



# Protocol for Monitoring Spring Communities at Ozark National Scenic Riverways, Missouri

*Version 2.0*

Natural Resource Report NPS/HTLN/NRR—2021/2231



**ON THE COVER**

Herbert Hoover birthplace cottage at Herbert Hoover NHS, prescribed fire at Tallgrass Prairie NPres, aquatic invertebrate monitoring at George Washington Carver NM, and the Mississippi River at Effigy Mounds, NM

Photography by NPS

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February 2021

U.S. Department of the Interior  
National Park Service  
Natural Resource Stewardship and Science  
Fort Collins, Colorado

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Please cite this publication as:

Dodd, H. R., D. E. Bowles, J. A. Hinsey, J. T. Cribbs, L. W. Morrison, M. D. DeBacker, G. A. Rowell, J. L. Haack-Gaynor, and J. M. Williams. 2021. Protocol for monitoring spring communities at Ozark National Scenic Riverways, Missouri: Version 2.0. Natural Resource Report NPS/HTLN/NRR—2021/2231. National Park Service, Fort Collins, Colorado. <https://doi.org/10.36967/nrr-2284630>.

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# Protocol Revision History

The original protocol narrative (version 1.0) was published in 2008 (Bowles, Dodd et al. 2008). This new protocol (version 2.0) incorporates the recommended changes described in DeBacker et al. (2012). The primary changes recommended by DeBacker et al. (2012) were to change the revisit design so that invertebrates were collected from each spring on a 3-year rotation (see Table 4 in the Sampling Design chapter) and adding Phillips Spring as a sampling site. The other major change included here is to place vegetation sampling on a 3-year rotation as well instead of annually. 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 # |
|-----------------|---------------|---|---|--|---------------|
| 1.00            | 1/2020        | Hope R. Dodd, David E. Bowles, Janice A. Hinsey, J. Tyler Cribbs, Lloyd W. Morrison, Michael D. DeBacker, Gareth A. Rowell, Jennifer L. Haack-Gaynor, Jeffrey M. Williams | Changes to habitat data collection, data analysis, and a new temporal sampling design for plants and invertebrates. | Recommendations described in DeBacker et al. (2012) in part. | 2.0           |

# Standard Operating Procedures

SOP 1. Preparation for Field Sampling and Laboratory Processing

SOP 2. Training for Field Sampling and Laboratory Processing

SOP 3. Reach Selection

SOP 4. Documenting CORE 5 Water Quality Variables

SOP 5. Aquatic Vegetation Sampling

SOP 6. Aquatic Invertebrate Sampling

SOP 7. Laboratory Processing and Identification of Invertebrates

SOP 8. Fish Community Sampling

SOP 9. Physical Habitat Measurements

SOP 10. Measuring Spring Discharge

SOP 11. Data Management

SOP 12. Data Analysis

SOP 13. Reporting

SOP 14. Procedures and Equipment Storage after the Field Season

SOP 15. Revising the Protocol

# **Abstract**

Ozark National Scenic Riverways (OZAR) was established to preserve and interpret the free-flowing Current and Jacks Fork rivers, as well as other aquatic resources in the park. The extensive karst topography in the area surrounding OZAR is conducive to the formation of springs. Within the karst terrain of OZAR there are 425 known springs, including several first and second magnitude springs. Because springs at OZAR receive their baseflows from groundwater and only 5% of the watershed is protected within OZAR boundaries, contamination of groundwater and its direct impact on biological communities in the springs is of special concern. Due to the rapid recharge and transport of contaminants through the soluble bedrock system of caves, springs, and sinkholes, land use and other anthropogenic disturbances threaten the integrity of these springs. Thus, the NPS monitors the springs for indication of potential impacts. This protocol describes the background, justification, and detailed procedures for monitoring and analysis of the seven large springs (first and second magnitude) at OZAR, including water quality, instream and riparian habitat condition, aquatic vegetation, aquatic invertebrates, and fish.

# **Acknowledgments**

We thank our coworkers and peer reviewers whose comments improved upon the original protocol (Bowles, Dodd et al. 2008). 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>



# I. Background and Objectives

## Issues Being Addressed and Rationale for Monitoring Springs

Ozark National Scenic Riverways (OZAR) was established to preserve and interpret the free-flowing Current and Jacks Fork rivers. The park was designated for river corridor protection with narrow National Park Service (NPS) jurisdictional boundaries around the Current and Jacks Fork rivers. The jurisdictional boundary of OZAR encompasses only about 5% of the watershed of the Current and Jacks Fork Rivers with over 50% of the watershed in private ownership. Because a large portion of the watershed lies outside park boundaries, much of the watershed is left unprotected, placing the water quality of the rivers and tributaries at risk (Panfil and Jacobson 2001). Aquatic ecosystem condition and health are dependent on processes occurring in the entire watershed as well as in riparian and floodplain areas and cannot be manipulated independently of this interrelationship (Doppelt et al. 1993). The extensive karst topography in the area surrounding OZAR is conducive to the formation of springs. Because springs at OZAR receive their baseflows from groundwater, contamination of groundwater is of special concern due to the rapid recharge and transport of contaminants through the soluble bedrock system of caves, springs, and sinkholes.

Land use, particularly land clearing practices and associated increases in sediment load, nutrient loading, and other point and nonpoint sources, has been reported as the largest long-term threat to streams and springs in the Ozark Highlands (Duchrow 1977; Mott 1997; Scott and Udouj 1999). Land use practices at the watershed level can 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 a Midwestern watershed (Roth et al. 1996). Land use activities in the Ozark Highlands include timber management, landfills, grazing, swine and poultry operations, urbanization, gravel mining, stream channelization, removal of riparian vegetation, and lead-zinc mining.

Aquatic communities can be impacted from land use practices in the watershed, particularly from the conversion of forestland to pasture (Sweeney 1995).

Impacts to stream integrity 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. Increased bank erosion rates and changes in channel morphology through time have also been correlated with increased land clearing of steep uplands within a tributary basin (Stephenson and Mott 1992) as well as historical riparian land clearing (Jacobson and Primm 1997).

The NPS has mandated that the managers of OZAR establish baseline data or *vital signs* and long-term monitoring programs for the natural resources found within the park (DeBacker et al. 2005). This information will be used to address any current resource problems, while allowing managers to anticipate and plan for future resource problems. A specific mandate of this legislation is the “preservation of springs.” Within the karst terrain of OZAR there are 425 known springs (Bowles and Dodd 2016a). Although most of these springs are relatively small, seven are considered 1<sup>st</sup> and 2<sup>nd</sup> magnitude, defined as having discharges of at least 2.8 m<sup>3</sup>/second and 0.28 to 2.8 m<sup>3</sup>/second, respectively (Table 2; Meinzer 1927). The largest (Big Spring) has a maximum recorded discharge greater than 36 m<sup>3</sup>/sec and is ranked among the five largest springs in North America.

Assessment of chemical/physical characteristics in lotic systems is a common practice used to monitor aquatic conditions and determine potential areas of degradation or resource problems. This type of water quality assessment gives investigators immediate results but requires that sampling occur during or soon after a disturbance (such as high inputs of sediment or nutrients). Thus, chemical analysis six months or a year after a major disturbance may not indicate a problem. Because the “most direct and effective measure of the integrity of a water body is the status of its living systems” (Karr and Chu 1999), it follows that the main focus for a system of vital sign monitoring of spring resources should be the living component—thus providing the rationale for this biological monitoring protocol. A comprehensive monitoring program should include biotic indicators (vegetation, invertebrates, and fish) that respond or

are linked to the physical and chemical conditions within the system. Information obtained from monitoring vegetation, invertebrates, and fish, together with chemical and physical data, provides the most integrated and robust assessment of water quality and ecosystem integrity.

In order to assess the natural and anthropogenic processes influencing aquatic vegetation, invertebrate, and fish communities within the larger springs at OZAR, this protocol has been designed to incorporate the spatial relationship of these biotic indicators with chemical constituents and physical habitat. Local variables, such as conductivity, water temperature, pH, dissolved oxygen, turbidity, current velocity, substrate size, and other habitat variables will be measured. Springs have a mosaic structure, a high degree of individuality, and typically an azonal character attributed to their physiochemical stability (Cantonati et al. 2006). Because of the previously mentioned threats to the ecological functioning of spring systems, these unique habitats are imperiled. Therefore, the framework for monitoring the large springs at OZAR is directed towards maintaining their ecological integrity and this will be assessed through periodic monitoring of aquatic vegetation, invertebrates, and fish communities.

Mosses, algae, and higher plants are particularly important structural and biological constituents of springs and aquatic systems in general (Hannan and Dorris 1970; Cushing and Wolf 1984; Carpenter and Lodge 1986; Durante and Canfield 1990; Stream Bryophyte Group 1999; Cantonati et al. 2006), and they often have complex relationships with some fish and aquatic invertebrates (Cyr and Downing 1988; Xie et al. 2005, 2006). Additionally, aquatic plants are important biological filters of a variety of chemical contaminants and nutrients (Demars and Harper 1998; Cantonati et al. 2006; Vardanyan and Ingok 2006).

Growth of aquatic vegetation is influenced by a number of factors, including light and nutrient availability, water chemistry and dissolved gases, temperature, herbivory, and a broad array of physical characteristics, including channel size, canopy cover, water depth, substrate size, and current velocity (Spence 1967; Haslam 1978; Dawson and Kern-Hansen 1978, 1979; Chambers et al. 1991; Onaindia et al. 1996; Barendregt and Bio 2003; Bernez et al. 2004a). As such, aquatic vegetation communities in

spring ecosystems are vulnerable to a broad variety of anthropogenic disturbances (Sanford 1979; Englund 1991; Schütz 1995; Preston et al. 2003). Because of these vulnerabilities, monitoring changes in aquatic vegetation has long been used as an indicator of anthropogenic disturbance throughout Europe, although this approach has received little attention in the United States (Romero and Onaindia 1995; Tremp and Kohler 1995; Small et al. 1996; Bartodziej and Ludlow 1997; Ali et al. 1999; Schorer et al. 2000; Bernez et al. 2001, 2004b; Haury et al. 2002; Scott et al. 2002; Daniel et al. 2005; Haslam 2006). Previous studies have found that certain hydrophytes and mosses are more sensitive to anthropogenic disturbance than others (Haslam 1982; Carbiener et al. 1990; Poole and Bowles 1999; Bernez et al. 2001, 2004b; Haury et al. 2002). As Haslam (2006) stated, “the use of river plants as bioindicators is often undervalued. If autecology and the country of origin are known, and the species list long enough, interpretation is astonishingly accurate, for both natural factors and human impact.”

Invertebrates are an important tool for understanding and detecting changes in aquatic 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 taxa richness, or appreciable shifts in community composition relative to the reference condition (Plafkin et al. 1989).

Fish communities of Ozark lotic systems are important components of their aquatic ecosystems. The Ozark Highlands is one of the richest areas of the United States for fish species. More than 175 native and introduced species occur in the Ozarks with several of these species being unique to this region (Petersen 1998). The Current River basin is considered a *hot spot* for fish species that are designated as vulnerable or imperiled by The Nature Conservancy and the Natural Heritage Network (Master et al. 1998). Because many of these species are considered intolerant of habitat alterations (Robison and Buchanan 1988; Pfieger 1997; Dauwalter et al. 2003), fish community assemblages serve as a monitoring tool to assess changes in water and habitat quality for a number of ecological studies within the park (Hoefs 1989; Petersen 1998, 2004; Dodd 2013; Dodd et al. 2018). In addition to their importance as environmental indicators, direct economic value can also be associated with several fish species that are actively sought by anglers. Because the public is familiar with fish as both an environmental indicator and as a recreational opportunity, the status and change in fish diversity is of concern to resource managers and is easily interpreted to park visitors.

Habitat conditions within a spring are based on its groundwater sources and a matrix of other factors (Danks and Williams 1991). Terrain features shaped by the geology and topography determine the amount and variability of the water supply and the levels and variability of the temperature and water chemistry. Conditions above the surface are modified by the local climate, size of the spring, habitat diversity, and vegetation (both within and surrounding the spring). Species compositions associated with springs are influenced by differences in surrounding vegetation, substrate, pH, and other factors such as water chemistry (Williams and Danks 1991). Studies of the possible effects of climate change indicate that spring communities may be impacted due to increases in annual average water temperature and changes in discharge (Erman and Erman 1995; Williams et al. 1995; Hogg and Williams 1996; Taylor et al. 2012; Bowles and Dodd 2016a).

## History of Monitoring Springs at Ozark National Scenic Riverways

Biomonitoring of springs has not received the level of attention given to wadeable streams in North America. Initial biomonitoring efforts on surface waterways focused on detecting point source disturbances

rather than vague non-point source problems. Karst systems are considered to be highly vulnerable to pollution and have limited self-purification potential due to reduced adsorption, making them vulnerable to biological and chemical degradation (Gibert 1990). Thus, due to their vast and often unknown recharge areas, karst springs may represent the ultimate challenge in water quality protection.

Despite a large amount of literature on groundwater contamination and its complexity, there is almost no information on what happens when contaminated groundwater emerges at the surface through a spring and how it may affect surface water biota (Williams 1991; Notenboom et al. 1994; van der Kamp 1995). Potential groundwater contaminants include toxic compounds such as heavy metals, pesticides and xenobiotic organics, nitrogen and phosphorous compounds, and organic matter from sewage or other animal wastes. All of these contaminants may cause adverse effects on groundwater fauna such as bioaccumulation, biotransformation, increased density, and distress by anoxia.

