction while substrate size decreased. In contrast to the RCC, richness, diversity, and other metrics associated with pollution intolerant taxa were lower in the middle-river reaches. Bryant's work showed that species richness and diversity were negatively correlated with nitrate concentrations at the sampling sites.

Usrey (2001) expanded upon Bryant's findings by examining possible causes for the decrease in species diversity and richness in the middle region of the Buffalo River. Analysis of 10 years of water quality data indicated that elevated levels of nitrogen occurred at the mid-reaches of the river and that these increased concentrations were due to nonpoint source loading through several tributaries. Nitrogen levels for four mid-reach tributaries (Mill Creek, Little Buffalo River, Big Creek, and Davis Creek) represented approximately 40% of the total nitrogen loading to the river and average nitrate values were two to four times higher in these tributaries than in the adjacent river (Usrey 2001). The highest nutrient loads came from the Little Buffalo River and upper Big Creek.

Usrey (2001) further suggested that declining water quality and increasing densities of Asian clams were the two disturbances that were most likely responsible for the shifts in invertebrate community species composition in the middle reaches of the river. To further investigate these mid-reach disturbances and their impacts on invertebrate community diversity, Usrey (2001) also sampled eight sites seasonally for one year. Physical habitat and water quality were measured at each site. While no seasonal relationship was apparent between increasing nitrate levels and invertebrate diversity and community integrity, data combined for all seasons showed that higher nitrate concentrations were correlated with decreasing abundance of pollution intolerant Ephemeroptera, Plecoptera, and Trichoptera (EPT) taxa (Usrey 2001). Further evidence of the effect of nutrient enrichment on the community was shown by correlation of the abundance of pollution tolerant Diptera with increasing orthophosphate concentrations (Usrey 2001).

Bradley (2001) sampled four tributary sites in the Buffalo River drainage to assess water quality and invertebrate communities in an effort to determine reference water quality conditions. The sites were Bear Creek, Tomahawk Creek, and Calf Creek, all of which represented disturbed streams in the mid to lower reaches of the Buffalo River watershed. Water Creek was used as the undisturbed reference site. Bradley (2001) found water quality characteristics—including dissolved oxygen, conductivity, temperature, and pH—were similar among all four sites and were typical for the Ozark Highlands ecoregion. Discharge was significantly higher for Bear Creek and Calf Creek in the spring, and fecal coliform and turbidity levels were also higher for these sites. This was expected due to the larger drainage area and more cleared land in their respective watersheds. The reference stream, Water Creek, had low turbidity during all seasons due to a higher proportion of forested land in its drainage area. Tomahawk Creek showed a consistent level of fecal coliform concentrations throughout the year while those of the other three sites fluctuated, indicating a possible point source pollutant like a septic tank or continuous direct access of livestock to the stream.

Bradley (2001) also reported seasonal and yearly variations in invertebrate community structure, density, and richness among the four tributaries (Bradley 2001). However, most of the seasonal variations in community composition are explained by differences in life histories of the organisms and not due to anthropogenic disturbances. In the disturbed tributaries, the largest component of the community was Diptera, a largely pollution tolerant group typically abundant in streams with organic enrichment. In comparison, the reference stream was dominated by largely intolerant Ephemeroptera.

In a similar study, Dick (1998) collected water quality and invertebrate data from four perennial and two intermittent headwater tributaries to the Buffalo River. The objectives of that study were to gather baseline information on invertebrate assemblages in regional headwater streams, determine significant differences in assemblages, and relate variation in community assemblages to environmental variables. As expected, significant differences in community attributes were observed between intermittent and perennial streams, and seasonal succession was most influential in structuring the communities. Flow regime was also more important than stream order (size) in structuring invertebrate communities. This study found significant natural differences exist in benthic invertebrate community structure among headwater streams in the Ozark physiographic region (Dick 1998).

Subsequent to initiation of long-term monitoring described in Bowles et al. (2007), status reports and analyses concerning aquatic invertebrate community structure in the Buffalo River and its tributaries were published in Bowles (2015) and Bowles et al. (2013, 2017).

Local Biomonitoring Programs

Two aquatic invertebrate monitoring programs have been previously proposed for OZAR and BUFF. Kathy Doisy and Charles Rabeni, formerly of the Missouri Cooperative Fish and Wildlife Research Unit at the University of Missouri, completed a report titled, "A Biological Monitoring Program for the Ozark National Scenic Riverways" (Doisy and Rabeni 1999). The late Mike Mathis and his associates at the University of Central Arkansas similarly prepared an invertebrate monitoring protocol titled, "Development of a Multi-metric System of Biological Water Quality Monitoring for the Buffalo National River" (Mathis 2001). Both reports are soundly based on previously collected data, extensive literature reviews, watershed and reach-specific issues, and statistical analysis of variability and changes in space and time among sites. These works led to the development of multimetric indices specific to the respective streams in BUFF and OZAR. Mathis (2001) developed the Index of Community Integrity (ICI) for the Buffalo River, and Doisy and Rabeni (1999) recommend the Stream Condition Index (SCI) for the Jacks Fork and Current rivers. These indices are further discussed below.

Measurable Objectives

Two broad measurable objectives are addressed by this protocol:

1. Determine the annual status and trends of invertebrate species diversity, abundance, and community metrics.
2. Relate the invertebrate community to overall water quality through quantification of metrics related to species richness, abundance, and diversity, and region-specific multimetric indices as indicators of water quality and habitat condition (DeBacker et al. 2005).

Justification/Rationale for these Objectives: BUFF and OZAR were created to preserve and interpret the free-flowing Buffalo, Jacks Fork and Current rivers. The Heartland Network Aquatic Resources Working Group formally agreed that the framework for aquatic monitoring at OZAR and BUFF would be directed specifically towards understanding and maintaining the ecological integrity of these river systems. Aquatic invertebrates are an important biomonitoring tool for understanding and detecting changes in ecosystem integrity over time. Aquatic invertebrates respond rapidly to different environmental stressors, are relatively easy to collect, and can be analyzed at many different levels of precision.

Operational Objectives

1. Communicate monitoring results to park natural resource managers, other park staff, and partners, including outreach efforts when appropriate. Furthermore, contributions to the scientific community may be valuable.
2. Conduct monitoring safely, ideally without accident or injury. Safe monitoring includes during transportation to/from parks as well as during field operations.

II. Sampling Design

A long-term monitoring program must specify how to efficiently sample numerous parameters through space and time. An overall sampling design must contain multiple components, including (1) a spatial design (how sample sites are located and the area of statistical inference), (2) a revisit design (how frequently sites are sampled); and (3) a response design (how and what data are collected). To effectively use limited monitoring resources, information derived from a relatively small number of sample sites must be used to infer changes over a much larger area. For the inference to be valid, a probability-based sample design within a defined reference frame is required.

After five years of monitoring the river systems, it became apparent that changes to this monitoring protocol were necessary, primarily due to workload limitations (DeBacker et al. 2012). The first five years of monitoring invertebrate data were analyzed and discussions were held with park management that resulted in changes to habitat data collection, invertebrate data analysis, and a new spatial and temporal sampling design of sites. The necessary changes to the original protocol (Bowles et al. 2007), due to the revisit plan, are reflected in spatial and temporal sections of this protocol. These changes were implemented with monitoring starting in 2011. Based on the new temporal and spatial design, the sampling frequency described below represents a change from the annual sampling schedule as described in Bowles et al. (2007) to a *biennial* sampling schedule. In addition, the revised plan reduced the number of tributaries with an increase in the frequency of their sampling (See the Temporal Design section in this chapter). Maps indicating mainstem stretches selected for annual monitoring are in Appendix A. Additional information for stretches selected for monitoring, including stretch identification number, UTMs, and tributary names, are presented in Appendix B.

Spatial Design

Establishing the Sample Frame

We developed an integrated aquatic monitoring plan for both BUFF and OZAR (Bowles et al. 2007), which included the co-location and potential co-visitation of multiple vital signs. The framework for this plan was conceived in a workshop of biologists, statisticians, and administrators held in July 2004 (McDonald 2004). This protocol focuses on one of these vital signs, the aquatic invertebrate communities. Specifically, we are interested in the invertebrates inhabiting riffles in the main stems and tributaries located within National Park Service jurisdictional boundaries at BUFF and OZAR.

We have defined the sample unit to accommodate the field protocols for all vital signs. Our common sample unit definition is a *stretch* of contiguous river of some minimum and maximum length. The geomorphology of these waterways (and the resulting biological processes) is scale dependent (e.g., as rivers become larger, the distances associated with pool-riffle sequences increase). A key characteristic of our overall design is that all aquatic studies should be capable of producing unbiased estimates that are applicable to the entire stretch. While stretches must be long enough to accommodate unbiased estimates for all studies, they do not have to be the same size. Once defined, sample unit boundaries remained fixed forever and are used by all studies under the unified monitoring design.

Two different categories of stretch sizes were established. In the tributaries and upper main stems, stretch lengths are 1–2 km. In the lower main stems, stretch lengths are 3–5 km. Within categories, stretch length is not fixed, but varies depending upon several factors. Stretches were broken at natural features, such as confluences and springs. They were also delimited based on Valley Segment Type (VST) information. The sample frames for BUFF and OZAR were determined based on similar criteria, with the differences reflecting the important biological variations in the river systems in each park. For both parks, the initial sample frame of stretches was constructed through a collaborative agreement with the Missouri Resource Assessment Partnership (MORAP). To determine the sample frame at OZAR, MORAP used Missouri Aquatic Gap datasets, the same datasets used by the Missouri Department of Natural Resources and Missouri Department of Conservation. This dataset did not exist for Arkansas, and MORAP developed a comparable stream network for BUFF.

MORAP used data from the 1:100,000 National Hydrography Dataset (NHD) that was developed by the USGS and EPA. The coverage included arcs representing the centerlines of wide streams, as well as the segments of single line streams. An Arc/Info macro was run on the arc segments to pull select attributes from various NHD tables and attach them directly to the arc component. These stream segments were classified according to a number of variables, including temperature, stream size, flow, geology, soil texture, relative gradient, valley wall interaction (a surrogate for potential bluff pool habitat), stream size discrepancy, and channel type (metadata for BUFF are available at https://irma.nps.gov/DataStore/Reference/Profile/1041268 and metadata for OZAR are available at https://irma.nps.gov/DataStore/Reference/Profile/1041269). The dataset was restricted to those stream segments that touched the park jurisdiction boundary or other public lands adjacent to the park. Additionally, tributaries to the main stem river were cut when they crossed the floodplain of the main stem river. This allowed these segments to be coded as *floodplain* segments.

For both BUFF and OZAR, the final sample frame consisted of all stretches of the main stem and tributaries that met our inclusion criteria described below. Each stretch in both frames has a large number of associated characteristics based on GIS data, which could be used in analyses as covariates or domains (i.e., subpopulations of interest for which we want parameter estimates).

To establish the final sample frame for each park, we used the following procedure. (1) We removed all stretches that were not entirely or partially within the park boundaries (the MORAP dataset included adjacent public lands). (2) All secondary channels were removed (secondary channels occur where a waterway splits and flows around an island; secondary channels transport the lesser volume of water). (3) Stretches were stratified as either main stem or tributaries (at OZAR, the Jacks Fork was considered a main stem).

Selecting the Sites to Be Sampled

Spatial balance among sampling sites is important because all responses are known to be spatially autocorrelated (i.e., units close to one another tend to yield correlated responses). When responses are correlated in space, spatial balance can greatly improve the precision of the resulting estimates. Thus, we employed the Generalized Random Tessellation Stratified (GRTS) method of sample selection (e.g., Stevens and Olsen 1999, 2004). The GRTS technique generates a random sample that is spatially balanced. It allows multiple studies to maximize overlap of selected streams by utilizing a common sample, and allows units to be added easily after an initial sample has been drawn. Additionally, because GRTS samples are not evenly spaced, it is not possible for sample locations to be in phase with a cyclic response.

Perhaps the most desirable characteristic of GRTS is that for any sample size, any subset of stretches in the ordered GRTS sample constitutes a spatially balanced sample. This characteristic is desirable because it allows multiple studies to maximize overlap and add stretches in a way that guarantees spatial balance. It also allows each rotating panel (i.e., in the case of the tributaries; see below) to represent a spatially balanced sample from the entire park.

