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

Natural Resource Report NPS/HTLN/NRR—2007/009

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, the Mississippi River at Effigy Mounds NM.

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

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I. Background and Objectives

Issues Being Addressed and Rationale for Monitoring Benthic Invertebrates

Ozark National Scenic Riverways (OZAR) and Buffalo National River (BUFF) were established to preserve and interpret the free-flowing Buffalo, Jacks Fork, and Current Rivers. The parks were designated for river corridor protection, but much of their respective watersheds outside of park boundaries were left unprotected, placing the water quality of the rivers and tributaries at risk (Panfil and Jacobson, 2001). The NPS jurisdictional boundaries around the Buffalo, Current, and Jacks Fork rivers are generally narrow corridors that encompass only a small area within the respective watersheds of these rivers. The jurisdictional boundary of OZAR encompasses only about 5% of the watershed of the Current and Jacks Fork Rivers, and the boundary of BUFF is only 11% of the Buffalo River's watershed. Over 50% of the watersheds in both parks are in private ownership. Both parks are also located in an area of extensive karst topography making the rivers vulnerable to contaminated groundwater recharge and interbasin transfer of groundwater from adjacent watersheds. Because streams at OZAR primarily receive their baseflows from groundwater, contamination of groundwater is of special concern due to the rapid nature of recharge and transport of contaminants through the soluble bedrock system of cave, springs, and sinkholes. Wadeable streams of the Ozarkian region, including those at BUFF and OZAR, are generally considered to be in good condition overall although they are threatened by numerous stressors (United States Environmental Protection Agency, 2006).

Stream condition and ecosystem health are dependent on processes occurring in the entire watershed as well as riparian and floodplain areas and therefore cannot be manipulated independently of this inter-relationship (Doppelt *et al.*, 1993). Land use, particularly land clearing practices and associated increases in sediment load, nutrient concentrations, nutrient enrichment and other point and nonpoint sources, have been reported as the largest long-term threat to streams in the Ozark Highlands (Duchrow, 1977; Mott, 1997; Scott and Udouj, 1999). Land use practices at the watershed level appear to overwhelm localized protection of stream corridors. For example, measures of land use and riparian vegetation at larger spatial scales (watershed level) were superior to local measures at predicting stream conditions within that watershed (Roth *et al.*, 1996). Since the establishment of Buffalo National River in 1972, more of the watershed has been deforested than is protected within the boundaries of the National River (Mott, 2000). Over a 27 year study period, the annual increase in pasture land in the BUFF watershed was almost equal to the annual decrease in forested land (Scott and Hofer, 1995). Land use activities in the Ozarks Highlands include timber management, landfills, grazing, swine and poultry operations, urbanization, gravel mining, stream channelization, removal of riparian vegetation, and lead-zinc mining. Impacts to stream integrity from land use include disruptions in channel geomorphology, increased suspended and deposited sediments, bank erosion, increased light penetration and water temperature, higher periphyton biomass, and decreases in leaf litter and woody debris.

The framework for aquatic monitoring at OZAR and BUFF is therefore directed towards maintaining the ecological integrity of the rivers and tributaries in those parks. Invertebrates are an important tool for understanding and detecting changes in ecosystem integrity, and they can be used to reflect cumulative impacts that cannot otherwise be detected through traditional water

quality monitoring. The broad diversity of invertebrate species occurring in aquatic systems similarly demonstrates a broad range of responses to different environmental stressors. Benthic invertebrates are relatively easy to collect, and they can be analyzed at many different levels of precision. They are sensitive to a wide variety of impacts that occur in the Ozark Highlands, such as changes in chemical constituents (including metals), hydrological alterations, sedimentation and bank erosion, and land use and other changes in the watershed. Furthermore, changes in the diversity and community structure of benthic invertebrates are relatively simple to communicate to resource managers, administrators, and park visitors because the loss of biological communities is of interest and concern to these groups. Benthic community structure can be quantified to reflect stream integrity in several ways, including the absence of pollution sensitive taxa, dominance by a particular taxon combined with low overall taxon richness, or appreciable shifts in community composition relative to reference condition (Plafkin *et al.*, 1989; Lazorchak *et al*., 1998; Barbour *et al*., 1999; United States Environmental Protection Agency, 2006).

Aquatic communities can be impacted from land use practices in the watershed, particularly from the conversion of forestland to pasture (Sweeney, 1995). For example, Quinn *et al.* (1997) found densities of mayflies, stoneflies, and caddisflies that were 2 to 3 times higher in forested streams versus streams dominated by pasture. Water-quality and invertebrate community monitoring at BUFF (Bryant, 1997; Mott, 1997, Usrey, 2001) have shown a strong negative correlation between agricultural nonpoint source chemical pollution (nitrates) and stream water quality and invertebrate community structure. Increased bank erosion rates and changes in channel morphology through time have been correlated with increased land clearing of steep uplands within a tributary basin (Stephenson and Mott, 1992) and as historical riparian land clearing (Jacobson and Primm, 1997).

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

History of Invertebrate Monitoring

Ozark National Scenic Riverways (OZAR)

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

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

More recent work at OZAR by Doisy *et al*. (1997) showed the habitat types having the highest diversity of invertebrate species were high gradient riffles, coarse runs, and low gradient riffles. Additionally, Doisy and Rabeni (2001) showed high variability occurs within invertebrate communities, including within individual sites. Their results showed that variation in community structure within any single segment of stream was actually greater than variation throughout the total stream system, indicating that local habitat conditions have the greatest influence on community structure. Doisy and Rabeni (2001) also identified current velocity as the best indicator of invertebrate community composition, and found that diversity was positively correlated with current velocity. Indeed, Rabeni *et al*. (2002) showed that fast water channel units (high gradient riffles) have the most consistent community structure with higher densities and less variability compared to the other habitat units. This is an important finding for planning a long term monitoring program because it provides justification for focused sampling on high gradient riffles rather than more time-consuming and expensive multiple habitat sampling protocols. Finally, Doisy *et al*. (2002) described potential impacts to invertebrate communities from timber harvest and other deforestation activities in headwater tributary streams of the Current River system. Invertebrate communities from every stream that was sampled fell within the range of normally functioning communities. On the local level, the variables showing the greatest influence on invertebrate communities included substrate size and organic matter, and on the reach level they included stream bank erosion and canopy cover. However, on the watershed level, road densities and un-buffered stream banks showed the greatest acute effects. Their study found that relative densities of stoneflies and caddisflies decreased with increased road density in the watershed because of increased fine sediment deposition and lower organic matter inputs (Doisy *et al*., 2002). Removal of riparian buffers resulted in decreased substrate size and less organic matter entering the system, resulting in acute negative effects on invertebrate community diversity.

Buffalo National River (BUFF)

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

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

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

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

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

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

Bradley (2001) sampled four tributary sites in the Buffalo River drainage to assess water quality and invertebrate communities in an effort to determine reference water quality conditions. The sites were Bear Creek, Tomahawk Creek, and Calf Creek, all of which represented disturbed streams in the mid to lower reaches of the Buffalo's watershed. Water Creek was used as the undisturbed reference site. Bradley (2001) found water quality characteristics--including dissolved oxygen, conductivity, temperature, and pH--were similar among all four sites and were typical for the Ozark Highlands ecoregion. Discharge was significantly higher for Bear Creek and Calf Creek in the spring, and fecal coliform and turbidity levels were also higher for these sites. This was expected due to the larger drainage area and more cleared land in their respective watersheds. The reference stream, Water Creek, had low turbidity during all seasons due to a higher proportion of forested land in its drainage. Tomahawk Creek showed a consistent level of fecal coliform concentrations throughout the year while those of the other three sites fluctuated, indicating a possible point source pollutant like a septic tank or continuous direct access of livestock to the stream. Bradley (2001) also reported seasonal and yearly variations in invertebrate community structure, density, and richness among the four tributaries (Bradley, 2001). Most of the seasonal variations in community composition are explained by differences in life histories of the organisms and not due to anthropogenic disturbances, however. In the disturbed tributaries, the largest component of the community was Diptera, a largely pollution tolerant group typically abundant in streams with organic enrichment. In comparison, the reference stream was dominated by largely intolerant Ephemeroptera.

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

Local Biomonitoring Programs

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

Monitoring Objectives Addressed by the Protocol

Two broad objectives are addressed by this protocol: 1) Determine the annual status and trends of invertebrate species diversity, abundance and community metrics, and 2) Relate the invertebrate community to overall water quality through quantification of metrics related to species richness, abundance and diversity and region specific multi-metric indices as indicators of water quality and habitat condition (DeBacker *et al*., 2005).

Justification/Rationale for these Objectives

BUFF and OZAR were created to preserve and interpret the free-flowing Buffalo, Jacks Fork and Current rivers. The Heartland Network Aquatic Resources Working Group formally agreed that the framework for aquatic monitoring at OZAR and BUFF would be directed specifically towards understanding and maintaining the ecological integrity of these river systems.

Aquatic invertebrates are an important biomonitoring tool for understanding and detecting changes in ecosystem integrity over time. Aquatic invertebrates respond rapidly to different environmental stressors, are relatively easy to collect, and can be analyzed at many different levels of precision.

II. Sampling Design

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

For the inference to be valid, a probability based sample design within a defined reference frame is required.

Spatial Design

Establishing the sample frame

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

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

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

MORAP used data from the 1:100,000 National Hydrography Dataset (NHD) that was developed by the USGS and EPA. The coverage included arcs representing the centerlines of wide streams, as well as the segments of single line streams. An Arc/Info macro was run on the arc segments to pull select attributes from various NHD tables and attach them directly to the arc component. These stream segments were classified according to a number of variables including temperature, stream size, flow, geology, soil texture, relative gradient, valley wall interaction (a surrogate for potential bluff pool habitat), stream size discrepancy, and channel type (metadata are available at http://science.nature.nps.gov/nrdata/). The dataset was restricted to those stream

segments that touched the park jurisdiction boundary or other public lands adjacent to the park. Additionally, tributaries to the main stem river were cut when they crossed the floodplain of the main stem river. This allowed these segments to be coded as 'floodplain' segments.

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

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

Selecting the sites to be sampled

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

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

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

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

case, one would simply move to the next site in the ordered GRTS list (sacrificing only a small degree of spatial balance).

The total number of stretches to be sampled annually is limited primarily by budget and personnel. It was determined that 12 stretches could be sampled at each park in each year. (This takes into account complete processing of all samples, and the number of other protocols that will need to be implemented at these sites). At BUFF (which has many tributaries), each year 6 main stem stretches will be sampled, and 6 tributary stretches will be sampled. At OZAR (which has fewer tributaries, but many springs), 9 main stem stretches and 3 tributary stretches will be sampled. Maps indicating mainstem stretches selected for annual monitoring are presented in Appendix A. Additional information for stretches selected for monitoring, including stretch identification number, UTMs, and tributary names, are presented in Appendix B. Sampling of springs at OZAR will be accomplished as part of a separate protocol.

Main stems

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

We also divided the Buffalo River into lower and upper sections prior to drawing the GRTS sample. The frame for the main stem consists of 74 total stretches. Stretches above the confluence of Mill Creek near Pruitt were ~1-2 km in length, whereas stretches below this point were ~3-5 km in length. Again, this change in stretch length reflects changing river morphology as flows increase and the riverbed widens. The Buffalo River within the park boundary is 198 km long, and crosses three major geologic formations: the Boston Mountains, the Springfield Plateau, and the Salem Plateau. There is a losing reach on the Buffalo below the confluence with Richland Creek where much of the water (all during most summers) runs underground for several km before resurfacing at White's Spring. Thus we divided the river into an upper section (n=47 stretches) above the natural break at the losing reach, and a lower section (n=27 stretches) below White's Spring. (This break also approximates a major geologic shift, as the upper section includes the Boston Mountain formation, and the lower primarily represents the Springfield and Salem Plateaus.) The losing reach was deleted from the frame because it dries seasonally and the invertebrate communities located there may not be reflective of other areas of the river. The distance in river miles for the two sections is similar (89 km for the upper, 109 km for the lower). The lower section contains fewer stretches because the stretches are longer.

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

Tributaries

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

We conducted reconnaissance surveys of selected tributaries at BUFF that, based on a study of maps and consultation with park staff, may have been too far in the floodplain of the main stem, or may not have had sufficient flow. This ground-truthing resulted in adjustment of the floodplain criteria for two tributaries, and elimination of one tributary due to insufficient flow. Ultimately, at BUFF a total of 34 tributary stretches satisfied our selection criteria, and constituted the sample frame. We plan to sample 30 of these on a five-year rotation (the first 30 as ordered by GRTS). If, during the first five years, it is determined that any of these first 30 tributaries have to be deleted from the frame, we will have 4 alternate tributary stretches.

At OZAR, an initial set of 34 tributaries satisfied the above criteria. Ground-truthing and consultation with park staff resulted in elimination of 18 tributaries that were determined to have insufficient flow during the time of year selected for sampling (autumn). Although many of the tributaries at OZAR do contain some water all year, during the summer and autumn much of the flow is underground, through the gravel substrate. A total of 16 tributary stretches satisfied our selection criteria at OZAR, and constituted the sample frame. We plan to sample 15 of these on a five-year rotation (the first 15 as ordered by GRTS). If, during the first five years, it is determined that any tributary has to be deleted from the frame, we will have an alternate tributary stretch.

Temporal design

At both parks, the revisit design will have an always revisit panel and a set of rotating panels (Table 1). To ensure sufficient representation of monitoring sites on the main stems, the always revisit panel will consist of main stem stretches that will be sampled each year (n=9 for OZAR,

n=6 for BUFF). The rotating panels will consist of tributaries (n=3 for OZAR, n=6 for BUFF) that will be sampled every 5 years. At OZAR, 15 total tributary stretches will be sampled while at BUFF (which has many more tributaries) 30 total tributary stretches will be sampled. Panel assignments for each park are in Appendix B. Given our limited sample size, this strategy will yield maximum information on trend for the main stems, and maximum spatial coverage for the tributaries. However, we would be able to sample only a small fraction of the total number of tributaries in each park if the alternative approach was applied for the tributaries (*i.e.*, maximizing information on trend).

Table 1. Revisit plans for monitoring studies proposed at BUFF and OZAR. An 'x' in the right-most columns indicates all sample units in that panel are to be visited that year.

Study	Revisit Notation	Panel #	% of Annual Effort	Year											
				2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017
BUFF	[1-0,1-4]	1	50%	X	X	X	X	X	X	X	X	X	X	X	X
		2	50%	X					X					X	
		3			X					X					X
		4				X					X				
		5					X					X			
		6						X					X		
OZAR	[1-0,1-4]	1	75%	X	X	X	X	X	X	X	X	X	X	X	X
		2	25%	X					X					X	
		3			X					X					X
		4				X					X				
		5					X					X			
		6						X					X		

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

Invertebrate samples collected during the winter season have been shown to have higher species abundance and fewer organisms per sample, thus making sample processing and identification more efficient and cost effective.

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

Response Design

Types of Data Collected in the Field

This monitoring program will collect benthic invertebrates from stream riffles and associated habitat and water quality data. Habitat features are major, often limiting, determinants of invertebrate community structure 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 filamentous algae and aquatic plants play key roles in the microhabitat structure and distribution of aquatic invertebrates (Hauer and Lamberti, 1996; Allan, 1995; Rosenberg and Resh, 1993). We propose to monitor all of the aforementioned habitat variables at our sampling sites.

Biological and environmental correlates of water quality and habitat structure compared across time are powerful tools for assessing disturbances related to natural and anthropogenic impacts on aquatic invertebrate communities, and they are useful further for detecting change and elucidating patterns and trends in long-term data sets (Moulton *et al*., 2002). For example, as habitat conditions degrade (*e.g.*, water quality decreases, embeddedness increases), degradation of the benthic invertebrate community are expected to follow. However, the relationship of cause and effect of these variables on aquatic invertebrate 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 withstanding, benthic community structure, when viewed in association with environmental variables can be an effective indicator of ecosystem change (Reice and Wohlenberg, 1993). In combination, such data are useful for providing managers an integrated assessment of water quality.

The sampling approach described here is most comparable to that of the United States Geological Survey, National Water-Quality Assessment Program (USGS NAWQA) (Moulton *et al*., 2002), and the framework of this protocol generally fits within that of the former. However, the protocol described here is adapted from the NAWQA protocol to account for program specific goals related to long-term monitoring, and limitations posed by staff size and logistical and budgetary constraints. The general basis of the NAWQA program is to collect biological, physical and chemical data at sites that represent major natural and anthropogenic factors considered responsible for controlling water quality in a river basin. The NAWQA sampling design for benthic invertebrates includes two types of sampling sites: basic fixed sites and synoptic sites. The fixed sites are those at which parameters are measured over long periods of time and, as such, they are analogous to the sampling sites used in this monitoring protocol. The NAWQA synoptic sites are used for one-time collections and therefore 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. However, by compositing the individual samples collected from a reach, no estimate of variability among samples can be obtained. In contrast, this protocol recommends collecting three semi-quantitative benthic invertebrate samples from each of three consecutive riffles in a reach to assess both inter-and intra-riffle variability of benthic invertebrate community structure. Further, this protocol does not recommend collecting composite qualitative benthic samples taken from throughout the reach. The single habitat sampling of invertebrates in riffles we propose in this protocol also follows the recommendations of Rabeni *et al*. (2002) who concluded that riffles of Ozark streams provided the most consistent estimates of community structure both spatially and temporally in comparison to pools which were least concordant. Moreover, the species rich habitats of riffles are expected to be highly sensitive to water-quality changes because they can support a diverse community displaying a wide range of sensitivities to water-quality changes (Moulton *et al*., 2002). The NAWQA protocol allows for location of sites based on the whether or not 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. This sampling design in this protocol, by comparison, is strictly probabilistic and sampling reaches are permanent. Collecting techniques and equipment described herein are analogous to those described in the NAWQA protocol (Moulton *et al*., 2002).

The U.S. Environmental Protection Agency (EPA) has two programs for assessing water quality using invertebrate communities in wadeable streams. These are the Rapid Bioassessment Protocols for Use in Streams and Rivers (Barbour *et al.*, 1999), and the Environmental Monitoring and Assessment Program-Surface Waters (EMAP) (Lazorchak *et al*., 1998). An

additional set of protocols designed for larger non-wadeable rivers (Flotemersch *et al*., 2006) generally are not applicable to the streams addressed in this protocol and are not further addressed here. The Rapid Bioassessment approach uses either single habitat (*e.g.*, riffles) or multi-habitat approaches and both involve collecting samples from a 100 m reach determined by the investigator to be representative of the characteristics of the stream. The single habitat approach involves sampling using a kick-net with a 1 meter area sampled in front of the net and taking 2-3 kicks using foot agitation. The samples are then composited for analysis. Benthic metrics for analyzing data are the same or comparable to those used in this protocol (Barbour *et al*., 1999). The multi-habitat approach uses 20 jabs or kicks taken from different representative habitat types using a D-frame dipnet. Samples are composited for analysis and metrics are the same or comparable to those used in this protocol (Barbour *et al*., 1999).

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

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

Placement of samples

Stretch boundaries are permanent and will not be changed, excluding natural changes to the bedform due to the dynamic nature of rivers. GPS coordinates for each stretch are found in Appendix B. Procedures for selection of riffles and sample points within each riffle are described in SOP# 3, "Sampling Invertebrates and Collecting Habitat Data." For each selected stretch, three consecutive riffles will be chosen to represent a reach (defined broadly here as three consecutive riffle/glide/pool sequences). Thus the sample reach will extend from the upper boundary of the upstream most riffle to the lower boundary of the downstream most riffle. Riffle

selection is determined *a priori*, with the three riffles being those located in consecutive order upstream of the first riffle above the lower boundary of the selected stretch (Figure 1). The specific locations of the riffles sampled in a given year may move naturally due to hydrological processes.

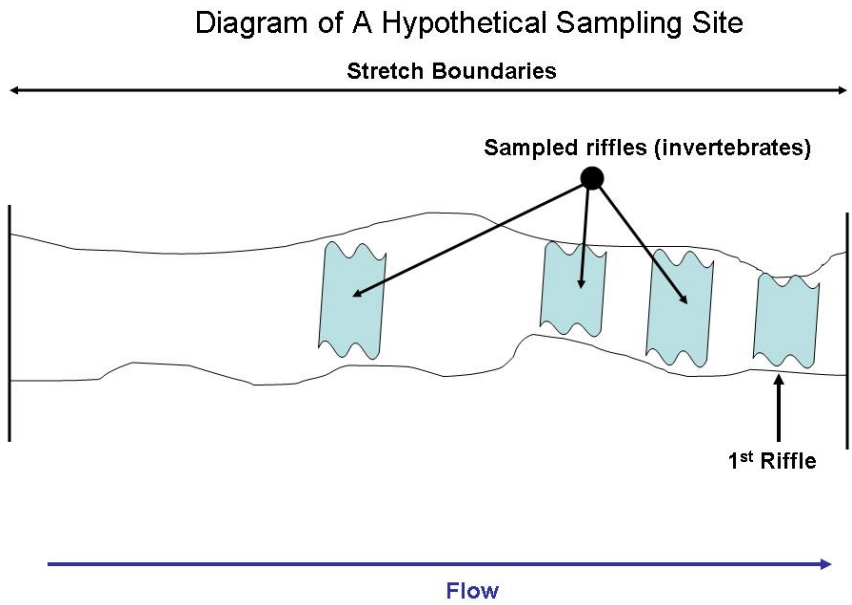

Figure 1. Riffle selection within a stretch.

Three benthic samples will be randomly collected from each selected riffle resulting in a total collection of nine separate samples per stretch. This sampling approach therefore provides an estimate of intra-stretch and intra- and inter-riffle variability. In total, 108 benthic samples will be collected annually from each park. The process of collecting benthic samples is described in SOP#3.

Number of Samples

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

samples, and then adding the fourth sample if results were inconclusive or showed extreme deviations.

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

Rationale for the Sampling Design

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

1. *Single habitat (riffle) sampling is appropriate for long-term monitoring of benthic invertebrates*. Sampling multiple habitats provides more comprehensive information about the invertebrate fauna compared to single habitat samples (Lenat and Barbour, 1994; Moulton *et al*., 2002). However, comparability between sites is necessary for accurate bioassessments and invertebrates collected from the same habitat types among sites are more similar than invertebrates collected from multiple habitats within the same site (Parsons and Norris, 1996; Rabeni *et al*., 1997). Indeed, Rabeni *et al*. (1997) showed metric sensitivity did not increase when comparing multiple versus single habitat sampling in Missouri streams. Furthermore, habitats in Ozark streams with the lowest community variability are high gradient riffles and coarse runs (Doisy and Rabeni, 1999). Therefore, single habitat sampling in riffle/run habitat is the focus of this protocol and will be employed at both BUFF and OZAR.

2. *Appropriate for Ozark streams*. The data generated from this study design will be directly comparable to those of Rabeni *et al*. (1997) and Mathis (2001) as well as other regional (state and federal) invertebrate monitoring programs that employ similar methodologies and rely largely on percentage-based metrics (*e.g*., Barbour *et al*., 1999).

3. *Accommodates habitats of varying size*. The sample design allows for unbiased estimates of invertebrate community condition that are applicable to the entire stretch regardless of length. While stretches must be long enough to accommodate unbiased estimates for all studies, they do not have to be the same size.

4. *Easy to learn and use*. Field procedures are easy to use and repeatable over time by

different sampling crews. Implementation does not require extensive time or costly equipment.

5. *The sequence of sampling events and revisit design for tributaries allows for the greatest amount of field work to be accomplished per year while minimizing cost.* Because staff available for manning field crews is limited, and travel costs associated with monitoring are high, this strategy allows cost effective monitoring for mainstem sites and further allows for maximum spatial coverage for the tributaries.

6. *The selected approach to monitoring is advantageous over other approaches.* The study design and methods selected for this protocol allow for an integration of community attributes and further allow us to characterize temporal changes and relative site quality. Additionally, our approach will allow us to correlate invertebrate community data with land use and habitat changes potentially arising from multiple stressors.

III. Field and Laboratory Methods

Field Season Preparations, Field Schedule, and Equipment Setup

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

Collecting Benthic Invertebrate Samples and Associated Habitat and Water Quality Data

Procedures for collecting benthic invertebrate samples and documenting habitat data are presented in SOP#3 (Sampling Invertebrates and Collecting Habitat Data), SOP#5 (Measuring Stream Discharge), and SOP#6 (Documenting CORE 5 Water Quality Variables)". Figure 2 shows a work flow diagram for collecting samples. Habitat variables will include an assessment of riffle length and width, and depth and current velocity measurements and dominant substrate for each sample at the specific locations benthic samples are collected. Several additional qualitative measurements of habitat condition will be taken from the area delineated by the sample net frame. Stream discharge will be measured at each site and preferably upstream of the sampling site after invertebrate collections have been completed. CORE 5 water quality parameters (temperature, dissolved oxygen, specific conductance, pH, turbidity) will be recorded for each riffle using hand-held instruments while data loggers will be used for other areas of the stream.

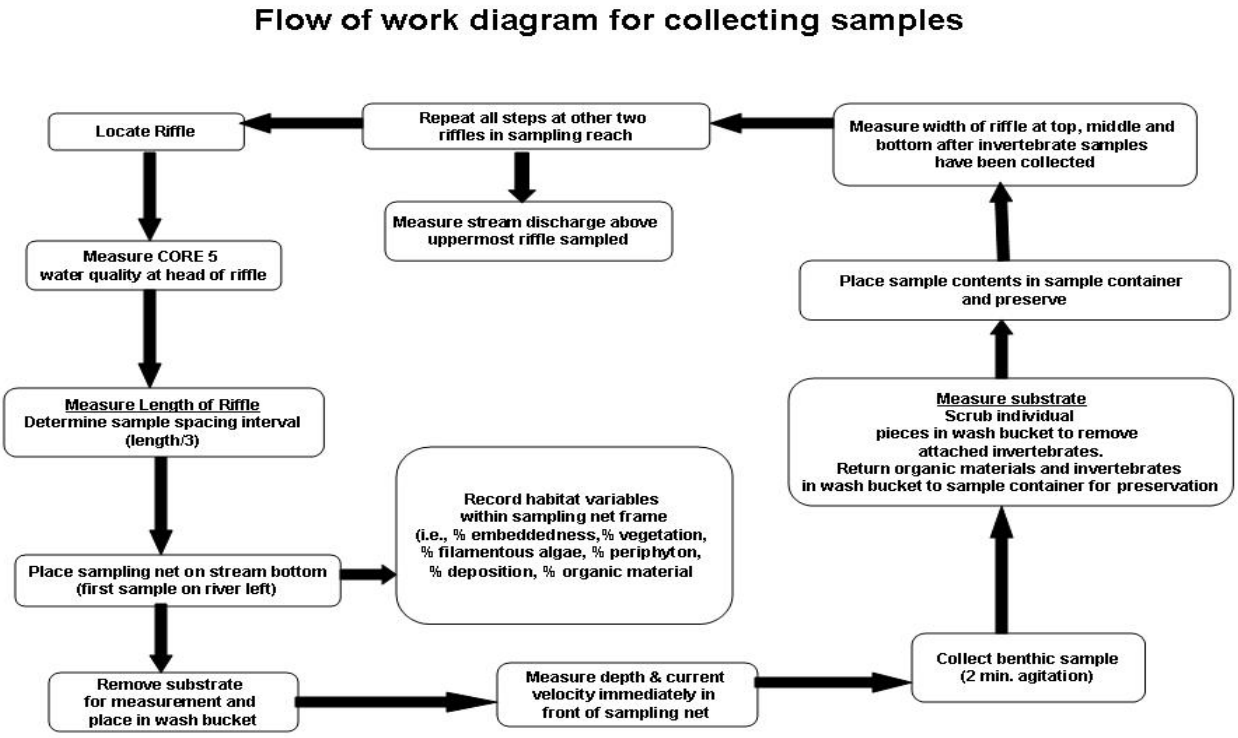

Figure 2. Flow of work diagram for collecting samples.

Riffle Measurements

Once the riffle to be sampled is located, its length (meters) will be measured to allow for equal spacing of invertebrate samples. Width should be measured (meters) for each transect (top, middle, bottom) after the benthic sample has been collected to avoid disturbing the sampling area. Once these steps are taken, the invertebrate sample and other associated habitat measurements can be taken.

Measurements within Sampling Net Frame

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

Substrate Size Assessment

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

Collecting Benthic Samples

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

Benthic Sample Processing and Specimen Identification

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

Subsampling benthic samples

Because of the relatively large number of samples that will be collected from BUFF and OZAR, subsampling individual samples will be necessary. The routine for subsampling benthic samples is presented in SOP#4. The method of subsampling will involve the fixed fraction approach with 25% of each sample being sorted following thorough washing, agitation, sieving, and elutriation of the entire sample (Moulton *et al.*, 2000). Additionally, a "large and/or rare" taxa component will be included where large or rare taxa that clearly are not in the sorted fraction are removed and stored in a separate vial for the purpose of reflecting accurate sample species richness estimates and calculating specific metrics such as EPT. A fixed fraction subsampling routine was selected over a fixed count routine because some the metrics to be calculated from samples

are related to specimen density which cannot be obtained with the latter method. Subsampled fraction debris will be subjected to QA/QC analysis (SOP#4) and should be kept until QA/QC is complete for that batch of samples and the Program Leader authorizes disposal of the debris.

Sample Storage and Reference Collection

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

Post Season Procedures

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

IV. Data Management

Overview of Database Design

All data management activities related to this protocol is described in SOP#7 "Data Management." Microsoft Access 2003 is the primary software environment for managing river invertebrate and habitat data. ESRI ArcInfo 9 serves as a tool for validation of spatial data residing in Access. Data products will be posted at the NPS I&M website: http://science.nature.nps.gov/im/monitor/protocoldb.cfm. Metadata for river invertebrate community monitoring will be available on the NPS I&M application server: http://science.nature.nps.gov/im/apps/index.cfm. QA/QC guidelines in this section are based on recommendations of Rowell *et al*. (2005) and S. Fancy at http://science.nature.nps.gov/im/monitor and citations therein. The general data model for river invertebrate community monitoring consists of two core sets of tables. One set manages species attribute data (species names, replicate number, count, *etc.*) the other associated habitat data. Species attribute and habitat data are linked in time and space by way of standardized event and location tables. The primary table for storing species attribute data contains information about the species. Supporting tables include taxonomic information, observers, and equipment information. A locations table provides detailed location information associated with each sampling point. Look-up tables are linked to relevant tables to provide the values for pick-lists on data-entry forms, thereby reducing possible error during data entry (see Data Verification and Editing below).

Data Entry

A number of features have been designed into the database to minimize errors that occur when field data is transcribed to the database for storage and analysis. Forms are used as portals for data entry into the database. Standardized identifiers (*e.g.*, sample location and event) are selected from a list of easily interpreted codes. Species and habitat data are entered into fields linked to appropriate tables. Look-up tables contain project-specific data and prohibit entry of data into a field if a corresponding value is not included in the look-up table. Consequently, only valid names or measures may be entered and spelling mistakes are eliminated. Species or habitat measures are selected using a pick list or by typing the beginning of the name.

Data Verification and Editing

Data verification immediately follows data entry and involves checking the accuracy of computerized records against the original source, usually paper field records. While the goal of data entry is to achieve 100% correct entries, this is rarely accomplished. To minimize transcription errors, our policy is to verify 100% of records to their original source by staff familiar with project design and field implementation. Further, 10% of records are reviewed a second time by the Project Manager and the results of that comparison reported with the data. If errors are found in the Project Manager's review, then the entire data set is verified again. Once the computerized data are verified as accurately reflecting the original field data, the paper forms are archived and the electronic version is used for all subsequent data activities.

Although data may be correctly transcribed from the original field forms, they may not be accurate or logical. For example, an invertebrate count of 3,325 instead of 325 may be illogical and almost certainly incorrect, whether or not it was properly transcribed from field forms. The process of reviewing computerized data for range and logic errors is the validation stage. Certain components of data validation are built into data entry forms (*e.g.*,. range limits). Data validation can also be extended into the design and structure of the database. As much as possible, values for data-entry forms have been limited to valid entries stored in the look-up tables.

Additional data validation can be accomplished during verification, if the operator is sufficiently knowledgeable about the data. The Project Manager will validate the data after verification is complete. Validation procedures seek to identify generic errors (*e.g.*, missing, mismatched, or duplicate records) as well as errors specific to particular projects. For example, one database query detects records with a location ID from a park and a period ID from a different park. Another query counts the number of riffles sampled per site to be sure all data were collected and entered.

During the entry, verification, and validation phases, the Project Manager is responsible for the data. The Project Manager must assure consistency between field forms and the database by noting how and why any changes were made to the data on the original field forms. In general, changes made to the field forms should not be made via erasure, but rather through marginal notes or attached explanations. Once validation is complete, the data set is turned over to the Data Manager for archiving and storage.

Spatial validation of database sample coordinates can be accomplished using ArcGIS (ESRI, Inc.). Because RMInverts is maintained as an Access database, it can be integrated directly with ArcCatalog (ArcGIS, ESRI, Inc.) as an OLE DB object. Coordinate data (UTM northing and easting) of the locations table can then be used to validate the UTM coordinate values for sample locations stored in Access against the original GPS coordinates.

Metadata Procedures

Metadata for project data are developed using ESRI ArcCatalog 9 and follow current Federal Geographic Data Committee (FGDC) standards. This requires conversion of the Access database to an ESRI personal geodatabase. Metadata are then exported into text and Extensible Markup Language (xml) format. Text-based metadata is then parsed using USGS metadata parser to check for errors in formal FGDC metadata.

Database Versions

Changes in database structure and functionality require a versioning system. This allows for the tracking of changes over time. With proper controls and communication, versioning ensures that only the most current version is used in any analysis. Versioning of archived data sets is handled by adding a two digit number separated by a period to the file name, with the first version being numbered XXXXXX1.0. Minor changes such as revisions in forms and report content should be noted by increase of the number to the right of the period. Major changes such as migration between Access versions or database normalization across multiple tables should be indicated by an increase in the number to the left of the period. Frequent users of the data are notified of the updates, and provided with a copy of the most recent archived version.

Database Security

Secure data archiving is essential for protecting data files from corruption. No versions of the database should be deleted under any circumstance. Monitoring databases are small and do not require significant computer drive space or resources. On the other hand, they represent primary data and are expensive to create and impossible to replace. Multiple backup copies of all program data are maintained at the HTLN offices, at the Wilson's Creek visitor center, and at the MSU campus offices. Tape backups of the invertebrate databases are made weekly. Each weekly full backup copy is maintained at the Wilson's Creek National Battlefield Visitor Center, Republic, MO. Once a month, one tape copy is stored offsite.

Currently, data are available for research and management applications on request, for database versions where all QA/QC has been completed and the data have been archived. Most data requests are currently met using FTP services. Portions of the invertebrate community monitoring data will be made available for download directly from the NPS I&M Monitoring webpage. Information related to location and persistence of species determined to be threatened or endangered will not be made available for download by the general public. In addition, metadata will be available directly from the NPS I&M NR-GIS Metadata and Data metadata server (http://science.nature.nps.gov/nrdata). Data requests should be directed to:

Data Manager
Wilson's Creek National Battlefield
6424 W. Farm Road 182
Republic, MO 65738-9514
(417) 732-6438

V. Analysis and Reporting

Metric Selection and Community Indices

Early biomonitoring programs tended to focus on one or two specific attributes or metrics of the community; the indicator species concept (Kremen, 1992) is an example. Individual metrics generally are chosen based on the specific and predictable response of organisms to landscape changes. Additionally, they are sensitive to a range of factors that stress biological systems and are relatively easy to measure and interpret (Karr and Chu, 1999). Barbour *et al*. (1999) lists and briefly describes many types of metrics used in assessing condition of stream condition. However, individual metrics in themselves often are not adequate for assessing complex systems with cumulative impacts (Karr, 1991). In comparison, 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. 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 for discriminating among different kinds of human impact. Multimetric approaches are favored by most aquatic resource agencies in the United States because they are based on sound scientific rationale, they are simple to implement, and they are among the most sound for assessing invertebrate community structure (Lenz and Miller, 1996; Bonada *et al*., 2006).

Ozark National Scenic Riverways

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

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

- Gross organic pollution- Hilsenhoff (1982) listed all of these as indicators of gross organic pollution.
- 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 Shannon Diversity Index typically decrease in response to increasing acidity (MacKay and Kersey, 1985; Hildrew *et al.*, 1984). Mayflies are especially sensitive to low pH (Peterson *et al.*, 1985).
- Effects of logging and clear cutting- Stone and Wallace (1998) found that the North Carolina Biotic Index (NCBI-a modification of the Biotic Index; Lenat, 1993) was the most sensitive to this type of disturbance.
- Heavy metal pollution- Taxa richness and EPT richness (Winner *et al.*, 1980, Chadwick *et al.*, 1986) have been shown to decrease in response to this type of pollution. However, further research indicates that mayflies may decrease in richness and abundance while caddisflies increase under these conditions, resulting in a static EPT. If no difference in the EPT is found, analysis of the richness and percent composition of mayfly taxa should be performed (Doisy and Rabeni, 1999).
- Insecticides- Wallace *et al.* (1996) found that both the EPT index and the NCBI easily detected disturbances to a stream treated with certain insecticides.
- Habitat degradation in the Ozark Highlands- Rabeni *et al.* (1997) found that Simpson's Index for high gradient riffles was a sound measure of disturbance. According to Doisy and Rabeni (1999), there are few strong relationships between the degree of habitat degradation and the biological condition of streams in the Ozarks. Doisy and Rabeni (1999) also found an increase in the percentage of collector/filterers with increased embeddedness in Ozark Highland streams.