Early works on springs in North America provided comprehensive information on single spring systems (Davidson and Wilding 1943; Sloan 1956; Odum 1957; Teal 1957; Minckley 1963; Minshall 1968; Tilly 1968; Stern and Stern 1969; Wilhm 1970), but there is a general lack of knowledge regarding the biological communities inhabiting different types of springs (Matthews et al. 1983; Ferrington 1995). This may have been because spring communities typically are represented by fewer species and have less diversity than streams due to relatively constant temperature regimes, mineralization (high dissolved solids), low dissolved oxygen, absence of plankton as a food source, and depauperate habitats (Van Gundy 1973; Williams and Danks 1991). However, over the previous two decades, there has been a resurgence of interest in the biodiversity of permanent springs in North America (Glazier and Gooch 1987; Danks and Williams 1991; Glazier 1991; Gooch and Glazier 1991; Williams and Danks 1991; Erman 1992; Blackwood et al. 1995; Erman and Erman 1995; Hargis 1995; Mattson et al. 1995; Webb et al. 1995, 1998; Williams et al. 1997; Poole and Bowles 1999; Williams and Williams 1999; Bowles et al. 2003, Bowles et al. 2007). This may be due to a growing interest in biodiversity and the accelerating loss of spring habitats worldwide (Erman and Erman 1995; Bowles et al. 2007).

Because of the complicated nature of monitoring large springs such as those at OZAR, most state agencies, including Missouri, have not addressed biomonitoring protocols for springs within their states. As of yet, there have not been any publications or protocols detailing relationships among the invertebrates, fish, vegetation, and water quality of the springs proposed for monitoring.

### **History of Aquatic Vegetation Monitoring**

No long-term biomonitoring programs for aquatic vegetation have been conducted for the springs at OZAR. However, there have been some studies of algae and aquatic vegetation in these springs that collectively serve as a useful baseline for developing this protocol. Drouet (1933) reported on the algae found in Alley, Big, and Round springs in addition to listing a few unsubstantiated records for vascular plants. Steyermark (1941) conducted the first known inventory of aquatic vegetation in Missouri springs including several springs at OZAR and corrected several misidentifications apparently made by Drouet (1933).

Currier (1990a, b) conducted floristic inventories for Alley, Big, Blue, and Round springs. These inventories were intended to compare the aquatic plant diversity of these springs to Steyermark's study (Steyermark 1941). Currier concluded that the plant composition in several springs had changed since Steyermark's inventory, with some species disappearing and others documented for the first time. For example, Currier (1990b) noted that *Zannichellia palustris*, *Potamogeton foliosus*, and submerged stands of the non-native *Poa annua* documented from Big Spring in Steyermark's study were absent in his study. Lipscomb (undated) also conducted a floristic inventory of the aquatic plants occurring in the large springs at OZAR. His findings were similar to those of the previous studies, and he found these three species were present. Steyermark (1941) did not record *Lemna trisulca* or *Ranunculus longirostris* from Big Spring, but these species are now present and abundant there (Currier 1990b; Lipscomb undated). The findings of Steyermark (1941), Currier (1990a, b) and Lipscomb (undated) suggest the aquatic plant community in these springs is dynamic.

Redfearn et al. (undated) conducted a botanical survey of OZAR and listed some aquatic species. However, their effort focused primarily on upland and wetland species. Conrad and Redfearn (1979),

in a broad treatment of the mosses and liverworts, addressed some aquatic species known to occur at OZAR. Redfearn (1981) addressed the rich diversity of bryophytes occurring in some Missouri springs, including all of the springs in this protocol. These springs each hold eight or more species of mosses and liverworts.

Converse (1994) conducted a study of macrophyte production in relation to water chemistry and nutrient dynamics at Big Spring. The focus of his research was on the production of three species: *Ranunculus longirostris*, *Nasturtium officinale*, and *Veronica comosa* (syn. *V. anagallis-aquatica*). Converse (1994) also reported water quality data for the large springs at OZAR, showing they were quite similar and stable (Table 1). Other constituents (i.e., total particulate phosphorus, soluble reactive phosphorus, particulate organic nitrogen, dissolved organic carbon, calcium, magnesium, sodium chloride, alkalinity, and turbidity) reported by Converse (1994) were not constant seasonally. These data suggest that the aquatic plant communities of Big Spring, and other springs at OZAR, are vulnerable to disturbances and nutrification associated with stormwater runoff. Converse (1994) further noted that the macrophyte production in Big Spring was one of the highest values ever reported for lotic ecosystems (15 g-dw m<sup>-2</sup> d<sup>-1</sup>), and the aquatic vegetation in all springs demonstrated a high biomass turnover.

Edwards (2002) surveyed the aquatic plants of the Jacks Fork River and included Alley Spring. He reported 19 species from Alley Spring; 11 were wetland type plants that were found on the banks and margins of the spring-run, while only eight species were truly aquatic. Edwards (2002) noted the occurrence of distinct plant communities occurring in the Jacks Fork River Basin, and he further described the community at Alley Springs as being characterized as a *Cardamine bulbosa*-*Veronica catenata* (junior synonym of *V. anagallis-aquatica*) community. However, pilot surveys by Heartland Network (HTLN) staff during 2005 and 2006 showed the diversity of aquatic plants occurring at Alley Spring is higher than reported by Edwards (2002) and the community there is better characterized as a *Veronica anagallis-aquatica*-*Elodea canadensis/nuttalli*-*Sparganium americanum* complex.

Padgett (2001) reported on the first occurrence of the exotic hydrophyte *Myriophyllum spicatum* (Eurasian

**Table 1.** Physical and chemical parameters for large springs at OZAR, 2007–2016, from program data (see Bowles et al. 2018 for more detail).

| Spring   | Statistical Metric | Temperature (°C) | Specific Conductance (µm/sec) | Dissolved Oxygen (mg/l) | pH        | Turbidity (NTU) |
|----------|--------------------|------------------|-------------------------------|-------------------------|-----------|-----------------|
| Alley    | Mean               | 13.78            | 259.37                        | 9.62                    | 7.25      | 1.14            |
|          | Standard Error     | 0.04             | 13.04                         | 0.37                    | 0.07      | 0.41            |
|          | Range              | 13.56–14.07      | 175.90–312.71                 | 8.31–11.85              | 6.83–7.52 | 0.02–3.34       |
|          | N                  | 10               | 10                            | 10                      | 10        | 10              |
| Big      | Mean               | 14.22            | 312.87                        | 9.20                    | 7.32      | 1.63            |
|          | Standard Error     | 0.04             | 10.37                         | 0.38                    | 0.10      | 0.42            |
|          | Range              | 13.94–14.40      | 251.67–349.74                 | 8.17–12.05              | 6.94–8.06 | 0.41–4.36       |
|          | N                  | 10               | 10                            | 10                      | 10        | 9               |
| Blue     | Mean               | 13.97            | 195.29                        | 8.96                    | 7.32      | 0.93            |
|          | Standard Error     | 0.23             | 15.70                         | 0.34                    | 0.09      | 0.51            |
|          | Range              | 13.38–15.35      | 127.93–272.67                 | 7.10–10.22              | 6.76–7.60 | 0.00–5.07       |
|          | N                  | 10               | 9                             | 10                      | 10        | 10              |
| Phillips | Mean               | 15.36            | 284.71                        | 7.59                    | 7.35      | 0.44            |
|          | Standard Error     | 0.18             | 10.35                         | 0.40                    | 0.15      | 0.19            |
|          | Range              | 14.50–15.65      | 255.17–314.94                 | 6.07–8.78               | 7.09–8.08 | 0–1.14          |
|          | N                  | 6                | 6                             | 6                       | 6         | 6               |
| Pulltite | Mean               | 13.83            | 275.49                        | 8.36                    | 7.29      | 2.30            |
|          | Standard Error     | 0.08             | 8.66                          | 0.19                    | 0.08      | 1.09            |
|          | Range              | 13.42–14.23      | 231.98–310.36                 | 7.46–9.48               | 6.83–7.81 | 0.01–10.93      |
|          | N                  | 10               | 10                            | 10                      | 10        | 10              |
| Round    | Mean               | 13.62            | 258.26                        | 8.77                    | 7.35      | 1.70            |
|          | Standard Error     | 0.12             | 19.28                         | 0.29                    | 0.07      | 0.60            |
|          | Range              | 12.90–14.02      | 181.00–353.45                 | 6.51–9.71               | 6.84–7.75 | 0.26–5.64       |
|          | N                  | 10               | 10                            | 10                      | 10        | 10              |
| Welch    | Mean               | 13.61            | 310.67                        | 9.40                    | 7.27      | 0.94            |
|          | Standard Error     | 0.03             | 10.42                         | 0.33                    | 0.09      | 0.59            |
|          | Range              | 13.46–13.80      | 238.00–355.87                 | 8.41–11.14              | 6.81–7.86 | 0–5.00          |
|          | N                  | 10               | 10                            | 9                       | 10        | 8               |

watermilfoil) from several locations in Missouri including some from OZAR. Other non-native species known to occur in the springs include *Nasturtium officinale*, *Poa annua*, (Steyermark 1941), and *Mentha piperita*.

Subsequent to Bowles et al. (2008), aquatic plant monitoring data were presented and analyzed in Bowles et al. (2011) and Bowles and Dodd (2015, 2016b). Bowles and Dodd (2015) listed 69 distinct

taxa with substantial overlap of species occurrences among springs, including 6 families, 6 genera, and 6 species of algae, and 9 families, 12 genera, and 19 species of mosses and liverworts. Among angiosperms, we reported 10 families, 13 genera, and 20 species of monocots, and 16 families, 23 genera, and 24 species of dicots. Individual sample cells typically contained four to six taxa, although Welch Spring generally had only two to three taxa represented.

Effective numbers of species were generally consistent among years for all springs, but the various species did not occur in equal abundance in the community within or among sample years. Taxa richness was slightly higher than Simpson's Diversity Index effective number ( $D_e$ ) and Shannon Diversity Index effective number ( $H_e$ ) for all years among springs.  $H_e$  ranged from 1.34 to 3.76 among sampling years and springs, with values for Alley and Blue springs of approximately 3.00 while those for Big, Pulltite, Round, and Welch springs were closer to 2.00.  $D_e$  ranged from 1.25 to 3.86 among sampling years and springs. Few nonnative plant species occurred in the springs, and they accounted for <15% of the foliar cover across transects.

### **History of Aquatic Invertebrate Monitoring**

Several studies have been done on invertebrates occurring in Missouri springs outside of OZAR (Sullivan 1928; Bonham 1962; Doisy 1984; Sarver and Kondratieff 1997; Mathis 1999) and in contiguous states such as Arkansas (Robison 1981; Mathis 1994; Hargis 1995; Bowles 1998; Jackson 2001; Usrey 2001), Iowa (Kennedy and Miller 1990), Illinois (Webb et al. 1995, 1998; Bade et al. 2002), Oklahoma (Matthews et al. 1983; Bass 2000; Gaskin and Bass 2000; Rudisill and Bass 2005), Kentucky (Minckley 1963; Rayburn and Freeze 1978), and Kansas (Blackwood et al. 1995; Ferrington et al. 1995). Other broader regional treatments of the Ozark Plateau include those of Holsinger (1989) and Koppelman and Figg (1995).

Published studies concerning invertebrates that include the springs and spring-runs of OZAR include information on their assemblages (Vineyard et al. 1974; Gardner 1984; Gardner and Taft 1984; Gardner 1986; Nielsen 1996; Ferro and Sites 2008) and specific families, orders, or classes, such as Chironomidae (Blackwood 2001), Ephemeroptera (Wiersema and Burian 1999), Plecoptera (Poulton and Stewart 1991), Trichoptera (Moulton and Stewart 1996), Odonata (Trial and Belshe 2002), Decapoda (Pfleiger 1996), Amphipoda (Sarver and Lister 2004), and Gastropoda (Wu et al. 1997). The Missouri Department of Conservation maintains a database with all known collection records for the state (Missouri Department of Conservation 2003), and their Missouri Species of Conservation Concern Checklist (Missouri Natural History Program 2003) lists several species of invertebrates from springs within the state.

Species listed as rare and uncommon within Missouri that are known to occur in either Shannon or Carter counties include a stonefly (*Allocapnia pygmaea*; collected from Round Spring, date unknown) and the Salem Cave Crayfish (*Cambarus hubrichti*). Although numerous studies have addressed aquatic invertebrates at OZAR, no previous long-term monitoring of aquatic invertebrate communities have been conducted for the large springs.

Subsequent to Bowles et al. (2008), aquatic invertebrate monitoring data were presented and analyzed in Bowles et al. (2011, 2018). Those studies showed that all water quality parameters monitored and the invertebrate communities indicate that conditions among the respective springs are good. The invertebrate fauna occurring in all springs was dominated by environmentally sensitive taxa. The dominance of intolerant taxa in the springs, mainly representatives of the caddisfly genus *Lepidostoma*, indicates their respective water quality conditions and habitat are not disturbed.