We used the S-Draw program developed by Trent McDonald (S-Draw is available at https://github.com/tmcd82070/SDraw/wiki/SDraw) to draw the GRTS samples. Main stem sites were weighted by stretch length. S-Draw allows for several options in drawing the sample. The hierarchical structure was randomized (Stevens and Olsen 1999). We employed the reverse hierarchical ordering option, which assures that any contiguous set of stretches will be spatially balanced (Stevens and Olsen 2004). We used a random number seed generated from the system clock (the default option).

All GRTS draws were *oversampled* (i.e., more sites were selected and ordered than will be immediately sampled). This will allow for an increase in site number in the future (if budget allows) without decreasing the overall degree of spatial balance. This will also provide flexibility not to sample certain sites if an issue arises and it is deemed appropriate. In such a case, one would simply move to the next site in the ordered GRTS list (sacrificing only a small degree of spatial balance).

The total number of stretches to be sampled is limited primarily by budget and personnel (DeBacker et al. 2012). This protocol proposes sampling 13 stretches every other year. This takes into account complete processing of all samples and the number of other protocols that will need to be implemented at these sites. At BUFF (which has many tributaries), 6 main stem stretches and 6 tributary stretches will be sampled on each sampling event. At OZAR (which has fewer tributaries, but many springs), 9 main stem stretches and 4 tributary stretches will be sampled.

Appendix B includes those mainstem and tributary stretches selected for monitoring in the original protocol, stretch identification number, UTMs, and associated information. Tributaries currently targeted for sampling are identified below in the BUFF revisit design.

Main Stems

At OZAR, a greater degree of control was desired for the main stem than was possible by selecting all stretches from the same pool with GRTS (which has a strong random element). The Jacks Fork, Upper Current, and Lower Current (upstream and downstream of the confluence with the Jacks Fork, respectively) are very different systems, primarily due to the influence of large springs. A total of 130 stretches comprised the sample frame for these main stems. Stretches on the Jacks Fork (n = 39) and Upper Current (n = 53) were ~1–2 km in length. Stretches on the Lower Current above the town of Van Buren (where a break in the park's boundary occurs) were ~1–2 km in length, but stretches below Van Buren were ~3–5 km in length. The river below Van Buren has higher flows, in large part due to the input of Big Spring (278 million gallons/day). A total of 38 stretches were identified on the Lower Current. It was desirable to have an equal number of sample sites on each of these three main stem sections. Thus, we divided the main stem of OZAR into three categories (Jacks Fork, Upper Current, and Lower Current) before selecting the GRTS sample.

We also divided the Buffalo River into lower and upper sections prior to drawing the GRTS sample. The frame for the main stem consisted of 74 total stretches. There is a losing reach on the Buffalo River below the confluence with Richland Creek where much of the water (all during most summers) runs underground for several km before resurfacing at White's Spring. Thus, we divided the river into an upper section (n = 47 stretches) above the natural break at the losing reach, and a lower section (n = 27 stretches) below White's Spring. This break also approximates a major geologic shift, as the upper section includes the Boston Mountain formation, and the lower primarily represents the Springfield and Salem Plateaus. The losing reach was deleted from the frame because it dries seasonally and the invertebrate communities located there may not be reflective of other areas of the river. The distance for the two sections is similar (89 km for the upper, 109 km for the lower). The lower section contains fewer stretches because the stretches are longer. Stretches above the confluence of Mill Creek near Pruitt were ~1–2 km in length, whereas stretches below this point were ~3–5 km in length. Again, this change in stretch length reflects changing river morphology as flows increase and the riverbed widens.

Following these criteria, we used GRTS to order 64 main stem stretches at OZAR and 37 main stem stretches at BUFF. Although we sampled only the first 9 and 6 (Bowles et al. 2007), respectively, this procedure will allow us to increase the number of stretches sampled in the future (if possible), or integrate other studies with a larger sample size, and still maintain a park-wide spatial balance.

Tributaries

To establish the tributary sample frame, all flood plain stretches were removed because those portions of tributaries within the floodplain of the main stem are likely to be more variable due to intermittent backwater inundation. These floodplain stretches represented a relatively short section of most tributaries in both parks. The resulting sample frame contained a large number of stretches. A number of tributaries, although indicated as perennial on USGS maps, drain relatively small watersheds and, according to park personnel, often have little or no flowing water. Thus, we revised our sample frame to include only tributaries of second order and above. Some of the tributaries had multiple stretches within park boundaries. Since we could not sample all tributaries, and sampling multiple stretches of the same tributary would yield relatively redundant information, we limited the frame to the most downstream stretch of each tributary. Because most of these tributaries were relatively small, it was determined that sampling could be accomplished in < 1 km, and we set the minimum acceptable distance for tributary stretches within the park boundary at 600 m.

Relatively few tributaries are sampled, and the ability to make overall inferences to an entire park is limited, because each watershed is unique. Thus, most inferences derived from tributary sampling pertain to the particular tributaries sampled. The original selection of tributaries described in the original sampling protocol (Bowles et al. 2007; Appendix B) was modified by DeBacker et al. (2012) based on the requests of BUFF and OZAR.

Because tributaries monitored by HTLN in the original protocol were selected at random, many of the tributaries formerly sampled were not of great interest to park management. Furthermore, ordination analyses revealed that the assessment of the tributaries depended on the year that they were sampled, and suggest a long time will be required at the current return interval before the natural variability can be described in these tributaries, much less detection of directional change (DeBacker et al. 2012). Stronger inferences could be made to tributaries sooner if they were to be sampled more frequently. Since sampling all tributaries specified in the original protocol would only result in an increasing workload, sampling fewer tributaries more frequently was deemed an appropriate option (DeBacker et al. 2012). Therefore, targeted tributaries for each park were selected by the park natural resource staff.

Temporal Design

At both parks, the new revisit design consists of a set of rotating panels (Tables 1 and 2). To ensure sufficient representation of monitoring sites on the main stems, the revisit panel will consist of main stem stretches that will be sampled every other year (n = 9 for OZAR, n = 6 for BUFF). In addition, one set of tributaries (n = 2 for OZAR, n = 4 for BUFF) will be sampled every 2 years, and another set of tributaries (see below) will be sampled every 4 years for each park. Given our limited sample size, this strategy will yield maximum information on trend for the main stems, and maximum spatial coverage for the tributaries. However, we would be able to sample only a small fraction of the total number of tributaries in each park if the alternative approach was applied for the tributaries (i.e., maximizing information on trend).

BUFF Revisit Design for Mainstems and Tributaries (4-year rotation):

Six mainstem sites (locations unchanged) are sampled every other year along with four targeted tributaries. Paneled tributaries will be sampled every four years.

<u>Year 1</u>

- All 6 mainstem sites
- 4 targeted tributaries (Mill Creek at Pruitt, Davis Creek, Calf Creek, Bear Creek)
- Panel 1 tributaries (Clabber Creek, Middle Creek, Leatherwood Creek)

<u>Year 2</u>

- No sampling

<u>Year 3</u>

- All 6 mainstem sites
- 4 targeted tributaries (Mill Creek at Pruitt, Davis Creek, Calf Creek, Bear Creek)
- Panel 2 tributaries (Cecil Creek, Little Buffalo River, Water Creek)

<u>Year 4</u>

- No sampling

OZAR Revisit Design for Mainstems and Tributaries (4-year rotation):

Nine mainstem sites (locations unchanged) are sampled every other year along with two targeted tributaries. Shawnee, Blair, and Rocky creeks were part of the original random GRTS panels. Sinking, Big (W), and Big (E) creeks are new additions. Paneled tributaries will be sampled every four years.

<u>Year 1</u>

- No sampling

<u>Year 2</u>

- 9 mainstem sites (6 Current River, 3 Jacks Fork River)
- 2 targeted tributaries (Sinking Creek, Shawnee Creek)
- Panel 1 tributaries (Big Creek West, Blair Creek)

<u>Year 3</u>

- No sampling

<u>Year 4</u>

- 9 mainstem sites (6 Current River, 3 Jacks Fork River)
- 2 targeted tributaries (Sinking Creek, Shawnee Creek)
- Panel 2 tributaries (Rocky Creek, Big Creek East)

Table 1. Revisit plans for monitoring studies at BUFF. An ‘X’ indicates all sample units in that panel are to be visited that year.

Type (% of Annual Effort)	Revisit Notation	Stream	Year											
			2017	2018	2019	2020	2021	2022	2023	2024	2025	2026	2027	2028
Mainstem River (50%)	[1-1]	Buffalo River	X	–	X	–	X	–	X	–	X	–	X	–
Targeted Tributaries (25%)	[1-1]	Davis Creek	X	–	X	–	X	–	X	–	X	–	X	–
	[1-1]	Mill Creek at Pruit	X	–	X	–	X	–	X	–	X	–	X	–
	[1-1]	Calf Creek	X	–	X	–	X	–	X	–	X	–	X	–
	[1-1]	Bear Creek	X	–	X	–	X	–	X	–	X	–	X	–
Panel Tributaries (25%)	[1-3]	Clabber Creek	X	–	–	–	X	–	–	–	X	–	–	–
	[1-3]	Middle Creek	X	–	–	–	X	–	–	–	X	–	–	–
	[1-3]	Leatherwood Creek	X	–	–	–	X	–	–	–	X	–	–	–
	[1-3]	Cecil Creek	–	–	X	–	–	–	X	–	–	–	X	–
	[1-3]	Little Buffalo River	–	–	X	–	–	–	X	–	–	–	X	–
	[1-3]	Water Creek	–	–	X	–	–	–	X	–	–	–	X	–

Table 2. Revisit plans for monitoring studies at OZAR. An ‘X’ indicates all sample units in that panel are to be visited that year.

Type (% of Annual Effort)	Revisit Notation	Stream	Year											
			2017	2018	2019	2020	2021	2022	2023	2024	2025	2026	2027	2028
Mainstem River (50%)	[1-1]	Current River, Jacks Fork	–	X	–	X	–	X	–	X	–	X	–	X
Targeted Tributaries (25%)	[1-1]	Sinking Creek	–	X	–	X	–	X	–	X	–	X	–	X
	[1-1]	Shawnee Creek	–	X	–	X	–	X	–	X	–	X	–	X
Panel Tributaries (25%)	[1-3]	Big Creek West	–	X	–	–	–	X	–	–	–	X	–	–
	[1-3]	Blair Creek	–	X	–		–	X	–	–	–	X	–	–
	[1-3]	Rocky Creek	–	–	–	X	–	–	–	X	–	–	–	X
	[1-3]	Big Creek East	–	–	–	X	–	–	–	X	–	–	–	X

The invertebrate community at any given site at BUFF and OZAR streams consists of a high diversity of species in various developmental stages. Therefore, temporal consistency in sample collection is essential to reducing the natural variability in invertebrate life cycles and community structure (Rabeni et al. 1997). Mathis (2001) found a higher similarity among sites in the summer compared to other seasons, suggesting that summer is the poorest time to sample invertebrates for the purpose of monitoring, at least when trying to detect only slight or moderate changes. The findings of Mathis (2001) are due in part to the summer seasonal fauna of Ozark streams being naturally tolerant of the harsh stream conditions that occur during this period including high water temperatures (up to 30°C) and low flows. Sampling during this period may give the incorrect appearance that the community is pollution tolerant. Variability among individual samples at a single site is generally lowest in the fall and winter, which indicates these seasons are best for detecting minimal differences between sites. Invertebrate samples collected during the winter season have been shown to have higher species abundance and fewer organisms per sample, thus making sample processing and identification more efficient and cost effective.

