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College of Graduate Studies and Research

MACROINVERTEBRATE ASSEMBLAGES AND WATER QUALITY ANALYSIS OF  
SPRING SYSTEMS ASSOCIATED WITH THE PONTOTOC RIDGE NATURE  
PRESERVE, OKLAHOMA

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Oklahoma City, Oklahoma

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MACROINVERTEBRATE ASSEMBLAGES AND WATER QUALITY ANALYSIS OF  
SPRING SYSTEMS ASSOCIATED WITH THE PONTOTOC RIDGE NATURE PRESERVE,  
OKLAHOMA

A THESIS

APPROVED FOR THE DEPARTMENT OF BIOLOGY

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By



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## ABSTRACT OF THESIS

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TITLE OF THESIS: Macroinvertebrate Assemblages and Water Quality Analysis  
of Spring Systems Associated with the Pontotoc Ridge Nature  
Preserve, Oklahoma

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### ABSTRACT:

Pontotoc Ridge Nature Preserve is located in southeastern Pontotoc County, Oklahoma, 20.7 miles south of Ada. This area consists of 2,900 acres of assorted vegetation with several springs, all of which emerge from the Arbuckle-Simpson Aquifer. Three springs, two located within the Nature Preserve and one on adjacent property, were surveyed during this study. Aquatic macroinvertebrates and physiochemical data were collected on a seasonal basis, every three months, beginning January 2011 and ending January 2012. With the exception of 16 dissolved oxygen readings and six orthophosphate readings, the physiochemical data meet standards that support and allow for aquatic life. A total of 127,048 individuals, representing 114 taxa, were collected throughout the course of this study. Non-hexapods, included amphipods, copepods, isopods, molluscs,

plathelminthes, nematodes, and various other taxa, were the dominant macroinvertebrates present. The amphipod, *Hyallela Azteca* complex, was the most numerous non-hexapod as well as the most numerous macroinvertebrate, having a total of 76,529 individuals. Hexapods, represented by collembolans, ephemeropterans, odonates, plecopterans, hemipterans, trichopterans, coleopterans, and dipterans, were more diverse in terms of taxa, with a total of 93 taxa collected and identified. Of the three springs studied, Smith Spring was the most diverse with an average of 44.8 taxa, followed by Canyon Spring with an average of 32.4 taxa, and Cave Spring with an average of only 21 taxa. Canyon Spring was the most populated (84,339 individuals), followed by Smith Spring (38,837 individuals), and Cave Spring (3,873 individuals). The April 2011 collection contained both the largest number of individuals, 34,368, as well as the highest number of taxa, 74, found. Similarity indices for combined collections between springs were similar, with the average indices above 0.425. Similarity indices for comparisons between upper and lower collection sites were lower, with average indices no greater than 0.349. Species diversity values were generally under 2.0, with a few exceptions in Cave Spring and Smith Spring, having averages no greater than 1.785. The results of this investigation indicate these springs are in nearly pristine condition and they play an important role in the Pontotoc Ridge ecosystem.

## INTRODUCTION

Springs are described as naturally occurring sources of emerging groundwater that have unique properties unto themselves, such as discrete habitats with relatively constant conditions (van der Kamp 1995). Although they are limited in terms of their dimensions and do not have homogeneous environments (Cantonati et al. 2006), they have been described as having mosaic structures that have the potential to support numerous microhabitats (Springer and Stevens 2008). Due to this relative uniformity, springs often support a very dense and diverse fauna (Lock and Williams 1981). Springs represent an environment that can be used to conduct extensive water quality analysis due to the interface between groundwater and surface water (Williams and Hogg 1988; Glazier and Gooch 1987). They also represent relationships between the organismal community and the environmental variables that influence these organisms (Wood et al. 2005; Smith et al. 2003). While several characteristics of springs influence the invertebrate community, temperature seems to be most important. Temperature effects can be seen in the collections made from the point of spring emergence to those points further downstream (Pflieger and Lipscomb 1974).

Spring studies have been conducted all over the world, examining faunal communities, physiochemical composition, and other influential abiotic factors. Von Fumetti et al. (2006) studied macrozoobenthic assemblages of twenty perennial springs in northwest Switzerland. The work examined which, and to what extent, abiotic factors influenced the macrozoobenthic assemblages

present. At each site, temperature, pH, conductivity, oxygen saturation, oxygen concentration, and discharge were measured, as well as substrate types. The results indicated that all 20 springs had similar physiochemical parameters, therefore having little influence on the macrozoobenthic composition. Leaf litter and discharge were the most important abiotic factors influencing the macrozoobenthic assemblages.

An investigation conducted in the United Kingdom focused on macroinvertebrate assemblage adaptability, comparing assemblages found in stable perennial springs to assemblages found in variable intermittent springs (Wood et al. 2005). The two regions studied in the English Peak District were White Peak and the River Lathkill catchment. Seventy-six taxa were recorded from White Peak while a total of 60 taxa were recorded from the River Lathkill catchment. A significant difference was seen between the intermittent and perennial assemblages when analyzing the springs on a regional scale. However, when combining both regions and analyzing all sites no significant difference was observed.

Ilmonen et al. (2009) examined 153 boreal springs in Finland, focusing on the key abiotic factors that influence macroinvertebrate assemblage variation. Over the course of this study, a total of 258 macroinvertebrate taxa were identified. Of these, dipteran larvae were the most dominant with 116 taxa recorded. Other groups included amphipods, isopods, ephemeropterans, odonates, plecopterans, heteropterans, trichopterans, and coleopterans. Little variation was seen between water chemistry and benthic macroinvertebrate

assemblages. The most influential factor on macroinvertebrate assemblages was the geographical gradient, due to the large scale at which this study was conducted.

Studies examining faunal composition, physiochemical parameters, and distribution of invertebrates have been conducted on various springs throughout the United States. Glazier and Gooch (1987) examined 15 Pennsylvania springs to determine distinct macroinvertebrate assemblage types. A total of 13 orders were recorded over the course of the study, resulting in the distinction of five assemblages. Each assemblage was based on the most distinct and representative taxa present. Over half the variation observed between these assemblages was due to pH and alkalinity, while the rest was influenced by other physiochemical parameters and substrate type.

Mattson et al. (1995) investigated benthic macroinvertebrate communities in spring-fed karst streams, focusing on the lower sections of the Suwannee River and Santa Fe River. Using Hester-Dendy multiplate samplers, it was determined that chironomids, ephemeropterans, and trichopterans were the dominant groups found on woody substrate. Sandy substrate was also analyzed from the lower reach of the Santa Fe River where it was determined that chironomids, oligochaetes, and molluscs were the dominant groups. Higher densities of benthic invertebrates were found in limestone shoal areas than were found in the sandy bottoms of these two rivers. The chironomids were the most dominant group seen throughout the whole study, both in the woody substrate and the sandy substrate.

According to Stevens and Meretsky (2008), Oregon has the highest density of named springs in the western United States, with a current inventory of 4,414. Anderson and Anderson (1995) studied five of these springs to determine habitat characteristics as well as aquatic insect composition. A total of 154 insect taxa was collected from the springs and a surrounding watershed. The dipterans were the most dominant group of insects, comprising almost 70% of identified taxa from the springs, as well as the watershed. Trichoptera were the second most dominant insect group as well as the most diverse of any other strictly aquatic order. The odonates were the only order with more species recorded from the springs than from the watershed. The differences seen between the spring and watershed insect composition was thought to be due to organic matter sources and processing rates.

Webb et al. (1995) examined seven Illinois springs, two saline springs and five hard water springs, to determine biodiversity. Non-insect groups dominated all seven springs, with one exception, Salt Well Spring, which was dominated by the dipteran, *Brachydeutera argentat*. Saline Spring had 34 taxa recorded and was dominated by the amphipods *Gammarus minus* and *G. pseudolimneaus* in terms of abundance. In terms of diversity, 12 taxa of oligochaetes were recorded from Saline Spring. For the remaining five springs, they were each dominated in abundance by amphipods and turbellarians, and dominated by oligochaetes in terms of diversity. A possible explanation for the low diversity of benthic macroinvertebrates in Salt Well Spring is thought to be due to the high chloride levels and the high dissolved solids. In many spring

studies, chironomids are the most dominant and diverse group in terms of diversity. However, in this study, oligochaetes were the most diverse group present.

Spring densities seen throughout the United States vary. According to Stevens and Meretsky (2008), the highest density of springs is found in and around the Rocky Mountains and the Intermountain West, while the lowest densities are seen within the Great Plains. Of the 17 western states Stevens and Meretsky (2008) discuss, Oklahoma has the third lowest density of named springs seen at 0.0006 springs per square kilometer ( $N/km^2$ ), just above North Dakota (0.0002  $N/km^2$ ), and Kansas (0.0001  $N/km^2$ ).

A study conducted in 1981 and 1982 for the Oklahoma Water Resources Research Institute (Matthews et al. 1983), examined 50 springs located throughout the state. The purpose was to determine the spring's community composition and indicate whether the organisms found would be helpful as biological indicators of groundwater quality. A total of 159 invertebrate taxa were reported, which included isopods, amphipods, gastropods, and various insect orders. It was determined that the use of invertebrates found in the springs as a biological monitoring tool would be impractical. This was due to the low similarity seen between the springs throughout the study.

Buckhorn Spring, located in the Arbuckle Mountains, was studied over a 17-month period, focusing on fluctuations of certain invertebrate populations and community interactions (Varza and Covich 1995). Various macroinvertebrates were collected over the course of the study, including

amphipods, gastropods, planarians, and crayfish. Although each of the above macroinvertebrates was seen throughout the study, the abundance varied. This variation was thought to be due mostly to food abundance and predation by crayfish.

Gaskin and Bass (2000) sampled seven Oklahoma springs to make interspring faunal comparisons and establish any patterns that might be occurring along a northwest to southeast transect across the state. During a three-month period, a total of 54 species were collected. Seventy percent of the taxa recorded were collected from only one site, suggesting that an exclusive spring fauna is not present. A direct correlation was seen between the numbers of organisms collected to the amount of microhabitat variation. No one species was collected from every spring, but a number of species were seen in over half of the springs, such as *Limnodrilus*, *Argia*, *Limnoporus*, and *Microvelia*. Other taxa collected included turbellarians, nematodes, gastropods, isopods, amphipods, decapods, and various insect orders.

A three-spring system located in Roman Nose State Park was sampled bi-monthly during 2002 to determine macroinvertebrate community structure (Rudisill and Bass 2005). Over the course of the study, a total of 21,268 individuals, representing 64 taxa, was collected. Of the 64 taxa collected, only 15 were recorded during all six collections times. Insects were the dominant invertebrates found in all three springs, with 15,111 individuals collected, representing 71%. Chironomidae represented the most dominant insect taxa seen. Of the three springs, Little Spring had the highest number of individuals



and taxa. Middle Spring was the second most populated, and Big Spring the least. Other macroinvertebrates collected included nematodes, gastropods, amphipods, decapods, and collembolans.

Bass (2000) conducted a limited one-time survey on two springs in the Pontotoc Ridge Nature Preserve to determine macroinvertebrate species composition and water quality. A total of 39 taxa was collected from both springs with the dominant macroinvertebrate groups present being the insects, particularly the dipterans. Other taxa observed were platyhelminthes, nematodes, oligochaetes, nematomorphans, gastropods, crustaceans, and hydrocarinans.

Other Oklahoma spring studies have been conducted throughout the state, many examining the same springs over time. This current survey involves two springs previously studied by Bass (2000) and an adjacent spring, from January 2011 to January 2012. Two of the three springs are located in the Pontotoc Ridge Nature Preserve (PRNP), the other located on private property adjacent to the Preserve.