### **History of Fish Monitoring**

The Ozark Plateau has a rich diversity of freshwater fishes with 112 species of fish reported to occur in or near OZAR (NPS 2005). Fish communities of Ozark streams and springs are important components of their respective ecosystems. Several species (including several darters, minnows, and madtoms) occurring at OZAR are considered intolerant of habitat alterations and poor water quality conditions (Robison and Buchanan 1988; Pfleiger 1997; Dauwalter et al. 2003). Therefore, fish assemblages are a useful monitoring tool to assess changes in water and habitat quality for a number of ecological studies within the park (Hoefs 1989; Petersen 1998, 2004). Large springs, such as those at OZAR, have been shown to serve as key thermal refugia for some stream fishes during winter and summer months when harsh temperature fluctuations occur in main-stem streams (Peterson and Rabeni 1996). Although the fish communities of the Current River Basin are generally well known, there have been no exhaustive surveys or long-term monitoring conducted on the fish assemblages of the large springs in this basin. Moreover, there have been few studies of spring-dwelling fish communities for the entire Ozark region (Matthews et al. 1985).

Subsequent to Bowles et al. (2008), fish community monitoring data were presented and analyzed in

Bowles et al. (2011). Fish communities consisted largely of species sensitive to poor water quality conditions and siltation. Knobfin sculpin (*Cottus hypselurus*) and banded sculpin (*Cottus carolinae*) were the dominant species in most springs, with the exception of Round Spring, which consisted largely of bleeding shiner (*Luxilus zonatus*).

## Rationale for Selecting this Resource to Monitor

OZAR lies within the Salem Plateau, one of three subdivisions of the Ozark Plateau, and is composed predominantly of dolomite, limestone, and sandstone, with elevations below 245 m (Nigh and Schroeder 2002). Accordingly, springs in this region typically are well-buffered with high pH, usually in the 7.5–8.5 range. Within the Ozark Plateau, the main factors affecting water quality include geology, land use, and population density. The geology of this area is dominated by mineral dissolution, ion exchange, and oxidation-reduction reactions (Adamski et al. 1995). The Ozark Plateau is characterized by *karst* topography (springs, sinkholes, and caves), meaning that the surface and groundwater are integrally connected. One difference in the groundwater from karst systems is that it may be oxygenated, due to contact with air spaces within channels and caves. Another difference is that unlike groundwater that is filtered through dense soil layers, groundwater in karst systems often moves rapidly through underground channels that fail to provide effective natural filtration and absorption. As a result, these waters often contain contaminants and pollutants not found in groundwater from other types of systems.

Several studies have addressed the water quality of various springs and groundwater within OZAR (Missouri Bureau of Geology and Mines 1926; Beckman and Hinckley 1944; Aley 1973a, b; Vineyard et al. 1974; Aley 1975, 1976a, b, 1978; Barks 1978; Aley and Foster 1979; Aley and Aley 1982; Aley 1987; Converse 1994; USGS 1995; Adamski 1996; Bell et al. 1996; Adamski 1997). Nutrient data from 395 groundwater samples in the Ozark Plateau showed that nutrient concentrations, in particular nitrite plus nitrate concentrations, were related to hydrogeology and land use. Approximately 4% of these samples had nitrite plus nitrate concentrations that exceeded the maximum concentration level allowed in drinking water by the EPA (Davis et al. 1995). Additional studies of this problem reported that nitrite plus nitrate

was the nutrient most often found and indicated a relationship between the nutrients in groundwater and land use in the area (Steele et al. 1987; Adamski 1997).

Aley and others have provided an extensive series of reports delineating the groundwater recharge areas and the resultant potential problems for many of the springs within OZAR (Aley 1973a, b, 1975, 1976a, b; Vineyard et al. 1974; Aley and Foster 1979; Aley and Aley 1982; Aley 1987). The crux of the problem for OZAR managers is that almost all of the recharge areas for the major springs within the park are outside the control of the NPS. A study of the OZAR watershed identified 378 point-source hazard areas within these privately held lands (Aley 1987). These included (1) sewage disposal facilities, (2) dumps, landfills, and salvage yards, (3) industrial sites, (4) transportation routes, including major pipelines, (5) petroleum storage sites, including service stations, and (6) chemical storage sites. Of specific concern are the effects of mining and waste disposal in the recharge zones, conversion of forestland to pasture, pipeline leakage, and impacts from recreational use such as increased nutrient input and bank erosion (Davis and Barr 2006).

Due to monetary, logistical, and staffing constraints, this protocol will focus on the spring-runs of the permanent, high discharge, and coldwater springs of OZAR. Reasons for the choice of the large springs include their value as major tourist attractions within the state, detailed mapping of recharge areas for the larger springs, and contribution of base flow to the Current and Jacks Fork rivers. The low variability in water chemistry and temperature of these spring-runs results in more stable biotic communities than those in smaller springs, aiding in higher statistical precision with less sampling effort.

Surveys of aquatic vegetation, fish, and invertebrate communities are important to researchers and managers to establish inventories and aid in the diagnosis of ecosystem disturbances such as water quality degradation and introduction of non-native (i.e., exotic) species. The methods we propose here for monitoring are repeatable, straight-forward, and easy to understand, thus allowing more precise information to be collected by various personnel in a time-effective manner. This protocol will be a primary aid in determining the ecological integrity of the large springs at OZAR. Specifically, data collected from

the springs will provide a baseline for assessing the potential for, or extent of, a variety of anthropogenic and natural disturbances.

## Measurable Objectives

Two broad objectives are addressed by this protocol:

1. determine the annual status and trends in species diversity, abundance, and community metrics for vegetation, invertebrates, and fish occurring in the large springs at OZAR, and
2. relate the community data to overall water quality and habitat condition (DeBacker et al. 2005).

*Justification/Rationale for These Objectives:* The structure of biotic communities of OZAR springs has not been consistently inventoried or monitored. The respective watersheds and recharge areas of the springs remain largely unprotected, leaving the springs vulnerable to disturbance. Estimating natural variability of the vegetation, invertebrate, and fish communities within the springs will further aid in defining sample precision, the number of samples required, and the minimum detectable difference in mean community metric values. The initial years

of data collection should adequately address the questions regarding natural variability and baseline water quality conditions. Measuring water quality, habitat structure and availability, and watershed land use patterns and correlating these with aquatic vegetation, invertebrate, and fish community composition will allow insight into the influences these variables have on the natural integrity of the springs at OZAR. A negative trend from the baseline data could be indicative of impairment or disturbance of the springs and could be a basis for more targeted studies.

## 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

### Spatial Design

#### *Establishing the Sample Frame*

We have developed an integrated aquatic monitoring plan for springs at OZAR, which includes the co-location and co-visitation of invertebrate, fish, and vegetation sampling. This protocol only addresses spring communities occurring within NPS jurisdictional boundaries (Table 2). This protocol is intended to evaluate the biological integrity of the springs downstream of the spring source and is not intended to assess the entire spring-run. Therefore, our common sample unit definition is a *reach* of the spring-run of some minimum and maximum length. Because of physical limitations of accessing the spring sources, including extreme depth and crew safety, sampling will be conducted on equally spaced transects beginning at the first accessible area of each spring. The effective sampling area of each spring-run will be based on safety of personnel, accessibility, ability to co-locate sampling areas for measured parameters, and other pertinent factors determined by the investigators at the time of sampling.

#### *Sampling Reaches within Springs*

The source or mouth of a spring usually maintains a constant or near-constant temperature, while the downstream spring-run may vary in temperature due to distance from the source, riparian canopy, and other factors. This increase in downstream temperature variability can impose very different constraints

on biological communities, resulting in longitudinal zonation of many of the taxa (Williams and Hogg 1988; Erman 1998; Cantonati et al. 2006). Such longitudinal effects may introduce confounding information into biological sampling programs. Although many studies have focused on the spring source in an effort to increase sampling consistency, the sources of the large springs at OZAR are too deep to sample practically or safely. Therefore, this protocol proposes that the spring-run or brook immediately below the source will be sampled.

At each spring, a sampling reach will be established that satisfies specific requirements necessary to obtain a representative and unbiased sample for all abiotic and biotic parameters being monitored (see SOP #3, Reach Selection, for details). The reach will be representative of the spring-run, containing various instream habitats and geomorphic channel units characteristic of the spring-run. Sampling multiple habitats often provides more comprehensive information about the invertebrate fauna compared to single habitat samples (Lenat and Barbour 1994). Many spring-runs consist primarily of run or riffle channel units. Therefore, the representative reach may only contain these channel units.

The portion of the spring-run to be sampled is unique for each spring because of their unique sizes and other physical characteristics. To ensure a uniform sampling effort that is representative of each spring's total area, we applied a weighting factor that

**Table 2.** OZAR springs chosen for the spring monitoring protocol.

| Spring Name | Spring Magnitude and Type (Meinzer 1927) | County  | Quad Map     | GPS Coordinates (UTME, UTMN)* | Rate of Flow (m <sup>3</sup> /sec) (Vineyard et al. 1974) |
|-------------|--|---------|--------------|-------------------------------|---|
| Alley       | 1 <sup>st</sup> , conduit                | Shannon | Alley Spring | 638380, 4113105               | 1.51–29.68  |
| Big         | 1 <sup>st</sup> , conduit                | Carter  | Big Spring   | 640182, 4002168               | 6.61–36.40 (est.)   |
| Blue        | 1 <sup>st</sup> , conduit                | Shannon | Powder Mill  | 663087, 4114859               | 1.74–6.61   |
| Phillips    | 2 <sup>nd</sup> , conduit                | Carter  | Big Spring   | 683229, 4078642               | 0.25–0.55   |
| Pulltite    | 2 <sup>nd</sup> , conduit                | Shannon | Round Spring | 633755, 4133111               | 0.16–3.98   |
| Round       | 2 <sup>nd</sup> , conduit                | Shannon | Round Spring | 641162, 4127406               | 0.28–14.56  |
| Welch       | 1 <sup>st</sup> , conduit                | Shannon | Cedar Grove  | 626198, 4139518               | 1.96–9.27   |

\* Universal Transverse Mercator (Zone 15), horizontal datum is North American Datum 1983.

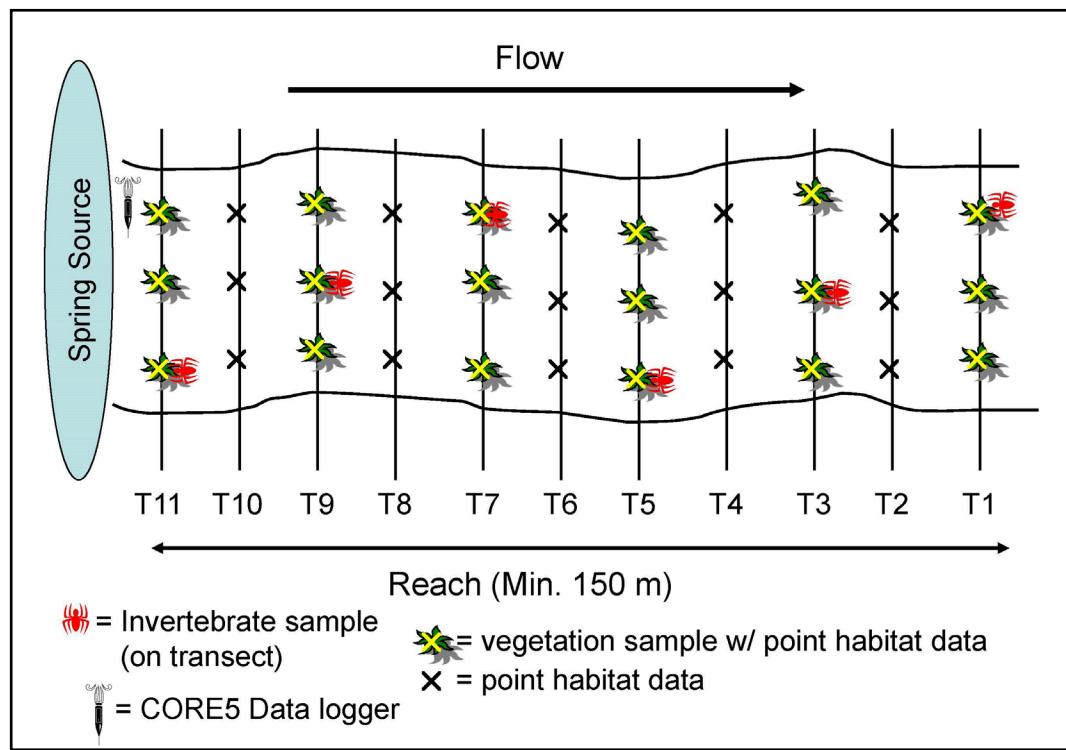
accounts for variation among average widths (Table 3). The weighting factor uses the minimum average width of 15 m recorded for Pulltite Spring and Welch Spring. To determine the weighting factor, the average width of the spring is divided by 15. This weighting factor is multiplied by 150 m, the minimum reach recommended by NAWQA (Moulton et al. 2002), to determine sampling reach length. The designated sampling reach lengths will allow for inclusion of representative macrohabitats (riffle, run, and pool habitats) present within the springs, although run habitats dominate all other habitat types. Because the lengths of the springs are relatively short, the reach will begin as close to the spring

source as possible. Once located, this reach will become a permanent sampling site barring dramatic alterations in spring morphology that would require re-establishing the sampling reach.

Once the sampling reach is established, it is divided into 11 equally spaced transects beginning at the first accessible area of each spring and proceeding downstream (Figure 1). The only exception to this will be Welch Spring, where only three transects will be used because the entire spring-run is less than 100 m in length. Procedures for establishing sample points within each spring-run are described in SOP #9 (Physical Habitat Measurements).