To reduce costs and increase efficiency and robustness of the community metrics that will be used, sampling will be conducted once per sampling year during a fall/winter index period between 1 November and 28 February, as generally recommended by Doisy and Rabeni (1999) and Mathis (2001). To the extent possible, temporal consistency should be maintained through successive years as well as between sample types. Samples from all sites should be collected within the shortest time frame possible (maximum of 3–4 weeks) to minimize the effects of seasonal change (Mathis 2001). If this is not possible, efforts should be made to collect all samples from the main channel sites during a consolidated time period and all samples from the tributary sites during another period (Mathis 2001). All efforts should be made to avoid collecting directly after a flood event or major disturbance. Samples should be collected only during baseflow conditions and a minimum of two weeks after flood waters recede to baseflow conditions (Mathis 2001).

Response Design

Types of Data Collected in the Field

This monitoring program will collect benthic invertebrates from stream riffles and associated habitat and water quality data. Habitat features are major, often limiting, determinants of invertebrate community structure; accordingly, they are especially important for proper determination of biomonitoring results and assessment of ecological integrity (Barbour et al. 1999). Although habitat incorporates all aspects of physical and chemical constituents and their interactions, variables such as current velocity, substrate size, embeddedness, water chemistry, and presence of filamentous algae and aquatic plants play key roles in the microhabitat structure and distribution of aquatic invertebrates (Hauer and Lamberti 1996; Allan 1995; Rosenberg and Resh 1993). We propose to monitor all of the aforementioned habitat variables at our sampling sites.

Biological and environmental correlates of water quality and habitat structure compared across time are powerful tools for assessing disturbances related to natural and anthropogenic impacts on aquatic invertebrate communities, and they are useful- for detecting change and elucidating patterns and trends in long-term data sets (Moulton et al. 2002). For example, as habitat conditions degrade (e.g., water quality decreases, embeddedness increases), degradation of the benthic invertebrate community is expected to follow. However, a cause and effect relationship between these variables and aquatic invertebrate community structure can be difficult to assess and analyze because there is often a broad response range among the resident species (Norris and Georges 1993). Therefore, any association of community structure with these variables or their combinations must be interpreted cautiously and be based on real biological properties. These limitations withstanding, benthic community structure, when viewed in association with environmental variables, can be an effective indicator of ecosystem change (Reice and Wohlenberg 1993). In combination, such data are useful for providing managers an integrated assessment of water quality.

The sampling approach described here is most comparable to that of the United States Geological Survey, National Water-Quality Assessment Program (USGS NAWQA; Moulton et al. 2002), and the framework of this protocol generally fits within the framework of the NAWQA protocol. However, the protocol described here is adapted from the NAWQA protocol to account for program specific goals related to long-term monitoring and limitations posed by staff size and logistical and budgetary constraints. The general basis of the NAWQA program is to collect biological, physical and chemical data at sites that have major natural and anthropogenic factors considered responsible for controlling water quality in a river basin. The NAWQA sampling design for benthic invertebrates includes two types of sampling sites: basic fixed sites and synoptic sites. The fixed sites are those at which parameters are measured over long periods of time, and as such, they are analogous to the sampling sites used in this monitoring protocol. The NAWQA synoptic sites are used for one-time collections. Therefore, they are not included in this protocol.

Additionally, the NAWQA program conducts water quality assessments in sampling reaches defined as the presence of two repeating geomorphic channel units, such as a sequence of pool-riffle-pool-riffle. From these sampling reaches two broad types of benthic samples are collected to characterize the invertebrate community: (1) semiquantitative benthic samples collected from targeted habitat types, and (2) a composite qualitative sample collected from a broad variety of habitats from throughout the reach. The semiquantitative benthic samples recommended by NAWQA are collected from richest-targeted habitat type (riffles for Ozark streams) using a Slack-Surber sampler (Moulton et al. 2002).

The number of individual benthic samples to be collected is not specified in the NAWQA protocol and depends on study objectives. Collected samples are partially processed in the field and subsequently composited into a single bulk sample. However, by compositing the individual samples collected from a reach, no estimate of variability among samples can be obtained. In contrast, this protocol recommends collecting three semiquantitative benthic invertebrate samples from each of three consecutive riffles in a reach to assess both inter-and intra-riffle variability of benthic invertebrate community structure.

Further, this protocol does not recommend collecting composite qualitative benthic samples taken from throughout the reach. The single habitat sampling of invertebrates in riffles we propose in this protocol also follows the recommendations of Rabeni et al. (2002) who concluded that riffles of Ozark streams provided the most consistent estimates of community structure, both spatially and temporally, in comparison to pools which were least concordant. Moreover, the species rich habitats of riffles are expected to be highly sensitive to water quality changes because they can support a diverse community displaying a wide range of sensitivities to water quality changes (Moulton et al. 2002).

The NAWQA protocol allows for location of sites based on whether or not the site is representative of the local area and supports objectives, thus giving the site investigator flexibility in establishing site boundaries depending on local conditions. The sampling design in this protocol, by comparison, is strictly probabilistic and sampling reaches are permanent. Collecting techniques and equipment described herein are analogous to those described in the NAWQA protocol (Moulton et al. 2002).

The U.S. Environmental Protection Agency (EPA) has two programs for assessing water quality using invertebrate communities in wadeable streams. These are the Rapid Bioassessment Protocols for Use in Streams and Rivers (Barbour et al. 1999) and the Environmental Monitoring and Assessment Program - Surface Waters (EMAP; Lazorchak et al. 1998). An additional set of protocols designed for larger non-wadeable rivers (Flotemersch et al. 2006) generally are not applicable to the streams addressed in this protocol and are not addressed here further. The Rapid Bioassessment approach uses either single habitat (e.g., riffles) or multihabitat approaches and both involve collecting samples from a 100-m reach determined by the investigator to be representative of the characteristics of the stream. The single habitat approach involves sampling using a kick-net with a 1-m area sampled in front of the net and taking 2–3 kicks using foot agitation. The samples are then composited for analysis. Benthic metrics for analyzing data are the same or comparable to those used in this protocol (Barbour et al. 1999). The multihabitat approach uses 20 jabs or kicks taken from different representative habitat types using a D-frame dipnet. Samples are composited for analysis and metrics are the same or comparable to those used in this protocol (Barbour et al. 1999).

The EMAP program uses probabilistically-selected sites and individual sampling sites are assessed using a transect-based design where community biological metrics are tied to habitat structure. Kick-net samples collected from flowing water habitats (e.g., riffles, runs) are combined into a single composite sample for the stream reach while kick-net samples collected from pool habitats are combined into a separate composite sample. The *kick-net* used in the EMAP method is effectively the same net as a Slack-Surber sampler minus the frame delineating the sampling area in front of the net. Data are analyzed following Barbour et al. (1999) and use either multimetric or multivariate approaches. In addition, some programs use O/E (Observed/Expected) Ratio of Taxa Loss to assess invertebrate community degradation. This tool is a ratio comparing the number of taxa expected (E) to exist at a site to the number that are actually observed (O). The taxa expected at individual sites are based on models developed from data collected at reference sites. This protocol does not use O/E ratios.

The EMAP approach focuses on evaluating ecological conditions on regional and national scales. The EPA's Wadeable Streams Assessment Program is based on the EMAP approach and is not considered separately here (United States Environmental Protection Agency 2004a, b, c, d). We opted to not use either EPA monitoring approach for some of the same reasons discussed above in reference to the USGS NAWQA program. However, there are some similarities among the EPA and USGS NAWQA approaches and the present protocol that will allow for comparison of data. Indeed, Peterson and Zumberge (2006) generally found no significant differences between invertebrate samples collected from riffles using the NAWQA and EMAP protocols. Because this protocol uses many of the same metrics employed in the former two protocols, we contend that the individual metrics and multimetric indices will be comparable among all three protocols.

Placement of Samples

Stretch boundaries are permanent and will not be changed, excluding natural changes to the bedform due to the dynamic nature of rivers. Global Navigation Satellite System (GNSS) coordinates for each stretch are found in Appendix B. Procedures for selection of riffles and sample points within each riffle are described in SOP# 3 (Sampling Invertebrates and Collecting Habitat Data). For each selected stretch, three consecutive riffles will be chosen to represent a reach (defined broadly here as three consecutive riffle/glide/pool sequences). Thus, the sample reach will extend from the upper boundary of the upstream-most riffle to the lower boundary of the downstream-most riffle. Riffle selection is determined *a priori*, with the three riffles being those located in consecutive order upstream of the first riffle above the lower boundary of the selected stretch (Figure 1). The specific locations of the riffles sampled in a given year may move naturally due to hydrological processes.

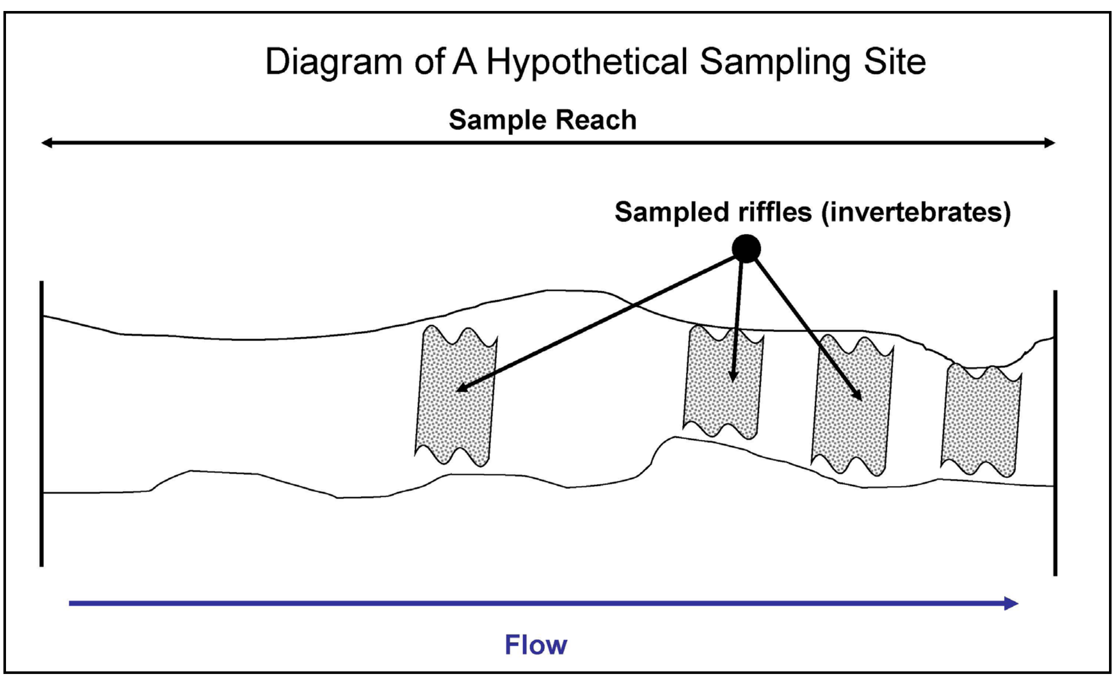

Figure 1. Riffle selection within a stretch.

Three benthic samples will be randomly collected from each selected riffle resulting in a total collection of nine separate samples per stretch. This sampling approach provides an estimate of intra-stretch and intra- and inter-riffle variability. In total, 117 benthic samples will be collected from among 13 sampling sites for each park on each sampling rotation. The process of collecting benthic samples is described in SOP#3.

Number of Samples

While developing an Index of Community Integrity (ICI) in his basin-wide study of the Buffalo River, Mathis (2001) showed that three samples per riffle are adequate to assess the invertebrate community in the Buffalo River. This sample size was determined by first collecting five samples at a site. The samples were then compiled into all possible combinations of groups ranging from two-sample pools to five-sample pools in a group. The various metrics and ICI values were then calculated for each group and compared to the results of the group with five samples using several techniques. Mean ICI values were compared using Pearson's correlation analysis to the five-sample pooled group, and the results showed Pearson's correlation coefficients tended to be higher with increasing sample size. Mathis (2001) indicated the minimum acceptable number of samples should have a statistically significant correlation coefficient that was > 0.90 ($p < 0.05$). Mathis (2001) indicated that three benthic samples met the aforementioned standards, but he suggested collecting four samples, analyzing three of the samples, and then adding the fourth sample if results were inconclusive or showed extreme deviations.