The Pontotoc Ridge Nature Preserve is located in southeastern Pontotoc County, Oklahoma near the town of Ada (Bass 2000) (Figures 1 & 2). It consists of 2,900 acres of assorted cross-timber and prairie vegetation, and limestone outcrops are quite common. Several springs and seeps are also located in the preserve, each draining from the Arbuckle-Simpson Aquifer (Jona A. Tucker, pers. comm.). The three study springs, Cave Spring, Smith Spring, and Canyon Spring, are all classified as rheocrene springs; flowing springs that emerge into

one or more stream channels (Springer and Stevens 2008). Cave Spring is also classified as a true cave spring, which is a spring whose emergence is entirely within a cave environment and is not directly connected to surface flow (Springer and Stevens 2008).

It is clear that more research examining spring communities is needed to obtain a better knowledge of Oklahoma spring systems in general, and in this case, the Pontotoc Ridge Nature Preserve. Since the initial survey in 1995 (Bass 2000), additional springs have been located on adjacent property surrounding the PRNP. With the new knowledge of more springs in the area, a-year long study was conducted to gather additional information. This study was conducted to 1) determine the macroinvertebrate community composition of the springs, 2) compare macroinvertebrate community composition between the springs, 3) compare composition these communities to previously collected data on these and other springs, and 4) determine the water quality of the Pontotoc Ridge springs.

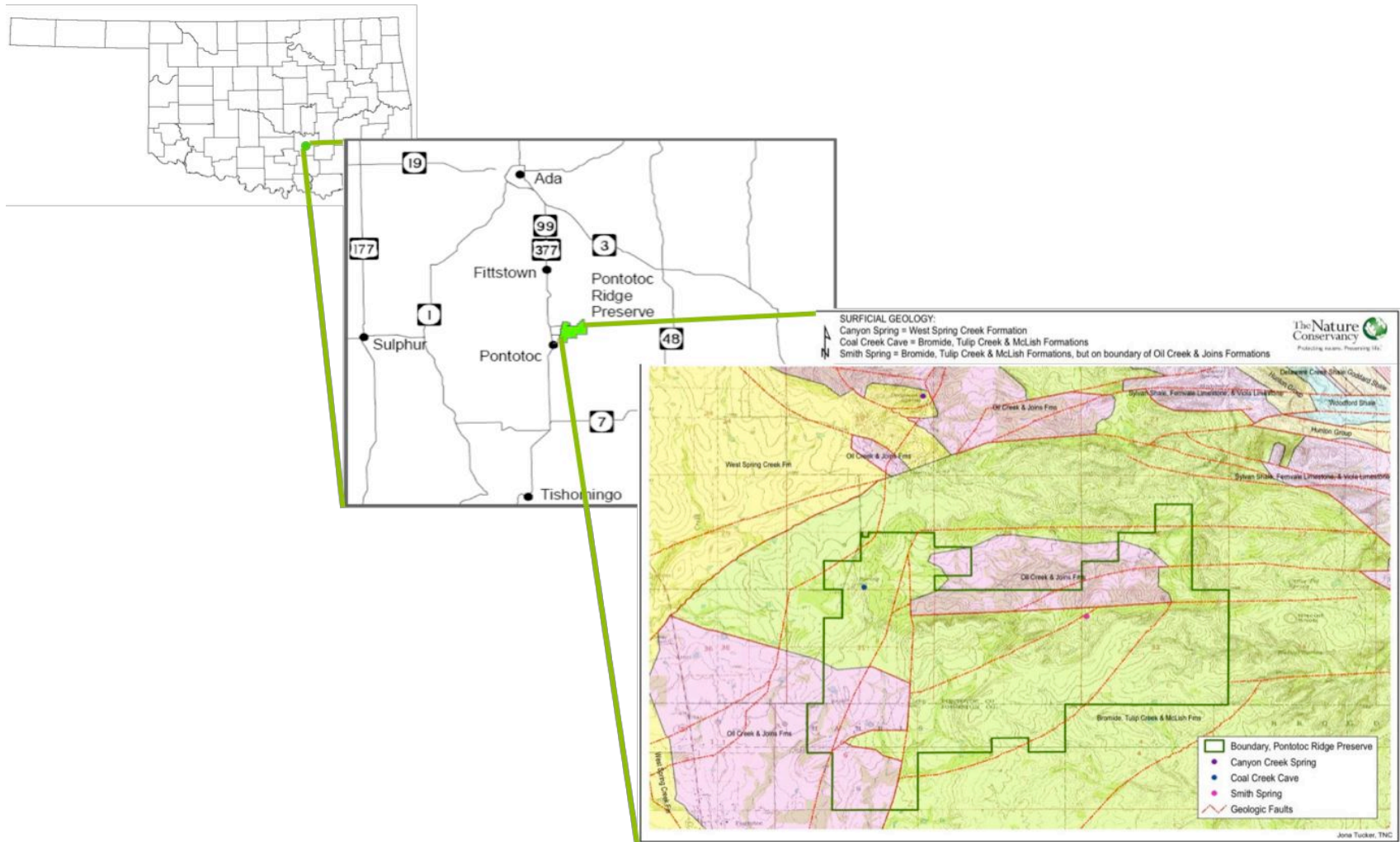


Figure 1. Pontotoc Ridge Nature Preserve, OK

## MATERIALS AND METHODS

Aquatic macroinvertebrates were collected and physiochemical conditions were measured on a seasonal basis from January 2011 to January 2012 (January 2011, April 2011, July 2011, October 2011, and January 2012). Macroinvertebrate samples were collected from the upstream site (three samples) near the emergence point and from a downstream site (three samples), roughly 10m away from the emergence point.

Physiochemical conditions were measured from each spring for each collection period. Temperature, dissolved oxygen (D.O.) concentration, and pH were measured at both sites per spring, while the alkalinity was measured only at the emergence point. A water sample, taken from the emergence point, was used to determine turbidity, conductivity, and concentrations of ammonia, nitrites, nitrates, and orthophosphates.

Water temperature and D.O. were measured in the field using a YSI meter. Alkalinity was determined by titration with 0.02N sulfuric acid to the phenolphthalein end point and pH was measured using a Hanna pH meter. Specific conductivity was measured using a Hanna Conductivity/TDS meter. Turbidity was measured using a Bausch & Lomb Spectrometer 20. Ammonia, nitrate, nitrite, and orthophosphate concentrations were analyzed using a DR 2800 Hach meter (Hach 2005). Percent oxygen saturation and carbon dioxide concentration were calculated using Rawson's Nomogram (Welch 1948) and Moore's Nomogram (Biotope: Specialist Veterinary Consultancy 2010).

Quantitative macroinvertebrate collections were conducted with a Surber net (Welch 1948). Six total samples, three near the emergence point and three at a downstream site, were collected from each spring during each season. The base of the net was placed on the bottom substrate of the spring and a 1ft<sup>2</sup> area within the base was disturbed allowing the flowing water to carry the sample into the net. Samples were then washed in a number 60 (0.250mm) U.S. standard sieve bucket, then transferred to a jar and preserved with a mixture of 10% formalin and Rose Bengal dye. The samples were washed in a number 60 (0.250mm) U.S. standard soil sieve, the macroinvertebrates were extracted, then stored in 70% ethanol until identified and counted.

Qualitative collections were conducted in the event the Surber net missed taxa within the quantitative collections. A dip net was used to sweep the submerged, floating, and hanging vegetation. Organisms found within the dip net were placed in vials containing 70% ethanol and transported back to the laboratory to be identified and counted. Identification of macroinvertebrates was determined using keys by Merritt et al. (2008), Pennak (1989), Epler (1995), and Wiederholm (1983). Upon completion, a synoptic collection was deposited in the University of Central Oklahoma Natural History Museum Invertebrate Collection.

Sorenson's index (Chao et al. 2006) of similarity was used to make comparisons between lower and upper collection sites of each spring, lower collection sites among the springs, upper collection sites among the springs, combined collection sites among springs for each of the collection periods, and combined collections for each spring. Comparisons were also made between the

previous study conducted by Bass (2000) on Cave Spring and Smith Spring to the current study. Comparisons were between the lower collection sites among each spring pair, upper collection sites among each spring pair, combined collection sites among each spring pair, and combined collections between each spring pair. The following formula was used to calculate the similarity indices:

$$S = \frac{2C}{A+B}$$

KEY:

A = the number of taxa at site A

B = the number of taxa at site B

C = the number of taxa common to both sites

S = the similarity index

Shannon's diversity index (Gotelli and Ellison 2004) was calculated for each sample collected, as well as for the lower collection sites, upper collection sites, and combined collection sites for each spring for each collection period.

Multi-response Permutation Procedures (MRPP) was used to compare the species composition between the head and downstream areas and species composition between each spring. Calculations were conducted using the program 'R' (R Core Team 2013). Rarefaction curves were also generated to evaluate species richness between the head and downstream as well as richness seen between each spring (Gotelli & Entsminger 2000).

## RESULTS

### **Physiochemical Conditions**

Water temperatures were fairly constant for two of the three springs sampled in the study, ranging from 17.2°C to 20.4°C (Appendix 1A). Smith and Canyon springs each had little variation between the lower and upper measurements, with one exception seen in Cave Spring. Cave Spring's temperature measurements varied largely, with readings from 13.5°C to 19.5°C. Six of the 26 measurements had temperatures that increased at the lower sites, from as little as 0.2°C to as much as 2.5°C. The lowest temperature readings were observed in Smith Spring in the coolest month of the study (January 2011 and 2012). The highest temperature reading, near 19°C, was the average observed temperature throughout Canyon Spring during the course of the study. Canyon and Smith springs had an average of 19.1°C and 17.9°C respectively, during the study period.

The dissolved oxygen content ranged from a minimum of 1.4 mg/L at Canyon Spring in October to a maximum of 7.6 mg/L at Cave Spring in January 2012 (Appendix 1B). The D.O. concentrations were always higher at the downstream sites; with the exception of the January 2011 readings from Cave Spring measured 6.1 mg/L.

The percent dissolved oxygen saturation ranged from 26% at Cave Spring (upstream) during April 2011 to 75% at Cave Spring (downstream) during January 2012 (Appendix 1C). Saturation always increased at the downstream sites, with two exceptions, one in Cave Spring during January 2011 and one in Smith Spring during January 2011 in which both readings were the same, at 57%.

Free carbon dioxide values varied throughout the springs, ranging from <10mg/L in Cave Spring during January 2011 and 2012 and in Smith Spring during January 2012 to 38 mg/L in Canyon Spring during July 2011 (Appendix 1D). Three of the 13 values were under measuring range, values given as <10 mg/L. During the collecting months of July and October 2011, Cave Spring was dry, so no measurements were taken. Cave Spring, having only three readings, and two of which were recorded as being <10 mg/L, varied compared to the third reading, being 37 mg/L. Smith and Canyon Spring were both consistent in CO<sub>2</sub> concentrations in January 2012 when both readings dropped drastically.

The pH varied minimally throughout the collection period (Appendix 1E). The lowest pH reading (7.1) was recorded in Canyon Spring during April 2011, whereas the highest reading (8.0) was found in Cave Spring during January 2012. Readings were typically lower at the head and higher downstream, with the exceptions of Cave Spring having the same reading during April 2011 and Smith Spring having the same reading during April and July 2011.

Alkalinity values ranged from a low of 248 mg/L to a high of 334 mg/L (Appendix 1F). Smith Spring had the lowest reading as well as the lowest overall alkalinity content with an average of 279 mg/L, Cave Spring had an average of 307 mg/L and Canyon Spring had the highest reading as well as the highest overall average at 322.2 mg/L.

Turbidity readings varied little. All 13 readings were <0.02 JTU, with four of the 13 readings recorded as zero JTU (Appendix 1G). The lowest readings were seen



in Cave Spring, during January and April 2011, while Canyon Spring had readings almost always near 100%T, a turbidity value of zero.

Conductivity readings varied greatly, ranging from 328  $\mu\text{mhos/cm}$  in Smith Spring during January 2012 to 906  $\mu\text{mhos/cm}$  in Canyon Spring during July 2011 (Appendix 1H). Smith Spring had the two lowest readings as well as the lowest overall average at 393  $\mu\text{mhos/cm}$ . Cave Spring had a higher overall average of 452  $\mu\text{mhos/cm}$ , and Canyon Spring had the highest overall average of 697.4  $\mu\text{mhos/cm}$ , as well as the highest overall reading.