**Table 3.** Weighting factors and length of sampling reaches for large springs at OZAR.

| Spring Name | Total Length (meters) | Average Width (meters) | Weight Factor (WF) | 150 m X WF | Transect Interval (m) |
|-------------|-----------------------|------------------------|--------------------|------------|-----------------------|
| Alley       | 800                   | 19                     | 19/15 ≈ 1.3        | 190 m      | 19                    |
| Big         | 600                   | 46                     | 46/15 ≈ 3.1        | 460 m      | 46                    |
| Blue        | 250                   | 16                     | 16/15 ≈ 1.1        | 160 m      | 16                    |
| Phillips    | 575                   | 15                     | 15/15 ≈ 1.0        | 150 m      | 15                    |
| Pulltite    | 250                   | 15                     | 15/15 ≈ 1.0        | 150 m      | 15                    |
| Round       | 300                   | 24                     | 24/15 ≈ 1.6        | 240 m      | 24                    |
| Welch       | 36                    | 15                     | 15/15 ≈ 1.0        | 36 m       | 18                    |



**Figure 1.** Transect location and layout within a spring-run.

**Table 4.** Proposed revised revisit plans for monitoring studies at seven large springs at OZAR. “X” indicates all sample units in that panel are to be visited that year.

| Study                             | Spring Name             | Year             |      |      |      |      |      |      |      |      |      |      |      |      |      |
|-----------------------------------|-------------------------|------------------|------|------|------|------|------|------|------|------|------|------|------|------|------|
|                                   |                         | Revisit Notation | 2018 | 2019 | 2020 | 2021 | 2022 | 2023 | 2024 | 2025 | 2026 | 2027 | 2028 | 2029 | 2030 |
| Vegetation, Invertebrates, & Fish | Alley, Phillips, Welch* | [1-2]            | -    | X    | -    | -    | X    | -    | -    | X    | -    | -    | X    | -    | -    |
|                                   | Blue, Round             | [1-2]            | -    | -    | X    | -    | -    | X    | -    | -    | X    | -    | -    | X    | -    |
|                                   | Big, Pulltite           | [1-2]            | X    | -    | -    | X    | -    | -    | X    | -    | -    | X    | -    | -    | X    |

\* Fish sampling will no longer occur in Welch Spring; only vegetation and invertebrate monitoring will be conducted at this spring.

## Temporal Design

In version 1.0 of the Springs Community Protocol (Bowles, Dodd et al. 2008), aquatic vegetation, invertebrates, instream habitat, and water quality data were collected annually at each spring. Fish communities and fish-related habitat data were collected on a three-year rotation at each spring. In this revised version (V 2.0) of the protocol, all springs will be placed on a three-year rotation for aquatic vegetation, invertebrates, fish, habitat, and water quality (Table 4). DeBacker et al. (2012) proposed shifting invertebrate sampling frequency from annually to triennially and that has been the approach used since 2012. Aquatic vegetation is now also placed on a three-year rotation based on concerns for environmental sensitivity of the springs as well as documented consistency of the aquatic plant communities in the springs (Bowles and Dodd 2015, 2016b). Due to the sensitivity of Welch Spring, fish sampling will no longer be conducted at this spring. This strategy will yield maximum information on trend without inflicting damage to ecologically sensitive springs.

Prior to HTLN sampling and protocol development for OZAR springs (Bowles, Dodd et al. 2008), little was known about the biotic communities of these systems. Although these communities are quite diverse, the dynamic nature of their respective populations has never been addressed prior to HTLN monitoring. Because of the thermal constancy of the springs and spring-runs, the sampling index period does not have to be constrained to any particular time period. Most of the aquatic hydrophytes occurring in the springs flower during mid-summer through early fall. Therefore, to ensure maximum

association of invertebrate and fish communities and to allow for finding hydrophytes in flower to aid field identifications, this protocol monitors aquatic vegetation concurrently with aquatic invertebrates and fish during the period 15 July–30 September. To the extent possible, temporal consistency will be maintained through successive years as well as between sample types.

## Response Design

The sampling approaches for collecting physical habitat, invertebrates, and fish in this protocol are comparable to those of the US Environmental Protection Agency (EPA) Environmental Monitoring and Assessment Program (EMAP; Lazorchak et al. 1998; McCormick and Hughes 1998, 2000) and the US Geological Survey (USGS) National Water Quality Assessment (NAWQA) program (Fitzpatrick et al. 1998; Moulton et al. 2002). However, this protocol has been adapted to meet the specific objectives of the long-term monitoring program while considering limitations of staff size and budget. Modifications from these national-level protocols for the purposes of this monitoring program were taken from literature pertaining to sampling of biotic communities in lotic systems.

## Habitat and Water Quality

Habitat incorporates all aspects of physical and chemical constituents and their interactions. Variables such as current velocity, discharge, dominant substrate size, embeddedness, water chemistry, and presence of periphyton, filamentous algae, and aquatic plants play key roles in the microhabitat structure and distribution of aquatic invertebrates

and fish (Rosenberg and Resh 1993; Allan 1995; Hauer and Lamberti 1996). Other habitat variables such as woody debris, boulders, canopy cover, and bank condition (e.g., height, angle, dominant substrate, degree of undercut, and vegetative cover) are also important for assessing spring condition. We will continue to monitor all of the aforementioned habitat variables at our sampling sites. Water quality parameters (temperature, pH, dissolved oxygen, specific conductance, and turbidity) will be collected at each spring during the sampling period.

### **Aquatic Vegetation**

We will collect cover estimates of the aquatic hydrophytes, mosses, and certain filamentous and globular algae among 1-m<sup>2</sup> sampling plots located along fixed transects (Figure 1) to evaluate vegetation community changes through time (SOP #5, Aquatic Vegetation Sampling). Cover estimates within the sample plots will be collected using the Daubenmire cover classes (Daubenmire 1959) that partition all possible percentage values into seven categories. Percent cover estimates allow us to obtain information on trends in vegetation communities and relate those to changes in habitat.

### **Aquatic Invertebrates**

The sampling approach described here for invertebrates is based in part on the EPA EMAP protocols (Lazorachak et al. 1998) and Bowles et al. (2007). Minor modifications to the approaches described in those protocols are made here to account for program-specific goals related to long-term monitoring of invertebrates, limitations posed by staff

size, and logistical and budgetary constraints. The EMAP approach focuses on evaluating ecological conditions on regional and national scales and such an application is not appropriate for the springs at OZAR, although the transect-based methods remain appropriate.

There is concern that collecting too many benthic samples annually from these delicate springs could cause long-term or permanent damage. For example, Englund (1991) found that the moss community in disturbed plots in a spring-run took over one year to recover following disturbance. Doisy and Rabeni (2004) collected six samples from each of the large OZAR springs but only analyzed three of those samples. They also used benthic subsampling procedures that were similar to those recommended in this protocol (SOP #7, Laboratory Processing and Identification of Invertebrates). Their analyses showed the various community metrics were fairly consistent among samples within spring-runs (Table 5). They also found that data from the subsamples adequately represented the contents of the entire samples (Table 6). However, Doisy and Rabeni (2004) restricted their sampling to the area of the spring-runs immediately downstream of the source. They did not collect samples from throughout the entire spring-run as proposed by Bowles et al. (2008) and in this protocol.

Other studies have similarly shown that three benthic samples are generally adequate to characterize the invertebrate communities occurring in a single habitat type (Canton and Chadwick 1988; Bowles 1989; Mathis 2001; Usrey and Hinsey 2006).

**Table 5.** Metrics for invertebrates based on three samples collected from each spring in Fall 2003 (mean and 95% confidence interval). Welch Spring data are based on only one sample.

| Spring Name | Taxa Richness       | EPT Richness        | Mayfly Richness   | Shannon Diversity Index | Biotic Index        | Ratio of Mayflies/Total Abundance | Ratio of Shredders/ Total Abundance |
|-------------|---------------------|---------------------|-------------------|-------------------------|---------------------|-----------------------------------|-------------------------------------|
| Alley       | 25.3<br>(23.0–27.7) | 11.3<br>(10.7–12.0) | 3.3<br>(2.7–4.0)  | 1.74<br>(1.58–1.90)     | 2.43<br>(2.23–2.63) | 35.3<br>(28.6–42.0)               | 46.8<br>(41.2–52.4)                 |
| Big         | 12.0<br>(9.7–14.3)  | 2.3<br>(1.7–3.0)    | 0.7<br>(0.01–1.3) | 0.94<br>(0.71–1.18)     | 2.02<br>(1.30–2.74) | 0.1<br>(0.0–0.2)                  | 74<br>(66.4–81.6)                   |
| Blue        | 14.3<br>(13.0–15.6) | 3.3<br>(2.0–7.4)    | 0.7<br>(0.01–1.3) | 1.37<br>(1.17–1.56)     | 2.35<br>(1.65–3.05) | 0.3<br>(0.04–0.5)                 | 49<br>(36.0–61.3)                   |
| Pulltite    | 20.0<br>(18.9–21.1) | 6.7<br>(4.3–9.0)    | 3.3<br>(2.7–4.0)  | 1.67<br>(1.58–1.77)     | 5.32<br>(5.08–5.56) | 16.2<br>(12.9–19.4)               | 4.9<br>(4.5–5.4)                    |
| Round       | 16.3<br>(15.0–17.6) | 4.0<br>(1.7–6.3)    | 1.0<br>(1.2–2.09) | 1.65<br>(1.20–2.09)     | 6.39<br>(5.72–7.05) | 0.9<br>(0.3–1.4)                  | 4.3<br>(0.0–8.7)                    |
| Welch       | 24.0                | 4.0                 | 3.0               | 1.80                    | 4.50                | 8.0                               | 40.0                                |

**Table 6.** Comparisons of benthic metrics for whole invertebrate samples and 400 specimen subsamples.

| Metric                                 | Sample #1    |                          | Sample #2    |                         |
|--|--------------|--------------------------|--------------|-------------------------|
|  | Whole Sample | Mean of Subsamples (CV*) | Whole Sample | Mean of Subsamples (CV) |
| Biotic Index                           | 2.34         | 2.34 (7%)                | 2.60         | 2.62 (4%)               |
| Shannon Diversity Index                | 1.53         | 1.49 (8%)                | 1.86         | 1.74 (7%)               |
| Ratio of Mayfly/Total Abundance (%)    | 36.5         | 36.8 (24%)               | 37.4         | 37.0 (13%)              |
| Ratio of Shredders/Total Abundance (%) | 50.0         | 49.8 (17%)               | 41.6         | 41.8 (13%)              |

\* Coefficient of variation.

**Table 7.** List of gear used and percent effort by gear for each spring reach sampled for fish communities.

| Spring Name | Panel | Reach Type   | Gear Used             | % Effort by Gear |
|-------------|-------|--------------|-----------------------|------------------|
| Alley       | 1     | Wadeable     | Towed Barge           | 100              |
| Round       | 2     | Wadeable     | Backpack              | 100              |
| Blue        | 2     | Wadeable     | Towed Barge, Backpack | 75, 25           |
| Big         | 3     | Non-wadeable | Boat, Backpack        | 65, 45           |
| Pulltite    | 3     | Wadeable     | Backpack              | 100              |

However, to better characterize the spring-runs and their available habitats along their respective continuums, this protocol recommends collecting five benthic invertebrate samples per spring with the individual samples being collected on transects 2, 4, 6, 8, and 10 (Figure 1). Sampling on successive transects begins at river left, then mid-channel, and then river right. This sequence is repeated until all five samples are completed. To the extent practical, the sampling location on the transect is shifted in subsequent sampling years to minimize long-term disturbance to the spring-run. During the first five years of monitoring (2007–2011), invertebrates were sampled annually at each spring. However, DeBacker et al. (2012) recommended sampling invertebrates every three years at each spring and on the same rotation schedule used for fish sampling. This approach has been used since 2012.

### Fish

Collection of fish community data will follow methods described in Petersen et al. (2008) and Dodd et al. (2018) for sampling river sites within OZAR. These methods closely follow USGS NAWQA protocols (Moulton et al. 2002). Fish will be collected within the same reach of each large OZAR spring where aquatic vegetation and invertebrates are sampled. However, unlike aquatic vegetation and invertebrates,

which are sampled on transects, fish will be collected from the entire reach.

Electrofishing techniques will be used to collect fish. Depending on the size of the spring-run (width and depth), communities will be sampled using backpack or towed barge gear in wadeable reaches and boat electrofishing equipment in non-wadeable reaches (see Table 7). Some portions of non-wadeable spring-runs may be shallow and require use of towed barge or backpack electrofishing equipment to collect small benthic species. A single pass will be used for wadeable reaches (those less than 1.5 m deep where backpack or tow barge gear are used) and two passes will be used in non-wadeable reaches (those deeper than 1.5 m where a boat will be used). Techniques for applying these sampling methods are presented in SOP #8 (Fish Community Sampling).

Fish processing will follow procedures described in Petersen et al. (2008) and Dodd et al. (2018) for OZAR river sites. At a sample reach, a subsample of fish from each species will be measured (lengths and weights) and anomalies will be recorded; the remaining fish of each species will be counted for abundance estimates. For details on fish processing and data recording see SOP #8.