Usrey and Hinsey (2006), following procedures similar to Mathis (2001), compared three different riffles, one each from an upper, middle, and lower site on a major tributary of the Buffalo River using various sample sizes from three samples per riffle to nine samples per riffle. An ANOVA with Bonferroni's adjustment was used to test for significance between community metrics for each of the three sites at various sample sizes. This study showed that three samples per riffle are sufficient to characterize the benthic invertebrate community with respect to calculation of metrics. Other published studies have shown the efficacy of collecting only three benthic samples per riffle. Canton and Chadwick (1988) found three benthic samples yielded reliable estimates of invertebrate density. Bowles (1989) found that single riffle sampling was inadequate and may produce misleading results, and that a multiple riffle sampling design is more appropriate. Bowles (1989) also found that estimates of benthic density and variability were not statistically different among sample sizes of 3, 6, and 12 samples per riffle.

Rationale for the Sampling Design

Monitoring objectives are integral to defining the sampling design. The sample design allows us to track changes in invertebrate communities over time by measuring net change in certain community metrics. For assessing annual status and trend through time of invertebrate communities, the overall survey design was deemed suitable for several reasons (a full account of the sample design is shown above).

1. *Single habitat (riffle) sampling is appropriate for long-term monitoring of benthic invertebrates*. Sampling multiple habitats provides more comprehensive information about the invertebrate fauna compared to single-habitat samples (Lenat and Barbour 1994; Moulton et al. 2002). However, comparability between sites is necessary for accurate bioassessments and invertebrates collected from the same habitat types among sites are more similar than invertebrates collected from multiple habitats within the same site (Parsons and Norris 1996; Rabeni et al. 1997). Indeed, Rabeni et al. (1997) showed metric sensitivity did not increase when comparing multiple versus single-habitat sampling in Missouri streams. Furthermore, habitats in Ozark streams with the lowest community variability are high gradient riffles and coarse runs (Doisy and Rabeni 1999). Therefore, single habitat sampling in riffle/run habitat is the focus of this protocol and will be employed at both BUFF and OZAR.
2. *Appropriate for Ozark streams*. The data generated from this study design will be directly comparable to those of Rabeni et al. (1997) and Mathis (2001) as well as other regional (state and federal) invertebrate monitoring programs that employ similar methodologies and rely largely on percentage-based metrics (e.g., Barbour et al. 1999).
3. *Accommodates habitats of varying size*. The sample design allows for unbiased estimates of invertebrate community condition that are ap-

plicable to the entire stretch regardless of length. While stretches must be long enough to accommodate unbiased estimates for all studies, they do not have to be the same size.

4. *Easy to learn and use*. Field procedures are easy to use and repeatable over time by different sampling crews. Implementation does not require extensive time or costly equipment.
5. *The sequence of sampling events and revisit design for tributaries allows for the greatest amount of field work to be accomplished per year while minimizing cost*. Because staff available for manning field crews is limited, and travel costs associated with monitoring are high, this strategy allows for cost-effective monitoring for mainstem sites and further allows for maximum spatial coverage for the tributaries.
6. *The selected approach to monitoring is advantageous over other approaches*. The study design and methods selected for this protocol allow for an integration of community attributes and further allow us to characterize temporal changes and relative site quality. Additionally, our approach will allow us to correlate invertebrate community data with land use and habitat changes potentially arising from multiple stressors.

III. Field and Laboratory Methods

Field Season Preparations, Field Schedule, and Equipment Setup

Procedures for field season preparations, including preparing a field sampling schedule, and equipment setup are described in SOP#1 (Preparation for Field Sampling and Laboratory Processing). Team leaders should ensure that team members have read and understand the protocol and supporting SOPs prior to sampling, and that all required equipment and supplies have been ordered and are in proper working conditions. They should also check stream staff gages to determine if sampling sites have recently flooded. The team leaders will prepare and maintain a field notebook detailing all sampling-related activities and staff participation during monitoring trips to ensure that trip reports are complete and accurate. Finally, the team leader should ensure that all required scientific collection permits have been obtained.

Collecting Benthic Invertebrate Samples and Associated Habitat and Water Quality Data

Procedures for collecting benthic invertebrate samples and documenting habitat data are presented in SOP#3 (Sampling Invertebrates and Collecting Habitat Data), SOP#5 (Measuring Stream Discharge), and SOP#6 (Documenting CORE 5 Water Quality Variables). Figure 2 shows a work flow diagram for collecting samples. Habitat variables will include depth and current velocity measurements and the dominant substrate for each sample at the specific locations benthic samples are collected. Several additional qualitative measurements of habitat condition will be taken from the area delineated by the sample net frame. Stream discharge will be measured at each site and preferably upstream of the sampling site after invertebrate collections have been completed. CORE 5 water quality parameters (temperature, dissolved

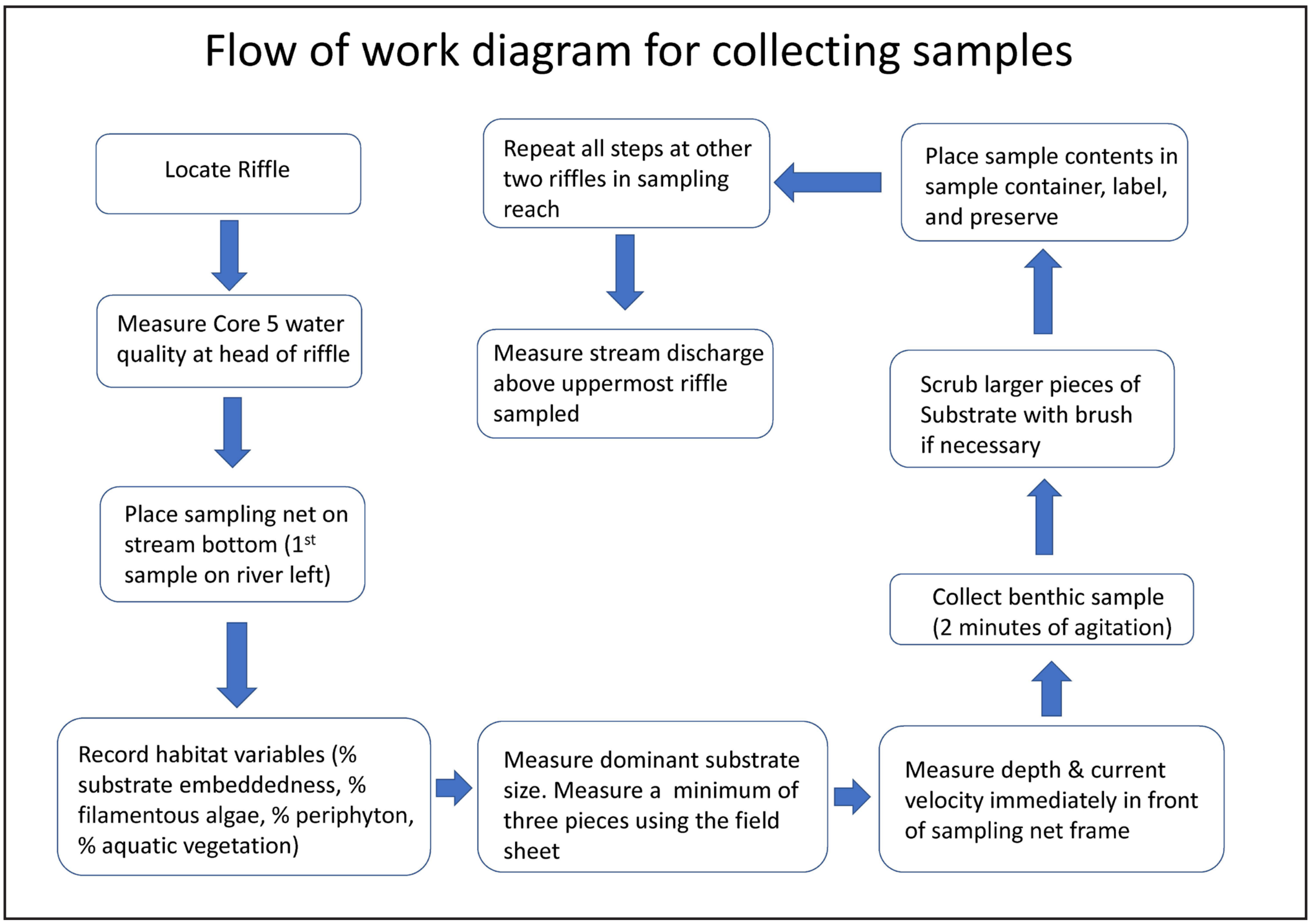

Figure 2. Flow of work diagram for collecting samples.

oxygen, specific conductance, pH, and turbidity) will be recorded for each riffle using hand-held instruments while data loggers will be used for other areas of the stream.

Measurements within Sampling Net Frame

Several variables will be assessed and recorded from within the sampling frame of the invertebrate collecting net after it is placed securely on the stream bottom and before disturbing the substrate. These variables include visual estimates of percent embeddedness of the substrate, percent periphyton, percent filamentous algae, percent sedimentation, and percent organic material. Standard classes for all percentage estimates will be as follows: 0 = Absent (0%), 1 = Sparse (<10%), 2 = Moderate (10–40%), 3 = Heavy (40–75%), and 4 = Very Heavy (>75%). Additionally, point velocity and depth (see SOP#5) are measured immediately in front of the sampling net prior to collecting the sample.

Substrate Size Assessment

When the habitat variables have been recorded, the process of removing substrate for size assessment can begin. Substrate assessments provide a unique characterization of the streambed composition at the time sampling takes place. Therefore, substrate will be measured from the area within the sampling frame of the net, and it will be measured for every sample. The intent of this substrate assessment is to characterize the dominant substrate for individual samples, not to fully characterize all sediments present. This assessment helps us describe the prevailing microhabitat conditions that influence the structure of invertebrate communities and may help explain variability between sample points. Substrate will be measured based on the standard Wentworth scale (Wentworth 1922). Procedures for collecting and measuring substrate samples are provided in SOP#3.

Collecting Benthic Samples

Three invertebrate samples will be collected from each riffle from randomly selected sample points as described in SOP#3. Samples will be collected with a Slack Surber sampler as described in Moulton et al. (2002). Water may flow over the top of the net in deep riffles, which potentially could allow some invertebrates dislodged from the substrate to wash over the net and not into it. Such losses must be considered relative to the restrictions quantitative sampling gear (i.e., Hess sampler) would impose on sampling effort. Moreover, this sampling approach allows for estimates of all of the main categories of community attributes including benthic densities. Each discrete sample is collected while progressing in an upstream direction. Sampling procedures will be the same for each riffle sampled, and whenever possible, samples should be collected by the same person to limit variability in sample techniques.

Benthic Sample Processing and Specimen Identification

Procedures for processing benthic samples and identifying specimens are described in SOP#4 (Laboratory Processing and Identification of Invertebrates). Methods for preparing samples for sorting and subsampling generally follow those presented in Moulton et al. (2000). A list of the aquatic invertebrate taxa known from BUFF and OZAR is shown in Appendix A.1 of SOP#8 (Data Analysis).

Subsampling Benthic Samples

Because of the relatively large number of samples that will be collected from BUFF and OZAR, subsampling individual samples will be necessary. The routine for subsampling benthic samples is presented in SOP#4 (Laboratory Processing and Identification of Invertebrates). The method of subsampling will involve the fixed fraction approach with 25% of each sample being sorted following thorough washing, agitation, sieving, and elutriation of the entire sample (Moulton et al. 2000). Additionally, a "large and/or rare" taxa component will be included where large or rare taxa that clearly are not in the sorted fraction are removed and stored in a separate vial for the purpose of reflecting accurate sample species richness estimates and calculating specific metrics such as EPT (Ephemeroptera, Plecoptera, Trichoptera) richness. A fixed-fraction subsampling routine was selected over a fixed-count routine because some the metrics to be calculated from samples are related to specimen density which cannot be obtained with the latter method. Subsampled fraction debris will be subjected to quality assurance/quality control (QA/QC) analysis (SOP#4) and should be kept until QA/QC is complete for that batch of samples and the program leader authorizes disposal of the debris.