Metric Scoring for the SCI

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

Buffalo National River

Mathis (2001) developed a multi-metric Index of Community Integrity (ICI), designed specifically for the Buffalo River. We hereafter refer to this index as the Buffalo River Index of Community Integrity (BRICI), to avoid confusion with other ICIs being used throughout the United States. The BRICI was designed using data collected by Bryant (1997) from eight sites on the main channel of the Buffalo River during the summer, fall, and winter of 1993 and spring of 1994. The BRICI is similar to that developed by the Ohio EPA (1987) and other state agencies. Mathis (2001) evaluated and selected 10 metrics for the BRICI that are sufficiently sensitive for detecting impairment. The metrics include: Margalef's Index of Taxa Richness,

Shannon's Taxa Diversity Index, Percent Dominant Taxa, Percent Chironomidae, Percent Plecoptera, Percent Trichoptera, Percent Elmidae, Percent Corbicula, Percent Intolerant, Percent Collector-Filterer. These and other community metrics are described in Barbour *et al*. (1999). Procedures for calculating and scoring these metrics are included in SOP#8.

Metric Scoring for the BRICI

The scoring method employed here is similar to the one used by Karr *et al.* (1986) to develop the Index of Biological Integrity (IBI) for fishes. This method involves dividing the data from all sites for a given metric into specific percentiles. Values in the highest percentile range are assumed to represent high water quality. Those in the lowest range are assumed to indicate low water quality, and intermediate percentiles represent water quality somewhere between high and poor water quality.

An assumption of this scoring technique is that when comparing sites that are similar in size, sites with higher water quality generally should have better values for each metric than sites with lower water quality. For metrics that decrease with increasing level of disturbance (Margalef's index, Shannon index, percent Plecoptera, percent Trichoptera, percent Elmidae, percent collector-filterer, and percent intolerant), higher metric values indicate higher water quality. For metrics that increase with increasing level of disturbance (percent Chironomidae, percent *Corbicula*, and percent dominant), lower metric values suggest higher water quality. Additional details of this method are presented in SOP#8.

Data Analysis

In determining the appropriate statistical approaches for this monitoring protocol, it is important to take into account the primary audience of the various reports that will result. This audience will consist of park resource managers, park superintendents, and other park staff. Park resource managers and staff may not have an in-depth background in statistical methods, and park superintendents may have limited time to devote to such reports. Additionally, protocols such as this may provide much data on many different types of variables. Thus it is important, to the extent possible, that 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 three main statistical approaches that could be employed with data from long-term monitoring projects such as this: (1) hypotheses testing, (2) parameter estimation, and (3) application of Bayesian methods. When analyzing ecological data, statisticians predominantly employ frequentist methods, and thus many resource managers are not familiar with the interpretation of Bayesian approaches. Bayesian methods are not widely used because they are often difficult to apply, and many researchers are not comfortable specifying subjective degrees of belief in their hypotheses (Utts, 1988; Hoenig and Heisey, 2001). Thus we do not advocate a Bayesian approach as our main method of data analysis.

Most hypothesis testing approaches involve a null hypothesis of no difference or no change. The problem with such approaches is that the hypothesis under test is thus trivial (Cherry, 1998; Johnson, 1999; Anderson *et al*., 2000, 2001). No populations or communities will be exactly the same at different times. Thus, we're 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 to any desired level.

Multivariate control charts may also be constructed, and although some of the above-mentioned texts describe multivariate control charts (using the Hotelling T^2 statistic), this approach is only practical for a small number of variables, and assumes a multivariate normal distribution. In general, species abundances are not distributed as multivariate normal (Taylor, 1961), and traditional multivariate procedures are frequently not robust to violations of this assumption (Mardia, 1971; Olson, 1974). A new type of multivariate control chart has recently been

described for use with complex ecological communities and a software application entitled *ControlChart.exe* is available for constructing these types of multivariate control chart (see Anderson and Thompson, 2004). Multivariate temporal autocorrelation will violate the assumption of stochasticity upon which this method is based, however, and it is important to test for temporal autocorrelation using Mantel correlograms prior to using this method. This new multivariate control chart appears to have promise but has not been widely applied nor thoroughly evaluated. Further evaluation of this method is warranted before application to the data of this protocol.

We did not conduct a formal power analysis for this protocol for three reasons: (1) The primary purpose of conducting a prospective power analysis is to determine whether the proposed sample size is adequate. There already exist a number of studies indicating that three samples per riffle is an appropriate number for calculation of the proposed metrics (see "Number of samples" under the Sample Design Section. Because our sample size will be determined primarily by budget, we would not be able to increase the number of riffles sampled per stretch or number of stretches regardless of the result of any power analysis. Furthermore, in many analyses sample size will equate with number of years; in this case, analyses will simply become more powerful over time. (2) Statistical power is dependent upon the hypothesis 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 mis-used and misinterpreted in ecological contexts (Morrison, 2007), and alternative approaches to evaluating the degree of uncertainty associated with our data will be evaluated and used when applicable.

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

Although our primary approach to organizing and analyzing data will consist of multimetric indices, we do not entirely rule out the use of any statistical methods at this time. Because of the nature of this long-term monitoring program, other approaches (some of which may not yet have even been developed!) may be appropriate at different points in time, depending upon the needs of the resource managers and questions of interest. At times, depending upon the question of

interest to resource managers, a hypothesis testing framework may be employed. Because data from studies of aquatic insects is often not normally distributed, non-parametric approaches may need to be employed. For example, if it is desirable to test for differences between riffles, a Kruskal-Wallace ANOVA, Friedman's non-parametric two-way ANOVA, or Cochran's Q test could be used. Of course, normality of the data will be evaluated prior to any tests, and transformations may be performed if useful prior to tests requiring normal distributions. These approaches and others are described in SOP#8.

Reporting

Reports summarizing monitoring data collected during the year should be prepared annually. Reports will include the overall SCI and ICI indices, as well as the individual metrics and indices that comprise these. These reports will include an update on the status of the resources in addition to documenting related data management activities and data summaries. In an effort to disseminate findings in a timely manner, annual summary reports should be completed by September 30th of the year following data collection. Summary reports may be used in place of annual reports for the year in which the last data is collected. Comprehensive trends analysis and synthesis reports will be completed every five to ten years depending on observed impacts in the watershed and how critical summary information is for setting management goals influencing stream condition. Executive summaries should be prepared for all types of reports. Refer to SOP#9 "Data Reporting" for details on types of reports and their primary audiences, report structure and style, and review procedures.

VI. Personnel Requirements and Training

Roles and Responsibilities

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

Qualifications and Training

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

VII. Operational Requirements

Annual Workload and Field Schedule

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

Laboratory processing time per benthic sample, including sorting, identification, counting, and entry into the database, will require approximately 6 hours per sample or 81 laboratory days to complete. These tasks will be accomplished by the Program Aquatic Ecologist stationed at each park.

Facility and Equipment Requirements

Field and lab equipment listed in SOP#1 "Preparation for Field Sampling and Laboratory Processing" are only for one sampling crew. Beyond normal office and equipment storage space, facility needs include access to a wet laboratory. Additional equipment requirements include access to a canoe and/or motorboat.

Startup Costs and Budget Considerations

Estimated costs for conducting invertebrate monitoring at BUFF and OZAR are shown in Table 2. Personnel expenses for fieldwork are based on a crew of three; a professional aquatic ecologist or fisheries biologist to oversee the fieldwork, data collection and to coordinate surveys, and two seasonal biological science technicians or others are required to assist in field data collection. Assistance with field work from other agencies is always welcome to the extent it is available. Field costs may vary somewhat from year to year depending on the skill level and

size of crew. Data management personnel expenses include staff time of biological science technicians, the aquatic ecologist and data manager.

Table. 2. Estimated costs for conducting annual invertebrate monitoring at BUFF and OZAR.

Budget Item	**Estimated Costs for BUFF and OZAR**
Coordinator	$6,857.59
Quantitative Ecologist	$6,920.02
Aquatic Program Leader	$17,300.05
Fisheries Ecologist	$14,434.56
GIS Specialist	$4,490.75
Data Manager	$5,132.29
Aquatic Ecologist STF (22 pay periods)	$8,252.50
Aquatic Ecologist STF (22 pay periods)	$8,252.50
Administrative Assistant	$3,510.76
Seasonal Biotechs (22 pay periods)	$4,492.78
Administrative support to WICR	$1,176.00
Overhead to Missouri State University	$500.00
Field Work Travel	$2,888.00
Computer Hardware & Software	$760.00
Vehicle Lease	$1,824.00
Field/Office Equipment	$1,368.00
Supplies	$912.00
Lab Fees	$2,000.00
TOTAL	$91,071.80

VIII. Procedures for Protocol Revision

Revisions to both the Protocol Narrative and to specific Standard Operating Procedures (SOPs) are to be expected. Careful documentation of changes to the protocol and a library of previous protocol versions are essential for maintaining consistency in data collection and for appropriate treatment of the data during data summary and analysis. The Microsoft Access® database for each monitoring component contains a field that identifies which version of the protocol was being used when the data were collected.

The rationale for dividing a sampling protocol into a Protocol Narrative with supporting SOPs is based on the following:

- The Protocol Narrative is a general overview of the protocol that gives the history and justification for doing the work and an overview of the sampling methods, but that does not provide all of the methodological details. The Protocol Narrative will only be revised if major changes are made to the protocol.
- SOPs, in contrast, are very specific step-by-step instructions for performing a given task. They are expected to be revised more frequently than the protocol narrative.

- When an SOP is revised it usually is not necessary to revise the Protocol Narrative to reflect the specific changes made to the SOP.
- All versions of the Protocol Narrative and SOPs will be archived in a Protocol Library.

The steps for changing the protocol (either the Protocol Narrative or the SOPs) are outlined in SOP#11, "Revising the Protocol." Each SOP contains a Revision History Log that should be filled out each time a SOP is revised to explain why the change was made, and to assign a new Version Number to the revised SOP. The new version of the SOP and/or Protocol Narrative should then be archived in the HTLN Protocol Library under the appropriate folder.

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X. Standard Operating Procedures (SOPs)

Protocol for Monitoring Aquatic Invertebrates at Ozark National Scenic Riverways, Missouri, and Buffalo National River, Arkansas. Heartland I&M Network and Prairie Cluster Prototype Monitoring Program

SOP 1: Preparation for Field Sampling and Laboratory Processing

Version 1.00 (07/01/2007)

Revision History Log:

Previous Version #	Revision Date	Author	Changes Made	Reason for Change	New Version #

This SOP provides information to prepare for the field season, including lists required of field and laboratory equipment. It also provides information on recording information on staff time spent on sampling trips, checking water levels at the parks, and obtaining collecting permits. A list of required data sheets with a brief explanation of their purpose is provided.

I. General Preparations

Prior to the field season all crew members should review the entire protocol, including SOPs. The following list includes key points to consider in preparing for the upcoming field season.

1. The team leader of each sampling crew must prepare a field notebook for the survey year. The notebook should contain entries for observer names, field hours and unique happenings that may influence how the data is reported. Information included in trip reports is based on what is recorded in field notebooks so it is imperative that they are clearly organized for ease of field note entry. Notebook entries should be recorded daily to ensure accuracy. An example of a notebook log is shown in Figure 1.

Date	Travel time (hours)	Field time (hours)	Non-project time (hours)	Lunch (hours)
30 Sep 2006	3	8	0	0.5
1 Oct 2006	0	8	1	0.5
2 October	3	8	0	0.5
Notes: J. Smith and H. Simpson traveled to OZAR to conduct fish monitoring in the Current River (CURRM01-03). Field assistance was provided by B. Jones and K. Adams. Returned to headquarters. Non-project time included discussing other projects with Park staff.				

Figure 1. Example of a field note book for recording scheduling, travel and field time, and personnel information.

2. Invertebrate community monitoring will be conducted during the period November 1-March 1. Inclement weather and personnel workloads will preclude the scheduling of sampling events to specific annual dates. Sampling dates should be scheduled and logistics organized prior to the start of each field season. Monitoring efforts will require a three person crew (two people to sample and one to record data and provide general assistance). Allow at least one day per site, or 12 days per park to complete annual sampling.

3. An equipment list will be compiled and equipment organized and made ready for the field season several weeks prior to the first sampling tour to make sure that all supplies are available and equipment in working condition. This allows time to make required repairs and order replacement equipment. Inspect the sample nets and wash bucket to ensure there are no tears in the nets or screen. Ensure water quality meters can be calibrated and are properly functioning as described in SOP#6. Equipment and supplies are for laboratory and field use are shown in (Tables 1 and 2).

Table 1. Laboratory equipment required for processing invertebrate samples.

Number Req.	**Description**
1	U.S. Standard sieve 500-µm mesh sieve; marked into 4 equal-sized sections
1	Shallow white pan for sorting; 8X10 inches or other suitable size
1	Petri dish or similar sorting container
3	Multi-well Petri dishes for separating sorted insects
1	Dissecting microscope and binocular microscope
Several	Forceps (fine point)
Several	Clean plastic bottles with labels inside and out for storage of processed samples
	Preservative (70% isopropyl alcohol)
Several	Storage vials with labels for reference collection specimens
Several	Pencils or fine point pen with waterproof ink
See below	Data sheets

Table 2. Field equipment required for monitoring aquatic invertebrates.

Number Req.	Description
1	Sample net: EPA style kick net (*i.e.*, Slack Surber) with 500µm mesh net fitted with 0.25 m^2 sample frame
1	Nylon scrub brush
1	Hand rake or the "Garden claw"
Varies per site	Labeled sample bottles (Plastic 500-ml wide mouth bottles; 9 bottles per stretch
Varies per # of samples	Preservative-99% isopropyl alcohol and 1-L plastic Nalgene bottles for carrying, bring extra quantities to ensure enough is available to preserve all samples. In total, approximately 12 gallons will be required per year for each park. Label container "flammable"
2	Forceps
2	Polypropylene wash bottles
1	U.S. Standard sieve, 500-µm mesh size
2	5-gal white buckets
1	Benthic sample wash bucket with 500-µm mesh size
1	Shallow white pan
2	Laminated plastic sheet with Wentworth scale codes for conducting substrate measurements
1	Clip board
5	Pencils
	Data sheets printed on waterproof paper, extra sample bottle labels and tape
1	Tape measure
1	Range finder
1	GPS unit
1	Digital camera
1	Field log book
	Directions to sample sites, sample site maps, list of GPS coordinates
	Waders and boots
	Life jackets
1	Velocity meter and wading rod
1	pH meter, conductivity meter, dissolved oxygen meter
	Extra batteries for water quality meters
	Buffer solution for pH meters (pH 7 and 10)
1	Backpack

II. Field and Laboratory Forms

Data sheets required are listed in Table 3. Print copies of field sheets and labels on waterproof paper. Data should be recorded on forms with waterproof ink or #2 lead pencil. Example data sheets are provided as attachments to their corresponding SOPs.

Table 3. List of data sheets required for invertebrate monitoring for the sampling season.

TITLE	PURPOSE	NUMBER OF COPIES NEEDED PER PARK
Stream field sampling form	Recording physical-chemical data for riffles	12
Sample cell substrate sheet	Recording substrate sizes from within sample cells	12
Discharge form	Recording stream discharge	12
Aquatic invertebrate identification & enumeration sheets	Recording names of identified taxa and their densities	Variable, but 12 minimum

III. Gages

Before deploying to a sample site, USGS stream gages should be checked to ensure that no floods have occurred in the last two weeks. If flooding has occurred, sampling should not take place until two weeks after flood waters have receded. Several USGS gages are located on the Current, Jacks Fork, and Buffalo Rivers as well as two tributaries of the Buffalo River. Buffalo National River also supports a flood warning system that provides water levels at four locations on the river and precipitation information throughout the watershed. Websites and locations for the USGS gages and the Buffalo River flood warning system can found in the Table 4. Gages should be checked prior to leaving for sampling.

Table 4. Information for gages in the Current, Jacks Fork, and Buffalo River watersheds.

USGS Gauge Number	Location	Information Provided	Website
		Current and Jacks Fork Watershed	
07064533	Current River above Akers, MO	Gage Height Discharge	http://waterdata.usgs.gov/mo/nwis/rt *Enter gage number or locate on map*
07067000	Current River at Van Buren, MO	Gage Height Discharge	
07068000	Current River at Doniphan, MO (outside NPS)	Gage height Discharge	
07065200	Jacks Fork River near Mountain View, MO	Gage height Discharge Water temperature	
07065495	Jacks Fork at Alley Spring, MO	Gage height Discharge Precipitation	
07066000	Jacks Fork at Eminence, MO	Gage height Discharge Precipitation	
		Buffalo River Watershed	
07055646	Buffalo River near Boxley, AR	Gage height Discharge Water temperature	http://waterdata.usgs.gov/ar/nwis/rt *Enter gage number or locate on map*
07055875	Buffalo River near St. Joe, AR	Gage height Discharge Precipitation	
07056700	Buffalo River near Harriet, AR	Gage height Discharge Precipitation	
0705587	Richland Creek near Witts Spring, AR	Gage height Discharge	
07056515	Bear Creek near Silver Hill, AR	Gage height Discharge Precipitation	

	19 precipitation gages in watershed		
Buffalo River Flood Warning System	Water level gages at Ponca, Pruitt, Highway 65 and Highway 14 bridges	Gage height Precipitation	www.buffaloriverandrain.com

IV. Collecting permits

Collection permits for taking invertebrates are required from the states of Arkansas and Missouri as appropriate. The state of Missouri does not currently require a collecting permit for stream invertebrates exclusive of crayfish.

Contact information for applying for permits is indicated below:

Arkansas

Arkansas Game & Fish Commission
Attn: Scientific Collector Permits
2 Natural Resources Drive
Little Rock AR 72205
(501) 223-6371

Missouri

Missouri Department of Conservation
P.O. Box 180
Jefferson City, MO 65102-0180
(573) 751-4115

Protocol for Monitoring Aquatic Invertebrates at Ozark National Scenic Riverways, Missouri, and Buffalo National River, Arkansas. Heartland I&M Network and Prairie Cluster Prototype Monitoring Program

SOP 2: Training for Field Sampling and Laboratory Processing

Version 1.00 (07/01/2007)

Revision History Log:

Previous Version #	Revision Date	Author	Changes Made	Reason for Change	New Version #

This SOP explains the training procedures for using field equipment properly, collecting habitat data, and processing invertebrates in the laboratory. This training ensures a high level of consistency among samplers and processors. Prior to training, all personnel should review the general protocol and each SOP. Someone familiar with the protocol and experience with the sampling and processing procedures should supervise the training.

I. Field Sampling and Habitat Data Collection

Procedure:

1. Find a nearby stream to practice collecting benthic samples and habitat data. Follow the procedures outlined in SOP#3 "Sampling Invertebrates and Collecting Habitat Data" and collect several practice samples for processing in the lab. Ensure that each person is comfortable with all aspects of the sampling routine.
2. After collecting the samples, practice using the water quality instruments. Each person should be familiar with the instruments and be able to calibrate each instrument as outlined in SOP#6 "Documenting CORE 5 Water Quality Variables". Practice discharge measurements following procedures described in SOP # 5 "Measuring Stream Discharge."

II. Laboratory Processing

Procedure:

1. Follow steps outlined in SOP#4 "Laboratory Processing and Identification of Invertebrates" using practice samples collected through field sampling training.

2. Material left over after sorting is completed should be checked by someone skilled in processing benthic samples to determine recovery efficiency. The number of invertebrates recovered should be expressed as a percentage of the total number of invertebrates. The standard for recovery is 95%.

Protocol for Monitoring Aquatic Invertebrates at Ozark National Scenic Riverways, Missouri, and Buffalo National River, Arkansas. Heartland I&M Network and Prairie Cluster Prototype Monitoring Program

SOP3: Sampling Invertebrates and Collecting Habitat Data

Version 1.00 (07/01/2007)

Revision History Log:

Previous Version #	Revision Date	Author	Changes Made	Reason for Change	New Version #

This SOP describes field procedures for selecting riffles to sample within a stretch and the process for randomly selecting sample points within a riffle. The SOP further explains procedures for collecting and processing invertebrate samples in streams, and for collecting associated habitat data. Appropriate QA/QC requirements are highlighted within the procedure descriptions.

I. Riffle Selection within a Stretch

1. The sample unit is a 'stretch' of contiguous river as defined in the protocol narrative. Sampling and habitat analysis will be restricted to a section of this stretch.

2. Locate the stretch of river to be sampled:

   a. Use GPS to find the lower geographic boundary of the stretch (beginning and ending points of stretches often correspond to tributary confluences which may help in locating the lower boundary in the field). For instructions on using the GPS, refer to the GPS SOP located at:
   http://www1.nature.nps.gov/im/units/htln/data_management/data_management.htm
   b. Locate the first riffle above the lower geographic boundary.
   c. The area that will be sampled includes the next 3 riffles located in consecutive order upstream of the first riffle located above the lower boundary of the stretch as shown in Figure 1.

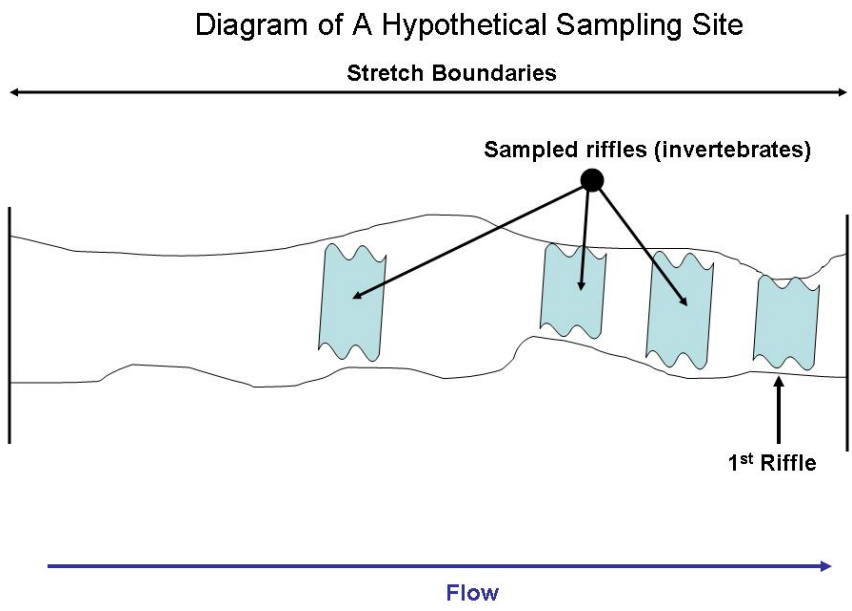


Figure 1. Diagram of riffle location within a stretch.

3. Site names will be designated by the first four characters of the river name (BUFF, CURR, JACK), Mainstem (M) or Tributary (T), and the site/tributary number. Mainstem sites and tributaries will be labeled in successive order from upstream to downstream. Example: JACKM03 is the 3rd mainstem site on the Jacks Fork River. CURRT10 is the 10th tributary sampled in the Current River Basin. Sample site codes and GPS coordinates can be found in Appendix B of this protocol.

II. Collecting Benthic Samples and Associated Habitat Data

Procedure:

1. Prior to collecting benthic samples and taking habitat measurements, always complete data sheet information for site name (*e.g.*, CURRM03), date and time of survey, and initials of personnel who collect the samples.

2. Measure CORE 5 water quality variables using the procedures described in SOP#6.

3. Measure the length of the riffle. Use a range finder for all measurements greater than 20 meters. Avoid walking in the riffle.

4. Collect three benthic samples per riffle. Benthic invertebrate samples will always be taken in an upstream direction. Individual benthic samples from riffles will be collected using the following *a priori* randomization procedure:

a) The sampling area is divided into three equal portions based on the measured length of the riffle as shown in Figure 2. First and foremost, the effective sampling area of the riffle will be based on safety of personnel, accessibility, and other pertinent factors determined by the investigators' best judgment at the time of sampling. The upper and lower riffle boundaries generally will be based on a visual assessment of gradient, velocity, and substrate characteristics. However, care should be used to avoid including the deeper, downstream portion of the riffle as it transitions in deeper run habitat with more of v-shaped channel.

b) Collect individual benthic samples starting at river left (1/4 point), then alternating to the middle, and finally the right (3/4 point). Samples will be collected at the approximate midpoint of each portion. Indicate sampling sequence on the data sheet (L, M, or R). Approximate position of the samples is shown in Figure 2.

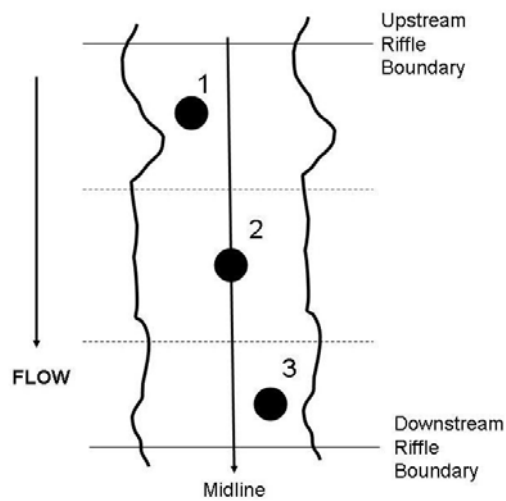


Figure 2. Diagram of sample locations within a riffle.

Note: Sample sequence should be altered only if the original starting point presents danger to the collector or if it is not accessible. Some riffles, especially those in the lower Buffalo River, may be wider than long and, in these instances, samples can be taken from left to right in equally spaced increments.

c) Samples will be collected with a Slack Surber sampler (analogous to the EPA-style rectangular benthic net) (~500 micron mesh) fitted with a sampling area guide (0.5 x 0.5 m^2 rectangle of PVC pipe placed immediately in front of the net) to delineate the approximate sampling area of 0.25 m^2 (Figure 3). Place the collection net and sample frame firmly against the stream substrate so that the opening is oriented directly into the stream current.

Figure 3. Invertebrate sampling net with sampling frame attached.

5. For each benthic sample, record habitat measurements based on visual estimates from within the sample frame on the habitat data form. Percentage categories are: 0= none (0%), 1= sparse (<10%), 2= moderate (10-40%), 3= heavy (40-75%), 4= very heavy (>75).

    a. percent embeddedness of the substrate (*e.g.*, bedrock & hardpan clay = 0% embeddedness; sand, clay, silt = 100% embededdness)
    b. percent periphyton
    c. percent filamentous algae
    d. percent sedimentation
    e. percent organic material.

6. Collect a sample of the substrate in the sampling area.

    a. After the net and sample frame has been placed, grab five handfuls of substrate from five evenly distributed locations within the sample frame (one from each corner, and one from the center) (Figure 4, 5). Place the substrate in a plastic bucket or wash bucket for processing <u>after</u> the invertebrate sample is collected (Step 8).

    <u>Note</u>: This technique is used to assess the dominant substrate in the sample area. It is not intended to fully characterize the substrate profile of the stream bottom.

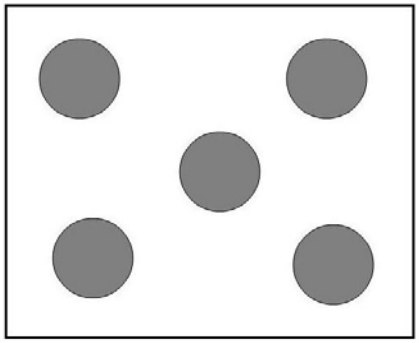


Figure 4. Location of substrate sample grabs within the sampling frame.

Figure 5. Collecting substrate from within the sampling frame.

b. Ensure the collected substrate pieces are washed and scrubbed with a soft brush before they are measured and discarded in order to dislodge any attached invertebrates (Figure 6). Forceps may be needed to remove snails and attached caddisflies. Shake the bucket so that the collected substrate is evenly distributed. Randomly (*i.e.*, blind touch) select a rock from the bucket. Measure the substrate piece using the Wentworth Scale (Table 1). A Wentworth scale category field sheet (provided at end of this SOP) is used for rapid measurement of substrate in the field (Figure 7). A piece of substrate belongs to the smallest size box through which it will fit on any axis. Record the corresponding substrate code on the data sheet. Twenty (20) pieces of substrate are selected and measured in this fashion. Discard the stone after it has been measured, recorded, and examined for attached invertebrates.

Figure 6. Scrubbing substrate with a brush to dislodge attached invertebrates.

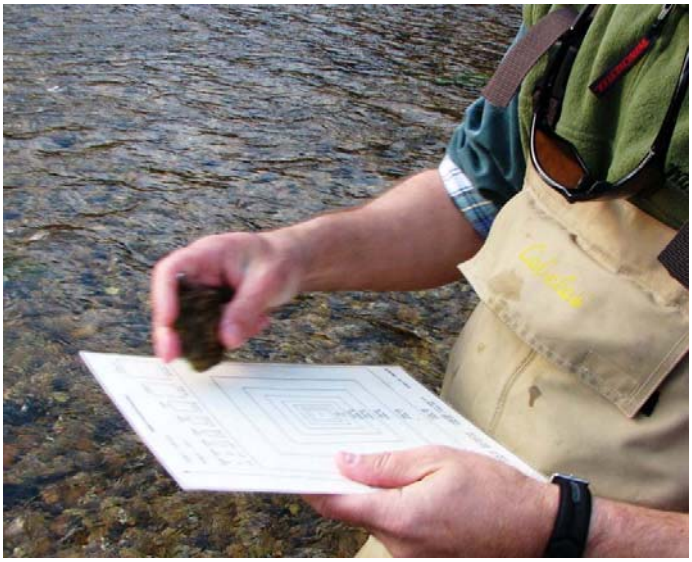


Figure 7. Measuring substrate with the field sheet.

c. Any remaining large pieces of substrate can be discarded after any attached invertebrates are removed. Debris and invertebrates remaining in the bucket after the 20 substrate pieces are measured should be added to the sample container for preservation.
d. In the event the sample area should contain predominantly fine substrates (Codes 1-6) the investigator will have to substitute 20 “pinches” of substrate in lieu of 20 individual pieces to characterized the relative substrate sizes.

7. Average velocity (meters/second) and depth (centimeters) are measured concurrently at each sample point and immediately in front of the sample frame. Depth and velocity can

be measured by a third team member while the other two members collect the benthic sample described in step 8 below. Measurements are done using a FLO-MATE Model 2000 current meter attached to a top-setting wading rod. The rod allows for quick and easy measurements of depth with incremental markings and an adjustable arm which places the current meter at the proper depth for measuring velocity (60% of the depth from the surface of the water). Velocity should be recorded in meters per second and increments on the wading rod are in centimeters. Greater detail regarding use of the FLO-MATE 2000 is provided in SOP#5 "Measuring Stream Discharge."

Table 1. Substrate size classes to be used for characterizing substrate based on the Wentworth Scale.

Size Code	Particle Diameter Range (mm)	Category
1	<0.062	Silt/clay
2	0.062-0.125	Very fine sand
3	0.125-0.25	Fine sand
4	0.25-0.50	Medium sand
5	0.50-1	Course sand
6	1-2	Coarse sand
7	2-4	Fine gravel
8	4-5.7	Medium gravel
9	5.7-8	Medium gravel
10	8-11.3	Coarse gravel
11	11.3-16	Coarse gravel
12	16-22.6	Small pebble
13	22.6-32	Small pebble
14	32-45	Large pebble
15	45-64	Large pebble
16	64-90	Small cobble
17	90-128	Small cobble
18	128-180	Large cobble
19	180-256	Large cobble
20	256-362	Boulder
21	362-512	Boulder
22	512-1024	Boulder
23	>1024	Boulder
24	Bedrock	Bedrock

8. Benthic sample collection

   a. While one team member holds the net firmly against the stream bottom facing the current, a second team member then uses a garden cultivation tool to agitate the entire area within the net sample frame for a timed period of 2 minutes (Figure 8). At the end of 2 minutes the net can be lifted from the stream bottom, but with sufficient caution so as not to spill the sample.

b. Remove any large debris or rocks from the net and inspect for attached organisms. These pieces can be discarded after any invertebrates have been removed. Pour and rinse the contents of the net into a wash bucket with a 500µm mesh sieve and rinse the sample by swirling the bucket in water using care not to submerge the bucket (Figure 8). Continue this process until all the fine silt and other sediments have been washed from the sample. Remove the sample contents from the wash bucket and place them in the sample container. Inspect the net, bucket, and sieve for any remaining organisms and carefully place them in the sample container (Figure 8). Excess water in the sample container can be drained into the sieve and inspected for organisms so that the preservative is not overly diluted.

Figure 8. Placement of net on stream bottom and agitating substrate with garden tool (top left), washing the fine sediments from the sample using the wash bucket (top right) and transferring sample from wash bucket to sample container (bottom). Note that sample container is always held over wash bucket to ensure that sample is not accidentally spilled.

c. Once all organisms have been removed from the net, fill the jar with preservative (99% isopropyl alcohol), ensure that the container is properly labeled, and tightly close the lid. Sample debris must be completely covered by preservative. Example sample labels are provided at the end of this SOP.

d. Repeat this procedure for each discrete sample. Prior to leaving the site, recheck the samples to ensure they have been properly labeled and tightly closed, and ensure data sheets are properly completed.

9. Measure the wetted width at the top, middle and bottom of each sampled riffle. Record this information on the habitat data sheet.

10. Record any necessary notes about the collection site or specific samples.

11. Take digital photographs of the riffles sampled from upstream and downstream perspectives at mid-channel.

The National Park Service

Heartland I&M- Aquatic Invertebrate Monitoring

Stream Field Sampling Form

Site Name/ID: _____

Date: _____ Team: _____

RIFFLE DATA	**RIFFLE #3**			**RIFFLE #2**			**RIFFLE #1**		
Sample #	**3**	**2**	**1**	**3**	**2**	**1**	**3**	**2**	**1**
Sampling sequence (L, M, R)									
Riffle Length (m):									
Width (m): (bottom-mid-top)									
Depth (cm) (each sample)									
Velocity (m/s) (@0.6 depth)									
Embeddedness (%)									
Vegetation (%)									
Filamentous algae (%)									
Periphyton (%)									
Deposition (%)									
Organics (%)									

0 = Absent (0%), 1 = Sparse (<10%), 2 = Moderate (10-40%), 3 = Heavy (40-75%), 4 = Very Heavy (>75%)

Temperature (C°)			
Specific conductance (µS)			
pH			
DO (mg/liter)			

NOTES:

Heartland I&M- Aquatic Invertebrate Monitoring
Sample Cell Substrate Assessment

Site Name/ID: _____ Date: _____

Team Members: _____

Wentworth Substrate Codes 1-24

	RIFFLE #3				RIFFLE #2				RIFFLE #1		
Sample #	3	2	1	Sample #	3	2	1	Sample #	3	2	1
1				1				1			
2				2				2			
3				3				3			
4				4				4			
5				5				5			
6				6				6			
7				7				7			
8				8				8			
9				9				9			
10				10				10			
11				11				11			
12				12				12			
13				13				13			
14				14				14			
15				15				15			
16				16				16			
17				17				17			
18				18				18			
19				19				19			
20				20				20			

1= <0.062 mm (silt/clay), 2= 0.062-0.125 mm (very fine sand), 3= 0.125-0.25 mm (fine sand), 4= -.25-0.50 mm (medium sand), 5= 0.5-1 mm, 6= 1-2 mm, 7= 2-4 mm, 8= 4-5.7 mm, 9= 5.7-8 mm, 10= 8-11.3 mm, 11= 11.3-16 mm, 12= 16-22.6 mm, 13= 22.6-32 mm, 14= 32-45 mm, 15= 45-64, 16= 64-90 mm, 17= 90-128, 18= 128-180 mm, 19= 180-256 mm, 20= 256-362 mm, 21= 362-51222= 512-1024 mm, 23= >1024 mm, 24= bedrock.

NOTES:

Protocol for Monitoring Aquatic Invertebrates at Ozark National Scenic Riverways, Missouri, and Buffalo National River, Arkansas. Heartland I&M Network and Prairie Cluster Prototype Monitoring Program

SOP 4: Laboratory Processing and Identification of Invertebrates

Version 1.00 (07/01/2007)

Revision History Log:

Previous Version #	Revision Date	Author	Changes Made	Reason for Change	New Version #

This SOP explains procedures for processing and storing samples after field collection as well as identification of specimens. Procedures for storing reference specimens are also described.

I. Preparing the Sample for Processing

Processing procedures apply to all benthic samples. This is an important and time consuming step. Particular care should be taken to ensure that samples are being processed thoroughly and efficiently. The purpose of sorting is to remove invertebrates from other material in the sample.

