COMPARATIVE ANALYSIS OF AQUATIC INSECT, AMPHIPOD, AND ISOPOD COMMUNITY COMPOSITION ALONG ENVIRONMENTAL GRADIENTS IN RHEOCRENE SPRING SYSTEMS OF MISSOURI

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by

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The undersigned, appointed by the Dean of the Graduate School, have examined the dissertation entitled

COMPARATIVE ANALYSIS OF AQUATIC INSECT, AMPHIPOD, AND ISOPOD COMMUNITY COMPOSITION ALONG ENVIRONMENTAL GRADIENTS IN RHEOCRENE SPRING SYSTEMS OF MISSOURI

Presented by Megan Mishell Zeller

A candidate for the degree of Doctor of Philosophy

And hereby certify that in their opinion it is worthy of acceptance.

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ABSTRACT

Spring systems in Missouri harbor a unique biota and provide critical initial discharge from subterranean aquifers to streams. However, little research has been conducted on the crenobiology or ecology in these systems. In this study, aquatic insect, amphipod, and isopod communities were examined in 16 spring systems in Missouri, in some of which associated environmental gradients were also measured. The goal of this study was to create a comprehensive list of species present in all studied systems, as well analyze changes in community composition among and within spring systems in relation to environmental gradients in selected springs. Sorenson's similarity coefficient and UPGMA cluster analysis showed that differences between high discharge spring systems may be related to the presence of trout and trout fisherman. Renkonen's similarity coefficient and UPGMA cluster analysis showed that differences between low to medium discharge spring systems may be related to the aquatic faunal region in which each is located, as species assemblages in Prairie and Big River faunal region springs were dissimilar from those in Ozark springs. Canonical correspondence analysis (CCA) showed that environmental conditions differ among springs and affect species differently in each aquatic faunal region, which may explain the observed differences in community composition. In addition, several state and federally listed species of conservation concern were collected, as well as several species endemic to the Interior Highlands.

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CHAPTER 1

Introduction

In North America, more than 8,600 species of insects are associated with freshwater environments during part of their life cycle (Voshell, 2003). In Missouri alone, 800-1,000 insect species are associated with aquatic environments during at least one stage of their life cycle (Sites and Poulton, in lit.). Additional undescribed aquatic species are likely to be discovered due to a lack of research conducted in certain aquatic habitats. For this reason, the status of many aquatic insect species is unknown. Few species of aquatic insects have been listed as endangered or threatened; however, in reality, many aquatic insect species are threatened and possibly even on the brink of extinction due to human activities, such as agriculture, urbanization, and pollution (Voshell, 2002). Therefore, it is imperative that the biology, ecology, and taxonomy of aquatic insect assemblages in understudied habitats be prioritized for research. In Missouri, springs are a prime example of a habitat that has been understudied, with many springs remaining unrecorded, unmeasured, and even unsampled (Vineyard and Feder, 1982). Very little research has been conducted to determine the aquatic insect species that compose spring communities and how these species are associated with environmental parameters.

Karst Topography

Southern Missouri, especially the Salem Plateau, is home to a vast landscape of rolling hills, deep valleys, caves, sinkholes, losing streams, natural bridges, and springs. This type of landscape is commonly referred to as karst topography. Karst is formed in areas with sufficient layers of carbonate rock, such as limestone and dolomite, adequate rainfall, vegetative cover, areas with openings in the bedrock, and areas with variable climate (MSS, 2007). As precipitation falls through the air and permeates through the soil, carbon dioxide is picked up, thus forming a weak carbonic acid (USGS, 2007). This solution infiltrates into the underlying carbonate rock and percolates through cracks and crevices, dissolving away the bedrock. As the bedrock is dissolved, caves and spring conduits are formed. Eventually, the pressure exerted on the groundwater forces water up through the spring conduits and other natural openings, thus forming springs. As a result of these karst processes, Missouri has at least 5,700 caves, thus earning the name "The Cave State". Missouri is also well-known for its large number of springs due to these same karst processes. Currently, a spring database maintained by the Missouri Department of Natural Resources lists more than 3,000 springs in the state (MDNR, 2006).

Spring Systems

A spring can be defined as any natural discharge of water from rock or soil onto the surface of the land or into a body of surface water (Vineyard and Feder, 1982). The majority of springs in Missouri occur south of the Missouri River in the Ozark Mountains region (Beckman and Hinchey, 1944; Vineyard and Feder, 1982) and can be classified as non-thermal springs, meaning they have temperatures that are approximately the same as the mean annual air temperature in the region in which they are found (Vineyard and Feder, 1982). Although most springs in Missouri are freshwater, numerous saline springs exist, which are the result of the chemical makeup of the rock layers through which they flow.

Springs generally fall into one of three categories: rheocrene, limnocrene, or helocrene. Rheocrene springs are those springs that emerge from the ground forming a free-flowing stream, or lotic system. Limnocrene springs are those springs that emerge from the ground to form a pond, or lentic system. Helocrene springs are those springs that emerge from the ground to form marshy areas. Spring size is usually classified based on the amount of water it discharges. First magnitude springs are the largest and have a discharge of at least 100 cubic feet per second (cfs) of water (Vineyard and Feder, 1982). Missouri has nine first magnitude springs and Missouri's largest spring, Big Spring, is one of the ten largest springs in the world (Vineyard and Feder, 1982). However, the majority of springs in Missouri are small rheocrene springs, many of which are unnamed.

Because each spring is different depending on the geology, topography, hydrology, and climate of the area where it exists, the physical and chemical characteristics of each spring system is unique. However, all freshwater rheocrene springs appear to share a common characteristic: physicochemical characteristics of the water change with distance downstream from the eucrene (spring source), with these characteristics becoming more like those of surface-fed streams in the same area. For example, the water temperature at the eucrene in Missouri springs averages 14.4˚C (58˚ F) year-round and changes with distance down the hypocrene (spring run) as it is exposed to the environment. Water chemistry also fluctuates depending upon surface water input, the topography of the catchment area in which the spring exists (McCabe, 1997), and the geology of the aquifer from which it emerges (Vineyard and Feder, 1982). These longitudinal changes in physicochemical characteristics, also called habitat gradients, most likely drive changes in biological community composition (McCabe, 1997).

Crenobiology

Crenobiology is the study of the biological organisms inhabiting springs and springbrooks. It is thought that spring systems provide habitat for biological communities different than those of surface-fed streams. Spring systems often hold endemic species close to the source (Nielsen, 1950; Michaelis, 1977; Sykora and Weaver, 1978), with other common stream species added downstream. However, not all species of organisms ocurring in springs are from the spring itself. In Missouri, a limited number of species of fish, turbellaria, salamanders, and other organisms that are subterranean have emerged to the surface through the eucrene (Pflieger et al., 1982). In Texas, much research has been conducted to explore new, endangered, and threatened subterranean aquifer species. Several species of aquatic insects have been studied, one of which exists in the Edwards Aquifer and occasionally rises to surface waters through one of two springs (Barr and Spangler, 1992; Barr, 1993). Similar research is needed in Missouri spring systems to begin building an understanding of the composition of our subterranean aquifer.

In addition to aquifer studies, it is also necessary to understand the macroinvertebrate communities that make up our spring systems and what conditions are necessary for their survival, especially since macroinvertebrates can be useful bioindicators. A limited number of studies has been conducted in the United States regarding aquatic insect assemblages in springs; however, the study of spring invertebrates has rapidly advanced in the United States over the past several decades. During this time, several symposia and compilations of papers have been organized focusing on spring research (Erman, 2002; Ferrington et al., 1995; Botosaneanu, 1998).

Many states have had little research conducted in the areas of crenobiology and spring exploration, although some states have had numerous spring studies conducted, some of which have focused on aquatic insect assemblages. For example, surveys focusing on the biodiversity, hydrogeology, and water quality of several springs in southern Illinois have been conducted (e.g., Webb et al., 1995, 1998). Long-term studies focusing on the biota and physical/chemical properties of cold springs have also been conducted in the Sierra Nevada Mountains of California (e.g., Erman, 1981, 1984, 1986, 1990, 1992, 1997, 1998 in Erman, 2002; Erman and Erman, 1992, 1995 in Erman, 2002). Extensive spring research has been and is currently being conducted in Texas, some of which focus directly on the aquatic insect species in these systems (e.g., Bosse et al., 1988; Brown and Barr, 1988; Barr and Spangler, 1992; Arsuffi, 1993; Barr, 1993). Florida has the largest number of first magnitude springs in the United States, and researchers are currently studying many aspects of springs, caves, and aquifers, including the biota (FDEP, 2002).

Although some states have had a great deal of research conducted in crenobiology, including assessments of aquatic insect assemblages, many states, including Missouri, are still behind. To date, a limited number of Missouri springs have been studied. Spring systems such as Big Spring (Nielsen, 1996), Bennett Spring (Sullivan, 1928), Stone Mill Spring (Doisy, 1984), Boone’s Lick Spring (Bonham, 1962), Greer Spring (B. Poulton, pers. comm.), and a small number of springs in southwestern Missouri (Blackwood, 2001; Sarver & Kondratieff, 1997) compose the majority of the springs and spring branches in Missouri that have been evaluated. A recent Ph.D. dissertation from the University of Kansas (Carroll, 2009) analyzed resource pulses and spatial subsidies in Ozark springs and their effects on community structure and food webs. The springs studied were Haseltine Spring, Steury Spring, and Danforth Spring, each of which is a fourth magnitude rheocrene spring located in the Springfield Plateau in southwest Missouri.

In addition to assessments of the subterranean aquifer and macroinvertebrate assemblages of spring systems, it is also important to understand the diversity and density of these organisms within the spring. Species richness in spring systems tends to be relatively low, although present species often exist in high densities (Vineyard et al., 1982). However, studies in different spring systems have shown that diversity of macroinvertebrates can increase downstream (Ward and Dufford, 1979; Meffe and Marsh, 1983; Danks and Williams, 1991; Ferrington et al., 1995), decrease downstream (Resh, 1983), peak in the intermediate reaches of the hypocrene (Sloan, 1956), or show no directional change in diversity (Noel, 1954; Williams and Hogg, 1988). Although Noel (1954) and Williams & Hogg (1988) found no changes in diversity, changes in abundance were found. Changes in physicochemical parameters are often blamed for shifts in macroinvertebrate communities within spring systems, with changes in temperature being cited most often (Ward and Stanford, 1982; Williams and Hogg, 1988; Williams, 1991; Erman, 1998); however, no similar studies have been published regarding community shifts in Missouri spring systems or the factors that drive these shifts. Studies investigating the longitudinal shift in aquatic insect, amphipod, and isopod communities in Missouri spring systems are needed to determine if certain species are dependent on spring conditions for survival.

Bioassessment of Spring Systems

Using the biological community of an aquatic system to assess the health of that system is often referred to as bioassessment or biomonitoring. This process is often used in surface-fed streams, however, no biomonitoring protocol currently exists for spring systems. As these unique systems are becoming of interest, and as urban, recreational, and agricultural pollution continue to plague waterways, the need for monitoring the quality of the water is necessary. Most states, including Missouri, have not addressed protocols for biomonitoring and protecting their spring resources; however, a handful of states have taken on this issue. Florida has taken great initiative to improve spring water quality and flow through improved research, monitoring, education, and landowner assistance (FDEP, 2007a). This special program involves a multi-agency task force (Florida Springs Task Force), which involves taking biological samples within the spring community (FDEP, 2007b). Montana has an aquatic macroinvertebrate inventory and assessment program in place for springs and seeps within the Bighorn Canyon National Recreation Area (Stagliano, 2008). The Ohio Environmental Protection Agency has also developed protocol and a field manual for evaluating headwater streams in the state (Ohio EPA, 2002). A manual of protocols for assessing terrestrial spring ecosystems in the Colorado Plateau has also been developed (Stevens et al., 2004). Arkansas has bioinventory and bioassessment protocol for caves and springs in the Sylamore Ranger District of the Ozark National Forest (Graening et al., 2003), although their analyses were open to all animal species and not restricted to macroinvertebrates. Although Missouri has never developed bioassessment protocol for spring systems, a small amount of work has been conducted in an attempt to establish baseline data and long-term monitoring programs for springs in the Ozark National Scenic Riverways (OZAR) (Doisy and Rabeni, 2004). These attempts to create new protocol using the biological community to assess the health of the spring system in question seem promising; however, it has never been addressed whether current biomonitoring protocol used in surface-fed stream systems could be used to monitor spring systems as well. In Missouri, the Missouri Department of Natural Resources currently uses the Semi-Quantitative Macroinvertebrate Stream Bioassessment protocol for sampling, which includes four primary metrics to assess the health of stream systems based on the aquatic insect taxa collected: Taxa Richness (TR) (count of all taxa), Ephemeroptera/Plecoptera/Trichoptera (EPT) Taxa Index (total number of distinct taxa within these orders), Biotic Index (BI) ($BI = \Sigma X_iT_i/n$; where X_i=number of individuals within each species, T_i=tolerance value of that species, and n=total number of organisms in the sample with tolerance values), and Shannon Diversity Index (SDI) ($H' = -\Sigma(p_i)(\log p_i)$; where p_i=proportion of total sample belonging to the i^{th} species). Although Doisy and Rabeni (2004) used these metrics, in addition to others, their sampling protocol differed from the bioassessment protocol used by MDNR for sampling aquatic insects in wadeable streams. Thus, it is uncertain whether there is a need to develop new protocol or if the current protocol will suffice.

Study Purpose

Missouri has approximately 85 state parks and historic sites, several of which contain springs. It is important for state-owned lands to have a comprehensive list of the species that occur in their spring systems. The presence of undescribed, threatened, or endangered species will provide park managers with important information that can be used to more effectively manage their parks and park resources. Also, it is unknown how macroinvertebrate assemblages differ in the eucrene compared to those downstream in the hypocrene. Information concerning this transition in community composition can be vital in determining if there are macroinvertebrate species that depend solely on spring conditions for survival and to determine which of these species are characteristic of spring systems. This can also be valuable information to determine if the current biomonitoring protocols using macroinvertebrates employed by the Missouri Department of Natural Resources for stream systems can also be applied to spring systems. Thus, the overall goals of this study were 1) to provide an inventory of the aquatic insect, amphipod, and isopod species present in Missouri eucrenes and hypocrenes in select state parks and historic sites, 2) to compare aquatic insect, amphipod, and isopod assemblages within and among Missouri spring systems, 3) to determine how the aquatic insect, amphipod, and isopod assemblages change in response to environmental gradients, and 4) to determine if it is feasible to apply current MDNR stream biomonitoring protocols to spring systems.

Study Sites

Twelve rheocrene springs, one spring-fed marsh, one spring-fed fen, a saline spring, and several seepage channels in 16 state parks and historic sites throughout Missouri were sampled and evaluated during this study. These springs vary in size, ranging from those with low discharge to those with high discharge. All springs studied were divided into one of three categories: quantitatively sampled rheocrene spring systems, qualitatively sampled rheocrene spring systems, and qualitatively sampled unique spring systems (Table 1). Each of the three categories of this study will be addressed in separate chapters.

CHAPTER 2

Comparative Analysis of Aquatic Insect, Amphipod, and Isopod Communities in Low to Medium Discharge Rheocrene Spring Systems in Missouri

Description of Study Sites

The quantitative portion of this study took place in eight low to medium discharge rheocrene springs (Figure 1). These spring systems included Cave Spring and Lone Spring at Cuivre River State Park (Figures 2, 3), Chickadee Spring at Meramec State Park (Figure 4), Mill Spring at Lake of the Ozarks State Park (Figure 5), Onondaga Spring at Onondaga Cave State Park (Figure 6), an unnamed spring at Dillard Mill State Historic Site (Figure 7), an unnamed spring at Rockbridge Memorial State Park (Figure 8), and an unnamed spring at Trail of Tears State Park (Figure 9). Below is a description of each individual spring.

Cave Spring (CSCR) and Lone Spring (LSCR) at Cuivre River State Park

N39˚03.259′ W090˚57.020′ and *N39˚04.135′ W090˚56.795′*

Cuivre River State Park is located north of the Missouri River in Lincoln County in the southern Lincoln Hills. Although this park is not located in the region of Missouri known for its karst topography, several low discharge springs exist in the park including Cave Spring and Lone Spring. Cave Spring is located in the Lincoln Hills Natural Area and consists of a small trickle that emerges from the base of a hill beneath Bear Den Cave, which cannot be accessed by trail. The amount of water discharged from this spring is unknown, but flow is permanent. Lone Spring is located in the Big Sugar Creek Natural Area and can be found emerging from a small cave along Lone Spring Trail. The discharge of this spring has been measured at 1.75 cubic feet per second (cfs) and typically becomes dry for part of the year (B. Schuette, pers. comm.). The hypocrene flows approximately 250 meters (m) before converging with Big Sugar Creek.

Chickadee Spring at Meramec State Park (CSM)

N38˚12.137′ W091˚07.234′

Meramec State Park is located on the tri-county border of Franklin, Washington, and Crawford counties and is named after the spring-fed Meramec River, a popular canoeing river. Within the park are many caves and several springs, including Chickadee Spring, which is located along Hickory Ridge. Chickadee Spring consists of water emerging from large cobble and boulder sized rocks. Although specific discharge information is unknown for the spring, discharge is low and it is subject to dry conditions during certain times of the year. According to state park personnel, a small study examining the discharge and flow of the spring started in 2008, which will provide much needed information about the spring and its discharge patterns. The hypocrene flows less than 200 m before converging with Beaver Creek, which eventually converges with the Meramec River approximately 0.8 kilometers (km) downstream.

Mill Spring at Lake of the Ozarks State Park (MSLO)

N38˚03.097′ W092˚34.889′

Mill Spring is located in Lake of the Ozarks State Park, Missouri’s largest state park, in Camden County. Mill Spring is a permanent spring that emerges from a low cave shortly before converging with a wet weather stream, which is a stream that flows only after prolonged or heavy precipitation events. The intermittent hypocrene flows less than 0.8 km before flowing into Coakley Hollow Stream, which eventually feeds into Lake of the Ozarks.

Onondaga Spring at Onondaga Cave State Park (OSOC)

N38˚03.584′   W091˚13.678′

Onondaga Spring is located in Onondaga Cave State Park in Crawford County and is named after the popular Onondaga Cave from which it exits. Although Onondaga Spring's base flow has not been well established, the spring's smallest flow was measured at 1.3 million gallons per day, whereas its largest measurable flow was 28 million gallons per day. Currently, park personnel are establishing an accurate base flow, although data are still being collected (T. Flynn, pers. comm.). This spring, which can be easily found along the Blue Heron Trail, appears to be a typical rheocrene spring emerging from a low cave into a large, dammed circular pool; however, the water discharged from the spring is channeled into a man-made concrete channel for approximately 25 m before entering an oxbow lake that feeds the Meramec River.

Unnamed spring at Dillard Mill State Historic Site (2DM)

N37˚43.226′   W091˚12.354′

Dillard Mill State Historic Site is found nestled within the Ozark Mountains in Crawford County. Within this park is a small, unnamed spring that has remained unstudied. This spring is located within a forested draw and emerges from a pipe located on the side of a semi-steep hill. The hypocrene flows approximately 30 m before flowing through several concrete slabs that lie parallel to the direction of water flow. These slabs are said to be the remnants of an old trout hatchery. Immediately after the concrete slabs is a large, silted pool. At the downstream end of the pool lies another pipe that feeds the spring water into Huzzah Creek.

Unnamed spring at Rockbridge Memorial State Park (1RB)

N38˚52.655′ W092˚18.930′

Rockbridge Memorial State Park is located in Boone County in the transition area between the northern prairie region and southern Ozark region of Missouri. This park contains a natural rock bridge, several sinkholes, caves, and four springs. One of these springs is a small unnamed spring located in Gans Creek Wild Area. This small spring emerges from two small openings at the base of a bluff. The hypocrene flows less than 400 m before converging with Gans Creek. Because this spring is secluded in a designated wild area and cannot be accessed by trail, the macroinvertebrate community within this unnamed spring has remained unexamined.

Unnamed spring at Trail of Tears State Park (3TT)

-no coordinates for this spring-

Trail of Tears State Park is located in Cape Girardeau County near the Mississippi River. There are two springs located within this state park: Moccasin Spring, which exists only on historical records and has not been found recently, and a small unnamed spring. This unnamed spring is located next to Moccasin Springs Road emerging from a small hole in the ground at the base of a tree. The silty hypocrene flows only 25 m before entering a culvert that runs under the roadway and into a small creek. This spring does not flow year round and is subject to dry conditions, although, it is also subject to flood waters from the Mississippi River. Although this small spring is easily accessible from the roadway, the invertebrate fauna within the spring has not been studied.

Study Questions

One of the goals of this portion of the study was to create a comprehensive list of aquatic insect, amphipod, and isopod taxa collected from each of these spring systems, with special notation of state and federally listed species, undescribed species, rare species, and/or new records for the state of Missouri. In addition to this goal, four specific questions were addressed:

1) Does aquatic insect, amphipod, and isopod community composition differ among spring systems?
2) Does the aquatic insect, amphipod, and isopod community composition change longitudinally within each spring system?
3) Is aquatic insect, amphipod, and isopod community composition in each spring related to environmental gradients?
4) Are longitudinal changes in community measures, such as species richness, diversity, dominance, and evenness, influenced by environmental gradients?

Methods

Quantitative sampling of the eight low to medium discharge rheocrene spring systems was conducted in May 2007 and August 2008. Because environmental conditions within spring systems are relatively stable and constant, they are less subject to the more drastic seasonal changes experienced in other aquatic systems; therefore, two strategically timed sampling periods wre considered sufficient to characterize the fauna. Collections were conducted once in the spring season and once in the fall to maximize the diversity of species and stages collected. Sampling was originally scheduled for consecutive spring and fall seasons, however, because the spring was dry during August 2007, it was sampled in August 2008 instead. Identification and other laboratory work took place during the winter and summer months each year.

Preceding physicochemical measurements and sampling, a distance of 400 m from the eucrene was measured by paces. Small surveying flags were put in place to mark ±2.5 m from 10 m, 25 m, 50 m, 100 m, 200 m, and 400 m downstream from the eucrene (Figure 10). All measuring and marking took place on the bank to prevent disturbance of the eucrene and hypocrene before it was sampled. After the hypocrene was measured and marked, an exact sampling location within the channel was chosen for taking physicochemical measurements and samples at each sampling increment. In order to standardize the location in which samples and measurements were taken among sites, the exact sampling location within the channel was chosen based on a number of factors, including 1) where the least amount of or no vegetation was present, 2) away from any structure that may cause unwanted bias, such as fallen logs or accumulated debris, 3) in the main flow (i.e. clear of backwash or pooled areas), 4) near the center of the channel away from the stream banks, and 5) where the substrate size was <256 mm (i.e. large cobble or smaller).

Following the initial measurements and marking of the hypocrene and determining the site for sampling within the designated area, a suite of environmental attributes was measured, including current velocity, canopy cover, temperature, pH, specific conductivity (SC), dissolved oxygen (DO), alkalinity, channel width, and maximum depth. Current velocity was taken using an Ohio Digital Stream Meter. The propeller of the meter was placed in the water where the sample was to be taken. If the value fluctuated between two values, the highest value was recorded. Forest overstory density was determined using a Model C spherical densitometer (Forest Densiometers). Temperature, pH, specific conductivity, and dissolved oxygen were all measured using a HQ40d Series Portable Meter (HACH). Probes used to measure each of these parameters were suspended in the water at the location in which the biological sample was to be taken. Alkalinity was determined by processing water samples from each sampling location using a HACH Digital Titrator. Both phenolphthalein alkalinity and total alkalinity were determined and recorded. Channel width (mm) was determined by using a tape measure across the wetted width of the channel at the location in which the sample was to be taken. Maximum depth (mm) was taken using a meter stick in the same location. In addition, substrate size (clay/silt, silt/sand, gravel, pebble, small cobble, large cobble) was visually categorized and recorded at each location where a sample was taken. Observations of the surroundings (human disturbances, dams, etc.), available habitat, and other physical characteristics that may have been pertinent to the study were also recorded. All measurements and observations were made from the bank, although entry into the water was necessary at times when measuring channel width and maximum depth. When entry into the water was necessary, access was made immediately downstream from the sampling location to prevent disturbance of the substrate that was to be sampled.

After physicochemical measurements and observations were completed at a location, biological sampling was conducted. A Surber sampler (30.5 x 30.5 cm (1 sq.ft), 1000 micrometer (µm) mesh) was used to sample at each sampling location. Once the Surber sampler was in place, the substrate within the frame of the sampler was agitated for one minute as the current carried organic matter, including organisms, into the net. During this one minute period, any large cobble included within the sampling frame of the Surber sampler was lightly scrubbed to remove any organisms clinging to the rock before the smaller substrate underneath was agitated. All aquatic insects, amphipods, and isopods were collected from each Surber sample and placed into a labeled vial to be taken to the laboratory. The net of the sampler was also examined to make certain that all target specimens were collected.

After the hypocrene had been sampled, each aquatic mesohabitat around the periphery of the eucrene was sampled qualitatively using an aquatic D-net (24 x 20 openings per inch mesh) until no recognizably new morphospecies were taken in two consecutive samples. Physicochemical measurements, observations, and sampling began downstream and progressed upstream to prevent contamination of subsequent samples by drifting sediment and biota; thus, 400 m was the first to be sampled and the eucrene was the last. If a spring converged with another stream before reaching 400 m, measurements and sampling began at the greatest distance increment before convergence.

Supplemental qualitative samples were taken near the eucrene using a blacklight trap at Cave Spring in Cuivre River State Park in May 2007. This additional qualitative sample was taken to collect adults to aid in larval identification. This spring was chosen for blacklight trapping because a blacklight trap was owned by, and available for use at, this state park. All samples were transported to the laboratory at the University of Missouri in Columbia in separate labeled containers containing 80% ethyl alcohol. In the laboratory, all samples were sorted and identified to the lowest possible taxonomic level.

Statistical Analysis

Taxa were counted to determine order, family, genus, and species richness for all springs together during each sampling season. Species richness values were also determined for each individual spring system. Shannon diversity index ($H' = -\Sigma p_i \ln p_i$) and evenness ($E = H'/\ln S$) equations (Magurran 1988) were used to compute a species diversity and evenness value for each spring system, where p_i=proportion of total sample belonging to the i^{th} species and S = species richness. These values were used to summarize the community data collected from the studied spring systems during this portion of the study.

To determine whether aquatic insect, amphipod, and isopod community composition differs among spring systems (question #1), a similarity and cluster analysis using quantitative data from the hypocrenes was conducted using Renkonen's similarity coefficient and the unweighted pair-group method using arithmetic means (UPGMA) clustering algorithm in NTSYSpc 2.11T (Rohlf, 2002). This method clusters springs together based on percent similarity of community composition. A second similarity and cluster analysis using qualitative data from the eucrene was conducted using Sorenson's similarity coefficient and the UPGMA clustering method in PC-ORD version 4.10 (McCune and Mefford, 1999). A third UPGMA cluster analysis using Renkonen's similarity coefficient was conducted to determine if the community composition of each spring system varied between sampling years.

To determine if community composition in each spring is related to environmental gradients (question #3), canonical correspondence analysis (CCA), a direct multivariate gradient technique, was used. This multivariate method ordinates sites, species, and environmental gradients simultaneously to show which environmental gradients are the most and least influential in determining species composition at a particular site. This analysis was also performed using PC-ORD version 4.1 (McCune and Mefford, 1999).

To determine if longitudinal changes in community measures, such as species richness, Shannon diversity index, Shannon evenness, and the density of dominant species, are influenced by environmental gradients (question #4), a stepwise regression was performed for each community measure against environmental variables in each spring using Minitab 15 (2007). This method identifies a subset of independent variables that best explains the overall variability in the response variable.

Results and Discussion

A total of 2,109 specimens was collected in 2007 (Table 2). Ten orders, 40 families, 71 genera, and 77 species were collected, of which 8 orders, 37 families, 66 genera, and 69 species were insects. A total of 2,267 specimens was collected in 2008 (Table 3). Ten orders, 38 families, 78 genera, and 88 species were collected, of which 8 orders, 34 families, 74 genera, and 81 species were insects. Special taxa collected from these springs include the blind, subterranean amphipod *Bactrurus brachycaudus* Hubricht & Mackin, which was collected from 1RB and 2DM in 2007. This species is listed on the Missouri species of conservation concern list as an S4 in the state of Missouri and a G4 globally, which implies that this species is uncommon, but not necessarily rare (MDC, 2010). The blind, subterranean isopod *Caecidotea salemensis* Lewis was collected from the eucrene of 1RB in 2008 and is ranked as an S2 in the state of Missouri, which implies that the species is imperiled in the state because of its rarity or because of some factor(s) making it vulnerable to extirpation from the state. This species is listed as a G4 globally (MDC, 2010) and is endemic to the Ozark Highlands. Last, the caddisfly species *Helicopsyche limnella* Ross, which was collected from MSLO in both sampling seasons, is not listed as a species of conservation concern, but it is a notable species because of its endemism to the Interior Highlands (Moulton and Stewart, 1996).

In both 2007 and 2008 (Figures 11, 12), MSLO had the highest species richness in the hypocrene and eucrene combined, whereas 3TT had the lowest species richness in 2007 and 2DM and 1RB had the lowest species richness in 2008. When considering species richness in the hypocrene only, 2DM had the lowest in 2007. In 2007, the eucrene community in 2DM and OSOC more than quadrupled the species richness in the entire spring, thus the hypocrene had much lower species richness than the eucrene. In 2008, the eucrene community in OSOC more than doubled the species richness in the entire spring. MSLO and CSCR had the highest species diversity and evenness in both sampling years (Figures 13, 14). Springs 2DM and 1RB had the lowest species diversity and evenness in 2007, whereas 2DM and CSM had the lowest species diversity and evenness in 2008.

Does aquatic insect, amphipod, and isopod community composition differ among spring systems?

Renkonen’s similarity coefficient and UPGMA cluster analysis showed that community composition within the hypocrene differs among spring systems. In 2007, communities within similar aquatic faunal regions (Pflieger, 1989; aquatic faunal regions depicted in figure 1) had the most similar aquatic insect, amphipod, and isopod communities (Figure 15). Springs 3TT and 1RB, both of which are located within the Big River Faunal Region of Missouri, were 95% similar in community composition. CSCR and LSCR, both in the Prairie Faunal Region of Missouri, had similar community composition to each another and to 3TT and 1RB. Those springs located within the Big

River Faunal Region and Prairie Faunal Region were dissimilar to the springs in the Ozark Faunal Region of Missouri, with only 2% similarity. Within the Ozark Faunal Region, springs 2DM and CSM showed 82% similarity in community composition. Spring OSOC had a community 72% similar to 2DM and CSM. Although MSLO is also in the Ozark Faunal Region of Missouri, it only had a 4% similarity to 2DM, CSM, and OSOC. Species richness in the hypocrene at MSLO was more than triple that of the other three Ozark hypocrenes, which explains why they had few species in common. In 2008, springs also clustered together based on the aquatic faunal region associations (Figure 16), with the exception of MSLO. The community of MSLO was dissimilar to all spring systems in 2007, but was 47% similar to the prairie spring CSCR in 2008. The communities at MSLO and CSCR had 26% similarity to the remaining two prairie springs, 1RB and LSCR. However, MSLO still had much higher species richness than did any of the other sampled springs. The communities at 1RB and LSCR had 86% similarity to each other. Spring 3TT was dry during the 2008 sampling period, thus it was not included in this analysis and it is unknown whether it would have had a similar community to the Big River spring 1RB, as it did in 2007. The three remaining Ozark springs clustered together as having similar aquatic insect, amphipod, and isopod communities. CSM and 2DM had communities that were 90% similar and OSOC had a community that was 75% similar to 2DM and CSM. Overall, in both sampling years, spring hypocrenes in the Prairie Faunal Region and Big River Faunal Region were most similar to one another in community composition of aquatic insects, amphipods, and isopods, as were springs in the Ozark Faunal Region, with the exception of MSLO which does not fit into one category or another due to its high species richness compared to the other spring systems.

Because the eucrenes were sampled qualitatively, whereas hypocrenes were sampled quantitatively, it was not possible to combine data from the entire spring system for analysis. Thus, a separate cluster analysis was conducted to determine if similarities in community composition follow the same patterns seen among hypocrenes. Sorenson's similarity coefficient and UPGMA cluster analysis showed similar patterns to those seen among spring hypocrenes (Figures 17, 18). In 2007, the community composition in the eucrenes of the springs in the Big River Faunal Region (3TT and 1RB) and the Prairie Faunal Region (LSCR and CSCR) were the most similar to one another. CSM and 2DM, both in the Ozark Faunal Region, also had similar communities in their eucrenes. However, instead of the community in the eucrene at OSOC having a similar community to the rest of the Ozark spring eucrenes as we saw with the hypocrenes, the eucrene community at OSOC was more similar to the Big River and Prairie spring communities. As with the community in the hypocrene, the community in the eucrene at MSLO was not similar to any other spring community. Results from 2008 are similar to those in 2007. CSM and 2DM, both Ozark springs, clustered together as having similar community composition in the eucrene and 1RB, CSCR, LSCR, and OSOC clustered together as having similar community composition. Instead of clustering alone, as it did in 2007, MSLO had a similar community to the Prairie and Big River springs, which is similar to those results obtained in 2008 in the hypocrene. Overall, similarity in aquatic insect, amphipod, and isopod community composition in the eucrenes was similar to those results obtained from the hypocrene for both sampling seasons, with the exception of OSOC. The community composition in the eucrene at OSOC appears to be more similar to that of Big River and Prairie springs than that of Ozark springs. It is unknown exactly why the eucrene community of OSOC is more similar to the eucrene communities of the Prairie and Big River springs rather than the Ozark springs.

The similarity and cluster analysis performed to determine if community composition within each spring system in each faunal region remained consistent between 2007 and 2008 shows results similar to the analyses performed for individual sampling years (Figure 19). Big River springs were most similar to one another, as were Prairie springs. Ozark springs were most similar to one another as well, with the exception of MSLO, which clustered as having a somewhat similar community composition to the Prairie springs. This was also shown in the 2008 cluster analysis. Overall, community composition was consistent between seasons, thus providing even stronger evidence for the separation of spring communities based on aquatic faunal region.

To date, no studies have evaluated differences in community composition of spring systems in different faunal regions of the state, primarily because Ozark springs have been the focus of research more so than springs in other faunal regions, possibly because they are more numerous. Because of this, it is difficult to determine if the patterns revealed are indeed true, especially since only eight springs were sampled. At the start of this study, it was unexpected that springs in different faunal regions of the state would have similar community compositions, which is why the number of springs sampled in each region is so small. This pattern was not expected because the aquatic faunal regions as proposed by Pflieger (1989) were based on fish communities in stream systems and were not necessarily intended for application with aquatic organisms in other types of aquatic systems. However, it has been shown that benthic macroinvertebrate communities in Missouri stream systems can also be classified by aquatic faunal region (Rabeni and Doisy, 2000). Studies of additional small, low discharge rheocrene spring systems are needed to see if similar patterns based on faunal region emerge.

The proportion of aquatic insects, amphipods, and isopods correspond with the results obtained from the cluster analyses, showing that community composition varies among spring systems and corresponds with the aquatic faunal regions. Pie charts were constructed using density data collected from the hypocrene. In 2007, the community in both Prairie springs, CSCR and LSCR, were amphipod and isopod dominated, with 85% and 91%, respectively, of the community being composed of these two groups. Thus, only 15% and 9%, respectively, of the community were aquatic insects (Figure 20). The dominant amphipod in these two springs was *Crangonyx forbesi* (Hubricht and Mackin) and the dominant and only isopod was *Caecidotea brevicauda* Forbes. In the Big River springs, 1RB and 3TT, the majority of the community was composed of isopods, with only 10% and 13%, respectively, of the community being composed of amphipods and aquatic insects (Figure 21). The dominant isopod species in these two springs was *C. brevicauda*. The majority of the amphipods that were present were *C. forbesi*, as in the Prairie springs. In the Ozark springs, the hypocrenes were primarily amphipod dominated, with 55%, 71%, 81%, and 93% of the community composed of this group in MSLO, OSOC, CSM, and 2DM, respectively (Figure 22). These springs had 3% or less of their community being composed of isopods and 45%, 26%, 19%, and 7%, respectively, of the community being composed of aquatic insects. Springs OSOC, CSM, and 2DM were dominated by the amphipod species *Gammarus minus* Say, whereas MSLO was dominated by another species, *Gammarus pseudolimnaeus* Bousfield. MSLO, which had the highest species richness overall, had the highest proportion of aquatic insects of any of the springs sampled.

In 2008, similar patterns in the proportion of aquatic insects, amphipods, and isopods were found, with just a few exceptions. The Prairie spring CSCR was still dominated by isopods and amphipods, with only 10% of the community being composed of aquatic insects; however, the second Prairie spring, LSCR, was dominated by isopods, with 88% of the community being isopods, 1% amphipods, and 11% aquatic insects (Figure 23). The Big River spring 1RB was also composed of a community similar to that sampled in 2007, with 85% of the community being composed of isopods (Figure 24). Again, 3TT was dry during the 2008 sampling period, thus there are no samples for comparison to the 2007 sample. Ozark springs MSLO, OSOC, CSM, and 2DM were also composed of communities similar to what was observed in 2007. These springs were composed of 64%, 71%, 92%, and 91% amphipods, respectively, and 34%, 27%, 8%, and 8% aquatic insects, respectively (Figure 25). As in 2007, MSLO had the highest species richness and the highest proportion of aquatic insects than any other spring sampled. In terms of dominant species in each spring system, patterns in dominance seen during the 2007 sampling period were also seen in 2008. The Big River spring 1RB was dominated by the isopod *C. brevicauda*, as was the Prairie spring LSCR. The Prairie spring CSCR was still dominated by *C. brevicauda*; however, both CSCR and LSCR had very few *C. forbesi* present. Instead, CSCR had the amphipod *G. pseudolimnaeus* present, which may explain why this particular spring had a somewhat similar community to the Ozark spring MSLO in 2008. It too was dominated by *G. pseudolimnaeus*. The three Ozark springs, CSM, OSOC, and 2DM, continued to be dominated by *G. minus*, as in the spring sampling season.

It is unknown why the dominant amphipod, *C. forbesi*, disappeared in LSCR or why another dominant amphipod species, *G. pseudolimnaeus,* took over in CSCR in 2008. Because samples were taken only twice, it is not possible to determine which year represents the typical community present in these springs. Measured environmental variables varied little between 2007 and 2008, thus it appears that this change may be due to some unmeasured variable.

Pie charts showing the proportion of individuals representing each aquatic insect order in each spring do not show a strong pattern (Figures 26-31). The proportion varies by spring system, and in many cases the proportions are not consistent among seasons. It should be noted that in several cases the proportion of aquatic insects present in the total sample was low. The total number of aquatic insects used to construct these pie charts ranged from 4 to 163 individuals.

In a paper summarizing research on spring invertebrate communities, with emphasis on North America, Williams and Williams (1998) suggested that the composition of spring communities varies depending on habitat persistence (glaciated vs. non-glaciated regions), although temperature regimes (cold vs. thermal springs), permanence (stable vs. intermittent springs), and salinity (freshwater vs. brackish vs. saline springs) also affect community composition. It is stated that permanently flowing, coldwater springs located in regions largely unaffected by recent (Quaternary) glacial activity are the most stable spring type and are said to be dominated by gastropods, amphipods, trichopterans, bivalves, oligochaetes, chironomids, and turbellarians, respectively. The springs that fall into this category in Missouri would be those springs in the Ozark faunal region. These springs are indeed amphipod dominated, with a high proportion of Trichoptera and/or Diptera in most cases, where the majority of Diptera collected in Ozark springs were chironomids. Since we did not sample other groups of aquatic macroinvertebrates, it is not possible to determine if there was also a high proportion of gastropods, bivalves, oligochaetes, or turbellarians; however, because there was such a high proportion present on vegetation, rocks, and the substrate, it is possible to say that the Ozark spring MSLO was indeed dominated by snails (gastropods) as well. Those springs located in the Prairie and Big River faunal regions of Missouri would be categorized as permanent, coldwater springs located in regions affected by recent (Quaternary) glacial activity. These springs are said to be dominated by arthropods, typically nemourid stoneflies, chironomids, trichopterans, mites, copepods, ostracods, and amphipods, respectively. Again, not all groups of aquatic invertebrates were sampled, but nemourids were collected in Prairie spring LSCR and Big River spring 1RB. However, with that said, nemourid stoneflies were also collected in the Ozark spring 2DM. Chironomids and trichopterans were also collected in the Prairie and Big River springs. The proportion of amphipods collected in these springs varied, but in most cases they were also isopod dominated, which was not listed as one of the dominating groups in glaciated springs. Thus, for both Ozark springs and Prairie and Big Rivers springs, the proposed community for non-glaciated versus glaciated springs partially hold true, indicating that glaciation may be used as an explanation for the observed differences in community composition in different faunal regions of Missouri. However, it would be necessary to survey the entire aquatic macroinvertebrate fauna to be sure. It is likely that physicochemical variables are also important in determining community composition, which is another question being addressed by this research. It should be noted that the communities proposed in the paper by Williams and Williams (1998) may not fit Missouri springs exactly because the communities presented were for “permanent” spring systems. Several of the Missouri springs sampled do dry up partially during times of drought. All springs that dried up during the fall of 2007, thus postponing our sampling efforts until the fall of 2008, still had moist substrate. The reason for rescheduling sampling efforts until the following fall season was due to the lack of flowing water, not due to the lack of water in general, thus the applicability of the spring types based on glaciations depend on the definition of “permanent”.

Although there are several hypotheses that attempt to explain the community composition of spring systems, based on the life history of the target organisms and the habitat from which they were collected, it appears that community composition may be related to the number of available mesohabitats. Those springs that were isopod dominated (eg. Prairie and Big River springs) had less aquatic vegetation than did the Ozark springs, which were dominated by amphipods, but also had a much higher proportion of aquatic insects. When making visual comparisons between small springs, as studied here, and larger springs, covered in the next chapter, larger springs have more mesohabitats and a higher proportion of aquatic insects, which too suggests that the availability of mesohabitats may influence whether a system is dominated by crustaceans or insects.