## Rationale for the Sampling Design

Biomonitoring methodologies are constantly being developed, refined, and debated in an effort to achieve the most efficient and effective assessments of the relations of water quality to invertebrate, fish, and vegetation communities. However, other than the HTLN Springs Community Protocol (Bowles, Dodd et al. 2008), there have not been any published protocols designed specifically for long-term biomonitoring of springs. While several biomonitoring methodologies have been developed for use on streams and rivers within the Ozarks (Rabeni et al. 1997; Doisy and Rabeni 1999; Rabeni et al. 1999; Mathis 2001; Rabeni and Wang 2001; Zweig and Rabeni 2001; Sarver et al. 2002), including HTLN protocols (Bowles et al. 2007; Bowles, Williams et al. 2008; Dodd et al. 2008; Petersen et al. 2008; Dodd et al. 2018), none are entirely appropriate for the springs at OZAR. However, portions of methodologies from HTLN invertebrate and fish protocols are used in this protocol. The sampling design described in this protocol is primarily based on existing national level stream biomonitoring protocols (Lazorak et al. 1998; Barbour et al. 1999; Moulton et al. 2002) and HTLN river monitoring protocols (Bowles et al. 2007; Petersen et al. 2008; Dodd et al. 2018) with minor modifications based on pilot data collected from the springs at OZAR in October of 2003 and 2005.

## Habitat and Water Quality

Habitat features are major, often limiting, determinants of invertebrate community structure and 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, sediment deposition, and presence of periphyton, filamentous algae, and aquatic plants play key roles in the microhabitat structure and distribution of aquatic invertebrates and fish (Rosenberg and Resh 1993; Allan 1995; Hauer and Lamberti 1996).

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 communities. As such, 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 spring communities are expected to follow. However, the relationship of cause and effect of these variables on aquatic community structure can be difficult to assess and analyze because there often is 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 notwithstanding, spring 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.

## Aquatic Vegetation

There are no existing national or regional-level protocols that address monitoring aquatic vegetation in springs. Those protocols developed for stream vegetation monitoring in the United States and Europe (Yin et al. 2000; Scott et al. 2002; Veit and Kohler 2003) often employ destructive sampling methods or are aimed at control or management rather than conservation. Because the springs at OZAR are fragile and prone to long-term damage from destructive sampling, we consider the previously referenced protocols inappropriate and will not use such methods.

Cover estimates allow for describing changes in vegetative cover and allow for exploration of the correlative relationships between compositional changes and habitat attributes. Use of cover estimates is essential for many species where individuals of a species can rarely be identified and counted. Specific estimation of percent cover done by eye can be subject to problems of observer bias. However, use of cover classes reduces the problem of observer bias through partitioning all possible values into percentage categories. To reduce potential observer bias, Daubenmire cover class estimates are used in this protocol for all cover estimates. Although cover class estimation is superior to percent cover estimates, the effect of different observers can be an important contributor to variability in the data set (Kercher et al. 2003). On the other hand, Klimeš (2003) noted that variation of total plant cover estimates among

observers tended to decrease as a function of increasing plot size. The 1-m<sup>2</sup> plots used in this study are well within the size range for plots that tended to exhibit the lowest amount of variation among observers (Klimeš 2003). Although Klimeš (2003) recommended that such error can be minimized by using the estimates of at least three observers, we feel that using multiple 1-m<sup>2</sup> plots coupled with annual training requirements and detailed guidance for aquatic vegetation monitoring (SOP #2, Training for Field Sampling and Laboratory Processing and SOP #5, Aquatic Vegetation Sampling) serves to minimize potential variation among observers.

### **Aquatic Invertebrates**

Biomonitoring methodologies are constantly being developed, refined, and debated in an effort to achieve the most efficient and effective assessments of the relationships between water quality and invertebrate, fish, and vegetation communities. However, prior to the original HTLN Springs Community Protocol (Bowles, Dodd et al. 2008) there have not been any protocols published that were designed specifically for biomonitoring of springs.

The EMAP program uses probabilistically selected sites where individual sampling sites are assessed using a transect-based design and 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. This protocol does not propose to composite samples by habitat; samples will be processed individually to gain a better estimate of intra-transect and inter-transect variability and allow a broader suite of analytical options. The *kick net* used in the EMAP method is effectively the same net as a Slack-Surber sampler used in the USGS NAWQA program (Moulton et al. 2002) minus the frame delineating the sampling area in front of the net. We have opted to use the NAWQA style Slack Surber sampler because it is more useful for delineating the sampling area. Data are analyzed following Barbour et al. (1999) and the use of 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. The current protocol does not use O/E ratios because there are no reference sites available for establishing expected taxa. The EPA's Wadeable Streams Assessment Program is based on the EMAP approach and is not considered separately here (USEPA 2004a, b, c, d, 2006).

EPA monitoring programs for assessing water quality using invertebrate communities in wadeable streams include the Rapid Bioassessment Protocols for Use in Streams and Rivers (Barbour et al. 1999). This approach uses either single habitat (e.g., riffles) or multi-habitat sampling 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<sup>2</sup> area sampled in front of the net and taking 2–3 kicks using foot agitation. The multi-habitat approach uses 20 jabs or kicks taken from different representative habitat types using a D-frame dipnet. For both approaches, samples are composited for analysis and metrics are the same or comparable to those used in this protocol (Barbour et al. 1999). An additional set of protocols designed for larger non-wadeable rivers (Flotemersch et al. 2006) are generally not applicable to the spring-runs addressed in this protocol and are not further addressed here.

The general basis of the NAWQA program is to collect biological, physical, and chemical data at sites that represent major natural and anthropogenic factors considered responsible for controlling water quality in a river basin (Moulton et al. 2002). The NAWQA sampling design for benthic invertebrates includes two types of sampling sites: basic fixed sites and synoptic sites. The fixed sites are those where 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) semi-quantitative

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 semi-quantitative 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. The NAWQA protocol allows for location of sites based on whether the site is representative of the local area and support objectives, thus giving the site investigator flexibility in establishing site boundaries depending on local conditions. The sampling design in this protocol, by comparison, employs a fixed transect design due to the limited relative length of the spring-runs.

Overall, our study design as described in the original Springs Monitoring Protocol (Bowles, Dodd et al. 2008) and here is closest to that of EMAP, and we opted not to use strict EPA or NAWQA monitoring approaches. However, there are some similarities among the EPA and NAWQA approaches and the present protocol 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.

### Fish

Prior to the original Springs Community Monitoring Protocol (Bowles, Dodd et al. 2008), there were no other existing protocols specifically for sampling fish in large spring systems. However, several different sampling approaches or protocols have been developed by state and federal agencies, including HTLN protocols (Dodd et al. 2008; Petersen et al. 2008; Dodd et al. 2018), to quantify status and trends of fish communities in streams and rivers. Rapid Bioassessment Protocols developed by the EPA (Barbour et al. 1999) have been used by many agencies to evaluate fish communities in streams. These protocols are designed to give a quick, broad picture

of stream quality and fish assemblages throughout a region with minimal field and laboratory efforts. Other monitoring groups use the EPA EMAP protocols for wadeable (McCormick and Hughes 1998) and non-wadeable streams (McCormick and Hughes 2000) and the USGS NAWQA protocols (Moulton et al. 2002). These latter two protocols have more rigorous data collection (i.e., collection of fish lengths and weights) and quantitative methods (i.e., designated reach length), giving a more complete picture of fish assemblage composition and structure.

The HTLN River Fish Community Protocol (Petersen et al. 2008; Dodd et al. 2018), which includes OZAR, uses methods similar to NAWQA protocols with minor modifications based on EMAP and other relevant literature. Therefore, this protocol for OZAR springs follows methods similar to the HTLN River Fish Community Protocol in terms of minimum reach length required, electrofishing gear and methods used, and data collection for fish communities and physical habitat. The two modifications of this protocol from the HTLN River Fish Community Protocol is reach length sampled, gear restrictions, and site selection. These modifications were necessary to meet specific requirements of this monitoring program under staffing and budgetary limitations to ensure that sampling effort (i.e., proportion of area sampled) was consistent across springs of different sizes, and to account for logistical constraints of sampling spring-runs.

The HTLN River Fish Community Protocol and the NAWQA protocol use a reach length of 20 times mean wetted stream width (at low flow) with a minimum length of 150 m and a maximum of 1000 m. This protocol also uses a minimum reach of 150 m; however, a weighting factor based on average width is used to establish the reach length. Applying this 20-times multiplier to springs at OZAR would result in reach lengths for some of over 1000 m. Because sampling a reach length of this size is logistically and monetarily impractical, and most spring-runs at OZAR are less than 500 m, this protocol uses the NAWQA minimum sampling reach of 150 m multiplied by a width-based weighting factor to allow for scale-dependent sampling among the springs.

The second modification from the HTLN River Fish Community Protocol and NAWQA protocol is the strict use of electrofishing techniques in this protocol. The former protocols allow for use of seining in

riffles and shallow margins of pools and run habitat, but dense aquatic vegetation in these springs precludes effective seining. Thus, only electrofishing methods will be used for OZAR springs. The OZAR springs protocol for sampling fish is similar to NAWQA in terms of site selection. NAWQA sites are selected based on professional judgment and other criteria such as access, presence of streamflow instrumentation, land use characteristics, and other specific objectives. Use of professional judgement and safe access to the spring-run is used here to determine the upstream boundary of the sample reach within a spring-run.

## Suitability of Survey Design to Meet Study Objectives

Monitoring objectives are integral to defining the sampling design. The sample design for spring community monitoring is driven by the study objectives discussed above. The overall survey design is deemed suitable for several reasons:

1. *Appropriate for long-term monitoring of aquatic communities.* A transect-based approach for sampling and monitoring aquatic communities is consistent with other national level protocols (Lazorachak et al. 1998; USEPA 2004a, b, c, d). Sampling multiple habitats provides more comprehensive information compared to single habi-

tat samples (Lenat and Barbour 1994; Moulton et al. 2002). However, run habitat is predominant in the large springs at OZAR.

2. *Appropriate for Ozark springs.* The data generated from this study design will be directly comparable to those of other regional (state and federal) monitoring programs, where applicable, that employ similar methodologies.
3. *Accommodates springs of varying size.* The sample design allows for unbiased estimates of community condition applicable to the entire spring-run regardless of length. While the sampled stretches must be long enough to accommodate unbiased estimates for all studies, they do not have to be the same size among springs.
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. *Sequence of sampling events and revisit design 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 cost effective monitoring of the springs.

### III. Field and Laboratory Methods

Field work conducted using this protocol will normally take two days per spring to accomplish. The first day for a spring-run will include deploying the datasonde for collecting CORE 5 water quality measurements and invertebrate samples, assessing instream and riparian habitat, and aquatic vegetation. The second day is devoted to collecting fish samples. A workflow diagram for collecting samples is shown in Figure 2.

#### Field Season Preparations, Field Schedule, and Equipment Setup

Procedures for field season preparations, including preparation of a field sampling schedule and equipment setup, are described in SOP #1 (Preparation for Sampling). 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 condition.

Fieldwork must be scheduled in advance so that crews can be assigned. Time spent at a sampling reach will vary, but eight or more hours are typical. Sampling should occur from July–August when spring flows are low allowing for efficient and safe sampling. 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.

#### Measuring Core 5 Water Quality and Spring Discharge

CORE 5 water quality parameters (temperature, dissolved oxygen, specific conductance, pH, and turbidity) will be recorded using a data logger or sonde. The datalogger will be deployed immediately upstream of the sampling area and allowed to

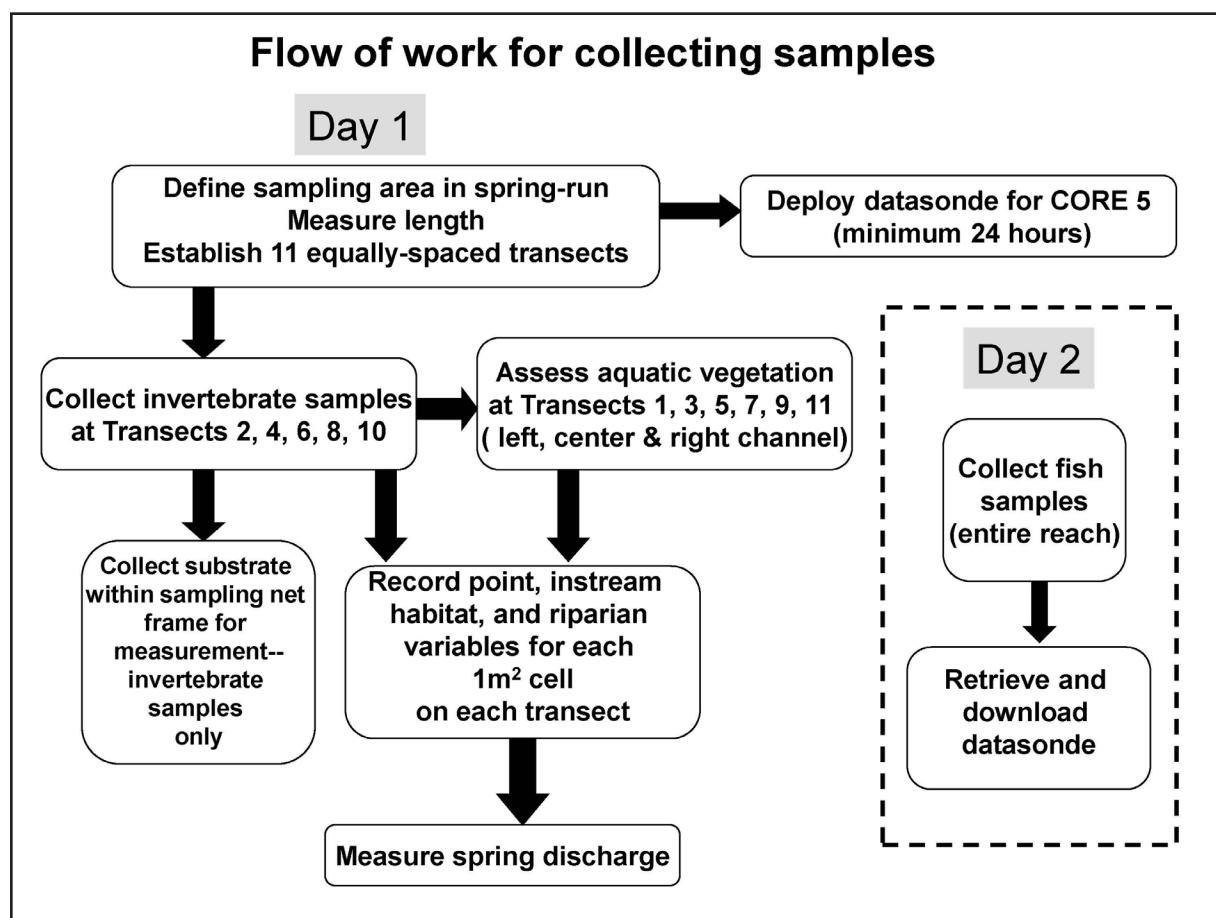


Figure 2. Flow of work diagram for collecting samples in the field.

operate for a minimum of 24 hours (to the extent practical). Only static water quality readings ( $n = 3$ ) are recorded at Welch Spring. Instructions for using the datalogger are located in SOP #4 (Documenting CORE 5 Water Quality Variables). Spring discharge will be measured at each site after invertebrate collections have been completed and preferably upstream of the sampling site. Instructions for measuring spring discharge are in SOP #10 (Measuring Spring Discharge).