The ammonia readings varied little, ranging from 0.093 mg/L in Canyon Spring during January 2011 to 0.177 in Smith Spring during January 2012 (Appendix 1I). Cave Spring had the lowest overall average at 0.128 mg/L. Smith Spring and Canyon Spring had an average differing by just 0.001 mg/L, 0.137 mg/L (Smith Spring) and 0.136 mg/L (Canyon). Although a low range test was used, 12 of the 13 nitrite readings were under measuring range, while one reading was recorded as a negative number (Appendix 1J).

Nitrate readings ranged from a low value of 0.252 mg/L in Cave Spring during January 2011 to a high of 0.779 mg/L in Smith Spring during January 2012 (Appendix 1K). Although a reading of 0.252 mg/L was the lowest reading recorded, one sample from Smith Spring during January 2011 was under measuring range.

Orthophosphates varied greatly throughout the collection period. Of the thirteen readings, four were under measuring range and three were recorded as negative numbers (Appendix 1L). Of the remaining six values, Cave Spring had the

lowest value at 0.109 mg/L during January 2011 and Smith Spring had the highest value of 0.204 mg/L during October 2011

### **Macroinvertebrates**

Over the course of this study a total 127,048 individuals, representing 114 taxa, were collected (Table 1). Eight of the 114 taxa were collected through qualitative sampling only. Seven taxa were collected at every site during every collecting period. Twenty-four taxa were collected using both collecting techniques.

Non-hexapods were the most abundant macroinvertebrate group collected throughout the study with a total of 87,675 individuals (Table 2), comprising 69% of macroinvertebrates collected throughout the study (Table 3). Only 21 taxa (19.13%) comprised non-hexapod macroinvertebrates collected both quantitatively and qualitatively (Tables 4 & 5). The amphipod, *Hyaella azteca complex*, was the most numerous non-hexapod (Tables 1 & 2) with total of 76,529 individuals, representing 60.24% of all individuals (Tables 1 & 6, Figure 2) and 87.29% of all non-hexapod individuals collected over the course of the study (Tables 1 & 2, Figure 3). The remaining non-hexapod taxa comprised only 12.7% of the total non-hexapod individuals found (Table 2).

Although non-hexapods represented 69% of all individuals, hexapods represent 80.86% of taxa diversity (Table 7). Ninety-three taxa of hexapods were collected in quantitative and qualitative samples (Table 5). Eight of the 93 taxa were found only in the qualitative samples.

Table 1. Percent composition of total individuals for each taxon found in qualitative collections.

Taxon	Cave		Smtih		Canyon		All	
	No.	Percent	No.	Percent	No.	Percent	No.	Percent
<b>Turbellaria</b>								
Planariidae								
<i>Dugesia</i> sp.	26	0.671	1432	3.687	1718	2.037	3176	2.500
<b>Nematoda</b>	136	3.511	1387	3.571	45	0.053	1568	1.234
<b>Oligochaeta</b>								
Lumbriculidae								
<i>Lumbriculus</i> sp.	13	0.336	265	0.682	83	0.098	361	0.284
Tubificidae								
<i>Limnodrilus</i> sp.	179	4.622	2148	5.531	235	0.279	2562	2.017
<b>Gastropoda</b>								
Planorbidae								
<i>Gyraulus</i> sp.	0	0	0	0	0	0	0	0
Physidae								
<i>Physa</i> sp.	99	2.556	9	0.023	0	0.000	108	0.085
<b>Pelecypoda</b>								
Unknown Pelecypoda 1	1	0.026	639	1.645	11	0.013	651	0.512
Sphaeriidae	0	0	25	0.064	4	0.005	29	0.023
<i>Sphaerium</i> sp.	0	0	114	0.294	20	0.024	134	0.105
<b>Copepoda</b>								
Cyclopoida	81	2.091	572	1.473	3	0.004	656	0.516
Ergasilidae								
<i>Ergasilus</i> sp.	0	0	1	0.003	0	0.000	1	0.001
Harpacticoidia	178	45.959	74	0.191	0	0.000	1854	1.459
<b>Isopoda</b>								
Unknown Isopoda 1	0	0	2	0.005	0	0.000	2	0.002
Asellidae								
<i>Caecidotea</i> sp.	2	0.052	13	0.033	10	0.012	25	0.020
<b>Amphipoda</b>								
<i>Hyallega azteca</i>	5	0.129	9	0.023	76515	90.726	76529	60.236
<b>Decopoda</b>								
Unknown Decopoda 1	7	0.181	0	0	0	0	7	0
Astacidae	1	0.026	0	0	0	0	1	0
Cambaridae	0	0	1	0.003	0	0.000	1	0.001
Cambarinae	8	0.207	0	0	0	0	8	0
<b>Acarina</b>								
Hydrachnidae								
<i>Sperchonopsis verrucosa</i>	0	0	1	0.003	0	0.000	1	0.001

Table 1, continued.

<b>Collembola</b>								
<i>Corynothrix</i> sp.	0	0	14	0.036	0	0.000	14	0.011
Isotomidae	0	0	2	0.005	0	0.000	2	0.002
<i>Spinactalets</i> sp.	0	0	8	0.021	0	0.000	8	0.006
<b>Ephemeroptera</b>								
Unknown								
Ephemeroptera 1	1	0.026	277	0.713	2	0.002	280	0.220
Baetidae	0	0	0	0	3	0	3	0
<i>Baetis</i> sp.	8	0.207	272	0.700	30	0.036	310	0.244
Heptageniidae								
<i>Stenonema femoratum</i>	0	0	0	0	1	0	1	0
Leptohiphidae	0	0	553	1.424	86	0.102	639	0.503
<i>Tricorythodes</i> sp.	0	0	10	0.026	28	0.033	38	0.030
Leptophlebiidae								
<i>Paraleptophlebia</i> sp.	21	0.542	0	0	0	0	21	0
<b>Odonata</b>								
Unknown Odonata 1	0	0	90	0.232	13	0.015	103	0.081
Anisoptera	0	0	1	0.003	0	0.000	1	0.001
Aeshnidae								
<i>Tricanthagyna</i> sp.	0	0	0	0	0	0	0	0
Cordulegastridae								
<i>Cordulegaster</i> sp.	0	0	1	0.003	0	0.000	1	0.001
Corduliidae								
<i>Neurocordulia</i> sp.	0	0	0	0	2	0	2	0
Zygoptera	0	0	127	0.327	0	0.000	127	0.100
Calopterygidae								
<i>Calopteryx</i> sp.	1	0.026	3	0.008	0	0.000	4	0.003
<i>Hetaerina</i> sp.	0	0	4	0.010	2	0.002	6	0.005
Coenagrionidae	0	0	2	0.005	0	0.000	2	0.002
<i>Argia</i> sp.	0	0	743	1.913	176	0.209	919	0.723
Lestidae								
<i>Archilestes</i> sp.	0	0	1	0.003	0	0.000	1	0.001
<i>Lestes</i> sp.	0	0	6	0.015	0	0.000	6	0.005
<b>Plecoptera</b>								
Pteronarcyidae								
<i>Pteronarcella</i> sp.	2	0.052	0	0	0	0	2	0
<i>Pteronarcys</i> sp.	1	0.026	0	0	0	0	1	0

Table 1. Percent composition of total individuals for each taxon found in qualitative collections.

<b>Hemiptera</b>								
Corixidae	0	0	1	0.003	0	0.000	1	0.001
<i>Glaenocorisa</i> sp.	0	0	1	0.003	0	0.000	1	0.001
<i>Graptocorixa</i> sp.	0	0	1	0.003	0	0.000	1	0.001
Gerridae								
<i>Trepobates</i> sp.	0	0	3	0.008	0	0.000	3	0.002
Veliidae								
<i>Rhagovelia</i> sp.	0	0	0	0	1	0	1	0
<b>Tricoptera</b>								
Helicopsychidae								
<i>Helicopsyche</i> sp.	2	0.052	701	1.805	3074	3.645	3777	2.973
Hydropsychidae	53	1.368	343	0.883	50	0.059	446	0.351
Hydroptilidae								
<i>Hydroptila</i> sp.	0	0	74	0.191	0	0.000	74	0.058
<i>Ochrotrichia</i> sp.	374	9.657	127	0.327	40	0.047	541	0.426
Leptoceridae								
<i>Nectopsyche</i> sp.	0	0	0	0	3	0	3	0
Polycentropodidae								
<i>Polycentropes</i> sp.	1	0.026	4	0.010	0	0.000	5	0.004
Philopotamidae								
<i>Dolophilodes</i> sp.	0	0	0	0	1	0	1	0
<b>Coleoptera</b>								
Dytiscidae	12	0.310	0	0	1	0	13	0
<i>Agabates</i> sp.	0	0	1	0.003	1	0.001	2	0.002
<i>Agabus</i> sp.	7	0.181	7	0.018	0	0.000	14	0.011
<i>Hydrotrupes</i> sp.	0	0	1	0.003	0	0.000	1	0.001
<i>Hygrotus</i> sp.	18	0.465	4	0.010	0	0.000	22	0.017
Laccophilinae	0	0	1	0.003	0	0.000	1	0.001
Dryopidae								
<i>Helicus</i> sp.	0	0	0	0	0	0	0	0
Elmidae								
<i>Ordobrevia</i> sp.	0	0	438	1.128	3	0.004	441	0.347
Haliplidae								
<i>Peltodytes</i> sp.	0	0	14	0.036	0	0.000	14	0.011
Hydrophilidae								
<i>Tropisternus</i> sp.	0	0	3	0.008	0	0.000	3	0.002

Table 1. Percent composition of total individuals for each taxon found in qualitative collections.

<b>Diptera</b>								
Brachycera								
Athericidae								
<i>Atherix</i> sp.	0	0	15	0.039	3	0.004	18	0.014
Empididae	0	0	6	0.015	0	0.000	6	0.005
<i>Hemerodromia</i> sp.	0	0	70	0.180	0	0.000	70	0.055
Stratiomyidae								
<i>Caloparyphus</i> sp.	0	0	3	0.008	18	0.021	21	0.017
<i>Euparyphus</i> sp.	0	0	0	0	9	0	9	0
<i>Myxosargus</i> sp.	0	0	2	0.005	0	0.000	2	0.002
Tabanidae								
<i>Silvus</i> sp.	0	0	0	0	1	0	1	0
<i>Chrysops</i> sp.	0	0	2	0.005	0	0.000	2	0.002
<i>Tabanus</i> sp.	0	0	0	0	2	0	2	0
Nematocera								
Ceratopogonidae								
<i>Culicoides</i> sp.	4	0.103	168	0.433	189	0.224	361	0.284
<i>Probezzia</i> sp.	17	0.439	272	0.700	4	0.005	293	0.231
<i>Dasyhelea</i> sp.	0	0	1	0.003	0	0.000	1	0.001
Chaoboridae								
<i>Eucorethra</i> sp.	0	0	1	0.003	0	0.000	1	0.001
Chironomidae								
<i>Ablabesmyia</i> sp.	1	0.026	0	0	0	0	1	0
<i>Cardiocladius</i> sp.	0	0	0	0	1	0	1	0
<i>Chironomus</i> sp.	23	0.594	3	0.008	0	0.000	26	0.020
<i>Corynoneura</i> sp.	695	17.945	532	1.370	504	0.598	1731	1.362
<i>Cricotopus</i> sp.	2	0.052	3	0.008	0	0.000	5	0.004
<i>Cryptochironomus</i> sp.	0	0	46	0.118	19	0.023	65	0.051
<i>Dicrotendipes</i> sp.	74	1.911	11	0.028	30	0.036	115	0.091
<i>Einfeldia</i> sp.	4	0.103	4	0.010	0	0.000	8	0.006
<i>Eukiefferiella</i> sp.	0	0	3	0.008	95	0.113	98	0.077
<i>Heleniella</i> sp.	0	0	215	0.554	0	0.000	215	0.169
<i>Larsia</i> sp.	61	1.575	498	1.282	12	0.014	571	0.449
<i>Microtendipes</i> sp.	47	1.214	6	0.015	0	0.000	53	0.042
<i>Orthocladius</i> sp.	9	0.232	101	0.260	349	0.414	459	0.361
<i>Parametrioctenus</i> sp.	26	0.671	1335	3.437	76	0.090	1437	1.131
<i>Paraspectra</i> sp.	8	0.207	0	0	0	0	8	0
<i>Paratanyarsus</i> sp.	5	0.129	58	0.149	8	0.009	71	0.056
<i>Paratendipes</i> sp.	0	0	279	0.718	4	0.005	283	0.223
<i>Paratrichocladius</i> sp.	0	0	2	0.005	88	0.104	90	0.071

Table 1. Percent composition of total individuals for each taxon found in qualitative collections.