Does the aquatic insect, amphipod, and isopod community composition change longitudinally within each spring system?

Line graphs showing longitudinal shifts in the proportion of aquatic insects, amphipods, and isopods do not appear to show a unidirectional change in all cases (Figures 32-37). The changes in these proportions appear to vary by spring system and are not consistent between sampling periods. In both sampling years for all springs, the dominant groups in each spring increase and decrease at various increments downstream. Springs 3TT and OSOC are not included because their hypocrenes were too short to construct line graphs. MSLO appears to be the only Ozark spring for which a pattern exists. In both 2007 and 2008, the proportion of aquatic insects increases with distance from the eucrene, whereas the proportion of amphipods decreases. Abiotic factors, such as environmental gradients, or biotic factors, such as competition between the dominant groups, may be hypothesized as possible reasons for these patterns, although the actual drivers of these longitudinal changes are unknown. CSCR appears to be the only Prairie spring for which an obvious pattern exists. In 2007, the proportion of amphipods decreases with distance from the eucrene, whereas isopods increase. This pattern also exists in LSCR, although not as strongly. In CSCR in 2008, the proportion of amphipods and isopods appear to increase and decrease at various increments downstream, possibly suggesting competitive interactions between them. Appendix A shows the abundances of aquatic insects, amphipods, and isopods at each site within each spring system. It is important to note that these trends are represented by a limited number of individuals, as aquatic insects were not abundant in these spring systems. Because of the paucity of aquatic insects present in these small spring systems, line graphs showing longitudinal shifts in the proportion of individuals in each insect order were not constructed. In all spring systems, fewer than 25 individual aquatic insects were collected, thus it would not be possible to detect a pattern.

Line graphs showing longitudinal shifts in species richness do not show strong patterns within spring systems, among spring systems, or between seasons within the same spring system (Figures 38-40). For previously stated reasons, line graphs were not constructed for springs 3TT and OSOC. Spring 2DM only flows 50 m before converging with another stream system, which is rather short for a pattern to emerge, however, in 2007 there is a decrease in species richness with distance from the eucrene. In MSLO in 2007, there is a gradual longitudinal increase in species richness down the hypocrene, but multiple peaks and troughs in species richness in 2008. In all other spring systems, species richness does not show a directional change, possibly because species richness is low overall. Appendix B shows species richness values for each site within each spring.

Line graphs showing longitudinal shifts in Shannon diversity and evenness show that strongest patterns in species diversity and evenness were seen in MSLO and CSCR in both 2007 and 2008. The Ozark spring MSLO exhibited a longitudinal increase in species diversity and evenness in both years (Figure 41), whereas the Prairie spring CSCR showed a longitudinal decrease in species diversity and evenness in both years (Figure 42). These two springs had the highest overall species richness, which may be why strong trends were more evident. The remaining springs, LSCR, 1RB, CSM and 2DM, had lower species richness (Figures 43-46), thus directional trends would be difficult to detect with few species present. Again, line graphs were not constructed for 3TT and OSOC due to their short hypocrenes. Individual Shannon diversity and evenness values for each site within each spring can be found in Appendix C.

Overall, the lack of strong patterns suggests that it may be necessary to sample further downstream. It is unknown at what point down the hypocrene spring systems become like that of a normal stream system, but it may be necessary to extend the sampling reach in order to pick up directional changes in richness, diversity, or evenness, as well as changes in the proportion of aquatic insects, amphipods, and isopods. However, it should be noted that several of the sampled spring systems did not flow the full 400 meters before converging with a higher order stream, thus it would not be possible to extend the sampling reach further downstream. It may be helpful to add sampling sites from the stream in which the spring system converges so that the spring community can be compared to the stream community.

When looking at the spring community as a whole, those springs that are located within the same aquatic faunal region had the most similar communities; however, when looking at longitudinal changes within spring systems, the results do not show a similar trend. For example, all Ozark springs do not display similar longitudinal changes, nor do Prairie and Big River springs. Based on these results, it may be that each individual spring is unique and the conditions within each spring determine what the community will consist of, thus examining the influence of various environmental variables on longitudinal changes in community measures is one of the objectives addressed later in this portion of the study. In addition, it is not always possible to fit entities into a clear, definitive category, which may be the case with spring communities. Thus, it is necessary to sample additional spring systems, as these analyses were conducted in only eight of the 3,000+ springs in Missouri.

Is aquatic insect, amphipod, and isopod community composition in each spring related to environmental gradients?

Canonical correspondence analysis (CCA) was used to relate community composition to measured environmental variables. The ordination graph resulting from CCA has three pieces of information plotted on it: sites, which are denoted by a closed dot; species, which are denoted by an X; and, environmental variables, which are represented by lines radiating from the center of the plot. Those sites which are plotted in close proximity to one another have similar community composition and are similar in their environmental conditions. Species points are plotted nearest sites at which they are found in maximum abundance. The further one gets from a species point, the lower the probability of finding that species. The measured environmental factors that are considered important are represented by those environmental lines that emanate toward or directly away from site or species points, thus if an environmental line was extended out in both directions across the entire plot it would represent a gradient. The direction in which an environmental line points indicates the end of the gradient with high values, whereas 180˚ from the direction the arrow is pointing indicates low values. Stated differently, the direction of an arrow indicates the direction of maximum change. If a site or species point is located perpendicular to an environmental line, it indicates that that particular environmental variable is not important at that site or to that species. The length of an environmental line represents its importance, thus longer lines indicate that an environmental variable is important at the sites and/or to the species at which it is radiating toward and a shorter line indicates the environmental variable is less important. The axes on which sites, species, and environmental variables are plotted are linear combinations of the environmental variables. Those environmental lines that are parallel or nearly parallel to an axis indicate what the axis represents. The angle between environmental lines indicates the correlation between environmental variables. In general, the point of CCA is to pick out species optima and identify ecological gradients.

In 2007, the total inertia was 4.8896 and the eigenvalues for the first three CCA axes were 0.891, 0.545, and 0.286, respectively (Table 4). Only 35.2% of the variation was explained within the first three axes. Although this number is rather low, meaningful gradients can be picked from the CCA plot (Figure 47). In addition, the Pearson correlation shows that the correlation between species data and environmental variables was 0.984, 0.825, and 0.888 for the first three axes, respectively, which is rather high, indicating that natural gradients do occur and affect species composition. On the CCA plot it is very easy to pick out patterns similar to those shown by the cluster analysis used to compare communities among spring systems. Those springs located in the Prairie and Big River Faunal Regions group together on the right side of the axis 2 line and those located in the Ozark Faunal Region group together on the left side of the axis 2 line, again indicating that the community composition in springs in each faunal region are dissimilar. In addition, MSLO, which is the Ozark spring that was shown by the cluster analysis to have a dissimilar community composition to all other spring systems, is plotted in the lower left corner of the CCA plot away from all other site and species points, again indicating that it has a dissimilar community to the other spring systems. It is also possible to pick out the dominant species associated with the springs in these faunal regions. The species points for *C. brevicauda* and *C. forbesi* are located near the site points for the Big River and Prairie spring sites, indicating that as you move away from these sites in the Prairie and Big River Faunal Regions you are less likely to find these species. The species point for *G. minus* is located near the centroid of the site points for the Ozark springs, again indicating that as you move away from these Ozark sites you are less likely to find this species. Last, the species point for *G. pseudolimnaeus* is located near the site points for the spring MSLO, indicating that this is where it is found in highest abundance. Environmental lines indicate that Big River and Prairie springs have opposite environmental characteristics of Ozark springs, which may explain the differences in community composition between the faunal regions. For example, the Prairie and Big River springs have high alkalinity, high specific conductivity, high temperature, shallow depth, low pH, narrow channels, low dissolved oxygen, and low velocity, whereas those springs in the Ozark faunal region have the exact opposite conditions. Overall, alkalinity, water velocity, and dissolved oxygen appear to be the most important factors, as the environmental lines for these variables on the plot are the longest. Specific conductivity, temperature, and channel width have moderately long lines, indicating that they may be influential as well, whereas the lines for canopy cover, pH, and maximum water depth are represented by the shortest lines, indicating that these environmental variables are not as important. When looking at species points in relation to environmental variables, it is also possible to pick out the environmental variable(s) that is most important to the dominant species. Low dissolved oxygen and high specific conductivity appear to be important to *C. brevicauda,* which is the dominant isopod species in the Prairie springs, LSCR and CSCR, and Big River springs, 1RB and 3TT. *C. forbesi* is the dominant amphipod in LSCR, CSCR, 1RB, and 3TT and appears to have the same environmental requirements and preferences as *C. brevicauda*. High current velocity appears to be most important to *G. minus*, whereas low alkalinity and high water depth appear to be most important to *G. pseudolimnaeus*. *G. minus* is the dominant amphipod species in the Ozark springs 2DM, CSM, and OSOC and *G. pseudolimnaeus* is the dominant amphipod species in the Ozark spring MSLO.

In 2008, the total inertia was 5.2022 and the eigenvalues for the first three CCA axes were 0.826, 0.665, and 0.353, respectively (Table 5). Only 35.4% of the variation was explained within the first three axes. Although this number is rather low, meaningful gradients can still be picked from the CCA plot (Figure 48). The Pearson correlation shows that the correlation between the species data and environmental variables was 0.958, 0.928, and 0.787 for the first three axes, respectively, which again indicates that gradients exist and these gradients affect species composition. As with the CCA results from the spring sampling season, it is possible to pick out patterns similar to those shown by the cluster analysis used to compare communities among spring systems. The Prairie and Big River springs, plus MSLO, are scattered throughout the right half of the CCA plot, indicating the communities in these springs are not extremely similar in terms of their community composition. However, there are indeed similarities. These springs clustered together loosely in the cluster analysis as well. LSCR and 1RB were most similar, which is also shown on the CCA plot, as these two springs are grouped in the upper right quadrant of the plot. CSCR and MSLO were also somewhat similar in community composition based on the cluster analysis, which too is shown on the plot. The majority of the sites in these springs group in the lower right quadrant of the plot, although there are a couple of the sites at CSCR that group with LSCR and 1RB. This explains why MSLO and CSCR are not more similar to one another because only the community composition in some of the CSCR sites are similar to the community in

MSLO, whereas the remainder of the sites are more similar in community composition to LSCR and 1RB. On the left side of the CCA plot just above axis 1 are the site points for the Ozark springs. These points are grouped together closely, indicating they have similar community composition, as shown in the cluster analysis. In the Prairie and Big River springs LSCR and 1RB, high temperature and low dissolved oxygen are the most important environmental variables. Canopy cover is also important, although less so than temperature and dissolved oxygen. In the Prairie spring CSCR and the Ozark spring MSLO, the environmental variables that are most important vary among sites within the spring. In the remaining Ozark springs CSM, OSOC, and 2DM, low specific conductivity is the most important variable, although high alkalinity is also somewhat influential. As in 2007, it is possible to determine which environmental variables are most important to the dominant species. Low dissolved oxygen and high temperature appear to be important to *C. brevicauda*, which is the dominant isopod species in the Prairie springs, LSCR and CSCR, and Big River spring 1RB. High current velocity and narrow channel width appears to be most important to *G. minus*, whereas low current velocity and high water depth appear to be most important to *G. pseudolimnaeus*. *G. minus* is the dominant amphipod species in the Ozark springs 2DM, CSM, and OSOC and *G. pseudolimnaeus* is the dominant amphipod species in the Ozark spring MSLO and the Prairie spring CSCR.

It is important to note that substrate types were not included in the analysis. Originally, substrate types were transformed into dummy variables and included in the CCA. Dummy variables were created because of the difficulty of making substrate types into continuous variables. The lines representing these substrate types on the CCA plot were short, indicating they were not as important as other environmental variables shown. Also, the addition of these eight lines made the CCA plot very crowded and difficult to interpret. For these reasons, substrate was removed from the analysis. The complete CCA output can be found in Appendix D.

Based on the CCA results from 2007 and 2008, the environmental conditions present in each spring system correspond with the faunal region in which each exists, which may explain why community composition also corresponds with the faunal region in which each spring can be found. This may also explain why spring systems in different faunal regions have different species dominating them. These species appear to require and/or prefer different environmental variables from one another, resulting in distinct community compositions. Those gradients that emerged as being important in each faunal region make valid ecological sense. Springs in the Prairie and Big River regions have lower water velocity because the slope of the land is less than that in the mountainous Ozark region of Missouri. Also, in general, mountain springs have cooler temperatures, which would result in higher dissolved oxygen levels, whereas the Prairie and Big River springs have shallower depth, which affects water temperature. Warmer water in these springs, therefore, results in lower dissolved oxygen levels. It is important to note that the environmental variables measured were only a few of the many variables that could be measured. Those variables picked for measurement were amongst the common physicochemical variables measured in aquatic studies. The majority of the habitat variables measured were physical variables, with few chemical variables measured; however, there are numerous chemical variables that could be measured, any of which could prove to be important to community composition.

It is known that the dominant species sampled in these spring systems are most commonly associated with springs and spring-fed streams; however, no research has studied the physicochemical preferences of these species, with the exception of *G. minus*. Based on a study in Pennsylvania rheocrene spring systems, Glazier et al. (1992) showed that *G. minus* is absent from springs with a pH less than 6.0 and with a conductivity less than 25µS cm^{-1}. Although the CCA did not specifically indicate that pH and conductivity were influential environmental variables affecting the distribution of this species among the spring systems, all springs, including those from which *G. minus* was collected, had a pH greater than 6.0 and a conductivity greater than 25µS cm^{-1}. Thus, these findings do correspond with the only data known to attempt to explain the habitat preferences of *G. minus*. There were, however, environmental variables that emerged as being influential to dominant species in both sampling seasons, which may be an indication of those variables that truly are important to the distribution of certain species. For example, in both sampling seasons, low dissolved oxygen showed to be important to *C. brevicauda*. High current velocity was found to be important to *G. minus* in both sampling seasons, whereas high water depth showed to be important to *G. pseudolimnaeus* in both sampling seasons. It would be helpful to sample these species in other similar spring systems in Missouri to determine if these particular environmental variables correspond with the highest abundance of these species collected as well.

Are longitudinal changes in community measures, such as species richness, Shannon diversity, Shannon evenness, and the density of dominant species, influenced by environmental gradients?

Stepwise regression is a method used to determine the minimum set of predictors required to explain the observed variation in response variables (McCune and Grace, 2002). In this case, environmental gradients are used to explain the observed longitudinal shifts in species richness, Shannon diversity, Shannon evenness, and density of the dominant species within each spring system individually. This procedure added environmental variables into the multiple regression model until the best subset of predictor variables were able to explain the greatest amount of variance possible. The method for which it does this can be found in the Minitab Help option. The alpha-to-enter and alpha-to-remove were set at 0.15, which is the default setting in Minitab 15. This alpha level provided criteria that were not too loose and not too stringent so that the best possible model could be determined. Table 6 lists the environmental variables that were indicated by the stepwise regression to be responsible for the observed changes in species richness, Shannon diversity, and Shannon evenness in each spring system for each sampling season. The corresponding adjusted R^2 value is also shown in this table. Table 7 lists the environmental variables that were indicated by the stepwise regression to be responsible for the observed changes in the density of dominant species in each spring system for each sampling season. The dominant species in each spring is listed, as well as the corresponding adjusted R^2 value. Note that the adjusted R^2 values are reported because of the number of independent variables included in each model; however, the complete output from these stepwise regressions, including p-values for each step, corresponding simple R^2 values, and other associated values, can be found in Appendix E.

Results do not reveal a solid pattern in influential environmental variables on species richness, Shannon diversity, or Shannon evenness. The environmental gradients influencing longitudinal changes in species richness, Shannon diversity, and Shannon evenness vary between spring systems, as well as between seasons within the same spring system. However, there were a few minor consistencies presented from the analyses and adjusted R^2 values were high in most instances, thus indicating that those environmental variables composing the model explained most of the variability within the response variables. For example, in the Prairie spring LSCR, DO and SC emerge in both seasons as being influential environmental variables, but with a different combination of variables in the model each time. These two environmental variables also appear to be important in this particular spring system in the CCA results in 2007, but not in 2008. In the Ozark spring MSLO, pH and DO appear in both seasons as being influential environmental variables, but also with a different combination of variables in the model each time. The CCA results for both 2007 and 2008 show pH to be somewhat influential to this particular spring system as well, however, the CCA results do not show DO as an influential environmental variable to this spring, with the exception of the 100 meter site in 2008.

When looking at the stepwise regression results for species richness for both sampling seasons, adjusted R-squared values ranged from 92.08% to 100%, with three exceptions. In the Prairie spring LSCR in 2007, DO was the only explanatory environmental variable in the model and it only accounted for 64.63% of the variation in species richness. In the Big River spring 1RB in 2008, maximum depth was the only variable in the model and it only accounted for 47.25% of the variation in species richness. Last, in the Ozark spring 2DM, none of the environmental variables could explain the observed changes in species richness. In the stepwise regression results for Shannon diversity for both sampling seasons, adjusted R-squared values ranged from

70.79% to 100%, with most values falling above 95%. In 2008, the Prairie and Big River springs LSCR, CSCR, and 1RB had adjusted R-squared values of 74.04%, 79.85%, and 70.79%, respectively. In addition, the Ozark spring CSM had no environmental variables that explained the observed changes in Shannon diversity in 2007. In the stepwise regression results for Shannon evenness, the adjusted R-squared values were scattered. In 2007, both LSCR and MSLO had no environmental variables that explained the observed changes in Shannon evenness. In 1RB, SC only explained 43.58% of the variation. In CSCR, CSM, and 2DM, 79.57%, 99.91%, and 99.02%, respectively, of the observed changes in Shannon evenness could be explained by a combination of the measured environmental variables. In 2008, all three Ozark springs had high adjusted R-squared values (99.66% - 99.97%) with models that included channel width. Two of the three models also included temperature. As in 2007, LSCR had no environmental variables that explained the observed changes in Shannon evenness, whereas canopy cover only explained 58.95% of the variation in 1RB and pH only explained 63.82% of the variation in CSCR.

Results also did not reveal a solid pattern in influential environmental variables on the density of the dominant species in each spring system either; however, once again there were some consistencies. For example, the stepwise regression showed current velocity to be an influential environmental variable to the longitudinal changes in density of *G. minus*, although it has shown to be influential in two different spring systems in different seasons. However, both springs from which *G. minus* were collected are in the Ozark faunal region. The CCA results for both sampling seasons also show current velocity to be an influential environmental variable to this species. As another example, the stepwise regression showed current velocity, DO, and pH to be influential environmental variables to the density of *C. brevicauda*, although not always within the same spring. Current velocity was one of the influential environmental variables to this species in 1RB in 2007 and in LSCR in 2008, whereas DO and pH are influential environmental variables in LSCR in both 2007 and 2008. In the CCA results for both sampling years, DO is also shown to be one of the environmental variables that is highly influential to *C. brevicauda*. Overall, patterns in influential environmental variables that emerged in the CCA are also shown in part for some springs by the stepwise regression.

Although the majority of R^2 values are rather high, indicating that a large portion of the overall variability is explained by the indicated environmental variables, it is important to note that the environmental variables measured make up only a small portion of the total number of variables that could be measured. The point of a stepwise regression is to simply whittle down a large number of predictor variables to those that appear to be the most significant. Of course, this works under the assumption that the most important environmental variables were measured. In some cases where the adjusted R-squared value is low, it may indicate that other variables not measured are also important to the measured community measures. However, with that said, even those that did have high adjusted R-squared values may have other environmental variables that are just as or more important. It should also be noted that substrate type was originally included in the analysis; however, in most cases the results were similar whether substrate type was included or not. For those instances where substrate type showed to be an important environmental variable, the results with substrate type excluded were stronger, thus substrate type was removed from all analyses. In addition, OSOC and 3TT are not included in these analyses because of their short hypocrenes and are therefore not listed in the result tables.

Conclusions

Several species of conservation concern and rare species were collected from these spring systems, thus emphasizing the importance of proper management and conservation plans. Overall, communities sampled in each hypocrene in 2007 and 2008 support that spring communities differ among spring systems and correspond with the aquatic faunal region in which each can be found. Further examination of the community also supports this conclusion. Springs located within the same faunal region have similar dominant species in their community, with the exception of the Ozark spring MSLO, which has such high diversity that it is dissimilar to all other spring systems. This information can be used to implement management and monitoring plans that are appropriate for the communities found in spring systems in the different faunal regions. The fact that spring systems in different faunal regions are dominated by particular groups of insects, which are associated with particular environmental conditions, can be used as a monitoring tool. If the density of these dominant species changes drastically during continuous long-term monitoring of these systems, it can be used as an indication of changing environmental conditions, which may indicate pollution, contamination, or alteration of the environment. Using these dominant species as indicator species within a system can also help natural resource scientists manage those rare species and species of conservation concern, whose abundance is not great enough to monitor directly. Further, this research is a stepping stone for future researchers and scientists who wish to understand the species composition of Missouri spring systems.

CHAPTER 3

Comparative Analysis of Aquatic Insect, Amphipod, and Isopod Communities in High Discharge Rheocrene Spring Systems in Missouri

Description of Study Sites

The first qualitative portion of this study took place in four high discharge rheocrene springs (Figure 49): Bennett Spring at Bennett Spring State Park (Figure 50), Ha Ha Tonka Spring at Ha Ha Tonka State Park (Figure 51), Montauk Spring at Montauk State Park (Figure 52), and Roaring River Spring at Roaring River State Park (Figure 53). Below is a description of each individual spring.

Bennett Spring at Bennett Spring State Park (BS)

Bennett Spring State Park, one of Missouri's many trout parks, is among the oldest and most popular parks in Missouri (MDNR, 2008). One of the main attractions in this park is Bennett Spring and its trout fishery. Bennett Spring is located in Dallas County and is Missouri's third largest spring, discharging over 100 million gallons of water daily into a circular pool to form Bennett Spring Creek (Vineyard and Feder, 1982). After flowing approximately 2.4 km, this tree-shaded creek converges with the Niangua River.

Although Bennett Spring is one of the most studied springs in Missouri in terms of hydrology (D. Tucker, pers. comm.), extensive studies of particular groups of organisms have not been conducted. However, catalogs of some of the plant, fish, amphibian, reptile, bird, mammal, and invertebrate species of the park do exist. Two of the more popular species of organisms in the park are the non-native rainbow trout, *Oncorhynchus mykiss* Walbaum, and brown trout, *Salmo trutta* Linnaeus, which are reared in the trout hatchery and stocked in the spring. Many trout fishermen come daily during trout season and are allowed to wade in the water while fishing.

Ha Ha Tonka Spring at Ha Ha Tonka State Park (HTS)

Ha Ha Tonka State Park is located in Camden County in the Osage River Hills region of the Ozark Mountains. This park is located near Lake of the Ozarks and exhibits many karst features, including sinkholes, caves, high bluffs, and springs. Ha Ha Tonka Spring, the largest spring in the park, is Missouri's twelfth largest spring (MDNR, 2008). The hypocrene flows approximately 0.54 km before entering the Lake of the Ozarks (L. Webb, pers. comm.). Vegetation is abundant in much of the hypocrene. There are no trout species in this spring and wading is not allowed.

Montauk Spring at Montauk State Park (MS)

Montauk Spring is located in Dent County in southeast Missouri within Montauk State Park, one of the many trout parks in Missouri. Montauk Spring is one of the ten largest springs in Missouri, discharging approximately 43 million gallons of water daily (MDNR, 2008). The cold waters of Montauk Spring flow approximately 100 m before converging with Pigeon Creek to form the headwaters of the Current River (MDNR, 2008), a popular and well known river in Missouri that is protected by the Ozark National Scenic Riverways. Like many spring systems, watercress is abundant in many areas of the hypocrene. The streams in this state park are stocked with non-native rainbow and brown trout from the trout hatchery in the state park, however, Montauk Spring is not directly stocked with trout. Trout can, however, swim upstream into the spring, thus trout are found in Montauk Spring. Neither trout fishing nor wading are allowed in Montauk

Spring, although trout fisherman are allowed to wade in those streamways with which Montauk Spring converges.

Roaring River Spring at Roaring River State Park (RRS)

Roaring River Spring is located in Roaring River State Park in Barry County in southwest Missouri. Roaring River Spring is the 20th largest spring in Missouri, discharging an annual average flow of 20.4 million gallons per day (Cassville Area Chamber of Commerce, 2008). The spring water emerges through a fault in the ground at the base of a high cliff, giving the blue water the appearance of emerging from a vaulted cave. In addition, a smaller spring emerges from the top of this fault, thus water trickles off the high bluff into the spring below. The water emerging from Roaring River Spring gathers in a large pool before flowing through and supplying water to the trout hatchery. This trout hatchery rears non-native rainbow and brown trout which are stocked in Roaring River. The water from the hatchery is eventually released into this river as well. Though Roaring River is a popular trout fishing location, wading is not allowed in the river, thus protecting the flora and fauna living there.

Study Questions

One of the goals of this portion of the study was to create a comprehensive list of aquatic insect, amphipod, and isopod taxa collected from each of these spring systems, with special notation of state and federally listed species, undescribed species, rare species, and/or new records for the state of Missouri. In addition to this goal, there was one main question addressed in this portion of the study: Does aquatic insect, amphipod, and isopod community composition differ among spring systems?

Methods

Qualitative sampling of the four high discharge rheocrene spring systems occurred in August 2007 and May 2008. Prior to biological sampling, observations of the surroundings (human disturbances, dams, etc.), available habitat, and other physical characteristics that may have been pertinent to the study were recorded. Once initial observations were recorded, the hypocrene was marked at ±2.5 m of 10 m, 25 m, 50 m, 100 m, 200 m, and 400 m from the eucrene in the same manner described in the previous chapter. At each sampling location, samples were taken with an aquatic D-net (24 x 20 openings per inch mesh). Samples were taken in each aquatic mesohabitat (e.g., gravel substrate, vegetation, leaf pack) at each marked increment until no recognizably new morphospecies were taken in two consecutive samples. If the hypocrene converged with another stream before reaching 400 m, sampling began at the greatest distance increment before convergence. Sampling began downstream and progressed upstream, thus the eucrene was the last to be sampled. No physicochemical parameters were measured in this portion of the study.

Supplemental qualitative samples were taken using drift nets at Bennett Spring, Montauk Spring, Ha Ha Tonka Spring, and Roaring River Spring in August 2007 and at Bennett Spring and Ha Ha Tonka Spring in May 2008. Drift nets were not placed in Montauk Spring and Roaring River Spring in May 2008 due to high water. A supplemental sample via blacklight trap was taken at Roaring River Spring in August 2007 because this state park had an available trap. Drift nets were placed at approximately 400 m downstream from the eucrene by Missouri Department of Natural Resources (MDNR) personnel. The blacklight trap was placed near the eucrene the evening before sampling occurred (~24 hours). Drift nets were used to take supplemental samples because the hypocrenes were deep enough to accommodate the drift nets.

All samples were transported to the laboratory at the University of Missouri in Columbia in separate labeled containers containing 80% ethyl alcohol. In the lab, all samples were sorted and identified to the lowest possible taxonomic level.

Statistical Analysis

Taxa were counted to determine order, family, genus, and species richness for all springs together during each sampling season. Species richness values were also determined for each individual spring system. These values were used to summarize the community data collected from the studied spring systems during this portion of the study.

To determine whether aquatic insect, amphipod, and isopod community composition varies among spring systems, a similarity and cluster analysis using qualtitative data from the eucrene and hypocrene was conducted using Sorenson's similarity coefficient and the unweighted pair-group method using arithmetic means (UPGMA) clustering algorithm in PC-ORD version 4.10 (McCune and Mefford, 1999). Because only qualitative data were collected, one cluster analysis was performed using the combined data from both sampling seasons.

Results and Discussion

In the fall of 2007, a total of 1,104 specimens was collected (Table 8). Eight orders, 39 families, 73 genera, and 83 species were collected, of which 6 orders, 35 families, 67 genera, and 76 species were insects. In the spring of 2008, a total of 1,493 specimens was collected (Table 9). Eight orders, 36 families, 61 genera, and 64 species were collected, of which 6 orders, 31 families, 55 genera, and 56 species were insects.

In both sampling seasons, the taxonomic richness (Table 10) of amphipods and isopods are similarly low at all springs, although these groups are characteristically low in diversity. In terms of aquatic insect taxonomic richness, Roaring River Spring had the highest family, genus, and species richness, whereas Ha Ha Tonka Spring had the lowest family, genus, and species richness in the fall of 2007. In the spring of 2008, the roles were reversed, where Ha Ha Tonka Spring had the highest family, genus, and species richness and Roaring River Spring had the lowest family, genus, and species richness. Overall, counting both sampling seasons together, Montauk Spring had the highest family, genus, and species richness, although all springs were very close in taxonomic richness. Montauk Spring may have a slightly higher taxonomic richness because it has several unique species that were not sampled from the other spring systems. For example, *Ceratopsyche piatrix* Ross (Hydropsychidae), which is endemic to the Interior Highlands and ranked as an S4 (apparently secure, but uncommon) on the Missouri species of conservation concern list, was sampled from Montauk Spring. *Caecidotea antricola* Creaser, a blind, subterranean species of isopod, is also ranked as an S4 on the Missouri species of conservation concern list and is endemic to the Ozark Highlands and *Serratella frisoni* (McDunnough), an ephemerellid mayfly, is ranked an S2 (imperiled) in the state of Missouri, both of which were also collected from Montauk Spring.

Although they are not ranked on the Missouri species of conservation concern list, three additional notable species were collected from other spring systems as well, all of which are endemic to the Interior Highlands. *Micrasema ozarkana* Ross and Unzicker was collected from Bennett Spring and *Helicopsyche limnella* Ross was collected from Ha Ha Tonka Spring, both of which are species of caddisfly (Trichoptera). *Stygobromus alabamensis* (Stout), a troglobitic amphipod, was collected from both Montauk and Roaring River Springs in the fall 2007 sampling season.

The aquatic insect, amphipod, and isopod community in these large rheocrene spring systems is composed primarily of aquatic insects. Although quantitative samples were not taken, it is estimated that less than 20% of the individuals collected were amphipods and isopods. Thus, the community composition of these larger rheocrene spring systems appears to differ greatly from that of smaller rheocrene spring systems in Missouri, which have a much higher proportion of amphipods and/or isopods. The reason could be related to the greater number of mesohabitats and increased habitat heterogeneity in large spring systems compared to small spring systems.

Sorenson’s similarity coefficient and UPGMA cluster analysis (Figure 54) shows that Montauk Spring and Roaring River Spring have the most similar community compositions. Both of these springs house rainbow and brown trout, however, wading is not allowed by trout fisherman. Bennett Spring shares many of its species with Montauk and Roaring River Springs, but also has its own distinct species. This spring also has brown trout and rainbow trout, however, wading is allowed in the spring by fisherman. The water of all three springs feed trout hatcheries in the state parks and are all stocked with trout from the hatchery. The fourth spring, Ha Ha Tonka Spring, is the most dissimilar and has few species shared with the other three spring systems. This spring system does not feed a trout hatchery and it is not stocked with trout, nor does it contain any species of trout. In addition, no wading is allowed in this spring.

Because trout feed on macroinvertebrates, it is likely that the presence of trout affects species composition of the macroinvertebrate community. It is often assumed that trout have an effect on macroinvertebrate communities because of top-down relationships, but this assumption is frequently made without scientific evidence to support it (Englund and Polhemus, 2001). There is a limited number of studies that examine the effect of trout species, especially non-native trout species as are found in Missouri spring systems, but those studies present conflicting evidence. Englund and Polhemus (2001) did not find a difference in density or taxonomic richness of native stream insects in Hawaiian streams with trout versus those without trout; however, Molineri (2008) found that subtropical mountain streams with rainbow trout in northwestern Argentina had a different aquatic invertebrate community structure than streams without trout. Studies have evaluated aquatic macroinvertebrate communities in lentic systems with trout, where community composition and/or density differences have been noted (Luecke, 1990; Finlay and Vredenburg, 2007). Based on these studies, it appears that the effect of trout on macroinvertebrate communities may differ depending on the type of aquatic system (lotic vs. lentic) and geographical region. Thus, based on our knowledge of top-down interactions, we can only speculate whether the presence or absence of non-native trout in large rheocrene spring systems in Missouri is influential on community composition. In addition, it is known that human disturbances have an effect on biological communities; however, no studies have examined the effect of wading by trout fisherman on benthic communities. The disturbance of the substrate by wading fisherman probably affects the benthic community, and as benthic organisms become suspended in the water, they are more vulnerable to trout and other predators. The extent to which this affects the community is unknown. The clustering of these four large rheocrene spring systems based on the presence of trout and wading by trout fisherman suggests that these variables may play a role in aquatic insect, amphipod, and isopod of community composition. It is necessary to sample other large rheocrene spring systems in Missouri that harbor trout and compare them with those that do not contain trout to determine if these variables play a role in shaping community composition.

Conclusions

Cluster analysis shows that three of the four sampled springs have differing amounts of dissimilarity in aquatic insect, amphipod, and isopod community composition, thus demonstrating the individuality of the spring systems. The presence of trout and trout fisherman may have an influence on community composition, as shown by the high similarity between Montauk and Roaring River Springs and the high dissimilarity of Ha Ha Tonka Spring, which is the only spring with no trout, and thus no trout fishing. The presence of uncommon and imperiled species and species of conservation concern provide state park personnel with vital information which can be used for educational and management purposes. The comprehensive list of species also provides park managers with information needed to determine which species may be important to monitor when tracking long-term changes in the aquatic insect, amphipod, and isopod community composition. Tracking these changes over time will help managers identify possible changes in water quality and other environmental conditions.

CHAPTER 4

Inventory of Aquatic Insect, Amphipod, and Isopod Species in Unique Spring Systems in Missouri

Description of Study Sites

The second qualitative portion of this study took place in four unique spring systems (Figure 55): a saline spring at Boone's Lick State Historic Site (Figure 56), seepage channels at Hawn State Park (Figure 57), spring-fed Oumessourit Marsh at Van Meter State Park (Figure 58), and a fen at St. Francois State Park (Figure 59). Unique spring systems are those defined by having physical and/or chemical properties different from those of typical rheocrene spring systems. Below is a description of each unique spring system.

Boone's Lick Spring at Boone's Lick State Historic Site

Boone's Lick Spring is a small saline spring located in Boone's Lick State Historic Site in Howard County. The waters of Boone's Lick Spring rise into a small circular pool before flowing into Salt Creek. The water discharged from the spring is of moderate salinity compared to seawater and is much more saline than that of Salt Creek. White salt crystals can be found forming on debris near the water. Surrounding the spring is the strong aroma of sulphur, which is the result of the mineralized water and occasional hydrogen sulphide bubbles rising to the surface. Little vegetation exists within the spring, with the dominant available habitats being silt and detritus. A Master's project conducted in this spring showed that the diversity of aquatic insects was low (Bonham, 1962), which is most often the case in brackish and saline waters. Saline seeps are ranked S1 on the Missouri communities of conservation concern list, which implies that these systems are critically imperiled in the state because of their extreme rarity or because of some factor(s) making it especially vulnerable to extirpation from the state; however, it is unknown if this small spring is classified as a "saline seep". If so, it underlines the importance of these types of systems.

Fen at St. Francois State Park

St. Francois State Park is located in east central Missouri in St. Francois County. One of the main attractions in this park is the Coonville Creek Natural Area, which houses a restored spring-fed fen. The main fen area at St. Francois State Park appears to be a 10 acre open prairie; however, within the grassy opening is a very diffuse network of springs and seeps which form very small drainages or channels that run through the grassy slopes to feed Coonville Creek. The ground between these small diffuse channels is soft, spongy, and saturated. The fen is home to many species of rare plants (MDC, 2010). Ozark fens are ranked S2 on the Missouri communities of conservation concern list, which implies that they are imperiled in the state because of their rarity or because of some factor(s) making them vulnerable to extirpation from the state, thus emphasizing the importance of this system.

Oumessourit Marsh at Van Meter State Park

Van Meter State Park is located in the Missouri River bottoms in Saline County. One of the main attractions at this park is the freshwater marsh in Oumessourit Natural Area. Oumessourit Marsh is a spring-fed marsh that is separated into two distinct sections: the south portion and the north, spring-fed portion. The spring that feeds the marsh can be accessed by Spring-Bluff Trail and flows year-round, although discharge is low. The spring water rises into a circular pool, flowing less than 50 meters before dispersing into the marsh. Although the aquatic insect community within the marsh has been studied briefly (unpublished), the aquatic insect community within the spring has not. Marshes are ranked S2 on the Missouri communities of conservation concern list, which implies that they are imperiled in the state because of their rarity of because of some factor(s) making them vulnerable to extirpation from the state, thus emphasizing the importance of this system.

Seepage Channels at Hawn State Park

Orchid Valley Natural Area is a 120 acre area of Hawn State Park in St. Genevieve County. Because this natural area houses many species of rare plants, including many species of orchids and ferns, the area is accessible by permit only. Orchid Valley Natural Area is characterized by steep valleys, cliffs, ledges, and ravines. Within these valleys one can find a dry, sand-bottomed stream bed with small, sparsely scattered pools formed by seeps. The discharge from the seeps depends upon the season and the amount of rainfall in the area. Acid seeps and saline seeps are ranked on the Missouri communities of conservation concern list as S2 and S1, respectively; however, it is unknown if these seepage channels fall into either of those categories.

Methods

Qualitative sampling of the four unique spring system was conducted in the spring and fall of 2008. The sampling protocol, including marking of the spring hypocrene, used at Boone’s Lick Spring and Oumessourit spring-fed marsh was identical to that used for the four high discharge rheocrene springs in the previous chapter; however, samples were also taken at the point where the spring enters the marsh at Oumessourit spring-fed marsh.

Since the seepage channels at Hawn State Park and the fen at St. Francois State Park are not typical rheocrene springs, the sampling protocol varied from that of typical rheocrene springs. At Hawn State Park, each main ravine within the natural area was followed until a seep was found. All mesohabitats within a particular seepage puddle were sampled. Some of the seeps sampled were present in both the spring and fall of 2008, however, some of the seeps were present during only one of the sampling periods. At St. Francois State Park, eight transects located 10 m apart were made across the width of the main fen area. All channels with water found across each transect (approximately 3-5 points per transect) were sampled for aquatic insects, amphipods, and isopods. Transect 7 fell along Coonville Creek, thus a small section of this creek was sampled and served as transect 7.

For all spring systems, each mesohabitat at each designated sampling location was sampled until no recognizably new morphospecies were taken in two consecutive samples. No physicochemical parameters were measured during this portion of the study. All samples were transported to the laboratory at the University of Missouri in Columbia in separate labeled containers containing 80% ethyl alcohol. In the lab, all samples were sorted and identified to the lowest possible taxonomic level.

Statistical Analysis

Because the spring systems in this portion of the study are unique and quite different from one another, in terms of the type of spring system they represent, there was no reason to perform statistical comparisons or any other statistical analyses. The purpose of examining these systems was solely to get a bioinventory of aquatic insect, amphipod, and isopod species present in these unstudied, unique habitats.

Results and Discussion

In the spring of 2008, a total of 464 specimens were collected (Table 11). In the fall of 2008, a total of 311 specimens were collected (Table 12). Few notable taxa were collected from these unique spring systems. *Helicopsyche limnella* Ross was collected from the spring-fed fen at St. Francois SP and is endemic to the Interior Highlands, as was *Dixa sp.* (Dixidae), which was identified by J.K. Moulton at the University of Tennessee in Knoxville as a possible new species. Further sampling in this fen aimed at collecting species of dixid larvae is currently being conducted so that additional larvae will be available for genetic work that may help determine if the *Dixa sp.* is indeed a new species.

In the spring of 2008, both the Boone's Lick Spring (saline spring) at Boone's Lick SHS and the seepage channels at Hawn SP had the lowest species richness. Both of these springs had a community composition composed entirely of aquatic insects. The spring-fed fen at St. Francois SP had the highest species richness. In the fall of 2008, Boone's Lick Spring again had the lowest species richness, whereas the seepage channels at Hawn SP had the highest species richness. As in the spring 2008 sampling season, no amphipods or isopods were collected from these two springs in the fall. Combining data from both sampling seasons, the spring-fed fen at St. Francois SP had the highest species richness, whereas the Boone's Lick Spring had the lowest species richness (Table 13). These results are logical because saline waters tend to have lower species richness than do freshwater systems and the fen at St. Francois SP covered a greater area than the other three systems.

Conclusions

The spring systems are unique for a variety of reasons; however, the aquatic insect, amphipod, and isopod communities sampled in these systems are not unique, although they are still important. All four of these systems either dry up periodically or are greatly reduced in flow during some part of the year, which may explain why common aquatic insect, amphipod, and isopod communities were found here. It would not make ecological sense for a rare species to inhabit a temporary habitat, as the species would then be extirpated once the system dries up, unless, of course, the species is rare because of its dependency on this type of habitat. Since these springs were sampled only twice, further sampling would increase the probability of sampling rare or uncommon species, especially if they exist in low abundance. Further sampling is needed in the fen at St. Francois SP, as the area over which this fen covers is very large and it is likely that additional species were simply not collected during the two sampling periods.

CHAPTER 5

Bioassessment of Missouri Spring Systems

One of the goals of this study was to provide MDNR with a report stating whether the current biomonitoring protocol used in Missouri streams involving the use of macroinvertebrates can be applied to rheocrene spring systems in the state as well. Species data collected from the quantitatively sampled low to medium discharge rheocrene spring systems (unnamed spring at Trail of Tears SP, unnamed spring at Rockbridge SP, Lone Spring at Cuivre River SP, Cave Spring at Cuivre River SP, unnamed spring at Dillard Mill SHS, Chickadee Spring at Meramec SP, Mill Spring at Lake of the Ozarks SP, and Onondaga Spring at Onondaga Cave SP) and qualitatively sampled high discharge rheocrene spring systems (Montauk Spring, Roaring River Spring, Bennett Spring, and Ha Ha Tonka Spring) were used to make these conclusions.