Procedure:

1. Sample processing begins by pouring the sample into a USGS standard sieve (500-µm) placed in a catch pan. The preservative should be kept and stored in a storage container for eventual rehydration of sample debris prior to QA/QC.
2. Rinse the sample contents in the sieve with tap water to flush the residual preservative. Large organic material (>2 cm) should be removed by hand and rinsed into the sieve. Each piece of debris removed from the bulk sample should be carefully inspected to ensure that all attached organisms are removed. The rinsed organic material should then be kept separate from the rest of the sample or placed in the original sample container.
3. Through elutriation, the organic debris should be separated from the inorganic content (sand and gravel). This may take several washes to accomplish. Carefully examine the inorganic content for the presence of remaining invertebrates (especially mollusk shells and Trichoptera cases). Add these specimens to the organic debris portion. Return the inorganic portion to the original sample container.
4. Inspect the sieve to ensure no invertebrates remain following rinsing. If present, remove any specimens and add them to the organic sample fraction.

5. The organic sample portion should then be returned to a clean 500-µm USGS-type sieve marked into four equal portions (Figure 1). The sieve should be placed in a shallow pan of water, and the contents floated until they are evenly distributed on the pan bottom. The sieve should then be carefully lifted from the water so that contents are not redistributed. The separated sample is now ready for sorting.

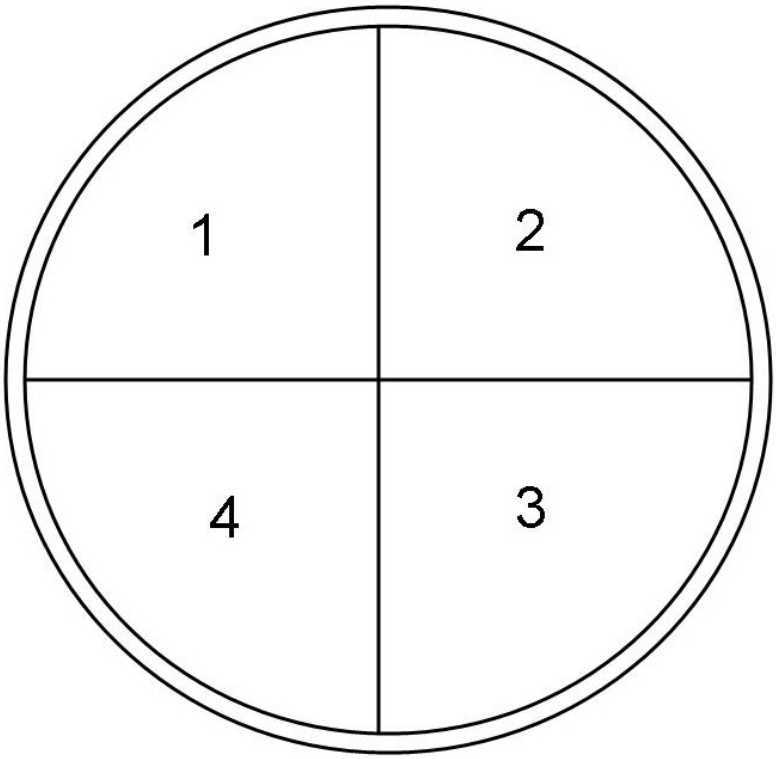


Figure 1. Diagram of a 500-µm USGS sieve marked into quarter fractions.

6. Notes regarding any difficulties with sample sorting should be written on the Aquatic Invertebrate Identification & Enumeration Sheet (an example is located at the end of this SOP). Also include the name of the person who sorted the sample, date sorted, and number of hours required to process and sort the sample.

II. Subsampling

In order to ensure that the subsample adequately represents the contents of the whole sample, a minimum of 200 organisms, if present, will be removed from the sample.

Procedure:

1. The sorter will randomly pick one of the quarter fractions of the sample in the sieve to represent a minimum 25% subsample using the random table below (Figure 2).

3	**4**	**1**	**2**
4	**3**	**2**	**1**
1	**4**	**3**	**2**
3	**1**	**4**	**2**
2	**4**	**1**	**3**
1	**2**	**3**	**4**
1	**3**	**2**	**4**
1	**3**	**4**	**2**
2	**3**	**1**	**4**
2	**1**	**4**	**3**
4	**3**	**1**	**2**
2	**1**	**3**	**4**
4	**1**	**3**	**2**
2	**3**	**4**	**1**
3	**2**	**1**	**4**
4	**1**	**2**	**3**
4	**2**	**3**	**1**
4	**2**	**1**	**3**

Figure 2. Randomly generated integer sequences between 1 and 4.

2. Using a putty knife or similar tool, the fraction contents are carefully scraped from the sieve and added to a white sorting pan containing water. The bottom of the sieve in the area where the subsample was removed should then be carefully inspected to ensure that no invertebrates remain.

3. All samples will be sorted under a minimum 10X magnification.

4. As invertebrates are removed from the fraction, they should be counted with a hand-held enumerator. When this fraction has been completely sorted, and 200 or more organisms have been removed, no additional sorting is necessary. If less than 200 organisms were removed, the sorter should remove another randomly selected quarter fraction from the sieve and sort it. This process is repeated until a minimum of 200 organisms have been removed or the entire sample has been sorted.

5. Always completed sort the removed sample fractions regardless of how many organisms are present in it. (*e.g.*, the first fraction removed possibly could contain 300 or more organisms).

> Note: The sorter need not record the number of specimens actually removed from the sorted fractions.

6. The sorter should clearly indicate on the specimen label and lab identification sheet how many fractions of the whole sample were sorted. **This information is critical for estimating benthic densities.** For example, to estimate density for the entire sample if only one quarter is sorted, the number of specimens in this fraction must be multiplied by a factor of 4; if 2 fractions are sorted the number of specimens must be multiplied by 2; if 3 fractions are sorted the number of specimens removed must be multiplied by 1.5.

7. Additionally, a "large and rare" taxa component is included in the subsampling routine. Large and/or rare taxa remaining in the sample that clearly were not in the sorted fraction are removed and stored in a separate vial. These specimens will be used fro reflecting accurate sample diversity estimates and calculating specific metrics such as EPT.

a. A large and/or rare additional taxa search will be completed following the subsample routine. Any large and/or clearly rare organisms (*e.g.*, *Corydalus cornutus*, *Pteronarcys picketii*, tabanids, tipulids, dragonfly larvae, crayfish, gordian worms, large beetles, other unusual species, *etc*.) in the sample that clearly were not in the subsampled fraction will be removed, placed in a separate vial, and labeled appropriately (Figure 3). There may be several or no specimens depending on the sample.

b. Just because a creature is large does not mean you should remove it during this process. It must fit the criterion that it was not present in the subsample.

c. A large and/or rare collection may not be necessary for all samples if those taxa are not present.

8. Any invertebrates present in the subsampled squares will be stored in a separate storage vial, preserved, and properly labeled (see below).

9. Organic debris from the subsampled portion will be retained in a separate container until QA/QC checks are completed.

III. Sample preservation and labeling

1. Invertebrates removed from the bulk samples will be stored in 75-80% isopropyl alcohol.
2. Labels will be written only on rag bond paper in permanent water proof ink (both supplied). Labels written in pencil are not acceptable.
3. Label data should be printed neatly and include the following: site number (*e.g.*, BUFFM02), riffle number (1-3), sample location (L, M, R), sampling date, and collector initials (Figure 3). All of this information is on the original sample label.
4. The vial label for "large and rare" taxa should be specifically labeled as such and also include the original sample data as well.
5. Do not crowd the collected specimens excessively as it inhibits long term preservation.

Bulk Sample:

Park Name: OZAR
Site Name: CURRM02
Sample: Riffle 1, Left
Collection Date: 12 Dec 06
Collector Initials: DEB, HRD

Large & Rare:

LARGE & RARE
Park Name: OZAR
Site Name: CURRM02
Sample: Riffle 1, Left
Collection Date: 12 Dec 06
Collector Initials: DEB, HRD

Identification:

Park Name: OZAR
Site Name: CURRM02
Sample: Riffle 1, Left
Collection Date: 12 Dec 06
Taxon Name: Chimarra
Det. D. E. Bowles

Figure 3. Example specimen labels.

IV. Identification of Invertebrates

To the extent possible, all invertebrates should be identified to genus exclusive of the groups and selected conditions indicated below.

Procedure:

1. A dissecting microscope and taxonomic keys are used to identify each specimen to the genus level whenever possible. For some taxa, only higher taxonomic levels can be obtained and some of these are listed below. In most cases, an entire sample can be identified to the required level, but an occasional sample may contain early instars or damaged specimens. In such cases, the specimen should be identified to the lowest level possible, as indicated in Table 1.

Table 1. Taxonomic levels for identification when not the Genus level.

Phylum Nematoda
Phylum Nematomorpha
Phylum Annelida, Class Hirudinea, Families Glossiphonidae, Piscicolidae, Hirudinidae, Erpobdellidae
Phylum Annelida, Class Oligochaeta, Families Aeolosomatidae, Opistocystidae, Naididae, Haplotaxidae, Enchytraeidae, Lumbriculidae, Tubificidae
Phylum Arthropoda, Class Arachnoidea, Order Hydracarina
Phylum Arthropoda, Class Crustacea, Order Ostracoda
Phylum Arthropoda, Class Insecta, Order Diptera, Family Chironomidae

2. The primary keys will be Merritt and Cummins (1996a) for identification of insects and Pennak (1989) for identification of non-insect invertebrates. Additional taxonomic references for specific orders are Brown (1972), Moulton and Stewart (1996), Pflieger (1996), Poulton and Stewart (1991), and Wiggins (1995).

3. Appendix C contains a list of taxa known to occur in OZAR and BUFF. Accuracy of scientific names should be checked at the Interagency Taxonomic Information System (IT IS) website at http://www.itis.usda.gov/advanced_search.html.

4. A running total of each taxon for each sample should be recorded on the laboratory bench sheet that is signed or initialed by the identifier. Final counts of each taxon should be entered on the bench sheet when a sample is complete.

5. If **damaged organisms** can be identified, they are counted ONLY if:

   (a) the fragment includes the head, and, in the case of arthropods, the thorax. For Oligochetes, a sufficient number of segments

   (b) the mollusk shell (bivalve or gastropod) is occupied by a specimen

   (c) the specimen is the sole representative of a taxon in the sample

6. If **early instar or juvenile** specimens can be identified, they are counted ONLY if:

   (a) with confidence, they can be associated with one or more mature specimens that have a more developed morphology

   (b) the specimen is the sole representative of a taxon in the sample

V. Sample Storage and Reference Collection

Procedure:

1. Identified samples are stored in vials with 75-80% isopropyl alcohol and labeled with the taxon, date collected, park and site names/code, and name of identifier. Organisms will be retained for at least three years and stored at the NPS HTLN office located at Missouri State University, Springfield, MO.
2. A reference collection consisting of a few representative specimens of each taxon should be prepared and stored in properly labeled vials containing 75-80% isopropyl alcohol. Vials should be labeled as above (Figure 3).
3. Regional or other taxonomist specialists should review the identifications for accuracy. One set of vials should be stored at the NPS HTLN office located at Missouri State University, Springfield, MO. Additional sets of specimens should be maintained in the laboratory where identifications are performed for use as reference and training.

VI. QA/QC

Procedure:

The following QA/QC procedure (adapted from United States Environmental Protection Agency, 2004) will be used on sorted invertebrate samples.

1. Initially, a QA officer (initially Aquatic Program Leader) will use a microscope to check **all** sorted grids from the first five samples processed by a sorter to ensure that all organisms were removed from the detritus. This will not only apply to inexperienced sorters, but also to those initially deemed as "experienced." Qualification will only occur when sorters are consistent in achieving >90% sorting efficiency after at least five samples have been checked. Samples will be checked using up 10x magnification.

2. The Aquatic Program Leader or other QA Officer will calculate percent sorting efficiency (PSE) for each sample as follows:

$$PSE = \frac{A}{A+B} \times 100$$

where A = number of organisms found by the primary sorter, and B = number of recoveries (organisms missed by the primary sort and found by the QC check).

If the sorting efficiency for each of these five consecutive samples is >90% for a particular individual, this individual is considered "experienced" and can serve as a QC Officer. In the event that an individual fails to achieve >90% sorting efficiency, they will be required to sort an additional five samples in order to continue to monitor their sorting efficiency. However, if they show marked improvement in their sorting efficiency prior to completion of the next five samples, whereby they acquire the >90% sorting efficiency, the QA Officer may, at his/her discretion, consider this individual to be "experienced." Sorting efficiency should not be calculated for samples processed by more than one individual.

3. After individuals acquire a >90% sorting efficiency, 10% (1 out of 10) of their samples will be checked.

4. If an "experienced" individual fails to maintain a >90% sorting efficiency as determined by QC checks, QC checks will be performed on every grid of five consecutive samples until a >90% sorting efficiency is achieved. During this time, that individual will not be able to perform QC checks.

5. Residues of sorted bulk samples should be returned to their original sample containers and rehydrated with the alcohol saved during sample preparation. All sorted samples will be kept until QA/QC checks are complete and permission is given by the Program Leader to discard the material.

6. A taxonomist not responsible for the original identification (*e.g.*, Program Leader) should randomly check all sample reference identifications and check the bench sheet for any errors. If a taxon appears to be especially difficult to identify, specimens will be sent to a person with taxonomic expertise in invertebrates of the region. If any reference specimens are incorrectly identified, all specimens assigned to that taxon will be reexamined. It is also important that the taxonomist maintains contact with other taxonomists through professional societies and other interactions, and stays current with the pertinent published literature.

VII. Bulk sample disposition

When the sample is finished, return the remainder of the bulk sample to its original container with its original label and old alcohol, and return to storage. Mark the top with an X or other mark to identify the sample as one that has been picked. These samples will be subjected to a QA/QC analysis by the Program Leader.

Aquatic Invertebrate Identification & Enumeration Sheet

Page ____ of ___

Site Name: _____

Riffle No, Sample No.: _____ State: _____

Sample date: _____ Date of Det: _____

Subsampled portion: 25 50 75 100 Determiner(s): _____

ORDER (or higher)	FAMILY	GENUS	SPECIES	TALLEY/COUNT	Tot Cnt	RC
Diptera						
Coleoptera						
Ephemeroptera						
Plecoptera						
Trichoptera						
Other						

Protocol for Monitoring Aquatic Invertebrates at Ozark National Scenic Riverways, Missouri, and Buffalo National River, Arkansas. Heartland I&M Network and Prairie Cluster Prototype Monitoring Program

SOP 5: Measuring Stream Discharge

Version 1.00 (07/01/2007)

Revision History Log:

Previous Version #	Revision Date	Author	Changes Made	Reason for Change	New Version #

This SOP is guidance for measuring discharge in rivers and streams, specifically in the Ozark Highlands area. This guidance is for using the FLO-MATE 2000 (Marsh-McBirney, Frederick, Maryland) although the SOP is generally applicable to other types of meters as well (*e.g.*, Price AA, pygmy meters). The SOP describes sampling procedures, calibration, and general maintenance procedures. Also, step by step guidance is provided for using the FLO-MATE meter. If other meters are used, field personnel should review the instruction manual for instrument-specific guidance on how to calibrate and operate those particular meters.

I. Background Information

Velocity and depth are measured using a current meter attached to a wading rod. The rod allows for quick and easy measurements of depth with incremental markings and an adjustable arm that places the current meter at the proper depth for measuring velocity (60% of the depth from the surface of the water). Some current meters have rotating cups (Pygmy and Price models) while others have a pair of electronic contacts on a small head (FLO-MATE 2000) to measure velocity. The sensor in the FLO-MATE 2000 is equipped with an electromagnetic coil that produces a magnetic field. A pair of carbon electrodes measure the voltage produced by the velocity of the conductor, which in this case is the flowing water. Internal electronics process measured voltages and output them as linear measurements of velocity. Velocity is displayed as either feet per second or meters per second.

Stream discharge (Q) is the volume of water passing a cross-section per unit of time and is generally expressed in cubic feet per second (ft^3/s) or cubic meters per second (m^3/sec). Discharge is estimated by multiplying current velocity by the cross-sectional area (Carter and Davidian, 1969). Cross sectional area is determined by first measuring the width of the stream channel. The cross section is then divided into smaller increments (usually 15 to 20 intervals)

and depth and velocity are measured at each increment. The depth and width of the interval are multiplied to get an area for each interval and then each interval area and velocity is multiplied to produce a discharge for each interval. These discharges are summed to produce a total discharge for that cross section of the stream. This process will be described in greater, step by step detail in the "Procedures" section.

II. Prior to the Field

Equipment List

- FLO-MATE Model 2000 (Marsh-McBirney) velocity meter
  Accuracy of ±2% operates in temperatures between 0°C and 72°C.
- Tape measure (in increments of feet and/or meters)
- Stakes for mounting tape measure if necessary
- Top-setting wading rod (in increments of feet or meters)*
- 3-5 gallon bucket
- Extra batteries (D alkaline)
- Carrying case
- Log book
- Data sheets on waterproof paper
- Instruction manual

*Standard wading rods come in both metric and English standard units (feet). Discharge measurements are generally recorded in English units as cubic feet per second. Whatever units are used, ensure that there is consistency between the settings on the velocity meter, the wading rod, and the tape measure and that the units are clearly recorded on the data sheet. English standard units are easily converted to metric units when required.

Key Function summary

One-key functions

ON/C----Turns unit on. Clears the display and restarts the meter.
OFF----Turns unit off.
UP ARROW----Increments **FPA**, **rC** and memory location.
DOWN ARROW----Decrements **FPA**, **rC** and memory location.
RCL----Alternates between recall and real-time operating modes. When in recall mode the instrument will not display the time bar cycling, the time bar will be stationary.
STO----Stores values in memory.

Two-key functions

ON/C—OFF----Alternates between units of measurement. Also turns beeper on and off.
UP ARROW—DOWN ARROW----Alternates between **FPA** and **rC** filtering.
ON/C—STO----Clears memory

RCL—STO----Initiates zero adjust sequence.

Preparation and Meter Calibration

Prior to using the flow meter, inspect the meter, cable, probe and standard wading rod for obvious defects or damage. If the meter has been stored for more than a couple of weeks the batteries should have been removed, they should be re-installed at this time.
Procedure:

1. With the meter off, remove it from its carrying case.
2. Locate the battery compartment cover on the bottom of the meter and remove the three captive screws.
3. Install two D-batteries as shown on the meter's battery compartment.
4. Reinstall the battery compartment cover using the three captive screws
5. Turn the unit on by pressing the **ON/C** key; you can select between ft/s and m/s by pressing the **ON/C** and **OFF** keys at the same time.
6. You can select between FPA and rC by pressing the UP ARROW and the DOWN ARROW keys at the same time.
7. To change the second interval for FPA or rC modes press the UP ARROW or the DOWN ARROW keys. After you have entered the desired time (20-seconds for this study) the instrument will automatically switch to data collecting mode.

Zero Adjustment

The FLO-MATE 2000 should be zero adjusted before measurements are taken. One calibration per day is needed if the meter is not turned off for a period of several hours. Turning the meter off for short periods of time will not affect the meter's zero calibration as this is stored internally by the meter. For other meters, check the instruction manual for calibration or zero adjustment procedures.

Procedure:

1. First clean the sensor because a thin film of oil on the electrodes can cause noisy readings (see "Maintenance" section).
2. Press the **ON/C** key to turn the meter on.
3. Attach the meter to the wading rod
4. Place the sensor in a plastic five-gallon bucket of water. Keep the sensor at least three inches away from the sides and bottom of the bucket. Wait 10 to 15 minutes to make sure the water is not moving before you take your zero reading.
5. Use a filter value of 5 seconds (**FPA** or **rC** mode). Use the **UP ARROW** or **DOWN ARROW** key to set the meter to 5 seconds.
6. To start the zero sequence, press the **STO** and **RCL** keys at the same time. The number 3 will appear on the display. Decrement to zero using the **DOWN ARROW** key.
7. The number 32 will be on the display; it will decrement itself to zero and turn off. The unit is now calibrated.

Note: Each key in the zero adjusts sequence must be pressed within 5 seconds of the previous key. If an ERR 3 comes on the display turn the unit off then back on and restart the zero sequence.

III. In the Field

Discharge measurements should be made after other measurements such as temperature, dissolved oxygen, pH, and conductivity are complete. Discharge measurements require wading across the stream and may stir-up sediments, disrupting accurate measurement of other parameters.

Note: Under normal flow conditions, discharge using the methods outlined below cannot be used for the three downstream most sampling sites on the Current River at OZAR (CURRM04, CURRM05, CURRM06). **Attempting to obtain a discharge measurement at these sites poses a significant safety risk for personnel**. To obtain an approximate measure of discharge at these locations, the mean daily discharge recorded at the USGS gaging station located at Van Buren, MO should be used. Information on this gaging station can be found in SOP#1. For CURRM06, a more accurate estimate of discharge can be obtained by measuring discharge at Big Spring and adding it to the daily mean discharge recorded at the Van Buren gaging station.

Quantitative Discharge Procedure:

1. Prior to taking any measurements, the location where discharge will be measured must be determined. An ideal cross-section in the sample reach will have the following qualities:
   - The stream channel directly above and below the cross-section is straight.
   - There is measurable stream flow, with a stream depth preferable greater than 10 cm and velocities generally greater than 0.15 meter/second.
   - The streambed is a uniform "U" shape, free of large boulders, woody debris, and dense aquatic vegetation
   - The stream flow is laminar and relatively uniform with no eddies, backwaters, or excessive turbulence.

     Note: The cross section will not likely meet all these qualifications but the best location should be selected based on these standards. Record (and/or draw a diagram) on the data sheet a description of any discrepancies with the cross section.
2. Once the cross section is established, measure the width of the stream with a tape measure to the nearest 0.1 meter and secure the tape across the stream for the duration of the discharge measurement.
3. Divide the stream into equal intervals across the width of the cross section, usually 15 to 20. A minimum of 10 intervals is recommended. A velocity and depth measurement will be recorded for each interval across the stream at the center of each interval. For example, if the stream is 10 meters wide, 10 velocity and depth measurements will be taken at one meter intervals. The first measurement will be taken a half foot from the water's edge, the second 1 and a half feet from the water's edge, *etc.*, as shown in Figure 1.

4. Turn the current meter on (**ON/C**) and check to ensure that the velocity units coincide with the measuring tape and wading rod units. For FLO-MATE 2000, set the meter to **FPA** with 20-second intervals.
5. Attach the sensor to the wading rod and ensure that the sensor is securely screwed onto the rod and facing upright.

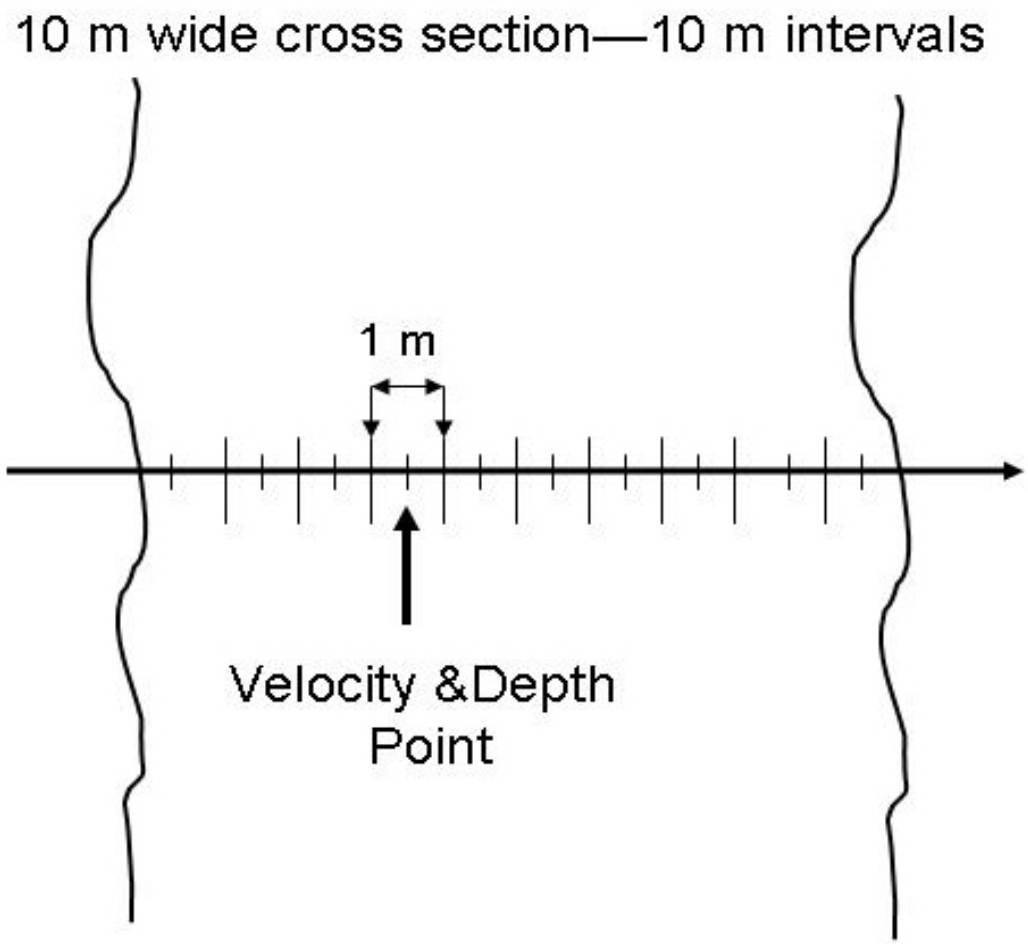


Figure 1. Cross section diagram.

6. One person should record discharge and one person should remain on the bank, recording data. Start at the water's edge and move to the center of the first interval. Place the wading rod as level as possible and hold perpendicular to the water level. Read depth from the wading rod to the nearest centimeter. The rod will have graduated marks along its length, with single marks indicating two centimeters, double marks indicating 10 centimeters, and triple marks indicating one-half meter increments.
7. Once depth has been read, adjust the arm of the sliding rod with the sensor attached to 60% of the water depth. The wading rod will place the sensor at 60% of the depth from the surface of the water when properly adjusted.

<u>Note</u>: For example, if the depth is 2.6 meters, line up the 2 on the meter scale (sliding rod) with the 6 on the tenth scale (increments on handle of fixed rod). The sensor is now located at 60% of the water depth.

8. Stand behind the sensor and make sure there is no disturbance (including the senor cord) around the sensor that interferes with the velocity measurement. The meter may be adjusted slightly up or downstream to avoid boulders or other interferences.

   <u>Note</u>: Make sure the sensor directly faces the flow of the water. This may not always be directly parallel with the water's edge, the rod and sensor may need to be turned slightly with each measurement.

9. Allow the instrument enough time to get an accurate reading, generally around a minute. Watch the time bar complete two full cycles and then take the velocity reading. If something happens during the measurement, such as accidental movement of the wading rod, the meter can be cleared and the reading started again by hitting **ON/C**.
10. Call out the distance from the water's edge, the depth, and then the velocity to the person recording data. Continue moving across the stream until measurements have been taken at all intervals.

    <u>Note:</u> If the water velocity increases greatly between intervals, additional measurements can be taken to shorten the width of the intervals within this area of high velocity. Be sure to change the interval width for these measurements in the calculation of discharge.

11. When finished, detach the sensor from the wading rod and place it back in the mesh side pocket for transportation. If you do not expect to use the meter for several days, turn the meter off, clean the sensor, and store properly.

IV. Equipment Maintenance and Storage

Maintenance

Clean the sensor whenever dirt or oil deposits appear on the sensor. If the sensor is dirty, readings become noisy or conductivity lost error appears on the display while taking measurements. Nonconductive coatings such as oil and grease can cause noisy readings or conductivity lost errors.

Procedure:

1. Clean the sensor with soap and water. If a problem still persists, clean the electrodes with 600 grit sandpaper. DO NOT USE HYDROCARBON SOLVENTS.
2. Change batteries when the low battery flag is displayed. The battery compartment is located in the bottom of the meter. Remove the instrument from its carrying case and install two D-batteries as described in the meter preparation procedures.

Storage

When storing the meter, clean and dry the sensor probe and cable, role the cable up and place both the cable and probe in the mesh pocket of the carrying case. When storing the meter for a period of more than two weeks, remove the batteries.

DISCHARGE

SITE NAME/ID: _____ **DATE:** _____

TEAM MEMBERS: _____

	Distance Units ft ☐ m ☐	Depth Units ft ☐ cm ☐	Velocity Units ☐ ft/s XX.X ☐ m/s X.XX	
	Start at River Right; Final measurement should be at river left bank			
	Distance from Bank	Depth	Velocity (@0.6 depth)	FLAG
1	0	0	0	
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				

Measurements are taken at 0.6 the water column depth

Flags:

Protocol for Monitoring Aquatic Invertebrates at Ozark National Scenic Riverways, Missouri, and Buffalo National River, Arkansas. Heartland I&M Network and Prairie Cluster Prototype Monitoring Program

SOP 6: Documenting CORE 5 Water Quality Variables

Version 1.00 (07/01/2007)

Revision History Log:

Previous Version #	Revision Date	Author	Changes Made	Reason for Change	New Version #

This SOP addresses the equipment and methods required to measure CORE 5 water quality variables (temperature, dissolved oxygen, specific conductance, pH, and turbidity) in association with aquatic invertebrate monitoring. Detailed guidance for measuring CORE 5 parameters, including training, calibration, QA/QC, data archiving, meter specifications, field measurements and trouble shooting, can be found in the Documenting CORE 5 Water Quality Variables SOP located at: http://www1.nature.nps.gov/im/units/htln/aquabugs.cfm. This SOP is based on guidance from the NPS Water Resources Division (2003) and Wagner *et al.* (2006).

Two approaches to recording CORE 5 data will be used in this protocol: 1) Discrete (*in situ*) measurements using hand-held instruments, and 2) Unattended measurements using data loggers or sondes.

I. Discrete CORE 5 measurements

Discrete (*in situ*) CORE 5 measurements do not reflect changes in water quality (*e.g.*, diurnal fluctuations or those associated with a hydrologic event) that are likely to have occurred in the stream. These measurements serve two general purposes: 1) They represent the natural condition of the surface water system at the time of sampling, although they are not intended to be a precise measure of water quality condition in the stream. 2) They serve as a cross-check for CORE5 parameter measures using unattended CORE 5 data sondes (see part II below).

II. Unattended CORE 5 measurements

CORE 5 water quality parameters measured with small intervals (*i.e.*, minutes to hours) between repeated measurements are considered continuous because few if any significant water quality

changes are likely to go unrecorded. When the goal is to characterize events of short duration, but such events are difficult to capture manually using discrete measurements (see above), continuous monitoring is appropriate. Continuous monitoring of core parameters helps address questions concerning daily or seasonal variability, or short-term changes (*e.g.*, precipitation related events) that might not be apparent or prevent accurate understanding of long-term data. Continuous monitoring also provides the most comprehensive temporal data set upon which to establish variability through time. Such information is necessary to document correlations, possible cause and effect relationships, and differentiate natural variability from anthropogenic-induced change to an aquatic system.

III. Analysis and Reporting

CORE 5 data will be analyzed using summary statistics (mean, median, range, standard deviation, standard error) for each site and date. This information will be presented in summary and synthesis reports to support invertebrate collection data.

IV. NPS STORET

Collected water quality data that has been successfully subjected to QA/QC will be exported to NPS STORET (see SOP#7 Data Management). Only summary data for a site and collection period in addition to pertinent metadata will be submitted. Instructions for preparing and exporting water quality data to this archival facility can be found at the following website: http://nrdata.nps.gov/Programs/Water/NPStoret/

Protocol for Monitoring Aquatic Invertebrates at Ozark National Scenic Riverways, Missouri, and Buffalo National River, Arkansas. Heartland I&M Network and Prairie Cluster Prototype Monitoring Program

SOP 7: Data Management

Version 1.00 (07/01/2007)

Revision History Log:

Previous Version #	Revision Date	Author	Changes Made	Reason for Change	New Version #

This SOP explains procedures for data management of Buffalo NR and Ozark NSR river invertebrate sampling data. It includes a general description of the data model and procedures for data entry, data verification and validation, and data integrity for the primary invertebrate data.

Data management can be divided into (a) the initial design phase that involves defining the data model, its entities and their relationships and (b) the procedures necessary to implement the database. Microsoft (MS) Access 2003 is the primary software used for maintaining invertebrate data. Water quality data will be stored in the National Park Service's NPStoret database: (http://www.nature.nps.gov/water/infoanddata/index.cfm#NPSTORET).

Environmental Systems Research Institute (ESRI) ArcInfo 9.x is used for managing spatial data associated with field sampling locations. Data products derived from this project will be available at the NPS I&M Data Store and EPA Storet National Data Warehouse (http://iaspub.epa.gov/storpubl/DW_home). QA/QC guidelines in this document are based on recommendations of Rowell *et al.* (2005) and citations therein.

I. Data Model

The NPS I&M program has designed the Natural Resource Database Template (NRDT) to be used as a database model for storing vital signs monitoring data in MS Access (National Park Service, 2006). The template has a core database structure that standardizes location and observation data to facilitate the integration of datasets. Developed in MS Access, the database allows users to enter, edit, display, summarize, and generate reports as well as integrate with other Natural Resource data systems such as NPStoret. Distributed databases, or replicas, allow data entry/modifications from remote locations (*i.e.*, at BUFF or OZAR) and subsequent synchronization with a master database archived on the HTLN server at Missouri State University. NPS WRD has also designed the NPStoret database to facilitate archiving NPS data

in the EPA Storet database. NPStoret is a series of Access-based templates patterned after the NRDT and includes data entry templates and an import module. It supports the core data management objectives of data entry and verification/validation in a referentially constrained environment (*i.e.*, related locations, events, and primary data elements (NPS/WRD, 2007).

A generalized NRDT entity relationship diagram of the invertebrate community database is given in Figure 1. The tables sampling events (tbl_SamplingEvents) and locations (tbl_Locations) are the two core tables of the database and contain general information pertaining to the field sample occasion (the when and where of the sample). This includes information such as date and time, river stretch ID and UTM coordinates, and park/project codes. Detailed information pertaining to the invertebrates sampled is maintained in tbl_Count. Other tables include habitat data (*e.g.* tbl_Substrate, tbl_Discharge), and associated lookup tables (*e.g.*, tlu_WentworthSubstrateCodes, tlu_Taxa).

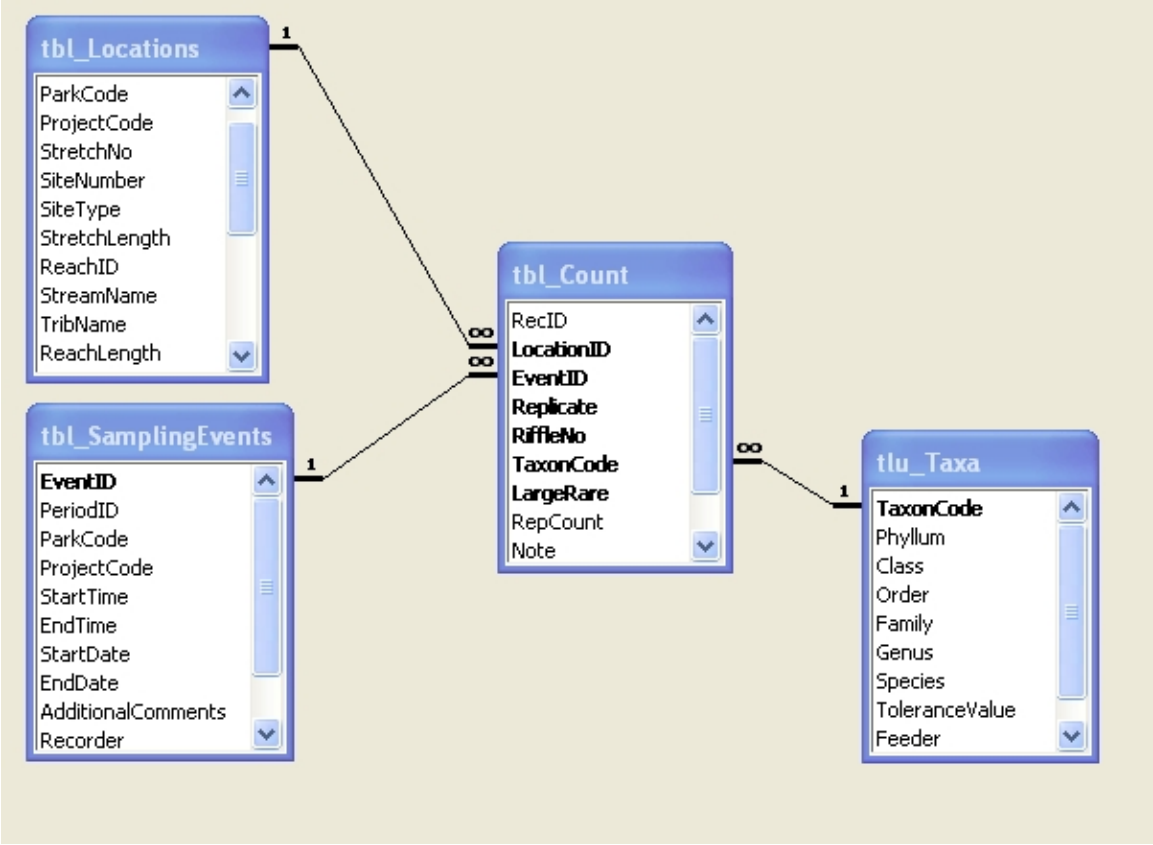


Figure 1. Data model for river invertebrate monitoring. Note tbl_Locations tracks GPS spatial and non-spatial field data while tbl_SamplingEvents tracks events. The data entry forms (below) follow these two levels of information organization.