<i>Phaenopsectra</i> sp.	28	0.723	91	0.234	0	0.000	119	0.094
<i>Polypedilum</i> sp.	0	0	12	0.031	2	0.002	14	0.011
<i>Procladius</i> sp.	0	0	4	0.010	0	0.000	4	0.003
<i>Rheotanytarsus</i> sp.	0	0	5	0.013	28	0.033	33	0.026
<i>Stenochironomus</i> sp.	0	0	21	0.054	0	0.000	21	0.017
<i>Sublettea</i> sp.	0	0	0	0	1	0	1	0
<i>Tanytarsus</i> sp.	25	0.645	24496	63.074	21	0.025	24542	19.317
<i>Thienemannimyia</i> sp.	0	0	13	0.033	6	0.007	19	0.015
<i>Tvetenia</i> sp.	5	0.129	1	0.003	702	0.832	708	0.557
Dixidae								
<i>Dixa</i> sp.	0	0	4	0.010	6	0.007	10	0.008
Psychodidae								
<i>Pericoma</i> sp.	0	0	7	0.018	0	0.000	7	0.006
Tanderidae								
<i>Protoplasa fitchii</i>	0	0	3	0.008	0	0.000	3	0.002
Tipulidae								
<i>Tipula</i> sp.	0	0	13	0.033	0	0.000	13	0.010
Total	3873		38836		84339		127048	

Table 2. Total percent composition of non-hexapod individuals for the collection period.

Taxon	Cave Spring		Smith Spring		Canyon Spring		All Springs	
	No.	Percent	No.	Percent	No.	Percent	No.	Percent
Amphipoda	5	0.21	9	0.13	76515	97.29	76529	87.29
Copepoda	1861	79.60	647	9.67	3	0.00	2511	2.86
Decapoda	16	0.68	1	0.01	0	0.00	17	0.02
Gastropoda	99	4.23	9	0.13	0	0.00	108	0.12
Isopoda	2	0.09	15	0.22	10	0.01	27	0.03
Turbellaria	26	1.11	1432	21.40	1718	2.18	3176	3.62
Pelecypoda	1	0.04	778	11.64	35	0.04	814	0.93
Nematoda	136	5.82	1387	20.72	45	0.06	1568	1.79
Oligochaeta	192	8.21	2413	36.05	318	0.40	2923	3.33
Acarina	0	0.00	1	0.01	0	0.00	1	0.00
Total	2338		6692		78644		87674	

Table 3. Total percent hexapod and non-hexapod individuals for the collection period.

Group	Individual Number	Percent
Hexapods	39,374	31
Non-hexapods	87,674	69
Total	127,048	

Table 4. Total percent composition of non-hexapod taxa for the collection period.

Taxon	Cave Spring		Smith Spring		Canyon Spring		All Springs	
	No.	Percent	No.	Percent	No.	Percent	No.	Percent
Amphipoda	1	7.69	1	5.88	1	8.33	1	4.55
Copepoda	2	15.38	3	17.65	1	8.33	3	13.64
Decapoda	4	30.77	1	5.88	1	8.33	5	22.73
Gastropoda	1	7.69	1	5.88	1	8.33	2	9.09
Isopoda	1	7.69	2	11.76	1	8.33	2	9.09
Turbellaria	0	0.00	1	5.88	1	8.33	1	4.55
Pelecypoda	1	7.69	4	23.53	3	25.00	4	18.18
Nematoda	1	7.69	1	5.88	1	8.33	1	4.55
Oligochaeta	2	15.38	2	11.76	2	16.67	2	9.09
Acarina	0	0.00	1	5.88	0	0.00	1	4.55
Total	13		17		12		22	



Table 5. Taxa found in quantitative and qualitative collections over the course of the study.

Taxon	Cave Spring Lower	Cave Spring Upper	Smith Spring Lower	Smith Spring Upper	Canyon Spring Lower	Canyon Spring Upper
<b>Turbellaria</b>						
<i>Dugesia</i> sp.			X	X	X	X
<b>Nematoda</b>	X	X	X	X	X	X
<b>Oligochaeta</b>						
<i>Limnodrilus</i> sp.	X	X	X	X	X	X
<i>Lumbriculus</i> sp.	X		X	X	X	X
<b>Gastropoda</b>						
<i>Gyraulus</i> sp.					X	
<i>Physa</i> sp.	X	X	X	X		
<b>Pelecypoda</b>						
Unknown Pelecypoda 1	X		X	X	X	X
Sphaeriidae			X	X	X	
<i>Sphaerium</i> sp.			X	X	X	
<b>Copepoda</b>						
Cyclopoida	X	X	X	X	X	X
<i>Ergasilus</i> sp.				X		
Harpacticoidia	X		X	X		
<b>Isopoda</b>						
Unknown Isopoda 1				X		
<i>Caecidotea</i> sp.		X	X	X		X
<b>Amphipoda</b>						
<i>Hyallela azteca</i>	X		X	X	X	X
<b>Decapoda</b>						
Unknown Decapoda 1		X			X	X
Astacidae		X				
<i>Astacus</i> sp.	X					
Cambaridae			X			
Cambarinae		X				
<b>Acarina</b>						
Hydrachnidae						
<i>Sperchonopsis verrucosa</i>			X			
<b>Collembola</b>						
<i>Corynothrix</i> sp.				X		
Isotomidae				X		
<i>Spinactalets</i> sp.				X		

Table 5, continued.

<b>Ephemeroptera</b>						
Unknown Ephemeroptera 1	X		X	X		
Baetidae					X	
<i>Baetis</i> sp.	X	X	X	X	X	
<i>Stenonema femoratum</i>					X	
Leptohyphidae			X	X	X	
<i>Tricorythodes</i> sp.			X		X	
<i>Paraleptophlebia</i> sp.	X	X				
<b>Odonata</b>						
Unknown Odonata 1			X	X	X	X
Anisoptera				X		
<i>Triacanthagyna</i> sp.			X			
<i>Cordulegaster</i> sp.			X			
<i>Neurocordulia</i> sp.					X	
Zygoptera				X		
<i>Calopteryx</i> sp.		X	X	X		
<i>Hetaerina</i> sp.			X	X	X	
Coenagrionidae				X		
<i>Argia</i> sp.			X	X	X	X
<i>Archilestes</i> sp.			X	X		
<i>Lestes</i> sp.			X			
<b>Plecoptera</b>						
<i>Pteronarcella</i> sp.	X					
<i>Pteronarcys</i> sp.	X					
<b>Hemiptera</b>						
Corixidae			X			
<i>Glaenocoris</i> sp.			X			
<i>Graptocorixa</i> sp.			X			
<i>Aquarius</i> sp.			X	X		X
<i>Trepobates</i> sp.			X		X	
<i>Buenoa</i> sp.			X			
<i>Rhagovelia</i> sp.		X	X		X	X
<b>Tricoptera</b>						
<i>Helicopsyche</i> sp.	X		X	X	X	X
Hydropsychidae	X		X	X	X	
<i>Hydroptila</i> sp.			X	X		
<i>Ochrotrichia</i> sp.	X	X	X	X	X	
<i>Nectopsyche</i> sp.					X	
<i>Polycentropes</i> sp.	X			X		
<i>Dolophilodes</i> sp.					X	

Table 5, continued.

<b>Coleoptera</b>						
<i>Agabates</i> sp.				X		
<i>Agabus</i> sp.	X	X	X	X		X
Dytiscidae	X	X				X
<i>Helicus</i> sp.					X	
<i>Hydrotropes</i> sp.				X		
<i>Hygrotus</i> sp.	X	X	X			
<i>Laccobius</i> sp.		X				
<i>Laccophilinae</i>			X			
<i>Ordobrevia</i> sp.			X	X	X	
<i>Peltodytes</i> sp.			X	X		
<i>Tropisternus</i> sp.			X	X		
<b>Diptera</b>						
<i>Atherix</i> sp.			X	X	X	
Empididae			X	X		
<i>Hemerodromia</i> sp.			X	X		
<i>Caloparyphus</i> sp.				X	X	X
<i>Euparyphus</i> sp.					X	X
<i>Myxosargus</i> sp.				X		
<i>Chrysops</i> sp.				X		
<i>Silvius</i> sp.						X
<i>Tabanus</i> sp.					X	X
<i>Culicoides</i> sp.	X		X	X	X	X
<i>Probezzia</i> sp.	X		X	X	X	X
<i>Dasyhelea</i> sp.			X			
<i>Eucoethra</i> sp.			X			
<i>Ablabesmyia</i> sp.	X					
<i>Cardiocladius</i> sp.					X	
<i>Chironomus</i> sp.	X	X	X	X		
<i>Corynoneura</i> sp.	X		X	X	X	X
<i>Cricotopus</i> sp.	X		X	X		
<i>Cryptochironomus</i> sp.			X	X	X	
<i>Dicrotendipes</i> sp.	X	X	X	X	X	
<i>Einfeldia</i> sp.		X	X	X		
<i>Eukiefferiella</i> sp.				X	X	X
<i>Heleniella</i> sp.			X	X		
<i>Larsia</i> sp.	X	X	X	X	X	X
<i>Microtendipes</i> sp.	X	X	X			

Table 5, continued.

<i>Orthocladus sp.</i>	X	X	X	X	X	X
<i>Parametriocnemus sp.</i>	X	X	X	X	X	X
<i>Paraspectra sp.</i>	X					
<i>Paratanytarsus sp.</i>	X	X	X	X	X	
<i>Paratendipes sp.</i>			X	X	X	X
<i>Paratrichocladius sp.</i>			X	X	X	X
<i>Phaenopsectra sp.</i>	X	X	X	X		
<i>Polypedilum sp.</i>			X	X	X	
<i>Procladius sp.</i>			X	X		
<i>Rheotanytarsus sp.</i>			X	X	X	
<i>Stenochironomus sp.</i>			X	X		
<i>Sublettea sp.</i>					X	
<i>Tanytarsus sp.</i>	X	X	X	X	X	X
<i>Thienemannimyia sp.</i>				X	X	X
<i>Tvetenia sp.</i>			X		X	
<i>Dixa sp.</i>			X	X	X	X
<i>Pericoma sp.</i>			X	X		
<i>Protoplasa fitchii</i>				X		
<i>Tipula sp.</i>			X	X		

Table 6. Percent individuals within each taxa represents for entire collection.

Number of Ind.	Taxa	Percent
3176	Turbellaria	2.500
1568	Nematoda	1.234
2923	Oligochaeta	2.301
108	Gastropoda	0.085
814	Pelecypoda	0.641
2511	Copepoda	1.976
27	Isopoda	0.021
76529	Amphipoda	60.236
17	Decapoda	0.013
1	Acarina	0.001
24	Collembola	0.019
1292	Ephemeroptera	1.017
511	Coleoptera	0.402
1172	Odonata	0.922
3	Plecoptera	0.002
7	Hemiptera	0.006
4847	Tricoptera	3.815
31518	Diptera	24.808

Table 7. Total number of hexapod and non-hexapod taxa for the collection period.