Results and Discussion

As stated in previous chapters, the sampled low to medium discharge rheocrene spring systems have a high proportion of amphipods and/or isopods and a lower proportion of aquatic insects in general. More specifically, those springs in the Prairie faunal region of Missouri are dominated by both amphipods and isopods, whereas those springs in the Big River faunal regions appear to be isopod dominated and Ozark springs appear to be amphipod dominated. The nature of the community composition in these springs may cause issues when trying to apply stream indices to spring systems using three of the four primary metrics. For example, it is generally thought that a system with a greater number of taxa is healthier, which is the basis for the Taxa Richness metric and

Shannon Diversity Index. However, in small rheocrene springs in Missouri that are dominated by only one or two species of amphipod and/or isopod, the lack of diversity may not actually imply an unhealthy system.

Various patterns have been revealed in this study regarding differences in biodiversity among spring systems, thus one cannot make the conclusion that low biodiversity in spring systems indicates an unhealthy system until patterns in community composition are better understood through further research. For example, if a certain community exists as it does in a spring system because of the chemical characteristics of the water resulting from the geology of the area in which the spring exists, low biodiversity is the result of the natural characteristics of the spring, not a disturbance or other phenomenon. In the MDNR report describing the protocol used for the bioassessment of stream systems, it is even stated that headwater streams may be less productive and support a limited number of taxa. This indicates that it is at least partially understood that these two metrics may be unsuitable for use in headwater streams.

The third metric that may also be unsuitable for use in these small rheocrene spring systems is the EPT (Ephemeroptera, Plecoptera, Trichoptera) Index. These three orders of insects are used for this particular index because they are considered to be pollution sensitive, thus if they are not found or are found in low density it indicates that the system may have been influenced by pollution of some sort. It may not be feasible to use this index in small rheocrene spring systems because they have a very low aquatic insect diversity overall. This low diversity, including very few, if any, EPT taxa, more than likely is not caused by a pollution or other disturbance event. This conclusion is based on the fact that even chironomid (midge) larvae were sparse and certain groups of chironomid larvae are generally considered to be tolerant of pollution. Again, the absence of EPT taxa, and aquatic insects in general, is more than likely not the result of poor system quality and health, but rather a physical or chemical variable related to the geology of the area in which the spring is found or biological interactions within the system.

The fourth primary metric, the Biotic Index (BI), is one metric that may be useful in small rheocrene spring systems in Missouri. With the Biotic Index, each taxon present is assigned a pollution tolerance value that ranges from 1-10. The overall tolerance value for the community is determined based on the assigned values for each taxon, which also ranges from 1-10, where 1 indicates an intolerant community and 10 indicates a community tolerant of pollution. Each taxon, regardless of the system in which it exists, is going to exhibit some level of tolerance to pollution, thus this index may be a feasible option for use in smaller spring systems. The one issue that may arise with the use of this index is the fact that it incorporates the abundance of individuals within each species. In a community dominated by only one or two species, this index may become skewed towards the tolerance value of those dominant species. It is important to keep in mind that not all groups of macroinvertebrates were sampled in these spring systems, thus it is unknown how those groups will affect these primary metrics.

In the large rheocrene spring systems studied, the majority of the sampled community was composed of aquatic insects, although there was also a small proportion of amphipods and isopods sampled as well. Again, other groups of macroinvertebrates were not sampled, thus it is unknown how those groups might affect different metrics. However, based on the data collected, the primary bioassessment metrics used by MDNR may be most practical for use in large rheocrene spring systems in Missouri. Because the spring systems sampled are so large, they have a variety of mesohabitats available, thus overall diversity is higher and more comparable to that of stream systems. Because of this, the Taxa Richness metric and Shannon Diversity Index would work well in these large spring systems. In addition, the EPT Index is also a viable option for use in large spring systems because the communities sampled in Montauk, Roaring River, Bennett, and Ha Ha Tonka Springs all had an array of EPT taxa present. The fourth metric, the Biotic Index, would also be applicable in large rheocrene spring systems. This index may be more applicable in these larger spring systems because there is more diversity, thus an overall community tolerance level can be calculated based on more taxa, which would provide a more accurate value. It is unknown if any one taxon dominates, which would skew the overall calculation, because only qualitative samples were taken; however, based on my experience there was not any one group that appeared to be overly abundant in comparison to other groups.

There are secondary metrics that use similarity coefficients to make comparisons in community composition among spring systems as well. These similarity coefficients are very much like those that were used in previous chapters to make comparisons. These comparisons were helpful in identifying patterns in community composition among spring systems, but attempting to use these metrics to identify environmental stress is not as simple because there are no reference springs for comparison. Further research is required to determine which spring systems have been negatively impacted and to determine what type of community is characteristic of these types of impacted springs. It is unknown if highly impacted springs exist in Missouri and how one should go about identifying them since the community composition of spring systems is relatively understudied and not well understood.

A final option for assessing the health of spring systems is the use of indicator species, as suggested by Doisy and Rabeni (2004) in their report evaluating and suggesting spring monitoring protocol for streams and springs in the Ozark Scenic National Riverways in Missouri. This method identifies indicator species in a community and notes the loss or replacement of these species with less sensitive species. If subsequent sampling signifies a change in the status of an indicator species, it may be necessary to take water quality samples and analyze the system further to determine why this change occurred. A high number of the sensitive indicator taxa mentioned in the report by Doisy and Rabeni were sampled in most of the spring systems in this study, including both small and large rheocrene systems, indicating that this index may be the best, and most useful, option for determining the health of spring systems.

Conclusions

The purpose of this bioassessment was not to provide an in depth look at why current protocol may or may not work, nor was it to suggest new or revised protocol, but to instead point researchers in a direction of whether using the current protocol is a feasible option for assessing the health of spring systems in Missouri. Because of the composition of the community in small rheocrene spring systems, it would be difficult to get meaningful and useful information from metrics created for use in wadeable streams in Missouri. However, these metrics may work well for large rheocrene spring systems in Missouri because of their higher diversity, larger size, and similarity to stream systems.

For both small and large rheocrene spring systems, the use of indicator species to signify changes within the system may be most useful. Overall, the study of spring communities should be continued so that additional baseline data will be available for researchers who continue to attempt to develop protocol for determining the health of Missouri spring systems.

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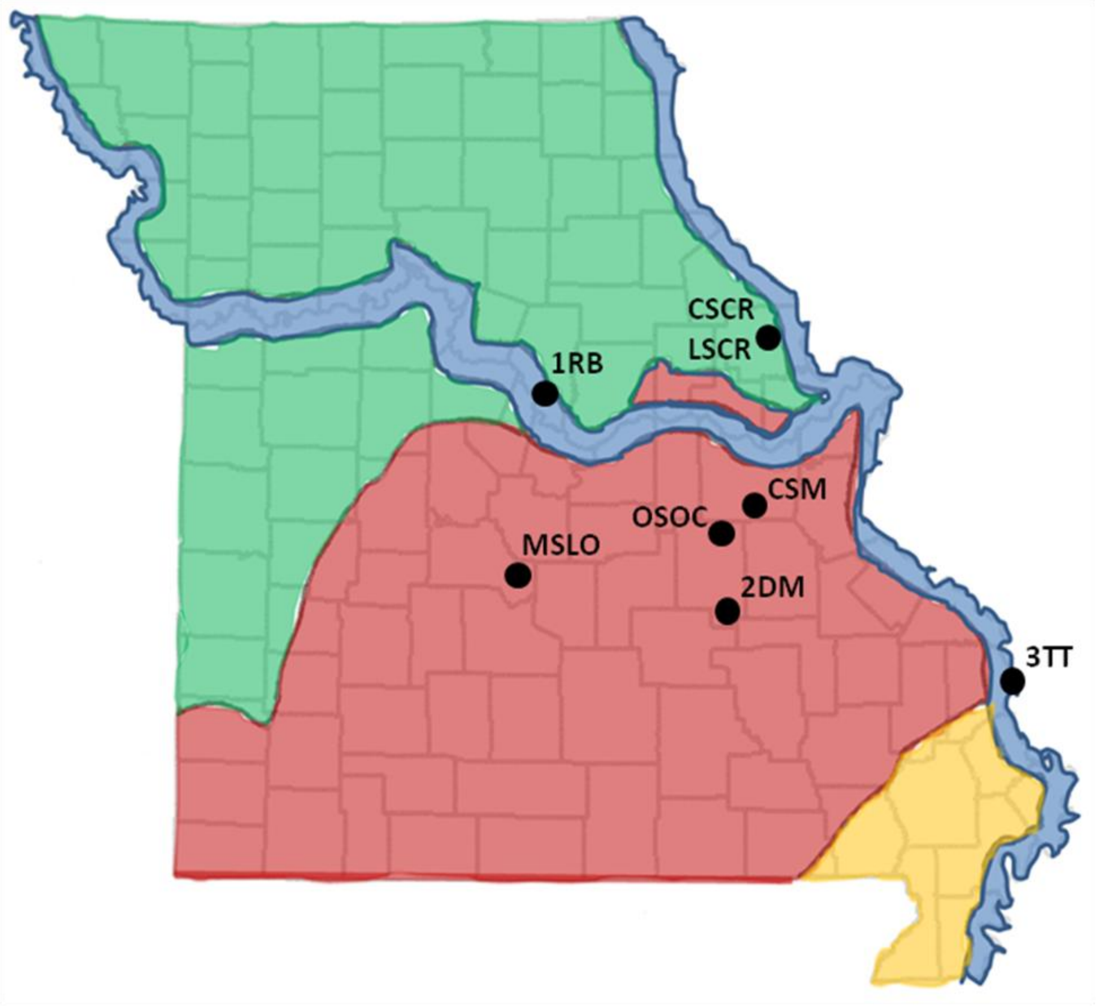

Figure 1. Aquatic faunal regions of Missouri, based on Pflieger (1989), and locations of quantitatively sampled low to medium discharge rheocrene spring systems within these regions [green, Prairie Aquatic Faunal Region; blue, Big River Aquatic Faunal Region; red, Ozark Aquatic Faunal Region; yellow, Lowland Aquatic Faunal Region; CSCR, Cave Spring at Cuivre River State Park; LSCR, Lone Spring at Cuivre River State Park; CSM, Chickadee Spring at Meramec State Park; MSLO, Mill Spring at Lake of the Ozarks State Park; OSOC, Onondaga Spring at Onondaga Cave State Park; 2DM, unnamed spring at Dillard Mill State Historic Site; 1RB, unnamed spring at Rockbridge State Park; 3TT, unnamed spring at Trail of Tears State Park].

Figure 2. Cave Spring at Cuivre River State Park (CSCR) [Note the quarter for scale].

Figure 3. Lone Spring at Cuivre River State Park (LSCR).


Figure 4. Chickadee Spring at Meramec State Park (CSM).


Figure 5. Mill Spring at Lake of the Ozarks State Park (MSLO).

Figure 6. Onondaga Spring at Onondaga Cave State Park (OSOC).

Figure 7. Unnamed spring at Dillard Mill State Historic Site (2DM).

Figure 8. Unnamed spring at Rockbridge State Park (1RB).

Figure 9. Unnamed spring at Trail of Tears State Park (3TT).

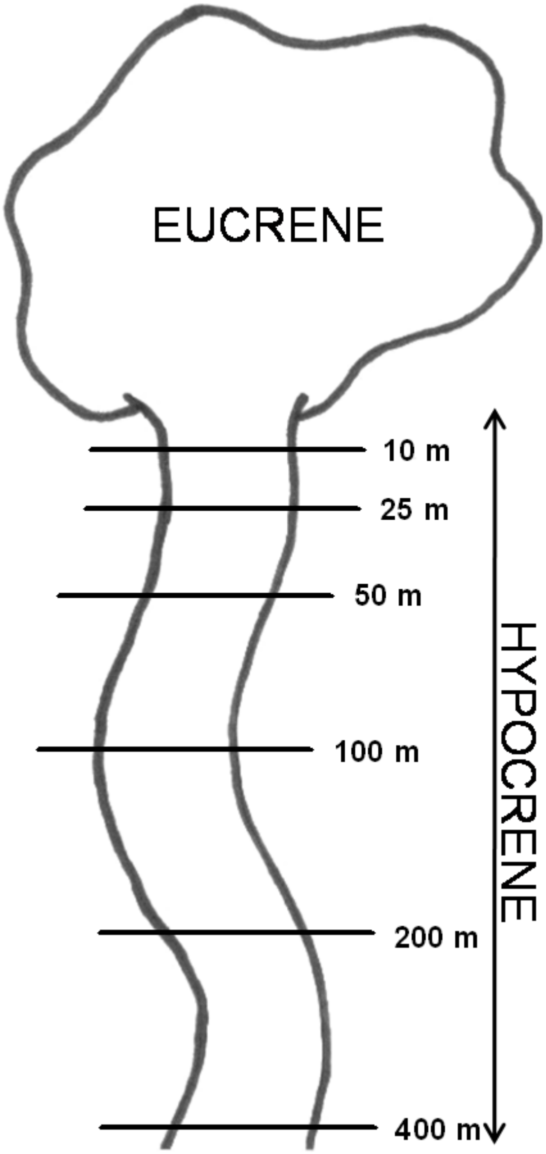

Figure 10. Sampling increments of the hypocrene.

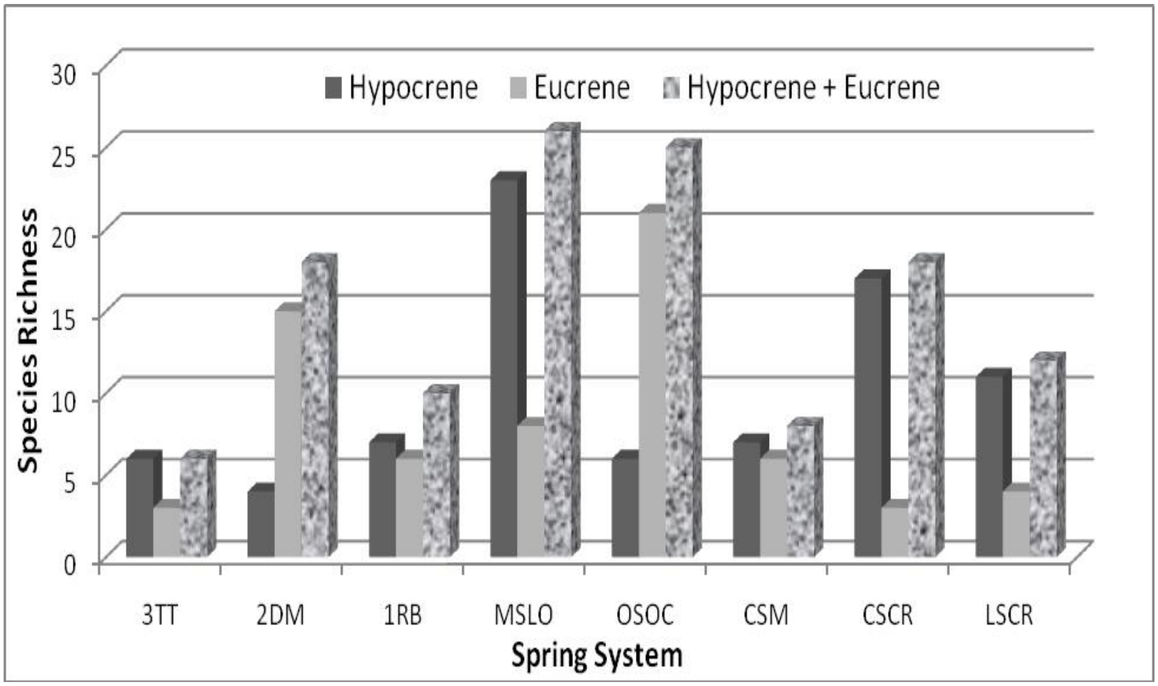

Figure 11. Species richness in the hypocrene, eucrene, and hypocrene + eucrene together during the 2007 sampling period in the quantitatively sampled low to medium discharge rheocrene spring systems [3TT, unnamed spring at Trail of Tears SP; 2DM, unnamed spring at Dillard Mill SHS; 1RB, unnamed spring at Rockbridge SP; MSLO, Mill Spring at Lake of the Ozarks SP; OSOC, Onondaga Spring at Onondaga Cave SP; CSM, Chickadee Spring at Meramec SP; CSCR, Cave Spring at Cuivre River SP; LSCR, Lone Spring at Cuivre River SP].

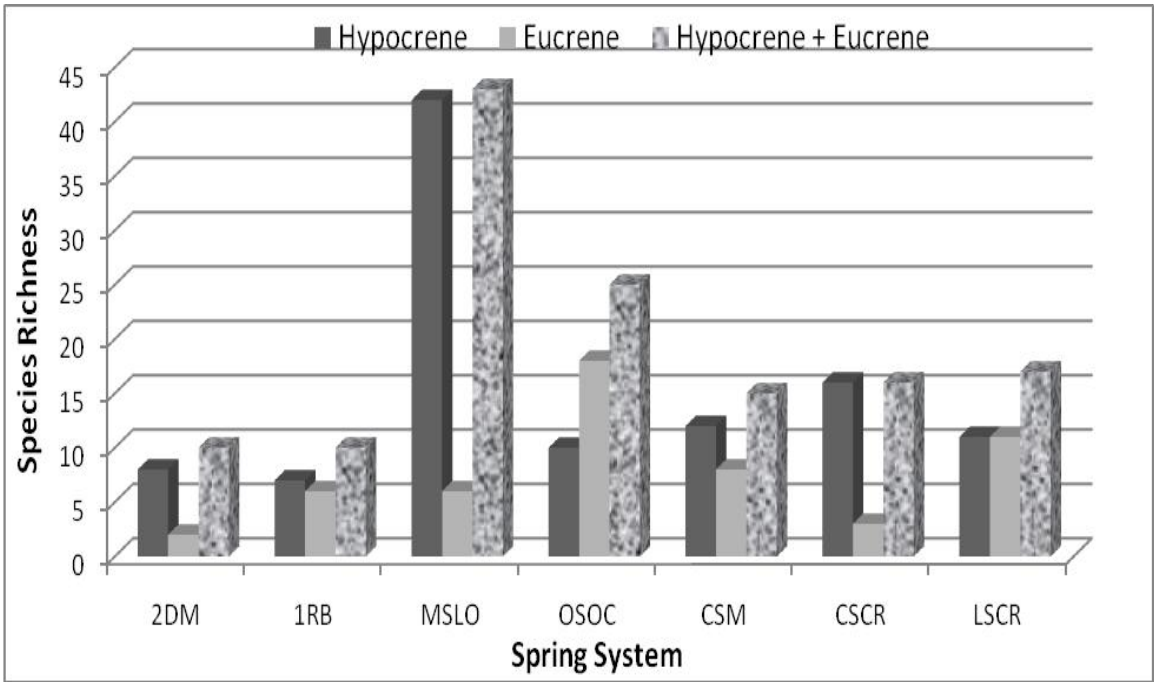

Figure 12. Species richness in the hypocrene, eucrene, and hypocrene + eucrene together during the 2008 sampling period in the quantitatively sampled low to medium discharge rheocrene spring systems [2DM, unnamed spring at Dillard Mill SHS; 1RB, unnamed spring at Rockbridge SP; MSLO, Mill Spring at Lake of the Ozarks SP; OSOC, Onondaga Spring at Onondaga Cave SP; CSM, Chickadee Spring at Meramec SP; CSCR, Cave Spring at Cuivre River SP; LSCR, Lone Spring at Cuivre River SP].

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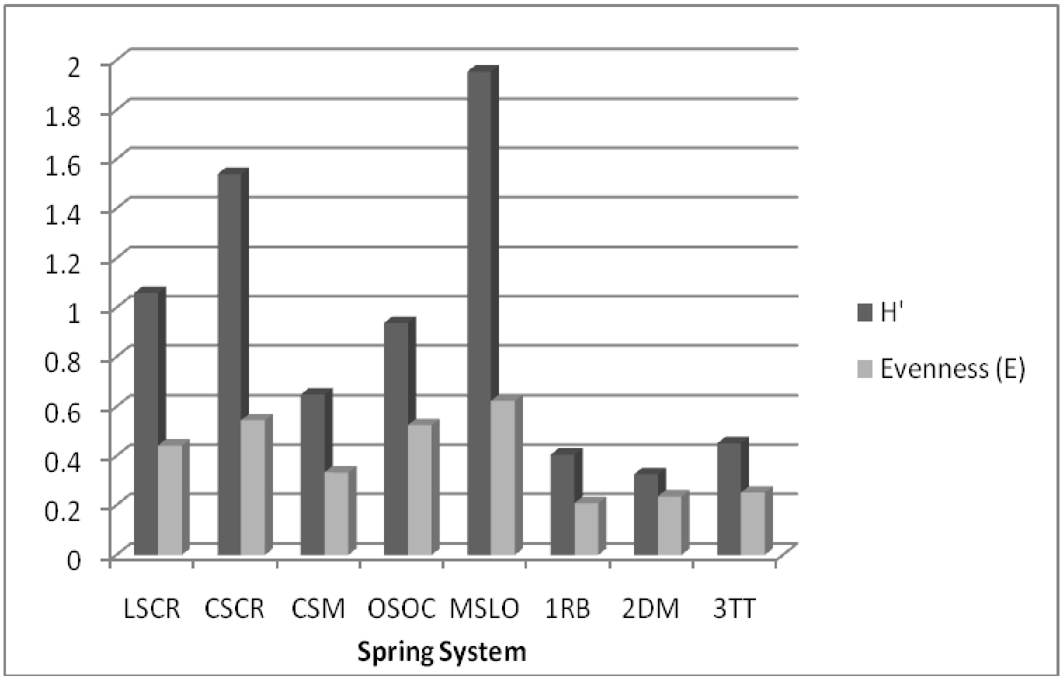

Figure 13. Shannon diversity and evenness values for the quantitatively sampled low to medium discharge rheocrene spring systems in the 2007 sampling period [H', Shannon diversity index; LSCR, Lone Spring at Cuivre River SP; CSCR, Cave Spring at Cuivre River SP; CSM, Chickadee Spring at Meramec SP; OSOC, Onondaga Spring at Onondaga Cave SP; MSLO, Mill Spring at Lake of the Ozarks SP; 1RB, unnamed spring at Rockbridge SP; 2DM, unnamed spring at Dillard Mill SHS; 3TT, unnamed spring at Trail of Tears SP].

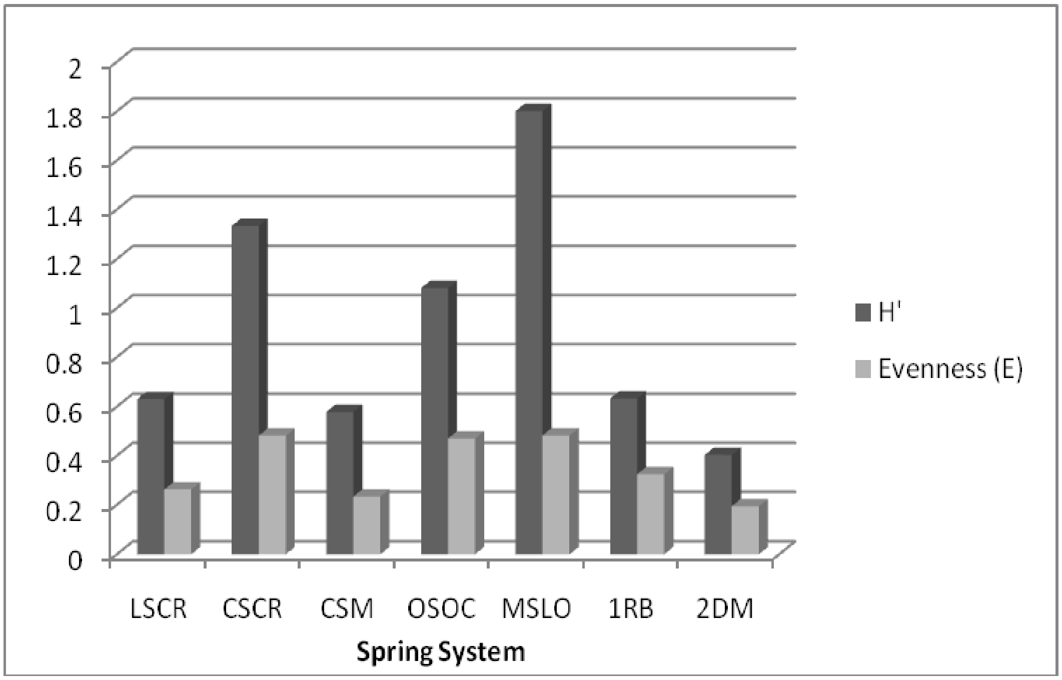

Figure 14. Shannon diversity and evenness values for the quantitatively sampled low to medium discharge rheocrene spring systems in the 2008 sampling period [H', Shannon diversity index; LSCR, Lone Spring at Cuivre River SP; CSCR, Cave Spring at Cuivre River SP; CSM, Chickadee Spring at Meramec SP; OSOC, Onondaga Spring at Onondaga Cave SP; MSLO, Mill Spring at Lake of the Ozarks SP; 1RB, unnamed spring at Rockbridge SP; 2DM, unnamed spring at Dillard Mill SHS].

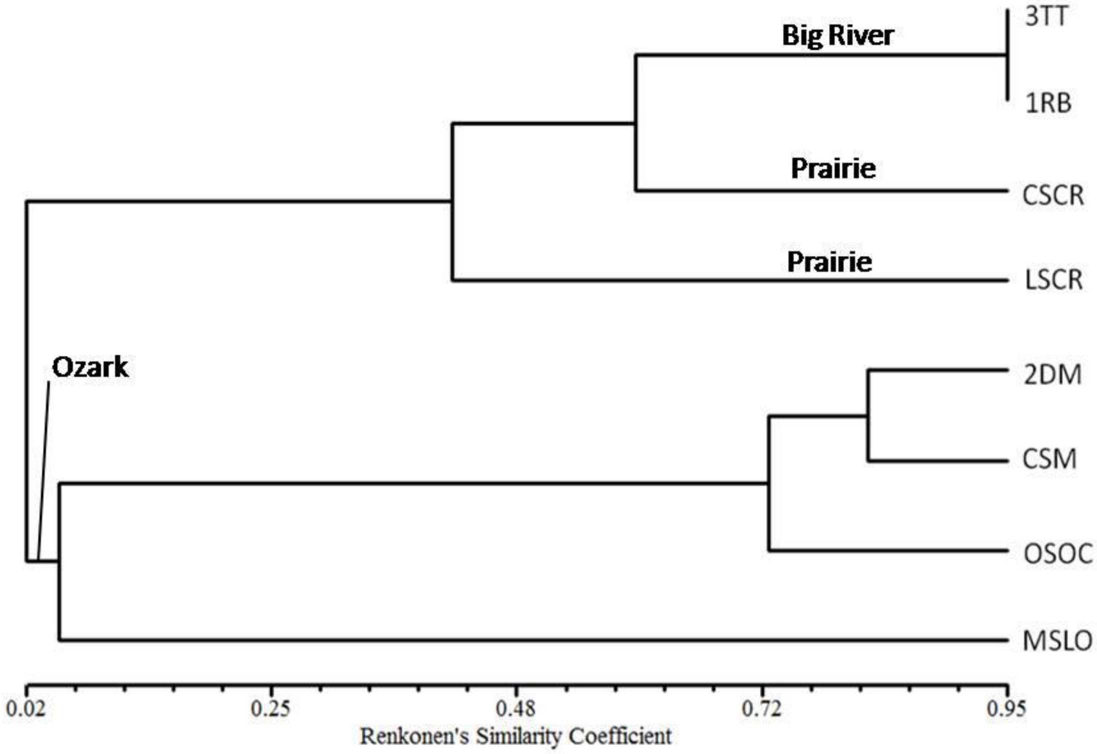

Figure 15. UPGMA cluster analysis using Renkonen's similarity coefficient showing percent similarity in aquatic insect, amphipod, and isopod community composition among the hypocrenes of the quantitatively sampled low to medium discharge rheocrene spring systems in the 2007 sampling period [3TT, unnamed spring at Trail of Tears SP; 2DM, unnamed spring at Dillard Mill SHS; 1RB, unnamed spring at Rockbridge SP; MSLO, Mill Spring at Lake of the Ozarks SP; OSOC, Onondaga Spring at Onondaga Cave SP; CSM, Chickadee Spring at Meramec SP; CSCR, Cave Spring at Cuivre River SP; LSCR, Lone Spring at Cuivre River SP; Big River, Big River Aquatic Faunal Region; Prairie, Prairie Aquatic Faunal Region; Ozark, Ozark Aquatic Faunal Region].

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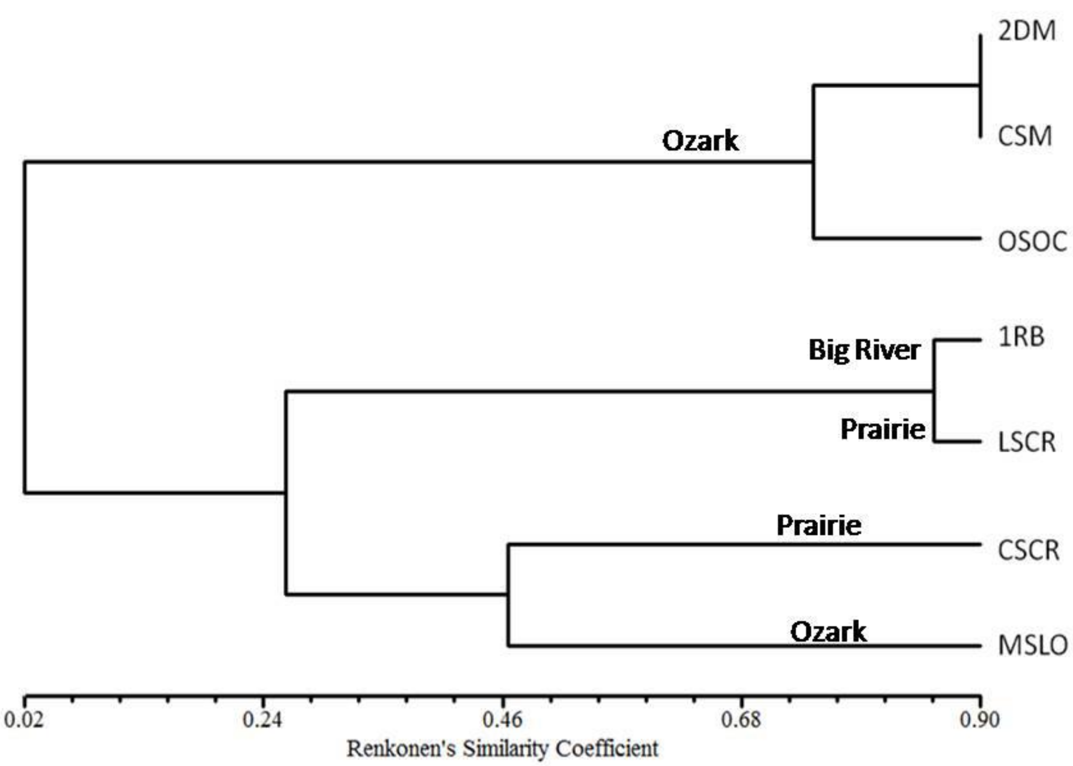


Figure 16. UPGMA cluster analysis using Renkonen's similarity coefficient showing percent similarity in aquatic insect, amphipod, and isopod community composition among the hypocrenes of the quantitatively sampled low to medium discharge rheocrene spring systems in the 2008 sampling period [2DM, unnamed spring at Dillard Mill SHS; 1RB, unnamed spring at Rockbridge SP; MSLO, Mill Spring at Lake of the Ozarks SP; OSOC, Onondaga Spring at Onondaga Cave SP; CSM, Chickadee Spring at Meramec SP; CSCR, Cave Spring at Cuivre River SP; LSCR, Lone Spring at Cuivre River SP; Big River, Big River Aquatic Faunal Region; Prairie, Prairie Aquatic Faunal Region; Ozark, Ozark Aquatic Faunal Region].

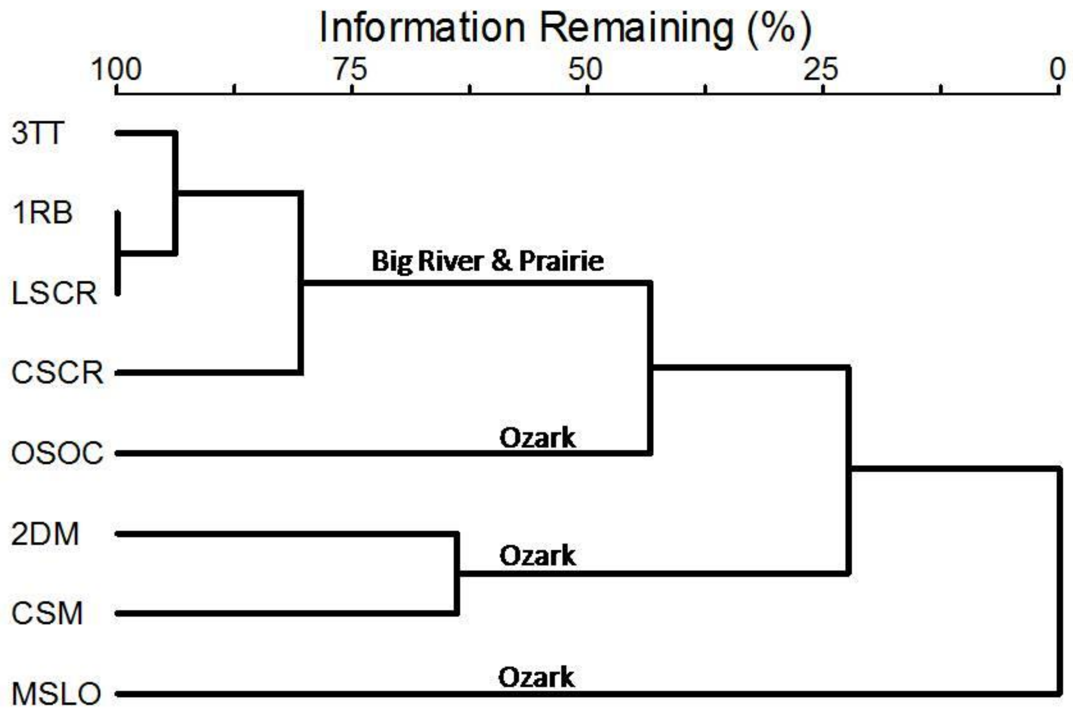


Figure 17. UPGMA cluster analysis using Sorenson's similarity coefficient showing similarity in aquatic insect, amphipod, and isopod community composition among the eucrenes of the quantitatively sampled low to medium discharge rheocrene spring systems in the 2007 sampling period [3TT, unnamed spring at Trail of Tears SP; 2DM, unnamed spring at Dillard Mill SHS; 1RB, unnamed spring at Rockbridge SP; MSLO, Mill Spring at Lake of the Ozarks SP; OSOC, Onondaga Spring at Onondaga Cave SP; CSM, Chickadee Spring at Meramec SP; CSCR, Cave Spring at Cuivre River SP; LSCR, Lone Spring at Cuivre River SP; Big River, Big River Aquatic Faunal Region; Prairie, Prairie Aquatic Faunal Region; Ozark, Ozark Aquatic Faunal Region].

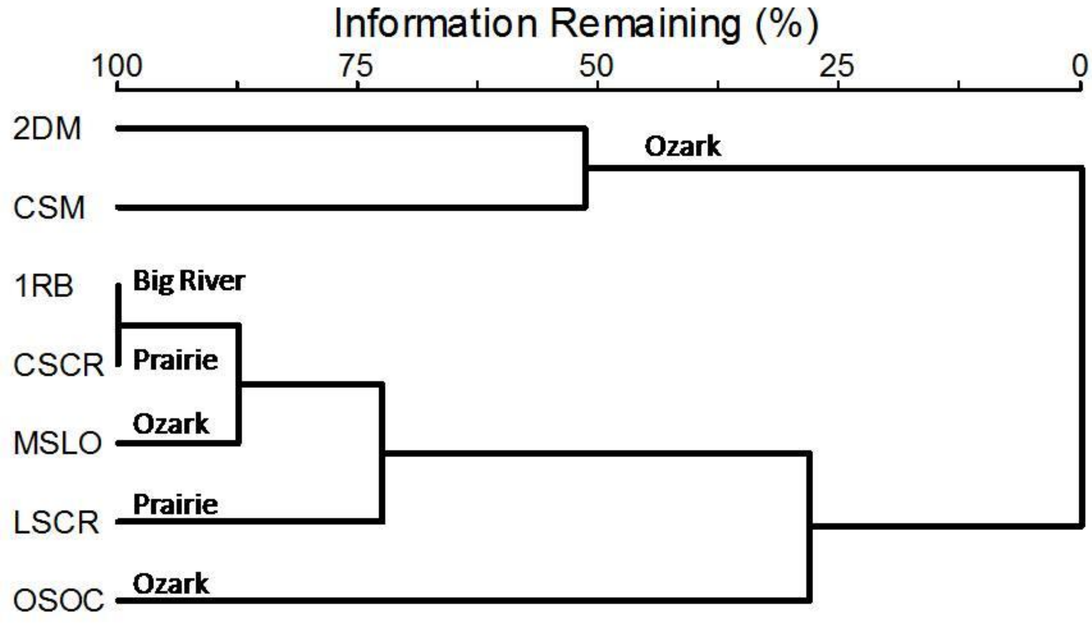


Figure 18. UPGMA cluster analysis using Sorenson's similarity coefficient showing similarity in aquatic insect, amphipod, and isopod community composition among the eucrenes of the quantitatively sampled low to medium discharge rheocrene spring systems in the 2008 sampling period [2DM, unnamed spring at Dillard Mill SHS; 1RB, unnamed spring at Rockbridge SP; MSLO, Mill Spring at Lake of the Ozarks SP; OSOC, Onondaga Spring at Onondaga Cave SP; CSM, Chickadee Spring at Meramec SP; CSCR, Cave Spring at Cuivre River SP; LSCR, Lone Spring at Cuivre River SP; Big River, Big River Aquatic Faunal Region; Prairie, Prairie Aquatic Faunal Region; Ozark, Ozark Aquatic Faunal Region].

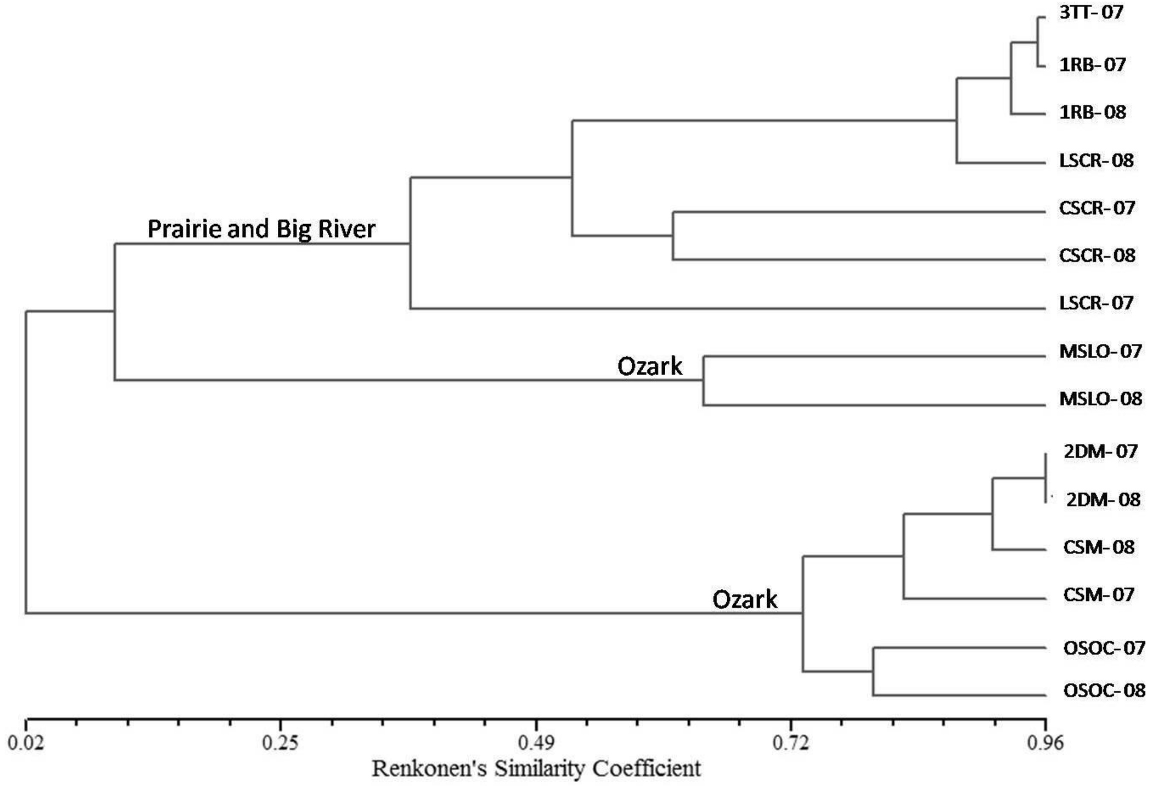


Figure 19. UPGMA cluster analysis using Renkonen’s similarity coefficient showing similarity in aquatic insect, amphipod, and isopod community composition among the hypocrenes of the quantitatively sampled low to medium discharge rheocrene spring systems in 2007 and 2008 [3TT, unnamed spring at Trail of Tears SP; 2DM, unnamed spring at Dillard Mill SHS; 1RB, unnamed spring at Rockbridge SP; MSLO, Mill Spring at Lake of the Ozarks SP; OSOC, Onondaga Spring at Onondaga Cave SP; CSM, Chickadee Spring at Meramec SP; CSCR, Cave Spring at Cuivre River SP; LSCR, Lone Spring at Cuivre River SP; 07, 2007 sampling period; 08, 2008 sampling period; Big River, Big River Aquatic Faunal Region; Prairie, Prairie Aquatic Faunal Region; Ozark, Ozark Aquatic Faunal Region].