Procedure:

4. Develop appropriate tables based on river invertebrate sampling protocols. Prior to table development, coordinate with project staff to determine data types (*e.g.* text, numeric,

memo), precision, and range of values. Acquiring field data forms assists with developing data logic (*i.e.*, how data components relate).

5. Develop appropriate indexes and identify primary key (s) to relate appropriate tables.

   Note: The NRDT data dictionary follows standards identified in the NRDT phase 2 and is modified where required. Naming standards follow I&M recommended procedures.

II. Data Recording

Quality assurance and quality control procedures related to data recording are important components of any project. Sampling data (*i.e.*, sample methods, effort, weather/water quality conditions, and species abundance data) are recorded and checked for completeness either before leaving a site or within 24 hours of data recording. This will aid in verification and validation of the data after entry into the database. To prevent the complete loss of field form data due to unforeseen circumstances (*i.e.*, fire or flood in the workplace), all field sheets are photocopied and a hard copy located in a separate location as the original. Field sheets are scanned into a computer and electronic copies of the data sheets stored on the HTLN server located at Missouri State University, Springfield, MO. This will ensure that at least one copy of the field sheets is available for data entry and verification.

1. Check field data sheets for completeness prior to data entry.
2. Photocopy and archive field data sheets.

III. Data Entry

Data entry is accomplished in replica databases using a tiered set of forms. Upon opening the replica, the user is presented with a switchboard (Figure 2). A preliminary set of forms define the sampling occasion and requires the input of location, period, and event IDs *prior* to entry of additional data. The other forms address the details of invertebrate occurrence, habitat, observers, *etc*. and have data entry instructions. Once all fields for the preliminary set of forms have been completed, data can be entered for the remaining forms. Additional forms document sampling personnel for each occasion and their specific hours related to the project (sampling hours, travel hours, *etc.*). The replicas are then synchronized with the master database and any subsequent data entry in a new replica.

Several features are “built-in” to form properties that enable the user to maximize data entry efforts while minimizing error. These include data input masks for ease of viewing multi-part data (*i.e.*, park/project codes and date in PeriodID, LocationID, or EventID), “fill-in-as-you-type” to automatically complete a field, default values to autopopulate common values, limiting input values to known ranges (or restricting null values) or providing “drop-down boxes”, and tab indexes to control the order of data entry. Forms also contain fields that require data input and/or are constrained to properties and integrity of related tables. The “Prevent Deletes” option is enforced in replica databases to ensure data are not inadvertently deleted.

Entering Sampling Occasion Data

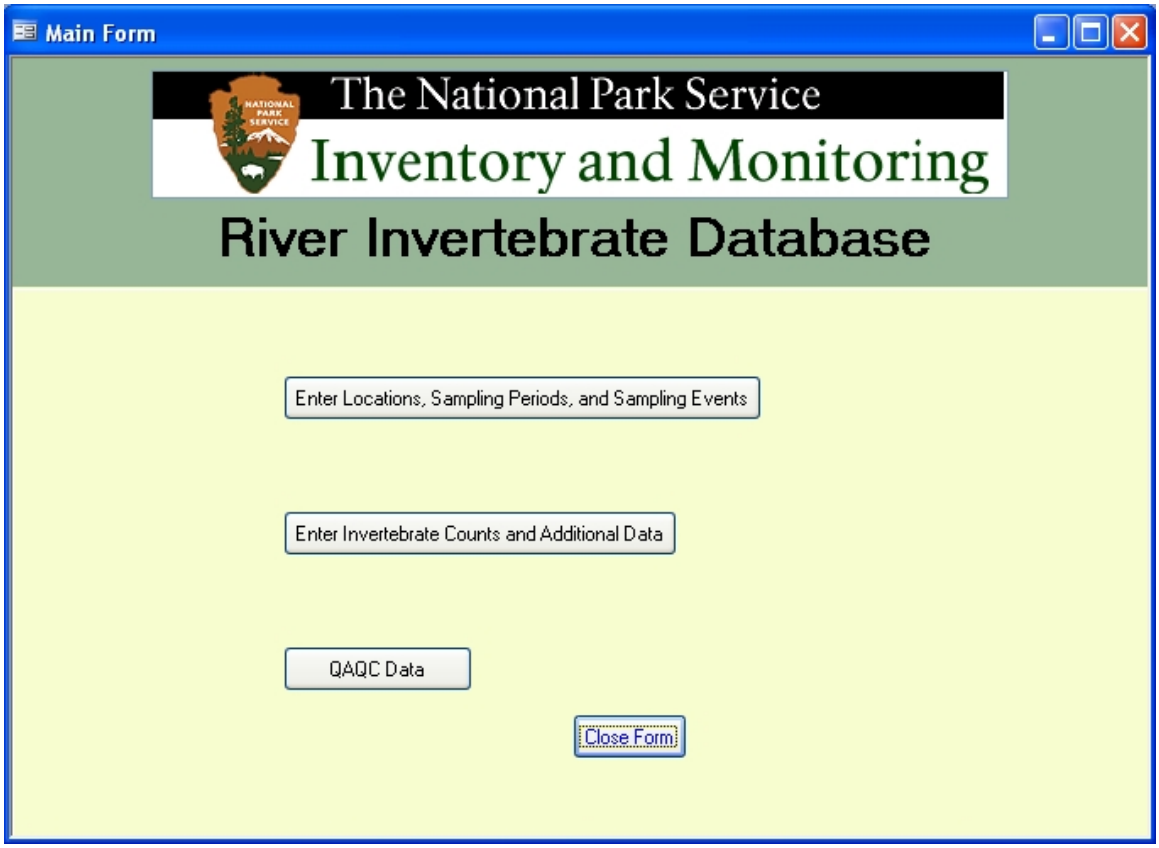


Figure 2. River invertebrate database switchboard. Note the primary data entry form button (top).

Procedure: Sampling Occasion Data

1. Open the Locations form (Figure 3, top) and select the appropriate Park Code, Site Type, and Sub Type and enter the Site Number and Stretch Number.

   Note: The Stretch Number refers to the Missouri Resource Assessment Partnership (MORAP) classified stream identifier and is transcribed from the appropriate MORAP StretchID table (see Appendix B for Stretch ID and related information).

2. Click the "Generate LocationID" button to generate a LocationID. This passes the user data to a secondary form where additional location data is entered. Note the user data on the first form are at the top of the secondary form and a LocationID has been generated with an input mask to separate Park Code, Project, and LocationID.

3. Enter additional location data for the specific sampling location. To exit the location form (as well as all other forms) the user clicks the “Close Form” button.

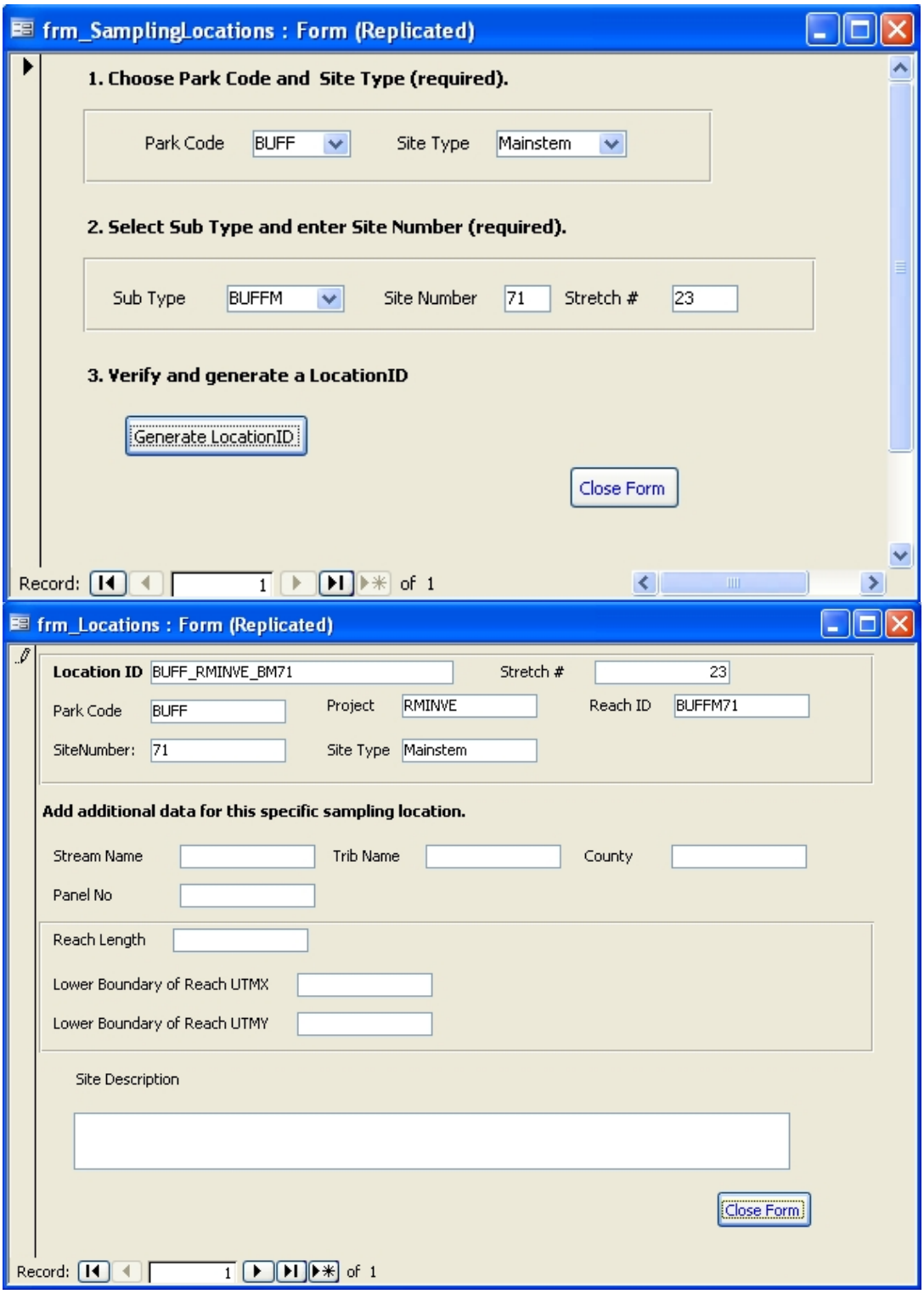
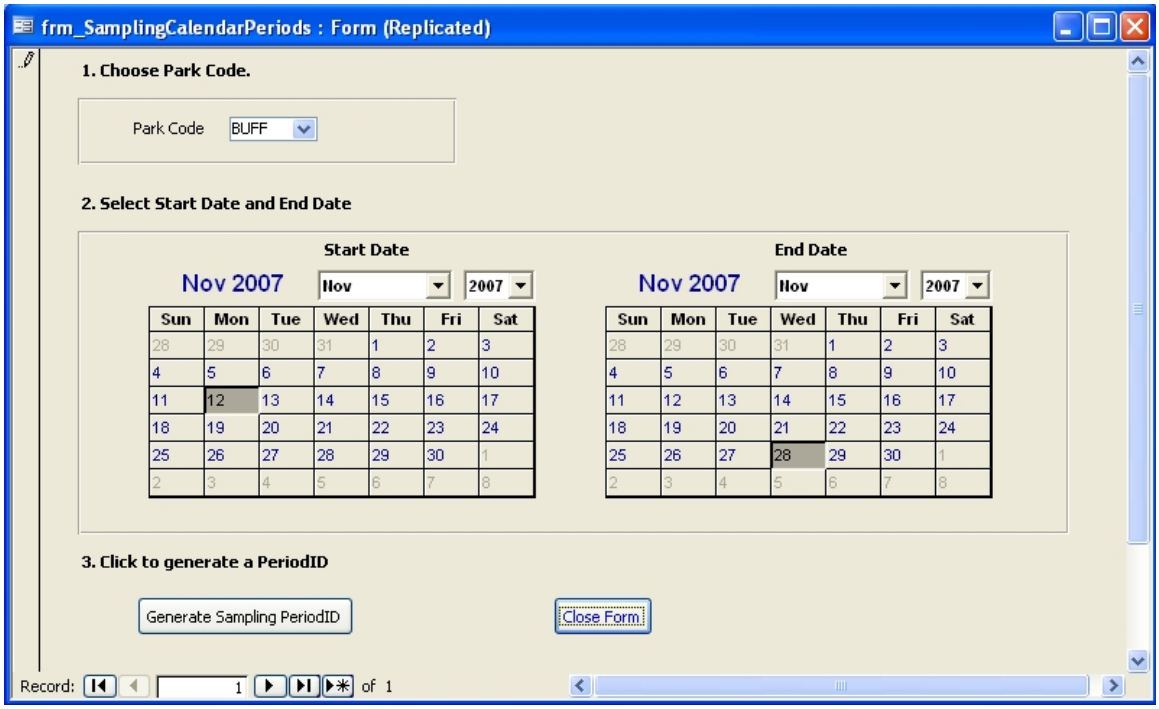


Figure 3. Location forms for invertebrate data.

4. Open the Sampling Period form (Figure 4) and select the appropriate Park Code and use the calendars to select the start and end dates of the sampling period time frame to develop a PeriodID.

5. Clicking the “Generate Sampling PeriodID” button passes the user data to a secondary form where the user reviews then accepts or revises the Protocol Version and clicks the “Verify” button.



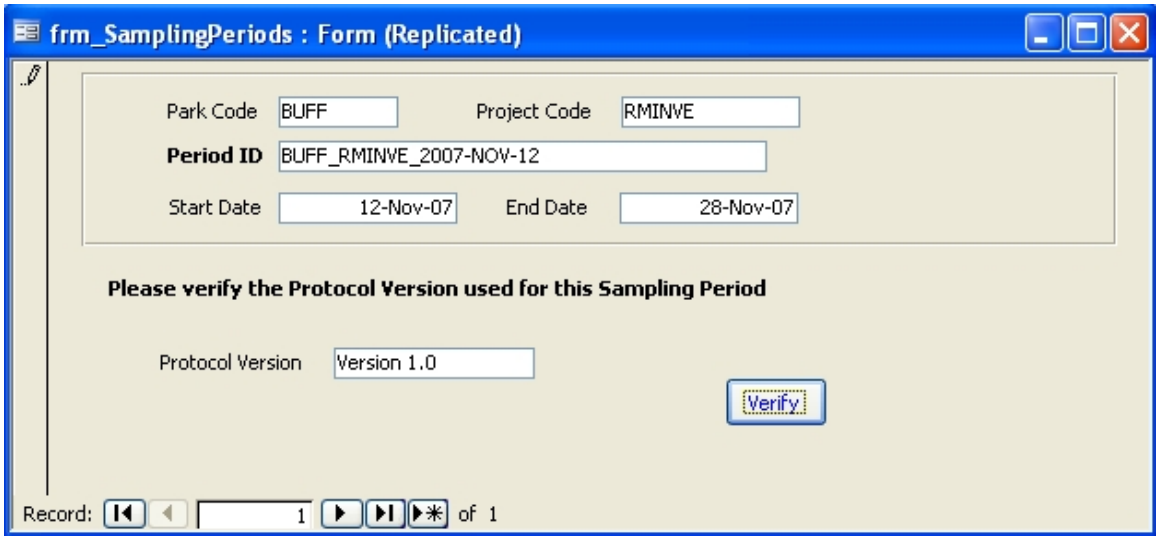
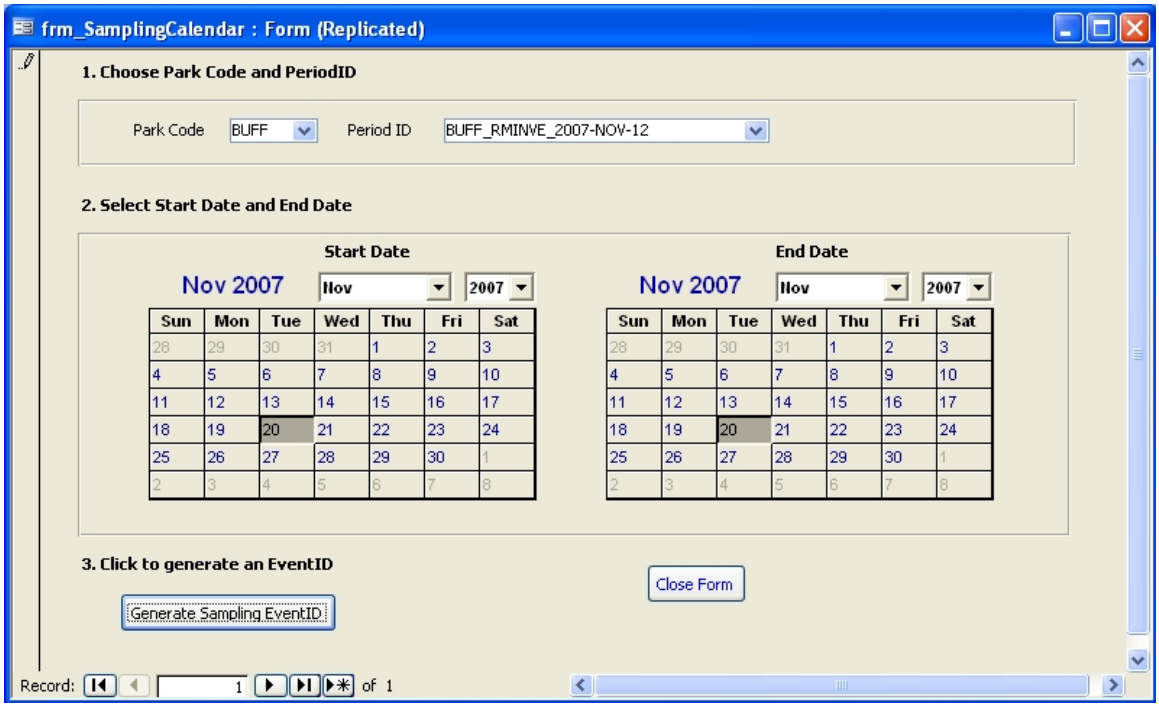


Figure 4. Sampling period forms for invertebrate data.

6. Open the Sampling Events forms (Figure 5) and select the Park Code and PeriodID and use the calendars to select the start and end dates of the sampling event to develop an EventID.

7. Clicking the "Generate Sampling EventID" button passes the user data to a secondary form where the user enters additional event data.



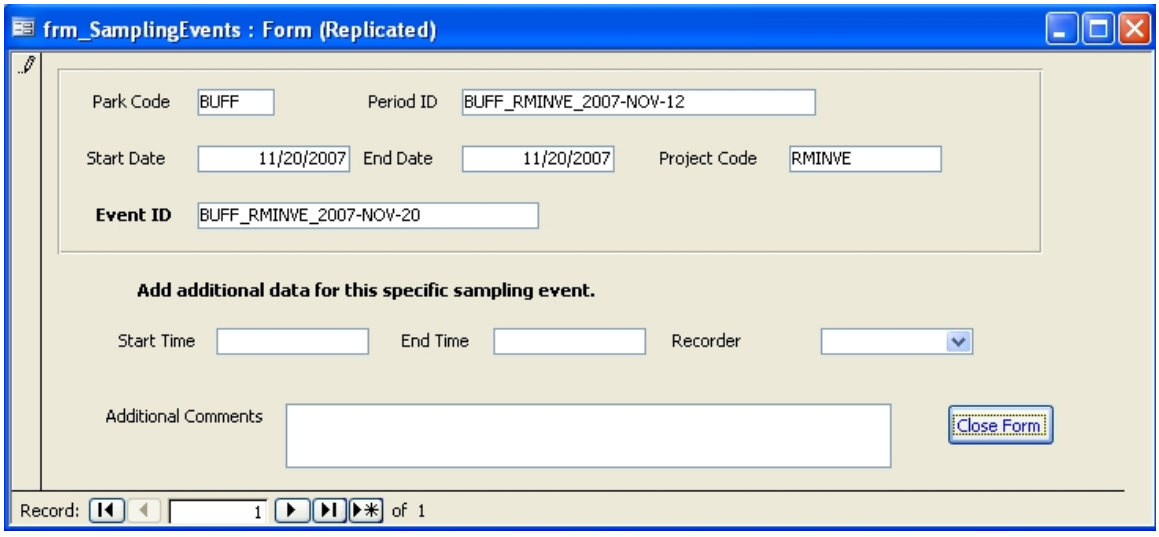


Figure 5. Sampling event forms for invertebrate data.

After inputting sampling occasion data (locations, periods, and events), the user can begin to enter additional invertebrate data. The following demonstrates data entry for the additional data and can be used in any order.

Procedure: Invertebrate Counts

1. Click the "Enter Invertebrate Counts and Additional Data" button on the main switchboard and select the "Invertebrate Counts" button to open the Invertebrate Counts form (Figure 6).
2. Select the appropriate Park Code, LocationID, Riffle #, Replicate, and EventID from the drop down box.
3. Click the "Open Count Form" button to enter occurrence data (Figure 7).

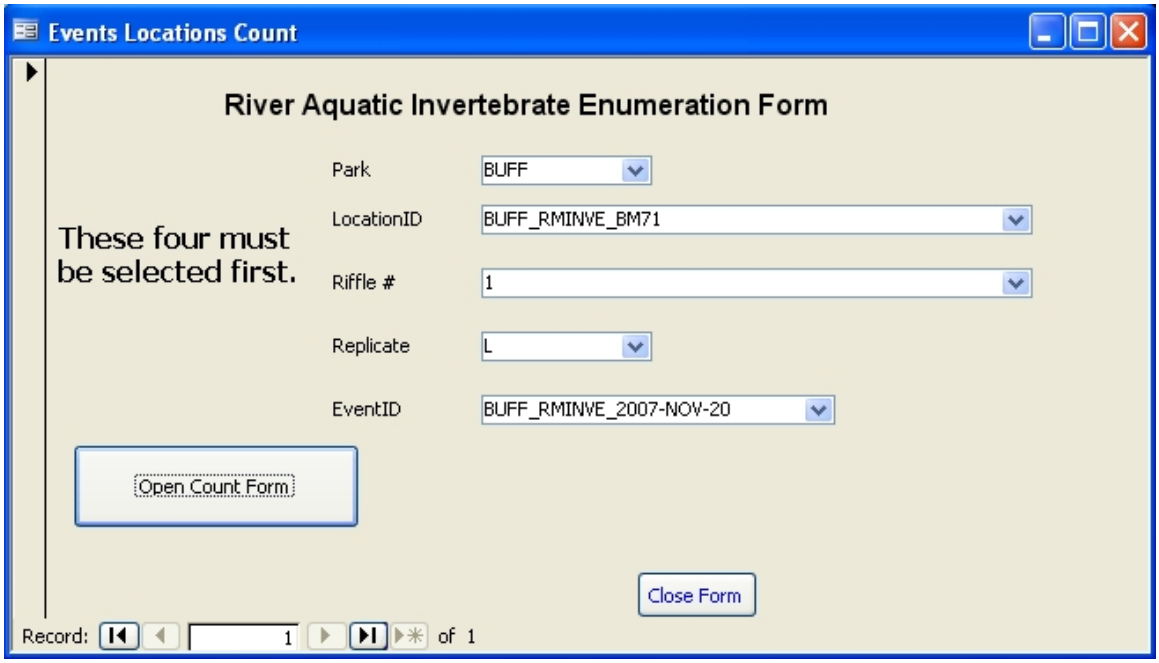


Figure 6. Preliminary data entry form for invertebrate counts. Location and event data are selected from drop down boxes and upon clicking the "Open Count Form" are passed to the invertebrate count data form (Figure 7).

4. Select the Taxa from the drop down box (Figure 7). Upon selection, the TaxonCode remains visible and the remaining values are hidden (inset of TaxonCode, Phylum, *etc*. added to demonstrate selection).
5. After selecting Taxa, enter the count.
6. Clicking the "Continue" button will prompt for the next Taxa.

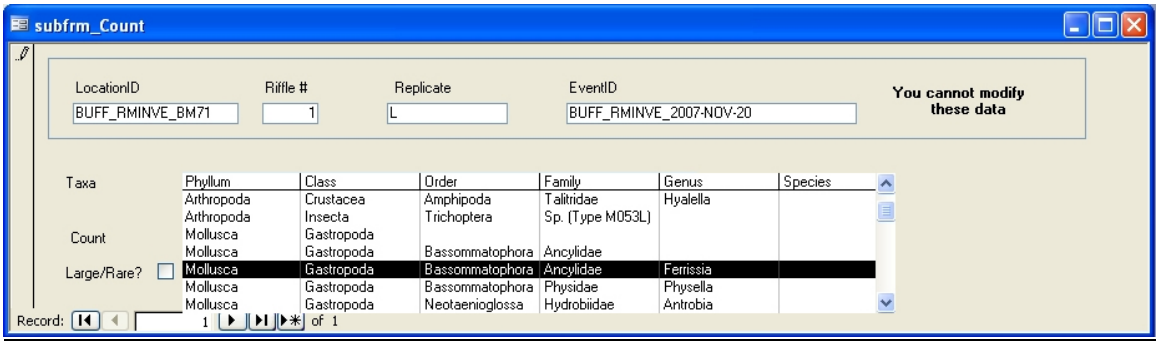


Figure 7. Taxa count form for invertebrate data.

Entering Habitat and Discharge Data

Habitat data are entered by clicking the "Enter Invertebrate Counts and Additional Data" button on the main switchboard and selecting the appropriate button for habitat type (see Figure 8). The user is then presented with selection boxes (LocationID, EventID, *etc.*) for the appropriate habitat type and this data is passed to subforms where specific replicate data is entered. Each habitat subform is provided with a "Continue" button that allows the user to advance to the next observation and the "Return" button to exit the form. When finished entering habitat data the user will be returned to the initial habitat form and exits the form by clicking the "Close Form" button.

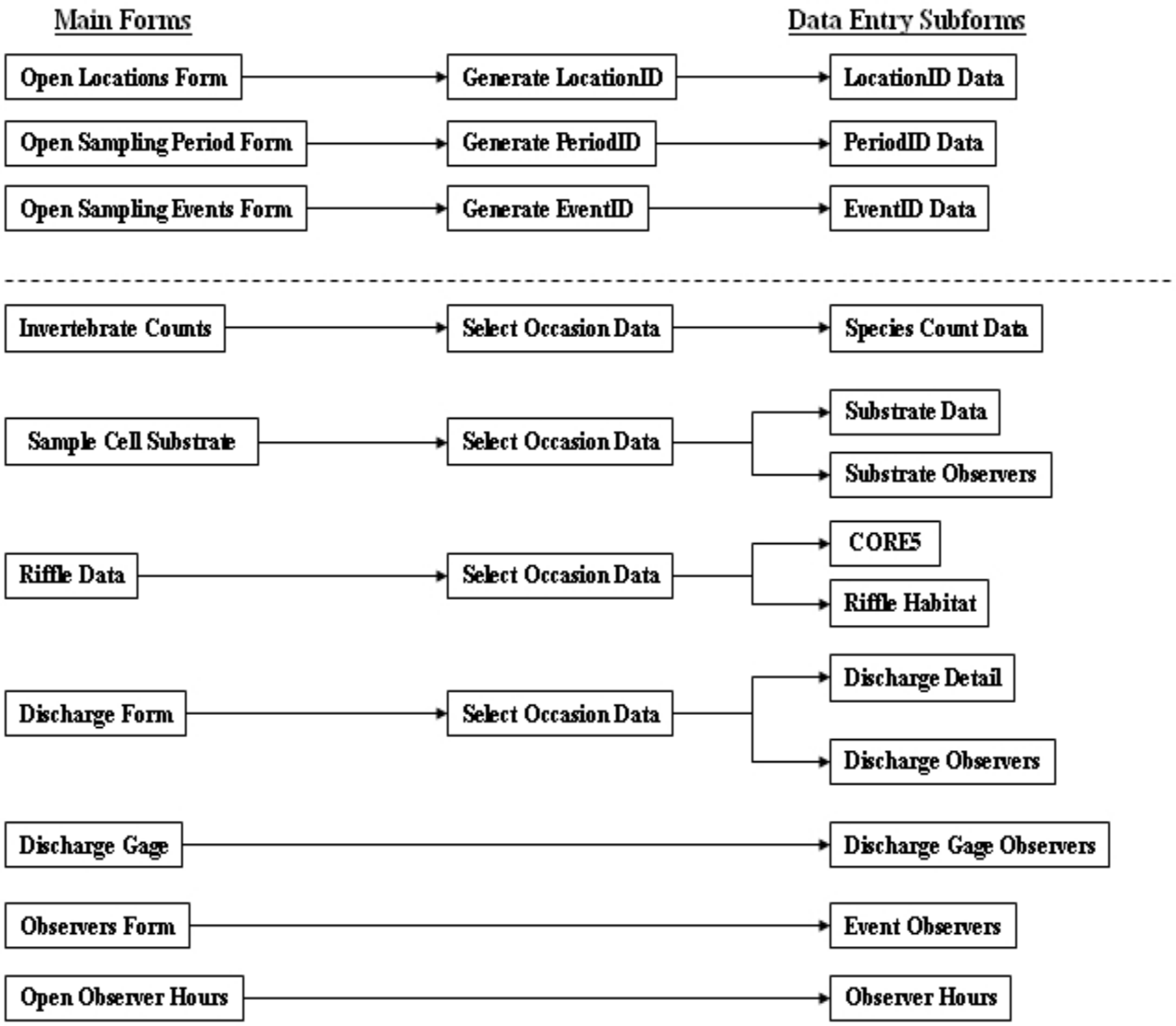


Figure 8. Outline of invertebrate community data forms. Note: Forms are selected from the main switchboard and data entered into data entry subforms.

Entering Water Quality Data

Water quality data (CORE 5) collected by hand-held meters are entered by clicking the "Riffle Data" button on the main habitat subform. The user selects the appropriate Park Code, LocationID and EventID and enters the CORE 5 data in another subform. Water quality data collected by unattended CORE 5 data loggers (Sondes) are uploaded from the logger using the manufacturer's accompanying software program and saved in MS Excel. Data are then edited to correct any missing data due to logger maintenance (down time) and validated to determine if the data meet the expected range requirements or critical limits. CORE 5 water quality summary data are then entered into NPStoret either by using the direct data entry templates or the import module. Metadata is then entered for each characteristic/parameter. Coordinate data for logger locations are collected in accordance with the current HTLN spatial data collection techniques

and entered into NPStoret. An NPStoret database is then sent to the WRD staff on an annual basis for initial QA/QC and subsequent upload into the WRD master copy of the EPA STORET.

IV. Data Verification

Data verification immediately follows data entry and involves checking the accuracy of computerized records against the original source, usually paper field records.
Data tables are queried to produce specific sets of data (*i.e.*, invertebrate data, habitat data, *etc.*) and exported to Excel worksheets (via the QAQC button, Figure 2). These worksheets can then be compared to the original source (*i.e.*, field data sheets) to identify missing, mismatched, or redundant records. The design master is then used to correct and/or delete errors via data edit forms. As data are edited, built in table triggers store the original record in a backup audit table and can be recovered where necessary (audit tables mirror the data table).

Procedure: Data Verification

1. Query pertinent data (tables and queries) and develop reports.
2. Develop switchboard to generate reports.
3. Instruct project manager on use of switchboard and export capability of reports.

V. Data Validation

Although data may be correctly transcribed from the original field forms, they may not be accurate or logical. For example, field crews collect data per occasion (location and event) and the resident data should reflect this. At any given occasion, invertebrate benthic sampling is conducted in three riffles per reach and three samples per riffle, totaling nine samples per stretch (location) during the sampling event. During the same occasion, one measurement of the CORE 5 water quality parameters are collected at each riffle, habitat parameters (% vegetation, % embeddedness, *etc.*) at three samples per riffle, and 20 replicates of substrate size classes within three samples at all three riffles. A query of these data should reflect these conditions and confirm the coincident (*i.e.*, relational) nature of the database. Missing, mismatched, or duplicate records can then be corrected.

As annual data are amassed in the database, validation is conducted via query and comparison among years to identify gross differences. For example, species A. may be recorded at a location this year, but not in previous years, thus representing a possible new locality. The design master is then used to correct and/or delete errors via data edit forms. Once verification and validation is complete, the data set is turned over to the Data Manager for archiving and storage. The data can then be used for all subsequent data activities.

Procedure: Data Validation

1. Query pertinent data to insure resident data match existing protocols for each parameter.
2. Archive validated database.

VI. Spatial Data Validation

Spatial validation of sample coordinates can be accomplished using the ArcMap component of ArcGIS. Coordinate data are maintained in the Access database and can be added to an ArcMap project and compared with existing features (*i.e.*, park boundaries, USGS DOQQ's, NHD hydrography, *etc*.) to confirm that coordinate data are valid.

Procedure: Spatial Data Validation

1. Develop testing project within ArcMap constrained to appropriate UTM zone and projection (NAD83).
2. Add park unit boundaries and any necessary spatial data (roads, water, contour, *etc.*).
3. Add relevant site coordinate data to testing project and validate against known features.
4. Correct mistakes, if necessary, and re-validate.
5. Develop metadata for final spatial dataset.

VII. File Organization

The various databases, reports and GIS coverages used and generated by the Heartland Network create a large number of files and folders to manage. Poor file organization can lead to confusion and data corruption. As a standard data management technique, files pertaining to the project are managed in their own folder: Analysis, for data analysis; Data, for copies of archived data as well as data sheets; Documents, for supporting materials related to the project; and, Spatial info, for various spatial data. The database is managed in the Databases folder and contains prior versions of the database in a subfolder.

VIII. Version Control

Prior to any major changes of a data set, a copy is stored with the appropriate version number. This allows for the tracking of changes over time. With proper controls and communication, versioning ensures that only the most current version is used in any analysis. Versioning of archived data sets is handled by adding a floating-point number to the file name, with the first version being numbered 1.00. Each major version is assigned a sequentially higher whole number. Each minor version is assigned a sequentially higher fractional number. Major version changes include migrations across Access versions and complete rebuilds of front-ends and analysis tools. Minor version changes include bug fixes in front-end and analysis tools. Frequent users of the data are notified of the updates, and provided with a copy of the most recent archived version.

IX. Replication

The aquatics program relies on distributed databases to allow remote users to enter/revise data and is accomplished via database replication consisting of a design master and replicas. The design master is stored on the server at Missouri State University and can be directly accessed for local data entry/revisions or design changes. A replica is created for users without access to

the server and is distributed for data entry/revision activities. When a user has finished data activities with the replica, it is returned and synchronized with the design master.

X. Backups

Secure data archiving is essential for protecting data files from corruption. Once a data set has passed the QA/QC procedures specified in the protocol, a new metadata record is created using the NPS Metadata Tools (stand alone or within ArcCatalog) and/or Dataset Catalog. Backup copies of the data are maintained at both on- and off-site locations. An additional digital copy is forwarded to the NPS Inventory and Monitoring Data Store. Tape backups of all data are made at regular intervals in accordance with current HTLN backup standard operating procedures and will be made minimally, once per week, with semi-annual tapes permanently archived.

Procedure: Backups

1. Create metadata record pursuant to data archiving.
2. Backup data.
3. Store backup copies on- and off-site and forward a copy to the I&M Data Store.
4. Administer regularly scheduled backups of data.

XI. Data Availability

Currently, data are available for research and management applications for those database versions where all QA/QC has been completed and the data have been archived. Data can be transferred using ftp or by e-mail (where files are smaller than a few megabytes). Monitoring data will become generally available for download directly from the NPS I&M Data Store. Metadata for the invertebrate community database are developed using ESRI ArcCatalog 9.x and the NPS Metadata Tools and Editor extension and will be available at the NPS I&M GIS server (http://science.nature.nps.gov/nrdata/). Water quality data will be stored at the EPA STORET National Data Warehouse and be publicly accessible via the Internet. Additionally, data requests can be directed to:

Heartland I&M Network
Attn. Data Manager
Wilson's Creek National Battlefield
6424 W. Farm Road 182
Republic, MO 65738-9514
417-836-5313 or 417-732-6438

Protocol for Monitoring Aquatic Invertebrates at Ozark National Scenic Riverways, Missouri, and Buffalo National River, Arkansas. Heartland I&M Network and Prairie Cluster Prototype Monitoring Program

SOP 8: Data Analysis

Version 1.00 (07/01/2007)

Revision History Log:

Previous Version #	Revision Date	Author	Changes Made	Reason for Change	New Version #

This SOP describes the metrics to be calculated for invertebrate data collected from BUFF and OZAR, the use of control charts, and other potential analytic approaches.

I. Multi-metrics

Ozark Scenic Riverways Stream Condition Index

The Stream Condition Index (SCI) is based on reference streams listed in Rabeni *et al*. (1997). This index uses four metrics.

Taxa Richness. This metric is a measure of the total number of genera or other designated taxonomic level of identification. Taxa richness generally increases with improving water quality, habitat diversity, or habitat suitability (Rabeni *et al*., 1997).

Calculate as the total number of genera or other designated taxonomic level of identification.

EPT Richness: This metric is a measure of the number of genera within the aquatic insect orders Ephemeroptera (mayflies), Plecoptera (Stoneflies), and Trichoptera (caddisflies). Members of these orders are generally considered pollution sensitive organisms and a decrease in their total number may indicate impairment.