Sample Storage and Reference Collection

Identified samples are stored in 4-dram glass vials with polycone caps and filled with 70% ethyl alcohol. Specimen vials will be labeled with the taxon name, date collected, park and site names/code, and name of identifier. Organisms will be retained for at least three years and stored at the NPS Heartland Inventory and Monitoring Network (HTLN) office located at Missouri State University, Springfield, MO. A reference collection consisting of a few representative specimens of each taxon will be prepared and stored in properly labeled vials containing 70% ethyl alcohol. Regional or other taxonomist specialists should review the identifications for accuracy. One set of vials will be stored at the NPS HTLN office located at Missouri State University, Springfield, MO. Additional sets of specimens should be maintained in the laboratory where identifications are performed for use as reference and training.

Post Season Procedures

Procedures for the end of the sample season are found in SOP#10 (Procedures and Equipment Storage after the Field Season) and are not further described here.

IV. Data Management

Data management procedures are an important part of any long-term monitoring program because they provide data consistency, data security, and availability over time. Therefore, care must be taken to ensure that adequate time and personnel are available for accurate data recording, data entry and verification, and analysis. At the core of data management is the monitoring database organized by primary and ancillary data.

Data processing typically involves the following steps: data entry, data verification, data validation and backups/storage; see SOP#7 (Data Management) for details on each step. Data entry consists of transferring field data from field sheets into a monitoring database using data entry forms. Data verification immediately follows data entry and involves checking the accuracy of computerized records against the original source, usually paper field records. Validation procedures seek to identify generic errors, such as missing, mismatched, or duplicate records, as well as logical errors specific to particular projects. Spatial validation of location coordinates can be accomplished using a Geographic Information System (GIS). Global Navigation Satellite System (GNSS) points are validated against high resolution imagery and/or LiDar for their general location.

Overview of Database Design

One tabular Microsoft Access database, henceforth referred to as the database, contains all data for the monitoring project. A generalized model of the invertebrate community database includes two primary tables for sampling events and locations. These two core tables contain general information pertaining to the field sampling occasion (the when and where of the sample). This includes information such as date and time, reach ID, and park/project codes. The invertebrate community tables serve as the organizing hub for invertebrate data. Other tables primarily address habitat or water quality conditions. The database also documents the protocol version and QA/QC results.

Quality Assurance and Quality Control

Quality Assurance (QA) includes all activities designed to ensure that data, products, or services meet specified requirements. Quality Assurance focuses on building in quality to prevent defects. Quality Control (QC) includes procedures for checking whether data meet standards and annotating or qualifying data that do not (DeVivo 2016).

QA/QC procedures and design elements occur throughout data collection, processing, and reporting. The database design includes fields to document the completion and results of QA/QC procedures and assessments.

- The Inventory and Monitoring Division Data Base Standards (Frakes et al. 2015) document requires every datum to be unambiguously traceable to a specific version of a monitoring protocol, a quality assurance plan (QAP) where available, and suite of standard operating procedures (SOPs).
- The certification guidelines for I&M data products (NPS 2016), and Minimum Implementation Standards for Network Projects v. 3.0 (Frakes and Kingston 2017) calls for every datum to have an associated QA/QC processing level (e.g., raw, provisional, certified).
- An annual operational review is required for all active monitoring protocols (Mitchell et al. 2018). Completion of an operational review, a summary of any flagged data, and a link to the review report are stored in the monitoring database.

Metadata Procedures

The Federal Geographic Data Committee (FGDC) now provides a range of options as guidance for metadata of spatial and non-spatial federal agency data. Most recommendations are variations of the ISO19115 standard, which is typically used for natural resource datasets. Creation of ISO metadata has been greatly facilitated by ESRI ArcGIS utilities that automatically generate spatial metadata. Once metadata are created, they should be saved in XML format following ISO metadata standards. Metadata are archived in the geodatabase and by Washington D.C. Area Support Office (WASO) I&M in the Integrated Resource Management Applications Data Store (IRMA DataStore). Metadata are archived by WASO with the submission of the monitoring protocol. Metadata will be updated with each protocol revision.

Data Archival Procedures

HTLN archives all spatial and non-spatial data (including tabular documents) on a weekly basis. Backups are incremental rather than mirrored so that files are never overwritten. Permanent data archives are created on a quarterly and annual basis and stored offsite in a bank safe box.

Like other monitoring databases/geodatabases, the aquatic invertebrate monitoring database is secured by file archives stored on the server. Databases are maintained under a directory called HTLNInvert under the heartlandcommon production drive. The database immediately below this directory is the production copy of the database. All backups are incremental rather than mirrored so that earlier versions are stored under this directory.

Annually, in fulfillment of the Data Analysis and Reporting Requirements (Gallo, K. memorandum dated 4/23/2018), the dataset will be uploaded to IRMA DataStore. The dataset is flagged as “read only” for all users except the Project Leader and Data Manager.

V. Data Summary, Analysis and Reporting

Metric Selection and Community Indices

Early biomonitoring programs tended to focus on one or two specific attributes or metrics of the community; the indicator species concept (Kremen 1992) is an example. Individual metrics generally are chosen based on the specific and predictable response of organisms to landscape changes. Additionally, they are sensitive to a range of factors that stress biological systems and are relatively easy to measure and interpret (Karr and Chu 1999). Barbour et al. (1999) lists and briefly describes many types of metrics used in assessing stream condition. However, individual metrics in themselves are often not adequate for assessing complex systems with cumulative impacts (Karr 1991).

In comparison, multimetric indices are designed to look at community structure through examination of multiple components of the invertebrate community and their level of change due to disturbance. Scores of individual metrics are normalized into a single integrated score, reducing the influence of one metric on the overall score and making results less ambiguous for resource managers. Bonada et al. (2006), in a comparative analysis of recent bioassessment approaches, showed that multimetric approaches rate among the best performers for 10 of 12 criteria they tested for discriminating among different kinds of human impact. Multimetric approaches are favored by most aquatic resource agencies in the United States because they are based on sound scientific rationale, they are simple to implement, and they are among the most sound for assessing invertebrate community structure (Lenz and Miller 1996; Bonada et al. 2006).

Stream Condition Index

A multimetric index was developed by Rabeni et al. (1997) for the state of Missouri. This index is called the Stream Condition Index (SCI). Doisy and Rabeni (1999) make recommendations specifically for monitoring invertebrates at OZAR with scoring criteria for the SCI based on a reference distribution generated from data collected in the Current River watershed. Doisy and Rabeni (1999) suggested four metrics as measures of community structure and balance. These metrics are taxa richness, EPT (Ephemeroptera, Plecoptera, Trichoptera) richness, Shannon's Diversity Index, and Biotic Index (BI). These and other community metrics are described in Barbour et al. (1999). Procedures for calculating and scoring these four metrics are included in SOP#8 (Data Analysis).

The four selected metrics are generally considered sufficiently sensitive to detect a variety of potential pollution problems in Ozark streams. Some of the potential disturbances that can be detected using these metrics include (after Doisy and Rabeni 1999) the following:

- Gross organic pollution: Hilsenhoff (1982) listed all four of the selected metrics as indicators of gross organic pollution.
- Agriculturally developed catchments: Ephemeroptera and Plecoptera have shown reductions in abundance or richness with these catchments (Quinn and Hickey 1990; Lenat and Crawford 1994).
- Increases in acidity : Taxa richness, EPT taxa, and Shannon Diversity Index typically decrease in response to increasing acidity (MacKay and Kersey 1985; Hildrew et al. 1984). Mayflies are especially sensitive to low pH (Peterson et al. 1985).
- Effects of logging and clear cutting: Stone and Wallace (1998) found that the North Carolina Biotic Index (NCBI, a modification of the Biotic Index; Lenat 1993) was the most sensitive to this type of disturbance.
- Heavy metal pollution: Taxa richness and EPT richness (Winner et al. 1980; Chadwick et al. 1986) have been shown to decrease in response to this type of pollution. However, further research indicates that mayflies may decrease in richness and abundance while caddisflies increase under these conditions, resulting in static EPT richness. If no difference in the EPT richness is found, analysis of the richness and percent composition of mayfly taxa should be performed (Doisy and Rabeni 1999).
- Insecticides: Wallace et al. (1996) found that both the EPT index and the NCBI easily detected disturbances to a stream treated with certain insecticides.

- Habitat degradation in the Ozark Highlands: Rabeni et al. (1997) found that Simpson's Index for high gradient riffles was a sound measure of disturbance. According to Doisy and Rabeni (1999), there are few strong relationships between the degree of habitat degradation and the biological condition of streams in the Ozarks. Doisy and Rabeni (1999) also found an increase in the percentage of collector/filterers with increased embeddedness in Ozark Highland streams.

<u>Metric Scoring for the SCI</u>

All metric values are normalized so that they become unitless and have equal influence on the SCI results following the suggestion of Barbour et al. (1996). Reference data provided in Doisy and Rabeni (1999), including four sites in the Current River watershed and other Ozarkian streams, were used to determine a range for each metric with one of three possible scores assigned to each range. The lower or upper quartile of the distribution for each metric is used as the minimum value representative of reference conditions. Details of this method are presented in SOP#8.

Ozark Rivers Stream Invertebrate Multimetric Index (ORSIMI)

Another multimetric index, Buffalo River Index of Community Integrity (BRICI), was formerly used to assess invertebrate communities at BUFF. This index is no longer used because several problems were found with its development and interpretation. Those deficiencies are described in DeBacker et al. (2012). Moreover, the BRICI was judged inadequate for the purpose of providing park managers meaningful information on change in the aquatic invertebrate communities at BUFF. The SCI was recommended as a replacement multimetric index for the BRICI. Although the SCI performed generally well for both parks, there are minor issues related to accurate scoring that must be resolved to maximize the benefit of that index. The Ozark Rivers Stream Invertebrate Multimetric Index (ORSIMI) was developed to fill this gap (DeBacker et al. 2012). Although the SCI is usually good for assessing overall impairment, the ORSIMI is useful for interpreting the degree of change in the invertebrate communities.

The ORSIMI is similar to the SCI in that it is based on four metrics: taxa richness, EPT richness, Shannon's Index, and the HBI. The ORSIMI index is arbitrarily scaled to 100 for the baseline period (in this case, 5 years). The average of each metric value over the baseline period is multiplied by a constant so that each metric contributes a total of 25 toward the 100 score total. (The HBI score is subtracted from 10, because a lower HBI score indicates better water quality and 10 is the maximum value for the HBI.) All future data would be compared to that baseline (i.e., the same constant is multiplied by each metric in all future years). Each site is calculated independently from the rest, because sites are not directly comparable. The index can be expanded if additional metrics are shown to be useful for interpreting change in community structure.

Similar to the SCI, each of the four metrics of the ORSIMI contributes the same weight to the overall index (based on baseline conditions), but the new index has greater resolution and sensitivity. For the SCI, any changes within 75% of the hypothesized distribution of values for any of the metrics would have no change on the index. In comparison, any change of any magnitude in any metric will result in a change in the overall ORSIMI. That change potentially could be negative or positive (i.e., total scores may be >100 if conditions improve). Unlike the SCI, there are no subjective judgments on what values indicate *impairment*; any comparisons will simply be to a baseline condition. Procedures for calculating and scoring these four metrics are included in SOP#8.

Data Analysis

In any long-term monitoring program, a consistent methodology and careful implementation of field sampling techniques are critical in obtaining comparable data. Once the field season is over, if data have not been correctly collected, they are lost forever. Therefore, the procedures for data collection must be specified and followed exactly. In contrast, data analysis techniques do not need to be specified in great detail. Many different analysis methods are available, and are documented in great detail elsewhere. Moreover, new methods are developed over time. Thus, absolute and detailed specification of data analysis techniques is not necessary or desirable. Due to the complexity of higher-level analyses, many options are available and step-by-step instructions will not be sufficient; a competent analyst will always need to be consulted. Moreover, data can always be reanalyzed if necessary. Thus, we present descriptions of various data analysis options, realizing that the most appropriate techniques will vary over time as sample sizes increase, and that the details of any analysis can be found in the relevant texts or literature.