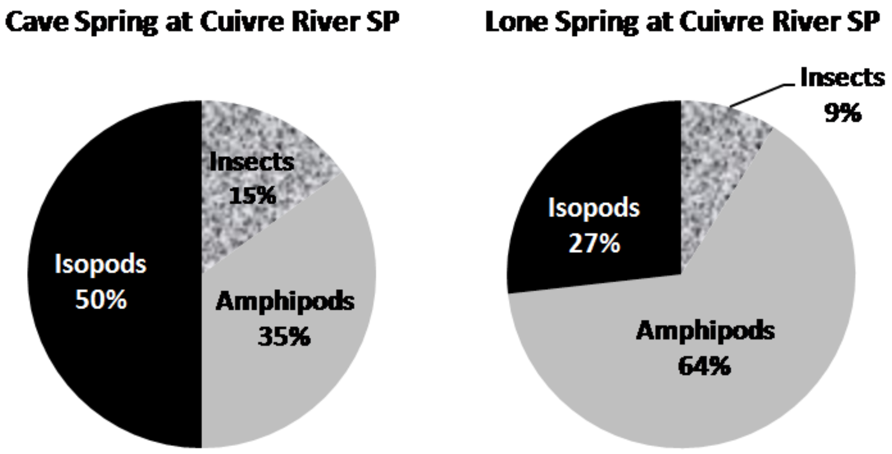


Figure 20. Proportion of aquatic insects, amphipods, and isopods in the Prairie faunal region springs, CSCR and LSCR, in the 2007 sampling period.

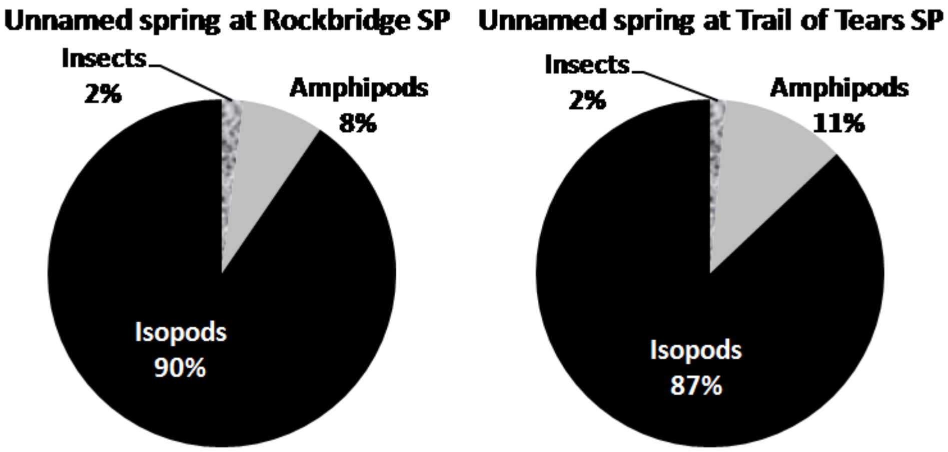


Figure 21. Proportion of aquatic insects, amphipods, and isopods in the Big River faunal region springs, 1RB and 3TT, in the 2007 sampling period.

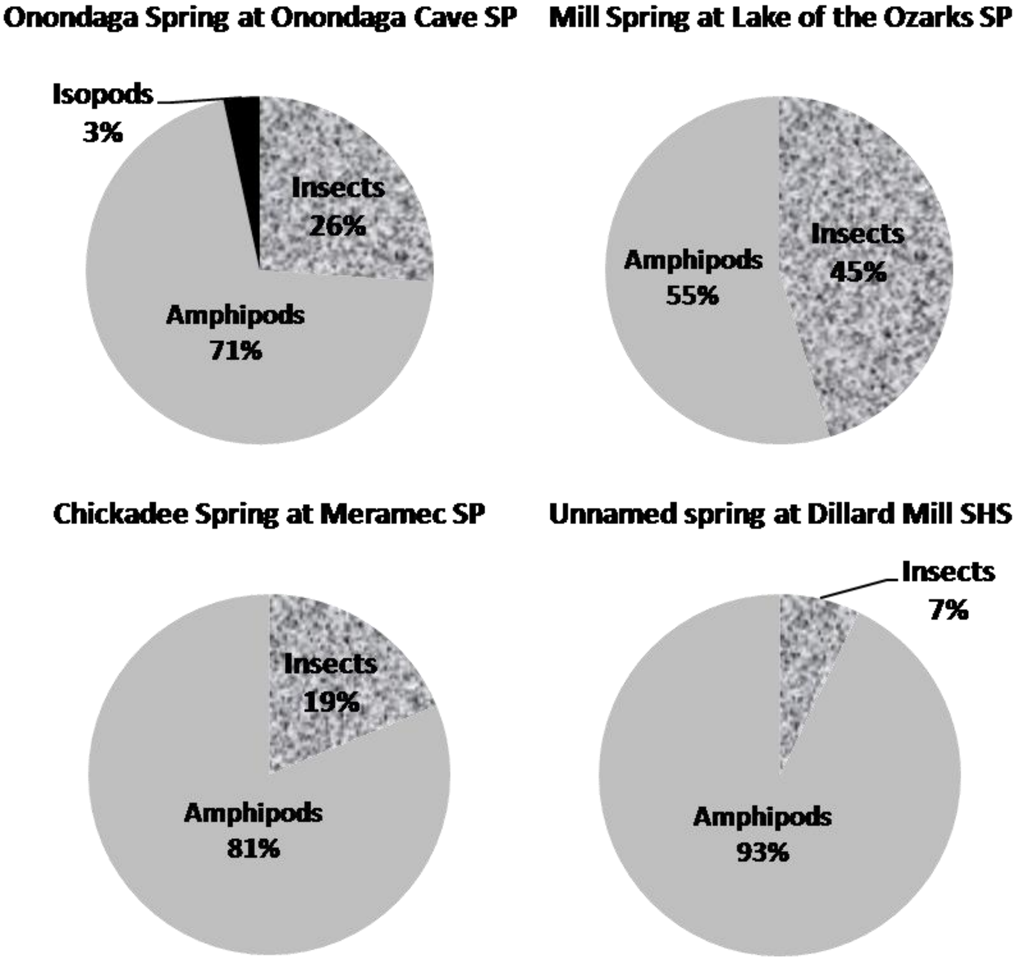


Figure 22. Proportion of aquatic insects, amphipods, and isopods in the Ozark faunal region springs, OSOC, MSLO, CSM, and 2DM, in the 2007 sampling period.

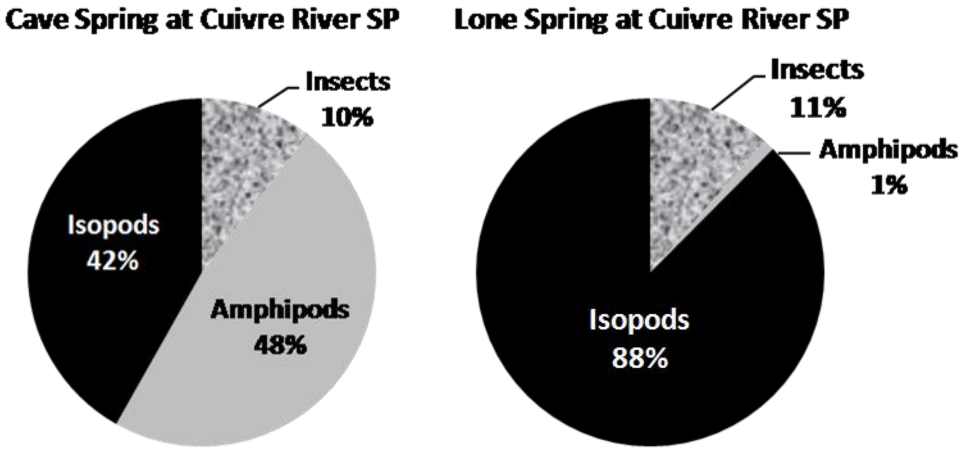


Figure 23. Proportion of aquatic insects, amphipods, and isopods in the Prairie faunal region springs, CSCR and LSCR, in the 2008 sampling period.

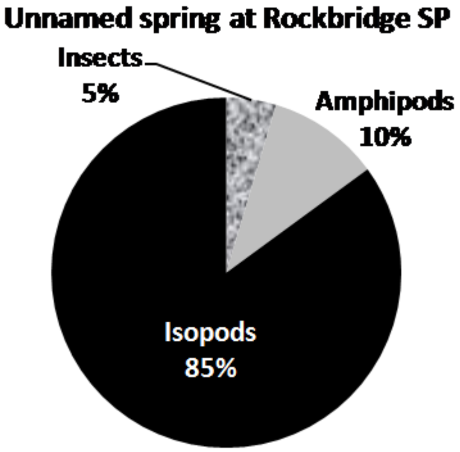


Figure 24. Proportion of aquatic insects, amphipods, and isopods in the Big River faunal region spring 1RB in the 2008 sampling period.

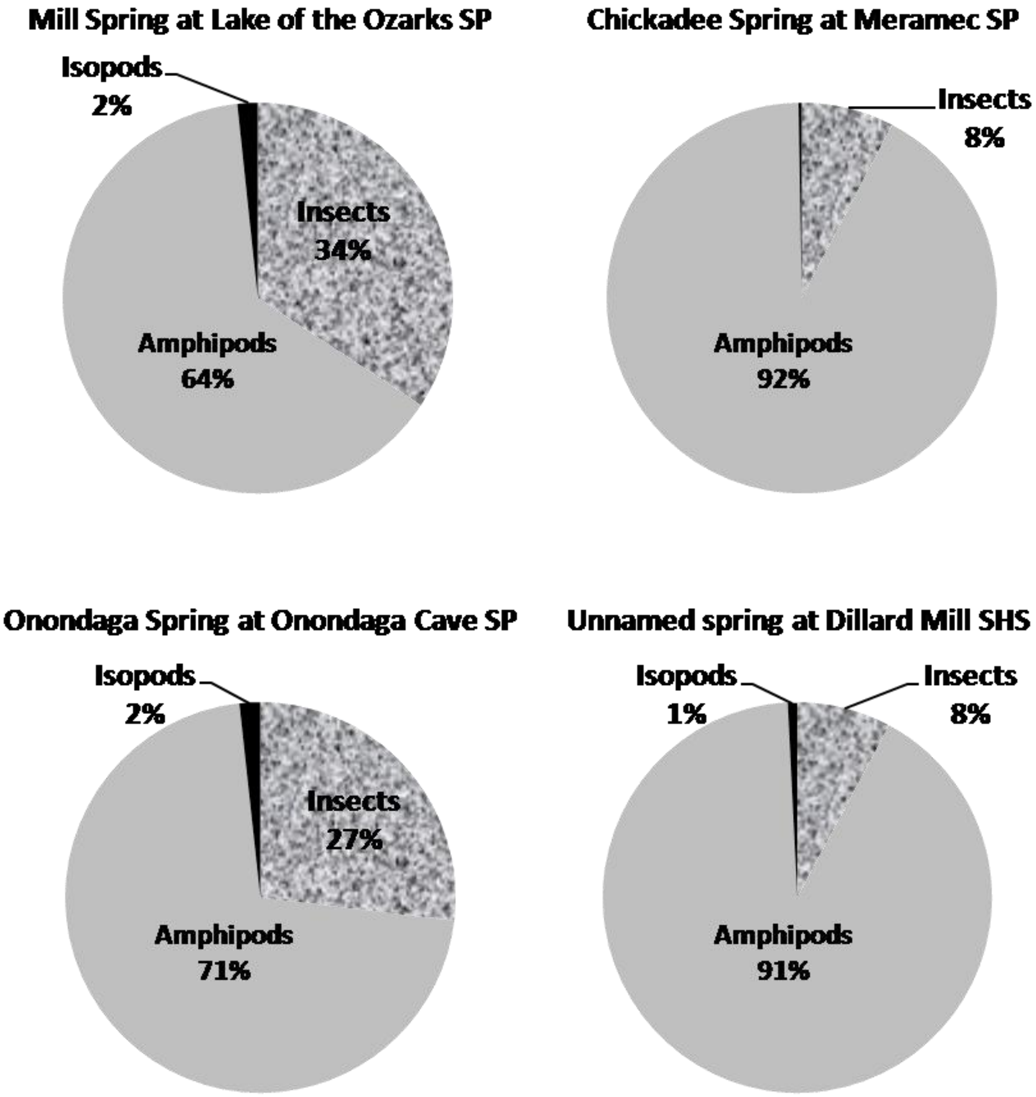


Figure 25. Proportion of aquatic insects, amphipods, and isopods in the Ozark faunal region springs, MSLO, CSM, OSOC, and 2DM, in the 2008 sampling period.

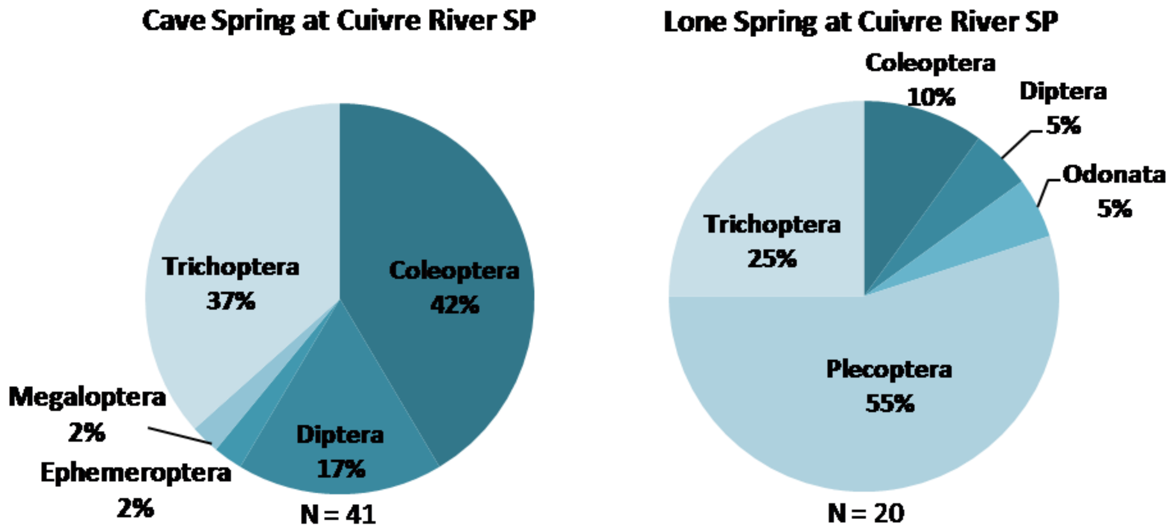


Figure 26. Proportion of aquatic insect orders in the Prairie faunal region springs, CSCR and LSCR, in the 2007 sampling period.

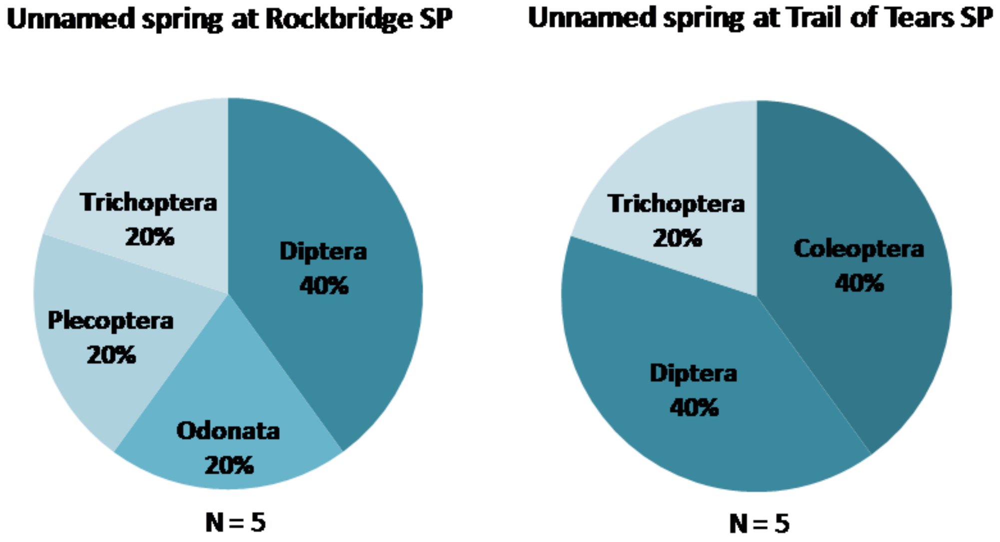
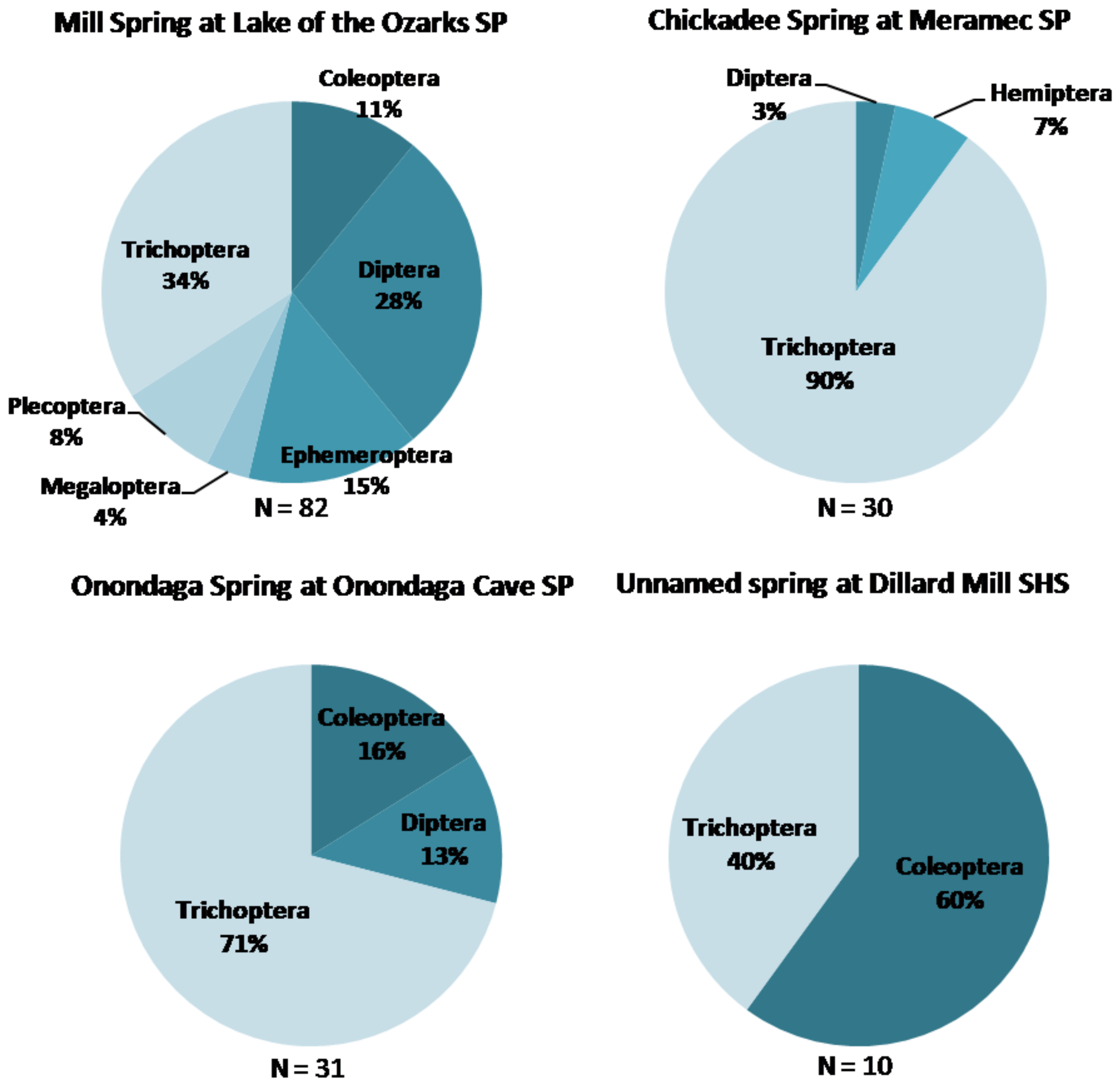


Figure 27. Proportion of aquatic insect orders in the Big River faunal region springs, 1RB and 3TT, in the 2007 sampling period.


Mill Spring at Lake of the Ozarks SP

Chickadee Spring at Meramec SP

Onondaga Spring at Onondaga Cave SP

Unnamed spring at Dillard Mill SHS

Figure 28. Proportion of aquatic insect orders in the Ozark faunal region springs, MSLO, CSM, OSOC, and 2DM, in the 2007 sampling period.

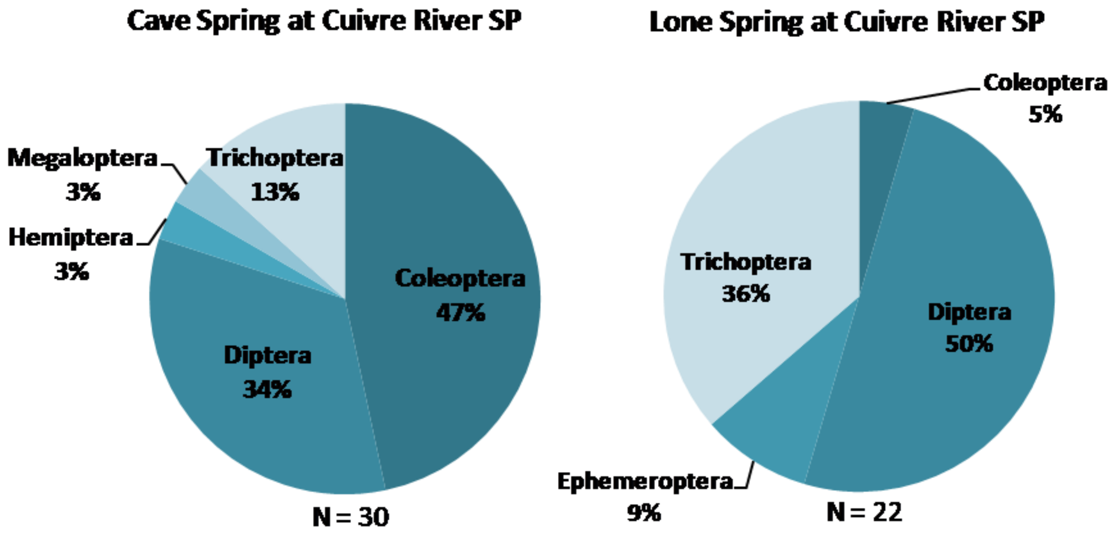


Figure 29. Proportion of aquatic insect orders in the Prairie faunal region springs, CSCR and LSCR, in the 2008 sampling period.

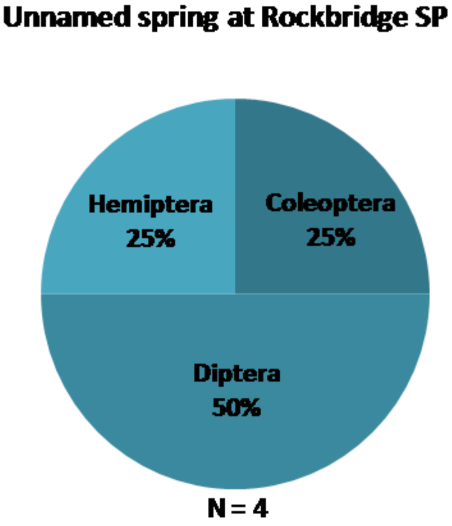


Figure 30. Proportion of aquatic insect orders in the Big River faunal region spring 1RB in the 2008 sampling period.

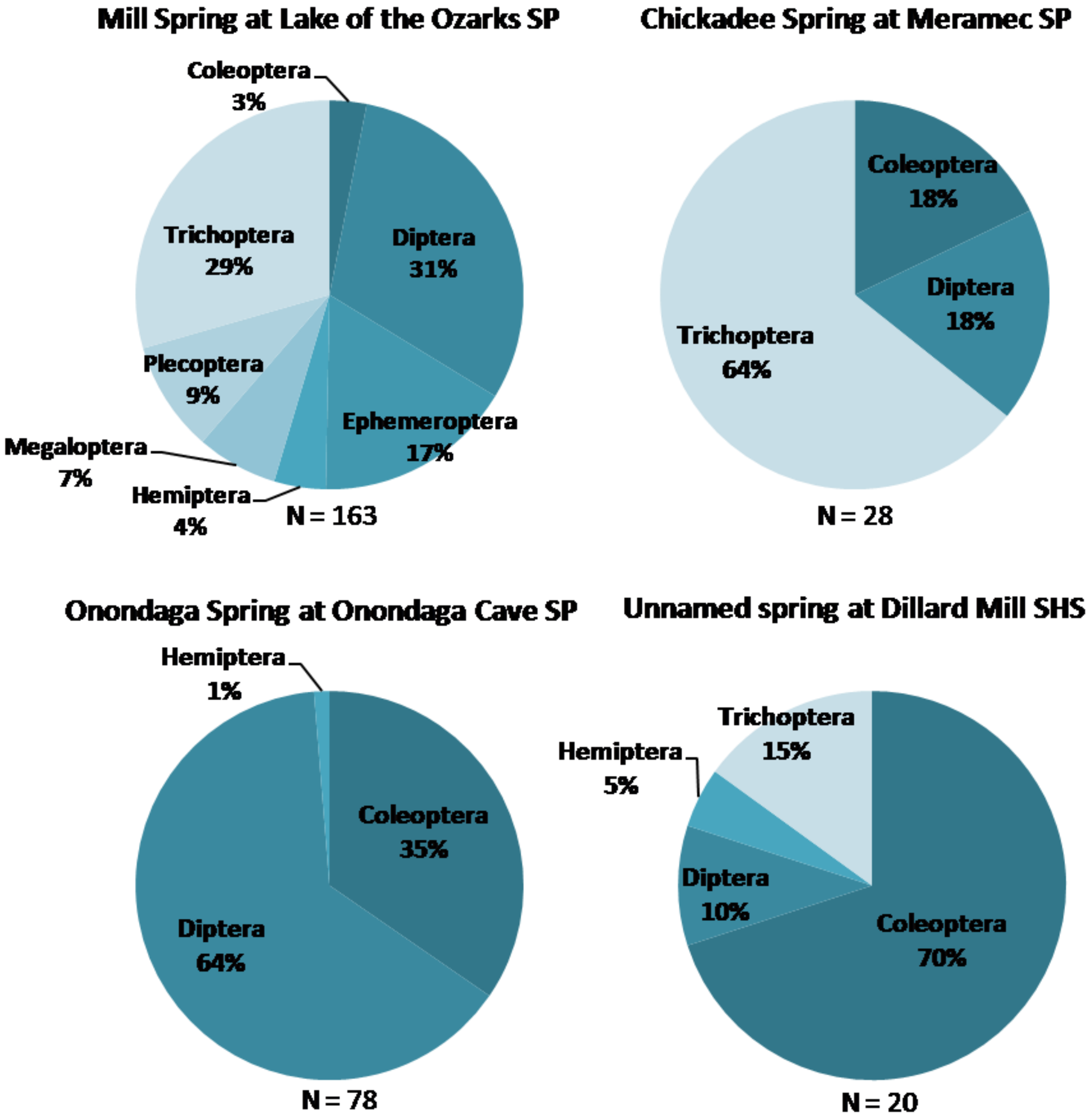


Figure 31. Proportion of aquatic insect orders in the Ozark faunal region springs, MSLO, CSM, OSOC, and 2DM, in the 2008 sampling period.

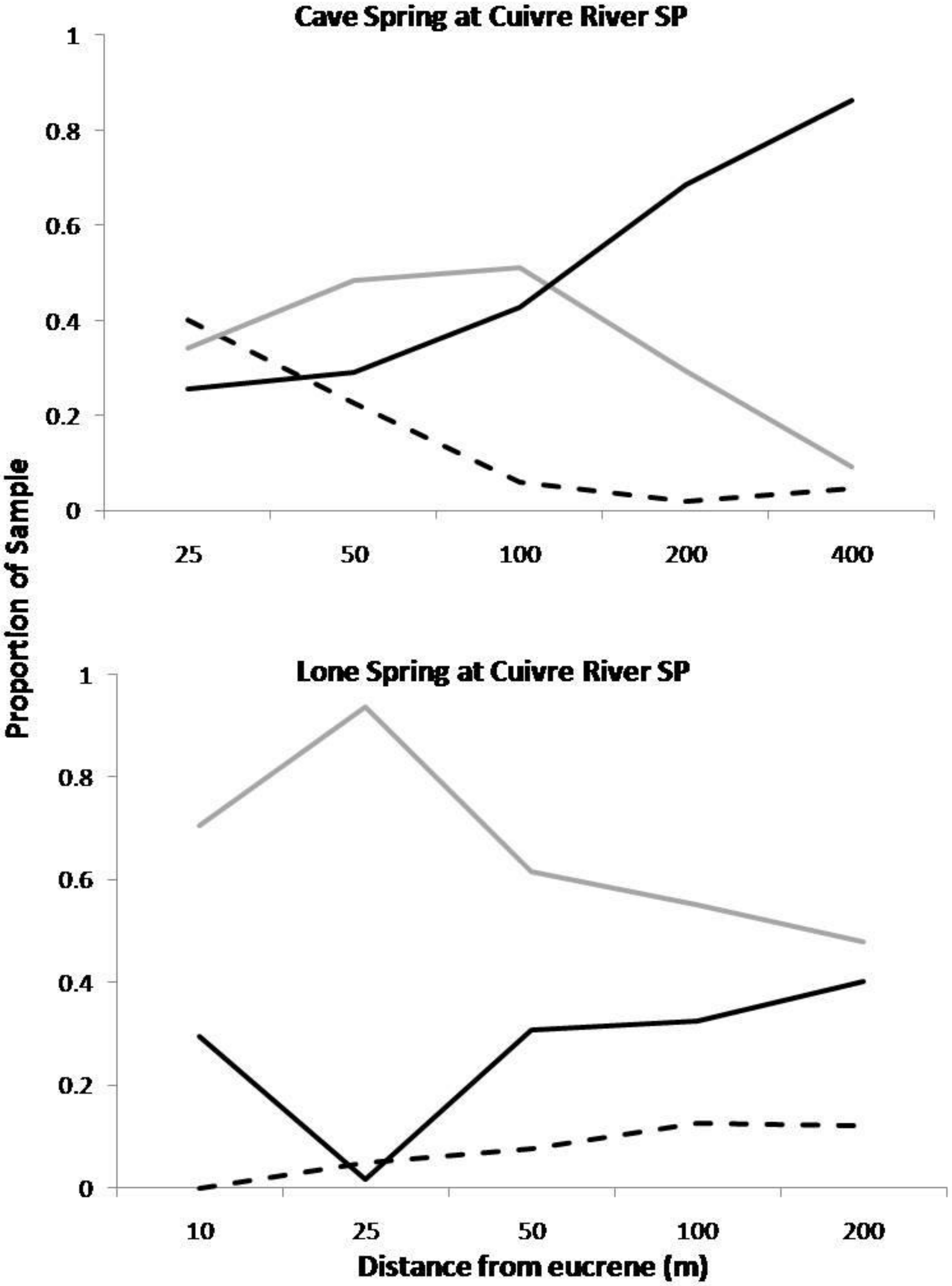


Figure 32. Longitudinal changes in the proportion of aquatic insects, amphipods, and isopods in the Prairie faunal region springs, CSCR and LSCR, in the 2007 sampling period [dashed line, aquatic insects; gray line, amphipods; black line, isopods].

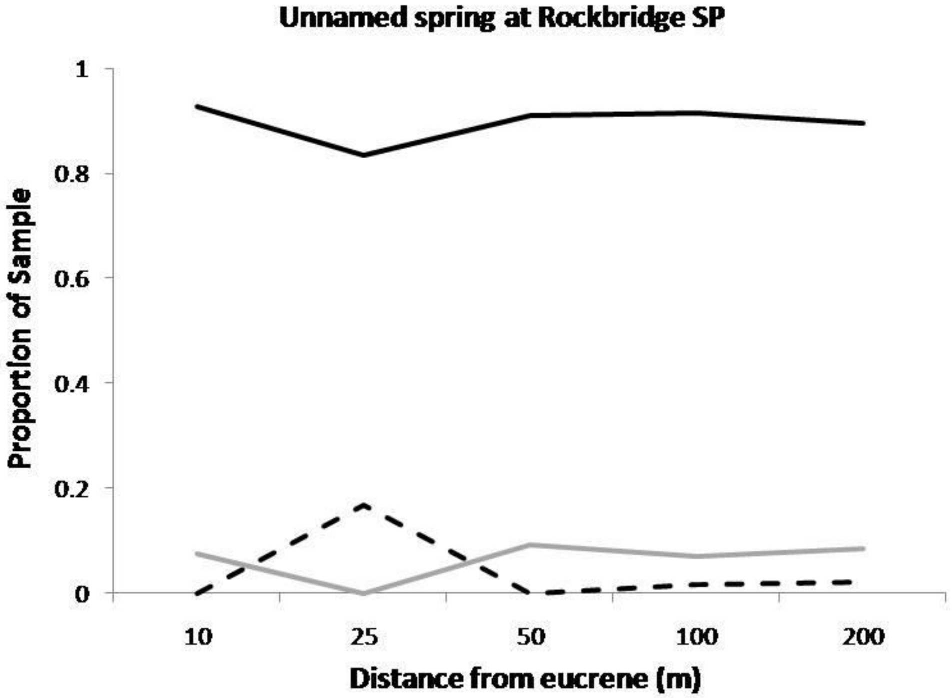


Figure 33. Longitudinal changes in the proportion of aquatic insects, amphipods, and isopods in the Big River faunal region spring 1RB in the 2007 sampling period [dashed line, aquatic insects; gray line, amphipods; black line, isopods].

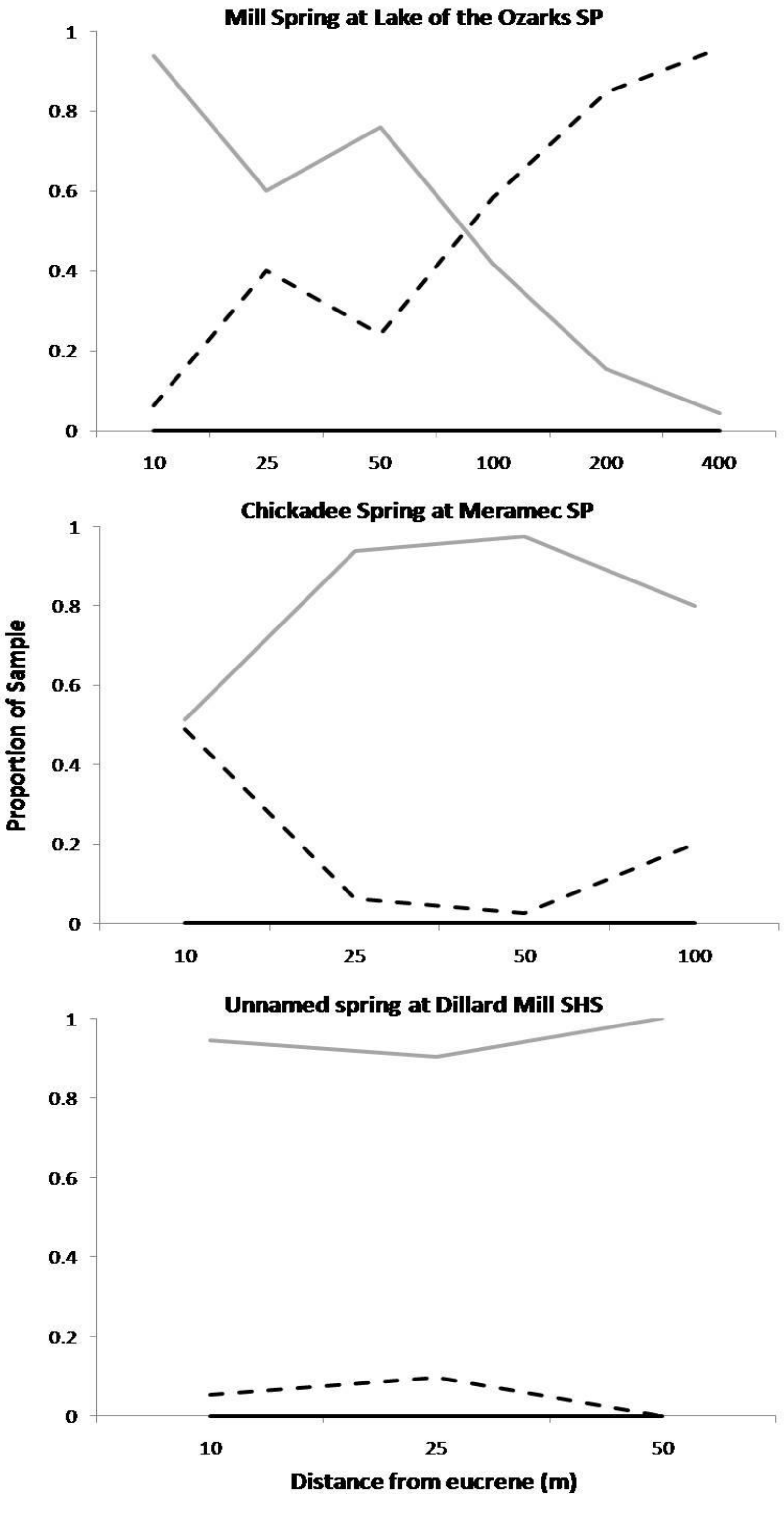


Figure 34. Longitudinal changes in the proportion of aquatic insects, amphipods, and isopods in the Ozark faunal region springs, MSLO, CSM, and 2DM, in the 2007 sampling period [dashed line, aquatic insects; gray line, amphipods; black line, isopods].

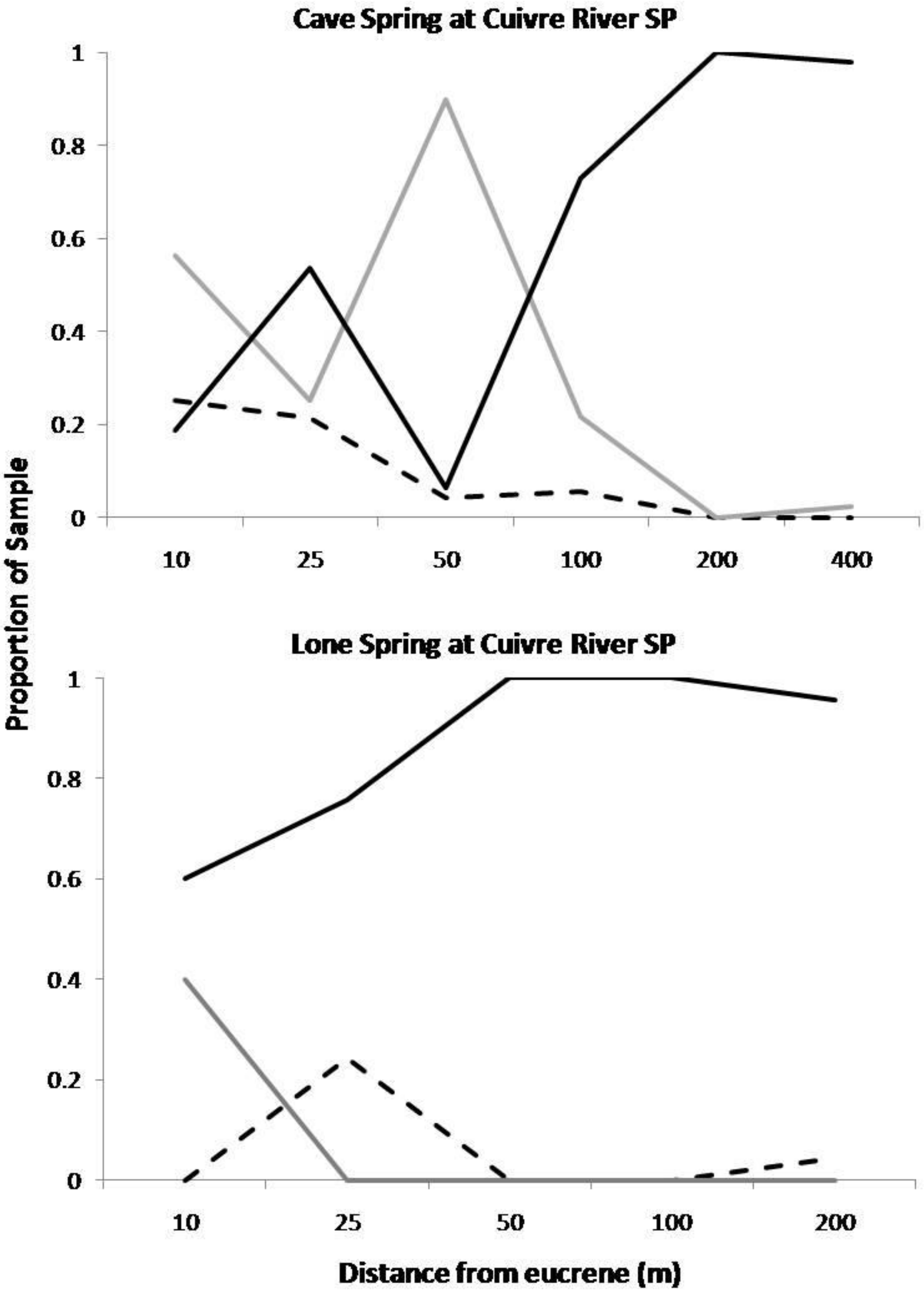


Figure 35. Longitudinal changes in the proportion of aquatic insects, amphipods, and isopods in the Prairie faunal region springs, CSCR and LSCR, in the 2008 sampling period [dashed line, aquatic insects; gray line, amphipods; black line, isopods].