Calculate as the total number of different genera within the aquatic insect orders Ephemeroptera, Plecoptera, and Trichoptera.

Shannon's Taxa Diversity Index. A variety of community diversity and similarity indices have been used in biological monitoring of water quality including Shannon's index, Simpson's index, the Coefficient of Community Loss, Jaccard Coefficient, and Pinkham-Pearson Community

Similarity index (Resh and Jackson, 1993). Due to a variety of theoretical concerns, diversity indices have been criticized recently, but Shannon's index of species (taxa) diversity is still one of the most commonly used evenness metrics both in terrestrial and aquatic ecosystems (Washington, 1984). This index is based on information theory where the higher the score (H), the greater is the degree of uncertainty that the next species will be the same as the previous one.

This metric proved useful in assessing water quality of the Buffalo River previously (Bryant, 1997) and was one of the few metrics that showed strong correlations with water quality measures such as nitrate nitrogen concentrations. Taxa diversity generally decreases with declining water quality because of reductions in both richness and evenness.

Calculate as:

$$H = -\sum_{i=1}^{S} p_i \log p_i$$

Or alternatively,

$$H' = -SUM\{ p_i * \ln(p_i)\}$$

P_i= relative abundance of each species, calculated as the proportion (decimal fraction) of individuals belonging to a given species.

<u>Note</u>: While any base may be used, the natural log (ln) is the most common.

Biotic Index (BI). The BI was first developed by Hilsenhoff (1982) and subsequently modified by Hilsenhoff (1988). Each taxon is assigned a pollution tolerance value related to its assumed or known tolerance of water quality degradation. Tolerance values for taxa occurring at OZAR will follow Lenant (1993) and Hilsenhoff (1987) and are presented in Appendix A.1 of this SOP. The BI increases with increasing impairment.

Calculate as:

$$BI = \sum (X_i T_i) / N$$

X_i = number of individuals within each genus
T_i = tolerance value of that species
N = total number of organisms in the sample

Metric Scoring for the SCI

All metric values are normalized so that they become unitless and can be comparable and have equal influence on the SCI results. Reference data provided in Doisy and Rabeni (1999) included four sites in the Current River watershed and other Ozarkian streams that were used to determine a range for each metric with one of three possible scores assigned to each range. The lower or

upper quartile of the distribution for each metric is used as the minimum value representative of reference conditions.

Because taxa richness, EPT richness, and the Shannon Index all decrease with increased impairment, any values above the lower quartile (25%) of the reference distribution receive the highest score of five. Values between the 25% quartile and the 1% quartile receive a score of 3 and values below the 1% quartile receive a score of 1. Ranges for each metric and the assigned scores based on the reference distribution generated are shown in Tables 1 and 2.

The Biotic Index increases with increased impairment so any value below the upper quartile (75%) of the reference distribution receives the highest score of 5. Values between the 75% quartile and the 99% quartile receive a score of 3 and values above the 99% quartile receive a score of 1. Ranges for each metric and the assigned scores for the Biotic Index are shown in Tables 1 and 2.

Each metric score is determined by averaging the metric values from the 9 samples. The four scores are then summed to generate the SCI score. Scores range from 16-20 for not impaired, 10-14 for impaired, and 4-8 for very impaired.

Table 1. Values for metrics using reference data collected during fall for the Current River watershed (after Rabeni *et al.*, 1997).

Community Metric	Mean	95% confidence levels
Taxa richness	17.5	12.1-22.9
EPT taxa	8.8	4.9-12.5
Biotic Index	3.21	2.92-3.50
Shannon's Index	6.1	2.69-9.54

Table 2. Descriptive statistics and scores for the metrics from data collected during from the fall for the Current River watershed (after Rabeni *et al.*, 1997).

	Statistics					Scores		
Metric	1%	25%	50%	75%	99%	5	3	1
Taxa richness	12	14	17	21	24	>=14	13-12	<12
EPT	5	6	9	12	13	>=6	5	<5
Biotic Index	2.97	2.97	3.16	3.4	3.55	<=3.40	3.41-3.55	>3.55
Shannon's Index	1.9	4.3	6.4	8.3	9.8	>=4.3	4.2-1.9	<1.9

Buffalo National River Index of Community Integrity

The Buffalo River Index of Community Integrity (BRICI) uses ten metrics.

Margalef's Index of Taxa Richness. Richness measures used in the past have included taxa richness (the total number of taxa at a given site and derivatives of this including Margalef's Index and Rarefracted Richness), family richness (the number of invertebrate families occurring at a particular site), EPT taxa richness, and niche occupant forms (the number of taxa occurring at a site that can be discerned by a novice) (Resh and Jackson, 1993). Of these, Margalef's index of taxa richness was selected for the BRICI. Margalef's index is not a true measure of diversity, as it does not include any component of evenness. However, this measure of richness is superior to raw richness values because it compensates for differences in sample size among the various sites and seasons (Washington, 1984). Richness and sample size are strongly correlated; increasing sample size results in higher richness (Washington, 1984). Resh and Jackson (1993) considered richness measures to be among the most reliable and accurate metrics because such measures rarely indicate impact when impacts have not occurred and generally show impact when impacts have occurred. The rationale behind the use of this metric is that taxa richness generally declines with decreasing water quality (Weber, 1973; Resh and Grodhaus, 1983; Resh and Jackson, 1993).

Calculate as:

$$d = s\text{-}1 / \mathit{ln}\ N$$

s = total number of taxa present
$\mathit{ln}\ N$ = natural log of the total abundance for the sample.

Shannon's Taxa Diversity Index. See description above.

Percent Dominant Taxa. This metric provides some insight into the evenness (relative abundance of the taxa to one another) of an invertebrate community. As water quality declines, a few taxa benefit from the disturbed conditions and increase dramatically in number. However, most other taxa decline in abundance. In grossly polluted environments, a single taxon may dominate the community, thus demonstrating a total imbalance of community structure and function. Percent dominance can be based on the single most abundant taxon, or the two or three most abundant taxa. For the BRICI described herein, percent dominance is based on the three most abundant taxa for two reasons. First, none of the habitats in the Buffalo River currently appear to be grossly polluted to the point where they would be totally dominated by a single taxon. Second, because the Chironomidae (midges) often is the dominant group in disturbed ecosystems and percent Chironomidae is another metric in the BRICI, using multiple taxa in this calculation minimizes the influence of the Chironomidae (midges) on percent dominance and prevents redundancy in the system.

Calculate as:

$$\text{Percent Dominance} = (D_1 + D_2 + D_3) / N * 100$$

D_1, D_2, and D_3 = abundances of the first, second, and third most numerous taxa in a sample

N = total abundance of invertebrates in that sample.

Percent Chironomidae. Immature midges (Order Diptera, Family Chironomidae) can be a rich and abundant component of any stream community, but they often become dominant in the benthos under disturbed conditions. The rationale of this metric is that as natural stream conditions degrade, the relative abundance of chironomids increases.

Calculate as a simple percentage of the total number of invertebrates collected in the sample.

Percent Plecoptera and Percent Trichoptera. The EPT (Ephemeroptera, Plecoptera, Trichoptera) group metrics (*e.g.*, EPT taxa richness, percent EPT, and EPT:D [Diptera]) are widely used in aquatic biological monitoring. Overall, these three orders of insects are the most sensitive groups to human disturbance (Lenat, 1993). Members of the order Plecoptera are the most intolerant to pollution, and the Trichoptera are the second most intolerant. However, many Ephemeroptera found in the Buffalo River belong to genera that have relatively high pollution tolerances and their occurrence in samples could be misleading with respect to detecting perturbation. Accordingly, the BRICI will use percent Plecoptera and percent Trichoptera separately instead of using the common EPT group metric. Their numbers should be relatively high under natural conditions and should decrease with increasing levels of human disturbance.

Calculate as a simple percentage of the total number of invertebrates collected in the sample.

Percent Elmidae. During an investigation of mid-river tributaries of the Buffalo River, Bradley (2001) found that riffle beetles (Elmidae) representing five genera-- *Dubiraphia*, *Macronychus*, *Microcylloepus*, *Optioservus*, and *Stenelmis*--were among the most abundant taxa at the reference site on Water Creek, often comprising >20% of the total invertebrates. The abundance of elmids at three disturbed sites was always much lower than at Water Creek, suggesting that riffle beetles inhabiting the Buffalo River watershed may be relatively intolerant to pollution. Although elmids are known to vary widely in their tolerance to pollution, this attribute is species-specific and may differ among populations of the same species (Hilsenhoff, 1982, 1988; Lenat, 1993). Because elmids are among the most dominant taxa in mid-river tributaries, they may significantly affect other metrics based on percentage composition. This metric is therefore included to compensate for these potential changes in the mid-river tributaries. The rationale on which this metric is based is that the proportion of the community comprised of elmids will decrease with increasing pollution.

Calculate as a simple percentage of the total number of invertebrates collected in the sample.

Percent *Corbicula*. Asian Clam (*Corbicula fluminea*) is the one of the few exotic animal species known from the Buffalo River. Like most invasive species, it usually reaches high numbers only if the natural community at a site is imbalanced (Sax and Brown, 2000). Metrics based on the number of exotic species have been used previously in monitoring fish populations (Karr *et al.*, 1986) and invertebrate communities (Gregory, 2005). The Environmental Protection Agency recently recommended that percent *Corbicula* be included as a community metric for assessing water quality of streams and wadeable rivers (Barbour *et al.*, 1999). The pretense on which this metric is based is that as the level of pollution increases, the number of *Corbicula* will also increase.

Calculate as a simple percentage of the total number of invertebrates collected in the sample.

Percent Intolerant. Some large-scale investigations have been able to assign tolerance values to invertebrate genera and species (*e.g.*, Hilsenhoff, 1982, 1988; Lenat, 1993). These values were used to calculate a Biotic Index that gives insight into the water quality of streams in the region. In order to calculate these tolerance values, large numbers of samples from many streams with known water quality are required. Therefore, the tolerance values given in Lenat (1993) will be used here and they are presented in Appendix A.1 of this SOP. Many of the taxa present in the Buffalo River are the same as those presented in Lenat (1993). Tolerance values for species not addressed by Lenat (1993) are presented as the mean tolerance value for all species in the genus. Genera from the Buffalo River not listed by Lenat (1993) are not assigned tolerance values until their specific tolerances can be determined. The basis of this metric is that as the level of pollution increases, the number of pollution intolerant species should decrease.

Calculate as a percentage of the number of specimens in the sample considered intolerant. Only those taxa with a tolerance value of zero or one are used to calculate this metric.

Percent Collector-Filterer. Analysis of functional feeding groups is a product of the River Continuum Concept (RCC) (Vannote *et al.*, 1980). The RCC suggests that there are predictable changes in the food resources along the length of a stream and that the community found in a given reach is structured to capitalize on the food resources available there. In the headwaters, shredders (organisms that consume organic matter larger than 1 mm) are the most abundant group. In the mid-reaches, scrapers (organisms that graze on microscopic algal growth called periphyton) and collectors (organisms that gather particles of organic matter smaller than 1 mm either from the bottom--collector gatherers--or filtered from the water column--collector filterers) dominate. Communities in downstream reaches are composed primarily of gatherers. All reaches have a predator component that is relatively similar in abundance (~10%).

By analyzing the functional feeding group structure of a given site, one can determine if energy flow through the system has been modified. For example, organic enrichment often results in increased growth of algae and periphyton because of release of nutrient limitations. If a headwater site is organically enriched, it may have a much larger scraper component than would occur naturally. Generally, functional metrics are expressed as ratios such as shredders/abundance or scrapers/collector-gatherers (Lenat and Barbour, 1994). However, the reason for including this metric is somewhat different than that normally given for incorporating metrics into a biological monitoring system. Recent research on the consequences of forestry

practices on invertebrate community structure suggests that the collector-filterer functional group is most negatively impacted by the increased sedimentation associated with logging. Agricultural and silvicultural practices occurring in the Buffalo River watershed not only increase nutrient loads, but also result in higher sedimentation rates (Mott, 1997). The rationale underlying this metric is that the number of collector-filterers will decrease with increasing levels of disturbance. Functional feeding groups were determined based on methods and information given in Merritt and Cummins (1996a, b) and are presented in Appendix A.1 of the SOP.

Calculate as a simple percentage of the total number of invertebrates collected in the sample.

Metric Scoring for the BRICI

For metrics that decrease with increasing level of disturbance (Margalef's index, Shannon index, percent Plecoptera, percent Trichoptera, percent Elmidae, percent collector-filterer, and percent intolerant), higher metric values indicate higher water quality.

For metrics that increase with increasing level of disturbance (percent Chironomidae, percent *Corbicula,* and percent dominant), lower metric values suggest higher water quality.

Scoring the metrics involves dividing the data from all sites for a given metric into specific percentiles (<25th percentile, 25th-50th percentile, 51th-75th percentile, >75th percentile). Values in the highest percentile range are assumed to represent high water quality. Those in the lowest range are assumed to indicate low water quality, and intermediate percentiles represent water quality somewhere between high and poor water quality. The quartile range in which each metric falls determines what score that metric receives. Data that falls into each of these ranges are scored 4, 6, 8, and 10, respectively for metrics that decrease with increasing disturbance, and 10, 8, 6, and 4 for percent Chironomidae, percent *Corbicula*, and percent dominant (Table 3). Each of these four scores for each of the four metrics are added together to determine the BRICI value for that site. Scores range from 16-20 for not impaired, 10-14 for impaired, and 4-8 for very impaired.

After results are calculated for the individual sites, they should be plotted as bar graphs so that trends in water quality can be observed. Sites with BRICI values that are 30-50% lower than sites with the highest BRICI values (reference sites) should be classified as slightly to moderately impacted. Sites with BRICI values that exhibit more than 50% change should be classified as severely impacted.

<u>Generating Summary Reports</u>

Upon opening the database, the user is presented with a switchboard and data analyses are initiated by selecting the Analyses button at the bottom of the form. The user is then presented with the individual data analyses (Figure 1). Since some metrics differ between the two parks, and in some cases are unique, the user chooses the specific data analysis option.

Table 3. Quartile scores for the Buffalo River Index of Community Integrity (BRICI) based on lower level identifications at eight main channel sites of the Buffalo National River (after Mathis, 2001).

Note: Margalef's and Shannon's indices were scored 3 pooled samples. Percent Plecoptera was scored independently for summer and fall communities versus winter and spring.

	ICI SCORES			
Metric	4	6	8	10
Margalef's Index	<3.83	3.83-4.39	4.40-4.94	>4.95
Shannon's Index	<2.13	2.13-2.34	2.35-2.53	>2.53
% Chironomidae	>34.72	26.24-34.72	15.28-26.23	<15.28
% Plecoptera (Summer & Fall)	<0.18	0.18-0.71	0.72-3.31	>3.31
% Plecoptera (Spring & Winter)	<13.76	13.76-23.76	23.77-32.80	>32.80
% Trichoptera	<1.37	1.37-4.06	4.07-9.55	>9.55
% Elmidae	<0.10	0.10-0.56	0.57-1.30	>1.30
% *Corbicula*	>1.46	0.05-1.46	0.01-0.04	0
% Collector-Filterer	<2.34	2.34-7.77	7.78-18.59	>18.59
% Intolerant	<2.29	2.29-9.24	9.25-18.28	>18.28
% Dominant	>66.58	58.59-66.58	50.89-58.58	<50.89

Procedure:

8. Open the main switchboard form and select the Analyses option to view specific analyses then choose the appropriate analysis (Figure 1).

   Note: Reports are generated either specific to park or grouping of parks depending on collection technique and/or metric used.

9. The Summary Invert Statistics summarizes a report for descriptive statistics on the numbers of organisms sampled (Figure 2).

10. The OZAR SCI generates scores (by LocationID, EventID, Riffle #, Replicate) for total richness, EPT, Shannon's Taxa Diversity Index, Biotic Index, and Stream Community Index (Figure 3). A similar report is generated for the BUFF BRICI for Margalef's Index of Taxa Richness, Shannon's Taxa Diversity Index, percent dominant taxa, percent Chironomidae, percent Plecoptera and percent Trichoptera, percent Elmidae, percent Corbicula, percent Intolerant, and percent Collector-Filterer.

11. Additional summary reports can be generated for total discharge, substrate, and riffle data.

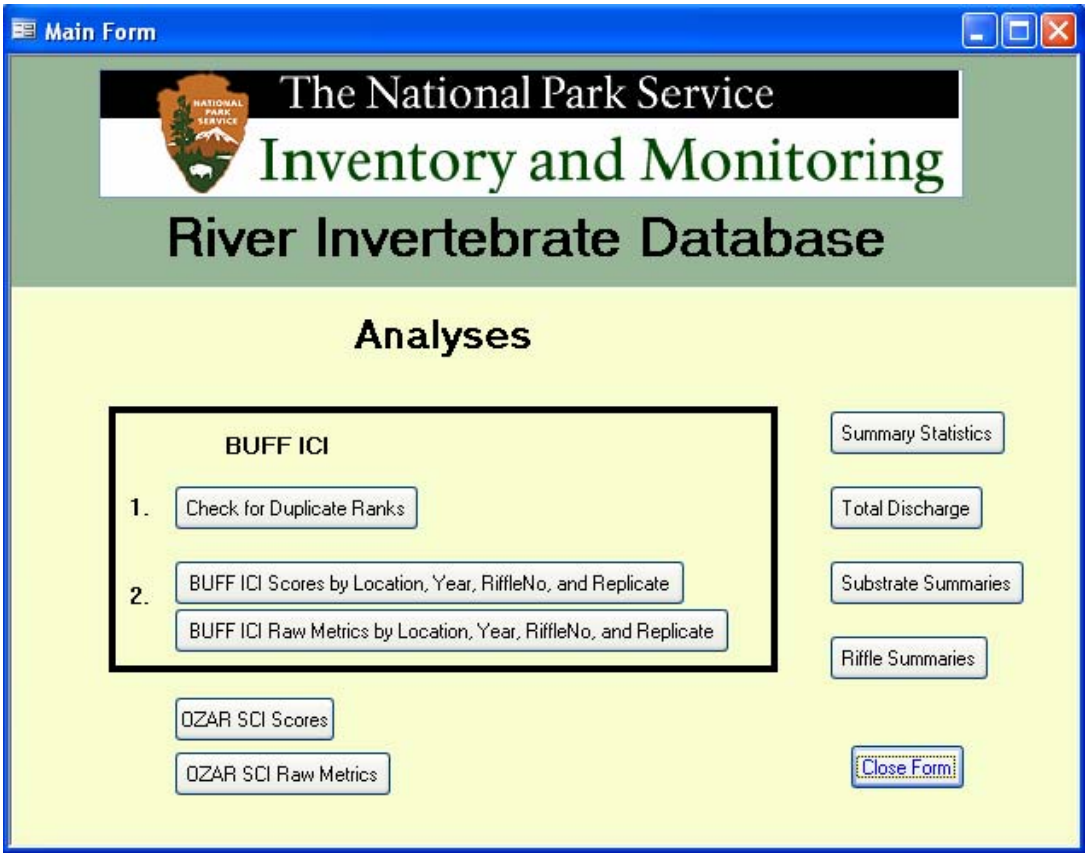


Figure 1. Individual analyses selection form.

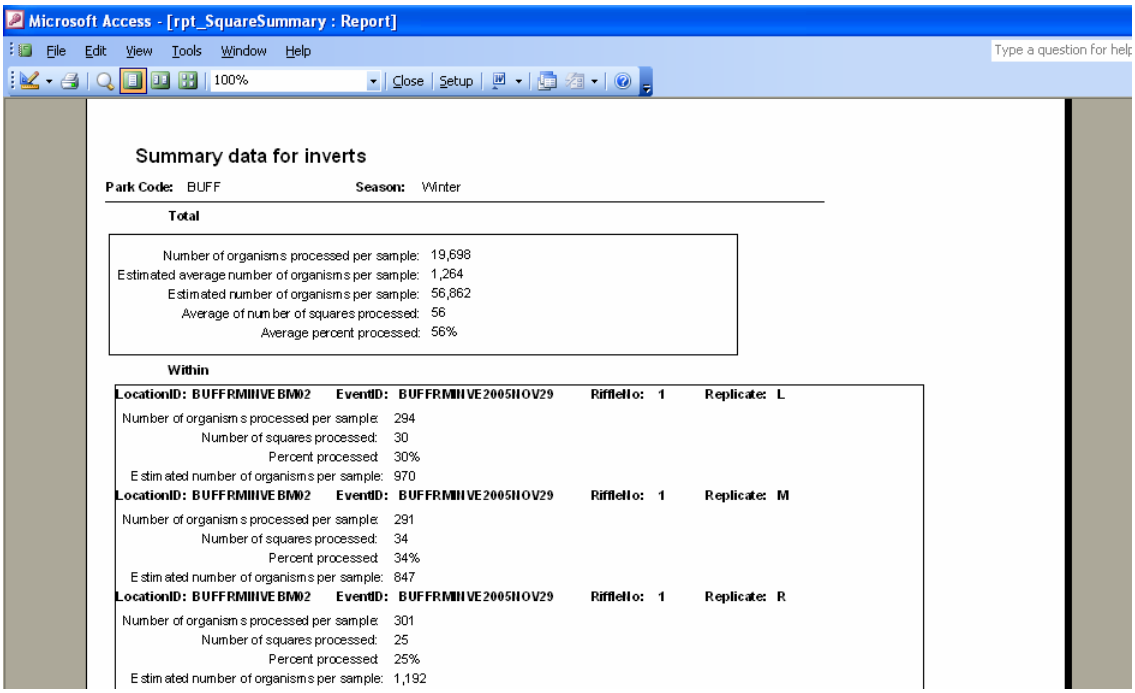
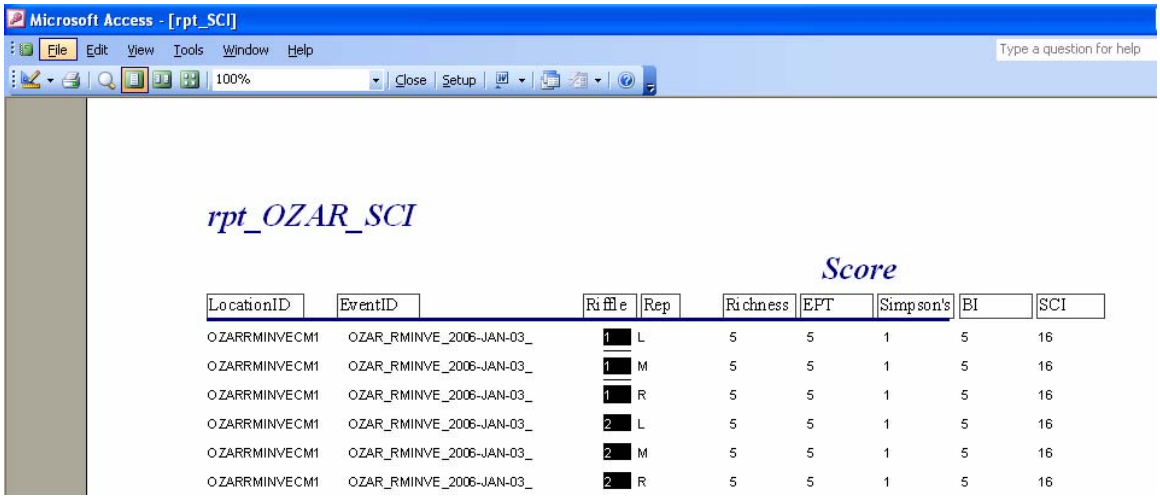


Figure 2. Summary statistics for invertebrates.



Microsoft Access - [rpt_SCI]

File Edit View Tools Window Help

Type a question for help

100% Close Setup

rpt_OZAR_SCI

LocationID	EventID	Riffle	Rep	Richness	EPT	Simpson's	BI	SCI
				Score				
OZARRMINVECM1	OZAR_RMINVE_2006-JAN-03_	1	L	5	5	1	5	16
OZARRMINVECM1	OZAR_RMINVE_2006-JAN-03_	1	M	5	5	1	5	16
OZARRMINVECM1	OZAR_RMINVE_2006-JAN-03_	1	R	5	5	1	5	16
OZARRMINVECM1	OZAR_RMINVE_2006-JAN-03_	2	L	5	5	1	5	16
OZARRMINVECM1	OZAR_RMINVE_2006-JAN-03_	2	M	5	5	1	5	16
OZARRMINVECM1	OZAR_RMINVE_2006-JAN-03_	2	R	5	5	1	5	16

Figure 3. Stream Community Index report for OZAR invertebrates.

II. Control Charts

The construction and interpretation of control charts is covered in many texts focusing on quality control in industry (*e.g.*, Beauregard *et al*., 1992; Gyrna, 2001; Montgomery, 2001). The application of control charts for ecological purposes, however, is relatively straightforward. The use of control charts in environmental monitoring is discussed in texts by McBean and Rovers (1998) and Manly (2001), although not in as great detail as the texts referenced above focusing on industrial applications. Many different types of control charts could be constructed, depending upon the type of information desired. For example, control charts can be used to evaluate variables or attributes (*i.e.*, count or frequency data), and focus on measures of central tendency or dispersion.

Most traditional control charts assume that observations come from a normal distribution, or that data can be transformed to normality. In industry, control limits are often set at a distance of 3 standard deviations on either side of the centerline (Wetherill and Brown, 1991; Beauregard *et al*., 1992; Montgomery, 2001). Thus, assuming a normal distribution centered at the centerline, the control limits would encompass 99.73 % of the distribution.

Control limits may be constructed so as to contain any desired proportion of the distribution (*i.e.*, representing [1-α] confidence intervals for any α. In this case, choosing control limits is equivalent to specifying a critical region for testing the hypothesis that a specific observation is statistically different from the proposed centerline value. (It is crucial that the centerline value is representative of the true population parameter.) Control limits could also be based on probabilistic thresholds other than confidence intervals (*e.g.*, McBean and Rovers, 1998).

If the observations cannot be assumed to come from a normal distribution, there are several options available beyond simple transformations of data. One option is to create subgroups of consecutive samples, and then use the subgroup averages, which will be approximately normally distributed in accordance with the central limit theorem (see Beauregard *et al*., 1992; Montgomery, 2001). It is possible to construct control charts based on other distributions (*e.g.*, a

Poisson distribution as in Atkinson *et al*., 2003), and construct analogous confidence limits, as long as the distributions are known. Distribution-free confidence limits may also be calculated, although these will usually be relatively wide and less sensitive to changes (Conover, 1999).

It is not absolutely necessary to use values from a statistical sampling process to determine centerlines and thresholds for action. It is possible to subjectively choose a centerline value as the desired state and set threshold limits to match the amount of variability with which one is comfortable for the variable of interest. It is crucial to realize that this approach has no statistical basis, and thus probabilities cannot be readily associated with the observations. This application also has a precedent in industry. Such charts, which plot observations without relevance to an underlying distribution, have been termed 'conformance charts'. Threshold values, which may be subjective, are termed 'action limits' (Beauregard *et al.*, 1992). If taking this approach, one should be very familiar with the system in question, and preferably select values that are defensible based on the data.

Although control charts have potentially wide applicability, each application may be different. A generic process for control chart construction is provided below, although decisions will always have to be made and an analyst familiar with control charts should ideally be consulted.

Steps in constructing a univariate control chart (see Figure 5):

1. Determine the parameter of interest. This may be the SCI, BRICI, any of the metrics or indices used to calculate the SCI or BRICI, or practically any other variable that may be derived from this protocol.

2. After several years of data are available, plot the values of the parameter of interest (on the y-axis) against time (on the x-axis).

3. Determined a "center-line" value for this parameter, which could represent a mean of the observations, a target value, or some other value. Determining an appropriate center-line contains inherent pitfalls, and an analyst who is familiar with control charts should be consulted.

4. Establish control limits around the center-line. It is possible that only an upper control limit, or only a lower control limit, or both will be necessary, depending upon the parameter of interest and management concerns. Control limits may be based on a probability distribution and thus allow one to make statistical inferences, or they may be based on target levels set by management. If the parameter of interest is the SCI or BRICI, a lower control limit could be set at 14, indicating impairment of water quality. Once again, determining appropriate control limits can be tricky, especially if statistical inferences are desired, and an analyst who is familiar with control charts should be consulted.

5. Continue to plot values of the parameter of interest over time as new data become available. If an observation exceeds the control limit(s), this is indicative of the potential need for management action, or a more focused study.

Control charts for the SCI or BRICI should be constructed (after several years of data are available) for the mainstem sites at BUFF and OZAR, and updated annually. A lower control limit should be set at 14 (indicating impairment of water quality) or potentially another level if

deemed appropriate. This use of a control chart is not based on any statistical distribution, and thus no statistical inferences are made, it is simply a management tool. Additional control charts can be constructed from other variables of interest as described above.

III. Other methods

If a hypothesis testing approach is deemed appropriate, many tests may be employed, depending upon the question being asked and the structure of the data. For example, a Kruskal-Wallace ANOVA may be used to test for significant differences between riffles using metric scores from each of the pseudoreplicates. If there is reason to compare more than two variables among samples, Friedman's non-parametric two-way analysis of variance should be used. Alternatively, Cochran's Q is particularly useful for measuring changes in frequencies (proportions) across time.

Quantitative Similarity analyses can be performed on each possible pairing of samples to produce a similarity matrix. Then spatial and temporal analysis with pervious year's data can be done using a Mean Similarity Analysis (Van Sickle, 1997) or Wilcoxon's matched pairs test.

A Detrended Correspondence Analysis (DCA) may be used to look for spatial relationships. This produces a two-dimensional graph in which samples of similar taxonomic composition are near each other and dissimilar entities are far apart. Equal distances in the ordination correspond to equal differences in species composition. Samples from the previous years can be compared over time and samples can be compared from different locations in the same year. In addition, environmental gradients can be inferred from the species composition data by performing a Spearman rank correlation between the rankings of the sample scores on the first two axes and rankings of the environmental variables (water quality and habitat data).

Regional invertebrate taxa list with functional feeding groups and tolerance values

Genus/Species	Tolerance Value	FFG	Subfamily	Family	Order	Class	Phylum
	6	C			Branchiobdellida	Hirudinea	Annelida
	7.8	Pa		Erpobdellidae	Arhynchobdellida	Hirudinea	Annelida
Erpobdella punctata		Pa		Erpobdellidae	Arhynchobdellida	Hirudinea	Annelida
Mooreobdella microstoma		Pa		Erpobdellidae	Arhynchobdellida	Hirudinea	Annelida
	7	Pr		Glossiphoniidae	Rhynchobdellida	Hirudinea	Annelida
Glossophonia complanata				Glossiphoniidae	Rhynchobdellida	Hirudinea	Annelida
Helobdella triserialis	8.9			Glossiphoniidae	Rhynchobdellida	Hirudinea	Annelida
Placobdella ornate				Glossiphoniidae	Rhynchobdellida	Hirudinea	Annelida
Placobdella parasitica	6.6	C		Glossiphoniidae	Rhynchobdellida	Hirudinea	Annelida
				Piscicolidae Pa	Rhynchobdellida	Hirudinea	Annelida
Piscicola punctata		Pa		Piscicolidae Pa	Rhynchobdellida	Hirudinea	Annelida
Haplotaxis		C		Haplotaxidae	Haplotaxida	Oligochaeta	Annelida
	7.3	C		Lumbricidae	Lumbricina	Oligochaeta	Annelida
Lumbriculus variegatus	8	C		Lumbriculidae	Lumbriculida	Oligochaeta	Annelida
	10	C		Enchytraeidae	Tubificida	Oligochaeta	Annelida
	9.2	C		Tubificidae	Tubificida	Oligochaeta	Annelida
Aulodrilus	8	C		Tubificidae	Tubificida	Oligochaeta	Annelida
Branchiura sowerbyi	8.4	C		Tubificidae	Tubificida	Oligochaeta	Annelida
Ilyodrilus templetoni	9.4	C		Tubificidae	Tubificida	Oligochaeta	Annelida
Limnodrilus angustipenis		C		Tubificidae	Tubificida	Oligochaeta	Annelida
Limnodrilus cervix	9.8	C		Tubificidae	Tubificida	Oligochaeta	Annelida
Limnodrilus claparedeianus	10	C		Tubificidae	Tubificida	Oligochaeta	Annelida
Limnodrilus hoffmeisteri	9.8	C		Tubificidae	Tubificida	Oligochaeta	Annelida
Potamothrix bavaricus		C		Tubificidae	Tubificida	Oligochaeta	Annelida
Quistadrilus multisetosus	10	C		Tubificidae	Tubificida	Oligochaeta	Annelida
Tubifex tubifex	10	C		Tubificidae	Tubificida	Oligochaeta	Annelida
	5.7	Pa, Pr			Hydracarina	Arachnoidea	Arthropoda
Allocrangonyx		C		Allocrangonyctidae	Amphipoda	Crustacea	Arthropoda
Allocrangonyx hubrichtti		C		Allocrangonyctidae	Amphipoda	Crustacea	Arthropoda
Bactrurus		C		Crangonyctidae	Amphipoda	Crustacea	Arthropoda
Bactrurus brachycaudus		C		Crangonyctidae	Amphipoda	Crustacea	Arthropoda
Crangonyx	8	C		Crangonyctidae	Amphipoda	Crustacea	Arthropoda
Crangonyx forbesi		C		Crangonyctidae	Amphipoda	Crustacea	Arthropoda
Stygobromus		C		Crangonyctidae	Amphipoda	Crustacea	Arthropoda

Stygobromus albamensis albamensis		C	Crangonyctidae	Amphipoda	Crustacea	Arthropoda
Stygobromus onandagensis		C	Crangonyctidae	Amphipoda	Crustacea	Arthropoda
Stygobromus ozarkensis		C	Crangonyctidae	Amphipoda	Crustacea	Arthropoda
Stygonectes		C	Crangonyctidae	Amphipoda	Crustacea	Arthropoda
Synurella		C	Crangonyctidae	Amphipoda	Crustacea	Arthropoda
Gammarus	6.9	C	Gammaridae	Amphipoda	Crustacea	Arthropoda
Gammarus fasciatus	6.9	C	Gammaridae	Amphipoda	Crustacea	Arthropoda
Gammarus minus		C	Gammaridae	Amphipoda	Crustacea	Arthropoda
Gammarus pseudolimnaeus		C	Gammaridae	Amphipoda	Crustacea	Arthropoda
Gammarus pulex		C	Gammaridae	Amphipoda	Crustacea	Arthropoda
Gammarus troglophilus		C	Gammaridae	Amphipoda	Crustacea	Arthropoda
Hyalella azteca Sassure	7.9	C	Hyalellidae	Amphipoda	Crustacea	Arthropoda
Caecidotea (blind & unpigmented)		C	Asellidae	Isopoda	Crustacea	Arthropoda
Caecidotea (epigean)	8	C	Asellidae	Isopoda	Crustacea	Arthropoda
Caecidotea antricola		C	Asellidae	Isopoda	Crustacea	Arthropoda
Caecidotea brevicauda		C	Asellidae	Isopoda	Crustacea	Arthropoda
Caecidotea ancyla		C	Asellidae	Isopoda	Crustacea	Arthropoda
Caecidotea dimorpha		C	Asellidae	Isopoda	Crustacea	Arthropoda
Caecidotea foxi		C	Asellidae	Isopoda	Crustacea	Arthropoda
Caecidotea fustis		C	Asellidae	Isopoda	Crustacea	Arthropoda
Caecidotea salemensis		C	Asellidae	Isopoda	Crustacea	Arthropoda
Caecidotea serrata		C	Asellidae	Isopoda	Crustacea	Arthropoda
Caecidotea stygia		C	Asellidae	Isopoda	Crustacea	Arthropoda
Lirceus garmani	7.7	C	Asellidae	Isopoda	Crustacea	Arthropoda
Lirceus hoppinae	7.7	C	Asellidae	Isopoda	Crustacea	Arthropoda
Cylisticus convexus		C	Cylisticidae	Isopoda	Crustacea	Arthropoda
Metroponorthus		C	Porcellionidae	Isopoda	Crustacea	Arthropoda
Porcellio		C	Porcellionidae	Isopoda	Crustacea	Arthropoda
Caucasonethes		C	Trichoniscidae	Isopoda	Crustacea	Arthropoda
Miktoniscus		C	Trichoniscidae	Isopoda	Crustacea	Arthropoda
Cambarellus puer		C	Cambaridae	Decapoda	Crustacea	Arthropoda
Cambarellus shufeldtii		C	Cambaridae	Decapoda	Crustacea	Arthropoda
Cambarus	8.1	C	Cambaridae	Decapoda	Crustacea	Arthropoda
Cambarus diogenes diogenes		C	Cambaridae	Decapoda	Crustacea	Arthropoda
Cambarus hubbsi		C	Cambaridae	Decapoda	Crustacea	Arthropoda
Cambarus hubrichti		C	Cambaridae	Decapoda	Crustacea	Arthropoda
Cambarus maculatus		C	Cambaridae	Decapoda	Crustacea	Arthropoda
Cambarus setosus		C	Cambaridae	Decapoda	Crustacea	Arthropoda
Fallicambarus fodiens		C	Cambaridae	Decapoda	Crustacea	Arthropoda
Faxonella clypeata		C	Cambaridae	Decapoda	Crustacea	Arthropoda
Orconectes	2.7	C	Cambaridae	Decapoda	Crustacea	Arthropoda