Group	Taxa Number	Percent
Hexapods	93	81
Non-hexapods	21	19
Total	114	

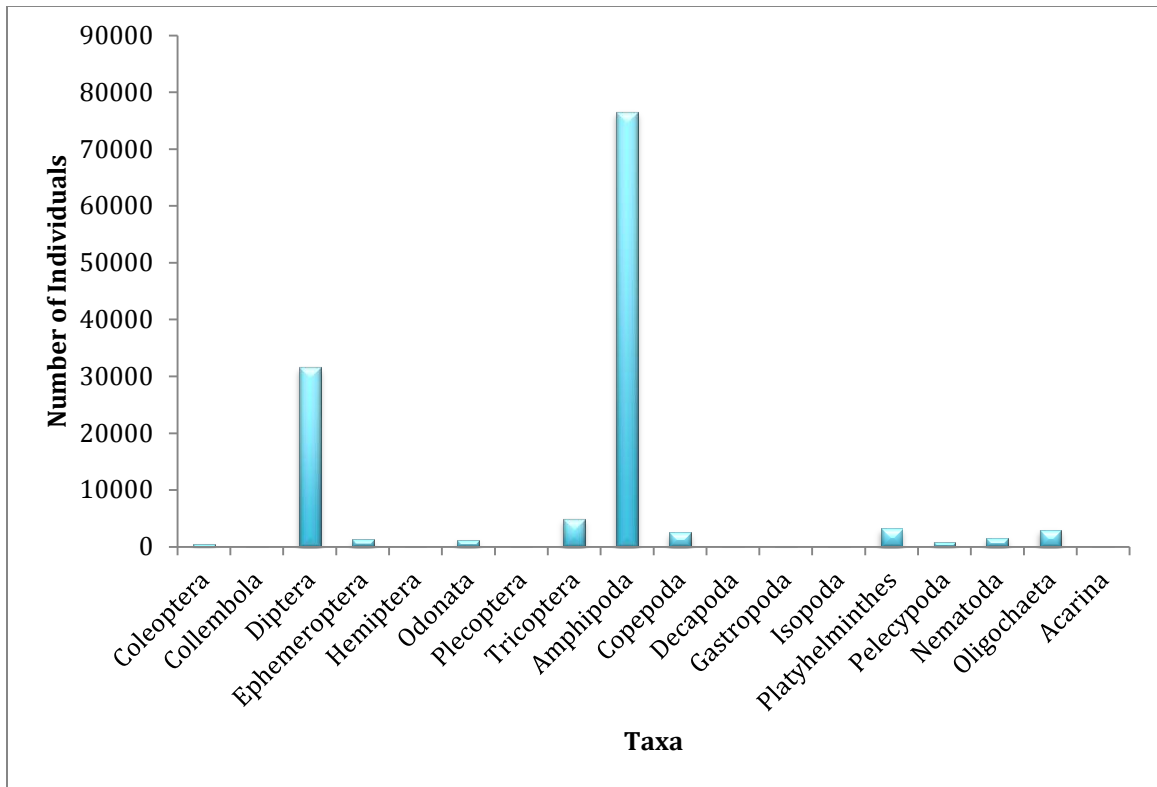


Figure 2. Total number of individuals for each taxa identified.

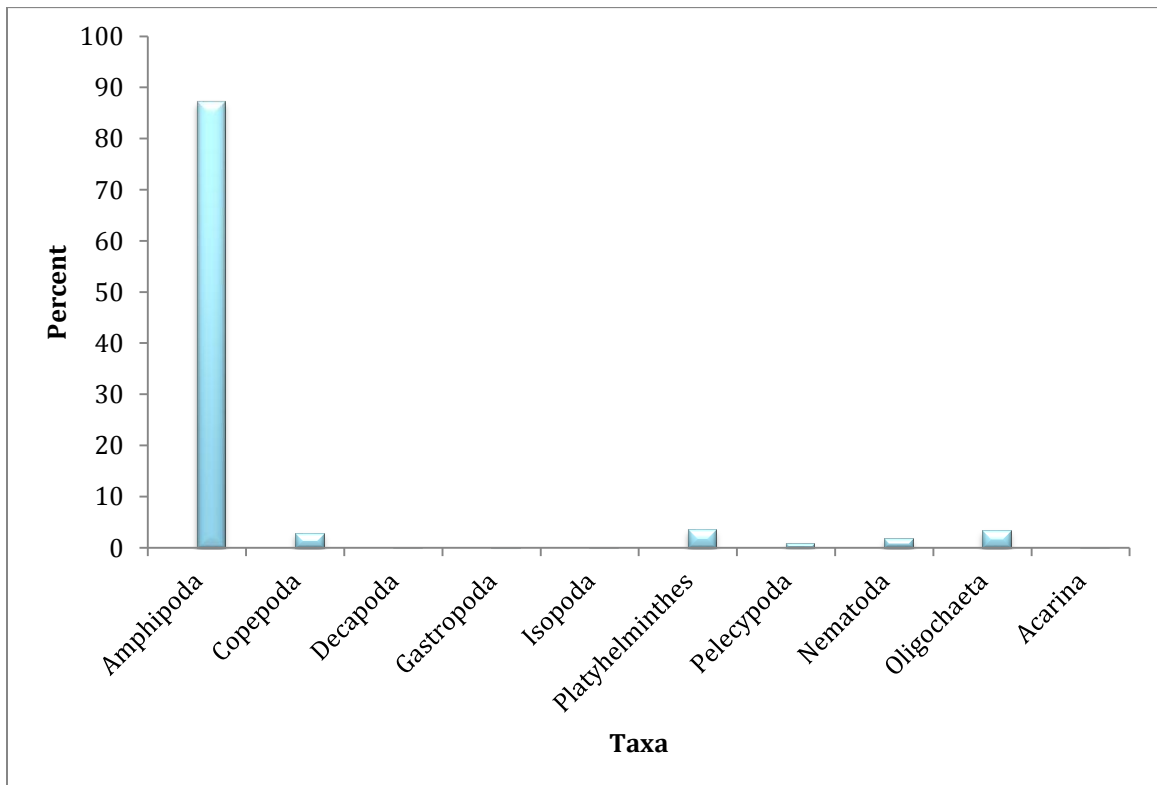


Figure 3. Percent of non-hexapod individuals.

Dipterans had the highest diversity of all hexapod taxa collected with a total of 44 taxa representing 47.31% (Table 8) of hexapods and 38.26% of all taxa collected (Table 9). The most dominant of the dipterans were the nematocerans, in particular, the Chironomidae. The dipterans represent 24.81% (31,518 individuals) of all individuals collected throughout the study (Table 6), but 80.05% of hexapod individuals collected (Table 10). Tricopterans were the second most numerous hexapod taxa seen, with a total of 4,847 individuals representing 12.31% of hexapods collected (Table 10). The remaining hexapod taxa collectively comprise only 7.65% of the hexapod individuals found (Table 10).

The amphipod *Hyaella azteca* complex was the most numerous organism found throughout the course of the study, almost exclusively from Canyon Spring. Of the 76,529 amphipod individuals found, 76,515 came from Canyon Spring (Table 2). Canyon Spring had the most individuals present at 84,339 (Figure 4) representing 66.38% of all individuals collected and a total of 58 taxa collected both quantitatively and qualitatively during the study (Figure 5).

Of the individuals found in Canyon Spring, 5,695 were hexapods. The most abundant hexapod order was the tricopterans with an individual count of 3,168 (55.62% of hexapods). The dipterans were the next most numerous order in Canyon Spring, having a total of 2,178 (38.24%) individuals (Table 10). All other hexapod orders collected in Canyon Spring (coleoptera, ephemeroptera, hemiptera, and odonata) constituted less than 7% of the remaining individuals (Table 10). The non-hexapod individuals found in Canyon Spring made up the majority of the individuals collected with a total of 78,644.

Table 8. Total percent composition of hexapod taxa for the collection period.

Taxon	Cave Spring		Smith Spring		Canyon Spring		All Springs	
	No.	Percent	No.	Percent	No.	Percent	No.	Percent
Coleoptera	4	12.90	8	10.67	4	8.70	11	11.83
Collembola	0	0.00	3	4.00	0	0.00	3	3.23
Diptera	16	51.61	37	49.33	25	54.35	44	47.31
Ephemeroptera	3	9.68	4	5.33	5	10.87	7	7.53
Hemiptera	1	3.23	7	9.33	3	6.52	7	7.53
Odonata	1	3.23	11	14.67	4	8.70	12	12.90
Plecoptera	2	6.45	0	0.00	0	0.00	2	2.15
Tricoptera	4	12.90	5	6.67	5	10.87	7	7.53
Total	31		75		46		93	

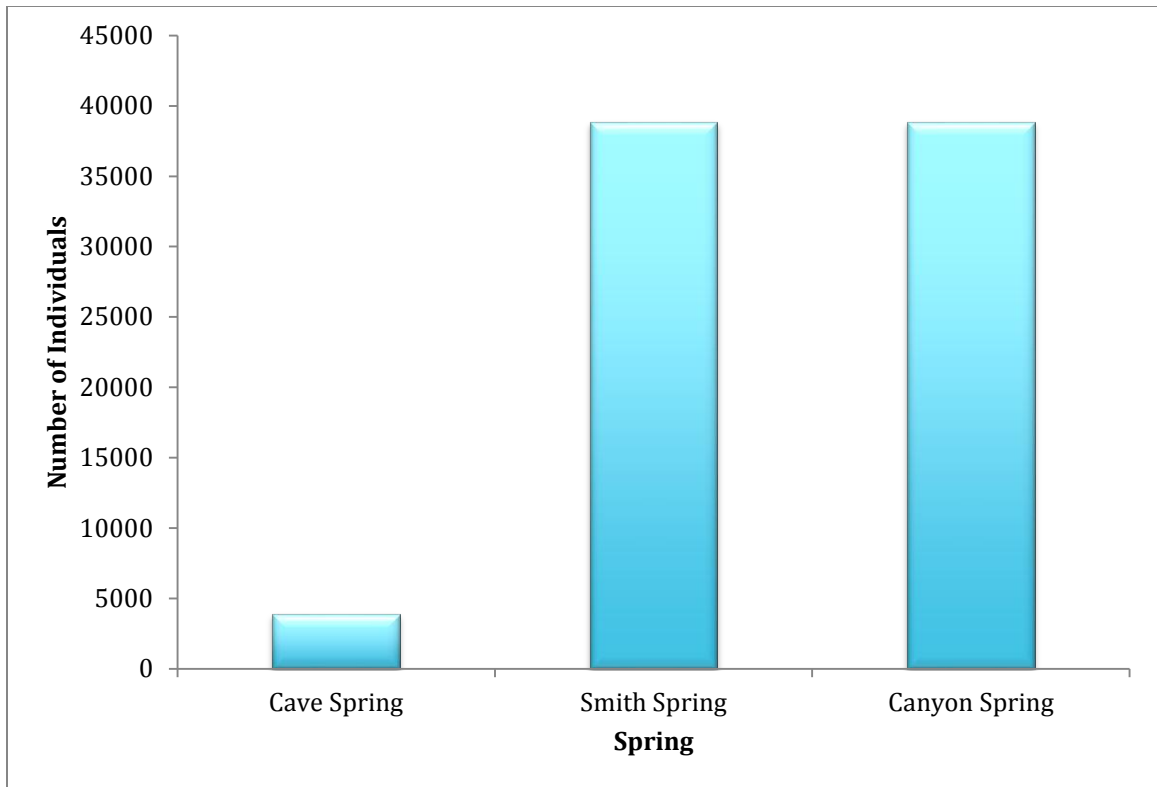
Table 9. Percent each taxa represents for entire collection.