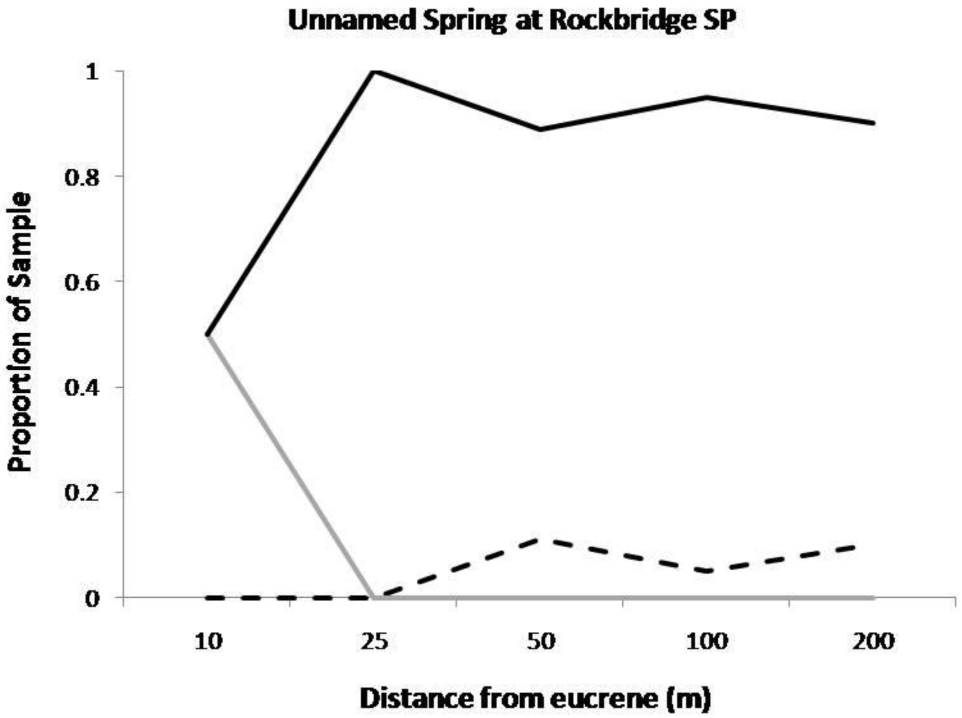


Figure 36. Longitudinal changes in the proportion of aquatic insects, amphipods, and isopods in the Big River faunal region spring 1RB in the 2008 sampling period [dashed line, aquatic insects; gray line, amphipods; black line, isopods].

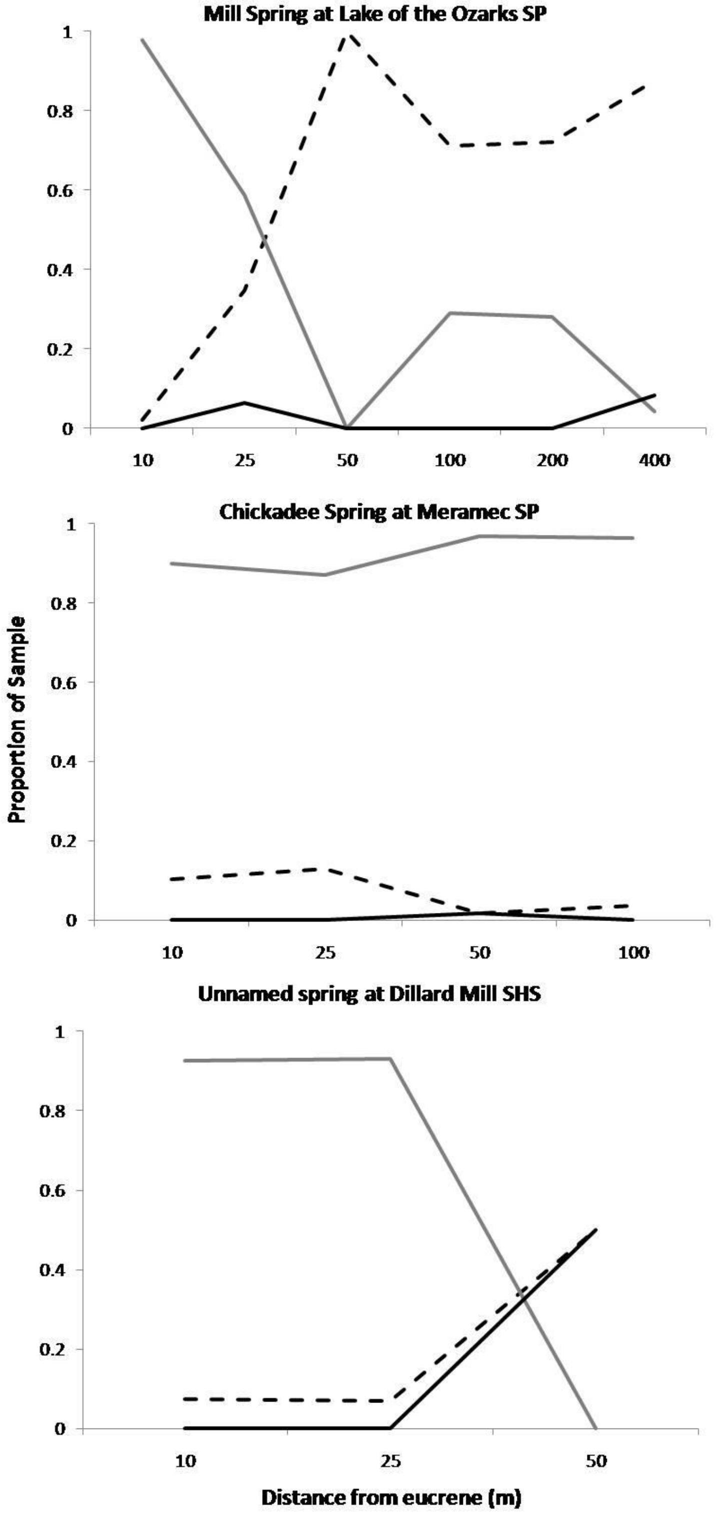


Figure 37. Longitudinal changes in the proportion of aquatic insects, amphipods, and isopods in the Ozark faunal region springs, MSLO, CSM, and 2DM, in the 2008 sampling period [dashed line, aquatic insects; gray line, amphipods; black line, isopods].

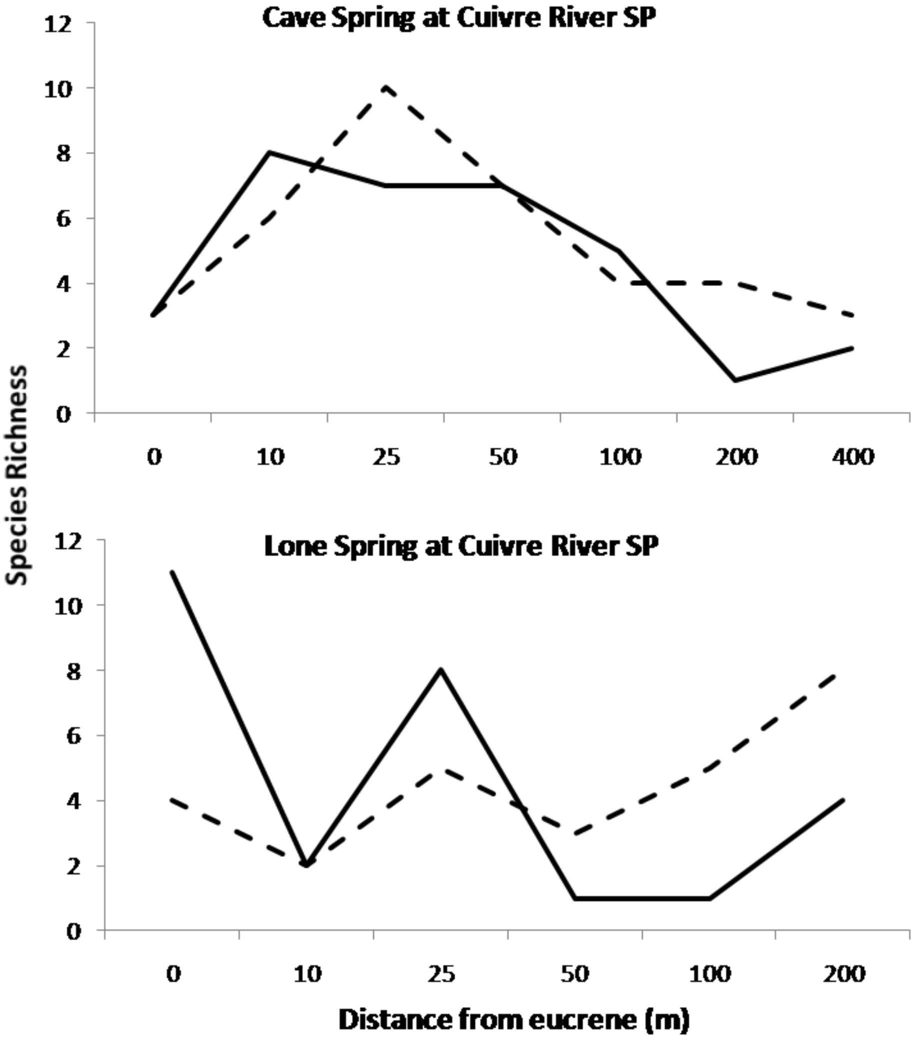


Figure 38. Longitudinal changes in species richness in the Prairie faunal region springs, CSCR and LSCR, in 2007 and 2008 [dashed line, 2007 sampling period; solid line, 2008 sampling period].

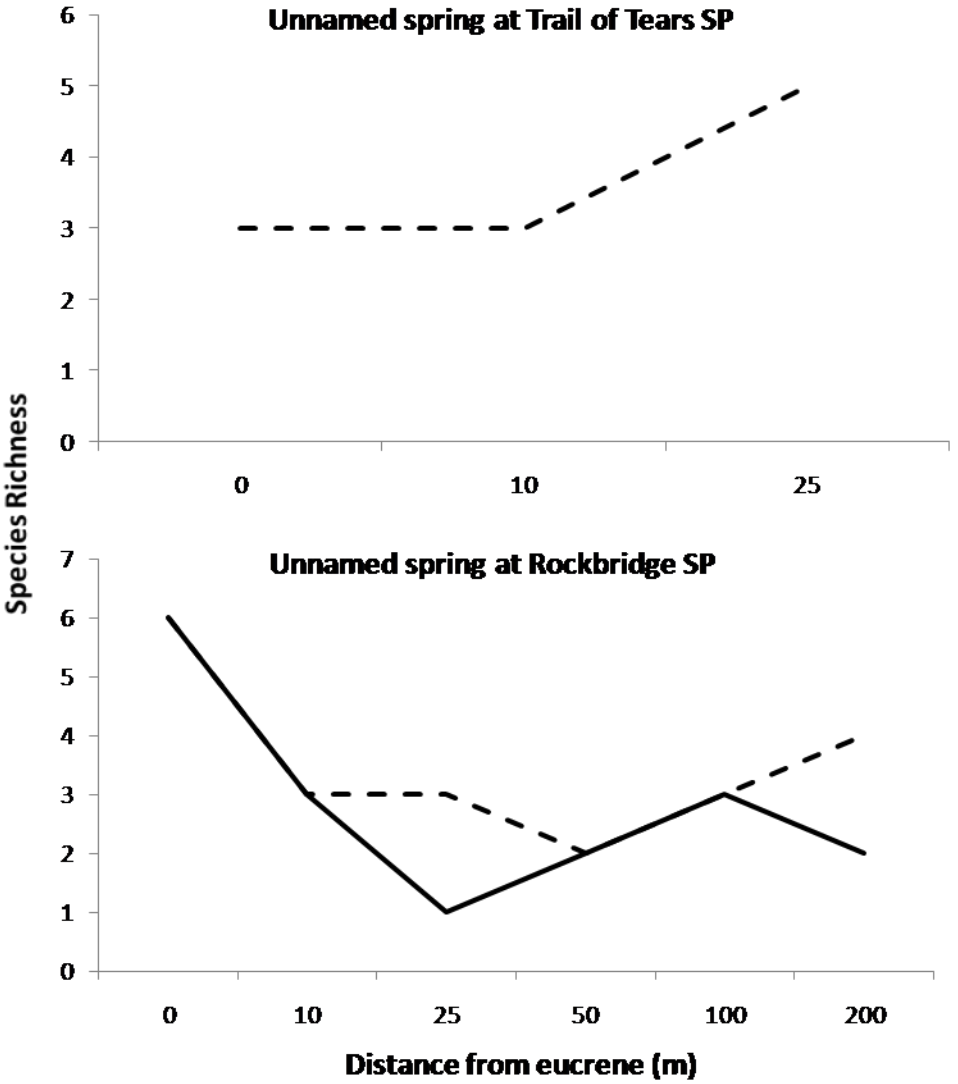


Figure 39. Longitudinal changes in species richness in the Big River faunal region springs, 3TT and 1RB, in 2007 and 2008 [dashed line, 2007 sampling period; solid line, 2008 sampling period].

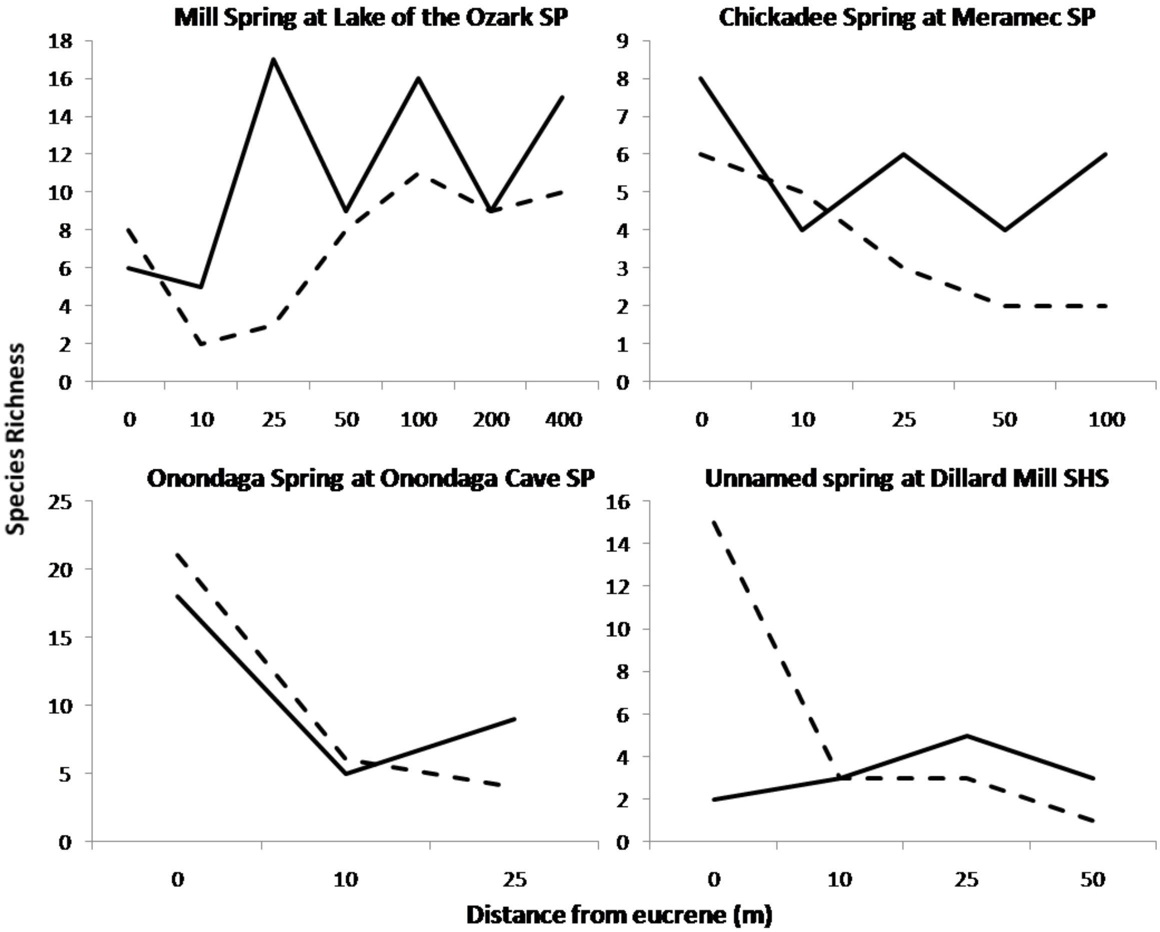


Figure 40. Longitudinal changes in species richness in the Ozark faunal region springs, MSLO, CSM, OSOC, 2DM, in 2007 and 2008 [dashed line, 2007 sampling period; solid line, 2008 sampling period].

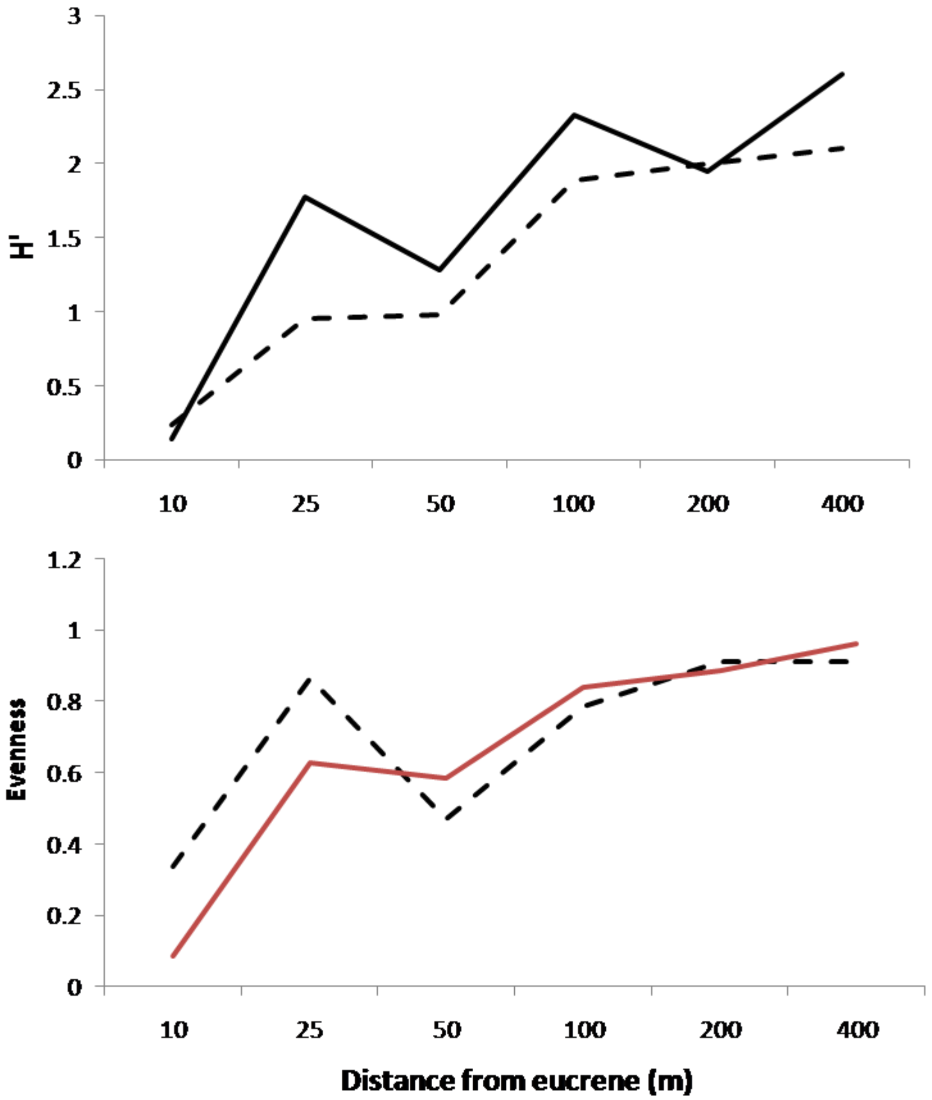


Figure 41. Longitudinal changes in Shannon diversity (H') and evenness in the Ozark spring MSLO in 2007 and 2008 [dashed line, 2007 sampling period; solid line, 2008 sampling period].

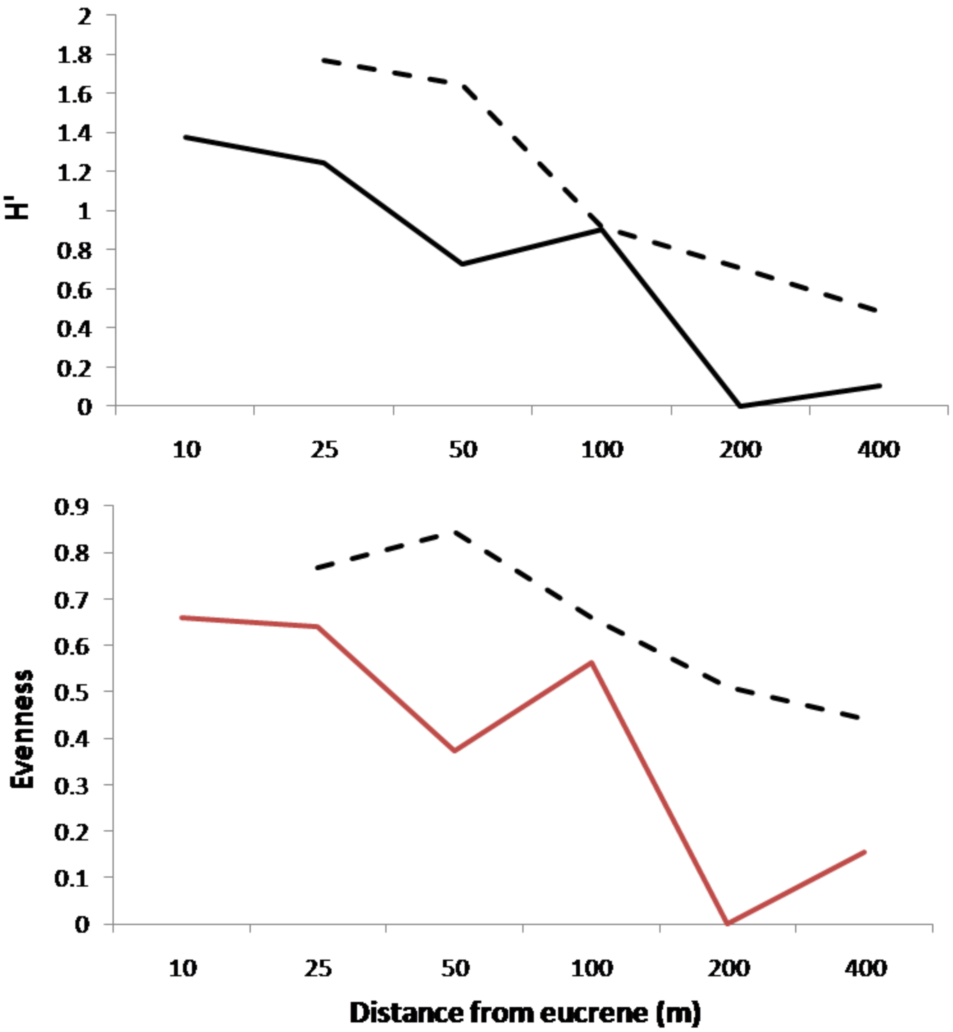


Figure 42. Longitudinal changes in Shannon diversity (H') and evenness in the Prairie spring CSCR in 2007 and 2008 [dashed line, 2007 sampling period; solid line, 2008 sampling period].

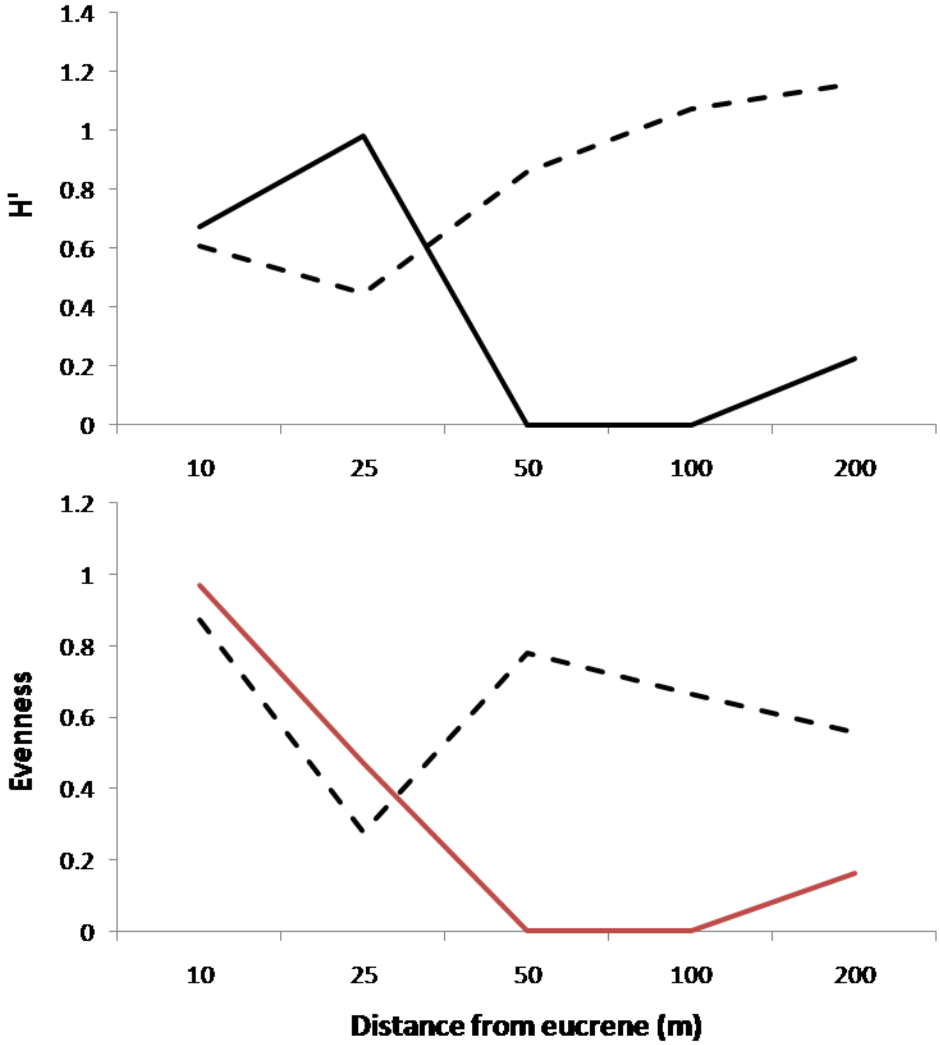


Figure 43. Longitudinal changes in Shannon diversity (H’) and evenness in the Prairie spring LSCR in 2007 and 2008 [dashed line, 2007 sampling period; solid line, 2008 sampling period].

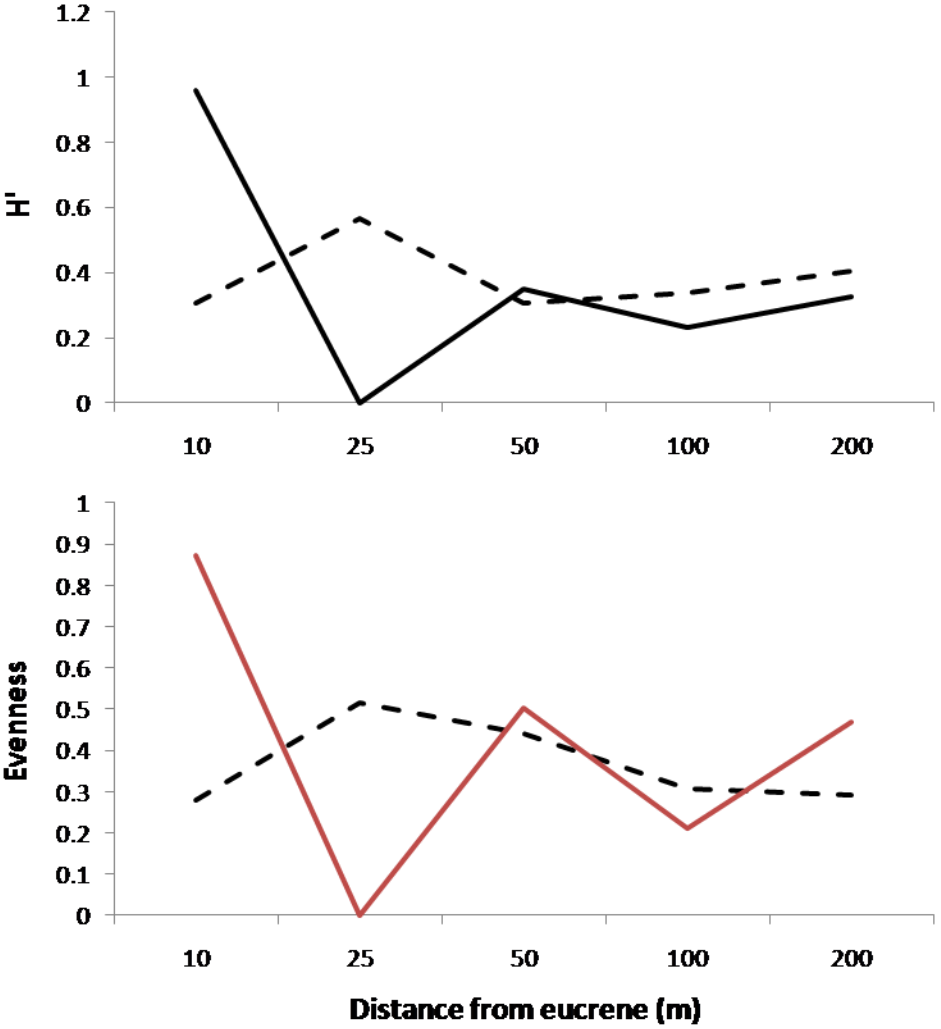


Figure 44. Longitudinal changes in Shannon diversity (H’) and evenness in the Big River spring 1RB in 2007 and 2008 [dashed line, 2007 sampling period; solid line, 2008 sampling period].

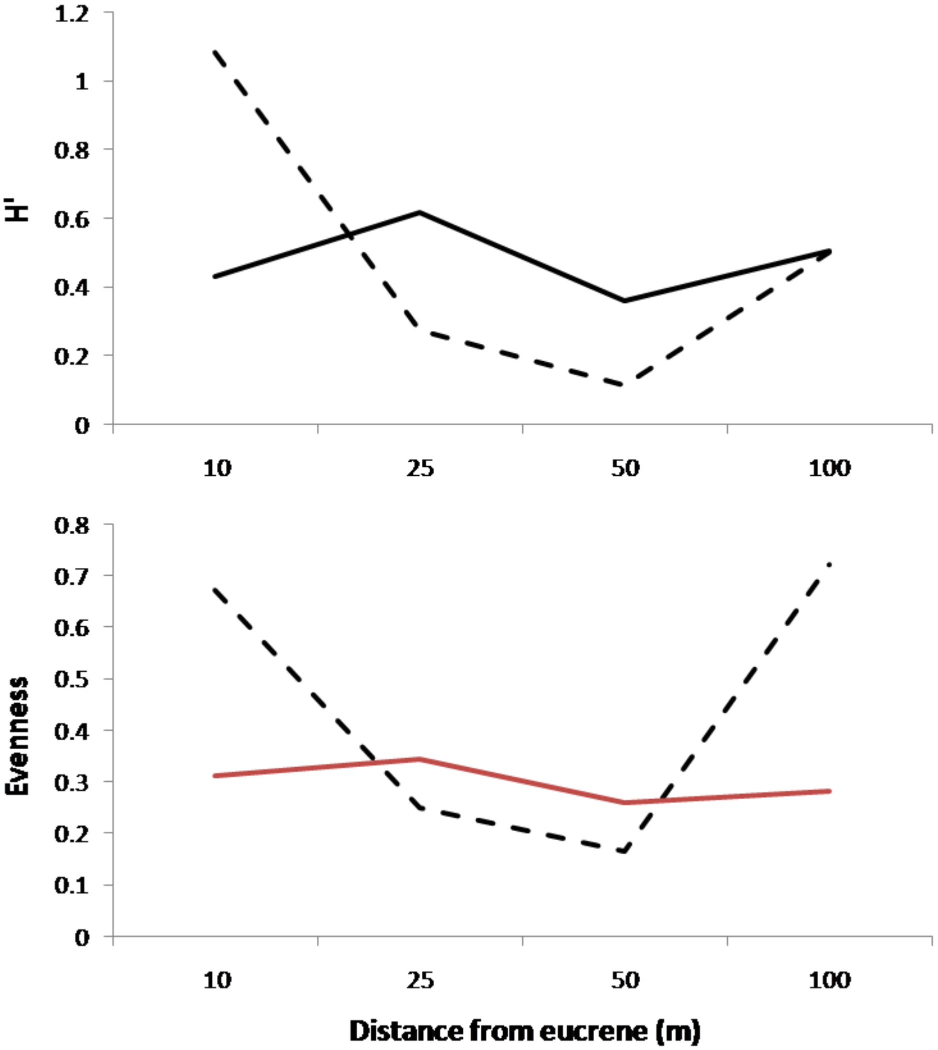


Figure 45. Longitudinal changes in Shannon diversity (H') and evenness in the Ozark spring CSM in 2007 and 2008 [dashed line, 2007 sampling period; solid line, 2008 sampling period].

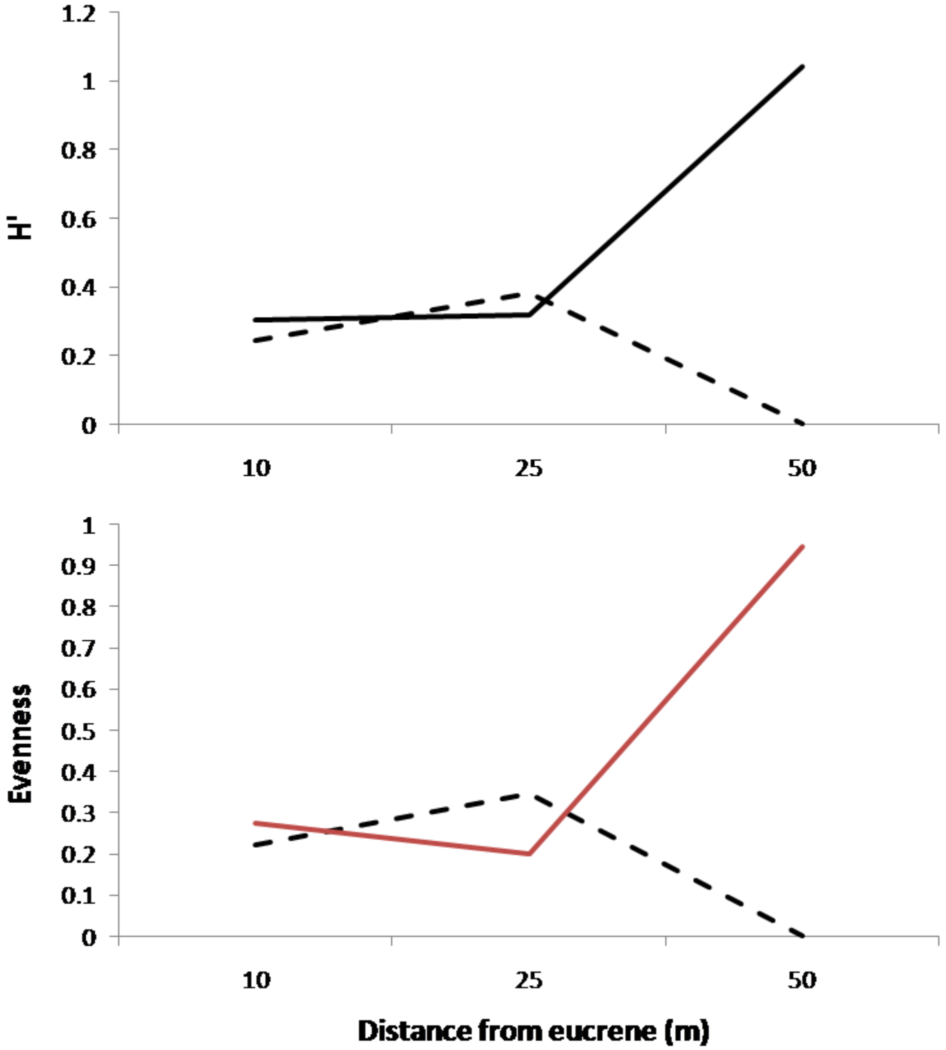


Figure 46. Longitudinal changes in Shannon diversity (H') and evenness in the Ozark spring 2DM in 2007 and 2008 [dashed line, 2007 sampling period; solid line, 2008 sampling period].

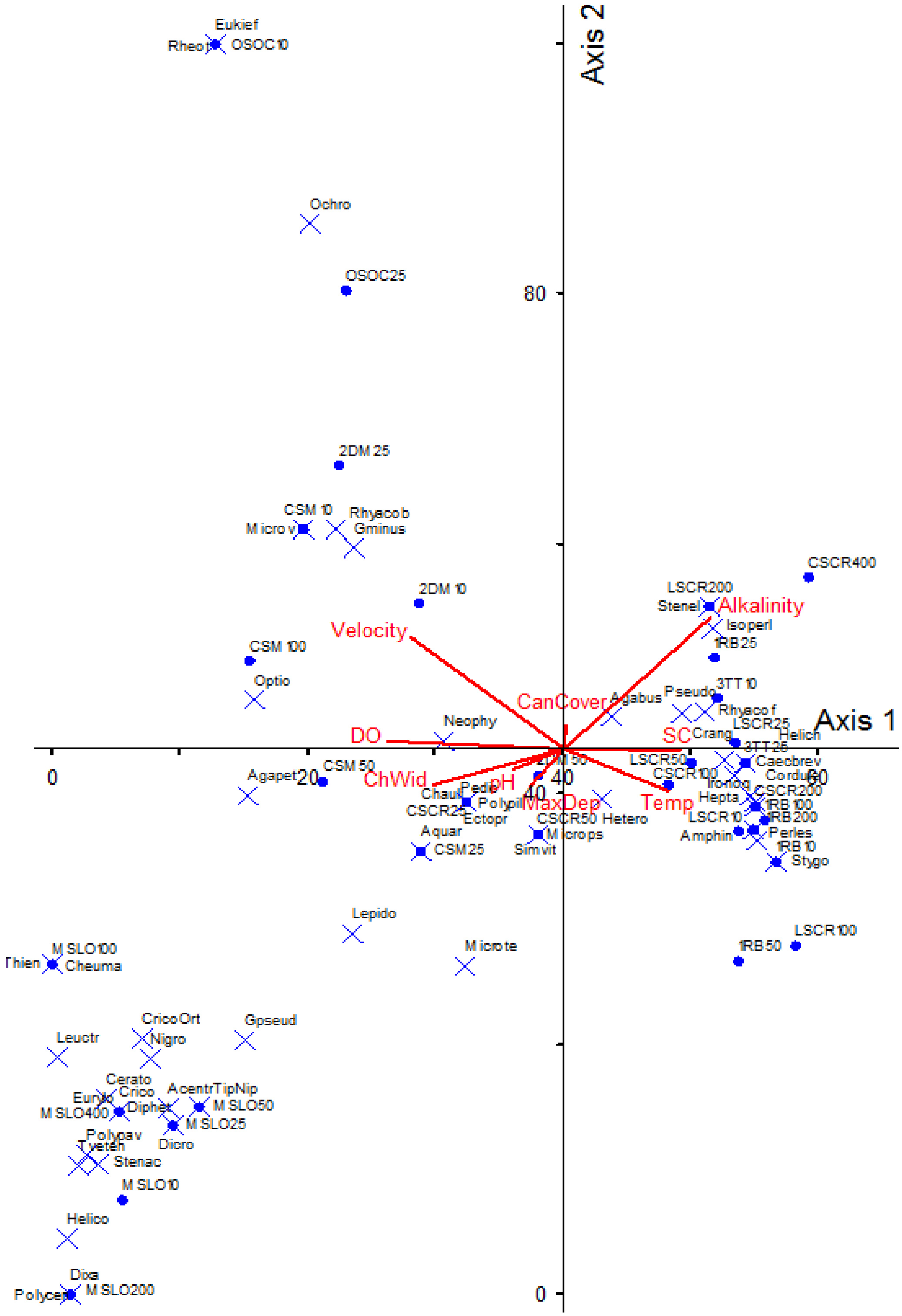


Figure 47. Canonical Correspondence Analysis (CCA) biplot for the 2007 sampling period showing the relationship among spring sites, species composition, and environmental variables [•, sampling sites; X, species; lines, environmental variables; DO, dissolved oxygen, ChWid, channel width; MaxDep, maximum depth; Temp, water temperature; SC, specific conductivity; CanCover; canopy cover].