Orconectes eupunctus		C	Cambaridae	Decapoda	Crustacea	Arthropoda
Orconectes harrisonii		C	Cambaridae	Decapoda	Crustacea	Arthropoda
Orconectes hylas		C	Cambaridae	Decapoda	Crustacea	Arthropoda
Orconectes immunis		C	Cambaridae	Decapoda	Crustacea	Arthropoda
Orconectes lancifer		C	Cambaridae	Decapoda	Crustacea	Arthropoda
Orconectes longidigitus		C	Cambaridae	Decapoda	Crustacea	Arthropoda
Orconectes luteus		C	Cambaridae	Decapoda	Crustacea	Arthropoda
Orconectes macrus		C	Cambaridae	Decapoda	Crustacea	Arthropoda
Orconectes marchandi		C	Cambaridae	Decapoda	Crustacea	Arthropoda
Orconectes medius		C	Cambaridae	Decapoda	Crustacea	Arthropoda
Orconectes meeki		C	Cambaridae	Decapoda	Crustacea	Arthropoda
Orconectes neglectus		C	Cambaridae	Decapoda	Crustacea	Arthropoda
Orconectes neglectus		C	Cambaridae	Decapoda	Crustacea	Arthropoda
Orconectes neglectus neglectus		C	Cambaridae	Decapoda	Crustacea	Arthropoda
Orconectes ozarkae		C	Cambaridae	Decapoda	Crustacea	Arthropoda
Orconectes palmeri palmeri		C	Cambaridae	Decapoda	Crustacea	Arthropoda
Orconectes peruncus		C	Cambaridae	Decapoda	Crustacea	Arthropoda
Orconectes punctimanus		C	Cambaridae	Decapoda	Crustacea	Arthropoda
Orconectes quadruncus		C	Cambaridae	Decapoda	Crustacea	Arthropoda
Orconectes rusticus		C	Cambaridae	Decapoda	Crustacea	Arthropoda
Orconectes virilis		C	Cambaridae	Decapoda	Crustacea	Arthropoda
Orconectes williamsi		C	Cambaridae	Decapoda	Crustacea	Arthropoda
Procambarus	9.5	C	Cambaridae	Decapoda	Crustacea	Arthropoda
Procambarus acutus acutus		C	Cambaridae	Decapoda	Crustacea	Arthropoda
Procambarus clarkii		C	Cambaridae	Decapoda	Crustacea	Arthropoda
Procambarus gracilis		C	Cambaridae	Decapoda	Crustacea	Arthropoda
Procambarus liberorum		C	Cambaridae	Decapoda	Crustacea	Arthropoda
Procambarus viaeveridus		C	Cambaridae	Decapoda	Crustacea	Arthropoda
Palaemonetes kadiakensis	6.7	C	Palaemonidae	Decapoda	Crustacea	Arthropoda
		C	Entomobrydiae	Collembola	Insecta	Arthropoda
		C	Isotomidae	Collembola	Insecta	Arthropoda
		C	Sminthuridae	Collembola	Insecta	Arthropoda
Helichus	5.4	C, Sc	Dryopidae	Coleoptera	Insecta	Arthropoda
Helichus basalis	5.5	C, Sc	Dryopidae	Coleoptera	Insecta	Arthropoda
Helichus fastigiatus	5.5	C, Sc	Dryopidae	Coleoptera	Insecta	Arthropoda
Helichus lithophilus	5.5	C, Sc	Dryopidae	Coleoptera	Insecta	Arthropoda
Helichus striatus	4.6	C, Sc	Dryopidae	Coleoptera	Insecta	Arthropoda
Acilius		C, Sc	Dryopidae	Coleoptera	Insecta	Arthropoda
Agabus	5	Pr	Dytiscidae	Coleoptera	Insecta	Arthropoda
Agabus amplus		Pr	Dytiscidae	Coleoptera	Insecta	Arthropoda
Brachyvatus		Pr	Dytiscidae	Coleoptera	Insecta	Arthropoda

Copelatus	9.1	Pr	Dytiscidae	Coleoptera	Insecta	Arthropoda
Coptotomus interrogatus	9	Pr	Dytiscidae	Coleoptera	Insecta	Arthropoda
Coptotomus		Pr	Dytiscidae	Coleoptera	Insecta	Arthropoda
Cybister	4.6	Pr	Dytiscidae	Coleoptera	Insecta	Arthropoda
Desmopachria	3.7	Pr	Dytiscidae	Coleoptera	Insecta	Arthropoda
Dytiscus	3.7	Pr	Dytiscidae	Coleoptera	Insecta	Arthropoda
Graphoderus	3.7	Pr	Dytiscidae	Coleoptera	Insecta	Arthropoda
Hoperius		Pr	Dytiscidae	Coleoptera	Insecta	Arthropoda
Hydaticus		Pr	Dytiscidae	Coleoptera	Insecta	Arthropoda
Hydroporus	8.9	Pr	Dytiscidae	Coleoptera	Insecta	Arthropoda
Hydroporus niger		Pr	Dytiscidae	Coleoptera	Insecta	Arthropoda
Hydroporus pulcher		Pr	Dytiscidae	Coleoptera	Insecta	Arthropoda
Hydroporus undulates		Pr	Dytiscidae	Coleoptera	Insecta	Arthropoda
Hydroporus vilis		Pr	Dytiscidae	Coleoptera	Insecta	Arthropoda
Hydrovatus	3.7	Pr	Dytiscidae	Coleoptera	Insecta	Arthropoda
Hygrotus	1.9	Pr	Dytiscidae	Coleoptera	Insecta	Arthropoda
Ilybius	3.7	Pr	Dytiscidae	Coleoptera	Insecta	Arthropoda
Laccophilus	10	Pr	Dytiscidae	Coleoptera	Insecta	Arthropoda
Liodessus		Pr	Dytiscidae	Coleoptera	Insecta	Arthropoda
Oreodytes	4.6	Pr	Dytiscidae	Coleoptera	Insecta	Arthropoda
Rhantus	3.7	Pr	Dytiscidae	Coleoptera	Insecta	Arthropoda
Thermonectus	3.7	Pr	Dytiscidae	Coleoptera	Insecta	Arthropoda
Uvarus	4.6	Pr	Dytiscidae	Coleoptera	Insecta	Arthropoda
Ancyronyx variegata	6.9	C, Sc	Elmidae	Coleoptera	Insecta	Arthropoda
Dubiraphia	6.4	C	Elmidae	Coleoptera	Insecta	Arthropoda
Dubiraphia vittata		C	Elmidae	Coleoptera	Insecta	Arthropoda
Gonielmis dietrichi		C	Elmidae	Coleoptera	Insecta	Arthropoda
Heterelmis		C	Elmidae	Coleoptera	Insecta	Arthropoda
Macronychus glabratus	4.7	C	Elmidae	Coleoptera	Insecta	Arthropoda
Microcylloepus	2.1	C	Elmidae	Coleoptera	Insecta	Arthropoda
Microcylloepus pusillus		C	Elmidae	Coleoptera	Insecta	Arthropoda
Optioservus	2.7	C, Sc	Elmidae	Coleoptera	Insecta	Arthropoda
Optioservus immunis		C, Sc	Elmidae	Coleoptera	Insecta	Arthropoda
Optioservus ozarkensis		C, Sc	Elmidae	Coleoptera	Insecta	Arthropoda
Stenelmis	5.4	C, Sc	Elmidae	Coleoptera	Insecta	Arthropoda
Stenelmis beameri	4.6	C, Sc	Elmidae	Coleoptera	Insecta	Arthropoda
Stenelmis bicarinata	5	C, Sc	Elmidae	Coleoptera	Insecta	Arthropoda
Stenelmis cheryl	5.5	C, Sc	Elmidae	Coleoptera	Insecta	Arthropoda
Stenelmis convexula		C, Sc	Elmidae	Coleoptera	Insecta	Arthropoda
Stenelmis crenata	5.5	C, Sc	Elmidae	Coleoptera	Insecta	Arthropoda
Stenelmis decorata	5.5	C, Sc	Elmidae	Coleoptera	Insecta	Arthropoda

Stenelmis exigua	5.5	C, Sc	Elmidae	Coleoptera	Insecta	Arthropoda
Stenelmis exilis	5	C, Sc	Elmidae	Coleoptera	Insecta	Arthropoda
Stenelmis grossa		C, Sc	Elmidae	Coleoptera	Insecta	Arthropoda
Stenelmis knobeli		C, Sc	Elmidae	Coleoptera	Insecta	Arthropoda
Stenelmis lateralis	4.6	C, Sc	Elmidae	Coleoptera	Insecta	Arthropoda
Stenelmis lignicola		C, Sc	Elmidae	Coleoptera	Insecta	Arthropoda
Stenelmis mera		C, Sc	Elmidae	Coleoptera	Insecta	Arthropoda
Stenelmis sandersoni	5.5	C, Sc	Elmidae	Coleoptera	Insecta	Arthropoda
Stenelmis sexlineata	6.4	C, Sc	Elmidae	Coleoptera	Insecta	Arthropoda
Stenelmis xylonastis		C, Sc	Elmidae	Coleoptera	Insecta	Arthropoda
Dineutus	5.5	Pr	Gyrinidae	Coleoptera	Insecta	Arthropoda
Gyretes	3.7	Pr	Gyrinidae	Coleoptera	Insecta	Arthropoda
Gyretes sinatus		Pr	Gyrinidae	Coleoptera	Insecta	Arthropoda
Gyrinus	6.3	Pr	Gyrinidae	Coleoptera	Insecta	Arthropoda
Haliplus	5		Haliplidae	Coleoptera	Insecta	Arthropoda
Peltodytes	8.5	He	Haliplidae	Coleoptera	Insecta	Arthropoda
Peltodytes edentulus			Haliplidae	Coleoptera	Insecta	Arthropoda
Peltodytes litoralis			Haliplidae	Coleoptera	Insecta	Arthropoda
Peltodytes lengi			Haliplidae	Coleoptera	Insecta	Arthropoda
Peltodytes muticus			Haliplidae	Coleoptera	Insecta	Arthropoda
Peltodytes sexmaculatus			Haliplidae	Coleoptera	Insecta	Arthropoda
Peltodytes tortulosus			Haliplidae	Coleoptera	Insecta	Arthropoda
Helophorus	7.9	Sh	Helophoridae	Coleoptera	Insecta	Arthropoda
Heterocerus			Heteroceridae	Coleoptera	Insecta	Arthropoda
Hydraena		C, Sc	Hydraenidae	Coleoptera	Insecta	Arthropoda
Ochthebus		C, Sc	Hydraenidae	Coleoptera	Insecta	Arthropoda
Hydrochus	4.6	Sh	Hydrochidae	Coleoptera	Insecta	Arthropoda
Berosus	8.6	He, C	Hydrophilidae	Coleoptera	Insecta	Arthropoda
Chaetarthria	5.5	C, Pr	Hydrophilidae	Coleoptera	Insecta	Arthropoda
Crenitis		C, Pr	Hydrophilidae	Coleoptera	Insecta	Arthropoda
Cymbiodyta	5.5	C, Pr	Hydrophilidae	Coleoptera	Insecta	Arthropoda
Dibolocelus	5.5	C, Pr	Hydrophilidae	Coleoptera	Insecta	Arthropoda
Enochrus	8.5	C, Pr	Hydrophilidae	Coleoptera	Insecta	Arthropoda
Helobata			Hydrophilidae	Coleoptera	Insecta	Arthropoda
Helochares	5	C, Pr	Hydrophilidae	Coleoptera	Insecta	Arthropoda
Helocombus	5.5	C, Pr	Hydrophilidae	Coleoptera	Insecta	Arthropoda
Hydrobius	5	C, Pr	Hydrophilidae	Coleoptera	Insecta	Arthropoda
Hydrochara	6	C	Hydrophilidae	Coleoptera	Insecta	Arthropoda
Hydrophilus	4.6	C, Pr	Hydrophilidae	Coleoptera	Insecta	Arthropoda
Laccobius	8	C, Pr	Hydrophilidae	Coleoptera	Insecta	Arthropoda
Paracymus	7.3	C, Pr	Hydrophilidae	Coleoptera	Insecta	Arthropoda

Sperchopsis	6.5	C, Pr		Hydrophilidae	Coleoptera	Insecta	Arthropoda
Tropisternus	9.8	C, Pr		Hydrophilidae	Coleoptera	Insecta	Arthropoda
Tropisternus lateralis		C, Pr		Hydrophilidae	Coleoptera	Insecta	Arthropoda
Lutrochus	2.75			Lutrochidae	Coleoptera	Insecta	Arthropoda
Lutrochus laticeps				Lutrochidae	Coleoptera	Insecta	Arthropoda
Hydrocanthus	6.9	Pr		Noteridae	Coleoptera	Insecta	Arthropoda
Suphisellus		Pr		Noteridae	Coleoptera	Insecta	Arthropoda
Ectopria nervosa	4.3	Sc		Psephenidae	Coleoptera	Insecta	Arthropoda
Psephenus herricki	2.5	Sc		Psephenidae	Coleoptera	Insecta	Arthropoda
Cyphon		Sh		Salpingidae	Coleoptera	Insecta	Arthropoda
Scirtes	5	Sh		Scirtidae	Coleoptera	Insecta	Arthropoda
Atherix	2.1	Pr		Athericidae	Diptera	Insecta	Arthropoda
Atherix lantha		Pr		Athericidae	Diptera	Insecta	Arthropoda
Atherix variegata		Pr		Athericidae	Diptera	Insecta	Arthropoda
	6	C, Pr, Sc	Ceratopogoninae	Ceratopogonidae	Diptera	Insecta	Arthropoda
Bezzia		Pr	Ceratopogoninae	Ceratopogonidae	Diptera	Insecta	Arthropoda
Culicoides		C, Pr	Ceratopogoninae	Ceratopogonidae	Diptera	Insecta	Arthropoda
Mallochohelea		Pr	Ceratopogoninae	Ceratopogonidae	Diptera	Insecta	Arthropoda
Palpomyia		C, Pr	Ceratopogoninae	Ceratopogonidae	Diptera	Insecta	Arthropoda
Probezzia		Pr	Ceratopogoninae	Ceratopogonidae	Diptera	Insecta	Arthropoda
	6	C, Sc	Dasyheleinae	Ceratopogonidae	Diptera	Insecta	Arthropoda
Dasyhelea		C, Sc	Dasyheleinae	Ceratopogonidae	Diptera	Insecta	Arthropoda
	6	C	Forcipomyiinae	Ceratopogonidae	Diptera	Insecta	Arthropoda
Atrichopogon		C	Forcipomyiinae	Ceratopogonidae	Diptera	Insecta	Arthropoda
Chaoborus	8.5	Pr		Chaoboridae	Diptera	Insecta	Arthropoda
Eucorethra underwoodi		Pr		Chaoboridae	Diptera	Insecta	Arthropoda
Acalcarella		C	Chironominae	Chironomidae	Diptera	Insecta	Arthropoda
Apedilum				Chironomidae	Diptera	Insecta	Arthropoda
Axarus	6	C		Chironomidae	Diptera	Insecta	Arthropoda
Chironomus	9.8	C, Sh		Chironomidae	Diptera	Insecta	Arthropoda
Cladopelma	2.5	C		Chironomidae	Diptera	Insecta	Arthropoda
Cladotanytarsus	3.7	C, F		Chironomidae	Diptera	Insecta	Arthropoda
Cladotanytarsus (*Cladotanytarsus*)	3.7	C, F		Chironomidae	Diptera	Insecta	Arthropoda
Cladotanytarsus (*Lenziella*)	5.5	C, F		Chironomidae	Diptera	Insecta	Arthropoda
Constempellina	4	C, F		Chironomidae	Diptera	Insecta	Arthropoda
Cryptochironomous	7.4	Pr		Chironomidae	Diptera	Insecta	Arthropoda
Cryptotendipes	6.1	C		Chironomidae	Diptera	Insecta	Arthropoda
Demicryptochironomus	2.1	C		Chironomidae	Diptera	Insecta	Arthropoda
Dicrotendipes	7.9	C, F		Chironomidae	Diptera	Insecta	Arthropoda
Endochironomus	7.5	C, Sh		Chironomidae	Diptera	Insecta	Arthropoda
Endotribelos				Chironomidae	Diptera	Insecta	Arthropoda

Glyptotendipes	8.5	C, Sh		Chironomidae	Diptera	Insecta	Arthropoda
Goeldichironomus	9			Chironomidae	Diptera	Insecta	Arthropoda
Harnischia	7.5			Chironomidae	Diptera	Insecta	Arthropoda
Hyporhygma		Sh		Chironomidae	Diptera	Insecta	Arthropoda
Kiefferulus	10	C		Chironomidae	Diptera	Insecta	Arthropoda
Lauterborniella	5.5	C		Chironomidae	Diptera	Insecta	Arthropoda
Lipiniella		C		Chironomidae	Diptera	Insecta	Arthropoda
Microchironomous	8	C		Chironomidae	Diptera	Insecta	Arthropoda
Micropsectra	1.4	C		Chironomidae	Diptera	Insecta	Arthropoda
Microtendipes	6.2	C, F		Chironomidae	Diptera	Insecta	Arthropoda
Nilothauma	5.5			Chironomidae	Diptera	Insecta	Arthropoda
Omisus				Chironomidae	Diptera	Insecta	Arthropoda
Pagastiella	2.6			Chironomidae	Diptera	Insecta	Arthropoda
Parachironomus	9.2	C, Pr		Chironomidae	Diptera	Insecta	Arthropoda
Paracladopelma	4.8	C		Chironomidae	Diptera	Insecta	Arthropoda
Paralauterborniella	8			Chironomidae	Diptera	Insecta	Arthropoda
Paratanytarsus	7.7	C, F		Chironomidae	Diptera	Insecta	Arthropoda
Paratendipes	5.3	C		Chironomidae	Diptera	Insecta	Arthropoda
Phaenopsectra	6.2	C, Sc		Chironomidae	Diptera	Insecta	Arthropoda
Polypedilum	7.4	C, Sh		Chironomidae	Diptera	Insecta	Arthropoda
Polypedilum convictum grp	5.3	C, Sh		Chironomidae	Diptera	Insecta	Arthropoda
Polypedilum fallax grp	6.7	C, Sh		Chironomidae	Diptera	Insecta	Arthropoda
Polypedilum halterale grp	7.2	C, Sh		Chironomidae	Diptera	Insecta	Arthropoda
Polypedilum illinoense grp	9.2	C, Sh		Chironomidae	Diptera	Insecta	Arthropoda
Polypedilum scaleanum grp	8.7	C, Sh		Chironomidae	Diptera	Insecta	Arthropoda
Pseudochironomus	4.2	Co		Chironomidae	Diptera	Insecta	Arthropoda
Rheotanytarsus	6.4	Fi		Chironomidae	Diptera	Insecta	Arthropoda
Robackia	3.4	C		Chironomidae	Diptera	Insecta	Arthropoda
Saetheria	8.1	C		Chironomidae	Diptera	Insecta	Arthropoda
Stelechomyia	4.6			Chironomidae	Diptera	Insecta	Arthropoda
Stempellina	2	C		Chironomidae	Diptera	Insecta	Arthropoda
Stempellinella	5.3	C		Chironomidae	Diptera	Insecta	Arthropoda
Stenochironomus	6.4	C, Sh		Chironomidae	Diptera	Insecta	Arthropoda
Stictochironomus	6.7	C, Sh		Chironomidae	Diptera	Insecta	Arthropoda
Sublettea	1.7	C, F		Chironomidae	Diptera	Insecta	Arthropoda
Tanytarsus	6.7	C, F		Chironomidae	Diptera	Insecta	Arthropoda
Thienemanniola				Chironomidae	Diptera	Insecta	Arthropoda
Tribelos	6.6	C		Chironomidae	Diptera	Insecta	Arthropoda
Xenochironomus	7	Pr		Chironomidae	Diptera	Insecta	Arthropoda
Zavreliella	7			Chironomidae	Diptera	Insecta	Arthropoda
Diamesa	7.7	C	Diamesinae	Chironomidae	Diptera	Insecta	Arthropoda

Potthastia	4.7	C		Chironomidae	Diptera	Insecta	Arthropoda
Pseudodiamesa	4.6	C		Chironomidae	Diptera	Insecta	Arthropoda
Sympotthastia	5.7	C		Chironomidae	Diptera	Insecta	Arthropoda
Syndiamesa		C		Chironomidae	Diptera	Insecta	Arthropoda
Brillia	5.2	C, Sh	Orthocladiinae	Chironomidae	Diptera	Insecta	Arthropoda
Cardiocladius	6.2	Pr		Chironomidae	Diptera	Insecta	Arthropoda
Corynoneura	6.2	C		Chironomidae	Diptera	Insecta	Arthropoda
Cricotopus bicintus	8.7	C, Sh		Chironomidae	Diptera	Insecta	Arthropoda
Cricotopus/Orthocladius	6.5	C, Sh		Chironomidae	Diptera	Insecta	Arthropoda
Diplocladius	7.7	C		Chironomidae	Diptera	Insecta	Arthropoda
Epoicocladius	4	C, Pr		Chironomidae	Diptera	Insecta	Arthropoda
Eukiefferiella	4	C, Pr		Chironomidae	Diptera	Insecta	Arthropoda
Eukiefferiella brehmi grp	3.7	C, Pr		Chironomidae	Diptera	Insecta	Arthropoda
Eukiefferiella brevicalcar grp	4	C, Pr		Chironomidae	Diptera	Insecta	Arthropoda
Eukiefferiella claripennis grp	5.7	C, Pr		Chironomidae	Diptera	Insecta	Arthropoda
Eukiefferiella coerulesens grp	4	C, Pr		Chironomidae	Diptera	Insecta	Arthropoda
Eukiefferiella cyanea grp		C, Pr		Chironomidae	Diptera	Insecta	Arthropoda
Eukiefferiella devonica grp	2.6	C, Pr		Chironomidae	Diptera	Insecta	Arthropoda
Eukiefferiella gracei grp	2.7	C, Pr		Chironomidae	Diptera	Insecta	Arthropoda
Eukiefferiella pseudomontana grp	8	C, Pr		Chironomidae	Diptera	Insecta	Arthropoda
Eukiefferiella rectangularis grp		C, Pr		Chironomidae	Diptera	Insecta	Arthropoda
Eukiefferiella similes grp		C, Pr		Chironomidae	Diptera	Insecta	Arthropoda
Heterotrissocladius	5.4	C, Pr		Chironomidae	Diptera	Insecta	Arthropoda
Hydrobaenus	9.6	C, Sc		Chironomidae	Diptera	Insecta	Arthropoda
Krenosmittia				Chironomidae	Diptera	Insecta	Arthropoda
Lymnophyes	8	C		Chironomidae	Diptera	Insecta	Arthropoda
Mesocricotopus		C		Chironomidae	Diptera	Insecta	Arthropoda
Mesosmittia	7			Chironomidae	Diptera	Insecta	Arthropoda
Nanocladius	7.2	C		Chironomidae	Diptera	Insecta	Arthropoda
Oliveridia				Chironomidae	Diptera	Insecta	Arthropoda
Orthocladius (*Euorthocladius*)	6.3	C		Chironomidae	Diptera	Insecta	Arthropoda
Orthocladius (*Symposiocladius*)	5.4	C		Chironomidae	Diptera	Insecta	Arthropoda
Parakiefferiella	5.9	C		Chironomidae	Diptera	Insecta	Arthropoda
Parametriocnemus	3.7	C		Chironomidae	Diptera	Insecta	Arthropoda
Paraphaenocladius	4	C		Chironomidae	Diptera	Insecta	Arthropoda
Parorthocladius		C		Chironomidae	Diptera	Insecta	Arthropoda
Psectrocladius	3.8	C		Chironomidae	Diptera	Insecta	Arthropoda
Pseudorthocladius		C, Sh		Chironomidae	Diptera	Insecta	Arthropoda
Pseudosmittia	4			Chironomidae	Diptera	Insecta	Arthropoda
Rheocricotopus	7.3	C, Sh		Chironomidae	Diptera	Insecta	Arthropoda
Smittia	4	C		Chironomidae	Diptera	Insecta	Arthropoda

Synorthocladius	4.7			Chironomidae	Diptera	Insecta	Arthropoda
Thienemanniella	6			Chironomidae	Diptera	Insecta	Arthropoda
Tvetenia	4	C		Chironomidae	Diptera	Insecta	Arthropoda
Tvetenia bavarica grp	3.9	C		Chironomidae	Diptera	Insecta	Arthropoda
Tvetenia discoloripes grp	4	C		Chironomidae	Diptera	Insecta	Arthropoda
Xylotopus	6.6	Sh		Chironomidae	Diptera	Insecta	Arthropoda
Monodiamesa	7	C	Prodiamesinae	Chironomidae	Diptera	Insecta	Arthropoda
Ablabesmyia	6.4	Pr	Tanypodinae	Chironomidae	Diptera	Insecta	Arthropoda
Clinotanypus	9.1	Pr		Chironomidae	Diptera	Insecta	Arthropoda
Coelotanypus	6.2	Pr		Chironomidae	Diptera	Insecta	Arthropoda
Djalmabatista	6.4	Pr		Chironomidae	Diptera	Insecta	Arthropoda
Krenopelopia		Pr		Chironomidae	Diptera	Insecta	Arthropoda
Labrundinia	5.3	Pr		Chironomidae	Diptera	Insecta	Arthropoda
Larsia	83	Pr		Chironomidae	Diptera	Insecta	Arthropoda
Monopelopia		Pr		Chironomidae	Diptera	Insecta	Arthropoda
Natarsia	8	Pr		Chironomidae	Diptera	Insecta	Arthropoda
Nilotanypus	6	Pr		Chironomidae	Diptera	Insecta	Arthropoda
Paramerina	2.8	Pr		Chironomidae	Diptera	Insecta	Arthropoda
Pentaneura	4.6	Pr		Chironomidae	Diptera	Insecta	Arthropoda
Procladius	9.3	Pr		Chironomidae	Diptera	Insecta	Arthropoda
Psectrotanypus	10	Pr		Chironomidae	Diptera	Insecta	Arthropoda
Tanypus	9.6	Pr		Chironomidae	Diptera	Insecta	Arthropoda
Thienemannimyia grp.	6	Pr		Chironomidae	Diptera	Insecta	Arthropoda
Zavrelymia	9.3	Pr		Chironomidae	Diptera	Insecta	Arthropoda
Aedes	5.5	Pr		Culicidae	Diptera	Insecta	Arthropoda
Anopheles	9.1	Fi		Culicidae	Diptera	Insecta	Arthropoda
Culex	10	Fi		Culicidae	Diptera	Insecta	Arthropoda
Culiseta	5.5	C, F		Culicidae	Diptera	Insecta	Arthropoda
Mansonia		C, F		Culicidae	Diptera	Insecta	Arthropoda
Dixa	2.8	C, Pr		Dixidae	Diptera	Insecta	Arthropoda
Dixella		C		Dixidae	Diptera	Insecta	Arthropoda
	9.7	Pr		Dolichopodidae	Diptera	Insecta	Arthropoda
Chelifera	6	Pr		Empididae	Diptera	Insecta	Arthropoda
Clinocera	6	Pr		Empididae	Diptera	Insecta	Arthropoda
Hemerodromia	6	C, Pr		Empididae	Diptera	Insecta	Arthropoda
Rhamphomyia		Pr		Empididae	Diptera	Insecta	Arthropoda
Roederiodes		Pr		Empididae	Diptera	Insecta	Arthropoda
	5.5	C, Sh		Ephydridae	Diptera	Insecta	Arthropoda
	6	Pr		Muscidae	Diptera	Insecta	Arthropoda
Pericoma	4	C		Psychodidae	Diptera	Insecta	Arthropoda
Pericoma fuliginosa		C		Psychodidae	Diptera	Insecta	Arthropoda

Pericoma freyii		C	Psychodidae	Diptera	Insecta	Arthropoda
Pericoma mutua		C	Psychodidae	Diptera	Insecta	Arthropoda
Psychoda	9.9	C	Psychodidae	Diptera	Insecta	Arthropoda
Telmatoscopus		C	Psychodidae	Diptera	Insecta	Arthropoda
		He, Sh	Scathophagidae	Diptera	Insecta	Arthropoda
		Pr	Sciomyzidae	Diptera	Insecta	Arthropoda
Cnephia	4	F	Simuliidae	Diptera	Insecta	Arthropoda
Prosimulium	2.6	F	Simuliidae	Diptera	Insecta	Arthropoda
Simulium	4.4	F	Simuliidae	Diptera	Insecta	Arthropoda
Simulium luggeri		F	Simuliidae	Diptera	Insecta	Arthropoda
Simulium venustum		F	Simuliidae	Diptera	Insecta	Arthropoda
Stegopterna	5	F	Simuliidae	Diptera	Insecta	Arthropoda
Allognosta			Stratiomyidae	Diptera	Insecta	Arthropoda
Caloparyphus		C	Stratiomyidae	Diptera	Insecta	Arthropoda
Euparyphus		C, Sc	Stratiomyidae	Diptera	Insecta	Arthropoda
Myxosargus		C	Stratiomyidae	Diptera	Insecta	Arthropoda
Nemotelus	7.3	C	Stratiomyidae	Diptera	Insecta	Arthropoda
Odontomyia	7.3	C	Stratiomyidae	Diptera	Insecta	Arthropoda
Oxycera		Sc	Stratiomyidae	Diptera	Insecta	Arthropoda
Stratiomys	7.3	C	Stratiomyidae	Diptera	Insecta	Arthropoda
	8.25	C	Syrphidae	Diptera	Insecta	Arthropoda
Chrysops	7.3	Pr	Tabanidae	Diptera	Insecta	Arthropoda
Hybomitra		Pr	Tabanidae	Diptera	Insecta	Arthropoda
Silvius		Pr	Tabanidae	Diptera	Insecta	Arthropoda
Tabanus	9.7	Pr	Tabanidae	Diptera	Insecta	Arthropoda
Protoplasa fitchii			Tanyderidae	Diptera	Insecta	Arthropoda
Antocha	4.6	C	Tipulidae	Diptera	Insecta	Arthropoda
Cryptolabis			Tipulidae	Diptera	Insecta	Arthropoda
Dactylolabis	3.7	C, Sh	Tipulidae	Diptera	Insecta	Arthropoda
Dicranota	0	Pr	Tipulidae	Diptera	Insecta	Arthropoda
Erioptera	5.5	C	Tipulidae	Diptera	Insecta	Arthropoda
Gonomyia	5.5	C	Tipulidae	Diptera	Insecta	Arthropoda
Holorusia		Sh	Tipulidae	Diptera	Insecta	Arthropoda
Hexatoma	4.7	Pr	Tipulidae	Diptera	Insecta	Arthropoda
Limnophila	4.6	Pr	Tipulidae	Diptera	Insecta	Arthropoda
Limonia	10	Sh	Tipulidae	Diptera	Insecta	Arthropoda
Lipsothrix		Sh	Tipulidae	Diptera	Insecta	Arthropoda
Molophilus			Tipulidae	Diptera	Insecta	Arthropoda
Ormosia	4.6	C	Tipulidae	Diptera	Insecta	Arthropoda
Paradelphomyia			Tipulidae	Diptera	Insecta	Arthropoda
Pedicia	4.6	Pr	Tipulidae	Diptera	Insecta	Arthropoda

Pilaria	7		Tipulidae	Diptera	Insecta	Arthropoda
Prionocera		Sh	Tipulidae	Diptera	Insecta	Arthropoda
Pseudolimnophila	7.3	C	Tipulidae	Diptera	Insecta	Arthropoda
Rhabdomastix		C	Tipulidae	Diptera	Insecta	Arthropoda
Tipula	7.7	C, Sh	Tipulidae	Diptera	Insecta	Arthropoda
Thaumalea		Sc	Thaumaleidae	Diptera	Insecta	Arthropoda
Ameletus	7	C, Sc	Ameletidae	Ephemeroptera	Insecta	Arthropoda
Ameletus lineatus	2.1	C, Sc	Ameletidae	Ephemeroptera	Insecta	Arthropoda
Ameletus ludens	0	C, Sc	Ameletidae	Ephemeroptera	Insecta	Arthropoda
	4	C	Baetidae	Ephemeroptera	Insecta	Arthropoda
Acentrella	3.6	C	Baetidae	Ephemeroptera	Insecta	Arthropoda
Acerpenna	3.7	C	Baetidae	Ephemeroptera	Insecta	Arthropoda
Apobaetis	6	C	Baetidae	Ephemeroptera	Insecta	Arthropoda
Baetis	6	C	Baetidae	Ephemeroptera	Insecta	Arthropoda
Barbaetis		C	Baetidae	Ephemeroptera	Insecta	Arthropoda
Callibaetis	9.3	C	Baetidae	Ephemeroptera	Insecta	Arthropoda
Camelobaetidius	9.3	C	Baetidae	Ephemeroptera	Insecta	Arthropoda
Centroptilum	6.3	C	Baetidae	Ephemeroptera	Insecta	Arthropoda
Centroptilum ozarkensum		C	Baetidae	Ephemeroptera	Insecta	Arthropoda
Cloeon	7.4	C	Baetidae	Ephemeroptera	Insecta	Arthropoda
Diphetor	5		Baetidae	Ephemeroptera	Insecta	Arthropoda
Fallceon	6	Pr	Baetidae	Ephemeroptera	Insecta	Arthropoda
Heterocloeon	2	Sc	Baetidae	Ephemeroptera	Insecta	Arthropoda
Labiobaetis	6	Pr	Baetidae	Ephemeroptera	Insecta	Arthropoda
Paracloeodes	5	Sc	Baetidae	Ephemeroptera	Insecta	Arthropoda
Plauditus	6	C	Baetidae	Ephemeroptera	Insecta	Arthropoda
Procloeon	6.3	C	Baetidae	Ephemeroptera	Insecta	Arthropoda
Pseudocentroptiloides		C	Baetidae	Ephemeroptera	Insecta	Arthropoda
Pseudocloeon		C	Baetidae	Ephemeroptera	Insecta	Arthropoda
Baetisca gibbera	1.4	C, Sc	Baetiscidae	Ephemeroptera	Insecta	Arthropoda
Baetisca lacustris	4	C, Sc	Baetiscidae	Ephemeroptera	Insecta	Arthropoda
Baetisca obesa	4	C, Sc	Baetiscidae	Ephemeroptera	Insecta	Arthropoda
Amercaenis		F	Caenidae	Ephemeroptera	Insecta	Arthropoda
Brachycercus	3.5	C	Caenidae	Ephemeroptera	Insecta	Arthropoda
Caenis	7.6	C, Sc	Caenidae	Ephemeroptera	Insecta	Arthropoda
Caenis amica	7.6	C, Sc	Caenidae	Ephemeroptera	Insecta	Arthropoda
Caenis anceps	7.6	C, Sc	Caenidae	Ephemeroptera	Insecta	Arthropoda
Caenis hilaris	7.6	C, Sc	Caenidae	Ephemeroptera	Insecta	Arthropoda
Caenis latipennis	7.6	C, Sc	Caenidae	Ephemeroptera	Insecta	Arthropoda
Caenis macafferti	7.6	C, Sc	Caenidae	Ephemeroptera	Insecta	Arthropoda
Caenis punctata	7.6	C, Sc	Caenidae	Ephemeroptera	Insecta	Arthropoda