Number of Ind.	Taxa	Percent
1	Turbellaria	0.870
1	Nematoda	0.870
2	Oligochaeta	1.739
2	Gastropoda	1.739
3	Pelecypoda	2.609
3	Copepoda	2.609
2	Isopoda	1.739
1	Amphipoda	0.870
5	Decapoda	4.348
1	Acarina	0.870
3	Collembola	2.609
7	Ephemeroptera	6.087
11	Coleoptera	9.565
12	Odonata	10.435
2	Plecoptera	1.739
7	Hemiptera	6.087
7	Tricoptera	6.087
44	Diptera	38.261

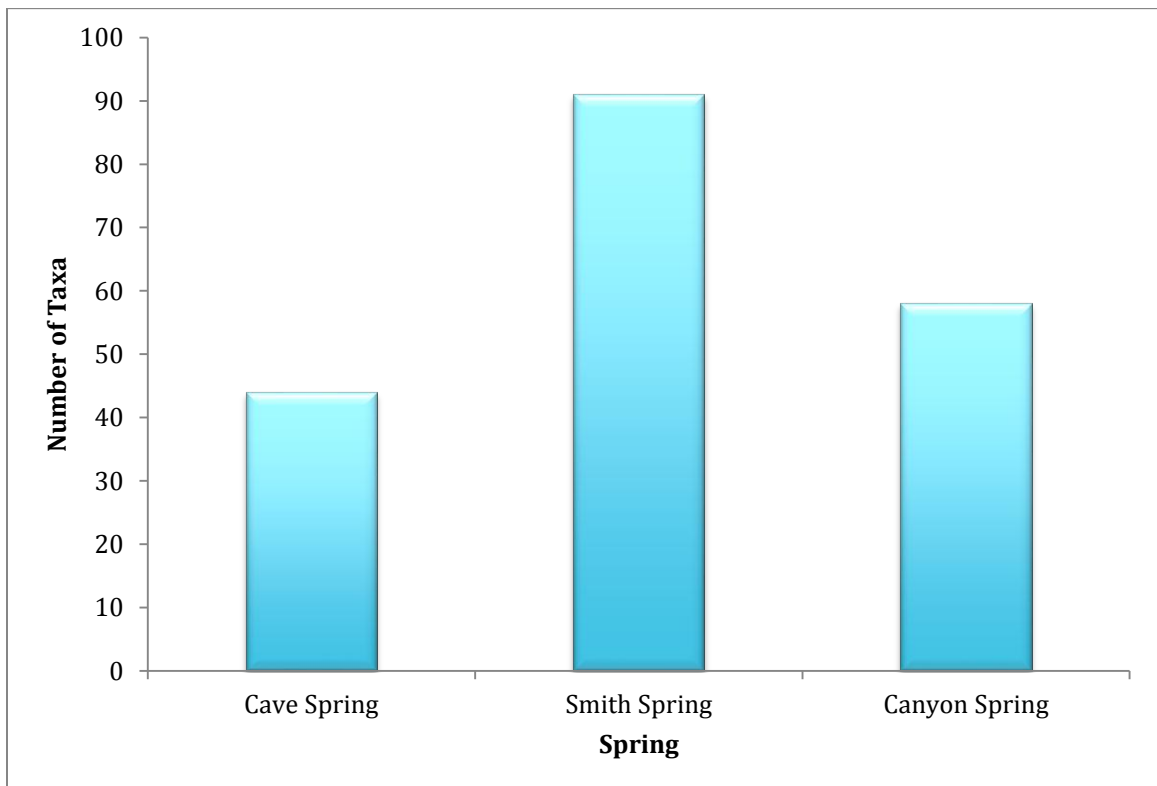


Table 10. Total percent composition of hexapod individuals for the collection period.

Taxon	Cave Spring		Smith Spring		Canyon Spring		All Springs	
	No.	Percent	No.	Percent	No.	Percent	No.	Percent
Coleoptera	37	2.41	469	1.46	5	0.09	511	1.30
Collembola	0	0.00	24	0.07	0	0.00	24	0.06
Diptera	1034	67.36	28306	88.06	2178	38.24	31518	80.05
Ephemeroptera	30	1.95	1112	3.46	150	2.63	1292	3.28
Hemiptera	0	0.00	6	0.02	1	0.02	7	0.02
Odonata	1	0.07	978	3.04	193	3.39	1172	2.98
Plecoptera	3	0.20	0	0.00	0	0.00	3	0.01
Tricoptera	430	28.01	1249	3.89	3168	55.62	4847	12.31
Total	1535		32144		5695		39374	



**Figure 4.** Total number of individuals at each spring over the course of the study.



**Figure 5.** Total number of taxa present at each spring over the course of the study.

Smith Spring had a total of 38,837 individuals (Figure 4), representing 30.57% of all individuals collected (Figure 2). Unlike Canyon Spring, where the non-hexapods were the most numerous, Smith Spring was dominated by hexapods with a total of 32,144 (82.77% of hexapods) individuals (Table 10). Smith Spring also had the greatest species richness with a total of 92 taxa collected both quantitatively and qualitatively (Figure 5). The most dominant order was the diptera, with a total of 37 taxa (Table 4) and 31,518 individuals (Table 10), representing 88.06% of hexapods (Table 10) and 72.88% of all individuals (Table 11) collected in Smith Spring.

The next most numerous hexapod group was the tricopterans, with a total of 1,249 individuals representing 3.89% of all individuals collected in Smith Spring (Table 10). The remaining hexapod groups (ephemeropterans, coleopterans, collembolans, hemipterans, and odonates) constituted only 8.05% of the remaining hexapod individuals found. The non-hexapod individuals only comprised 17.23% of the individuals collected in Smith Spring.

The oligochaetes were the most numerous non-hexapod group totaling 2,413 individuals, or 36.05% of non-hexapod individuals (Table 2) and 6.21% of all individuals collected from Smith Spring (Table 11). Nematodes (1,387 individuals) and platyhelminthes (1,432 individuals) each comprised around 20% of the total non-hexapod individuals collected in Smith Spring (Table 2). Pelecypods made up 11.64% (779 individuals) and copepods made up 9.67% (647 individuals) (Table 2). The remaining non-hexapod groups (amphipods, decapods, gastropods, isopods, and acarinids) comprised <1% of the individuals collected in Smith Spring (Table 2; Figure 6).

Table 11. Total percent composition of individuals found in each spring for the collection period.

Taxon	Cave Spring		Smith Spring		Canyon Spring		All Springs	
	No.	Percent	No.	Percent	No.	Percent	No.	Percent
Turbellaria	26	0.678	1432	3.687	1718	2.037	3176	2.500
Nematoda	136	3.544	1387	3.571	45	0.053	1568	1.234
Oligochaeta	192	5.004	2413	6.213	318	0.377	2923	2.301
Gastropoda	99	2.580	9	0.023	0	0.000	108	0.085
Pelecypoda	1	0.026	778	2.003	35	0.041	815	0.641
Copepoda	1861	48.501	647	1.666	3	0.004	2511	1.976
Isopoda	2	0.052	15	0.039	10	0.012	27	0.021
Amphipoda	5	0.130	9	0.023	76515	90.723	76529	60.236
Decapoda	16	0.417	1	0.003	0	0.000	17	0.013
Acarina	0	0.000	1	0.003	0	0.000	1	0.001
Collembola	0	0.000	24	0.062	0	0.000	24	0.019
Ephemeroptera	30	0.782	1112	2.863	150	0.178	1292	1.017
Odonata	1	0.026	978	2.518	193	0.229	1172	0.922
Plecoptera	3	0.078	0	0.000	0	0.000	3	0.002
Hemiptera	0	0.000	6	0.015	1	0.001	7	0.006
Tricoptera	430	11.207	1249	3.216	3168	3.756	4847	3.815
Coleoptera	37	0.964	469	1.208	5	0.006	511	0.402
Diptera	1034	26.948	28306	72.884	2178	2.582	31518	24.808
Total	3873		38836		84339		127049	

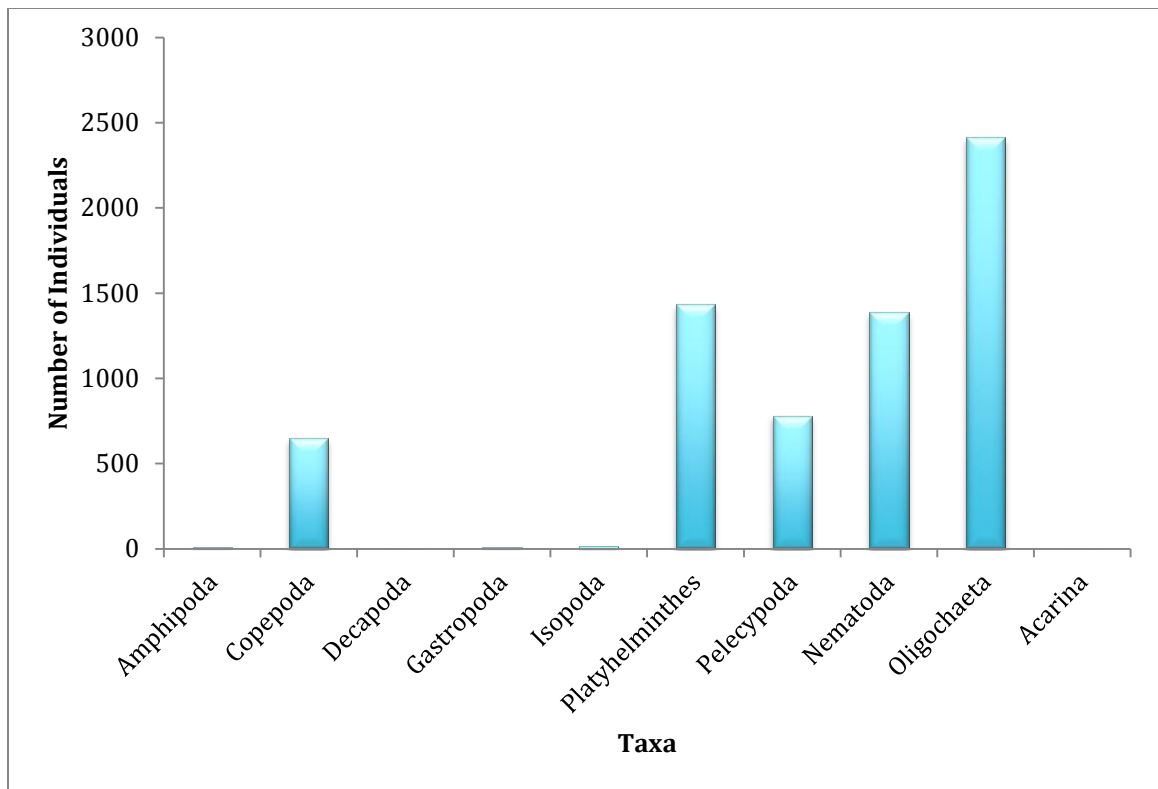
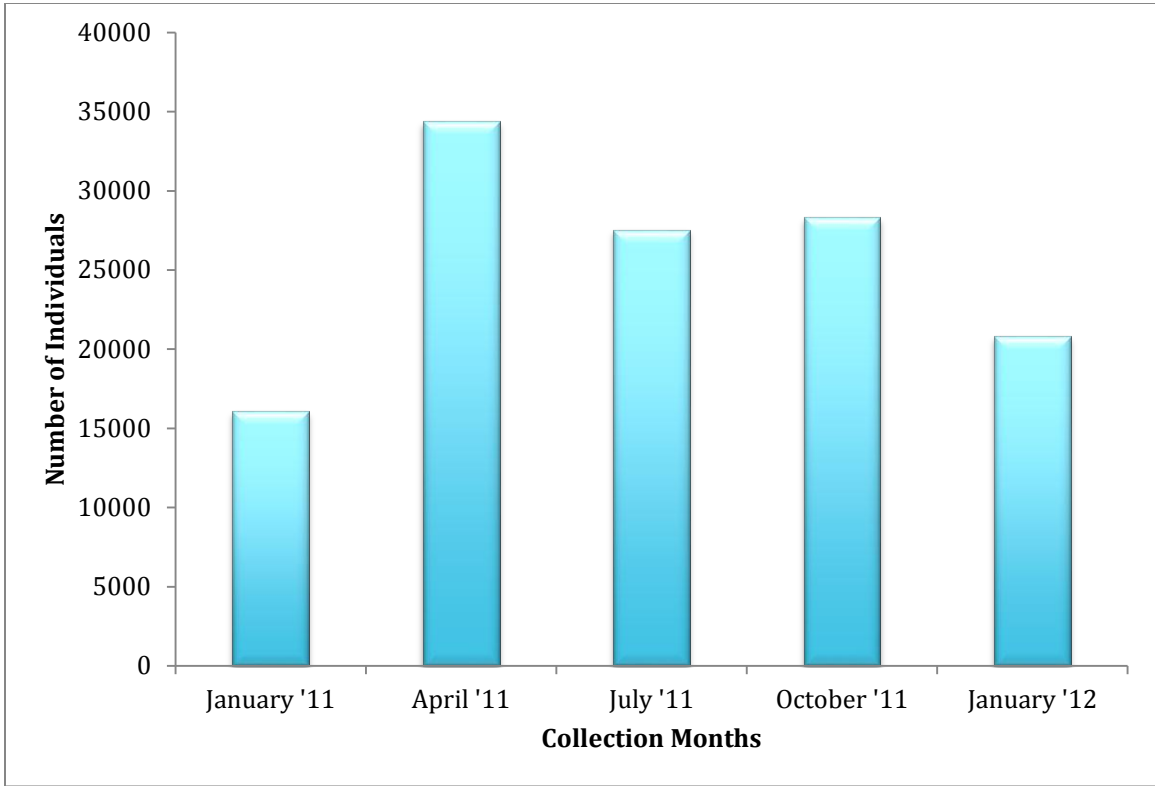