In determining the appropriate statistical approaches for this monitoring protocol, it is important to take into account the primary audience of the various reports that will result. This audience will consist of park resource managers, park superintendents, and other park staff. Park resource managers and staff may not have an in-depth background in statistical methods, and park superintendents may have limited time to devote to such reports. Additionally, protocols such as this may provide much data on many different types of variables. To the extent possible, our core data analyses and presentation methods provide a standard format for evaluation of numerous variables, are relatively straightforward to interpret, can be quickly updated whenever additional data become available, and can be used for many different types of indicators, whether univariate or multivariate. Additionally, the type and magnitude of variability or uncertainty associated with the results should be easily discernible, and a threshold for potential management action will ideally be indicated.

There are three main statistical approaches that could be employed with data from long-term monitoring projects such as this: (1) hypotheses testing, (2) parameter estimation, and (3) application of Bayesian methods. When analyzing ecological data, statisticians predominantly employ frequentist methods, and thus many resource managers are not familiar with the interpretation of Bayesian approaches. Bayesian methods are not widely used because they are often difficult to apply, and many researchers are not comfortable specifying subjective degrees of belief in their hypotheses (Utts 1988; Hoenig and Heisey 2001). Thus we do not advocate a Bayesian approach as our main method of data analysis.

Most hypothesis testing approaches involve a null hypothesis of no difference or no change. The problem with such approaches is that the hypothesis under test is thus trivial (Cherry 1998; Johnson 1999; Anderson et al. 2000, 2001). No populations or communities will be exactly the same at different times. Therefore, we are not really interested in whether these are changing per se, but rather in the magnitude of change, and whether it represents something biologically important. Null hypothesis significance testing relies heavily on *P*-values, and results primarily in yes/no decisions (reject or fail to reject the null hypothesis). *P*-values are strongly influenced by sample size, however, and one may, with a large enough sample size, obtain a statistically 'significant' result that is not biologically important. Alternatively, with a small sample size, one may determine that a biologically important result is not statistically significant (Yoccoz 1991). Thus, traditional null hypothesis testing places the emphasis on the *P*-value (which is dependent on sample size) and rejection of the null hypothesis, whereas we should be more concerned whether the data support our scientific hypotheses and are practically (i.e., biologically) significant (Kirk 1996; Hoenig and Heisey 2001).

Parameter estimation provides more information than hypothesis testing, is more straightforward to interpret, and is easier to compute (e.g., Steidl et al. 1997; Gerard et al. 1998; Johnson 1999; Anderson et al. 2000, 2001; Colegrave and Ruxton 2003; Nakagawa and Foster 2004). Parameter estimation emphasizes the magnitude of effects and the biological significance of the results, rather than making binary decisions (Shaver 1993; Stoehr 1999). One of the primary recommendations from a workshop on environmental monitoring organized by the Ecological Society of America was that trend studies should focus on description of trends and their uncertainty, rather than hypothesis testing (Olsen et al. 1997). Thus, most of our data analyses will take the form of parameter estimation rather than null hypothesis significance testing.

We will also employ control charts in data organization and analysis. Control charts represent a basic summary for almost any data set, a sort of *quick look* for busy managers to determine which variables are in the greatest need of more in-depth analyses or management action. Developed for industrial applications, control charts indicate when a system is going *out of control* by plotting through time some measure of a stochastic process with reference to its expected value (e.g., Beauregard et al. 1992; Gyrna 2001; Montgomery 2001). Control charts may be univariate or multivariate, and can represent many different types of variables. They have been applied to ecological data (McBean and Rovers 1998; Manly 2001), including fish communities (Pettersson 1998; Anderson and Thompson 2004) and natural resources within the I&M program (Atkinson et al. 2003). Control charts contain upper and lower control limits specifying thresholds beyond which variability in the indicator reveals a biologically important change is occurring and warns that management may need to act. Control limits can be set to any desired level.

Multivariate control charts may also be constructed, and although some of the above-mentioned texts describe multivariate control charts (using the Hotelling T^2 statistic), this approach is only practical for a small number of variables, and assumes a multivariate normal distribution. In general, species abundances are not distributed as multivariate normal (Taylor 1961), and traditional multivariate procedures are frequently not robust to violations of this assumption (Mardia 1971; Olson 1974). A new type of multivariate control chart has recently been described for use with complex ecological communities and a software application entitled *ControlChart.exe* is available for constructing these types of multivariate control charts (see Anderson and Thompson 2004). Multivariate temporal autocorrelation will violate the assumption of stochasticity upon which this method is based, however, and it is important to test for temporal autocorrelation using Mantel correlograms prior to using this method. This new multivariate control chart appears to have promise, but has not been widely applied nor thoroughly evaluated. Further evaluation of this method is warranted before application to the data of this protocol.

We did not conduct a formal power analysis for this protocol for three reasons. (1) The primary purpose of conducting a prospective power analysis is to determine whether the proposed sample size is adequate. A number of studies already exist indicating that three samples per riffle is an appropriate number for calculation of the proposed metrics (see Number of Samples under the Sample Design Section). Because our sample size will be determined primarily by budget, we would not be able to increase the number of riffles sampled per stretch or number of stretches regardless of the result of any power analysis. Furthermore, in many analyses sample size will equate with number of years; in this case, analyses will simply become more powerful over time. (2) Statistical power is dependent upon the hypothesis being tested and the statistical test used. Over the course of this long-term monitoring program, we will be interested in many different questions and could potentially evaluate a number of different hypotheses. Thus, there is no single *power* relevant to the overall protocol. Estimating power at this point in the context of such a long-term, multifaceted monitoring program could be potentially misleading, as the test this power is based upon may rarely (or never) actually be employed. (3) Most of our data analyses will take the form of parameter estimation rather than null hypothesis significance testing. When estimating parameters, there is no associated statistical power. In general, statistical power analyses are frequently misused and misinterpreted in ecological contexts (Morrison 2007), and alternative approaches to evaluating the degree of uncertainty associated with our data will be evaluated and used when applicable.

Because of the extent of the river systems in these parks (i.e., the main stem of the Buffalo River covers almost 200 km within the park boundary, and the main stems of the Current and Jacks Fork rivers within their respective park boundary are even more extensive), we will usually not attempt to average over all stretches to obtain a single, park-wide estimate of a given parameter. The biological characteristics of these rivers are constantly changing along their length. The patterns and processes that characterize the upper sections of these rivers will be very different from those that characterize the lower sections of the same rivers. Thus, any given point estimate would not be representative of most of the river. Moreover, the variability associated with this estimate would be very large (because of the great differences among stretches). Different tributaries are likely to be even more different than different stretches along a mainstem. Thus we will primarily evaluate indices and metrics for each stretch over time.

Although our primary approach to organizing and analyzing data will consist of multimetric indices, we do not entirely rule out the use of any statistical methods at this time. Because of the nature of this long-term monitoring program, other approaches (some of which may not yet have even been developed!) may be appropriate at different points in time, depending upon the needs of the resource managers and questions of interest. At times, depending upon the question of interest to resource managers, a hypothesis testing framework may be employed. Because data from studies of aquatic insects is often not normally distributed, non-parametric approaches may need to be employed. For example, if it is desirable to test for differences between riffles, a Kruskal-Wallace ANOVA, Friedman's non-parametric two-way ANOVA, or Cochran's Q test could be used. Of course, normality of the data will be evaluated prior to any tests, and transformations may be performed if useful prior to tests requiring normal distributions. These approaches and others are described in SOP#8.

Reporting

Annual reporting updates should be completed by May 1 of the year data are collected. Annual reporting requirements include an informal trip report, and an operational review report. The updates may be in the form of a web article or data visualizer. Trend reports are updated every four years (2 sampling cycles). Trend reports explore correlations among the data over time. Trend reports are published as Natural Resource Reports in the NPS Natural Resource Publication Series and Uploaded to IRMA, or published in peer-reviewed scientific literature. Refer to SOP#9 (Data Reporting) for details on reporting.

VI. Personnel Requirements and Training

Roles and Responsibilities

The project manager is the aquatic program leader for the HTLN and this person bears responsibility for implementing this monitoring protocol. Because consistency is essential to implementation of the protocol, the project manager or an aquatic ecologist with several years of experience collecting the data related to this protocol will lead field data collections. The project manager will oversee all laboratory work including all QA/QC requirements.

The data management aspect of the monitoring effort is the shared responsibility of the project manager and the data manager. Typically, the project manager is responsible for data collection, data entry, data verification and validation, data summary, analysis, and reporting. The data manager is responsible for data archiving, data security, dissemination, and database design. The data manager, in collaboration with the project manager, also develops data entry forms and other database features as part of quality assurance and automates report generation. The data manager is ultimately responsible to ensure that adequate QA/QC procedures are built into the database management system and appropriate data handling procedures are followed. Technicians will be responsible for field collection and laboratory processing, equipment maintenance, purchasing of supplies, and sample storage. At least one technician with taxonomic experience will be responsible for the identification of specimens to the genus level.

Qualifications and Training

Training is an essential component for collection of credible data. Training for consistency and accuracy should be emphasized for both the field and laboratory aspects of the protocol. SOP#2 (Training for Field Sampling and Laboratory Processing) describes the training requirements for new technicians. The project manager should oversee this training and ensure that each technician is adequately prepared to collect data. Taxonomic identifications may be performed by a technician with several years of experience but initial identifications should be checked by expert taxonomists.

VII. Operational Requirements

Field Schedule

Samples will be taken during the fall-winter index period (1 November to 28 February). Sampling should begin as soon as possible in November due to the unpredictable weather in the Ozarks during the winter months. Samples should be collected within the shortest time frame possible to minimize the effects of seasonal change. If flood events or other problems interrupt the sampling schedule, the river sites should be sampled as a group and the tributary sites should be sampled as a group. At a minimum, two people will be required to complete the field sampling portion of the protocol; however, three people make the process much more efficient. Because of travel considerations, only one site can be sampled per day under normal circumstances. Sampling trips to the Lower Wilderness area of Buffalo National River require a minimum of two field days including overnight camping at remote, primitive locations. All benthic invertebrate sampling can be completed in 13 field days under normal conditions for each park.

Facility and Equipment Requirements

Field and lab equipment listed in SOP#1 (Preparation for Field Sampling and Laboratory Processing) are only for one sampling crew. Beyond normal office and equipment storage space, facility needs include access to a wet laboratory. Additional equipment requirements include access to a canoe and/or motorboat, as well as maintenance and/or replacement of equipment shared among multiple projects (e.g. Global Navigation Satellite System [GNSS] units, cameras, vehicles, server). Network vehicles are shared and fuel/maintenance costs are incurred at the Network level.

Budget Considerations

Approximately one day, including travel, is required to complete the sampling for each sampling site. Personnel expenses for fieldwork are based on a preferred crew size of three people: program leader, aquatic ecologist, and biological science technician. Field costs will vary from year to year depending on the skill level and size of the crew, and parks to be sampled (number and distance from work domain). Laboratory processing time per benthic sample, including sorting, identification, counting, and entry into the database, will require approximately 6 hours per sample.

Data management personnel expenses include staff time of biological science technicians, the project manager, and the data manager. The project leader also invests time in preparation for field trips (two or more days) and data evaluation and reporting. These steps can include a month or more of the project leader's time per report, not including peer reviewer's time. Additional shared support staff include the quantitative ecologist and geographic information specialist.

VIII. Procedures for Protocol Revision

Over time, revisions to both the protocol narrative and to specific SOPs are expected. Careful documentation of changes to the protocol, and a library of previous protocol versions are essential for maintaining consistency in data collection and for appropriate treatment of the data during data summary and analysis. Mitchell et al. (2018) describe the necessary review and documentation for modifying the monitoring protocol. The steps for changing the protocol (either the protocol narrative or the SOPs) are outlined in SOP#11 (Revising the Protocol).

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Appendix A. Maps of Mainstem Sampling Locations for BUFF and OZAR

Maps A.1 through A.5 show mainstem sampling locations in Buffalo National River and Ozark National Scenic Riverways.