Caenis tardata	7.6	C, Sc	Caenidae	Ephemeroptera	Insecta	Arthropoda
Cercobrachys		C	Caenidae	Ephemeroptera	Insecta	Arthropoda
Cercobrachys serpentis		C	Caenidae	Ephemeroptera	Insecta	Arthropoda
Attenella attenuata	1	C	Ephemerellidae	Ephemeroptera	Insecta	Arthropoda
Ephemerella	1.7	C, Sc	Ephemerellidae	Ephemeroptera	Insecta	Arthropoda
Ephemerella argo		C, Sc	Ephemerellidae	Ephemeroptera	Insecta	Arthropoda
Ephemerella aurivillii	0	C, Sc	Ephemerellidae	Ephemeroptera	Insecta	Arthropoda
Ephemerella catawba	4	C, Sc	Ephemerellidae	Ephemeroptera	Insecta	Arthropoda
Ephemerella dorothea	1	C, Sc	Ephemerellidae	Ephemeroptera	Insecta	Arthropoda
Ephemerella excrucians	2	C, Sc	Ephemerellidae	Ephemeroptera	Insecta	Arthropoda
Ephemerella invaria	2.2	C, Sc	Ephemerellidae	Ephemeroptera	Insecta	Arthropoda
Ephemerella needhami	0	C, Sc	Ephemerellidae	Ephemeroptera	Insecta	Arthropoda
Ephemerella subvaria	1	C, Sc	Ephemerellidae	Ephemeroptera	Insecta	Arthropoda
Eurylophella	3	C	Ephemerellidae	Ephemeroptera	Insecta	Arthropoda
Eurylophella aestiva	5	C	Ephemerellidae	Ephemeroptera	Insecta	Arthropoda
Eurylophella bicolor	5.1	C	Ephemerellidae	Ephemeroptera	Insecta	Arthropoda
Eurylophella enoensis	5	C	Ephemerellidae	Ephemeroptera	Insecta	Arthropoda
Eurylophella funeralis	2.3	Sh	Ephemerellidae	Ephemeroptera	Insecta	Arthropoda
Eurylophella macdunnoughi		C	Ephemerellidae	Ephemeroptera	Insecta	Arthropoda
Eurylophella versimilis	0.3	C	Ephemerellidae	Ephemeroptera	Insecta	Arthropoda
Serratella	1.9	C	Ephemerellidae	Ephemeroptera	Insecta	Arthropoda
Serratella deficiens	2.7	C	Ephemerellidae	Ephemeroptera	Insecta	Arthropoda
Serratella frisoni		C	Ephemerellidae	Ephemeroptera	Insecta	Arthropoda
Serratella serratoides	1.5	C	Ephemerellidae	Ephemeroptera	Insecta	Arthropoda
Serratella sordida	2	C	Ephemerellidae	Ephemeroptera	Insecta	Arthropoda
Timpanoga	2	C	Ephemerellidae	Ephemeroptera	Insecta	Arthropoda
Timpanoga lita	0	C	Ephemerellidae	Ephemeroptera	Insecta	Arthropoda
Timpanoga provonshali		C	Ephemerellidae	Ephemeroptera	Insecta	Arthropoda
Timpanoga simplex	3.9	C	Ephemerellidae	Ephemeroptera	Insecta	Arthropoda
Ephemera	2.2	C, Pr	Ephemeridae	Ephemeroptera	Insecta	Arthropoda
Ephemera guttulata	2	C, Pr	Ephemeridae	Ephemeroptera	Insecta	Arthropoda
Ephemera simulans	3.7	C, Pr	Ephemeridae	Ephemeroptera	Insecta	Arthropoda
Ephemera traverae		C, Pr	Ephemeridae	Ephemeroptera	Insecta	Arthropoda
Ephemera varia		C, Pr	Ephemeridae	Ephemeroptera	Insecta	Arthropoda
Hexagenia	4.7	C	Ephemeridae	Ephemeroptera	Insecta	Arthropoda
Hexagenia atrocaudata	3.7	C	Ephemeridae	Ephemeroptera	Insecta	Arthropoda
Hexagenia bilineata	3.7	C	Ephemeridae	Ephemeroptera	Insecta	Arthropoda
Hexagenia limbata	4.6	C	Ephemeridae	Ephemeroptera	Insecta	Arthropoda
Hexagenia rigida	5.5	C	Ephemeridae	Ephemeroptera	Insecta	Arthropoda
Anepeorus		Pr	Heptageniidae	Ephemeroptera	Insecta	Arthropoda
Epeorus	1.2	C	Heptageniidae	Ephemeroptera	Insecta	Arthropoda

Heptagenia	2.8	C, Sc	Heptageniidae	Ephemeroptera	Insecta	Arthropoda
Leucrocuta	0	C, Sc	Heptageniidae	Ephemeroptera	Insecta	Arthropoda
Macdunnoa	4.6	C, Sc	Heptageniidae	Ephemeroptera	Insecta	Arthropoda
Nixe	2	C, Sc	Heptageniidae	Ephemeroptera	Insecta	Arthropoda
Rhithrogena	0.4	C	Heptageniidae	Ephemeroptera	Insecta	Arthropoda
Stenacron	7.1	C	Heptageniidae	Ephemeroptera	Insecta	Arthropoda
Stenonema	3.4	C, Sc	Heptageniidae	Ephemeroptera	Insecta	Arthropoda
Stenonema bednariki	3.4	C, Sc	Heptageniidae	Ephemeroptera	Insecta	Arthropoda
Stenonema exiguum	5	C, Sc	Heptageniidae	Ephemeroptera	Insecta	Arthropoda
Stenonema femoratum	7.5	C, Sc	Heptageniidae	Ephemeroptera	Insecta	Arthropoda
Stenonema integrum	5.5	C, Sc	Heptageniidae	Ephemeroptera	Insecta	Arthropoda
Stenonema luteum		C, Sc	Heptageniidae	Ephemeroptera	Insecta	Arthropoda
Stenonema mediopunctatum	1.7	C, Sc	Heptageniidae	Ephemeroptera	Insecta	Arthropoda
Stenonema modestum	5.8	C, Sc	Heptageniidae	Ephemeroptera	Insecta	Arthropoda
Stenonema pulchellum	3	C, Sc	Heptageniidae	Ephemeroptera	Insecta	Arthropoda
Stenonema terminatum	4.5	C, Sc	Heptageniidae	Ephemeroptera	Insecta	Arthropoda
Stenonema vicarium	1	C, Sc	Heptageniidae	Ephemeroptera	Insecta	Arthropoda
Isonychia	3.8	F	Isonychidae	Ephemeroptera	Insecta	Arthropoda
Isonychia bicolor	2	F	Isonychidae	Ephemeroptera	Insecta	Arthropoda
Isonychia rufa	3.7	F	Isonychidae	Ephemeroptera	Insecta	Arthropoda
Isonychia sayi		F	Isonychidae	Ephemeroptera	Insecta	Arthropoda
Isonychia sicca	3.7	F	Isonychidae	Ephemeroptera	Insecta	Arthropoda
Choroterpes	2	C, Sc	Leptophlebiidae	Ephemeroptera	Insecta	Arthropoda
Habrophlebiodes	6	C, Sc	Leptophlebiidae	Ephemeroptera	Insecta	Arthropoda
Leptophlebia	6.4	C	Leptophlebiidae	Ephemeroptera	Insecta	Arthropoda
Neochoroterpes		C, Sc	Leptophlebiidae	Ephemeroptera	Insecta	Arthropoda
Paraleptophlebia	1.2	C, Sh	Leptophlebiidae	Ephemeroptera	Insecta	Arthropoda
Neoephemera		C	Neoephemeridae	Ephemeroptera	Insecta	Arthropoda
Pentagenia vittigera	6.4	C	Palingeniidae	Ephemeroptera	Insecta	Arthropoda
Ephoron	4.6	C	Polymitarcyidae	Ephemeroptera	Insecta	Arthropoda
Ephoron album	4.6	C	Polymitarcyidae	Ephemeroptera	Insecta	Arthropoda
Ephoron leukon	1.5	C	Polymitarcyidae	Ephemeroptera	Insecta	Arthropoda
Tortopus primus	5.5	C	Polymitarcyidae	Ephemeroptera	Insecta	Arthropoda
Anthopotamus	1.6	F	Potamanthidae	Ephemeroptera	Insecta	Arthropoda
Pseudiron centralis	5	Pr	Pseudironidae	Ephemeroptera	Insecta	Arthropoda
Siphlonurus	2.6	C, Sc	Siphlonuridae	Ephemeroptera	Insecta	Arthropoda
Tricorythodes	5.4	C	Tricorythidae	Ephemeroptera	Insecta	Arthropoda
Belostoma	9.8	Pr	Belostomatidae	Hemiptera	Insecta	Arthropoda
Lethocerus	4.6	Pr	Belostomatidae	Hemiptera	Insecta	Arthropoda
Corisella	6.4	Pr	Corixidae	Hemiptera	Insecta	Arthropoda
Hesperocorixa	5	Mp	Corixidae	Hemiptera	Insecta	Arthropoda

Palmacorixa	5.5		Corixidae	Hemiptera	Insecta	Arthropoda
Sigara	4.6	Mp, C	Corixidae	Hemiptera	Insecta	Arthropoda
Trichocorixa	5.5	C, Pr	Corixidae	Hemiptera	Insecta	Arthropoda
Gelastocoris	7.3	Pr	Gelastocoridae	Hemiptera	Insecta	Arthropoda
Aquarius	6.4	Pr	Gerridae	Hemiptera	Insecta	Arthropoda
Gerris	6.4	Pr	Gerridae	Hemiptera	Insecta	Arthropoda
Metrobates	6.4	Pr	Gerridae	Hemiptera	Insecta	Arthropoda
Rheumatobates	6.4	Pr	Gerridae	Hemiptera	Insecta	Arthropoda
Trepobates	6.4	Pr	Gerridae	Hemiptera	Insecta	Arthropoda
Hebrus	6.4	Pr	Hebridae	Hemiptera	Insecta	Arthropoda
Merragata	7.3	Pr	Hebridae	Hemiptera	Insecta	Arthropoda
Hydrometra	7.3	Pr	Hydrometridae	Hemiptera	Insecta	Arthropoda
Mesovelia	6.4	Pr	Mesoveliidae	Hemiptera	Insecta	Arthropoda
Pelocoris		Pr	Naucoridae	Hemiptera	Insecta	Arthropoda
Nepa	4.6	Pr	Nepidae	Hemiptera	Insecta	Arthropoda
Ranatra	7.5	Pr	Nepidae	Hemiptera	Insecta	Arthropoda
Ranatra fusca	7.3	Pr	Nepidae	Hemiptera	Insecta	Arthropoda
Ranatra kirkaldyi	5.5	Pr	Nepidae	Hemiptera	Insecta	Arthropoda
Ranatra nigra	6.4	Pr	Nepidae	Hemiptera	Insecta	Arthropoda
Buenoa	5.5	Pr	Notonectidae	Hemiptera	Insecta	Arthropoda
Notonecta	5.5	Pr	Notonectidae	Hemiptera	Insecta	Arthropoda
Neoplea	5.5	Pr	Pleidae	Hemiptera	Insecta	Arthropoda
Micracanthia	5.5	Pr	Saldidae	Hemiptera	Insecta	Arthropoda
Pentacora	6.4	Pr	Saldidae	Hemiptera	Insecta	Arthropoda
Saldula	6.4	Pr	Saldidae	Hemiptera	Insecta	Arthropoda
Microvelia	6.4	Pr	Veliidae	Hemiptera	Insecta	Arthropoda
Platyvelia		Pr	Veliidae	Hemiptera	Insecta	Arthropoda
Rhagovelia	7.3	Pr	Veliidae	Hemiptera	Insecta	Arthropoda
Steinovelia		Pr	Veliidae	Hemiptera	Insecta	Arthropoda
		He	Cossidae	Lepidoptera	Insecta	Arthropoda
Nepticula		He	Nepticulidae	Lepidoptera	Insecta	Arthropoda
Bellura		He	Noctuidae	Lepidoptera	Insecta	Arthropoda
Simyra		He	Noctuidae	Lepidoptera	Insecta	Arthropoda
Nymphula	7	He	Pyralidae	Lepidoptera	Insecta	Arthropoda
Parapoynx	5	He	Pyralidae	Lepidoptera	Insecta	Arthropoda
Petrophila	1.8	He, Sc	Pyralidae	Lepidoptera	Insecta	Arthropoda
Schoenobius		He	Pyralidae	Lepidoptera	Insecta	Arthropoda
Archips		Sh	Toticidae	Lepidoptera	Insecta	Arthropoda
Chauliodes	4	Pr	Corydalidae	Megaloptera	Insecta	Arthropoda
Chauliodes pectinicornis	4	Pr	Corydalidae	Megaloptera	Insecta	Arthropoda
Chauliodes rastricornis	4	Pr	Corydalidae	Megaloptera	Insecta	Arthropoda

Corydalus	5.6	Pr		Corydalidae	Megaloptera	Insecta	Arthropoda
Corydalus cornutus	5.6	Pr		Corydalidae	Megaloptera	Insecta	Arthropoda
Nigronia fasciatus	6.2	Pr		Corydalidae	Megaloptera	Insecta	Arthropoda
Nigronia serricornis	5.5	Pr		Corydalidae	Megaloptera	Insecta	Arthropoda
Sialis	7.5	Pr		Sialidae	Megaloptera	Insecta	Arthropoda
Sialis velata		Pr		Sialidae	Megaloptera	Insecta	Arthropoda
Climacia	6.5	Pr		Sisyridae	Neuroptera	Insecta	Arthropoda
Sisyra		Pr		Sisyridae	Neuroptera	Insecta	Arthropoda
Sisyra vicaria		Pr		Sisyridae	Neuroptera	Insecta	Arthropoda
Aeshna	6.4	Pr		Aeshnidae	Odonata	Insecta	Arthropoda
Anax	6.4	Pr		Aeshnidae	Odonata	Insecta	Arthropoda
Basiaeschna janata	7.7	Pr		Aeshnidae	Odonata	Insecta	Arthropoda
Boyeria	6.3	Pr		Aeshnidae	Odonata	Insecta	Arthropoda
Epiaeschna heros	3.7	Pr		Aeshnidae	Odonata	Insecta	Arthropoda
Nasiaeschna pentacantha	8	Pr		Aeshnidae	Odonata	Insecta	Arthropoda
Calopteryx	8.3	Pr		Calopterygidae	Odonata	Insecta	Arthropoda
Hetaerina	6.2	Pr		Calopterygidae	Odonata	Insecta	Arthropoda
Amphiagrion	2.8	Pr		Coenagrionidae	Odonata	Insecta	Arthropoda
Argia	8.7	Pr		Coenagrionidae	Odonata	Insecta	Arthropoda
Chromagrion		Pr		Coenagrionidae	Odonata	Insecta	Arthropoda
Enallagma	9	Pr		Coenagrionidae	Odonata	Insecta	Arthropoda
Ischnura	9.4	Pr		Coenagrionidae	Odonata	Insecta	Arthropoda
Nehalennia		Pr		Coenagrionidae	Odonata	Insecta	Arthropoda
Telebasis		Pr		Coenagrionidae	Odonata	Insecta	Arthropoda
Cordulegaster	6.1	Pr		Cordulegastridae	Odonata	Insecta	Arthropoda
	7	Pr		Gomphidae	Odonata	Insecta	Arthropoda
Arigomphus	6.4	Pr		Gomphidae	Odonata	Insecta	Arthropoda
Dromogomphus	6.3	Pr		Gomphidae	Odonata	Insecta	Arthropoda
Erpetogomphus	5.5	Pr		Gomphidae	Odonata	Insecta	Arthropoda
Gomphus	6.2	Pr		Gomphidae	Odonata	Insecta	Arthropoda
Hagenius brevistylus	4	Pr		Gomphidae	Odonata	Insecta	Arthropoda
Lanthus parvulus	2.7	Pr		Gomphidae	Odonata	Insecta	Arthropoda
Lanthus vernalis	2.7	Pr		Gomphidae	Odonata	Insecta	Arthropoda
Ophiogomphus	6.2	Pr		Gomphidae	Odonata	Insecta	Arthropoda
Progomphus obscurus	8.7	Pr		Gomphidae	Odonata	Insecta	Arthropoda
Stylogomphus albistylus	4.8	Pr		Gomphidae	Odonata	Insecta	Arthropoda
Stylurus	4	Pr		Gomphidae	Odonata	Insecta	Arthropoda
Archilestes	6.4	Pr		Lestidae	Odonata	Insecta	Arthropoda
Lestes	6.4	Pr		Lestidae	Odonata	Insecta	Arthropoda
Epitheca	5.5	Pr	Corduliinae	Libellulidae	Odonata	Insecta	Arthropoda
Epitheca	8.5	Pr	Corduliinae	Libellulidae	Odonata	Insecta	Arthropoda

Helocordulia		Pr	Corduliinae	Libellulidae	Odonata	Insecta	Arthropoda
Neurocordulia	4	Pr	Corduliinae	Libellulidae	Odonata	Insecta	Arthropoda
Somatochlora	8.9	Pr	Corduliinae	Libellulidae	Odonata	Insecta	Arthropoda
Brachymesia		Pr	Libellulinae	Libellulidae	Odonata	Insecta	Arthropoda
Celithemis	3.7	Pr	Libellulinae	Libellulidae	Odonata	Insecta	Arthropoda
Dythemis	3.7	Pr	Libellulinae	Libellulidae	Odonata	Insecta	Arthropoda
Erythemis	7.7	Pr	Libellulinae	Libellulidae	Odonata	Insecta	Arthropoda
Erythrodiplax		Pr	Libellulinae	Libellulidae	Odonata	Insecta	Arthropoda
Leucorrhinia	6.4	Pr	Libellulinae	Libellulidae	Odonata	Insecta	Arthropoda
Libellula	9.8	Pr	Libellulinae	Libellulidae	Odonata	Insecta	Arthropoda
Orthemis ferruginea	4.6	Pr	Libellulinae	Libellulidae	Odonata	Insecta	Arthropoda
Pachydiplax longipennis	9.6	Pr	Libellulinae	Libellulidae	Odonata	Insecta	Arthropoda
Pantala	6.4	Pr	Libellulinae	Libellulidae	Odonata	Insecta	Arthropoda
Perithemis	10	Pr	Libellulinae	Libellulidae	Odonata	Insecta	Arthropoda
Plathemis	10	Pr	Libellulinae	Libellulidae	Odonata	Insecta	Arthropoda
Sympetrum	7.3	Pr	Libellulinae	Libellulidae	Odonata	Insecta	Arthropoda
Tramea		Pr	Libellulinae	Libellulidae	Odonata	Insecta	Arthropoda
Didymops	5.5	Pr	Macromiinae	Libellulidae	Odonata	Insecta	Arthropoda
Macromia	6.7	Pr	Macromiinae	Libellulidae	Odonata	Insecta	Arthropoda
Tachopteryx thoreyi	3.7	Pr		Petaluridae	Odonata	Insecta	Arthropoda
Allocapnia	2.8	Sh		Capniidae	Plecoptera	Insecta	Arthropoda
Allocania forbesi		Sh		Capniidae	Plecoptera	Insecta	Arthropoda
Allocapnia granulate		Sh		Capniidae	Plecoptera	Insecta	Arthropoda
Allocapnia jeanae		Sh		Capniidae	Plecoptera	Insecta	Arthropoda
Allocapnia malverna		Sh		Capniidae	Plecoptera	Insecta	Arthropoda
Allocapnia mohri		Sh		Capniidae	Plecoptera	Insecta	Arthropoda
Allocapnia mystica		Sh		Capniidae	Plecoptera	Insecta	Arthropoda
Allocapnia oribata		Sh		Capniidae	Plecoptera	Insecta	Arthropoda
Allocapnia ozarkana		Sh		Capniidae	Plecoptera	Insecta	Arthropoda
Allocapnia peltoides		Sh		Capniidae	Plecoptera	Insecta	Arthropoda
Allocania pygmaea		Sh		Capniidae	Plecoptera	Insecta	Arthropoda
Allocapnia rickeri		Sh		Capniidae	Plecoptera	Insecta	Arthropoda
Allocapnia sandersoni		Sh		Capniidae	Plecoptera	Insecta	Arthropoda
Allocapnia smithi		Sh		Capniidae	Plecoptera	Insecta	Arthropoda
Allocapnia vivipara		Sh		Capniidae	Plecoptera	Insecta	Arthropoda
Allocapnia warreni		Sh		Capniidae	Plecoptera	Insecta	Arthropoda
Nemocapnia carolina		Sh		Capniidae	Plecoptera	Insecta	Arthropoda
Paracapnia angulata	0.2	Sh		Capniidae	Plecoptera	Insecta	Arthropoda
Alloperla	1.4	Pr		Chloroperlidae	Plecoptera	Insecta	Arthropoda
Alloperla caddo		Pr		Chloroperlidae	Plecoptera	Insecta	Arthropoda
Alloperla caudate		Pr		Chloroperlidae	Plecoptera	Insecta	Arthropoda

Alloperla hamata		Pr	Chloroperlidae	Plecoptera	Insecta	Arthropoda
Alloperla leonarda		Pr	Chloroperlidae	Plecoptera	Insecta	Arthropoda
Alloperla Ouachita		Pr	Chloroperlidae	Plecoptera	Insecta	Arthropoda
Haploperla	1.4	C, Pr	Chloroperlidae	Plecoptera	Insecta	Arthropoda
Haploperla brevis	1.3	C, Pr	Chloroperlidae	Plecoptera	Insecta	Arthropoda
Leuctra	0.7	Sh	Leuctridae	Plecoptera	Insecta	Arthropoda
Leuctra paleo		Sh	Leuctridae	Plecoptera	Insecta	Arthropoda
Leuctra rickeri		Sh	Leuctridae	Plecoptera	Insecta	Arthropoda
Leuctra tenuis		Sh	Leuctridae	Plecoptera	Insecta	Arthropoda
Zealeuctra	0	Sh	Leuctridae	Plecoptera	Insecta	Arthropoda
Zealeuctra Cherokee		Sh	Leuctridae	Plecoptera	Insecta	Arthropoda
Zealuctra claasseni		Sh	Leuctridae	Plecoptera	Insecta	Arthropoda
Zealuctra fraxina		Sh	Leuctridae	Plecoptera	Insecta	Arthropoda
Zealuctra narfi		Sh	Leuctridae	Plecoptera	Insecta	Arthropoda
Zealuctra warreni		Sh	Leuctridae	Plecoptera	Insecta	Arthropoda
Zealuctra wachita		Sh	Leuctridae	Plecoptera	Insecta	Arthropoda
Amphinemura	3.4	Sh	Nemouridae	Plecoptera	Insecta	Arthropoda
Amphinemura delosa		Sh	Nemouridae	Plecoptera	Insecta	Arthropoda
Amphinemura nigritta		Sh	Nemouridae	Plecoptera	Insecta	Arthropoda
Prostoia	6.1	Sc, Sh	Nemouridae	Plecoptera	Insecta	Arthropoda
Prostoia completa	6.1	Sc, Sh	Nemouridae	Plecoptera	Insecta	Arthropoda
Prostoia similes	6.1	Sc, Sh	Nemouridae	Plecoptera	Insecta	Arthropoda
Shipsa rotunda	0.3	Sc, Sh	Nemouridae	Plecoptera	Insecta	Arthropoda
Acroneuria	1.4	Pr	Perlidae	Plecoptera	Insecta	Arthropoda
Acroneuria abnormis		Pr	Perlidae	Plecoptera	Insecta	Arthropoda
Acroneuria evoluta		Pr	Perlidae	Plecoptera	Insecta	Arthropoda
Acroneuria filicis		Pr	Perlidae	Plecoptera	Insecta	Arthropoda
Acroneuria internata		Pr	Perlidae	Plecoptera	Insecta	Arthropoda
Acroneuria mela		Pr	Perlidae	Plecoptera	Insecta	Arthropoda
Acroneuria ozarkensis		Pr	Perlidae	Plecoptera	Insecta	Arthropoda
Acroneuria perplexa		Pr	Perlidae	Plecoptera	Insecta	Arthropoda
Agnetina capitata	1.4	Pr	Perlidae	Plecoptera	Insecta	Arthropoda
Agnetina flavescens	0	Pr	Perlidae	Plecoptera	Insecta	Arthropoda
Attaneuria ruralis	2.75	Pr	Perlidae	Plecoptera	Insecta	Arthropoda
Neoperla	1.6	Pr	Perlidae	Plecoptera	Insecta	Arthropoda
Neoperla carlsoni		Pr	Perlidae	Plecoptera	Insecta	Arthropoda
Neoperla catharae		Pr	Perlidae	Plecoptera	Insecta	Arthropoda
Neoperla choctaw		Pr	Perlidae	Plecoptera	Insecta	Arthropoda
Neoperla falayah		Pr	Perlidae	Plecoptera	Insecta	Arthropoda
Neoperla harpi		Pr	Perlidae	Plecoptera	Insecta	Arthropoda
Neoperla osage		Pr	Perlidae	Plecoptera	Insecta	Arthropoda

Paragnetina	1.8	Pr	Perlidae	Plecoptera	Insecta	Arthropoda
Paragnetina kansensis	2	Pr	Perlidae	Plecoptera	Insecta	Arthropoda
Paragnetina media	1	Pr	Perlidae	Plecoptera	Insecta	Arthropoda
Perlesta	0	C, Pr	Perlidae	Plecoptera	Insecta	Arthropoda
Perlesta baumanni		C, Pr	Perlidae	Plecoptera	Insecta	Arthropoda
Perlesta browni		C, Pr	Perlidae	Plecoptera	Insecta	Arthropoda
Perlesta cinctipes		C, Pr				
Perlesta decipiens		C, Pr	Perlidae	Plecoptera	Insecta	Arthropoda
Perlesta fusca		C, Pr	Perlidae	Plecoptera	Insecta	Arthropoda
Perlesta shubata		C, Pr	Perlidae	Plecoptera	Insecta	Arthropoda
Perlinella drymo	0	Pr	Perlidae	Plecoptera	Insecta	Arthropoda
Perlinella ephyre	0	Pr	Perlidae	Plecoptera	Insecta	Arthropoda
Clioperla clio	4.8	Pr	Perlodidae	Plecoptera	Insecta	Arthropoda
Helopicus nalatus	5.75	Pr	Perlodidae	Plecoptera	Insecta	Arthropoda
Hydroperla	2.75	Pr	Perlodidae	Plecoptera	Insecta	Arthropoda
Hydroperla crosbyi	3.7	Pr	Perlodidae	Plecoptera	Insecta	Arthropoda
Hydroperla fugitans	2.75	Pr	Perlodidae	Plecoptera	Insecta	Arthropoda
Isoperla	2	C, Pr	Perlodidae	Plecoptera	Insecta	Arthropoda
Isoperla bilineata	5.5	C, Pr	Perlodidae	Plecoptera	Insecta	Arthropoda
Isoperla buski		C, Pr	Perlodidae	Plecoptera	Insecta	Arthropoda
Isoperla coushatta		C, Pr	Perlodidae	Plecoptera	Insecta	Arthropoda
Isoperla decepta		C, Pr	Perlodidae	Plecoptera	Insecta	Arthropoda
Isoperla dicala	2.2	C, Pr				
Isoperla mohri		C, Pr	Perlodidae	Plecoptera	Insecta	Arthropoda
Isoperla namata	1.8	C, Pr	Perlodidae	Plecoptera	Insecta	Arthropoda
Isoperla mohri		C, Pr	Perlodidae	Plecoptera	Insecta	Arthropoda
Isoperla signata		C, Pr	Perlodidae	Plecoptera	Insecta	Arthropoda
Isoperla szczytkoi		C, Pr	Perlodidae	Plecoptera	Insecta	Arthropoda
Pteronarcys pictetii	1.7	Sh	Pteronarcyidae	Plecoptera	Insecta	Arthropoda
Strophopteryx	2.5	Sh	Taeniopterygidae	Plecoptera	Insecta	Arthropoda
Strophopteryx arkansae		Sh	Taeniopterygidae	Plecoptera	Insecta	Arthropoda
Strophopteryx cucullata		Sh	Taeniopterygidae	Plecoptera	Insecta	Arthropoda
Strophopteryx fasciata	3	Sh	Taeniopterygidae	Plecoptera	Insecta	Arthropoda
Taeniopteryx	6.3	C, Sh	Taeniopterygidae	Plecoptera	Insecta	Arthropoda
Taeniopteryx burksi	5.8	C, Sh	Taeniopterygidae	Plecoptera	Insecta	Arthropoda
Taeniopteryx lita		C, Sh	Taeniopterygidae	Plecoptera	Insecta	Arthropoda
Taeniopteryx lonicera	2	C, Sh	Taeniopterygidae	Plecoptera	Insecta	Arthropoda
Taeniopteryx maura		C, Sh	Taeniopterygidae	Plecoptera	Insecta	Arthropoda
Taeniopteryx metequi	1.4	C, Sh	Taeniopterygidae	Plecoptera	Insecta	Arthropoda
Taeniopteryx parvula	2	C, SH	Taeniopterygidae	Plecoptera	Insecta	Arthropoda
Brachycentrus	2.2	F	Brachycentridae	Trichoptera	Insecta	Arthropoda

Brachycentrus americanus	1	F	Brachycentridae	Trichoptera	Insecta	Arthropoda
Brachycentrus lateralis	0.4	F	Brachycentridae	Trichoptera	Insecta	Arthropoda
Brachycentrus numerosus	1.8	F	Brachycentridae	Trichoptera	Insecta	Arthropoda
Brachycentrus occidentalis	1	F	Brachycentridae	Trichoptera	Insecta	Arthropoda
Micrasema	0.6	Sh	Brachycentridae	Trichoptera	Insecta	Arthropoda
Agapetus	0	C, Sc	Glossosomatidae	Trichoptera	Insecta	Arthropoda
Agapetus illini	0	C, Sc	Glossosomatidae	Trichoptera	Insecta	Arthropoda
Glossosoma	1.5	C, Sc	Glossosomatidae	Trichoptera	Insecta	Arthropoda
Protoptila	2.8	Sc	Glossosomatidae	Trichoptera	Insecta	Arthropoda
Helicopsyche	0	Sc	Helicopsychidae	Trichoptera	Insecta	Arthropoda
Ceratopsyche	1.4	F	Hydropsychidae	Trichoptera	Insecta	Arthropoda
Ceratopsyche morosa grp	2.95	F	Hydropsychidae	Trichoptera	Insecta	Arthropoda
Ceratopsyche piatrix		F	Hydropsychidae	Trichoptera	Insecta	Arthropoda
Ceratopsyche slossonae	0	F	Hydropsychidae	Trichoptera	Insecta	Arthropoda
Cheumatopsyche	6.6	F	Hydropsychidae	Trichoptera	Insecta	Arthropoda
Diplectrona	2.2	F	Hydropsychidae	Trichoptera	Insecta	Arthropoda
Hydropsyche	4	F	Hydropsychidae	Trichoptera	Insecta	Arthropoda
Macrostemum	3.6	F	Hydropsychidae	Trichoptera	Insecta	Arthropoda
Potamyia flava	5	F	Hydropsychidae	Trichoptera	Insecta	Arthropoda
Agraylea	8	He, Sc	Hydroptilidae	Trichoptera	Insecta	Arthropoda
Dibusa angata		Sc	Hydroptilidae	Trichoptera	Insecta	Arthropoda
Hydroptila	6.2	He, Sc	Hydroptilidae	Trichoptera	Insecta	Arthropoda
Ithytrichia	4	Sc	Hydroptilidae	Trichoptera	Insecta	Arthropoda
Leucotrichia	4.3	C, Sc	Hydroptilidae	Trichoptera	Insecta	Arthropoda
Neotrichia	2	Sc	Hydroptilidae	Trichoptera	Insecta	Arthropoda
Ochrotrichia	6.4	C, He	Hydroptilidae	Trichoptera	Insecta	Arthropoda
Orthotrichia	7.2	C, He	Hydroptilidae	Trichoptera	Insecta	Arthropoda
Oxyethira	3	C, He	Hydroptilidae	Trichoptera	Insecta	Arthropoda
Stactobiella	2.75	Sh	Hydroptilidae	Trichoptera	Insecta	Arthropoda
Lepidostoma	1	Sh	Lepidostomatidae	Trichoptera	Insecta	Arthropoda
Ceraclea	2.3	C, Sh	Leptoceridae	Trichoptera	Insecta	Arthropoda
Leptocerus americanus	4.6	Sh	Leptoceridae	Trichoptera	Insecta	Arthropoda
Mystacides	3.5	C, Sh	Leptoceridae	Trichoptera	Insecta	Arthropoda
Nectopsyche	4.1	C, Sh	Leptoceridae	Trichoptera	Insecta	Arthropoda
Nectopsyche albida	5.5	C, Sh	Leptoceridae	Trichoptera	Insecta	Arthropoda
Nectopsyche candida	3.8	C, Sh	Leptoceridae	Trichoptera	Insecta	Arthropoda
Nectopsyche diarina	5.5	C, Sh	Leptoceridae	Trichoptera	Insecta	Arthropoda
Nectopsyche exquisita	4.2	C, Sh	Leptoceridae	Trichoptera	Insecta	Arthropoda
Nectopsyche pavida	4.2	C, Sh	Leptoceridae	Trichoptera	Insecta	Arthropoda
Nectopsyche spiloma	4.6	C, Sh	Leptoceridae	Trichoptera	Insecta	Arthropoda
Oecetis	5.7	He, Pr	Leptoceridae	Trichoptera	Insecta	Arthropoda

Setodes	0.9	C, Pr	Leptoceridae	Trichoptera	Insecta	Arthropoda
Triaenodes	3.7	Sh	Leptoceridae	Trichoptera	Insecta	Arthropoda
Frenesia	0	Sh	Limnephilidae	Trichoptera	Insecta	Arthropoda
Hesperophylax	3	He, Sh	Limnephilidae	Trichoptera	Insecta	Arthropoda
Hydatophylax	2.3	C, Sh	Limnephilidae	Trichoptera	Insecta	Arthropoda
Ironoquia	7.3	C, Sh	Limnephilidae	Trichoptera	Insecta	Arthropoda
Leptophylax		He, Sh	Limnephilidae	Trichoptera	Insecta	Arthropoda
Limnephilus	2.75	He, Sh	Limnephilidae	Trichoptera	Insecta	Arthropoda
Platycentropus	4	Sh	Limnephilidae	Trichoptera	Insecta	Arthropoda
Pseudostenophylax	0	C, Sh	Limnephilidae	Trichoptera	Insecta	Arthropoda
Pycnopsyche	2.3	C, Sh	Limnephilidae	Trichoptera	Insecta	Arthropoda
Molanna	3.9	C, Sh	Molannidae	Trichoptera	Insecta	Arthropoda
Marilia		Sh	Odontoceridae	Trichoptera	Insecta	Arthropoda
Psilotreta	0	C, Sc	Odontoceridae	Trichoptera	Insecta	Arthropoda
Chimarra	2.8	F	Philopotamidae	Trichoptera	Insecta	Arthropoda
Dolophilodes	1	F	Philopotamidae	Trichoptera	Insecta	Arthropoda
Wormaldia	0.4	F	Philopotamidae	Trichoptera	Insecta	Arthropoda
Agrypnia	4.6	Sh	Phryganeidae	Trichoptera	Insecta	Arthropoda
Banksiola		Sh	Phryganeidae	Trichoptera	Insecta	Arthropoda
Phryganea	4.6	Sh, Pr	Phryganeidae	Trichoptera	Insecta	Arthropoda
Ptilostomis	6.7	Sh, Pr	Phryganeidae	Trichoptera	Insecta	Arthropoda
Cernotina	4.6	Pr	Polycentropodidae	Trichoptera	Insecta	Arthropoda
Cyrnellus fraternus	7.4	F	Polycentropodidae	Trichoptera	Insecta	Arthropoda
Neureclipsis	4.4	F, Sh	Polycentropodidae	Trichoptera	Insecta	Arthropoda
Paranyctiophylax	0.9	C, Pr	Polycentropodidae	Trichoptera	Insecta	Arthropoda
Phylocentropus	5.6	F	Polycentropodidae	Trichoptera	Insecta	Arthropoda
Polycentropus	3.5	C, Pr	Polycentropodidae	Trichoptera	Insecta	Arthropoda
Lype diversa	4.3	Sc	Psychomyiidae	Trichoptera	Insecta	Arthropoda
Paduniella neartica	0	C, Sc	Psychomyiidae	Trichoptera	Insecta	Arthropoda
Psychomyia flavida	3.3	C, Sc	Psychomyiidae	Trichoptera	Insecta	Arthropoda
Rhyacophila	0.8	Pr	Rhyacophilidae	Trichoptera	Insecta	Arthropoda
Neophylax	1.6	Sc	Uenoidae	Trichoptera	Insecta	Arthropoda
Neophylax concinnus	1.6	Sc	Uenoidae	Trichoptera	Insecta	Arthropoda
	0	F	Unionidae	Unionida	Bivalvia	Mollusca
Dreissena polymorpha	8	F	Dreissenidae	Cardiida	Bivalvia	Mollusca
Corbicula	6.3	F	Corbiculidae	Veneroidea	Bivalvia	Mollusca
Pisidium	6.8	F	Sphaeriidae	Veneroidea	Bivalvia	Mollusca
Sphaerium/Musculium	7.7	F	Sphaeriidae	Veneroidea	Bivalvia	Mollusca
Ferrissia	6.9	Sc	Ancylidae	Limnophila	Gastropoda	Mollusca
Ferrissia rivularis		Sc	Ancylidae	Limnophila	Gastropoda	Mollusca
Laevapex	7.3	Sc	Ancylidae	Limnophila	Gastropoda	Mollusca

Laevapex fuscus		Sc	Ancylidae	Limnophila	Gastropoda	Mollusca
Rhodaemea		Sc	Ancylidae	Limnophila	Gastropoda	Mollusca
Lymnaea	6	Sc	Lymnaeidae	Limnophila	Gastropoda	Mollusca
Lymnaea (*Fossaria*)		Sc	Lymnaeidae	Limnophila	Gastropoda	Mollusca
Lymnaea (*Stagnicola*)	8	Sc	Lymnaeidae	Limnophila	Gastropoda	Mollusca
Lymnaea humilis		Sc	Lymnaeidae	Limnophila	Gastropoda	Mollusca
Lymnaea modicella		Sc	Lymnaeidae	Limnophila	Gastropoda	Mollusca
Lymnaea obrussa		Sc	Lymnaeidae	Limnophila	Gastropoda	Mollusca
Pseudosuccinea	7.2	Sc	Lymnaeidae	Limnophila	Gastropoda	Mollusca
Pseudosuccinea columella		Sc	Lymnaeidae	Limnophila	Gastropoda	Mollusca
Physa	9.1	Sc	Physidae	Limnophila	Gastropoda	Mollusca
Physa anatine		Sc	Physidae	Limnophila	Gastropoda	Mollusca
Physa goodrichi		Sc	Physidae	Limnophila	Gastropoda	Mollusca
Physa gyrina		Sc	Physidae	Limnophila	Gastropoda	Mollusca
Physa heterostropha		Sc	Physidae	Limnophila	Gastropoda	Mollusca
Physa pomilia		Sc	Physidae	Limnophila	Gastropoda	Mollusca
Carychium exile		Sc	Planorbidae	Limnophila	Gastropoda	Mollusca
Gyraulus	8	Sc	Planorbidae	Limnophila	Gastropoda	Mollusca
Helisoma	6.5	Sc	Planorbidae	Limnophila	Gastropoda	Mollusca
Helisoma subcrenatum		Sc	Planorbidae	Limnophila	Gastropoda	Mollusca
Helisoma trivolvis		Sc	Planorbidae	Limnophila	Gastropoda	Mollusca
Menetus	8.4	Sc	Planorbidae	Limnophila	Gastropoda	Mollusca
Menetus sampsoni		Sc	Planorbidae	Limnophila	Gastropoda	Mollusca
Planorbella		Sc	Planorbidae	Limnophila	Gastropoda	Mollusca
Planorbula		Sc	Planorbidae	Limnophila	Gastropoda	Mollusca
Promenetus		Sc	Planorbidae	Limnophila	Gastropoda	Mollusca
Pomatiopsis lapidaria		Sc	Pomatiopsidae	Limnophila	Gastropoda	Mollusca
		Sc	Hydrobiidae	Limnophila	Gastropoda	Mollusca
Fontigens aldrichi		Sc	Hydrobiidae	Limnophila	Gastropoda	Mollusca
Elimia	2.5	Sc	Pleuroceridae	Limnophila	Gastropoda	Mollusca
Elimia protosiensis ozarkensis		Sc	Pleuroceridae	Limnophila	Gastropoda	Mollusca
Elimia protosiensis		Sc	Pleuroceridae	Limnophila	Gastropoda	Mollusca
Elimia protosiensis plebeius		Sc	Pleuroceridae	Limnophila	Gastropoda	Mollusca
Goniobasis		Sc	Pleuroceridae	Mesogastropoda	Gastropoda	Mollusca
Goniobasis protosiensis		Sc	Pleuroceridae	Mesogastropoda	Gastropoda	Mollusca
Goniobasis ozarkensis		Sc	Pleuroceridae	Mesogastropoda	Gastropoda	Mollusca
Goniobasis protosiensis ozarkensis		Sc	Pleuroceridae	Mesogastropoda	Gastropoda	Mollusca
Leptoxis	1.6	Sc	Pleuroceridae	Mesogastropoda	Gastropoda	Mollusca
Lithasia		Sc	Pleuroceridae	Mesogastropoda	Gastropoda	Mollusca
Pleurocera	6	Sc	Pleuroceridae	Mesogastropoda	Gastropoda	Mollusca
Pleurocera acuta		Sc	Pleuroceridae	Mesogastropoda	Gastropoda	Mollusca

Pleurocera alveare		Sc	Pleuroceridae	Mesogastropoda	Gastropoda	Mollusca
Valvata	8	Sc	Valvatidae	Mesogastropoda	Gastropoda	Mollusca
Campeloma	6.7	Sc	Viviparidae	Mesogastropoda	Gastropoda	Mollusca
Campeloma subsolidum		Sc	Viviparidae	Mesogastropoda	Gastropoda	Mollusca
Cipangopaludina		Sc	Viviparidae	Mesogastropoda	Gastropoda	Mollusca
Lioplax		Sc	Viviparidae	Mesogastropoda	Gastropoda	Mollusca
Viviparus	6	Sc	Viviparidae	Mesogastropoda	Gastropoda	Mollusca
Viviparus georgianus		Sc	Viviparidae	Mesogastropoda	Gastropoda	Mollusca
		Pa	Chordodidae			Nematomorpha
		Pa	Gordiidae			Nematomorpha
Macrocotyla grandulosa		C, Pr	Dendrocoelidae	Tricladida		Platyhelminthes
Dugesia	7.5	C, Pr	Planariidae	Tricladida		Platyhelminthes

Protocol for Monitoring Aquatic Invertebrates at Ozark National Scenic Riverways, Missouri, and Buffalo National River, Arkansas. Heartland I&M Network and Prairie Cluster Prototype Monitoring Program

SOP 9: Data Reporting

Version 1.00 (07/01/2007)

Revision History Log:

Previous Version #	Revision Date	Author	Changes Made	Reason for Change	New Version #

This SOP gives instructions for reporting on invertebrate data collected at Buffalo National River and Ozark National Scenic Riverways. The SOP describes the procedure for formatting a report, the review process, and distribution of completed reports. Efficient reporting of monitoring results is critical in assisting park Resource Managers in management decisions.