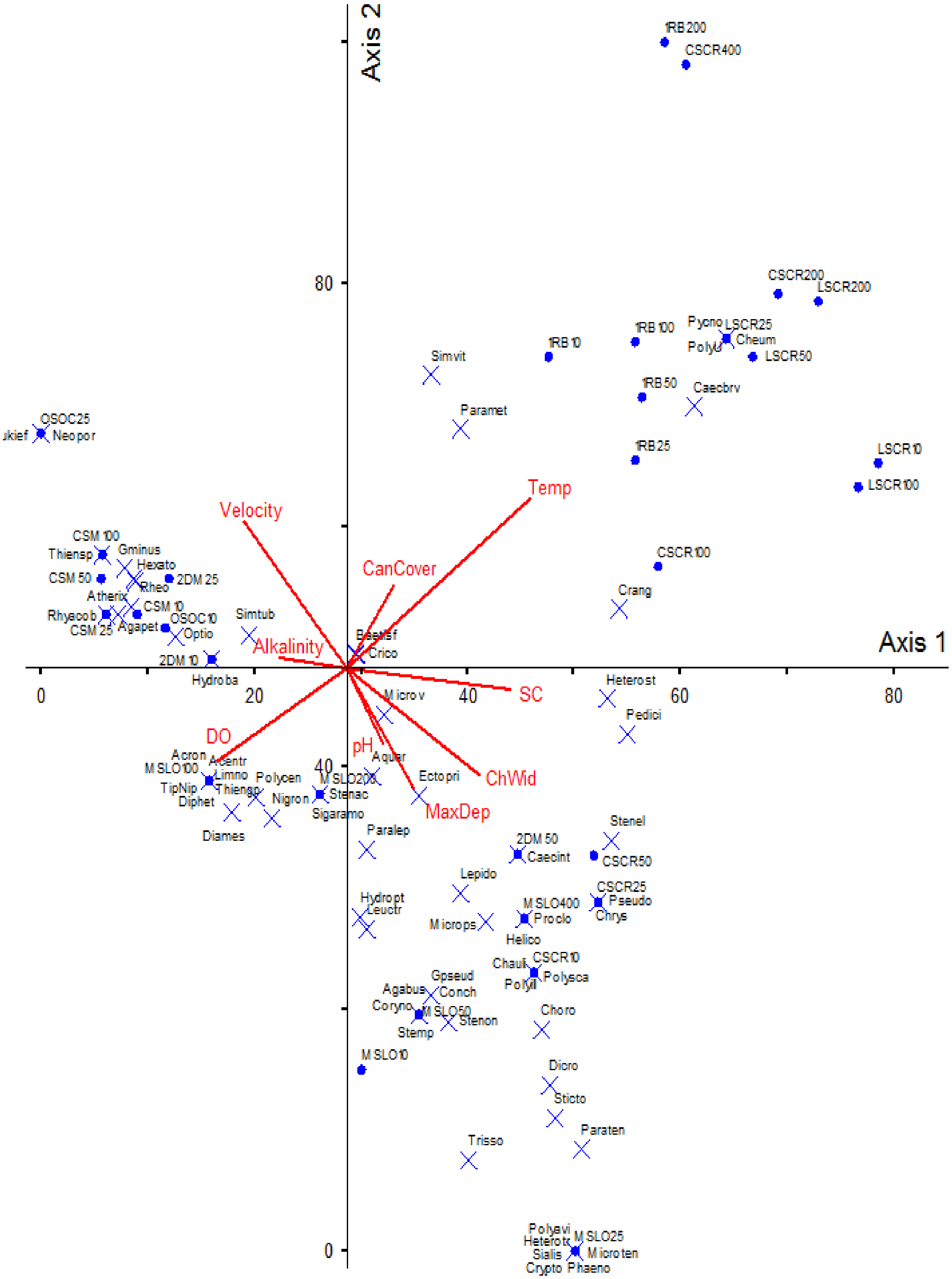


Figure 48. Canonical Correspondence Analysis (CCA) biplot for the 2008 sampling period showing the relationship among spring sites, species composition, and environmental variables [•, sampling sites; X, species; lines, environmental variables; DO, dissolved oxygen, ChWid, channel width; MaxDep, maximum depth; Temp, water temperature; SC, specific conductivity; CanCover; canopy cover].

Figure 49. Locations of qualitatively sampled high discharge rheocrene spring systems in Missouri [A, Bennett Spring at Bennett Spring SP (BS); B, Ha Ha Tonka Spring at Ha Ha Tonka SP (HTS); C, Montauk Spring at Montauk SP (MS); D, Roaring River Spring at Roaring River SP (RRS)].

Figure 50. Bennett Spring at Bennett Spring State Park (BS).

Figure 51. Ha Ha Tonka Spring at Ha Ha Tonka State Park (HTS).

Figure 52. Montauk Spring at Montauk State Park (MS).

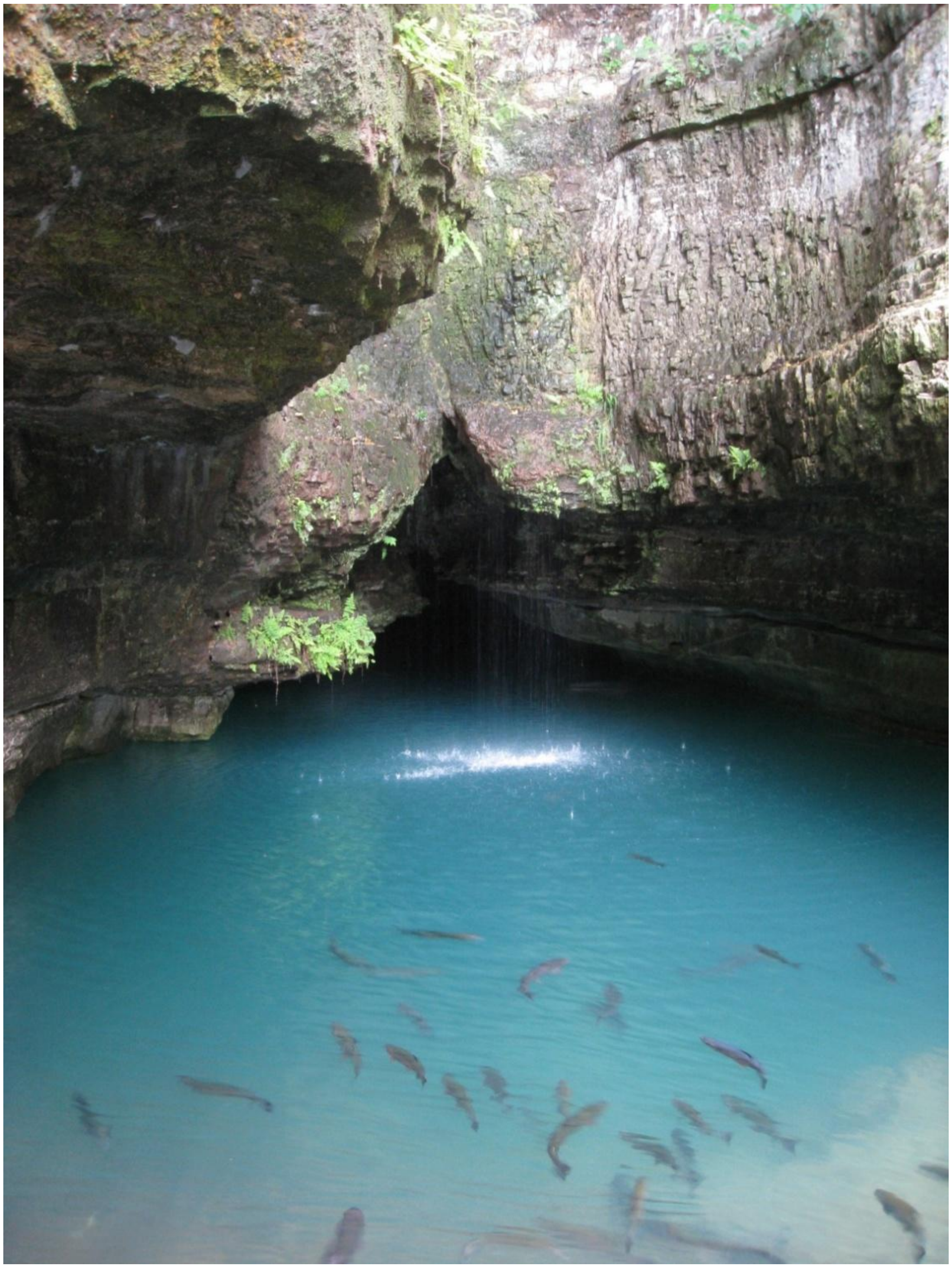


Figure 53. Roaring River Spring at Roaring River State Park (RRS).

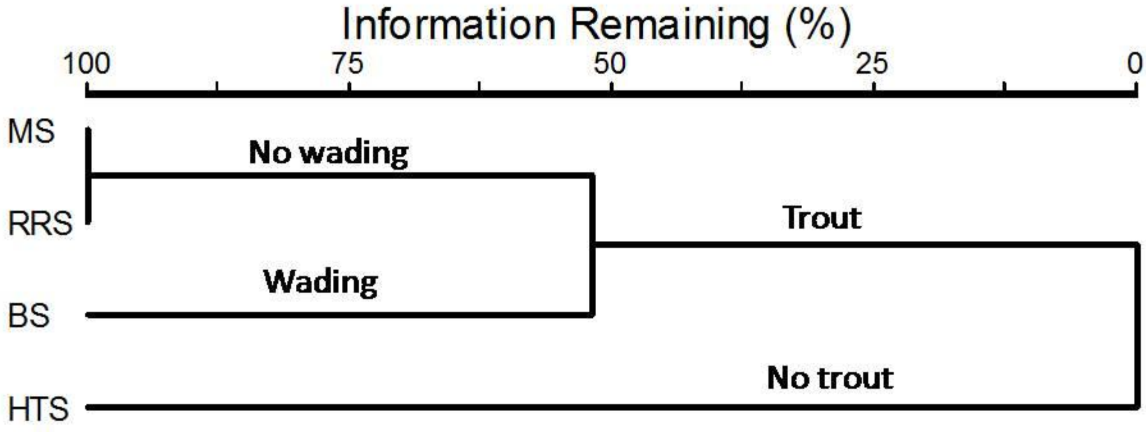


Figure 54. UPGMA cluster analysis using Sorenson's similarity coefficient showing similarity in aquatic insect, amphipod, and isopod community composition among the qualitatively sampled high discharge rheocrene spring systems [MS, Montauk Spring at Montauk SP; RRS, Roaring River Spring at Roaring River SP; BS, Bennett Spring at Bennett Spring SP; HTS, Ha Ha Tonka Spring at Ha Ha Tonka SP; No wading, no wading allowed by fisherman; wading, wading allowed by fisherman; trout, trout present in spring system; no trout, trout absent from spring system].

Figure 55. Locations of qualitatively sampled unique spring systems in Missouri [A, Boone's Lick Spring at Boone's Lick SHS (SSBL); B, seepage channels at Hawn SP (SCHS); C, spring-fed Oumessourit Marsh at Van Meter SP (SMVM); D, spring-fed fen at St. Francois SP (FSFS)].

Figure 56. Boone’s Lick Spring at Boone’s Lick State Historic Site (SSBL).

Figure 57. Seeps at Hawn State Park (SCHS).

Figure 58. A portion of the spring that feeds Oumessourit Marsh at Van Meter State Park (SMVM).

Figure 59. Spring-fed fen at St. Francois State Park (FSFS).

Table 1. Springs sampled and the state park and county in which each is located [SP, state park; SHS, state historic site].

SPRING	STATE PARK	COUNTY
Quantitative Rheocrene Spring Systems		
Cave Spring	Cuivre River SP	Lincoln
Chickadee Spring	Meramec SP	Crawford and Franklin
Lone Spring	Cuivre River SP	Lincoln
Mill Spring	Lake of the Ozarks SP	Camden
Onondaga Spring	Onondaga Cave SP	Crawford
unnamed spring	Dillard Mill SHS	Crawford
unnamed spring	Rockbridge Memorial SP	Boone
unnamed spring	Trail of Tears SP	Cape Girardeau
Qualitative Rheocrene Spring Systems		
Bennett Spring	Bennett Springs SP	Dallas and Laclede
Ha Ha Tonka Spring	Ha Ha Tonka SP	Camden
Montauk Spring	Montauk SP	Dent
Roaring River Spring	Roaring River SP	Barry
Qualitative Unique Spring Systems		
Boone’s Lick Spring (saline)	Boone's Lick SHS	Howard
seepage channels	Hawn SP	St. Genevieve
spring-fed fen	Saint Francois SP	St. Francois
spring-fed marsh	Van Meter SP	Saline

Table 2. Taxa collected from the eight quantitatively sampled low to medium discharge rheocrene spring systems in the 2007 sampling period [EIH, endemic to the Interior Highlands].

Order	Family	Taxon	Status
Unnamed spring at Trail of Tears SP			
Amphipoda	Crangonyctidae	*Crangonyx forbesi* (Hubricht & Mackin)	
Coleoptera	Dryopidae	*Helichus* sp.	
	Dytiscidae	*Agabus* sp.	
Diptera	Tipulidae	*Pseudolimnophila* sp.	
Isopoda	Asellidae	*Caecidotea brevicauda* Forbes	
Trichoptera	Limnephilidae	*Ironoquia punctatissima* (Walker)	
Unnamed spring at Dillard Mill SHS			
Amphipoda	Crangonyctidae	*Bactrurus brachycaudus* Hubricht & Mackin	S4, G4
		Crangonyx forbesi (Hubricht & Mackin)	
	Gammaridae	*Gammarus minus Say*	
Coleoptera	Elmidae	*Optioservus sandersoni* Collier	
	Dytiscidae	*Agabus* sp.	
	Hydrophilidae	*Hydrobius melaenus* (Germar)	
Diptera	Chironomidae	*Hydrobaenus* sp.	
		Thienemanniella sp.	
		Tvetenia sp.	
	Ephydridae	*Parydra* sp.	
	Tipulidae	*Tipula* sp.	
Ephemeroptera	Ameletidae	*Ameletus lineatus* Traver	
	Baetidae	*Baetis flavistriga* McDunnough	
Hemiptera	Veliidae	*Microvelia americana* Uhler	
Isopoda	Asellidae	*Caecidotea intermedia* Forbes	
Plecoptera	Nemouridae	*Amphinemura* sp.	
Trichoptera	Lepidostomatidae	*Lepidostoma* sp.	
	Rhyacophilidae	*Rhyacophila banksi* Ross	
Unnamed spring at Rockbridge SP			
Amphipoda	Crangonyctidae	*Bactrurus brachycaudus* Hubricht & Mackin	S4, G4
		Crangonyx forbesi (Hubricht & Mackin)	
		Stygobromus sp.	
Diptera	Tipulidae	*Pseudolimnophila* sp.	
Isopoda	Asellidae	*Caecidotea brevicauda* Forbes	
Odonata	Cordulegastridae	*Cordulegaster obliqua* (Say)	
Plecoptera	Nemouridae	*Amphinemura* sp.	
Trichoptera	Limnephilidae	*Ironoquia punctatissima* (Walker)	
	Rhyacophilidae	*Rhyacophila fenestra* Ross	
Mill Spring at Lake of the Ozarks SP			
Amphipoda	Gammaridae	*Gammarus pseudolimnaeus* Bousfield	
Coleoptera	Elmidae	*Optioservus sandersoni* Collier	

Table 2. Continued

Order	Family	Taxon	Status
Diptera	Chironomidae	*Cricotopus (C.) trifascia* Edwards	
		Cricotopus/Orthocladius sp.	
		Dicrotendipes sp.	
		Microtendipes sp.	
		Polypedilum (Uresipedilum) aviceps Townes	
		Psilometriocnemus sp.	
		Thienemanniella sp.	
		Tvetenia sp.	
	Dixidae	*Dixa* sp.	
	Tipulidae	*Tipula (Nippotipula)* sp.	
Ephemeroptera	Baetidae	*Acentrella turbida* (McDunnough)	
		Diphetor hageni (Eaton)	
	Ephemerellidae	*Eurylophella lutulenta* (Clemens)	
	Heptageniidae	*Stenacron interpunctatum* (Say)	
Megaloptera	Corydalidae	*Nigronia serriconus* (Say)	
Plecoptera	Leuctridae	*Leuctra* sp.	
Trichoptera	Glossosomatidae	*Agapetus illini Ross*	
	Helicopsychidae	*Helicopsyche limnella* Ross	EIH
	Hydropsychidae	*Ceratopsyche slossonae* Banks	
		Cheumatopsyche sp.	
	Hydroptilidae	*Hydroptila* sp.	
	Lepidostomatidae	*Lepidostoma* sp.	
	Polycentropodidae	*Polycentropus* sp.	
	Rhyacophilidae	*Rhyacophila* sp.	

Onondaga Spring at Onondaga Cave SP

Order	Family	Taxon	Status
Amphipoda	Gammaridae	*Gammarus minus* Say	
		Gammarus pseudolimnaeus Bousfield	
Coleoptera	Dytiscidae	*Hydroporus rufilabris* Sharp	
		Laccophilus maculosus (Say)	
		Neoporus dimidiatus Gemminger & Harold	
	Elmidae	*Optioservus sandersoni* Collier	
	Gyrinidae	*Dineutus emarginatus* Say	
	Haliplidae	*Haliplus variomaculatus* Brigham & Sanderson	
Diptera	Ceratopogonidae	*Tribe Palpomyiini*	
	Chironomidae	*Dicrotendipes* sp.	
		Eukiefferiella sp.	
		Micropsectra sp.	
		Paracladopelma sp.	
		Paratanytarsus sp.	
		Psectrocladius sp.	
		Rheotanytarsus sp.	
Hemiptera	Corixidae	*Sigara mathesoni* Hungerford	
	Gerridae	*Aquarius remigis* (Say)	
		Gerris marginatus Say	
	Veliidae	*Microvelia americana* Uhler	
Isopoda	Asellidae	*Caecidotea brevicauda* Forbes	
Odonata	Coenagrionidae	*Argia apicalis* (Say)	

Table 2. Continued.

Order	Family	Taxon	Status
		Ischnura sp.	
Trichoptera	Hydroptilidae	*Ochrotrichia* sp.	
	Leptoceridae	*Triaenodes* sp.	
Chickadee Spring at Meramec SP			
Amphipoda	Gammaridae	*Gammarus minus* Say	
Diptera	Chironomidae	*Cricotopus/Orthocladius* sp.	
Hemiptera	Gerridae	*Aquarius remigis* (Say)	
	Veliidae	*Microvelia americana* Uhler	
Trichoptera	Glossosomatidae	*Agapetus illini* Ross	
	Hydropsychidae	*Ceratopsyche slossonae* Banks	
	Rhyacophilidae	*Rhyacophila banksi* Ross	
	Uenoidae	*Neophylax concinnus* MacLachlan	
Cave Spring at Cuivre River SP			
Amphipoda	Crangonyctidae	*Crangonyx forbesi* (Hubricht & Mackin)	
	Gammaridae	*Gammarus pseudolimnaeus* Bousfield	
Coleoptera	Dytiscidae	*Agabus* sp.	
		Heterosternuta ohionis (Fall)	
	Psephenidae	*Ectopria* sp.	
Diptera	Chironomidae	*Micropsectra* sp.	
		Microtendipes sp.	
		Polypedilum (s.str.) illinoense (Malloch)	
	Simuliidae	*Simulium vittatum complex*	
	Tipulidae	*Pedicia* sp.	
		Pseudolimnophila sp.	
Ephemeroptera	Heptageniidae	*Heptagenia flavescens* Walsh	
Hemiptera	Veliidae	*Microvelia americana* Uhler	
Isopoda	Asellidae	*Caecidotea brevicauda* Forbes	
Megaloptera	Corydalidae	*Chauliodes pectinicoris* (Linnaeus)	
Trichoptera	Lepidostomatidae	*Lepidostoma* sp.	
	Rhyacophilidae	*Rhyacophila fenestra* Ross	
	Uenoidae	*Neophylax concinnus* MacLachlan	
Lone Spring at Cuivre River SP			
Amphipoda	Crangonyctidae	*Crangonyx forbesi* (Hubricht & Mackin)	
	Gammaridae	*Gammarus pseudolimnaeus* Bousfield	
Coleoptera	Dytiscidae	*Heterosternuta ohionis* (Fall)	
	Elmidae	*Stenelmis* sp.	
Diptera	Tipulidae	*Pseudolimnophila* sp.	
Isopoda	Asellidae	*Caecidotea brevicauda* Forbes	
Odonata	Cordulegastridae	*Cordulegaster obliqua* (Say)	
Plecoptera	Nemouridae	*Amphinemura* sp.	
	Perlidae	*Perlesta cinctipes* (Banks)	
	Perlodidae	*Isoperla decepta* Frison	

Table 2. Continued.

Order	Family	Taxon	Status
Trichoptera	Glossosomatidae	*Agapetus illini* Ross	
	Rhyacophilidae	*Rhyacophila fenestra* Ross	

Table 3. Taxa collected from the eight quantitatively sampled low to medium discharge rheocrene spring systems in the 2008 sampling period [EIH, endemic to the Interior Highlands; EOH, endemic to the Ozark Highlands].

Order	Family	Taxon	Status
Unnamed spring at Dillard Mill SHS			
Amphipoda	Gammaridae	*Gammarus minus* Say	
Coleoptera	Elmidae	*Optioservus sandersoni* Collier	
Diptera	Chironomidae	*Hydrobaenus* sp.	
	Tipulidae	*Hexatoma* sp.	
Hemiptera	Veliidae	*Microvelia americana* Uhler	
Isopoda	Asellidae	*Caecidotea intermedia* Forbes	
Trichoptera	Lepidostomatidae	*Lepidostoma* sp.	
	Rhyacophilidae	*Rhyacophila banksi* Ross	
		Rhyacophila fenestra Ross	
	Uenoidae	*Neophylax concinnus* MacLachlan	
Unnamed spring at Rockbridge SP			
Amphipoda	Crangonyctidae	*Crangonyx forbesi* (Hubricht & Mackin)	
	Gammaridae	*Gammarus pseudolimnaeus* Bousfield	
Coleoptera	Dytiscidae	*Heterosternuta* sp.	
	Hydrophilidae	*Anacaena limbata* (Fabricius)	
Diptera	Chironomidae	*Micropsectra* sp.	
		Paratendipes sp.	
Hemiptera	Gerridae	*Aquarius remigis* (Say)	
Isopoda	Asellidae	*Caecidotea brevicauda* Forbes	
		Caecidotea salemensis Lewis	S2, G4, EOH
Odonata	Cordulegastridae	*Cordulegaster obliqua* (Say)	
Mill Spring at Lake of the Ozarks SP			
Amphipoda	Crangonyctidae	*Crangonyx forbesi* (Hubricht & Mackin)	
	Gammaridae	*Gammarus pseudolimnaeus* Bousfield	
		Gammarus sp.	
Coleoptera	Dytiscidae	*Agabus* sp.	
	Elmidae	*Optioservus sandersoni* Collier	
	Psephenidae	*Ectopria* sp.	
Diptera	Chironomidae	*Conchapelopia* sp.	
		Corynoneura sp.	
		Cricotopus/Orthocladius sp.	
		Cryptochironomus sp.	
		Diamesa sp.	
		Dicrotendipes sp.	
		Heterotrissocladius sp.	
		Micropsectra sp.	
		Microtendipes sp.	
		Paratendipes sp.	
		Phaenopsectra sp.	

Table 3. Continued

Order	Family	Taxon	Status
		Polypedilum (Uresipedilum) aviceps Townes	
		Stempellinella sp.	
		Stictochironomus sp.	
		Thienemannimyia group	
		Trissopelopia sp.	
	Simuliidae	*Simuliium tuberosum complex*	
	Tipulidae	*Limnophila* sp.	
		Tipula (Nippotipula) sp.	
Ephemeroptera	Baetidae	*Acentrella turbida* (McDunnough)	
		Baetis flavistriga McDunnough	
		Diphetor hageni (Eaton)	
		Procloeon nr. rubropictum (McDunnough)	
	Heptageniidae	*Stenacron interpunctatum* (Say)	
		Stenonema femoratum (Say)	
	Leptophlebiidae	*Choroterpes basalis* (Banks)	
		Paraleptophlebia assimilis (Banks)	
Hemiptera	Corixidae	*Sigara modesta* (Abbott)	
	Gerridae	*Aquarius remigis* (Say)	
Isopoda	Asellidae	*Caecidotea brevicauda* Forbes	
Megaloptera	Corydalidae	*Nigronia serriconus* (Say)	
	Sialidae	*Sialis* sp.	
Plecoptera	Leuctridae	*Leuctra* sp.	
	Perlidae	*Acroneuria frisoni* (Stark)	
Trichoptera	Glossosomatidae		
	Helicopsychidae	*Helicopsyche limnella* Ross	EIH
	Hydroptilidae	*Hydroptila* sp.	
	Lepidostomatidae	*Lepidostoma* sp.	
	Polycentropodidae	*Polycentropus* sp.	
Onondaga Spring at Onondaga Cave SP			
Amphipoda	Gammaridae	*Gammarus minus* Say	
		Gammarus pseudolimnaeus Bousfield	
		Gammarus sp.	
	Hyalellidae	*Hyalella azteca* Saussure	
Coleoptera	Dytiscidae	*Coptotomus loticus* Hilsenhoff	
		Heterosternuta sp.	
		Neoporus dimidiatus Gemminger & Harold	
	Elmidae	*Optioservus sandersoni* Collier	
	Haliplidae	*Haliplus variomaculatus* Brigham & Sanderson	
	Helophoridae	*Helophorus* sp.	
	Hydrophilidae	*Berosus* sp.	
		Tropisternus lateralis nimbatus (Say)	
Diptera	Ceratopogonidae	*Tribe Palpomyiini*	
	Chironomidae	*Cricotopus/Orthocladius* sp.	
		Eukiefferiella sp.	
		Paratanytarsus sp.	
		Procladius sp.	
		Rheotanytarsus sp.	

Table 3. Continued.

Order	Family	Taxon	Status
	Simuliidae	*Simulium tuberosum complex*	
		Simulium vittatum complex	
Ephemeroptera	Baetidae	*Callibaetis* sp.	
Hemiptera	Corixidae	*Sigara mathesoni* Hungerford	
	Gerridae	*Aquarius remigis* (Say)	
	Veliidae	*Microvelia americana* Uhler	
Isopoda	Asellidae	*Caecidotea brevicauda* Forbes	
Odonata	Coenagrionidae	*Ischnura* sp.	
Chickadee Spring at Meramec SP			
Amphipoda	Gammaridae	*Gammarus minus Say*	
		Gammarus pseudolimnaeus Bousfield	
Coleoptera	Elmidae	*Optioservus sandersoni* Collier	
	Psephenidae	*Ectopria leechi* Brigham	
Diptera	Athericidae	*Atherix* sp.	
	Chironomidae	*Diamesa* sp.	
		Parametriocnemus sp.	
		Thienemanniella sp.	
	Tipulidae	*Hexatoma* sp.	
Isopoda	Asellidae	*Caecidotea brevicauda* Forbes	
Trichoptera	Brachycentridae	*Micrasema rusticum* (Hagen)	
	Glossosomatidae	*Agapetus illini* Ross	
	Hydroptilidae		
	Lepidostomatidae	*Lepidostoma* sp.	
	Rhyacophilidae	*Rhyacophila banksi* Ross	
	Uenoidae	*Neophylax concinnus* MacLachlan	
Cave Spring at Cuivre River SP			
Amphipoda	Crangonyctidae	*Crangonyx forbesi* (Hubricht & Mackin)	
	Gammaridae	*Gammarus minus Say*	
		Gammarus pseudolimnaeus Bousfield	
Coleoptera	Dytiscidae	*Heterosternuta ohionis* (Fall)	
	Elmidae	*Stenelmis* sp.	
	Psephenidae	*Ectopria* sp.	
Diptera	Chironomidae	*Micropsectra* sp.	
		Polypedilum (s.str.) illinoense (Malloch)	
		Polypedilum (Tripodura) scalaenum (Schrank)	
	Tabanidae	*Chrysops* sp.	
	Tipulidae	*Pedicia* sp.	
		Pseudolimnophila sp.	
Hemiptera	Veliidae	*Microvelia* sp.	
Isopoda	Asellidae	*Caecidotea brevicauda* Forbes	
Megaloptera	Corydalidae	*Chauliodes pectinicoris* (Linnaeus)	
Trichoptera	Lepidostomatidae	*Lepidostoma* sp.	

Table 3. Continued.

Order	Family	Taxon	Status
Lone Spring at Cuivre River SP			
Amphipoda	Crangonyctidae	*Crangonyx forbesi* (Hubricht & Mackin)	
	Gammaridae	*Gammarus pseudolimnaeus* Bousfield	
Coleoptera	Elmidae	*Stenelmis* sp.	
Diptera	Chironomidae	*Cricotopus/Orthocladius* sp.	
		Parametriocnemus sp.	
		Paratendipes sp.	
		Polypedilum (Uresipedilum) sp.	
	Simuliidae	*Simulium tuberosum complex*	
		Simulium vittatum complex	
Ephemeroptera	Baetidae	*Acerpenna pygmaea* (Hagen)	
		Baetis flavistriga McDunnough	
	Heptageniidae	*Stenonema femoratum* (Say)	
Hemiptera	Gerridae	*Aquarius remigis* (Say)	
	Veliidae	*Microvelia americana* Uhler	
Isopoda	Asellidae	*Caecidotea brevicauda* Forbes	
Trichoptera	Hydropsychidae	*Cheumatopsyche* sp.	
	Leptoceridae	*Pycnopsyche* sp.	

Table 4. Canonical Correspondence Analysis (CCA) summary statistics for 2007, including eigenvalues, variance explained, and Pearson and Kendall Correlation values for the first three canonical axes.

Number of canonical axes: 3
Total variance ("inertia") in the species data: 4.8896

	Axis 1	Axis 2	Axis 3
Eigenvalue	0.891	0.545	0.286
Variance in species data			
% of variance explained	18.2	11.1	5.8
Cumulative % explained	18.2	29.4	35.2
Pearson Correlation, Spp-Envt*	0.984	0.825	0.888
Kendall (Rank) Corr., Spp-Envt	0.774	0.589	0.653

Table 5. Canonical Correspondence Analysis (CCA) summary statistics for 2008, including eigenvalues, variance explained, and Pearson and Kendall Correlation values for the first three canonical axes.

Number of canonical axes: 3
Total variance ("inertia") in the species data: 5.2022

	Axis 1	Axis 2	Axis 3
Eigenvalue	0.826	0.665	0.353
Variance in species data			
% of variance explained	15.9	12.8	6.8
Cumulative % explained	15.9	28.7	35.4
Pearson Correlation, Spp-Envt*	0.958	0.928	0.787
Kendall (Rank) Corr., Spp-Envt	0.760	0.708	0.522

Table 6. Summary table of the stepwise regression results showing the subset of environmental variables that best explains the variance in species richness, diversity, and evenness in 2007 and 2008 and the corresponding adjusted R^2 value [ChWid, channel width; Temp, water temperature; CurVel, water current velocity; DO, dissolved oxygen; SC, specific conductivity; Alk, alkalinity; MaxDep, maximum water depth; CanCov, canopy cover; p-value to enter and p-value to remove from model = 0.15].

Spring System	Community Measure	Influential Environmental Variable(s)	Adjusted R^2
2007			
1RB	Species Richness	pH, ChWid, Temp	99.86
	Shannon Diversity Index	CurVel, Temp, DO	99.78
	Shannon Evenness	SC	43.58
CSCR	Species Richness	DO	92.08
	Shannon Diversity Index	DO	97.82
	Shannon Evenness	Alk	79.57
LSCR	Species Richness	DO	64.63
	Shannon Diversity Index	Temp, SC, Alk	100
	Shannon Evenness	none	*
2DM	Species Richness	Alk	97.37
	Shannon Diversity Index	CurVel	99.02
	Shannon Evenness	CurVel	99.02
CSM	Species Richness	SC	95.45
	Shannon Diversity Index	none	*
	Shannon Evenness	MaxDep, CanCov	99.91
MSLO	Species Richness	pH, CurVel, Alk	93.79
	Shannon Diversity Index	pH, CurVel, DO	95.04
	Shannon Evenness	none	*

Table 6. Continued.

Spring System	Community Measure	Influential Environmental Variable(s)	Adjusted R^2
2008			
1RB	Species Richness	MaxDep	47.25
	Shannon Diversity Index	CanCov	70.79
	Shannon Evenness	CanCov	58.95
CSCR	Species Richness	Temp, CurVel, CanCov, ChWid	100
	Shannon Diversity Index	Temp	79.85
	Shannon Evenness	pH	63.82
LSCR	Species Richness	SC, DO	94.3
	Shannon Diversity Index	SC	74.04
	Shannon Evenness	none	*
2DM	Species Richness	none	*
	Shannon Diversity Index	pH	99.91
	Shannon Evenness	ChWid	99.93
CSM	Species Richness	CurVel	97.22
	Shannon Diversity Index	CurVel, pH	99.91
	Shannon Evenness	ChWid, Temp	99.97
MSLO	Species Richness	ChWid, pH, SC, DO	99.93
	Shannon Diversity Index	ChWid, pH, Temp, CanCov	99.71
	Shannon Evenness	ChWid, pH, Temp	99.66

Table 7. Summary table of the stepwise regression results showing the subset of environmental variables that best explains the variance in the density of the dominant species in 2007 and 2008 and the corresponding adjusted R^2 value [CurVel, water current velocity; Alk, alkalinity; MaxDep, maximum water depth; ChWid, channel width; CanCov, camopy cover; DO, dissolved oxygen; SC, specific conductivity; Temp, water temperature; p-value to enter and p-value to remove from model = 0.15].

Spring System	Dominant Species	Influential Environmental Variable(s)	Adjusted R^2
2007			
1RB	*Caecidotea brevicauda* Forbes	pH, CurVel, Alk	99.29
CSCR	*Caecidotea brevicauda* Forbes	MaxDep	43.22
	Crangonyx forbesi (Hubricht & Mackin)	ChWid, CanCov	94.67
LSCR	*Caecidotea brevicauda* Forbes	DO, pH, SC	100
	Crangonyx forbesi (Hubricht & Mackin)	none	*
2DM	*Gammarus minus* Say	CurVel	98.94
CSM	*Gammarus minus* Say	MaxDep	94.03
MSLO	*Gammarus pseudolimnaeus* Bousfield	CanCov, MaxDep, Alk	95.67
2008			
1RB	*Caecidotea brevicauda* Forbes	none	*
CSCR	*Caecidotea brevicauda* Forbes	CanCov, ChWid, DO, Temp	99.99
	Gammarus pseudolimnaeus Bousfield	CanCov	44.03
LSCR	*Caecidotea brevicauda* Forbes	DO, CurVel, pH	99.87
2DM	*Gammarus minus* Say	SC	99.82
CSM	*Gammarus minus* Say	CurVel, CanCov	99.88
MSLO	*Gammarus pseudolimnaeus* Bousfield	pH	55.03

Table 8. Taxa collected from the four qualitatively sampled high discharge rheocrene spring systems in 2007 [EIH, endemic to the Interior Highlands; EOH, endemic to the Ozark Highlands].

Order	Family	Taxon	Status
Montauk Spring at Montauk SP			
Amphipoda	Crangonyctidae	*Stygobromus alabamensis* (Stout)	EIH
	Hyalellidae	*Hyalella azteca* Saussure	
Coleoptera	Dytiscidae	*Heterosternuta wickhami* (Zaitzev)	
	Hydrophilidae	*Enochrus pygmaeus nebulosus* (Say)	
		Tropisternus sp.	
Diptera	Ceratopogonidae	Tribe Palpomyiini	
	Chironomidae	*Conchapelopia* sp.	
		Cricotopus sp.	
		Cricotopus/Orthocladius sp.	
		Cryptochironomus sp.	
		Dicrotendipes sp.	
		Eukiefferiella sp.	
		Micropsectra sp.	
		Microtendipes sp.	
		Parametriocnemus sp.	
		Paratanytarsus sp.	
		Procladius sp.	
		Rheotanytarsus sp.	
		Stictochironomus sp.	
	Culicidae	*Anopheles punctipennis* (Say)	
	Dixidae	*Dixa* sp.	
	Simuliidae	*Simulium vittatum complex*	
		Simulium tuberosum complex	
	Stratiomyidae	*Allognosta* sp.	
Ephemeroptera	Baetidae	*Baetis tricaudatus* Dodds	
		Iswaeon anoka (Daggy)	
		Iswaeon anoka (Daggy) variant	
	Ephemerellidae	*Serratella frisoni* (McDunnough)	S2, G4
	Leptohyphidae	*Tricorythodes* sp.	
Hemiptera	Gerridae	*Aquarius remigis* (Say)	
	Veliidae	*Microvelia americana* Uhler	
Odonata	Coenagrionidae	*Ischnura* sp.	
Trichoptera	Glossosomatidae	*Glossosoma intermedium* Klapalek	
	Hydropsychidae	*Ceratopsyche piatrix* Ross	S4, G4, EIH
	Lepidostomatidae	*Lepidostoma* sp.	
Roaring River Spring at Roaring River SP			
Amphipoda	Crangonyctidae	*Crangonyx forbesi* (Hubricht & Mackin)	
		Stygobromus alabamensis (Stout)	EIH
	Gammaridae	*Gammarus minus* Say	
		Gammarus pseudolimnaeus Bousfield	
Coleoptera	Dytiscidae	*Laccophilus proximus* Say	
	Hydrophilidae	*Enochrus pygmaeus nebulosus* (Say)	
		Tropisternus sp.	

Table 8. Continued.

Order	Family	Taxon	Status
Diptera	Ceratopogonidae	*Bezzia* sp.	
		Tribe Palpomyiini	
	Chironomidae	*Cricotopus* sp.	
		Cricotopus/Orthocladius sp.	
		Cryptochironomus sp.	
		Dicrotendipes sp.	
		Eukiefferiella sp.	
		Nanocladius sp.	
		Paramerina sp.	
		Paratanytarsus sp.	
		Paratendipes sp.	
		Rheotanytarsus sp.	
		Thienemanniella sp.	
		Thienemannimyia group	
	Dolichopodidae	*Dolichopus* sp.	
	Muscidae	*Spilogona* sp.	
	Simuliidae	*Simulium claricentrum* Adler	
		Simulium tuberosum complex	
		Simulium vittatum complex	
	Tipulidae	*Limonia (Dicranomyia)* sp.	
Ephemeroptera	Baetidae	*Baetis* sp.	
		Baetis tricaudatus Dodds	
		Callibaetis fluctuans (Walsh)	
		Centroptilum ozarkensum Wiersema & Burian	
	Heptageniidae	*Stenacron interpunctatum* (Say)	
Hemiptera	Corixidae	*Sigara mathesoni* Hungerford	
	Gerridae	*Aquarius remigis* (Say)	
	Pleidae	*Neoplea striola* (Fieber)	
	Veliidae	*Microvelia americana* Uhler	
Isopoda	Asellidae	*Caecidotea brevicauda* Forbes	
		Lirceus sp.	
Odonata	Aeshnidae	*Basiaeschna janata* (Say)	
	Coenagrionidae	*Ischnura* sp.	
Trichoptera	Hydropsychidae	*Ceratopsyche slossonae* Banks	
	Hydroptilidae	*Hydroptila* sp.	
		Oxyethira sp.	
	Polycentropodidae	*Polycentropus* sp.	
Bennett Spring at Bennett Spring SP			
Amphipoda	Gammaridae	*Gammarus pseudolimnaeus* Bousfield	
	Hyalellidae	*Hyalella azteca* Saussure	
Coleoptera	Dytiscidae	*Laccophilus maculosus* Say	
	Elmidae	*Optioservus sandersoni* Collier	
	Hydrophilidae	*Anacaena suturalis* (LeConte)	
		Enochrus pygmaeus nebulosus (Say)	
		Tropisternus collaris (Fabricius)	
		Tropisternus sp.	
Diptera	Ceratopogonidae	Tribe Palpomyiini	
	Chironomidae	*Conchapelopia* sp.	
		Cricotopus sp.	
		Cricotopus/Orthocladius sp.	
		Cryptochironomus sp.	

Table 8. Continued.

Order	Family	Taxon	Status
		Dicrotendipes sp.	
		Micropsectra sp.	
		Paratanytarsus sp.	
		Rheotanytarsus sp.	
		Synorthocladius sp.	
		Thienemannimyia sp.	
	Culicidae	*Anopheles punctipennis* (Say)	
	Simuliidae	*Simulium vittatum complex*	
	Syrphidae	*Eristalis* sp.	
Ephemeroptera	Baetidae	*Centroptilum ozarkensum* Wiersema & Burian	
Hemiptera	Belastomatidae	*Belastoma flumineum* Say	
	Corixidae	*Sigara mathesoni* Hungerford	
	Gerridae	*Aquarius remigis* (Say)	
	Mesoveliidae	*Mesovelia mulsanti* White	
	Saldidae	*Micracanthia humilis* (Say)	
	Veliidae	*Microvelia americana* Uhler	
Odonata	Aeshnidae	*Basiaeschna janata* (Say)	
Trichoptera	Brachycentridae	*Micrasema ozarkana* Ross & Unzicker	EIH
	Hydroptilidae	*Oxyethira* sp.	
Ha Ha Tonka Spring at Ha Ha Tonka SP			
Amphipoda	Gammaridae	*Gammarus pseudolimnaeus* Bousfield	
Coleoptera	Dytiscidae	*Neoporus dimidiatus* Gemminger & Harold	
		Thermonectus basilaris (Harris)	
	Elmidae	*Optioservus sandersoni* Collier	
	Haliplidae	*Peltodytes lengi* Roberts	
	Hydrophilidae	*Enochrus ochraceus* (Melsheimer)	
Diptera	Ceratopogonidae	Tribe Palpomyiini	
	Chironomidae	*Cricotopus* sp.	
		Cryptochironomus sp.	
		Dicrotendipes sp.	
		Eukiefferiella sp.	
		Paratanytarsus sp.	
		Paratendipes sp.	
		Procladius sp.	
		Rheotanytarsus sp.	
		Synorthocladius sp.	
		Thienemannimyia sp.	
	Simuliidae	*Simulium tuberosum complex*	
Ephemeroptera	Baetidae	*Baetis tricaudatus* Dodds	
		Centroptilum ozarkensum Wiersema & Burian	
	Ephemerellidae	*Eurylophella lutulenta* (Clemens)	
Hemiptera	Corixidae	*Trichocorixa calva* (Say)	
	Veliidae	*Microvelia americana* Uhler	
Isopoda	Asellidae	*Caecidotea brevicauda* Forbes	
Trichoptera	Helicopsychidae	*Helicopsyche limnella* Ross	EIH
	Hydropsychidae	*Cheumatopsyche* sp.	
	Hydroptilidae	*Oxyethira* sp.	
	Leptoceridae	*Ceraclea nepha* (Ross)	
		Triaenodes injustus (Hagen)	

Table 9. Taxa collected from the four qualitatively sampled high discharge rheocrene spring systems in 2008 [EIH, endemic to the Interior Highlands; EOH, endemic to the Ozark Highlands].