Figure 6. Total number of non-hexapod individuals in Smith Spring.

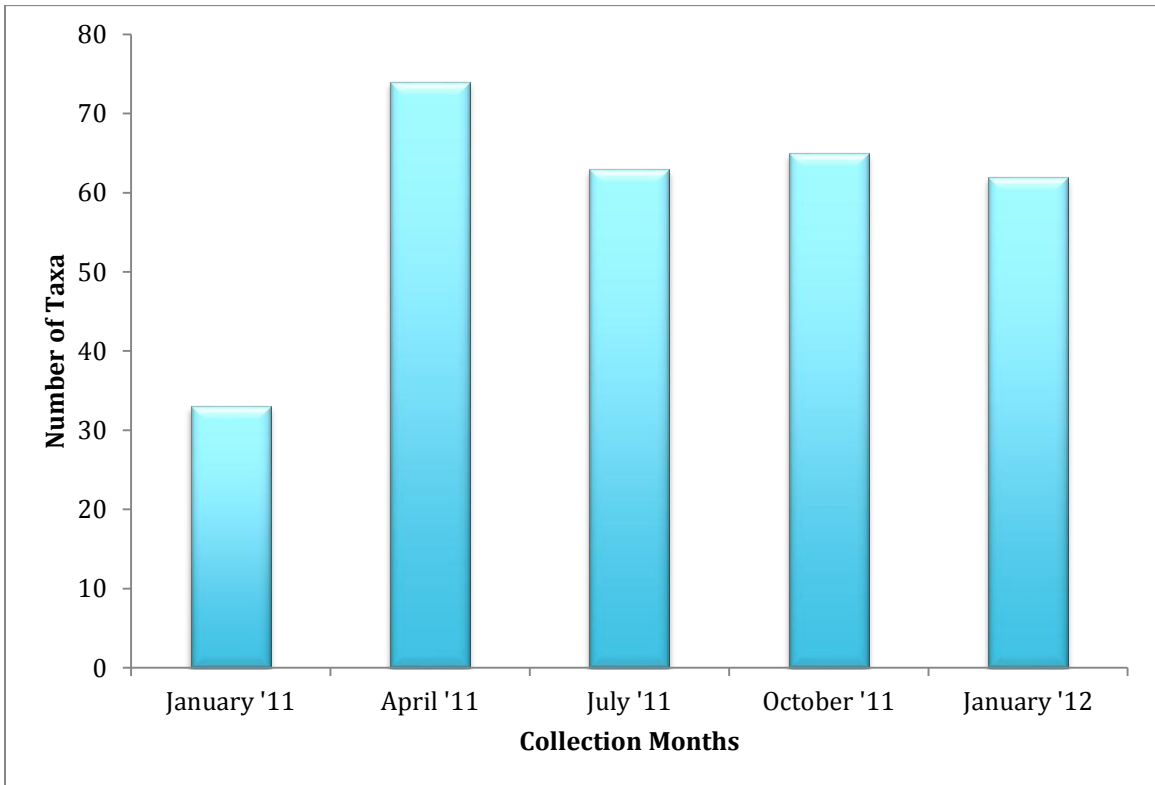
Cave Spring was the least species rich of the springs investigated, with only 44 taxa (Table 4; Figure 5). Cave Spring was dominated by hexapods in terms of taxa, with a total of 31 (70.45%) but only comprising 1,535 (39.6%) individuals (Tables 8 & 10). Dipterans were the most numerous group with 16 taxa (51.61%) and a total of 1,034 individuals (67.36% of hexapod individuals) (Tables 8 & 10). The next most numerous groups were the coleopterans and the tricopterans, both having four taxa (12.90%). However, tricopterans were the more numerous of the two with a total of 430 individuals while coleopterans had a total of 37 individuals (Tables 8 & 10). The remaining hexapod groups (ephemeroptera, odonata, and plecoptera) constituted less than 3% of the remaining individuals collected from Cave Spring (Table 10).

Although Cave Spring had more non-hexapod individuals, 2,338 (60.4% of individuals in Cave Spring), it had only 13 taxa (29.55% of all taxa collected in Cave Spring) (Tables 2 & 4). The most dominant non-hexapod in terms of abundance were the copepods, with a total of 1,861 individuals (79.60% of non-hexapod individuals) (Table 2). The next most numerous taxa were the oligochaetes with a total of 192 individuals, representing only 8.21% of the non-hexapod individuals collected (Table 2). The remaining groups (amphipods, decapods, isopods, nematodes, gastropods, and pelecypoda) represented 12.18% of the remaining individuals collected throughout the study (Table 2).

Of the five collections conducted, the April 2011 collection yielded the most individuals, 34,368 (Figure 7), as well as the highest diversity (74) (Figure 8). This



**Figure 7. Total number of individuals for each collection month.**



**Figure 8. Total number of taxa for each collection month.**

trend held for the remaining months. The next most numerous and species rich collection was October 2011, with a total of 28,299 individuals and 65 taxa. The third most numerous and diverse collection was July 2011, with a total of 27,509 individuals and 63 taxa. The last two collections were the least numerous and least diverse. The January 2012 collection was the more numerous and diverse of the January collections, with a total of 20,814 individuals and 62 taxa collected, while the January 2011 collection had totals of 16,059 individuals and 33 taxa.

Two of the three upper collection sites within Cave Spring were more species rich than the lower collection sites (Table 12). Cave Spring also had the lowest species richness seen throughout the study. The upper collection sites of Smith Spring were the most diverse and species richness. Smith Spring had the highest species richness seen throughout the study, with one exception, the January 2011 collection produced 19 taxa, which is the same as the January 2011 collection for Canyon Spring. Contrary to Cave Spring and Smith Spring, the lower collection sites were more species rich within Canyon Spring.

The species similarity for Cave Spring between months was highly impacted by the drought, seen during much of the year in 2011. Due to this, only three comparisons were made. The highest similarity (0.571) observed between the months of April 2011 and January 2012, with the lowest similarity (0.125) between the months of January and April 2011. The highest similarity (0.692) for Smith Spring occurred between the months of July and October 2011, and the lowest similarity (0.247) occurred between the months of January and October 2011 (Table 13). Canyon Spring had a high similarity value of 0.822, between the months of April



Table 12. Species richness for lower, upper, and combined sites at Cave Spring, Smith Spring, and Canyon Spring

	January '11	April	July	October	January '12	Average
<b>Cave Spring</b>						
Lower	3	20	0	0	28	17.0
Upper	4	21	0	0	11	12.0
Combined	6	28	0	0	29	21.0
<b>Smith Spring</b>						
Lower	15	35	32	45	32	31.8
Upper	14	41	45	49	35	36.8
Combined	19	53	52	56	44	44.8
<b>Canyon Spring</b>						
Lower	19	37	35	23	32	29.2
Upper	10	18	22	17	16	16.6
Combined	19	37	41	31	34	32.4
Total Spring System	33	74	63	65	62	59.4

Table 13. Combined Smith Spring species similarity between months.

CII. April	0.375			
CIII. July	0.294	0.673		
CIV. Oct	0.247	0.660	0.692	
CV. Jan '12	0.310	0.591	0.587	0.639
	CI. Jan '11	CII. April	CIII. July	CIV. Oct

Table 14. Combined Canyon Spring species similarity between months.

CII. April	0.481			
CIII. July	0.377	0.822		
CIV. Oct	0.553	0.687	0.706	
CV. Jan '12	0.431	0.732	0.806	0.750
	CI. Jan '11	CII. April	CIII. July	CIV. Oct

and July 2011, and a low value of 0.377, seen between the months of January and July 2011 (Table 14). The combined springs species similarity between months was also calculated, resulting in a high of 0.750, between the months of July 2011 and January 2012 (Table 15). The lowest value, 0.333, was seen between the months of January and July 2011.

Similarity indices between the upper and lower collection sites within each spring were also calculated (Table 16). Cave Spring had the lowest similarity, 0.286, during January 2011 and Smith Spring had the highest similarity, 0.343. During April 2011, Cave Spring had the highest similarity, 0.406, and Canyon Spring had the lowest, 0.306. There were no similarity comparisons for Cave Spring during the months of July and October 2011. The highest similarity value, for both months, was Smith Spring, with a value of 0.301 during July 2011 and 0.380 during October 2011. Canyon Spring had the lower values at 0.238 during July 2011 and 0.239 during October 2011. Smith Spring had the highest similarity values during January 2012 at 0.393 while Cave Spring had the lowest value at 0.271.

Similarity indices were also calculated for comparisons between springs (Table 17). The lowest similarity value was during January 2011 between Cave Spring and Canyon Spring, at the lower collection sites with a value of 0.105. The highest similarity value, 0.476, was between Smith Spring and Canyon Spring, during January 2011 at the upper collection sites. The lowest value during April 2011 was between Cave Spring and Canyon Spring at the lower collection site, with a value of 0.327. The highest value, 0.531, observed during April 2011, was between Smith Spring and Canyon Spring at the lower collection sites. Due to the on-going

Table 15. Combined springs species similarity between months.

CII. April	0.371			
CIII. July	0.333	0.698		
CIV. Oct	0.348	0.626	0.694	
CV. Jan '12	0.386	0.709	0.750	0.721
	CI. Jan '11	CII. April	CIII. July	CIV. Oct

Table 16. Sorenson's species similarity indices between upper and lower sites within Cave Spring, Smith Spring, and Canyon Spring.

	Jan-11	Apr-11	Jul-11	Oct-11	Jan-12	Average
Cave Spring	0.286	0.406	N/A	N/A	0.271	0.321
Smith Spring	0.343	0.328	0.301	0.380	0.393	0.349
Canyon Spring	0.340	0.306	0.238	0.239	0.286	0.282

Table 17. Sorenson's species similarity indices between upper sites between pairs of springs, lower sites between pairs of springs, and combined sites between pairs of springs.