I. Report Format

Template

The report template for regional natural resource technical reports should be followed (http://www.nature.nps.gov/publications/NRPM/index.cfm). Natural resource reports are the designated medium for disseminating high priority, current natural resource management information with managerial application. The natural resource technical reports series is used to disseminate the results of scientific studies in the physical, biological, and social sciences for both the advancement of science and the achievement of the National Park Service's mission.

Style

Standards for scientific writing as recommended in the CBE Style Manual (1994) should be followed. Reports should be direct and concise. Refer to CBE Style Manual (CBE Style Manual Committee 1994) or Writing with Precision, Clarity and Economy (Mack 1986), Strunk and White (2000), Day (1983), and Batzli (1986).

II. Types of Reports and Review Procedure

Table 1. Summary of types of reports produced and review process. Adapted from Debacker *et al*. 2005.

Type of Report	Purpose of Report	Primary Audience	Review Process	Frequency
Annual Status Reports for Specific Protocols	Summarize monitoring data collected during the year and provide an update on the status of selected natural resources. Document related data management activities and data summaries.	Park resource managers and external scientists	Internal peer review by HTLN staff	Annually
Executive Summary of Annual Reports for Specific Protocols	Same as Annual Status Reports but summarized to highlight key points for non-technical audiences.	Superintendents, interpreters, and the general public	Internal peer review by HTLN staff	Simultaneous with Annual Status Reports
Comprehensive Trends and Analysis and Synthesis Reports	Describe and interpret trends in individual vital signs. Describe and interpret relationships among observed trends and park management, known stressors, climate, *etc*. Highlight resources of concern that may require management action.	Park resource managers and external scientists	Internal peer review by HTLN staff	Every 5-7 years
Executive	Same as	Superintendents,	Internal peer	Simultaneous

Summary of Comprehensive Trends and Analysis and Synthesis Reports	Comprehensive Trends and Analysis and Synthesis Reports, but summarized to highlight findings and recommendations for non-technical audiences.	interpreters, and the general public	review by HTLN staff	with Comprehensive Trends Analysis and Synthesis Reports

III. Distribution Procedure

Annual reports will be provided to the respective parks, Buffalo National River and Ozark National Scenic Riverways, where aquatic invertebrate monitoring was done. Additionally, a copy will be kept on file with the HTLN office of the National Park Service, Republic, Missouri, and made available to all interested parties upon request. All data collected by the HTLN is public property and subject to requests under the Freedom of Information Act (FOIA). Reports also are converted to Adobe .pdf files and posted on the HTLN website: (**http://www1.nature.nps.gov/im/units/htln/index.htm**).

Protocol for Monitoring Aquatic Invertebrates at Ozark National Scenic Riverways, Missouri, and Buffalo National River, Arkansas. Heartland I&M Network and Prairie Cluster Prototype Monitoring Program

SOP 10: Procedures and Equipment Storage After the Field Season

Version 1.00 (07/01/2007)

Revision History Log:

Previous Version #	Revision Date	Author	Changes Made	Reason for Change	New Version #

This Standard Operating Procedure explains procedures that all field observers using the Aquatic Invertebrate Monitoring Protocol at Buffalo National River, Arkansas and Ozark National Scenic Riverways, Missouri should be familiar with and follow after the field season is completed.

Procedures:

Equipment

1. Clean and repair all equipment prior to return to the proper storage areas.
2. Check sampling nets and determine if new nets must be ordered prior to the next field season.
3. Batteries must be removed from all equipment.
4. Change oil in boat motors, lubricate grease fittings, and determine if motors require other maintenance or repair prior to the next field season.
5. Examine boats and canoes to determine if other than normal maintenance is required prior to the next field season.

Paperwork and Reports

1. All references manuals should be re-shelved on their appropriate bookshelf. Other reference materials and extra data sheets need to be filed in their appropriate filing cabinet. Clean the insides and outsides of all vehicles used in the field.
2. At the end of each field season, after all sampling has been completed file the project manager will a trip report with the data manager outlining hours worked, field-crew members and their responsibilities on the project, and any unique situations encountered. This information is incorporated in the database and used during data analysis, and it may be useful in identifying causes for discrepancies and inconsistencies in the data.

Protocol for Monitoring Aquatic Invertebrates at Ozark National Scenic Riverways, Missouri, and Buffalo National River, Arkansas. Heartland I&M Network and Prairie Cluster Prototype Monitoring Program

SOP 11: Revising the Protocol

Version 1.00 (07/01/2007)

Revision History Log:

Previous Version #	Revision Date	Author	Changes Made	Reason for Change	New Version #

This SOP explains how to make changes to the Invertebrate Monitoring Protocol Narrative and accompanying SOPs for the Buffalo National River, Arkansas and Ozark National Scenic Riverways, Missouri, and how to track these changes. Anyone asked to edit the Protocol Narrative or SOPs must follow this outlined procedure in order to eliminate confusion in how data is collected and analyzed.

Procedures:

1. The Aquatic Monitoring Protocol and accompanying SOPs for Buffalo National River, Arkansas and Ozark National Scenic Riverways, Missouri have used sound methodologies for collecting and analyzing aquatic invertebrate data. However, all protocols require editing as new and different information becomes available. Required edits should be made in a timely manner and appropriate reviews undertaken.
2. All edits require review for clarity and technical soundness. Small changes or additions to existing methods will be reviewed in-house by the Heartland Network and Prairie Cluster LTEM staff. However, if a complete change in methods is sought, then an outside review is required. Regional and national staff of the National Park Service with familiarity in aquatic invertebrate research and data analysis will be utilized as reviewers. Also, experts in aquatic invertebrate research and statistical methodologies outside of the Park Service will be used in the review process.
3. Document edits and protocol versioning in the Revision History Log that accompanies the Protocol Narrative and each SOP. Log changes in the Protocol Narrative or SOP being edited. Version numbers increase incrementally by hundredths (*e.g.*, version 1.1, version 1.2, …*etc.*) for minor changes. Major revisions should be designated with the next whole number (*e.g.*, version 2.0, 3.0, 4.0 …*etc.*). Record the previous version number, date of revision, author of the revision, identify paragraphs and pages where changes are made, and the reason for making the changes along with the new version number.

4. Inform the Data Manager about changes to the Protocol Narrative or SOP so the new version number can be incorporated in the Metadata of the project database. The database may have to be edited by the Data Manager to accompany changes in the Protocol Narrative and SOPs.
5. Post new versions of the protocol on the Heartland Network internet website and forward copies to all individuals with a previous version of the affected Protocol Narrative or SOP.

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

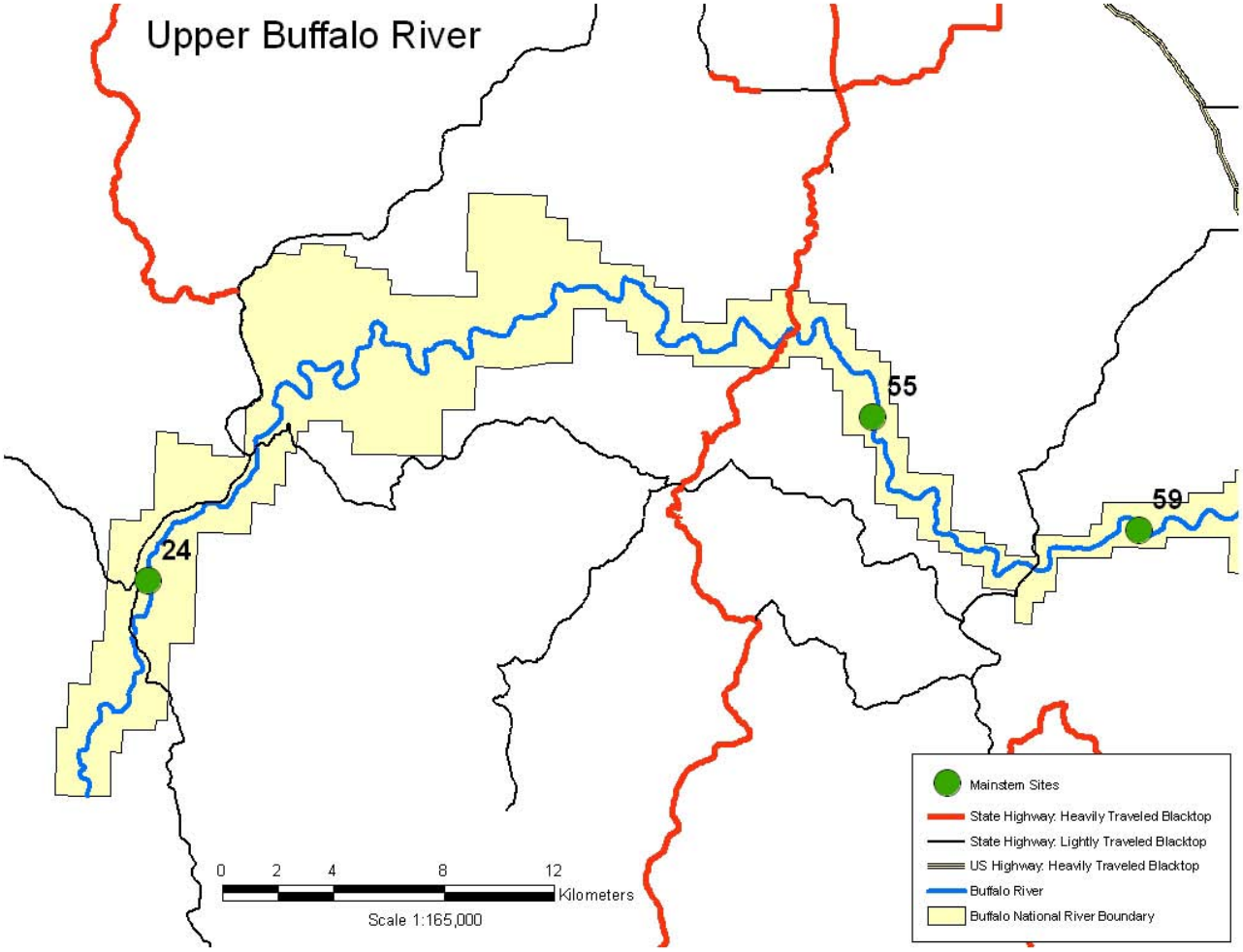


Figure A.1. – Location of stretches selected for monitoring on the upper Buffalo National River.

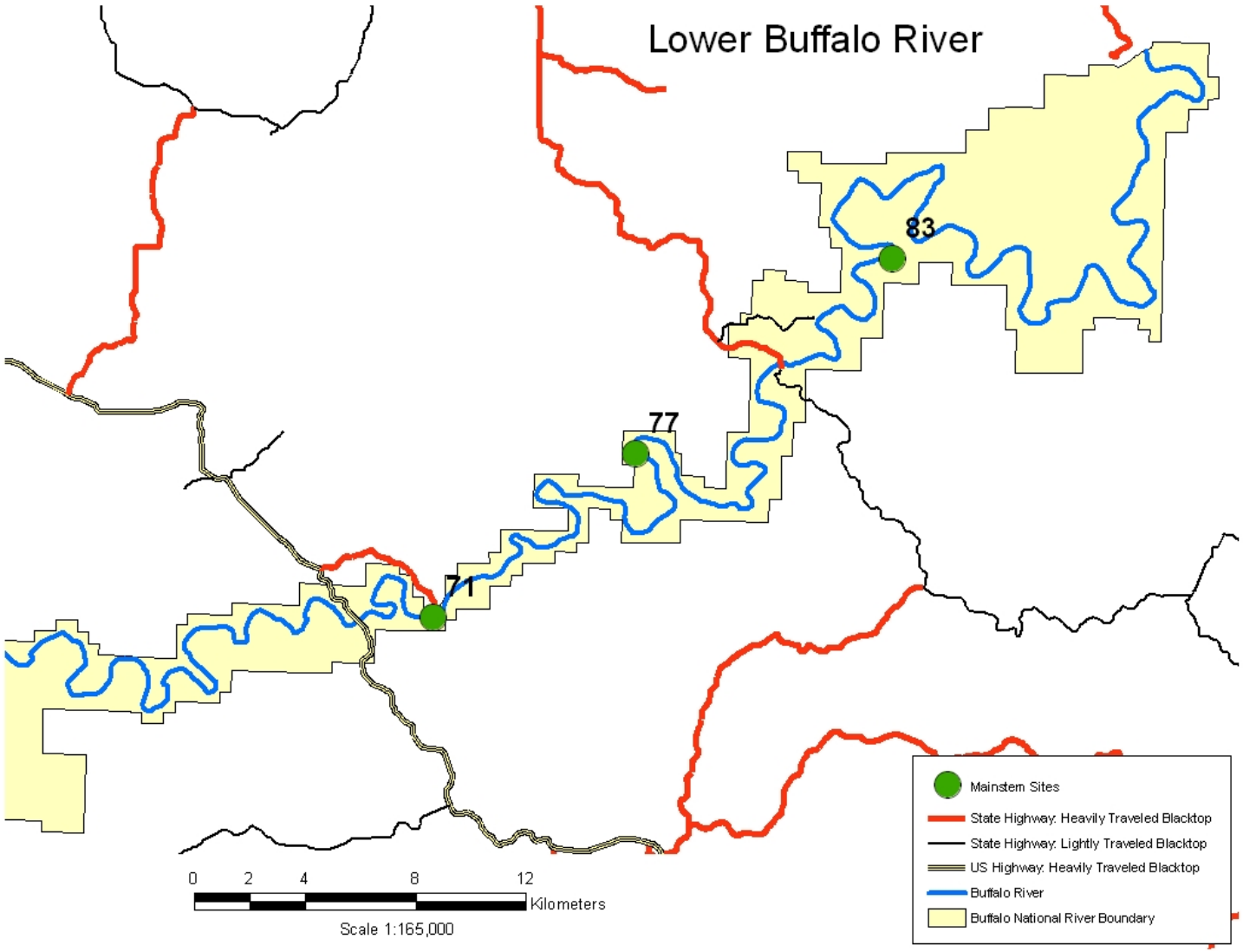


Figure A.2. – Location of stretches selected for monitoring on the lower Buffalo National River.

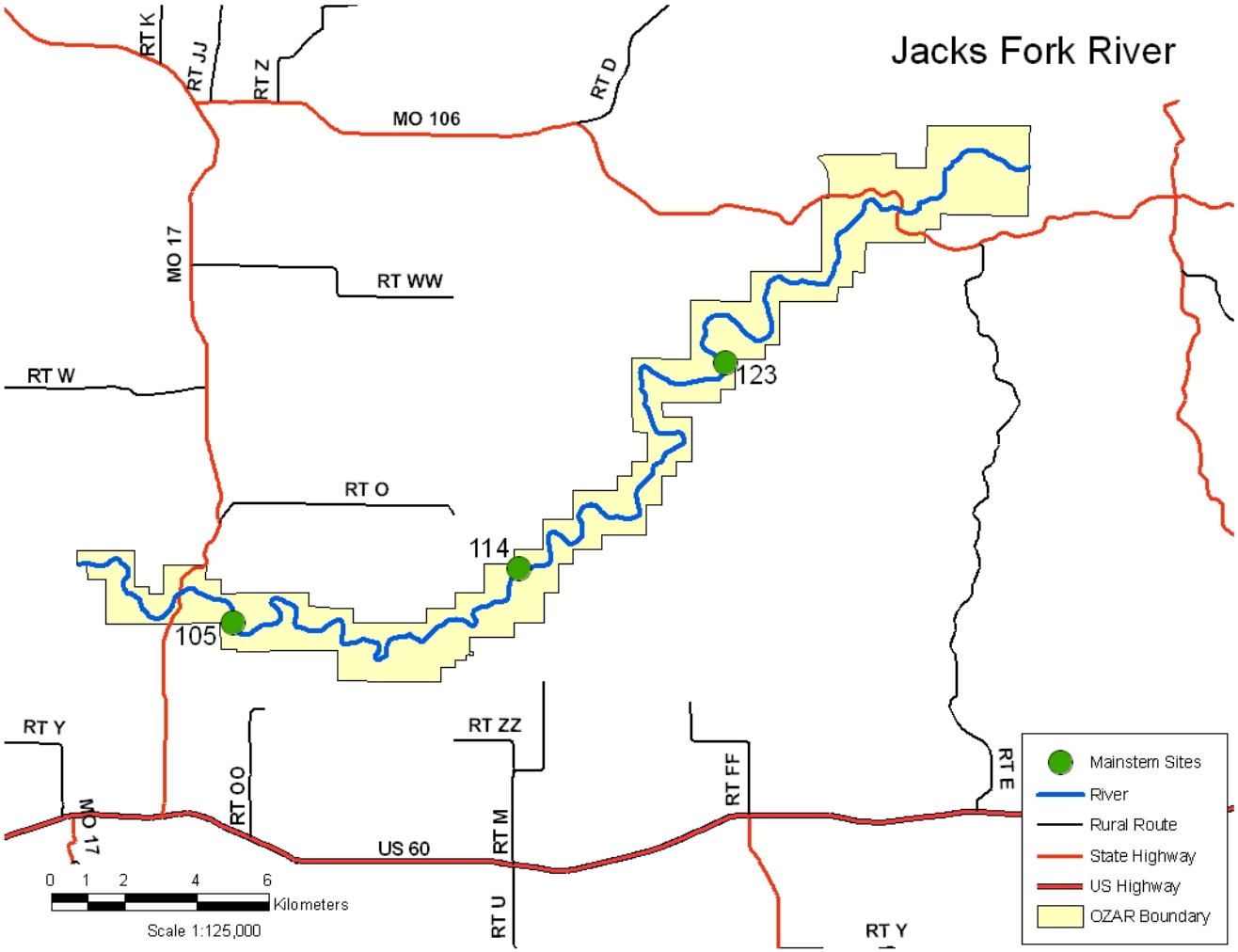


Figure A.3. – Location of stretches selected for monitoring on the Jacks Fork River.

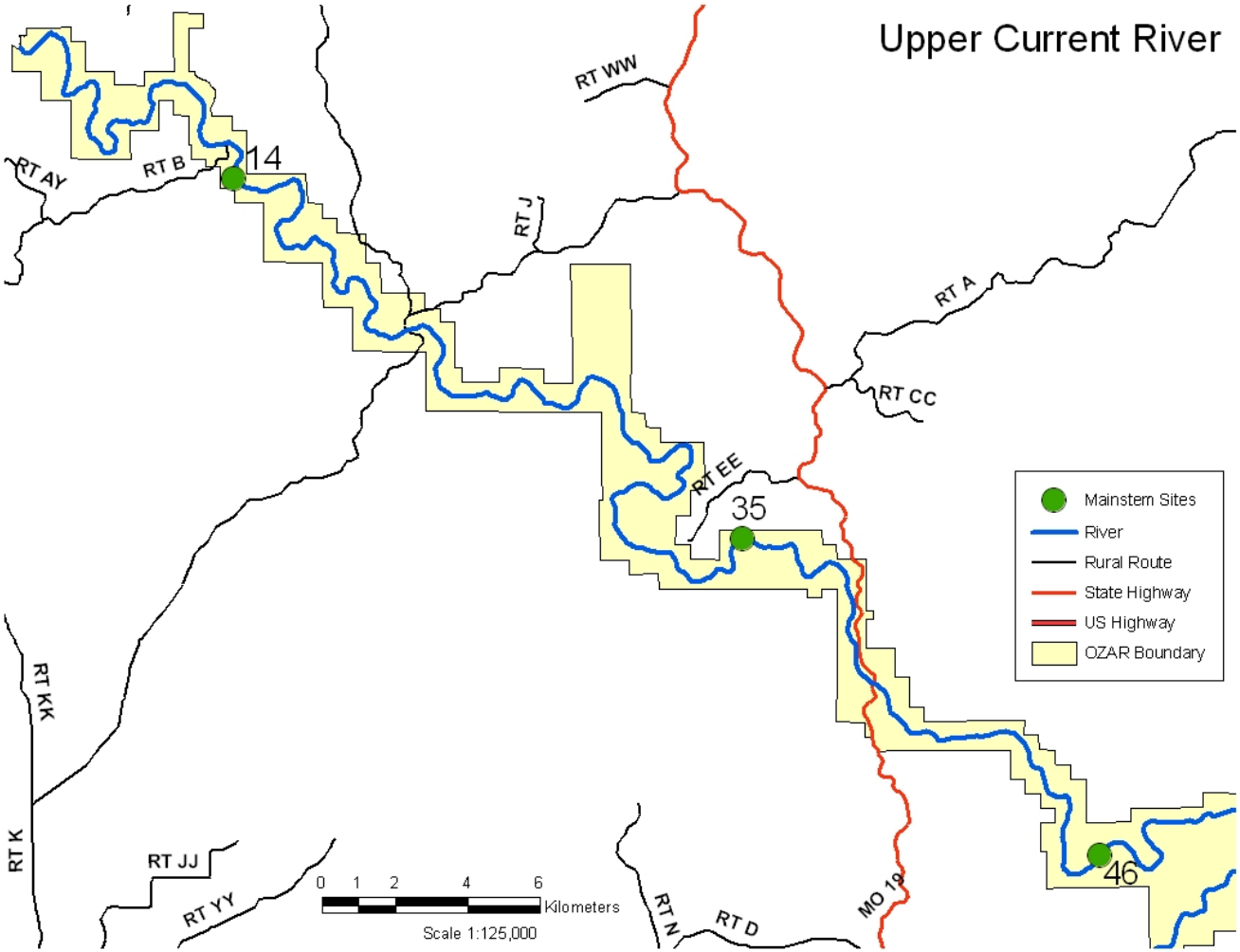


Figure A.4. – Location of stretches selected for monitoring on the upper Current River.

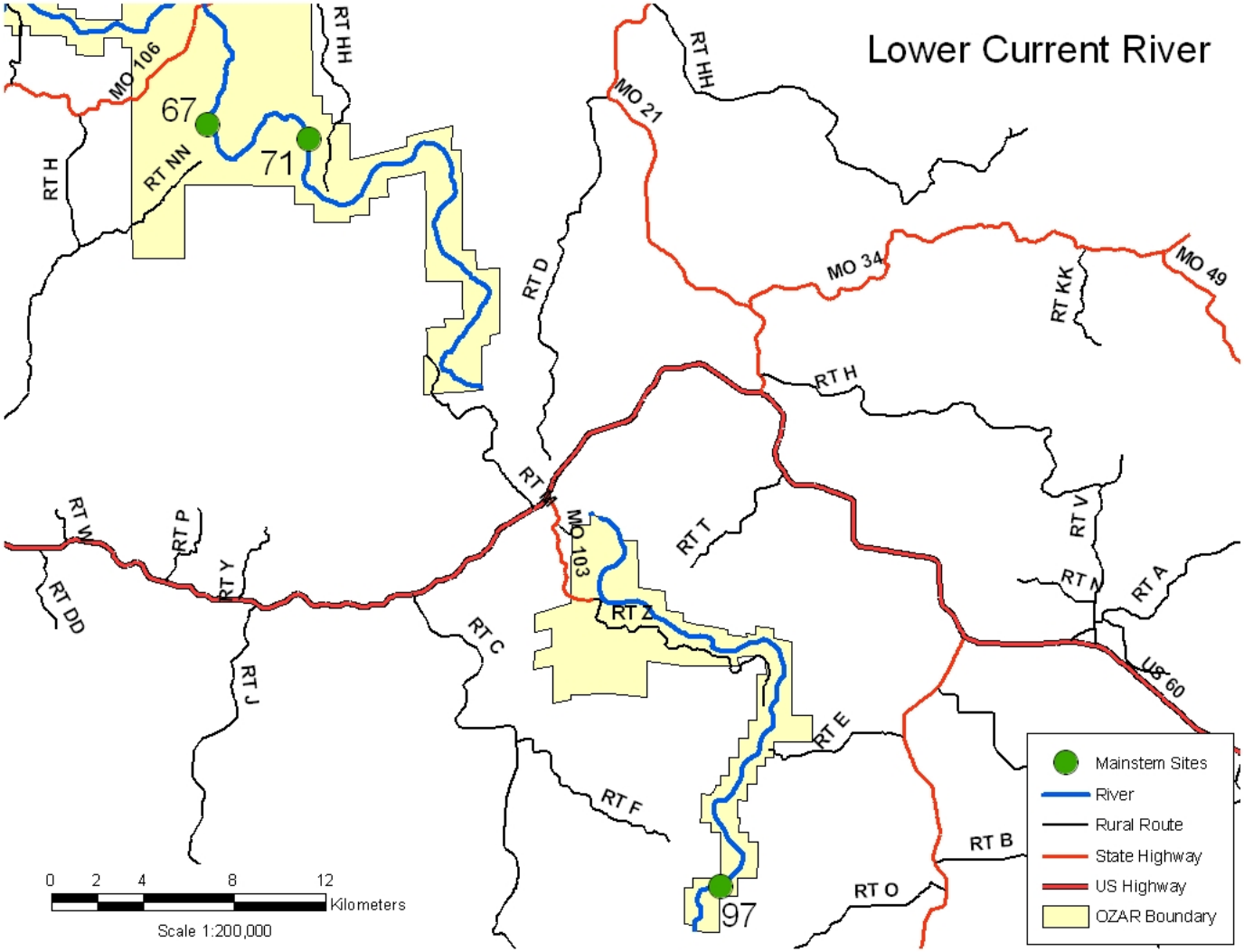


Figure A.5. – Location of stretches selected for monitoring on the lower Current River.

XII. Appendix B. Sample Site Codes and GPS Coordinates

Reach ID	River Basin	Site Type	Site/Trib Number	Trib Name	Panel No.	County	Stretch ID	Lower Stretch UTMX	Lower Stretch UTMY
Buffalo National River									
BUFFM01	Buffalo	Mainstem	01		Annual	Newton	24	464088.50	3981659.30
BUFFM02	Buffalo	Mainstem	02		Annual	Newton	55	490340.90	3987599.90
BUFFM03	Buffalo	Mainstem	03		Annual	Newton	59	499985.28	3983483.70
BUFFM04	Buffalo	Mainstem	04		Annual	Searcy	69	520484.74	3981679.66
BUFFM05	Buffalo	Mainstem	05		Annual	Searcy	73	529619.95	3984878.60
BUFFM06	Buffalo	Mainstem	06		Annual	Marion	87	545997.97	3995359.40
BUFFT03	Buffalo	Tributary	03	Whiteley	1	Newton	640	463933.84	3982976.75
BUFFT09	Buffalo	Tributary	09	Little Buffalo	1	Newton	420	490340.91	3987600.00
BUFFT22	Buffalo	Tributary	22	Spring	1	Searcy	300	536995.44	3986311.00
BUFFT24	Buffalo	Tributary	24	Hickory	1	Marion	649	540092.81	3992069.50
BUFFT30	Buffalo	Tributary	30	Middle	1	Marion	603	551428.31	3993556.50
BUFFT31	Buffalo	Tributary	31	Leatherwood	1	Marion	623	551307.69	3996258.00
BUFFT05	Buffalo	Tributary	05	Cecil	2	Newton	462	479905.44	3992743.25
BUFFT07	Buffalo	Tributary	07	Mill	2	Newton	646	487979.09	3990501.25
BUFFT12	Buffalo	Tributary	12	Sheldon Branch	2	Newton	242	492781.88	3983234.50
BUFFT21	Buffalo	Tributary	21	Brush	2	Searcy	241	527984.75	3983963.00
BUFFT25	Buffalo	Tributary	25	Little Panther	2	Marion	238	540006.31	3993475.25
BUFFT33	Buffalo	Tributary	33	Stewart	2	Marion	390	552646.50	4000976.50
BUFFT04	Buffalo	Tributary	04	Sneeds	3	Newton	441	472172.12	3990497.25
BUFFT13	Buffalo	Tributary	13	Big	3	Newton	244	495709.59	3981030.75
BUFFT15	Buffalo	Tributary	15	Davis	3	Newton	383	504216.16	3984923.25
BUFFT16	Buffalo	Tributary	16	Mill Branch	3	Newton	225	504310.34	3984978.25
BUFFT19	Buffalo	Tributary	19	Calf	3	Searcy	378	520463.22	3981045.50
BUFFT28	Buffalo	Tributary	28	Boat	3	Marion	484	543933.19	3998512.25
BUFFT01	Buffalo	Tributary	01	Smith	4	Newton	279	464098.72	3978179.75
BUFFT06	Buffalo	Tributary	06	Glade	4	Newton	403	481332.88	3992648.50
BUFFT10	Buffalo	Tributary	10	Wells	4	Newton	638	490814.66	3986624.00

BUFFT11	Buffalo	Tributary	11	Rock	4	Newton	317	492478.22	3984111.00
BUFFT20	Buffalo	Tributary	20	Bear	4	Searcy	472	526905.38	3983413.50
BUFFT23	Buffalo	Tributary	23	Water	4	Searcy	574	538186.50	3989492.75
BUFFT08	Buffalo	Tributary	08	Vanishing	5	Newton	229	489406.03	3989463.00
BUFFT14	Buffalo	Tributary	14	Lick	5	Newton	367	499899.69	3983426.50
BUFFT17	Buffalo	Tributary	17	Richland	5	Searcy	304	509734.38	3975988.00
BUFFT27	Buffalo	Tributary	27	Clabber	5	Marion	476	540925.44	3998147.75
BUFFT29	Buffalo	Tributary	29	Big	5	Marion	366	547400.69	3992751.25
BUFFT32	Buffalo	Tributary	32	Cow	5	Marion	275	551138.19	3998892.75
BUFFT18	Buffalo	Tributary	18	Slay Branch	Extra	Searcy	232	514572.59	3979212.00
BUFFT26	Buffalo	Tributary	26	Rush	Extra	Marion	694	540617.75	39978810.50
Ozark Scenic Riverways									
CURRM01	Current	Mainstem	01		Annual	Shannon	14	623336.28	4141730.82
CURRM02	Current	Mainstem	02		Annual	Shannon	35	637468.41	4131822.17
CURRM03	Current	Mainstem	03		Annual	Shannon	42	643252.57	4126300.08
CURRM04	Current	Mainstem	04		Annual	Shannon	67	661785.40	4111783.86
CURRM05	Current	Mainstem	05		Annual	Shannon	71	666195.85	4111128.94
CURRM06	Current	Mainstem	06		Annual	Carter	97	684220.63	4078321.12
JACKM01	Jacks Fork	Mainstem	01		Annual	Shannon	105	619895.88	4101117.92
JACKM02	Jacks Fork	Mainstem	02		Annual	Shannon	114	627768.31	4102604.03
JACKM03	Jacks Fork	Mainstem	03		Annual	Shannon	123	633244.58	4108431.69
JACKT01	Jacks Fork	Tributary	01	Flat Rock Hollow	1	Shannon	907	627068.19	4101683.25
CURRT08	Current	Tributary	08	Rocky	1	Shannon	657	661958.31	4110833.50
CURRT13	Current	Tributary	13	Mill	1	Carter	685	672241.31	4100790.25
JACKT02	Jacks Fork	Tributary	02	Water Branch	2	Shannon	542	639856.63	4114033.50
CURRT02	Current	Tributary	02	Sutton	2	Shannon	850	649132.00	4118740.25
CURRT11	Current	Tributary	11	Chilton	2	Carter	930	673293.31	4103594.50
CURRT07	Current	Tributary	07	Powder Mill	3	Shannon	494	662035.12	4116885.50
CURRT09	Current	Tributary	09	Carr	3	Shannon	695	666007.31	4111876.50

CURRT10	Current	Tributary	10	Thorny	3	Shannon	711	666068.69	4111169.25
JACKT03	Jacks Fork	Tributary	03	Shawnee	4	Shannon	699	650824.38	4115207.50
CURRT01	Current	Tributary	01	Shafer Spring	4	Dent	819	622056.00	4144309.50
CURRT03	Current	Tributary	03	Thompson	4	Shannon	576	652336.88	4117663.00
CURRT04	Current	Tributary	04	Prairie Hollow	5	Shannon	920	654127.81	4116782.50
CURRT06	Current	Tributary	06	Blair	5	Shannon	917	659132.19	4116340.75
CURRT12	Current	Tributary	12	Rogers	5	Carter	950	672338.69	4102176.50
CURRT05	Current	Tributary	05	Thorny Hollow	Extra	Shannon	473	657045.31	4115739.25

The NPS has organized its parks with significant natural resources into 32 networks linked by geography and shared natural resource characteristics. HTLN is composed of 15 National Park Service (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 Heartland, 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. Though 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.

www.nature.nps.gov/im/units/htln

Heartland Network
Natural Resource Monitoring

The Department of the Interior protects and manages the nation's natural resources and cultural heritage; provides scientific and other information about those resources; and honors its special responsibilities to American Indians, Alaska Natives, and affiliated Island Communities.

NPS D-163, June 2007

National Park Service
U.S. Department of the Interior

Natural Resource Program Center

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