## Assessing Point, Instream, and Riparian Habitat

Habitat composition within a stream is an important component in shaping aquatic communities. The type and abundance of specific habitats (i.e., woody debris, undercut banks, overhanging vegetation, etc.) will influence species presence and relative abundance, as well as size structure of the populations. Therefore, physical habitat data related to fish cover and riparian areas will also be collected at spring-runs. These methods have been modified from NAWQA protocols (Fitzpatrick et al. 1998) to meet the objectives of the NPS. At each transect, channel unit type (i.e., riffle, run, glide, pool) and pool form (if applicable) are recorded. Instream habitat is collected within three 1-m<sup>2</sup> plots at center channel and half the distance between center and the left and right banks (Figure 1). Procedures for locating sample points within each spring-run are described in SOP #3 (Reach Selection). Wetted width of each transect is measured and depth and current velocity are measured immediately in front of the sampling frame (see SOP #9, Physical Habitat Measurements). Additional categorical variables to be assessed include substrate embeddedness, periphyton, filamentous algae, aquatic vegetation, organic debris, and canopy cover. 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%).

Dominant substrate (Wentworth Scale, Wentworth 1922) is assessed within the sample plot. Substrate assessments provide a unique characterization of the streambed composition at the time sampling takes place. Therefore, average substrate size will be assessed from the area within the sampling frame

of the net (0.25 m<sup>2</sup>) following collection of habitat data listed above, and it will be assessed for every sample cell. The intent of this substrate assessment is to characterize the dominant substrate for individual samples, not to fully characterize all sediments present. This assessment will help us describe the prevailing microhabitat conditions that influence the structure of invertebrate communities and may help explain variability between sample points. Substrate size will be assessed using the standard Wentworth scale. Procedures for collecting and measuring substrate samples are provided in SOP #9 (Physical Habitat Measurements).

Fish cover is assessed at each transect. Boulders and any artificial cover are assessed at each plot where depth, velocity, and substrate are collected. Small and large woody debris are assessed within a 1-m band on either side of a transect on the left and right sides of center channel. Cover along the banks is assessed within 1 m upstream and downstream of a transect and includes trees/roots, overhanging vegetation, undercut banks, and bluffs (within 5 m of wetted edge). Bank characteristics are observed at each transect to determine bank stability. Bank angle and dominant substrate are observed from the bottom of the bank (i.e., at wetted edge or at the top base of the bar if one is present), and the category code is recorded. Percent vegetative cover, bank height, and bank cover are assessed from the bank bottom to 10 m into the bank. Bank cover categories include large trees, small trees/shrubs, grass/forbs, bare sediment, and artificial cover.

## Assessing the Aquatic Vegetation Community

Percentage composition of the aquatic vegetation community will be assessed in three, equally spaced 1-m<sup>2</sup> sample cells located along each of 6 transects (Figure 1). Welch Spring will have only three transects because of its short length. A modified Daubenmire scale (Daubenmire 1959) is used to categorically estimate species areal coverage. Standard classes for all cover estimates will be as follows: 1 = 0–0.99%, 2 = 1–5%, 3 = 5–25%, 4 = 25–50%, 5 = 50–75%, 6 = 75–95%, and 7 = 95–100%. Refer to SOP #5 (Aquatic Vegetation Sampling) for further details related to assessing the spring plant communities.

## Collecting Benthic Invertebrate Samples

Procedures for collecting benthic invertebrate samples and documenting habitat data are presented in SOP #6 (Aquatic Invertebrate Sampling). Additionally, we propose collecting a substrate sample from within the invertebrate net sampling frame for measurement (SOP #9, Physical Habitat Measurements).

One invertebrate sample will be collected from sample points located on each of five transects in each spring as described in SOP #6. Only three benthic samples will be collected from Welch Spring because of the short spring-run. Samples will be collected with a Slack Surber sampler (Moulton et al. 2002). Water may flow over the top of the net in deep runs potentially allowing some invertebrates dislodged from the substrate to wash over the net. Because the metrics to be calculated are largely percentage-based, such losses of invertebrates should not negatively affect the metric scores and their loss must be considered relative to the restrictions quantitative sampling gear (i.e., Hess sampler) would impose on sampling effort. Each discrete sample is collected while progressing in an upstream direction. Sampling procedures will be the same for each sampling point 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 #7 (Laboratory Processing and Identification of Invertebrates). Methods for preparing samples for sorting and subsampling generally follow those presented in Moulton et al. (2002). A list of the aquatic invertebrate taxa known from the greater Ozarks region is shown in SOP #12 (Data Analysis).

### Subsampling Benthic Samples

Because of the relatively high densities of aquatic invertebrates occurring in the samples, subsampling individual samples will be necessary to process the samples in a timely and cost-effective manner. The routine for subsampling benthic samples is presented in SOP #7. The method of subsampling will involve the fixed fraction approach, with 12.5% of each

sample being sorted following thorough washing, agitation, sieving, and elutriation of the entire sample (Moulton et al. 2002). 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. A fixed fraction subsampling routine was selected over a fixed count routine because some metrics to be calculated from samples are related to specimen density that cannot be obtained with the latter method. Subsampled fraction debris will be subjected to QA/QC analysis (SOP #7) and should be kept until QA/QC is complete for that batch of samples and the Program Leader authorizes disposal of the debris.

## Collecting Fish Samples

Fish communities will be sampled using various electrofishing methods (SOP #8, Fish Community Sampling) and associated fish cover and bank/riparian habitat will be measured. The size of the spring-run (width and depth) will determine the type of electrofishing gear used. Wadeable reaches will be sampled using backpack or towed barge electrofishing units. Non-wadeable reaches will be sampled with boat electrofishing equipment. At sites where depth requires that boat electrofishing be used, towed barge or backpack electrofishing will also be used to collect fish in shallow areas to obtain a representative sample of the reach.

When monitoring, it is important to note that gear type and gear efficiency have been shown to affect fish community data. In a study of fish data from 55 NAWQA sites, Meador and McIntyre (2003) found that among electrofishing methods (backpack, towed barge, and boat), Jaccard's (similarity) index and percent similarity index values between years and between multiple reaches were significantly greatest for backpack electrofishing. These results suggest that data collected using different gear (or different combinations of multiple types of gear) will be affected by gear type.

There are three alternatives to resolve the problem of analyzing data collected by different gear. First, the data can be considered to be affected primarily by the size of the spring-run when gear usage is based on the spring-run size; and therefore, data are treated as

equivalent across gear types and combined for analysis. Second, data can be compared only with other data collected using the same gear types. Third, the raw data can be corrected for differing gear efficiencies before making comparisons across sites associated with different gear types.

This protocol is concerned with monitoring each spring-run over time, not comparing across springs that may use different gears or combination of gears. Therefore, when monitoring temporal changes in communities, it is imperative that gear type (or combination of gear types) used in a spring-run and sampling effort for each gear type be consistent across years (see Table 7). For reaches where multiple gears are used, we will combine the data across gear types for analysis because samples collected with electrofishing gear are based on time (i.e., effort) and percent effort by gear is consistent across years. However, there may be specific monitoring questions where analyzing data by electrofishing gear is necessary. Therefore, in the field, data from different electrofishing gear will be kept separate.

When processing samples and recording data, all sample data (gear used, time spent sampling, electrofishing settings, length of the spring-run sampled, and species data collected with the gear type) will be recorded separately for each method. To the extent practical, individuals will be identified in the field using appropriate fish identification keys and other information. Specimens that cannot be reliably identified in the field will be preserved for identification in the laboratory (see SOP #8). Individual lengths and weights will be collected on a subsample of each species at a site to estimate the size structure and community composition; anomalies will also be recorded to determine the occurrence of diseases and deformities in the fish populations.

## **Sample Storage and Reference Collection**

### ***Aquatic Vegetation***

A reference collection of each species identified in the field is mounted and labeled on herbarium sheets and stored at the HTLN reference collection presently located at Missouri State University, Springfield, Missouri.

### ***Aquatic Invertebrates***

Identified invertebrate 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 five years and stored at the NPS HTLN office located at Missouri State University, Springfield, Missouri. 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. One set of vials will be stored at the NPS HTLN laboratory presently located at Missouri State University, Springfield, Missouri.

### ***Fish***

A reference collection of identified fish species is kept at the NPS HTLN laboratory presently located at Missouri State University, Springfield, Missouri. All other fish collected during monitoring will be returned to the springs from which they were collected.

## **Post Season Procedures**

Procedures for the end of the sample season are found in SOP #14 (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 in that 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.

Data processing typically involves the following steps: data entry, data verification, data validation and backups/storage (see SOP #11, 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 GIS. Global Navigation Satellite System (GNSS) points are validated against DRGs (digital raster graphic files) or DOQQs (digital ortho-quarter quadrangles) for their general location.

## Overview of Database Design

There are three tabular Microsoft Access databases, henceforth referred to as the databases, containing all data (fish, aquatic invertebrates, and aquatic vegetation) for the springs monitoring project. Under the original Springs Monitoring Protocol, all biotic and abiotic measurements collected for springs was entered into one springs database. Because the fish and invertebrate sampling and field forms for springs follow those of the HTLN fish and invertebrate protocols for rivers (Bowles, Williams et al. 2007; Petersen et al. 2008; Dodd et al. 2018) and streams (Bowles, Williams et al. 2008; Dodd et al. 2008), fish and invertebrate data collected from the springs are now entered into the fish and invertebrate databases. A separate aquatic vegetation database was created to hold all springs vegetation monitoring data. The general data model for spring community monitoring consists of two core sets of tables. 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 taxa

related tables serve as the organizing hub for taxa data. Other tables primarily address habitat or water quality conditions. The database also documents the protocol version and QA/QC results. All data management activities related to this protocol are described in SOP #11.

### ***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).

Quality Assurance (QA) and Quality Control (QC) procedures and design elements occur throughout data collection, processing, and reporting and are addressed in the SOPs.

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 ISO191xx 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 WASO I&M (IRMA). 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 databases for springs community data are stored and secured by file archives stored on the server (Aquatic Vegetation, Invertebrates, and Fish databases). The databases are maintained under a directory named 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 2018), the three databases containing springs community data will be uploaded to IRMA DataStore. The dataset is flagged as “read only” for all users except the Project Leader and Data Manager. If at anytime protected species are identified, they will not be included in the uploaded dataset.

# V. Analysis and Reporting

## Aquatic Vegetation

The intent of this monitoring protocol is to track aquatic vegetation community composition and species occurrences through time along fixed transects within a defined sampling reach. Degradation of aquatic vegetation in the springs could signal anthropogenic disturbance, particularly in light of analogous changes in invertebrate and fish communities. Evidence suggests that the aquatic vegetation communities of the springs at OZAR have changed little during the past century (Drouet 1933; Steyermark 1941; Currier 1990a, b; Converse 1994; Edwards 2002; Lipscomb undated).

We propose to analyze aquatic vegetation community data from the springs using several metrics. Once estimates for all parameters have been obtained for each plot, they are averaged to obtain a measure of variability (standard deviation) for a transect and then averaged across transects to obtain an estimate of variability (standard error of the mean) for the sample reach. Procedures for calculating these metrics are shown in SOP # 12 (Data Analysis). Summary indices and variables will provide information to park managers on the status of the target communities.