Order	Family	Taxon	Status
Montauk Spring at Montauk SP			
Amphipoda	Crangonyctidae	*Crangonyx forbesi* (Hubricht & Mackin)	
	Hyalellidae	*Hyalella azteca* Saussure	
Coleoptera	Dytiscidae	*Agabus* sp.	
	Elmidae	*Stenelmis* sp.	
Diptera	Chironomidae	*Cricotopus/Orthocladius* sp.	
		Cryptochironomus sp.	
		Dicrotendipes sp.	
		Eukiefferiella sp.	
		Micropsectra sp.	
		Paratanytarsus sp.	
		Polypedilum sp.	
		Rheotanytarsus sp.	
		Stictochironomus sp.	
	Empididae	*Roederiodes* sp.	
	Ephydridae	*Parydra* sp.	
	Stratiomyidae	*Nemotelus* sp.	
Ephemeroptera	Baetidae	*Baetis tricaudatus* Dodds	
		Centroptilum ozarkensum Wiersema & Burian	
	Caenidae	*Caenis latipennis* Banks	
	Ephemerellidae	*Serratella frisoni* (McDunnough)	S2, G4
Hemiptera	Corixidae	*Sigara mathesoni* Hungerford	
	Veliidae	*Microvelia americana* Uhler	
Isopoda	Asellidae	*Caecidotea antricola* Creaser	S4, G5, EOH
Trichoptera	Glossosomatidae	*Glossosoma intermedium* Klapalek	
	Hydropsychidae	*Ceratopsyche piatrix* Ross	S4, G4, EIH
	Hydroptilidae	*Ochrotrichia* sp.	
	Lepidostomatidae	*Lepidostoma* sp.	
	Rhyacophilidae	*Rhyacophila banksi* Ross	
Roaring River Spring at Roaring River SP			
Amphipoda	Gammaridae	*Gammarus minus* Say	
		Gammarus pseudolimnaeus Bousfield	
Coleoptera	Haliplidae	*Peltodytes lengi* Roberts	
Diptera	Ceratopogonidae	Tribe Palpomyiini	
	Chironomidae	*Cricotopus/Orthocladius* sp.	
		Cryptochironomus sp.	
		Dicrotendipes sp.	
		Eukiefferiella sp.	
		Microtendipes sp.	
		Rheotanytarsus sp.	
		Thienemanniella sp.	
	Empididae		
	Simuliidae	*Simulium vittatum complex*	
Ephemeroptera	Baetidae	*Baetis tricaudatus* Dodds	

Table 9. Continued.

Order	Family	Taxon	Status
	Baetidae	*Centroptilum ozarkensum* Wiersema & Burian	
	Heptageniidae	*Stenacron interpunctatum* (Say)	
		Stenonema femoratum (Say)	
	Leptophlebiidae	*Paraleptophlebia assimilis* (Banks)	
Hemiptera	Corixidae	*Sigara modesta* (Abbott)	
	Gerridae	*Aquarius remigis* (Say)	
	Veliidae	*Microvelia americana* Uhler	
Isopoda	Asellidae	*Caecidotea brevicauda* Forbes	
		Lirceus sp.	
Bennett Spring at Bennett Spring SP			
Amphipoda	Gammaridae	*Gammarus pseudolimnaeus* Bousfield	
	Hyalellidae	*Hyalella azteca* Saussure	
Coleoptera	Dytiscidae	*Agabus* sp.	
	Dytiscidae	*Hydroporus rufilabris* Sharp	
	Elmidae	*Optioservus sandersoni* Collier	
	Haliplidae	*Peltodytes lengi* Roberts	
	Hydrophilidae	*Tropisternus collaris* (Fabricius)	
Diptera	Ceratopogonidae	Tribe Palpomyiini	
	Chironomidae	*Cricotopus* sp.	
		Cricotopus/Orthocladius sp.	
		Cryptochironomus sp.	
		Dicrotendipes sp.	
		Paratanytarsus sp.	
		Rheotanytarsus sp.	
		Thienemanniella sp.	
		Thienemannimyia group	
	Empididae		
	Ephydridae		
	Sciomyzidae	*Sepedon* sp.	
	Stratiomyidae	*Caloparyphus* sp.	
Ephemeroptera	Baetidae	*Centroptilum ozarkensum* Wiersema & Burian	
Hemiptera	Corixidae	*Sigara mathesoni* Hungerford	
		Trichocorixa calva (Say)	
	Gerridae	*Aquarius remigis* (Say)	
	Mesoveliidae	*Mesovelia mulsanti* White	
Trichoptera	Brachycentridae	*Micrasema ozarkana* Ross & Unzicker	EIH
	Hydroptilidae	*Hydroptila* sp.	
Ha Ha Tonka Spring at Ha Ha Tonka SP			
Amphipoda	Crangonyctidae	*Crangonyx forbesi* (Hubricht & Mackin)	
	Gammaridae	*Gammarus pseudolimnaeus* Bousfield	
		Gammarus sp.	
	Hyalellidae	*Hyalella azteca* Saussure	
Coleoptera	Dytiscidae	*Neoporus dimidiatus* Gemminger & Harold	
	Elmidae	*Optioservus sandersoni* Collier	
	Gyrinidae	*Gyrinus* sp.	

Table 9. Continued.

Order	Family	Taxon	Status
Diptera	Ceratopogonidae	Tribe Palpomyiini	
	Chironomidae	*Cricotopus* sp.	
		Cricotopus/Orthocladius sp.	
		Dicrotendipes sp.	
		Eukiefferiella sp.	
		Paratanytarsus sp.	
		Polypedilum sp.	
		Rheotanytarsus sp.	
Ephemeroptera	Baetidae	*Baetis tricaudatus* Dodds	
		Centroptilum ozarkensum Wiersema & Burian	
	Ephemerellidae	*Eurylophella lutulenta* (Clemens)	
Hemiptera	Corixidae	*Trichocorixa calva* (Say)	
	Gerridae	*Aquarius remigis* (Say)	
	Veliidae	*Microvelia americana* Uhler	
Isopoda	Asellidae	*Caecidotea brevicauda* Forbes	
Odonata	Calopterygidae	*Calopteryx maculata* (Beauvois)	
Trichoptera	Glossosomatidae	*Agapetus illini* Ross	
	Helicopsychidae	*Helicopsyche limnella* Ross	EIH
	Hydroptilidae	*Ochrotrichia* sp.	
	Leptoceridae	*Ceraclea nepha* (Ross)	
		Nectopsyche diarina (Ross)	
		Triaenodes injustus (Hagen)	
	Molannidae	*Molanna blenda* Sibley	

Table 10. Taxonomic richness of aquatic macroinvertebrates in the four qualitatively sampled high discharge rheocrene spring systems in 2007 and 2008.

		2007				2008			
		Order	Family	Genus	Species	Order	Family	Genus	Species
Montauk Spring	Insects	6	17	31	33	5	16	25	25
	Total	7	19	33	35	7	19	28	28
Roaring River Spring	Insects	6	19	35	38	4	12	19	19
	Total	8	22	40	44	6	14	22	23
Bennett Spring	Insects	7	20	32	32	5	17	23	23
	Total	7	20	32	32	6	19	25	25
Ha Ha Tonka Spring	Insects	5	15	27	27	6	17	26	26
	Total	7	17	29	29	8	20	29	30

Table 10. Continued.

		COMBINED			
		Order	Family	Genus	Species
Montauk Spring	Insects	6	24	42	44
	Total	8	27	46	48
Roaring River Spring	Insects	6	22	39	43
	Total	8	25	44	49
Bennett Spring	Insects	6	23	37	39
	Total	7	25	39	41
Ha Ha Tonka Spring	Insects	6	20	36	36
	Total	8	24	40	41

Table 11. Taxa collected from the four qualitatively sampled unique spring systems in the spring 2008 sampling season [EIH, endemic to the Interior Highlands; nsp, possible undescribed species].

Order	Family	Taxon	Status
Boone's Lick Spring at Boone's Lick SHS			
Coleoptera	Dytiscidae	*Agabus* sp.	
Diptera	Ceratopogonidae	*Culicoides* sp.	
	Chironomidae	*Chironomus* sp.	
		Hydrobaenus sp. O	
		Stictochironomus sp.	
		Zavrelimyia sp.	
	Culicidae	*Anopheles punctipennis* (Say)	
		Culex erraticus (Dyar & Knab)	
		Culex restuans Theobald	
	Tipulidae	*Pseudolimnophila* sp.	
Ephemeroptera	Heptageniidae	*Nixe perfida* (McDunnough)	
	Leptophlebiidae	*Paraleptophlebia praepedita* (Eaton)	
Hemiptera	Veliidae	*Microvelia americana* Uhler	
Spring-fed fen at St. Francois SP			
Amphipoda	Gammaridae	*Gammarus pseudolimnaeus* Bousfield	
Coleoptera	Elmidae	*Optioservus ovalis* (LeConte)	
	Haliplidae	*Peltodytes lengi* Roberts	
Diptera	Chironomidae	*Thienemaniella* sp.	
		Tvetenia sp.	
		Zavrelimyia sp.	
	Dixidae	*Dixa* sp.	nsp
	Ptychopteridae	*Ptychoptera* sp.	
	Simuliidae	*Simulium tuberosum complex*	
	Stratiomyidae	*Stratiomys* sp.	
	Tipulidae	*Pseudolimnophila* sp.	
		Tipula (Nippotipula) sp.	
Ephemeroptera	Caenidae	*Caenis latipennis* Banks	
Hemiptera	Nepidae	*Nepa apiculata* Uhler	
Isopoda	Asellidae	*Caecidotea brevicauda* Forbes	
Odonata	Calopterygidae	*Calopteryx maculata* (Beauvois)	
Plecoptera	Leuctridae	*Leuctra* sp.	
	Perlidae	*Acroneuria frisoni* (Stark)	
Trichoptera	Glossosomatidae	*Agapetus illini* Ross	
	Helicopsychidae	*Helicopsyche limnella* Ross	EIH
	Hydropsychidae	*Ceratopsyche slossonae* (Banks)	
		Hydropsyche scalaris Hagen	
	Limnephilidae	*Frenesia missa* (Milne)	
		Pycnopsyche sp.	
	Molannidae	*Molanna blenda* Sibley	
	Philopotamidae		

Table 11. Continued.

Order	Family	Taxon	Status
Seepage channels at Hawn SP			
Coleoptera	Dytiscidae	*Hydrocolus* sp.	
	Hydrophilidae	*Cymbiodyta chamberlaini* Smetana	
Diptera	Cecidomyiidae		
	Chironomidae	*Chironomus* sp.	
		Paramerina sp.	
		Zavrelimyia sp.	
	Tipulidae	*Pseudolimnophila* sp.	
Ephemeroptera	Leptophlebiidae	*Paraleptophlebia assimilis* (Banks)	
	Siphlonuridae	*Siphlonurus marshalli* Traver	
Hemiptera	Corixidae	*Sigara modesta* (Abbott)	
Odonata	Cordulegastridae	*Cordulegaster maculata* Selys	
Trichoptera	Lepidostomatidae	*Lepidostoma* sp.	
	Polycentropodidae		
Oumessourit Marsh (spring-fed) at Van Meter SP			
Amphipoda	Crangonyctidae	*Crangonyx forbesi* (Hubricht & Mackin)	
	Hyalellidae	*Hyalella azteca* Saussure	
Coleoptera	Dytiscidae	*Agabus* sp.	
		Heterosternuta sp.	
		Hydroporus rufilabris Sharp	
		Neoporus sp.	
	Hydrophilidae	*Cymbiodyta chamberlaini* Smetana	
	Lampyridae	*?Pyractonema* sp.	
Diptera	Stratiomyidae	*Odontomyia* sp.	
		Stratiomys sp.	
	Tabanidae	*Chrysops* sp.	
	Tipulidae	*Tipula* sp.	
Hemiptera	Gelastocoridae	*Ochterus americanus* (Uhler)	
	Veliidae	*Microvelia americana* Uhler	
Isopoda	Asellidae	*Caecidotea forbesi* Williams	
Trichoptera	Limnephilidae	*Frenesia missa* Milne	

Table 12. Taxa collected from the four qualitatively sampled unique spring systems in the fall 2008 sampling season [EIH, endemic to the Interior Highlands].

Order	Family	Taxon	Status
Boone's Lick Spring at Boone's Lick SHS			
Coleoptera	Dryopidae	*Helichus basalis* LeConte	
	Dytiscidae	*Acilius mediatus* (Say)	
		Agabus sp.	
		Copelatus glyphicus (Say)	
		Laccophilus fasciatus Aube'	
Diptera	Chaoboridae	*Chaoborus americanus* (Johannsen)	
		Chaoborus flavicans (Meigen)	
	Chironomidae	*Chironomus* sp.	
		Parametriocnemus sp.	
		Psectrotanypus sp.	
	Dolichopodidae	*Hydrophorus* sp.	
	Tipulidae	*Erioptera (Symplecta)* sp.	
Spring-fed fen at St. Francois SP			
Amphipoda	Gammaridae	*Gammarus pseudolimnaeus* Bousfield	
Coleoptera	Elmidae	*Optioservus ovalis* (LeConte)	
	Haliplidae	*Peltodytes duodecimpunctatus* (Say)	
		Peltodytes lengi Roberts	
Diptera	Chironomidae	*Psilometriocnemus* sp.	
		Zavrelimyia sp.	
	Ptychopteridae	*Ptychoptera* sp.	
	Tipulidae	*Pedicia* sp.	
Ephemeroptera	Caenidae	*Caenis latipennis* Banks	
Hemiptera	Veliidae	*Microvelia americana* Uhler	
Isopoda	Asellidae	*Caecidotea brevicauda* Forbes	
Odonata	Aeshnidae	*Boyeria vinosa* (Say)	
	Coenagriondae	*Argia* sp.	
Trichoptera	Helicopsychidae	*Helicopsyche limnella* Ross	EIH
	Lepidostomatidae	*Lepidostoma* sp.	
	Limnephilidae	*Frenesia missa* (Milne)	
		Pycnopsyche sp.	
Seepage channels at Hawn SP			
Coleoptera	Dytiscidae	*Agabus gagates* Aube'	
		Hydrocolus sp.	
		Neoporus striatopunctatus (Melsheimer)	
	Hydrophilidae	*Cymbiodyta vindicata* Fall	
		Hydrobius sp.	
Diptera	Chironomidae	*Chironomus* sp.	
		Micropsectra sp.	
		Microtendipes sp.	

Table 12. Continued.

Order	Family	Taxon	Status
		Procladius sp.	
		Tribelos sp.	
		Zavrelimyia sp.	
	Culicidae	*Anopheles punctipennis* (Say)	
		*Culex erraticus (*Dyar & Knab)	
	Dixidae	*Dixella* sp.	
	Tipulidae	*Pseudolimnophila* sp.	
Ephemeroptera	Leptophlebiidae	*Paraleptophlebia assimilis* (Banks)	
Hemiptera	Corixidae	*Sigara modesta* (Abbott)	
	Gerridae	*Aquarius remigis* (Say)	
	Notonectidae	*Notonecta irrorata* Uhler	
	Veliidae	*Microvelia americana* Uhler	
Megaloptera	Sialidae	*Sialis* sp.	
Odonata	Aeshnidae	*Aeshna umbrosa* Walker	
	Cordulegastridae	*Cordulegaster maculata* Selys	
	Libellulidae	*Libellula* sp.	
Trichoptera	Molannidae	*Molanna blenda* Sibley	
Oumessourit Marsh (spring-fed) at Van Meter SP			
Amphipoda	Crangonyctidae	*Crangonyx forbesi* (Hubricht & Mackin)	
	Hyalellidae	*Hyalella azteca* Saussure	
Coleoptera	Hydrophilidae	*Anacaena limbata* (Fabricius)	
		Tropisternus sp.	
		Tropisternus lateralis nimbatus (Say)	
	Noteridae	*Hydrocanthus iricolor* Say	
Diptera	Stratiomyidae	*Nemotelus* sp.	
	Tabanidae	*Tabanus/Whitneyomyia/Atylotus* sp.	
	Tipulidae	*Pedicia* sp.	
Hemiptera	Belastomatidae	*Belastoma* sp.	
	Ochteridae	*Ochterus americanus (*Uhler)	
	Veliidae	*Microvelia americana* Uhler	
Isopoda	Asellidae	*Caecidotea forbesi* Williams	
Trichoptera	Limnephilidae	*Frenesia missa* Milne	

Table 13. Taxonomic richness of aquatic macroinvertebrates in unique spring systems in the spring and fall of 2008.

		SPRING 2008				**Fall 2008**			
		Order	**Family**	**Genus**	**Species**	**Order**	**Family**	**Genus**	**Species**
Boone's Lick Spring at	Insects	4	8	12	13	2	6	12	12
Boone's Lick SHS	Total	4	8	12	13	2	6	12	12
Spring-fed fen at	Insects	7	19	24	24	6	12	14	15
St. Francois SP	Total	9	21	26	26	8	14	16	17
Seepage channels at	Insects	6	11	13	13	7	16	25	25
Hawn SP	Total	6	11	13	13	7	16	25	25
Oumessourit Marsh at	Insects	4	6	13	13	4	9	10	11
Van Meter SP	Total	6	9	16	16	6	12	13	14

Table 13. Continued.

		COMBINED			
		Order	**Family**	**Genus**	**Species**
Boone's Lick Spring at	Insects	4	11	21	23
Boone's Lick SHS	Total	4	11	21	23
Spring-fed fen at	Insects	7	23	31	31
St. Francois SP	Total	9	25	33	33
Seepage channels at	Insects	7	20	29	30
Hawn SP	Total	7	20	29	30
Oumessourit Marsh at	Insects	4	12	20	21
Van Meter SP	Total	6	15	23	24

Appendix A. Raw numbers showing the number of aquatic insects, amphipods, and isopods at each site within each quantitatively sampled low to medium discharge rheocrene spring system in 2007 and 2008 [*, indicates that quantitative samples were not taken at that particular site].

2007		**Site (meters from eucrene)**								**2008**	**Site**						
Spring System		**10**	**25**	**50**	**100**	**200**	**400**	**Total**	**TOTAL**	**10**	**25**	**50**	**100**	**200**	**400**	**Total**	**TOTAL**
CSCR	Insects	*	28	7	3	2	1	41		12	12	4	2	0	0	30	
	Amphipods	*	24	15	25	30	2	96		27	14	87	8	0	1	137	
	Isopods	*	18	9	21	70	19	137	**274**	9	30	6	27	4	44	120	**287**
LSCR	Insects	0	3	1	5	11	*	20		0	18	0	0	4	*	22	
	Amphipods	12	58	8	22	44	*	144		2	0	0	0	0	*	2	
	Isopods	5	1	4	13	37	*	60	**224**	3	56	18	4	88	*	169	**193**
1RB	Insects	0	2	0	1	2	*	5		0	0	1	2	1	*	4	
	Amphipods	4	0	3	4	8	*	19		9	0	0	0	0	*	9	
	Isopods	50	10	30	53	85	*	228	**252**	9	10	8	38	9	*	74	**87**
3TT	Insects	2	3	*	*	*	*	5		*	*	*	*	*	*	*	
	Amphipods	20	19	*	*	*	*	39		*	*	*	*	*	*	*	
	Isopods	77	219	*	*	*	*	296	**340**	*	*	*	*	*	*	*	**DRY**
MSLO	Insects	1	2	21	14	22	22	82		5	32	28	59	18	21	163	
	Amphipods	15	3	66	10	4	1	99		219	54	0	24	7	1	305	
	Isopods	0	0	0	0	0	0	0	**181**	0	6	0	0	0	2	8	**476**
OSOC	Insects	13	18	*	*	*	*	31		48	30	*	*	*	*	78	
	Amphipods	15	69	*	*	*	*	84	**119**	66	141	*	*	*	*	207	**290**

Appendix A. Continued.

Spring 2007		Site (meters from eucrene)								Fall 2008			Site				
Spring System		10	25	50	100	200	400	Total	TOTAL	10	25	50	100	200	400	Total	TOTAL
	Isopods	1	3	*	*	*	*	4		5	0	*	*	*	*	5	
CSM	Insects	20	3	1	6	*	*	30		8	15	1	4	*	*	28	
	Amphipods	21	45	40	24	*	*	130		70	101	59	107	*	*	337	
	Isopods	0	0	0	0	*	*	0	**160**	0	0	1	0	*	*	1	**366**
2DM	Insects	3	7	0	*	*	*	10		5	13	2	*	*	*	20	
	Amphipods	53	66	10	*	*	*	129		62	172	0	*	*	*	234	
	Isopods	0	0	0	*	*	*	0	**139**	0	0	2	*	*	*	2	**256**

Appendix B. Species richness at each site in the quantitatively sampled low to medium discharge rheocrene spring systems in 2007 and 2008 [*, indicates that samples were not taken at these locations due to a short hypocrene].

	Sites (meters from eucrene)						
Spring System	**0**	**10**	**25**	**50**	**100**	**200**	**400**
2007							
3TT	3	3	5	*	*	*	*
2DM	15	3	3	1	*	*	*
1RB	6	3	3	2	3	4	*
MSLO	8	2	3	8	11	9	10
OSOC	21	6	4	*	*	*	*
CSM	6	5	3	2	2	*	*
CSCR	3	6	10	7	4	4	*
LSCR	4	2	5	3	5	8	3
2008							
2DM	2	3	5	3	*	*	*
1RB	6	3	1	2	3	2	*
MSLO	6	5	17	9	16	9	15
OSOC	18	5	9	*	*	*	*
CSM	8	4	6	4	6	*	*
CSCR	3	8	7	7	5	1	*
LSCR	11	2	8	1	1	4	2

Appendix C. Shannon diversity (H') and evenness (E) values at each site within each quantitatively sampled low to medium discharge spring system in 2007 and 2008 [*, indicates that quantitative samples were not taken at a site, thus values could not be calculated].

		Site (meters from eucrene)					
Spring System		**10**	**25**	**50**	**100**	**200**	**400**
2007							
3TT	H'	0.597403	0.355539	*	*	*	*
	E	0.54378	0.220909	*	*	*	*
2DM	H'	0.242999	0.381443	0	*	*	*
	E	0.221187	0.347204	0	*	*	*
1RB	H'	0.305707	0.566086	0.304636	0.336811	0.403762	*
	E	0.278266	0.515273	0.439497	0.306579	0.291253	*
MSLO	H'	0.233792	0.950271	0.975543	1.883344	1.998138	2.098595
	E	0.33729	0.864974	0.469137	0.785415	0.909392	0.911408
OSOC	H'	1.368572	0.708733	*	*	*	*
	E	0.763815	0.511243	*	*	*	*
CSM	H'	1.083046	0.273574	0.114665	0.500402	*	*
	E	0.672934	0.249018	0.165427	0.721928	*	*
CSCR	H'	*	1.766422	1.642691	0.91645	0.708987	0.485105
	E	*	0.767147	0.844176	0.661079	0.511426	0.441561
LSCR	H'	0.605797	0.446614	0.858741	1.072802	1.157204	*
	E	0.873981	0.277497	0.78166	0.666569	0.556497	*
2008							
2DM	H'	0.302789	0.320192	1.039721	*	*	*
	E	0.275611	0.198946	0.946395	*	*	*
1RB	H'	0.958	0	0.348832	0.233173	0.325083	*

Appendix C. Continued.

		Site (meters from eucrene)					
Spring System		**10**	**25**	**50**	**100**	**200**	**400**
	E	0.872009	0	0.503258	0.212243	0.468996	*
MSLO	H'	0.136677	1.77785	1.284682	2.329656	1.943041	2.600431
	E	0.084922	0.627503	0.584684	0.840246	0.884316	0.96026
OSOC	H'	1.163771	0.726183	*	*	*	*
	E	0.723092	0.3305	*	*	*	*
CSM	H'	0.43061	0.617682	0.361439	0.50437	*	*
	E	0.31062	0.344735	0.260723	0.281494	*	*
CSCR	H'	1.374264	1.246454	0.724716	0.906108	0	0.106566
	E	0.660881	0.640551	0.37243	0.562997	0	0.153742
LSCR	H'	0.673012	0.981439	0	0	0.22405	*
	E	0.970951	0.471973	0	0	0.161618	*

Appendix D. PC-ORD output showing canonical coefficients, site, species, and environmental variable scores, and inter-set correlations from the canonical correspondence analysis (CCA) performed using species and environmental data collected in eight quantitatively sampled low to medium discharge rheocrene spring systems in 2007 and 2008.

Canonical Correspondence Analysis Output (2007)

```
MULTIPLE REGRESSION RESULTS:
Regression of sites in species space on Environ
-----------------------------------------------------------------------
                              Canonical Coefficients
               --------------------------------------------------------
                     Standardized                    Original Units
               ------------------------  ------------------------------
    Variable    Axis 1  Axis 2  Axis 3     Axis 1     Axis 2     Axis 3
S.Dev
-----------------------------------------------------------------------
-----------
   1 Temp       0.365  -0.185  -0.496       0.209     -0.106     -0.283
0.175E+01
   2 DO        -0.160  -0.292  -0.084      -0.112     -0.204     -0.058
0.144E+01
   3 SC         0.199   0.076  -0.468       0.002      0.001     -0.005
0.102E+03
   4 pH        -0.331   0.052  -0.195      -1.129      0.176     -0.667
0.293E+00
   5 Vel       -0.381   0.878  -0.371      -2.927      6.752     -2.848
0.130E+00
   6 ChWid     -0.081   0.309  -0.104      -0.001      0.004     -0.001
0.838E+02
   7 MaxDep     0.017  -0.168  -0.350       0.003     -0.030     -0.063
0.554E+01
   8 Cancov     0.152   0.027   0.030       0.012      0.002      0.002
0.124E+02
   9 Alk        0.358   0.895  -0.004       0.011      0.028      0.000
0.324E+02
-----------------------------------------------------------------------


Scores that are derived from the scores of species  (WA Scores)
FINAL SCORES and raw data totals (weights) for  32 sites
--------------------------------------------------------------------
                                                            Raw Data
                   Axis 1       Axis 2       Axis 3          Totals
--------------------------------------------------------------------
   1 3TT25        0.831437    -0.216072     0.984029        241.0000
   2 3TT10        0.822648    -0.190917     0.372394         99.0000
   3 2DM50       -1.040658     1.717481     0.004829         10.0000
   4 2DM25       -1.065606     1.672782    -0.072618         73.0000
   5 2DM10       -1.035521     1.645520    -0.000899         56.0000
   6 1RB200       0.835031    -0.223799     0.984592         95.0000
   7 1RB100       0.829334    -0.210052     1.028563         58.0000
   8 1RB50        0.832826    -0.216329     0.916404         33.0000
   9 1RB25        0.823793    -0.201930     1.096144         12.0000
  10 1RB10        0.838326    -0.234484     1.150685         54.0000
```

Appendix D. Continued.

```
  11 MSLO400     -2.091434    -2.599529    -0.011183          23.0000
  12 MSLO200     -2.059198    -2.660533    -0.205582          26.0000
  13 MSLO100     -2.031387    -2.596779    -0.034227          24.0000
  14 MSLO50      -1.591474    -2.161487    -0.159772          87.0000
  15 MSLO25      -1.433024    -2.497871    -0.578717           5.0000
  16 MSLO10      -1.622502    -2.511342    -0.049486          16.0000
  17 OSOC25      -1.027278     2.114022     0.349083          90.0000
  18 OSOC10      -1.165798     2.677391     0.269126          29.0000
  19 CSM100      -1.144482     1.293983    -0.163538          30.0000
  20 CSM50       -1.042766     1.721262    -0.018845          41.0000
  21 CSM25       -1.055381     1.575305    -0.028456          48.0000
  22 CSM10       -1.229764     0.723532    -0.210291          41.0000
  23 CSCR400      0.803206    -0.225260     0.843980          22.0000
  24 CSCR200      0.811816    -0.207856    -0.162320         102.0000
  25 CSCR100      0.795824    -0.128195    -1.394651          49.0000
  26 CSCR50       0.189288    -0.565253    -0.636002          31.0000
  27 CSCR25      -0.528577    -1.097777     1.282684          70.0000
  28 LSCR200      0.777997    -0.071533    -1.766877          92.0000
  29 LSCR100      0.807324    -0.207527    -2.174947          40.0000
  30 LSCR50       0.818372    -0.189668    -2.389539          13.0000
  31 LSCR25       0.712385    -0.143781    -3.453002          62.0000
  32 LSCR10       0.805472    -0.135166    -2.119425          17.0000
---------------------------------------------------------------------


Scores that are linear combinations of Environ  (LC Scores)
FINAL SCORES and raw data totals (weights) for  32 sites
---------------------------------------------------------------------
                                                             Raw Data
                   Axis 1       Axis 2       Axis 3            Totals
---------------------------------------------------------------------
   1 3TT25        0.800316    -0.063222     0.673841         241.0000
   2 3TT10        0.672026     0.239680     0.924767          99.0000
   3 2DM50       -0.114289    -0.118750     0.498782          10.0000
   4 2DM25       -0.989998     1.312681    -0.305078          73.0000
   5 2DM10       -0.640201     0.677399    -0.297304          56.0000
   6 1RB200       0.835298    -0.371966     0.930656          95.0000
   7 1RB100       0.884786    -0.330217     0.793284          58.0000
   8 1RB50        0.770846    -0.982054     0.917248          33.0000
   9 1RB25        0.657486     0.425847     0.968635          12.0000
  10 1RB10        0.934833    -0.521156     1.311670          54.0000
  11 MSLO400     -1.962022    -1.678464     0.046715          23.0000
  12 MSLO200     -2.175864    -2.526539     0.016191          26.0000
  13 MSLO100     -2.251991    -0.994452     0.058301          24.0000
  14 MSLO50      -1.605506    -1.658816    -0.393190          87.0000
  15 MSLO25      -1.722319    -1.745818    -0.216384           5.0000
  16 MSLO10      -1.947820    -2.089678     0.269587          16.0000
  17 OSOC25      -0.962435     2.126196     0.756474          90.0000
  18 OSOC10      -1.537810     3.267386    -0.173973          29.0000
  19 CSM100      -1.383634     0.408109    -0.350534          30.0000
  20 CSM50       -1.062396    -0.150759    -0.159059          41.0000
  21 CSM25       -0.631964    -0.474997     0.025932          48.0000
  22 CSM10       -1.147419     1.020573    -0.219109          41.0000
  23 CSCR400      1.077553     0.798534    -0.181879          22.0000
```

Appendix D. Continued.

```
  24 CSCR200      0.843044    -0.263433    -0.875970        102.0000
  25 CSCR100      0.456962    -0.162453     0.364469         49.0000
  26 CSCR50      -0.117705    -0.392452    -0.001627         31.0000
  27 CSCR25      -0.434076    -0.246434     0.977109         70.0000
  28 LSCR200      0.636998     0.664103    -1.867791         92.0000
  29 LSCR100      1.016474    -0.909138    -2.160089         40.0000
  30 LSCR50       0.556994    -0.065392    -3.011664         13.0000
  31 LSCR25       0.751719     0.025888    -2.176172         62.0000
  32 LSCR10       0.769278    -0.383412    -2.228392         17.0000
--------------------------------------------------------------------


FINAL SCORES and raw data totals (weights) for  52 species
--------------------------------------------------------------------
                                                            Raw Data
                   Axis 1       Axis 2       Axis 3          Totals
--------------------------------------------------------------------
   1 Acentr      -1.748113    -1.666675    -0.217228          5.0000
   2 Agabus       0.205692     0.150574     0.247002          3.0000
   3 Agapet      -1.390141    -0.217892    -0.239080         36.0000
   4 Amphin       0.835298    -0.371966     0.930656          1.0000
   5 Aquar       -0.631965    -0.474997     0.025932          1.0000
   6 Caecbrev     0.745855    -0.124374     0.389945        725.0000
   7 Cerato      -2.017176    -1.623904     0.093607          5.0000
   8 Chauli      -0.434076    -0.246433     0.977108          1.0000
   9 Cheuma      -2.251992    -0.994452     0.058302          1.0000
  10 Cordule      0.836980    -0.241646    -0.595727          2.0000
  11 Crang        0.706212    -0.052483    -1.020107        266.0000
  12 Crico       -1.962023    -1.678464     0.046715          2.0000
  13 CricoOrt    -1.860197    -1.341084     0.013487          8.0000
  14 Dicro       -1.722320    -1.745818    -0.216384          1.0000
  15 Diphet      -1.962023    -1.678464     0.046715          1.0000
  16 Dixa        -2.175864    -2.526539     0.016191          1.0000
  17 Ectopr      -0.434076    -0.246433     0.977109         11.0000
  18 Eukief      -1.537809     3.267386    -0.173973          1.0000
  19 Eurylo      -1.962023    -1.678464     0.046715          2.0000
  20 Gminus      -0.927480     0.935543     0.001379        343.0000
  21 Gpseud      -1.407969    -1.350912    -0.021318        130.0000
  22 Helich       0.800315    -0.063222     0.673841          1.0000
  23 Helico      -2.188552    -2.271191     0.023210          6.0000
  24 Hepta        0.843044    -0.263433    -0.875970          1.0000
  25 Hetero       0.165077    -0.231396    -0.065168          6.0000
  26 Ironoq       0.817807    -0.217594     0.802249          2.0000
  27 Isoperl      0.656118     0.557733    -1.919188          6.0000
  28 Lepido      -0.936118    -0.851740     0.389838          7.0000
  29 Leuctr      -2.230241    -1.432191     0.046270          7.0000
  30 Microps     -0.117706    -0.392452    -0.001628          1.0000
  31 Microte     -0.439638    -1.004625    -0.546177          2.0000
  32 Microv      -1.147419     1.020573    -0.219109          1.0000
  33 Neophy      -0.533319     0.033814     0.433370         12.0000
  34 Nigro       -1.821001    -1.437362    -0.242693          3.0000
  35 Ochro       -1.119355     2.437430     0.502716         22.0000
  36 Optio       -1.365857     0.230460    -0.167248         19.0000
  37 Pedic       -0.434076    -0.246433     0.977108          2.0000
```

Appendix D. Continued.

```
  38 Perles       0.848683    -0.425741    -2.271945          5.0000
  39 Polycen     -2.175864    -2.526539     0.016191          1.0000
  40 Polypill    -0.434076    -0.246433     0.977108          1.0000
  41 Polypav     -2.105553    -1.880892     0.036823          5.0000
  42 Pseudo       0.514874     0.165443     0.453462          6.0000
  43 Rheot       -1.537809     3.267386    -0.173973          3.0000
  44 Rhyacob     -1.004477     1.019993    -0.275874          5.0000
  45 Rhyacof      0.615226     0.175846    -1.226498          6.0000
  46 Simvit      -0.117706    -0.392452    -0.001628          1.0000
  47 Stenac      -2.052307    -1.926586    -0.075627          4.0000
  48 Stenel       0.636998     0.664103    -1.867791          1.0000
  49 Stygo        0.934833    -0.521156     1.311670          1.0000
  50 Thien       -2.251992    -0.994452     0.058302          1.0000
  51 TipNip      -1.605506    -1.658816    -0.393190          1.0000
  52 Tveten      -2.141436    -1.931498     0.034350          4.0000
--------------------------------------------------------------------


CORRELATIONS AND BIPLOT SCORES for   9 Environ
--------------------------------------------------------------------
                       Correlations*            Biplot Scores
    Variable      Axis 1  Axis 2  Axis 3     Axis 1  Axis 2  Axis 3
--------------------------------------------------------------------
   1 Temp          0.461  -0.192  -0.787      0.461  -0.192  -0.787
   2 DO           -0.772   0.037  -0.253     -0.772   0.037  -0.253
   3 SC            0.511  -0.007  -0.320      0.511  -0.007  -0.320
   4 pH           -0.225  -0.094  -0.679     -0.225  -0.094  -0.679
   5 Vel          -0.673   0.520  -0.274     -0.673   0.520  -0.274
   6 ChWid        -0.575  -0.168  -0.048     -0.575  -0.168  -0.048
   7 MaxDep       -0.148  -0.174  -0.577     -0.148  -0.174  -0.577
   8 Cancov        0.006   0.108  -0.081      0.006   0.108  -0.081
   9 Alk           0.644   0.607   0.000      0.644   0.607   0.000
------------------------------------------------------------------
* Correlations are "intraset correlations" of ter Braak (1986)


INTER-SET CORRELATIONS for   9 Environ
-----------------------------------------
                       Correlations
    Variable      Axis 1  Axis 2  Axis 3
-----------------------------------------
   1 Temp          0.453  -0.158  -0.699
   2 DO           -0.760   0.030  -0.225
   3 SC            0.502  -0.006  -0.285
   4 pH           -0.221  -0.078  -0.602
   5 Vel          -0.662   0.429  -0.244
   6 ChWid        -0.566  -0.139  -0.042
   7 MaxDep       -0.146  -0.143  -0.512
   8 Cancov        0.006   0.089  -0.072
   9 Alk           0.633   0.501   0.000
```

Appendix D. Continued.