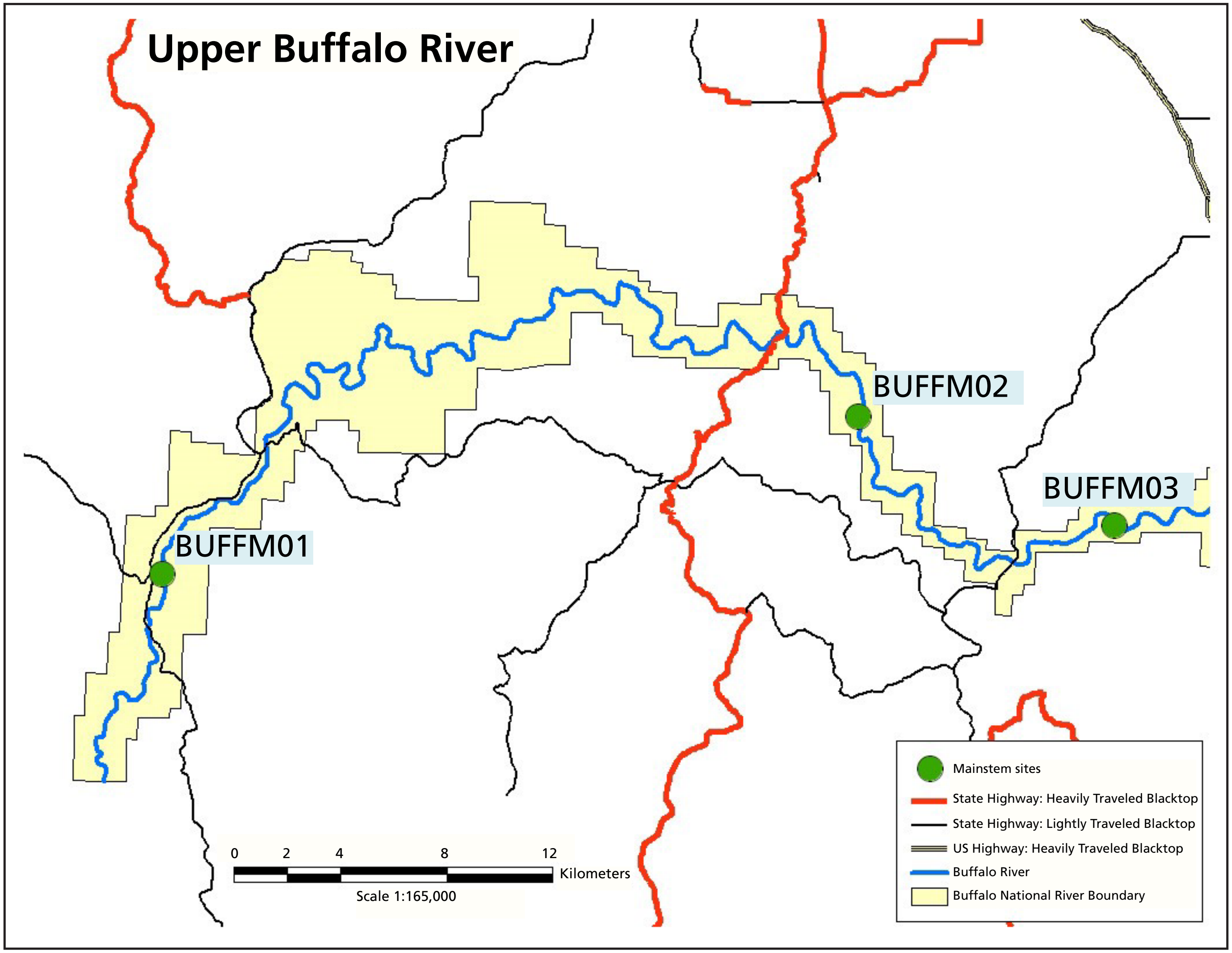

Figure A.1. Location of mainstem stretches selected for monitoring on the upper Buffalo National River.

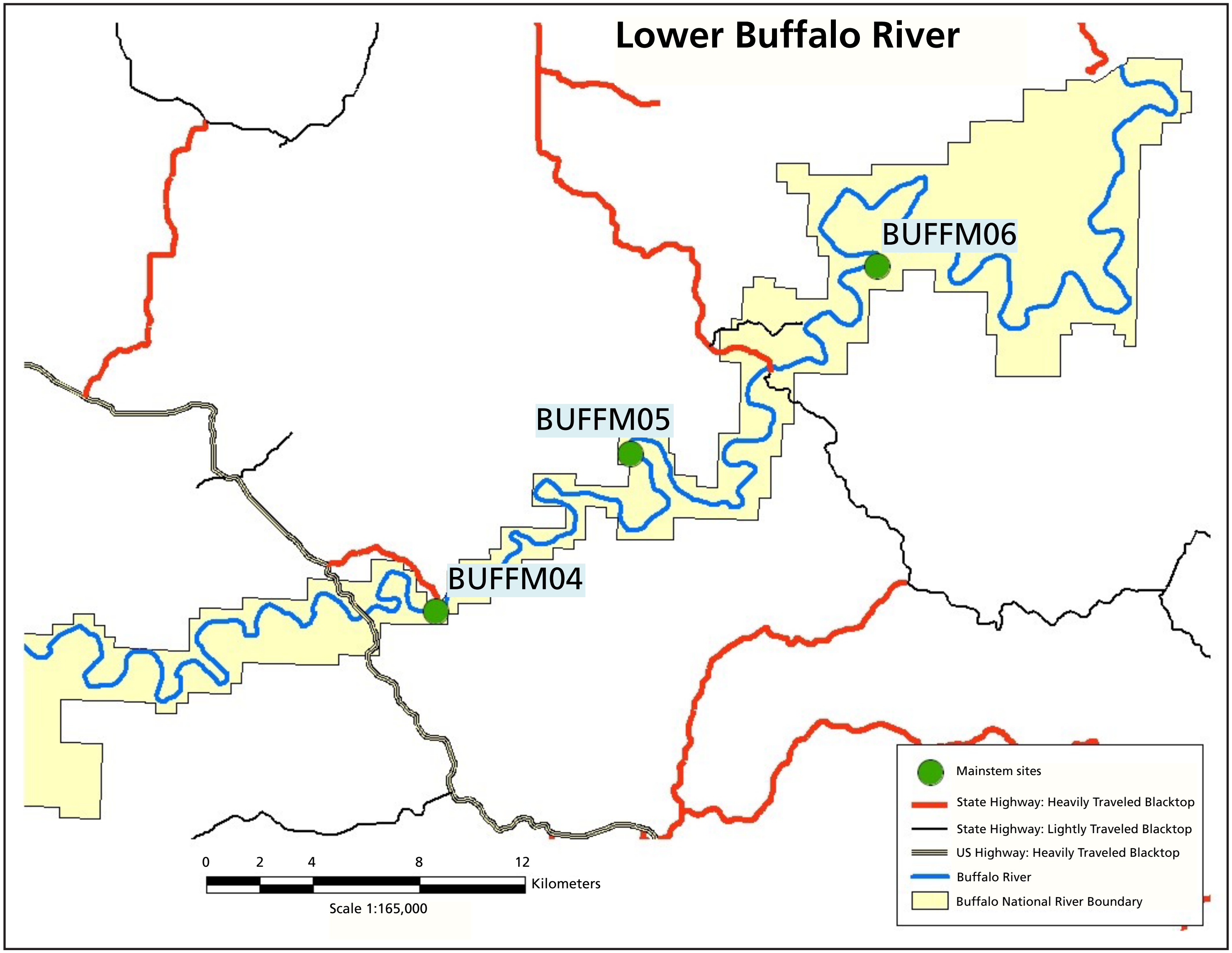

Figure A.2. Location of mainstem stretches selected for monitoring on the lower Buffalo National River.

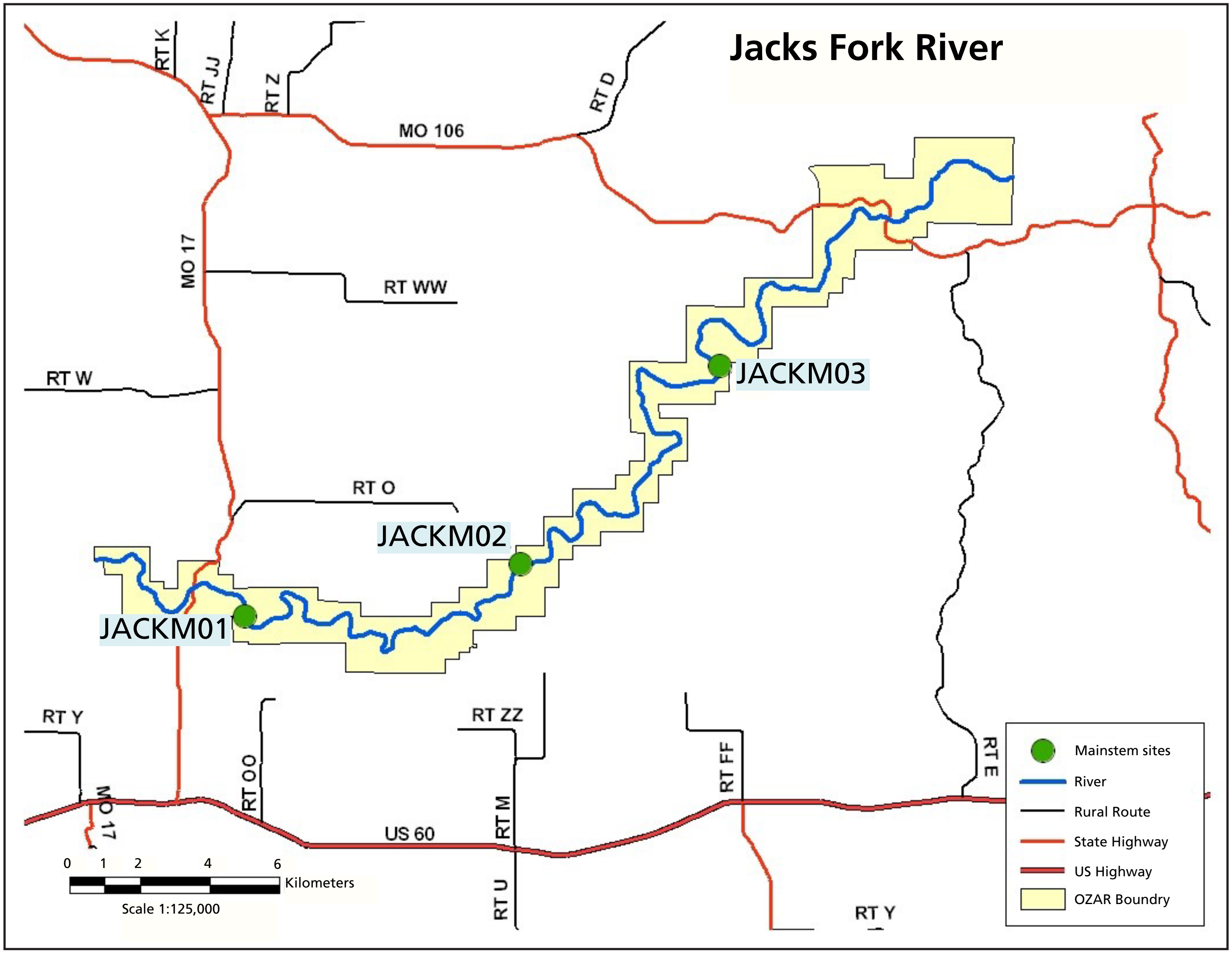

Figure A.3. Location of mainstem stretches selected for monitoring on the Jacks Fork River.