Frequency and percent foliar cover are calculated for each individual species collected within the spring to assess community composition. Frequency is defined as the number of times a species is present in a given number of plots of a particular size (Raunkiaer 1934). Individual species frequency at a transect (percentage of a species occurrence within the three plots) is calculated and then averaged across the transects ( $n = 6$ ) to assess occurrence within the reach. Percent foliar cover serves as an estimate of abundance for herbaceous species. To calculate this metric, the cover class intervals are first converted to median values to estimate percent cover for each species within each cell. Mean percent cover is then averaged as the species percent cover for a transect ( $n = 3$ ), and for all transects in the reach ( $n = 6$ ). From these basic estimates of foliar cover and frequency, the following metrics are generated for each transect and are then averaged: (1) species relative cover, (2) species relative frequency, and (3) species importance value.

Diversity of aquatic vegetation is calculated at each plot within the transect and then averaged for each transect ( $n = 3$ ) and averaged across the sample reach ( $n = 6$ ) using three measures: species richness ( $S$ ), Simpson's Diversity Index ( $D$ ), and the Shannon Diversity Index ( $H$ ). Species richness is calculated as the total number of plant species recorded per plot and includes all species (native and exotic) in the estimate. Species richness is then averaged for a transect ( $n = 3$ ) and then averaged across all transects ( $n = 6$ ) for a reach. Simpson's index of diversity ( $D$ ) is best described as a dominance index because it weights toward the abundance of the most common species. It gives the probability of any two individuals drawn at random from an infinitely large community belonging to different species (McCune and Grace 2002). The Shannon Diversity Index accounts for both abundance and evenness of the species present, and it is more robust when all species are equally abundant or have high evenness. An additional metric, Species Distribution Evenness ( $E'$ ; also known as the Shannon's Evenness Index) is a measure of the distribution of species within a community as compared to equal distribution and maximum diversity (Pielou 1969). It is calculated using the values of the Shannon Diversity Index (SOP #12).

When interested in measuring diversity in a single community it is best to use all three diversity measures to most accurately reflect diversity (Joust 2006). At the most basic level of species diversity, species richness provides a total number of distinct species sampled per unit area. Richness is insensitive to species abundance. Thus, a single individual species occurring only once in a community is treated the same as a species with thousands of individuals in the community. This measure is an indicator of species diversity but does not provide any information about the composition of species within the community. Shannon Diversity Index weights species by their abundance and evenness. It is an intermediate between species richness and Simpson's Diversity Index in its sensitivity to rare species. Therefore, this diversity measure provides information on both the count of unique species and their abundance or density in the community. Simpson's Diversity Index goes one step further by disproportionately favoring

dominant species based on species abundance and is little affected by gain or loss of rare species.

Shannon and Simpson's diversity index values are converted into effective number of species for each community ( $H_e$  and  $D_e$ , respectively; Hill 1973; see SOP #12). This allows for both diversity measures to be compared directly to species richness of the sites within and among sample years based on count of distinct species in the community (Joust 2006). Dominance takes into account the species abundance and evenness of species distribution in the community. The degree of species dominance in the community is reflected by the degree that species richness of the site is greater than the effective number of species for each community based on the Shannon and Simpson's indices ( $S > H_e > D_e$ ) when evenness remains constant in a single community. The difference in number of species between the diversity measures reflects both how each metric considers uncommon species and how species diversity is partitioned within the community among years. If all species occurred in equal abundance in the community within and among sample years, then  $S = H_e = D_e$ . Therefore, effective number of species for each diversity measure reflects the number of species found in a similar community when all species occur in equal density.

## Aquatic Invertebrates

There is little information on the best methods for monitoring and assessing invertebrate communities of springs. Consequently, the metrics that we chose to use are based on evaluations done for stream biomonitoring studies, with a focus on metrics that are sensitive to factors such as organic pollution and inorganic toxicants rather than fine sediment. Early biomonitoring programs focused on one or two specific attributes of the communities within a system. For example, the Indicator Species concept that dominated biological evaluations for a long time (Kremen 1992) operated on the premise that a particular perturbation could be detected by monitoring for a single sensitive species. This view of a system may be of value in detecting selected anthropogenic effects, while being unsuccessful in detecting complex, cumulative impacts (Karr 1991). This is because an aquatic system is composed of an array of communities, each clearly defined by many different attributes. This has led to the development of the alternative, multimetric approach that uses an

array of measures or metrics, each of which reflects the health of a particular biological attribute such as community structure, community balance, or biological condition. These metrics may be grouped into several categories (Resh and Jackson 1993):

- Richness measures that count the number of distinct specified taxonomic units such as families or species. Examples include taxa richness and number of Ephemeroptera, Plecoptera, and Trichoptera (EPT) taxa.
- Enumeration measures that count the total number of individuals or estimate the relative abundance of all the individuals (or a particular group) from the collection. Examples include total abundance and % dominant taxon.
- Community diversity indices, such as the Shannon Diversity Index, combine richness and relative abundance into a summary statistic.
- Similarity indices that use either qualitative or quantitative data to estimate the percentage similarity of two different communities. Examples include the Sørenson Similarity Index (qualitative) and the Quantitative Similarity Index (quantitative).
- Biotic indices that combine pre-established, water-quality tolerance values for the collected taxa with their relative abundance into a summary statistic. Examples include the Hilsenhoff Biotic Index (Biotic Index) and Family Biotic Index.
- Functional feeding group ratios that divide the individuals into different groups based on the morphological structure of their mouth parts and food acquisition behavior. Examples include the ratio of scrapers to collector-filterers and the ratio of shredders to the total abundance.

Dozens of metrics have been developed for each of these various categories resulting in difficulty in attempts to compare results. In recent years, several authors have evaluated the usefulness of many of these metrics in an effort to standardize their use. Barbour et al. (1992) analyzed data from 10 ecoregions within the United States to assess the variation found in the rapid bioassessment metrics used by the EPA. They found that EPT richness was the best (least variable and most sensitive) measure of community structure, the Biotic Index was the best measure of community balance, and the ratio of Total Shredders/Total Abundance was the best measure of

the functional feeding groups. Rabeni et al. (1997), using data from the major ecoregions of Missouri, determined that various metrics had less variability and were more sensitive to either organically enriched or habitat degraded impacts. Organically affected sites were best detected with Taxa Richness, EPT Richness, the Biotic Index, and the Shannon Diversity Index. Habitat degraded sites were more difficult to detect with metrics, but the most sensitive were the Shannon Diversity Index and the Percent Dominant Taxon. The assumption behind the assessment of the Percent Dominant Taxon is that a community that is heavily dominated by one species is impaired. Since dominance by a few taxa is a common and normal occurrence in these springs, this metric will not be used for this protocol (see Table 7).

Multi-metric 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. Individual metrics 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). 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 multi-metric approaches

rate among the best performers for 10 of 12 criteria they tested. However, we are unable to recommend an index-based scoring system for OZAR springs because of insufficient regional data on which to base such an index.

Doisy and Rabeni (2004) suggested seven metrics as measures of community structure and balance in springs at OZAR. The procedures for calculating and scoring these metrics are included in SOP #12 (Data Analysis). They include Taxa Richness, EPT (Ephemeroptera, Plecoptera, Trichoptera) Richness, Ephemeroptera Richness, Ratio of Total Ephemeroptera Abundance/Total Abundance, Shannon Diversity Index, Biotic Index (BI), and Ratio of Shredder Abundance/Total Abundance. We are including Percent Dominant Taxon and Percent Intolerant Taxa as additional metrics. The basis of the Percent Intolerant metric is that as the level of pollution increases, the number of pollution intolerant species should decrease.

The assumption behind the assessment of Percent Dominant Taxon is that a community that is heavily dominated by one species is impaired. This metric was selected because none of the Springs at OZAR currently appear to be grossly polluted to the point where they would be totally dominated by a single taxon. The invertebrate communities of the large springs at OZAR are dominated by largely intolerant taxa making these two metrics valuable for evaluating these systems (Table 8).

**Table 8.** The five dominant taxa and their relative mean percentages (% composition of dominant taxa) among three samples collected at OZAR springs during Fall 2003. Note: specimen identification was done by Dr. Charles Rabeni's lab, University of Missouri. Some specimens they identified as *Gammarus* were likely another amphipod, *Crangonyx*. Both taxa commonly occur in the springs. However, this finding does not negate the general findings of this table.

| Spring   | 1   | 2  | 3  | 4   | 5  |
|----------|---|--|--|---|--|
| Alley    | <b><i>Lepidostoma*</i></b><br><b>(44.7)</b> | <b><i>Serratella*</i></b><br><b>(24.9)</b> | Diphetor<br>(7)                            | Hydrobiidae/ <i>Elimia</i><br>(7)         | Chironomidae<br>(5)                        |
| Big      | <b><i>Lepidostoma*</i></b><br><b>(74)</b>   | <i>Gammarus</i> (12)                       | <i>Gammarus</i><br>(6)                     | Oligochaeta<br>(4)                        | Nematoda<br>(3)                            |
| Blue     | <b><i>Lepidostoma*</i></b><br><b>(48.6)</b> | Hydrobiidae/ <i>Elimia</i><br>(31)         | <b><i>Optioservus*</i></b><br><b>(6.4)</b> | Hydracarina<br>(5)                        | <i>Gammarus</i><br>(3)                     |
| Pulltite | Hydrobiidae/ <i>Elimia</i><br>(51)          | <i>Gammarus</i><br>(16)                    | <i>Baetis</i><br>(8)                       | <b><i>Serratella*</i></b><br><b>(6.4)</b> | <b><i>Lepidostoma*</i></b><br><b>(4.7)</b> |
| Round    | <i>Gammarus</i><br>(50)                     | Hydrobiidae/ <i>Elimia</i><br>(14)         | Hydracarina<br>(10)                        | Oligochaeta<br>(5)                        | <b><i>Optioservus*</i></b><br><b>(4.7)</b> |
| Welch    | <b><i>Lepidostoma*</i></b><br><b>(38)</b>   | <i>Gammarus</i><br>(27)                    | <i>Baetis</i><br>(7)                       | Chironomidae<br>(6)                       | Hydracarina<br>(5)                         |

\* Taxa considered pollution intolerant (also in bold).

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

- Gross organic pollution: Hilsenhoff (1982) listed all of these as indicators of gross organic pollution.
- Thermal impairment: Resh and Jackson (1993) analyzed data from California streams and found that measures such as Taxa Richness and EPT Richness were quite accurate in detecting impairment.
- Agriculturally developed catchments: Ephemeroptera and Plecoptera have shown reductions in abundance or richness (Quinn and Hickey 1990; Lenat and Crawford 1994).
- Increases in acidity: Taxa richness, EPT taxa, and the Shannon Diversity Index typically decrease in response to increasing acidity (Hildrew et al. 1984; MacKay and Kersey 1985; Resh and Jackson 1993). Mayflies are especially sensitive to low pH (Peterson et al. 1985).
- 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 a static EPT. Hickey and Clements (1998) found that abundance and species richness of mayflies, EPT Richness, and Taxa Richness were strong indicators of heavy metal contamination in streams. Yuan and Norton (2003) analyzed the effects of common anthropogenic stressors on streams and reported that Ephemeroptera (mayfly) richness was the most sensitive indicator of elevated metals or ion concentrations (aluminum and conductivity). Other studies have shown that Taxa Richness, EPT Richness, and Abundance were not affected by heavy metals because of the replacement of sensitive taxa by tolerant taxa (Clements and Kiffney 1994).
- Insecticides: Wallace et al. (1996) found that the EPT index detected disturbances to a stream treated with certain insecticides.

- Habitat degradation in the Ozark Highlands: Rabeni et al. (1997) found that the Shannon Diversity Index for high gradient riffles typically decreased with disturbance.
- Increased embeddedness in Ozark streams: Rabeni et al. (1999) found that increased embeddedness in Ozark streams has been associated with an increased percentage of collector/filterers.
- Chronic and acute conditions: the reduction in relative abundance of indicator species is a sign of a chronic condition, whereas the replacement of indicator species by less sensitive ones may indicate an acute problem.

## Fish

Several parameters and analysis techniques have been used to detect trends in fish communities and investigate the relationships between fish communities and environmental conditions. Two common approaches are parameter estimations/metric calculations (or combining metrics to form a biological index; Plafkin et al. 1989; Hughes and Oberdorff 1998; Barbour et al. 1999; Simon 1999) and multivariate statistics (for examples applying to Ozark fish communities see Petersen 1998, 2004). Using multiple analytic approaches will provide multiple lines of evidence, increasing the validity and confidence of study conclusions. A detailed summary of calculated metrics and data analyses are given in SOP #12 (Data Analysis).

Biological metrics are commonly used by scientists to compare the condition of the biological community at multiple sites (Simon 1999) or across time. A metric is a characteristic of the biota that changes in a predictable way with increased human disturbance (Barbour et al. 1999). Attributes of the fish community such as habitat and substrate preferences, trophic guilds, spawning preferences, and degree of tolerance to disturbance are measures frequently reflected in metrics making it possible to determine relationships between biological communities and environmental conditions. Metrics used for analysis and reporting are listed in SOP #12 (Data Analysis).

An extension of the metric approach is to combine multiple metrics into an Index of Biotic Integrity (IBI). This index is used as an indicator of overall stream quality, enabling investigators to compare conditions at multiple sites (Karr 1981; Barbour et al.

1999; Simon 1999) or at a single site across time. Prior to use of fish communities as bioindicators, aquatic invertebrate communities were (and still are) used as indicators of stream quality (Hilsenhoff 1977).