Canonical Correspondence Analysis Output (2008)

```
MULTIPLE REGRESSION RESULTS:
Regression of Sites in species space on Environ
----------------------------------------------------------------------
                              Canonical Coefficients
               -------------------------------------------------------
                     Standardized               Original Units
               ------------------------  -----------------------------
    Variable    Axis 1  Axis 2  Axis 3     Axis 1     Axis 2     Axis 3
S.Dev
----------------------------------------------------------------------
-----------
   1 Temp       0.558   0.971   1.009      0.190      0.331      0.343
0.294E+01
   2 DO         0.002   0.145  -0.253      0.001      0.094     -0.163
0.155E+01
   3 SC         0.254  -0.299  -3.030      0.003     -0.003     -0.032
0.938E+02
   4 pH        -0.012  -0.510   2.927     -0.032     -1.351      7.760
0.377E+00
   5 Vel       -0.322   0.219   0.152     -2.195      1.492      1.036
0.147E+00
   6 ChWid      0.322  -0.241   1.349      0.003     -0.002      0.012
0.116E+03
   7 MaxDep    -0.013  -0.074  -1.088     -0.002     -0.010     -0.153
0.709E+01
   8 Cancov    -0.064   0.113  -0.390     -0.010      0.018     -0.064
0.613E+01
   9 Alk       -0.477   0.560  -1.486     -0.012      0.015     -0.039
0.384E+02
----------------------------------------------------------------------
```

```
Scores that are derived from the scores of species  (WA Scores)
FINAL SCORES and raw data totals (weights) for  31 Sites
-------------------------------------------------------------------
                                                          Raw Data
                   Axis 1       Axis 2       Axis 3        Totals
-------------------------------------------------------------------
   1 2DM50        0.257570    -0.525053     8.697472        4.0000
   2 2DM25       -1.070235     0.517004    -0.243246      185.0000
   3 2DM10       -1.070528     0.517531    -0.210098       67.0000
   4 1RB200       1.662147     1.268874    -0.341265       10.0000
   5 1RB100       1.665547     1.257152     0.194564       40.0000
   6 1RB50        1.529244     1.204290     0.075835        9.0000
   7 1RB25        1.704795     1.428395     0.043682       10.0000
   8 1RB10        1.419781     0.643693    -0.457774       18.0000
   9 MSLO400      0.551129    -1.212602     4.136340       24.0000
  10 MSLO200      0.184433    -1.284856     1.392471       25.0000
  11 MSLO100     -0.060896    -1.034163     3.392735       83.0000
  12 MSLO50       0.080876    -1.323094     5.238140       28.0000
  13 MSLO25       0.685079    -1.746823     0.497989       92.0000
  14 MSLO10       0.399779    -1.754769    -1.265223      224.0000
  15 OSOC25      -1.038606     0.597527    -0.119224      171.0000
```

Appendix D. Continued.

```
  16 OSOC10      -0.894160     0.486422     0.270497         119.0000
  17 CSM100      -0.935388     0.330224    -0.318281         111.0000
  18 CSM50       -0.971375     0.446790    -0.352522          61.0000
  19 CSM25       -1.046812     0.455851    -0.357021         114.0000
  20 CSM10       -1.073167     0.511230    -0.363802          78.0000
  21 CSCR400      1.696692     1.403913     0.023604          45.0000
  22 CSCR200      1.704795     1.428395     0.043682           4.0000
  23 CSCR100      1.505716     0.871182    -0.391740          37.0000
  24 CSCR50       0.551962    -1.365271    -1.175109          97.0000
  25 CSCR25       1.294869     0.132416    -0.860652          56.0000
  26 CSCR10       0.741060    -1.055954    -0.676152          48.0000
  27 LSCR200      1.646207     1.416003     0.098069          92.0000
  28 LSCR100      1.704795     1.428395     0.043682           4.0000
  29 LSCR50       1.704795     1.428395     0.043682          18.0000
  30 LSCR25       1.544966     1.379832     0.214754          74.0000
  31 LSCR10       1.558947     0.987706    -0.317727           5.0000
------------------------------------------------------------------


Scores that are linear combinations of Environ  (LC Scores)
FINAL SCORES and raw data totals (weights) for  31 Sites
------------------------------------------------------------------
                                                         Raw Data
                  Axis 1       Axis 2       Axis 3         Totals
------------------------------------------------------------------
   1 2DM50        0.694883    -0.671625     5.362177           4.0000
   2 2DM25       -0.723643     0.325501    -0.180431         185.0000
   3 2DM10       -0.546205     0.031495    -0.053179          67.0000
   4 1RB200       1.289184     2.268072    -2.678835          10.0000
   5 1RB100       1.168353     1.180962     0.724453          40.0000
   6 1RB50        1.197748     0.982253     1.094696           9.0000
   7 1RB25        1.170001     0.751485     0.624077          10.0000
   8 1RB10        0.818400     1.130253    -0.093212          18.0000
   9 MSLO400      0.722500    -0.907998     1.999231          24.0000
  10 MSLO200     -0.106683    -0.454307     0.126026          25.0000
  11 MSLO100     -0.561008    -0.404003     2.355468          83.0000
  12 MSLO50       0.291407    -1.252870     1.904583          28.0000
  13 MSLO25       0.927156    -2.106863     1.306687          92.0000
  14 MSLO10       0.057260    -1.452060    -0.901883         224.0000
  15 OSOC25      -1.242491     0.849765    -0.303017         171.0000
  16 OSOC10      -0.734782     0.144722     0.555261         119.0000
  17 CSM100      -0.990583     0.411296     0.852107         111.0000
  18 CSM50       -0.999980     0.326790    -0.823386          61.0000
  19 CSM25       -0.976029     0.197805    -0.332941         114.0000
  20 CSM10       -0.848494     0.197651    -0.571239          78.0000
  21 CSCR400      1.375996     2.186671     0.172733          45.0000
  22 CSCR200      1.750606     1.356830    -0.252637           4.0000
  23 CSCR100      1.260440     0.367363    -1.324545          37.0000
  24 CSCR50       1.004303    -0.676114    -1.454105          97.0000
  25 CSCR25       1.018131    -0.848076    -0.934753          56.0000
  26 CSCR10       0.757185    -1.101137    -0.213988          48.0000
  27 LSCR200      1.911485     1.326976     0.886606          92.0000
  28 LSCR100      2.078328     0.656227    -0.865942           4.0000
```

Appendix D. Continued.

```
  29 LSCR50       1.647373     1.126025    -2.134362          18.0000
  30 LSCR25       1.537961     1.193672    -0.029435          74.0000
  31 LSCR10       2.160039     0.742282     2.605973           5.0000
--------------------------------------------------------------------


FINAL SCORES and raw data totals (weights) for  68 species
--------------------------------------------------------------------
                                                            Raw Data
                   Axis 1       Axis 2       Axis 3          Totals
--------------------------------------------------------------------
   1 Acentr      -0.561008    -0.404003     2.355468           4.0000
   2 Acron       -0.561008    -0.404003     2.355468           2.0000
   3 Agabus       0.291407    -1.252869     1.904582           1.0000
   4 Agapet      -0.928203     0.197747    -0.422302           8.0000
   5 Aquar        0.103085    -0.391620     0.117563           7.0000
   6 Atherix     -0.976029     0.197805    -0.332941           1.0000
   7 Baetisf      0.038698     0.052476     1.674067           7.0000
   8 Caecbrv      1.407764     0.950445     0.015419         377.0000
   9 Caecint      0.694883    -0.671624     5.362176           2.0000
  10 Chauli       0.757184    -1.101137    -0.213988           1.0000
  11 Cheum        1.537961     1.193672    -0.029435           2.0000
  12 Choro        0.790718    -1.307619     1.768383           3.0000
  13 Chrys        1.018131    -0.848076    -0.934753           1.0000
  14 Conch        0.291407    -1.252869     1.904582           1.0000
  15 Coryno       0.291407    -1.252869     1.904582           1.0000
  16 Crang        1.106671     0.217366    -0.303511          22.0000
  17 Crico        0.035556     0.050886     0.932987           6.0000
  18 Crypto       0.927155    -2.106862     1.306687           1.0000
  19 Diames      -0.466661    -0.520382    -0.024888           2.0000
  20 Dicro        0.824827    -1.507430     1.652959           4.0000
  21 Diphet      -0.561008    -0.404003     2.355468           2.0000
  22 Ectopri      0.292770    -0.462154    -0.008704           3.0000
  23 Eukief      -1.242490     0.849765    -0.303017           9.0000
  24 Gminus      -0.901893     0.364414    -0.102141         763.0000
  25 Gpseud       0.343386    -1.183001    -0.461384         446.0000
  26 Helico       0.722500    -0.907997     1.999231           2.0000
  27 Heterost     1.055591    -0.111002    -1.343389           6.0000
  28 Heterotr     0.927155    -2.106862     1.306687           1.0000
  29 Hexato      -0.861812     0.326145    -0.501908           2.0000
  30 Hydroba     -0.546205     0.031495    -0.053179           1.0000
  31 Hydropt      0.052314    -0.898472     1.904654          37.0000
  32 Lepido       0.461019    -0.815809    -0.207277           5.0000
  33 Leuctr       0.080396    -0.943675     1.852457          13.0000
  34 Limno       -0.561008    -0.404003     2.355468           2.0000
  35 Microps      0.565188    -0.915331     0.919204          10.0000
  36 Microten     0.927155    -2.106862     1.306687           3.0000
  37 Microv       0.152232    -0.165991     1.201685           3.0000
  38 Neopor      -1.242490     0.849765    -0.303017           1.0000
  39 Nigron      -0.301983    -0.544319     2.051811          10.0000
  40 Optio       -0.691226     0.111773     0.354272          47.0000
  41 Paralep      0.080746    -0.656000     2.177350           2.0000
  42 Paramet      0.460451     0.869136     0.869356           2.0000
  43 Paraten      0.953955    -1.741548     1.241994           9.0000
```

Appendix D. Continued.

```
  44 Pedici       1.139286    -0.240357    -1.129649          2.0000
  45 Phaeno       0.927155    -2.106862     1.306687          1.0000
  46 Polycen     -0.367915    -0.465591     2.068170          9.0000
  47 Polyill      0.757184    -1.101137    -0.213988          1.0000
  48 Polysca      0.757184    -1.101137    -0.213988          1.0000
  49 Polyavi      0.927155    -2.106862     1.306687          1.0000
  50 PolyU        1.537961     1.193672    -0.029435          1.0000
  51 Proclo       0.722500    -0.907997     1.999231          1.0000
  52 Pseudo       1.018131    -0.848076    -0.934753          1.0000
  53 Pycno        1.537961     1.193672    -0.029435          6.0000
  54 Rheo        -0.857332     0.314905     0.348091         29.0000
  55 Rhyacob     -0.874538     0.223282    -0.397758         10.0000
  56 Sialis       0.927155    -2.106862     1.306687          1.0000
  57 Sigaramo    -0.106683    -0.454307     0.126026          1.0000
  58 Simtub      -0.394978     0.121823     1.222994         14.0000
  59 Simvit       0.342033     1.061588     0.012763         11.0000
  60 Stemp        0.291407    -1.252869     1.904582          2.0000
  61 Stenac      -0.106683    -0.454307     0.126026          2.0000
  62 Stenel       1.075890    -0.621215    -0.834162          9.0000
  63 Stenon       0.410236    -1.280585     0.716357          8.0000
  64 Sticto       0.845293    -1.627316     1.583705          5.0000
  65 Thiensp     -0.990582     0.411296     0.852107          1.0000
  66 Thiengp     -0.561008    -0.404003     2.355468          1.0000
  67 TipNip      -0.561008    -0.404003     2.355468          1.0000
  68 Trisso       0.492208    -1.779461     0.202402          2.0000
--------------------------------------------------------------------


CORRELATIONS AND BIPLOT SCORES for   9 Environ
--------------------------------------------------------------------
                       Correlations*              Biplot Scores
    Variable      Axis 1  Axis 2  Axis 3     Axis 1  Axis 2  Axis 3
--------------------------------------------------------------------
   1 Temp          0.741   0.612   0.044      0.741   0.612   0.044
   2 DO           -0.525  -0.338   0.006     -0.525  -0.338   0.006
   3 SC            0.661  -0.074  -0.102      0.661  -0.074  -0.102
   4 pH            0.145  -0.271   0.159      0.145  -0.271   0.159
   5 Vel          -0.417   0.530   0.237     -0.417   0.530   0.237
   6 ChWid         0.539  -0.386   0.490      0.539  -0.386   0.490
   7 MaxDep        0.271  -0.435   0.245      0.271  -0.435   0.245
   8 Cancov        0.189   0.300   0.088      0.189   0.300   0.088
   9 Alk          -0.272   0.036   0.145     -0.272   0.036   0.145
------------------------------------------------------------------
* Correlations are "intraset correlations" of ter Braak (1986)


INTER-SET CORRELATIONS for   9 Environ
-----------------------------------------
                       Correlations
    Variable      Axis 1  Axis 2  Axis 3
-----------------------------------------
   1 Temp          0.710   0.568   0.035
   2 DO           -0.503  -0.314   0.004
   3 SC            0.633  -0.069  -0.080
```

Appendix D. Continued.

```
4 pH            0.138  -0.251   0.126
5 Vel          -0.399   0.492   0.187
6 ChWid         0.516  -0.358   0.386
7 MaxDep        0.260  -0.404   0.193
8 Cancov        0.181   0.279   0.069
9 Alk          -0.261   0.033   0.114
```

Appendix E. Minitab output from the stepwise regressions performed on the eight quantitatively sampled low to medium discharge rheocrene spring systems showing the subset of environmental variables that best explains the variation in longitudinal changes of species richness, Shannon diversity, Shannon evenness, and density of the dominant species, as well as the corresponding regression equation, T-value, P-value, and R-squared values.

1RB- 2007

Stepwise Regression: Species Richness versus Environmental Variables

```
  Alpha-to-Enter: 0.15  Alpha-to-Remove: 0.15

Species Richness = 36.61 - 0.368 Temp + 0.017 Channel Width - 4.16 pH

Response is Species Richness on 9 predictors, with N = 5

Step                    1        2        3
Constant            32.86    27.74    36.61

pH                  -4.22    -3.69    -4.16
T-Value             -2.91    -6.29   -37.81
P-Value             0.062    0.024    0.017

Channel Width (cm)         0.01718  0.01719
T-Value                       4.14    25.43
P-Value                      0.054    0.025

Temp. (˚C)                           -0.368
T-Value                               -8.62
P-Value                               0.074

S                   0.417    0.165   0.0269
R-Sq                73.90    97.27    99.96
R-Sq(adj)           65.20    94.55    99.86
```

2DM- 2007

Stepwise Regression: Species Richness versus Environmental Variables

```
  Alpha-to-Enter: 0.15  Alpha-to-Remove: 0.15

Species Richness = 37.07 - 0.132 (T) Alkalinity

Response is Species Richness on 9 predictors, with N = 3

Step                 1
Constant         37.07

(T) Alkalinity  -0.132
T-Value          -8.66
P-Value          0.073

S                0.187
R-Sq             98.68
R-Sq(adj)        97.37
```

Appendix E. Continued.

CSCR- 2007

Stepwise Regression: Species Richness versus Environmental Variables

```
  Alpha-to-Enter: 0.15  Alpha-to-Remove: 0.15

Species Richness = -12.48 + 2.30 DO

Response is Species Richness on 9 predictors, with N = 5

Step            1
Constant   -12.48

DO (mg/L)    2.30
T-Value      6.89
P-Value     0.006

S           0.811
R-Sq        94.06
R-Sq(adj)   92.08
```

CSM- 2007

Stepwise Regression: Species Richness versus Environmental Variables

```
  Alpha-to-Enter: 0.15  Alpha-to-Remove: 0.15

Species Richness = 682.6 -1.45 SC

Response is Species Richness on 9 predictors, with N = 4


Step           1
Constant   682.6

SC (µs)    -1.45
T-Value    -8.00
P-Value    0.015

S          0.302
R-Sq       96.97
R-Sq(adj)  95.45
```

Appendix E. Continued.

LSCR- 2007

Stepwise Regression: Species Richness versus Environmental Variables

```
  Alpha-to-Enter: 0.15  Alpha-to-Remove: 0.15

Species Richness = -57.30 + 7.8 DO

Response is Species Richness on 9 predictors, with N = 5

Step            1
Constant   -57.30

DO (mg/L)     7.8
T-Value      2.88
P-Value     0.063

S            1.37
R-Sq        73.47
R-Sq(adj)   64.63
```

MSLO-2007

Stepwise Regression: Species Richness versus Environmental Variables

```
  Alpha-to-Enter: 0.15  Alpha-to-Remove: 0.15

Species Richness = -135.2 – 0.262 (T)Alkalinity + 95 Current Velocity + 22.3 pH

Response is Species Richness on 9 predictors, with N = 6

Step                        1       2       3
Constant               -136.8  -178.3  -135.2

pH                       19.1    23.1    22.3
T-Value                  2.12    4.04    7.34
P-Value                 0.101   0.027   0.018

Current Velocity (m/s)             53      95
T-Value                          2.76    5.43
P-Value                         0.070   0.032

(T) Alkalinity                         -0.262
T-Value                                 -2.95
P-Value                                 0.099

S                        2.89    1.77   0.938
R-Sq                    52.93   86.74   97.52
R-Sq(adj)               41.17   77.89   93.79
```

Appendix E. Continued.

1RB- 2008

Stepwise Regression: Species Richness versus Environmental Variables

```
  Alpha-to-Enter: 0.15  Alpha-to-Remove: 0.15

Species Richness = 4.687 -0.28 Max. Depth

Response is Species Richness on 8 predictors, with N = 5

Step                 1
Constant         4.687

Max. Depth (cm)  -0.28
T-Value          -2.14
P-Value          0.122

S                0.608
R-Sq             60.43
R-Sq(adj)        47.25
```

2DM- 2008

Stepwise Regression: Species Richness versus Environmental Variables

```
  Alpha-to-Enter: 0.15  Alpha-to-Remove: 0.15


Response is Species Richness on 9 predictors, with N = 3


No variables entered or removed
```

Appendix E. Continued.

CSCR- 2008

Stepwise Regression: Species Richness versus Environmental Variables

```
  Alpha-to-Enter: 0.15  Alpha-to-Remove: 0.15

Species Richness = 23.32 - 0.81 Temp – 196.1 Current Velocity – 0.107 Canopy
Cover + 0.00293 Channel Width

Response is Species Richness on 9 predictors, with N = 6

Step                        1        2        3         4
Constant                23.00    22.90    22.59     23.32

Temp. (˚C)            -0.9547  -0.8200  -0.7699   -0.8127
T-Val                   -7.15   -20.04   -54.39    -90.36
P-Value                 0.002    0.000    0.000     0.007

Current Velocity (m/s)          -209.4   -230.6    -196.1
T-Value                          -7.15   -25.84    -28.07
P-Value                          0.006    0.001     0.023

Canopy Cover (Avg. % open)                -0.149    -0.107
T-Value                                    -6.02    -10.35
P-Value                                    0.026     0.061

Channel Width (cm)                                 0.00293
T-Value                                              -5.24
P-Value                                              0.120

S                       0.873    0.237   0.0664    0.0176
R-Sq                    92.74    99.60    99.98    100.00
R-Sq(adj)               90.93    99.33    99.95    100.00
```

CSM- 2008

Stepwise Regression: Species Richness versus Environmental Variables

```
  Alpha-to-Enter: 0.15  Alpha-to-Remove: 0.15

Species Richness = 2.056 + 17.1 Current Velocity

Response is Species Richness on 9 predictors, with N = 4

Step                         1
Constant                 2.056

Current Velocity (m/s)    17.1
T-Value                  10.29
P-Value                  0.009

S                        0.193
R-Sq                     98.14
R-Sq(adj)                97.22
```

Appendix E. Continued.

LSCR- 2008

Stepwise Regression: Species Richness versus Environmental Variables

```
  Alpha-to-Enter: 0.15  Alpha-to-Remove: 0.15

Species Richness = -744.2 + 1.08 SC + 11.1 DO

Response is Species Richness on 9 predictors, with N = 5

Step            1       2
Constant   -559.7  -744.2

SC (µs)      0.90    1.08
T-Value      2.56    7.93
P-Value     0.083   0.016

DO (mg/L)            11.1
T-Value              4.48
P-Value             0.046

S            1.91   0.704
R-Sq        68.59   97.15
R-Sq(adj)   58.12   94.30
```

MSLO- 2008

Stepwise Regression: Species Richness versus Environmental Variables

```
  Alpha-to-Enter: 0.15  Alpha-to-Remove: 0.15

Species Richness = -4.22.6 + 0.18 DO + 0.31 SC + 36.29 pH + 0.024 Channel Width

Response is Species Richness on 9 predictors, with N = 6

Step                     1         2         3         4
Constant             3.235  -239.103  -377.169  -423.563

Channel Width (cm) 0.02771   0.02049   0.02379   0.02358
T-Value               2.94      5.53     12.00     48.75
P-Value              0.042     0.012     0.007     0.013

pH                             31.69     34.75     36.29
T-Value                         5.21     11.66     46.92
P-Value                        0.014     0.007     0.014

SC (µs)                                  0.239     0.309
T-Value                                   3.42     14.76
P-Value                                  0.076     0.043

DO (mg/L)                                          0.179
T-Value                                             5.73
P-Value                                            0.110

S                     3.04      1.11     0.518     0.126
R-Sq                 68.36     96.85     99.54     99.99
R-Sq(adj)            60.45     94.75     98.85     99.93
```

Appendix E. Continued.

1RB- 2007

Stepwise Regression: Shannon Diversity versus Environmental Variables

```
  Alpha-to-Enter: 0.15  Alpha-to-Remove: 0.15

Shannon Diversity = 1.8197 - 0.015 DO - 0.105 Temp + 2.94 Current Velocity

Response is Shannon Diversity on 9 predictors, with N= 5

Step                          1       2        3
Constant                 0.1875  2.7351   1.8197

Current Velocity (m/s)    2.332   2.963    2.939
T-Value                    2.73   12.05    41.84
P-Value                   0.072   0.007    0.015

Temp. (˚C)                       -0.172   -0.105
T-Value                           -6.41    -6.75
P-Value                           0.023    0.094

DO (mg/L)                                -0.0147
T-Value                                    -4.86
P-Value                                    0.129

S                        0.0679  0.0179  0.00511
R-Sq                      71.32   98.67    99.95
R-Sq(adj)                 61.76   97.34    99.78
```

2DM- 2007

Stepwise Regression: Shannon Diversity versus Environmental Variables

```
  Alpha-to-Enter: 0.15  Alpha-to-Remove: 0.15

Shannon Diversity = -0.0796 + 1.251 Current Velocity

Response is Shannon Diversity on 9 predictors, with N = 3

Step                            1
Constant                 -0.07963

Current Velocity (m/s)      1.251
T-Value                     14.26
P-Value                     0.045

S                          0.0191
R-Sq                        99.51
R-Sq(adj)                   99.02
```

Appendix E. Continued.

CSCR- 2007

Stepwise Regression: Shannon Diversity versus Environmental Variables

```
  Alpha-to-Enter: 0.15  Alpha-to-Remove: 0.15

Shannon Diversity = -2.559 + 0.467 DO

Response is Shannon Diversity on 9 predictors, with N= 5

Step            1
Constant   -2.559

DO (mg/L)   0.467
T-Value     13.45
P-Value     0.001

S          0.0842
R-Sq        98.37
R-Sq(adj)   97.82
```

CSM- 2007

Stepwise Regression: Shannon Diversity versus Environmental Variables

```
  Alpha-to-Enter: 0.15  Alpha-to-Remove: 0.15

Response is Shannon Diversity on 9 predictors, with N= 4

No variables entered or removed
```

LSCR- 2007

Stepwise Regression: Shannon Diversity versus Environmental Variables

```
  Alpha-to-Enter: 0.15  Alpha-to-Remove: 0.15

Shannon Diversity = 57.70 – 0.02 (T)Alkalinity + 0.032 SC – 3.83 Temp

Response is Shannon Diversity on 9 predictors, with N= 5

Step                 1        2         3
Constant         32.84    53.02     57.70

Temp. (˚C)     -1.7396  -4.4164   -3.8336
T-Value          -2.44    -9.05  -1504.55
P-Value          0.093    0.012     0.000

SC (µs)                 0.04867   0.03181
T-Value                    6.01    553.35
P-Value                   0.027     0.001

(T) Alkalinity                   -0.01968
T-Value                           -354.50
P-Value                             0.002
```

Appendix E. Continued.

```
S                 0.202   0.0565  0.000226
R-Sq              66.49    98.24    100.00
R-Sq(adj)         55.32    96.49    100.00
```

MSLO- 2007

Stepwise Regression: Shannon Diversity versus Environmental Variables

```
  Alpha-to-Enter: 0.15  Alpha-to-Remove: 0.15

Shannon Diversity = -32.54 +5.9 Current Velocity – 0.44 DO + 4.90 pH

Response is Shannon Diversity on 9 predictors, with N= 6

Step                        1       2       3
Constant               -31.42  -27.96  -32.54

pH                       4.36    4.45    4.90
T-Value                  2.99    4.14    9.07
P-Value                 0.040   0.026   0.012

DO (mg/L)                       -0.43   -0.44
T-Value                         -2.09   -4.36
P-Value                         0.128   0.049

Current Velocity (m/s)                    5.9
T-Value                                  3.27
P-Value                                 0.082

S                       0.466   0.343   0.167
R-Sq                    69.11   87.42   98.02
R-Sq(adj)               61.39   79.03   95.04
```

MSLO- 2008

Stepwise Regression: Shannon Diversity versus Environmental Variables

```
  Alpha-to-Enter: 0.15  Alpha-to-Remove: 0.15

Shannon Diversity = -38.55 – 0.060 Canopy Cover + 0.003 Channel Width + 0.470
Temp + 4.20 pH

Response is Shannon Diversity on 9 predictors, with N= 6

Step                        1       2         3         4
Constant               -62.67  -51.66    -41.65    -38.55

pH                       8.34    6.08      4.62      4.20
T-Value                  2.98    2.87      4.21     13.84
P-Value                 0.041   0.064     0.052     0.046

Temp. (˚C)                      0.421     0.463     0.470
T-Value                          2.38      5.46     20.79
P-Value                         0.097     0.032     0.031
```

Appendix E. Continued.

```
Channel Width (cm)                            0.00201  0.00304
T-Value                                          3.36    12.00
P-Value                                         0.078    0.053

Canopy Cover (Avg. % open)                              -0.060
T-Value                                                  -5.22
P-Value                                                  0.121

S                         0.549   0.373    0.177   0.0471
R-Sq                      68.94   89.27    98.39    99.94
R-Sq(adj)                 61.18   82.12    95.97    99.71
```

LSCR- 2008

Stepwise Regression: Shannon Diversity versus Environmental Variables

```
Alpha-to-Enter: 0.15  Alpha-to-Remove: 0.15

Shannon Diversity = -89.80 + 0.145 SC

Response is Shannon Diversity on 9 predictors, with N= 5

Step            1
Constant   -89.80

SC (µs)     0.145
T-Value      3.52
P-Value     0.039

S           0.222
R-Sq        80.53
R-Sq(adj)   74.04
```

CSM- 2008

Stepwise Regression: Shannon DIversity versus Environmental Variables

```
  Alpha-to-Enter: 0.15  Alpha-to-Remove: 0.15

Shannon Diversity = 3.44 – 0.406 pH + 1.555 Current Velocity

Response is Shannon DIversity on 9 predictors, with N= 4

Step                         1        2
Constant                0.2255   3.4363

Current Velocity (m/s)   1.467    1.555
T-Value                   2.87    54.06
P-Value                  0.103    0.012

pH                               -0.406
T-Value                          -25.32
P-Value                           0.025

S                       0.0594  0.00331
R-Sq                     80.43    99.97
R-Sq(adj)                70.64    99.91
```

Appendix E. Continued.

CSCR- 2008

Stepwise Regression: Shannon DIversity versus Environmental Variables

```
Alpha-to-Enter: 0.15  Alpha-to-Remove: 0.15

Shannon Diversity = 4.102 - 0.179 Temp

Response is SHannon DIversity on 9 predictors, with N= 6

Step             1
Constant     4.102

Temp. (˚C)  -0.179
T-Value      -4.56
P-Value      0.010

S            0.257
R-Sq         83.88
R-Sq(adj)    79.85
```

2DM- 2008

Stepwise Regression: Shannon Diversity versus Environmental Variables

```
Alpha-to-Enter: 0.15  Alpha-to-Remove: 0.15

Shannon Diversity = -32.05 + 4.551 pH

Response is Shannon Diversity on 9 predictors, with N= 3

Step            1
Constant   -32.05

pH          4.551
T-Value     48.32
P-Value     0.013

S          0.0123
R-Sq        99.96
R-Sq(adj)   99.91
```

Appendix E. Continued.

1RB- 2008

Stepwise Regression: Shannon DIversity versus Environmental Variables

```
  Alpha-to-Enter: 0.15  Alpha-to-Remove: 0.15

Shannon Diversity = 0.163 + 0.67 Canopy Cover

Response is Shannon DIversity on 8 predictors, with N= 5

Step                           1
Constant                  0.1626

Canopy Cover (Avg. % open)    0.67
T-Value                     3.27
P-Value                    0.047

S                          0.192
R-Sq                       78.09
R-Sq(adj)                  70.79
```

2DM- 2007

Stepwise Regression: Shannon Evenness versus Environmental Variables

```
  Alpha-to-Enter: 0.15  Alpha-to-Remove: 0.15

Shannon Evenness = -0.0725 + 1.139 Current Velocity

Response is Evenness (E) on 9 predictors, with N=3

Step                         1
Constant              -0.07248

Current Velocity (m/s)     1.139
T-Value                  14.26
P-Value                  0.045

S                       0.0174
R-Sq                     99.51
R-Sq(adj)                99.02
```

1RB- 2007

Stepwise Regression: Shannon Evenness versus Environmental Variables

```
  Alpha-to-Enter: 0.15  Alpha-to-Remove: 0.15

Shannon Evenness = -1.248 + 0.0041 SC

Response is Evenness (E) on 9 predictors, with N = 5

Step            1
Constant   -1.248

SC (µs)    0.0041
T-Value      2.02
P-Value     0.136

S          0.0792
R-Sq        57.68
R-Sq(adj)   43.58
```

MSLO- 2007

Stepwise Regression: Shannon Evenness versus Environmental Variables

```
  Alpha-to-Enter: 0.15  Alpha-to-Remove: 0.15

Response is Evenness (E) on 9 predictors, with N = 6

No variables entered or removed
```

CSM- 2007

Stepwise Regression: Shannon Evenness versus Environmental Variables

```
 Alpha-to-Enter: 0.15  Alpha-to-Remove: 0.15

Shannon Evenness = 5.146 + 0.117 Canopy Cover – 0.920 Max Depth

Response is Evenness (E) on 9 predictors, with N = 4

Step                              1        2
Constant                      1.643    5.146

Max. Depth (cm)              -0.193   -0.920
T-Value                       -2.77   -33.31
P-Value                       0.109    0.019

Canopy Cover (Avg. % open)            0.1168
T-Value                                26.57
P-Value                                0.024
```

```
S                             0.159  0.00846
R-Sq                          79.33    99.97
R-Sq(adj)                     69.00    99.91
```

Appendix E. Continued.

CSCR- 2007

Stepwise Regression: Shannon Evenness versus Environmental Variables

```
  Alpha-to-Enter: 0.15  Alpha-to-Remove: 0.15

Shannon Evenness = 5.515 – 0.0166 (T) Alkalinity

Response is Evenness (E) on 9 predictors, with N = 5

Step                1
Constant        5.515

(T) Alkalinity  -0.0166
T-Value           -4.07
P-Value           0.027

S               0.0763
R-Sq             84.68
R-Sq(adj)        79.57
```

LSCR- 2007

Stepwise Regression: Shannon Evenness versus Temp. (˚C), DO (mg/L), ...

```
  Alpha-to-Enter: 0.15  Alpha-to-Remove: 0.15

Response is Evenness (E) on 9 predictors, with N = 5

No variables entered or removed
```

2DM- 2008

Stepwise Regression: Shannon Evenness versus Environmental Variables

```
  Alpha-to-Enter: 0.15  Alpha-to-Remove: 0.15

Shannon Evenness = 0.093 + 0.00218 Channel Width

Response is Evenness (E) on 9 predictors, with N = 3

Step                      1
Constant            0.09317

Channel Width (cm)  0.00218
T-Value               52.32
P-Value               0.012

S                    0.0111
R-Sq                  99.96
R-Sq(adj)             99.93
```

Appendix E. Continued.

1RB- 2008

Stepwise Regression: Shannon Evenness versus Environmental Variables

```
  Alpha-to-Enter: 0.15  Alpha-to-Remove: 0.15

Shannon Evenness = 0.2277 + 0.59 Canopy Cover

Response is Evenness (E) on 8 predictors, with N = 5

Step                             1
Constant                    0.2277

Canopy Cover (Avg. % open)    0.59
T-Value                       2.60
P-Value                      0.081

S                            0.211
R-Sq                         69.21
R-Sq(adj)                    58.95
```

MSLO- 2008

Stepwise Regression: Shannon Evenness versus Environmental Variables

```
  Alpha-to-Enter: 0.15  Alpha-to-Remove: 0.15

Shannon Evenness = -14.58 + 0.00035 Channel Width + 1.55 pH + 0.2079 Temp

Response is Evenness (E) on 9 predictors, with N = 6

Step                     1        2        3
Constant            -3.411  -16.305  -14.579

Temp. (˚C)          0.2675   0.2006   0.2079
T-Value               3.78     6.94    23.44
P-Value              0.019    0.006    0.002

pH                             1.80     1.55
T-Value                        5.18    13.50
P-Value                       0.014    0.005

Channel Width (cm)                   0.00035
T-Value                                 5.53
P-Value                                0.031

S                    0.167   0.0611   0.0185
R-Sq                 78.14    97.80    99.87
R-Sq(adj)            72.68    96.34    99.66
```

Appendix E. Continued.

CSM- 2008

Stepwise Regression: Shannon Evenness versus Environmental Variables

```
  Alpha-to-Enter: 0.15  Alpha-to-Remove: 0.15

Shannon Evenness = 0.0398 + 0.0090 Temp + 0.00088 Channel Width

Response is Evenness (E) on 9 predictors, with N = 4

Step                       1         2
Constant             0.19350   0.03978

Channel Width (cm)   0.00080   0.00088
T-Value                18.58     61.10
P-Value                0.003     0.010

Temp. (˚C)                      0.0090
T-Value                           6.98
P-Value                          0.091

S                    0.00339  0.000681
R-Sq                   99.42     99.99
R-Sq(adj)              99.14     99.97
```

CSCR- 2008

Stepwise Regression: Shannon Evenness versus Environmental Variables

```
  Alpha-to-Enter: 0.15  Alpha-to-Remove: 0.15

Shannon Evenness = -9.081 + 1.28 pH

Response is Evenness (E) on 9 predictors, with N = 6

Step            1
Constant   -9.081

pH           1.28
T-Value      3.13
P-Value     0.035

S           0.164
R-Sq        71.06
R-Sq(adj)   63.82
```

LSCR- 2008

Stepwise Regression: Shannon Evenness versus Environmental Variables

```
  Alpha-to-Enter: 0.15  Alpha-to-Remove: 0.15

Response is Evenness (E) on 9 predictors, with N = 5

No variables entered or removed
```

Appendix E. Continued.

2DM- 2007

Stepwise Regression: G.minus versus Environmental Variables

```
  Alpha-to-Enter: 0.15  Alpha-to-Remove: 0.15

Density of G. minus = -0.671 + 190 Veloc

Response is G.minus on 9 predictors, with N = 3

Step             1
Constant   -0.6709

Veloc          190
T-Value      13.67
P-Value      0.046

S             3.02
R-Sq         99.47
R-Sq(adj)    98.94
```

1RB- 2007

Stepwise Regression: C.brevicauda versus Environmental Variables

```
  Alpha-to-Enter: 0.15  Alpha-to-Remove: 0.15

Density of C. brevicauda = 83.84 + 2.60 Alk – 647 Veloc – 91 pH

Response is C.brevi on 9 predictors, with N = 5

Step             1        2       3
Constant   1227.05  1492.03   83.84

pH            -167     -201     -91
T-Value      -2.90    -5.17   -4.55
P-Value      0.062    0.035   0.138

Veloc                  -333    -647
T-Value                -2.37  -10.75
P-Value                0.141   0.059

Alk                             2.60
T-Value                         6.15
P-Value                        0.103

S             16.6     10.4    2.36
R-Sq         73.73    93.11   99.82
R-Sq(adj)    64.98    86.22   99.29
```

Appendix E. Continued.

MSLO- 2007

Stepwise Regression: G.pseudolimnaeus versus Environmental Variables

```
  Alpha-to-Enter: 0.15  Alpha-to-Remove: 0.15

Density of G. pseudolimnaeus = 364.3 – 1.39 Alk – 11.5 Depth + 8.42 CanCov

Response is G.pseud on 9 predictors, with N = 6

Step             1       2        3
Constant    -3.313  78.423  364.300

CanCov        2.41    5.82     8.42
T-Value       2.44    4.92     7.63
P-Value      0.072   0.016    0.017

Depth                 -7.5    -11.5
T-Value              -3.24    -6.15
P-Value              0.048    0.025

Alk                           -1.39
T-Value                       -2.88
P-Value                       0.102

S             17.6    9.57     5.16
R-Sq         59.73   91.06    98.27
R-Sq(adj)    49.66   85.09    95.67
```

CSM- 2007

Stepwise Regression: G.minus versus Environmental Variables

```
  Alpha-to-Enter: 0.15  Alpha-to-Remove: 0.15

Density of G. minus = -21.54 + 8.8 Depth

Response is G.minus on 9 predictors, with N = 4

Step             1
Constant    -21.54

Depth          8.8
T-Value       6.94
P-Value      0.020

S             2.88
R-Sq         96.02
R-Sq(adj)    94.03
```

Appendix E. Continued.

CSCR- 2007

Stepwise Regression: C.forbesi versus Environmental Variables

```
  Alpha-to-Enter: 0.15  Alpha-to-Remove: 0.15

Density of C. forbesi = 50.19 + 3.13 CanCov – 0.700 Width

Response is C.forb on 9 predictors, with N = 5

Step            1       2
Constant    41.90   50.19

Width      -0.399  -0.700
T-Value     -2.76   -8.00
P-Value     0.070   0.015

CanCov               3.13
T-Value              4.39
P-Value             0.048

S            8.30    3.12
R-Sq        71.72   97.34
R-Sq(adj)   62.30   94.67
```

CSCR- 2007

Stepwise Regression: G.pseudolimnaeus versus Environmental Variables

```
  Alpha-to-Enter: 0.15  Alpha-to-Remove: 0.15

Density of G. pseudolimnaeus = 77.94 – 4.6 Temp

Response is G.pseud on 9 predictors, with N = 5

Step           1
Constant   77.94

Temp        -4.6
T-Value    -2.85
P-Value    0.065

S           6.23
R-Sq       73.07
R-Sq(adj)  64.10
```

Appendix E. Continued.

CSCR- 2007

Stepwise Regression: C.brevicauda versus Environmental Variables

```
  Alpha-to-Enter: 0.15  Alpha-to-Remove: 0.15

Density of C. brevicauda = -10.84 + 3.3 Depth

Response is C.brevi on 9 predictors, with N = 5

Step            1
Constant   -10.84

Depth         3.3
T-Value      2.01
P-Value     0.138

S            18.3
R-Sq        57.41
R-Sq(adj)   43.22
```

1RB- 2007

Stepwise Regression: C.forbesi versus Environmental Variables

```
  Alpha-to-Enter: 0.15  Alpha-to-Remove: 0.15

Density of C. forbesi = 161.9 + 0.302 Depth + 0.197 CanCov – 23.42 pH

Response is C.forb on 9 predictors, with N = 5

Step             1        2        3
Constant     129.5    170.8    161.9

pH         -17.809  -24.347  -23.415
T-Value      -3.38    -9.36  -322.39
P-Value      0.043    0.011    0.002

CanCov              0.2140   0.1974
T-Value               4.17   138.55
P-Value              0.053    0.005

Depth                        0.3016
T-Value                       52.21
P-Value                       0.012

S             1.52    0.597   0.0162
R-Sq         79.17    97.85   100.00
R-Sq(adj)    72.23    95.70   100.00
```

Appendix E. Continued.

LSCR- 2007

Stepwise Regression: C.forb versus Environmental Variables

```
  Alpha-to-Enter: 0.15  Alpha-to-Remove: 0.15

Response is C.forb on 9 predictors, with N = 5

No variables entered or removed
```

LSCR- 2007

Stepwise Regression: C.brevicauda versus Environmental Variables

```
  Alpha-to-Enter: 0.15  Alpha-to-Remove: 0.15

Density of C. brevicauda = -526.4 + 51.76 DO + 22.05 pH – 0.074 SC

Response is C.brevi on 9 predictors, with N = 5

Step            1       2        3
Constant   -446.2  -557.7   -526.4

DO         57.594  53.416   51.756
T-Value     19.91   25.84   522.18
P-Value     0.000   0.001    0.001

pH                  18.72    22.05
T-Value              2.98    78.84
P-Value             0.097    0.008

SC                         -0.0735
T-Value                     -33.90
P-Value                      0.019

S            1.47   0.771   0.0321
R-Sq        99.25   99.86   100.00
R-Sq(adj)   99.00   99.72   100.00
```

2DM- 2008

Stepwise Regression: G.minus versus Environmental Variables

```
  Alpha-to-Enter: 0.15  Alpha-to-Remove: 0.15

Density of G. minus = 21035 – 57.0 SC

Response is G.minus(2DM) on 9 predictors, with N = 3

Step            1
Constant    21035

SC          -57.0
T-Value    -32.91
P-Value     0.019
```

Appendix E. Continued.

```
S            3.74
R-Sq        99.91
R-Sq(adj)   99.82
```

1RB- 2008

Stepwise Regression: C.forbesi versus Environmental Variables

```
  Alpha-to-Enter: 0.15  Alpha-to-Remove: 0.15

Response is C.forb(1RB) on 8 predictors, with N = 5

No variables entered or removed
```

MSLO- 2008

Stepwise Regression: G.pseudolimnaeus versus Environmental Variables

```
  Alpha-to-Enter: 0.15  Alpha-to-Remove: 0.15

Density of G. pseudolimnaeus = 6025 – 774 pH

Response is G.pseud(MSLO) on 9 predictors, with N = 6

Step           1
Constant    6025

pH          -774
T-Value    -2.67
P-Value    0.056

S           57.0
R-Sq       64.02
R-Sq(adj)  55.03
```

CSM- 2008

Stepwise Regression: G.minus versus Environmental Variables

```
  Alpha-to-Enter: 0.15  Alpha-to-Remove: 0.15

Density of G. minus = 41.44 – 2.51 CanCov + 263.7 Vel

Response is G.minus(CSM) on 9 predictors, with N = 4

Step           1       2
Constant   30.23   41.44

Vel        290.0   263.7
T-Value     3.82   41.87
P-Value    0.062   0.015
```

Appendix E. Continued.

```
CanCov             -2.51
T-Value           -17.54
P-Value            0.036

S           8.82   0.710
R-Sq       87.92   99.96
R-Sq(adj)  81.88   99.88
```

CSCR- 2008

Stepwise Regression: G.pseudolimnaeus versus Environmental Variables

```
  Alpha-to-Enter: 0.15  Alpha-to-Remove: 0.15

Density of G. pseudolimnaeus = 60.77 – 15.4 CanCov

Response is G.pseud(CSCR) on 9 predictors, with N = 6

Step           1
Constant   60.77

CanCov     -15.4
T-Value    -2.22
P-Value    0.090

S           23.0
R-Sq       55.23
R-Sq(adj)  44.03
```

CSCR- 2008

Stepwise Regression: C.brevicauda versus Environmental Variables

```
  Alpha-to-Enter: 0.15  Alpha-to-Remove: 0.15

Density of C. brevicauda = -61.32 + 1.64 Temp + 5.68 DO – 0.091 Width + 8.25
CanCov

Response is C.brevi(CSCR) on 9 predictors, with N = 6

Step             1        2        3         4
Constant   -4.1463  23.1485  -0.5040  -61.3204

CanCov       9.287    8.894    9.265     8.245
T-Value       3.35     6.88    12.83    134.08
P-Value      0.029    0.006    0.006     0.005

Width              -0.1765  -0.1490   -0.0906
T-Value              -3.95    -5.63    -30.68
P-Value              0.029    0.030     0.021

DO                            2.49      5.68
T-Value                       2.82     38.88
P-Value                      0.106     0.016
```

Appendix E. Continued.

```
Temp                                    1.641
T-Value                                 23.46
P-Value                                 0.027

S             9.24     4.29     2.36    0.142
R-Sq         73.67    95.75    99.14   100.00
R-Sq(adj)    67.08    92.91    97.86    99.99
```

LSCR- 2008

Stepwise Regression: C.brevicauda versus Environmental Variables

```
  Alpha-to-Enter: 0.15  Alpha-to-Remove: 0.15

Density of C. brevicauda = -4527 + 283 pH + 247.2 Vel + 335 DO

Response is C.brevi(LSCR) on 9 predictors, with N = 5

Step           1      2      3
Constant   -1426  -1078  -4527

DO           214    153    335
T-Value     2.85   5.72  13.56
P-Value    0.065  0.029  0.047

Vel                268.1  247.2
T-Value             5.23  24.86
P-Value            0.035  0.026

pH                          283
T-Value                    7.53
P-Value                   0.084

S           22.3   7.12   1.33
R-Sq       73.03  98.16  99.97
R-Sq(adj)  64.05  96.33  99.87
```

VITA

Megan Mishell (Hedrick) Zeller was born to Timothy and Tamara Hedrick on September 30, 1981 in Wichita, Kansas. After moving to Missouri as a young child, she graduated from Lexington High School in Lexington, Missouri in May 2000. Immediately following her graduation from high school, she attended Longview Community College in Lee's Summit, Missouri on an A+ Scholarship. It was there she received her Associate of Arts degree in May 2002. She then transferred to the University of Central Missouri in Warrensburg to study biology and earth sciences, graduating magna cum laude with her Bachelor of Science in Biology in May 2004. Following her graduation in 2004, she was offered a graduate teaching assistantship and began graduate school at the University of Central Missouri. In May 2006 she graduated summa cum laude with her Master of Science in Biology. In the fall of 2006 she began the Plant, Insect, and Microbial Sciences Ph.D. program in the entomology program area at the University of Missouri in Columbia on a Division of Plant Sciences Doctoral Fellowship. She graduated with her Ph.D. in May 2010. Over the course of her studies, she has worked for various state and federal agencies, such as the Missouri Department of Conservation and the U.S. Geological Survey, to gain relevant experience in her field. Also during this time, she married her high school sweetheart, Lucas Zeller, on January 5, 2008.