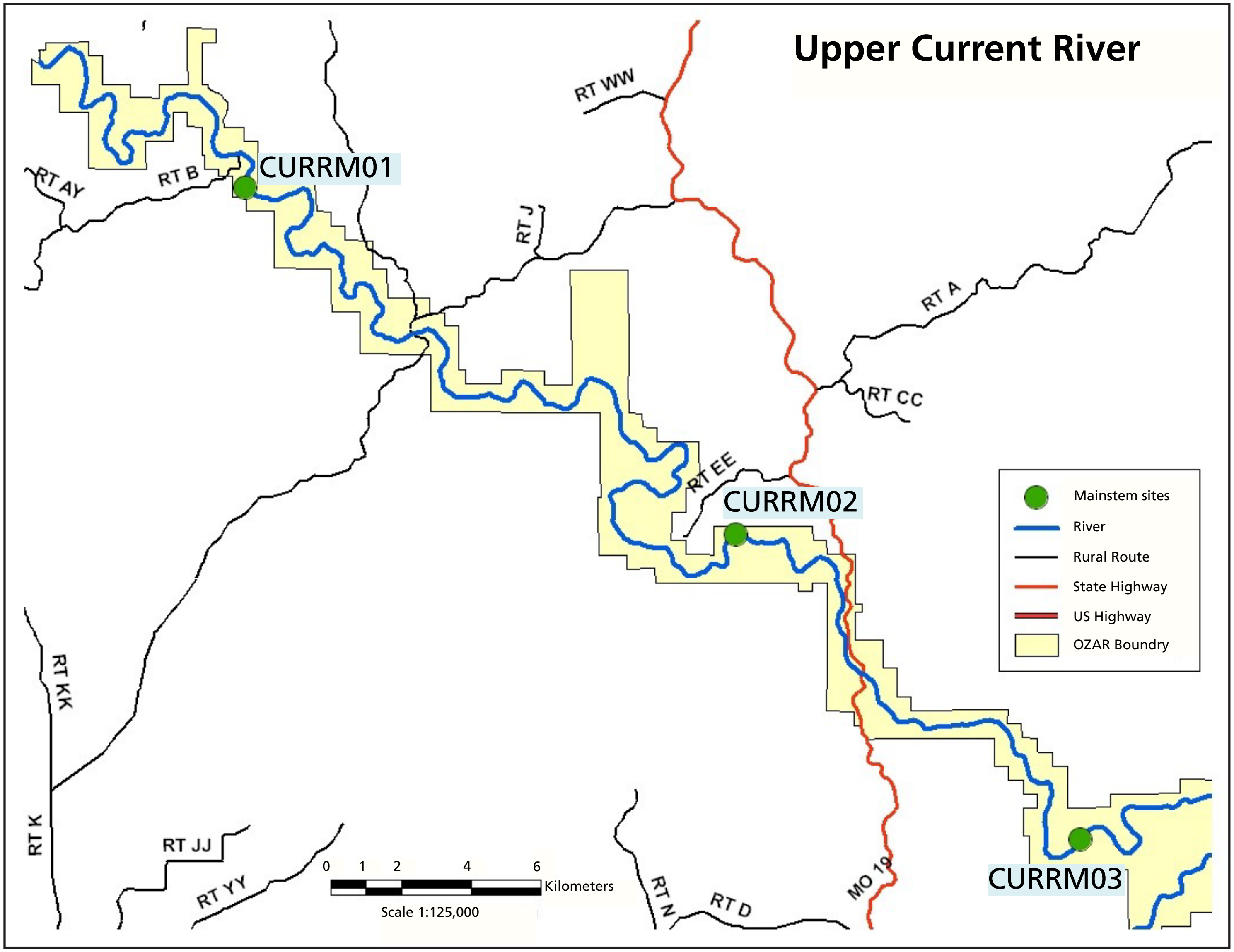

Figure A.4. Location of mainstem stretches selected for monitoring on the upper Current River.

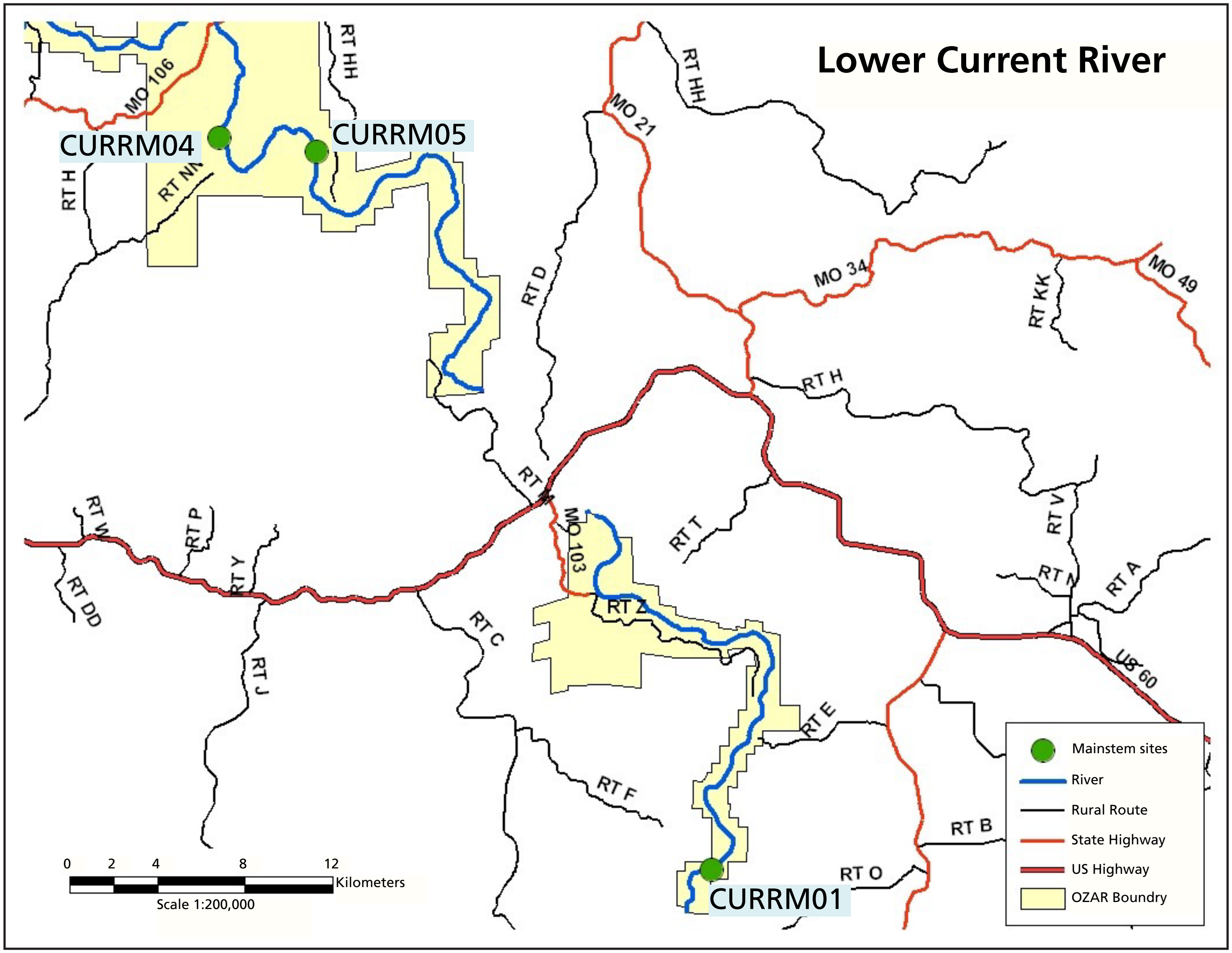

Figure A.5. Location of mainstem stretches selected for monitoring on the lower Current River.

Appendix B. Sample Site Codes and Global Navigation Satellite System (GNSS) Coordinates

Tables B.1 and B.2 provide details on sample sites at Buffalo National River and Ozark National Scenic Riverways.

Table B.1. Sample sites at Buffalo National River.

Reach ID	River Basin	Site Type	Site/Trib Number	Trib Name	Panel No.	County	Stretch ID	GRTS Easting Coordinate	GRTS Northing Coordinate	Lower Stretch Easting Coordinate	Lower Stretch Northing Coordinate
BUFFM01	Buffalo	Mainstem	01	–	Biennial	Newton	24	464088.50	3981659.30	464259.03	3981461.48
BUFFM02	Buffalo	Mainstem	02	–	Biennial	Newton	55	490340.90	3987599.90	490387.38	3987727.62
BUFFM03	Buffalo	Mainstem	03	–	Biennial	Newton	59	499985.28	3983483.70	499922.29	3983702.40
BUFFM04	Buffalo	Mainstem	04	–	Biennial	Searcy	69	520484.74	3981679.66	520396.87	3981741.88
BUFFM05	Buffalo	Mainstem	05	–	Biennial	Searcy	73	529619.95	3984878.60	529076.11	3984910.53
BUFFM06	Buffalo	Mainstem	06	–	Biennial	Marion	87	545997.97	3995359.40	545843.90	3995174.57
BUFFT09	Buffalo	Tributary	09	Little Buffalo	2	Newton	420	490340.91	3987600.00	490169.94	3987680.94
BUFFT30	Buffalo	Tributary	30	Middle	1	Marion	603	551428.31	3993556.50	551487.94	3993528.20
BUFFT31	Buffalo	Tributary	31	Leatherwood	1	Marion	623	551307.69	3996258.00	551410.83	3996233.10
BUFFT05	Buffalo	Tributary	05	Cecil	2	Newton	462	479905.44	3992743.25	479870.76	3992741.03
BUFFT07	Buffalo	Tributary	07	Mill	Biennial	Newton	646	487979.09	3990501.25	487933.18	3990589.49
BUFFT15	Buffalo	Tributary	15	Davis	Biennial	Newton	383	504216.16	3984923.25	504131.92	3985033.52
BUFFT19	Buffalo	Tributary	19	Calf	Biennial	Searcy	378	520463.22	3981045.50	520421.06	3981049.44
BUFFT20	Buffalo	Tributary	20	Bear	Biennial	Searcy	472	526905.38	3983413.50	526899.05	3983227.75
BUFFT23	Buffalo	Tributary	23	Water	2	Searcy	574	538186.50	3989492.75	537965.09	3989702.35
BUFFT27	Buffalo	Tributary	27	Clabber	1	Marion	476	540925.44	3998147.75	540821.63	3998527.10

Table B-2. Sample sites at Ozark National Scenic Riverways.

Reach ID	River Basin	Site Type	Site/Trib Number	Trib Name	Panel No.	County	Stretch ID	GRTS Easting Coordinate	GRTS Northing Coordinate	Lower Stretch Easting Coordinate	Lower Stretch Northing Coordinate
CURRM01	Current	Mainstem	01	–	Biennial	Shannon	14	623336.28	4141730.82	623330.398	4141819.863
CURRM02	Current	Mainstem	02	–	Biennial	Shannon	35	637468.41	4131822.17	637113.180	4131465.688
CURRM03	Current	Mainstem	03	–	Biennial	Shannon	42	643252.57	4126300.08	642631.838	4126422.535
CURRM04	Current	Mainstem	04	–	Biennial	Shannon	67	661785.40	4111783.86	662104.165	4112724.645
CURRM05	Current	Mainstem	05	–	Biennial	Shannon	71	666195.85	4111128.94	666087.431	4111411.528
CURRM06	Current	Mainstem	06	–	Biennial	Carter	97	684220.63	4078321.12	685209.621	4079792.188
JACKM01	Jacks Fork	Mainstem	01	–	Biennial	Shannon	105	619895.88	4101117.92	619901.956	4101344.874
JACKM02	Jacks Fork	Mainstem	02	–	Biennial	Shannon	114	627768.31	4102604.03	627579.024	4102191.921
JACKM03	Jacks Fork	Mainstem	03	–	Biennial	Shannon	123	633244.58	4108431.69	633476.295	4108173.626
CURRT08	Current	Tributary	08	Rocky	2	Shannon	657	661958.31	4110833.50	661931.524	4110852.285
JACKT03	Jacks Fork	Tributary	03	Shawnee	Biennial	Shannon	699	650824.38	4115207.50	650814.727	4115153.041
CURRT06	Current	Tributary	06	Blair	1	Shannon	917	659132.19	4116340.75	658985.647	4116351.733
CURRT14	Current	Tributary	16	Sinking Creek	Biennial	Shannon	n/a	640797.00	4129669.00	640779.257	4129682.694
CURRT15	Current	Tributary	15	Big Creek (W)	1	Shannon	n/a	623119.00	4141831.00	623206.262	4141756.210
CURRT16	Current	Tributary	16	Big Creek (E)	2	Shannon	n/a	648007.00	4124292.00	648341.558	4123747.999

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National Park Service
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