Although fish communities have been largely ignored in spring-runs, the popularity of fish with the general public and stakeholders have made them the most commonly used bioindicator for investigating ecological relationships using the IBI approach in streams (Barbour et al. 1999; Simon 1999).

One of the first fish IBIs developed by Karr (1981) has been modified for use in rivers and streams in many other regions and countries (Hughes and Oberdorff 1998; Simon 1999). Three IBIs have been created for Ozark Highland streams (Hoefs 1989; Dauwalter et al. 2003 ; Matt Combes, Missouri Department of Conservation, written communication, 2006). The Dauwalter IBI (Dauwalter et al. 2003) was chosen for assessment of biotic integrity of fish communities in the river and tributaries at OZAR (see Dodd et al. 2018) and was considered for use in the springs. However, all three Ozarks region IBIs were found to be inappropriate for use in the cool-water spring-runs because these IBIs were developed for warm-water systems which have higher expected diversity and richness than cool-water systems. Therefore, an IBI will not be used to assess fish communities of springs within OZAR.

## 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 as much detail. Many different analysis methods are available and are documented in 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 a large amount of 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 ideally will be indicated.

There are four main statistical approaches that could be employed with data from long-term monitoring projects such as this: (1) hypotheses testing, (2) parameter estimation, (3) multivariate approaches, and (4) 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 Heissey 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 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. Control charts 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 using a desired confidence interval around the data, a desired management goal, or a regulatory threshold for the metric of interest.

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.

Multivariate analyses are another commonly used statistical method to explain variability in community data and attribute that variability to specific environmental variables or gradients (Gauch 1982; Jongman et al. 1995; Petersen 1998; Everitt and Dunn 2001; Timm 2002; Petersen 2004). Multivariate techniques differ from univariate or bivariate analyses in that the former techniques are generally more descriptive and generate hypotheses from the biological data rather than attempt to disprove a null hypothesis, and the effectiveness improves as the number of variables increases (Williams and Gillard 1971). Two multivariate techniques commonly used to analyze community data include ordination and classification (Gauch 1982; Jongman et al. 1995; Everitt and Dunn 2001; McCune and Grace 2002; Timm 2002).

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. There are already studies indicating that three samples from the same habitat is an appropriate number for calculation of the proposed invertebrate metrics. Because our sample size will be determined primarily by budget, we would not be able to increase the number of samples taken from each spring 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 under test 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.

Although our primary approach to organizing and analyzing data will consist of metric estimation combined with control charts and potentially

multivariate analysis, 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 among samples or springs, 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#12 (Data Analysis).

## Reporting

Reports and updates should be completed the calendar year in which the data were collected and should include an informal trip report and an operational review report. Updates of the data may be in the form of a web article or data visualizer. Trend reports are updated every six 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 Report Series and Uploaded to IRMA or published in peer-reviewed scientific literature. Refer to SOP#13 (Data Reporting) for details on reporting.

# VI. Personnel Requirements and Training

## Roles and Responsibilities

The project manager bears responsibility for implementing this monitoring protocol. Because consistency is essential to implementation of the protocol, the project manager will usually lead field data collection efforts unless technicians have several years of experience collecting the data related to this protocol as determined by the project manager. 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 overseeing 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.

For invertebrate samples, at least one technician certified with taxonomic experience will be responsible for identification to the genus level. For fish monitoring, the fisheries biologist (or a technician certified in taxonomic identification by the fisheries biologist) will be responsible for identifying fish to the species level in the field and the laboratory.

## Qualifications and Training

Critical to the success of a monitoring program is a high level of consistency in field collection and data analysis from year to year. To obtain this consistency, it is necessary to have a competently trained staff.

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 or fisheries biologist should oversee this training and ensure that each technician is adequately prepared to collect data. Training should be done prior to each field season with each crew-member reviewing the SOPs associated with this protocol. Training should include discussions with crewmembers on safety protocols for fieldwork, demonstrations on proper use of water quality meters, GNSS units, and electrofishing/seining equipment, and practice of proper sampling techniques. Taxonomic identifications for plants, invertebrates and fish may be performed by a certified technician with several years of experience (see appropriate SOPs for details). Initial identifications will be checked by expert taxonomists.

The fish crew will be made up of at least two staff who are familiar with electrofishing and motorboat use. For safety of the crew, at least two members of the crew (HTLN fisheries biologist or aquatic ecologist and one other technician) must have successfully completed the U.S. Fish & Wildlife Service (USFWS) electrofishing course and the Department of the Interior (DOI) motorboat operator course.

# VII. Operational Requirements

## Field Schedule

Samples will be taken once a year during the summer index period of 15 July–30 September. Sampling should begin at relatively the same time each year and samples should be collected within the shortest time frame possible (4 weeks) to minimize the effects of seasonal change. For aquatic vegetation, invertebrate, and habitat sampling, a minimum of two people will be required, but three people make the process much more efficient. For fish monitoring a minimum crew of four to five people will be needed depending on the gear used. Because of travel considerations, only one site can be sampled per day under normal circumstances.

## 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. GNSS units, cameras, vehicles, server). Network vehicles are shared and fuel/maintenance costs are incurred at the Network level.

## Budget Considerations

Laboratory processing time per benthic invertebrate sample, including sorting, identification, counting, and entry into the database, will require approximately 8 hours per sample ( $n = 5$  samples per spring), or a total of 10 laboratory days to complete all samples. These tasks primarily will be accomplished by certified technicians. Processing of all fish samples preserved for laboratory identification will take approximately 5 laboratory days and will be conducted by the fisheries biologist or certified aquatic ecologists.

Data management personnel expenses include staff time of biological science technicians, project managers, and the data manager. The project leaders also invest time in preparation for field trips (2 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. Literature Cited

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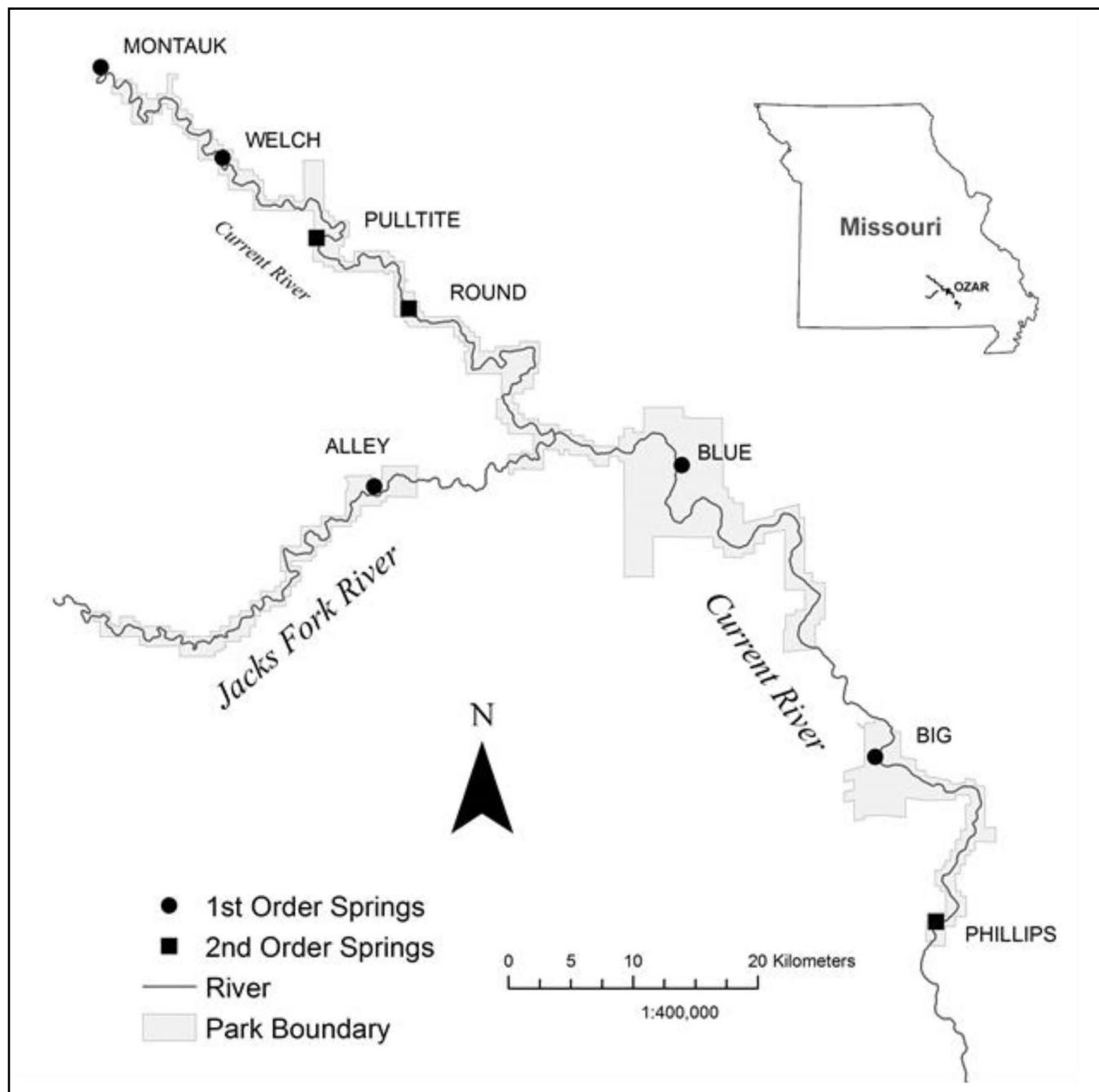
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## Appendix A. Map and Photographs

A map of spring locations at Ozark National Scenic Riverways, Missouri, is shown in Figure A1 and photographs of the large springs are shown in Figures A2–A4.



**Figure A1.** Map showing the general locations of large springs at Ozark National Scenic Riverways, Missouri. Montauk Spring is located at Montauk State Park and is not included in this protocol.



Alley Spring-Downstream



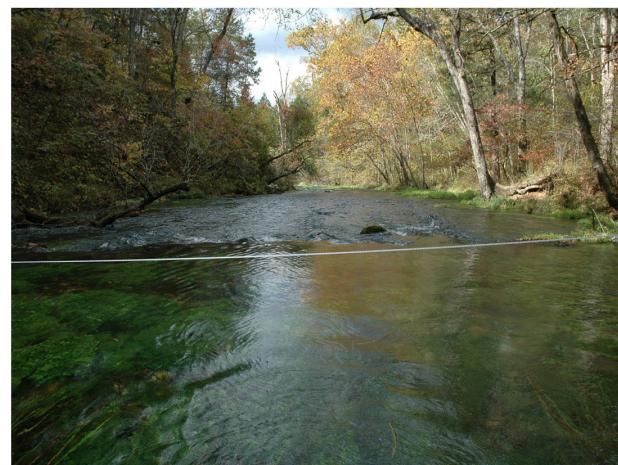
Alley Spring-Upstream



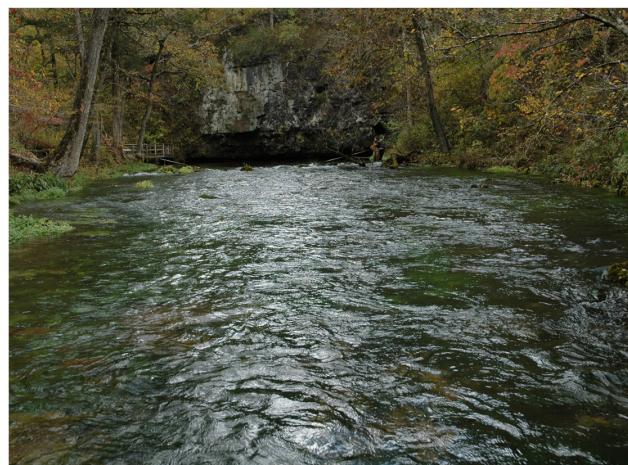
Big Spring-Downstream



Big Spring-Upstream



Blue Spring-Downstream



Blue Spring-Upstream

Figure A2. Photographs of Alley, Big, and Blue springs, Ozark National Scenic Riverways, Missouri.



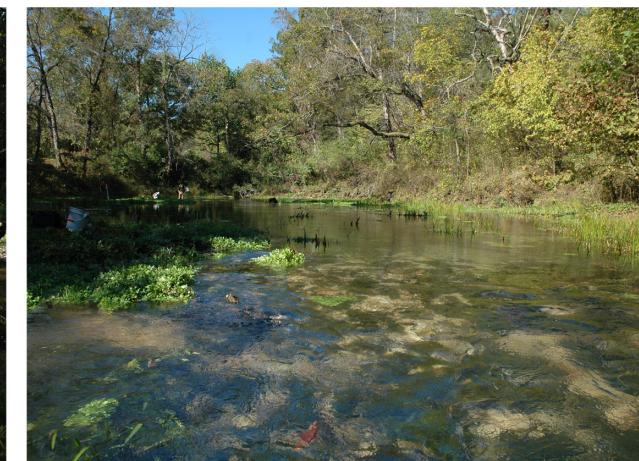
Pulltite Spring-Downstream



Pulltite Spring-Upstream



Round Spring-Downstream



Round Spring-Upstream



Welch Spring-Downstream



Welch Spring-Upstream

**Figure A3.** Photographs of Pulltite, Round, and Welch springs, Ozark National Scenic Riverways, Missouri.



Phillips Spring-Source



Phillips Spring-Downstream

**Figure A4.** Photograph of Phillips Spring, Ozark National Scenic Riverways, Missouri.



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NPS 920/175080, February 2021

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