		Jan-11	Apr-11	Jul-11	Oct-11	Jan-12	Average
<b>Cave Spring and Smith Spring</b>	Lower	0.308	0.449			0.517	1.274
	Upper	0.154	0.407	N/A	N/A	0.318	0.879
	Combined	0.231	0.426			0.431	1.088
<b>Cave Spring and Canyon Spring</b>	Lower	0.105	0.327			0.517	0.317
	Upper	0.154	0.389	N/A	N/A	0.400	0.314
	Combined	0.118	0.352			0.482	0.317
<b>Smith Spring and Canyon Spring</b>	Lower	0.444	0.531	0.615	0.471	0.516	0.516
	Upper	0.476	0.473	0.400	0.339	0.531	0.444
	Combined	0.458	0.504	0.550	0.409	0.523	0.489

drought affecting the July and October 2011 collections, no comparisons were made between Cave Spring and Smith Spring, and between Cave Spring and Canyon Spring. Therefore, both the highest and lowest similarity values observed during July and October 2011 were seen between Smith Spring and Canyon Spring. The highest similarity value, 0.53, for January 2012 was between Smith Spring and Canyon Spring at the upper collection sites, while the lowest value, 0.318, was between Cave Spring and Smith Spring at the upper collection sites.

Combined collections of Cave Spring, Smith Spring, and Canyon Spring were compared to determine similarity between sites (Table 18). Cave Spring and Smith Spring had the lowest similarity value (0.484) between the upper collection sites, and Cave Spring and Canyon Spring had the highest similarity value (0.541) between the upper collection sites. The highest similarity value (0.614) between the lower collection sites was seen between Cave Spring and Smith Spring, and the lowest value (0.311) was seen between Smith Spring and Canyon Spring. For the combined lower and upper collection sites, the highest similarity value (0.545) was between Cave Spring and Smith Spring, and the lowest value (0.446) was between Smith Spring and Canyon Spring.

Species diversity in each spring was calculated for the lower, upper, and combined collection sites (Table 19). Diversity values ranged from a high of 2.671, in Cave Spring during April 2011, to a low of 0.101 in Canyon Spring during January 2011. Canyon Spring had the lowest overall means, never averaging above 1.00. Cave Spring had the highest overall means, averaging 1.785, while Smith Spring had overall means of 1.571.

Table 18. Sorenson's species similarity for the combined collections of Cave Spring, Smith Spring, and Canyon Spring between spring pairs.

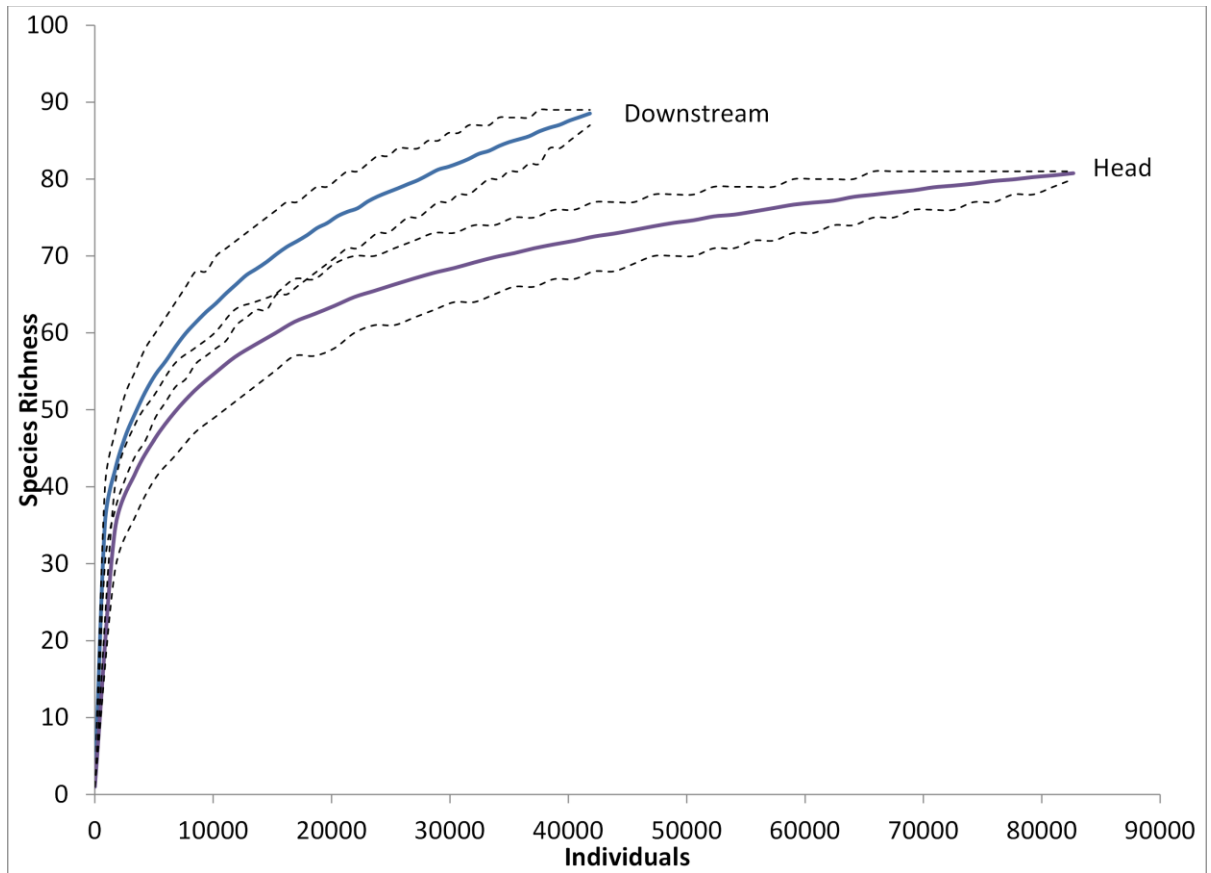
	Upper Sites	Lower Sites	Combined Sites	Average
Cave Spring and Smith Spring	0.484	0.614	0.545	0.548
Cave Spring and Canyon Spring	0.541	0.385	0.466	0.464
Smith Spring and Canyon Spring	0.518	0.311	0.446	0.425

Table 19. Shannon's diversity for lower, upper, and combined collection sites at Cave Spring, Smith Spring, and Canyon Spring.

	January '11	April	July	October	January '12	Average	Spring Average
<b>Cave Spring</b>							
Lower	0.598	2.399	N/A	N/A	1.602	1.533	
Upper	1.386	2.624	N/A	N/A	1.856	1.955	1.785
Combined	1.295	2.671	N/A	N/A	1.630	1.865	
<b>Smith Spring</b>							
Lower	1.627	1.569	1.383	1.907	0.527	1.402	
Upper	1.902	0.594	1.665	2.531	1.563	1.651	1.571
Combined	1.994	0.797	1.655	2.609	1.241	1.659	
<b>Canyon Spring</b>							
Lower	0.810	1.216	1.383	0.417	1.164	0.998	
Upper	0.101	0.157	1.665	0.113	0.144	0.436	0.709
Combined	0.340	0.802	1.655	0.267	0.396	0.692	

Multi-response permutation procedure (mrpp) was used to determine community composition. A significant difference was seen between the head and downstream regions (mrpp,  $p=0.005$ ) as well as between Cave Spring, Smith Spring, and Canyon Spring (mrpp,  $p=0.001$ ). Rarefaction curves generated also support these findings. Figure 9 indicates a significant difference ( $p<0.05$ ) is seen between the species composition found between the head and downstream regions. Figure 10 indicates a significant difference ( $p<0.05$ ) existed between Smith Spring and Canyon Spring and Smith Spring and Cave Spring while also showing no significant difference ( $p>0.05$ ) existed between Canyon Spring and Cave Spring. The detrended correspondence analysis (DCA) (Figure 11) shows which taxa were predominantly found in a particular spring.

Sorenson's similarity indices were calculated comparing Smith Spring and Cave Spring from the 1995 (Bass 2000) survey to the current 2011/2012 survey. The lower collection sites of Cave Spring displayed the lowest similarity value (0.292), while the upper collection sites displayed the highest similarity value (0.320) (Table 20). This is the opposite for Smith Spring. The highest similarity value (0.419) was seen in the lower collection sites and the lower similarity value (0.222) was seen in the upper collection sites (Table 20). Similarity indices were also calculated for comparisons between springs. Cave Spring had a similarity value of 0.286 and Smith Spring had a similarity value of 0.333 between the two investigations.



**Figure 9. Rarefaction curve comparing species richness between the head and downstream collection sites.**

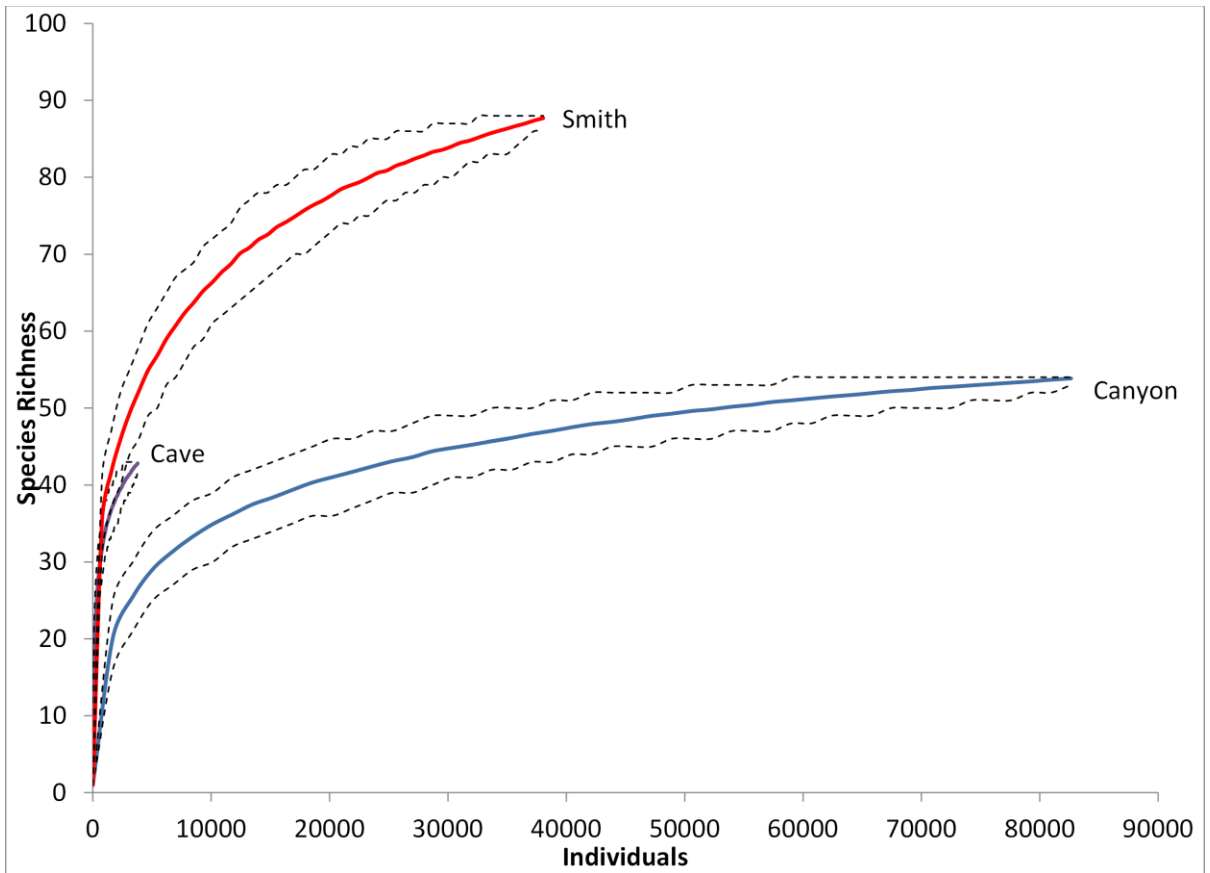


Figure 10. Rarefaction curve comparing species richness between Cave Spring, Smith Spring, and Canyon Spring.



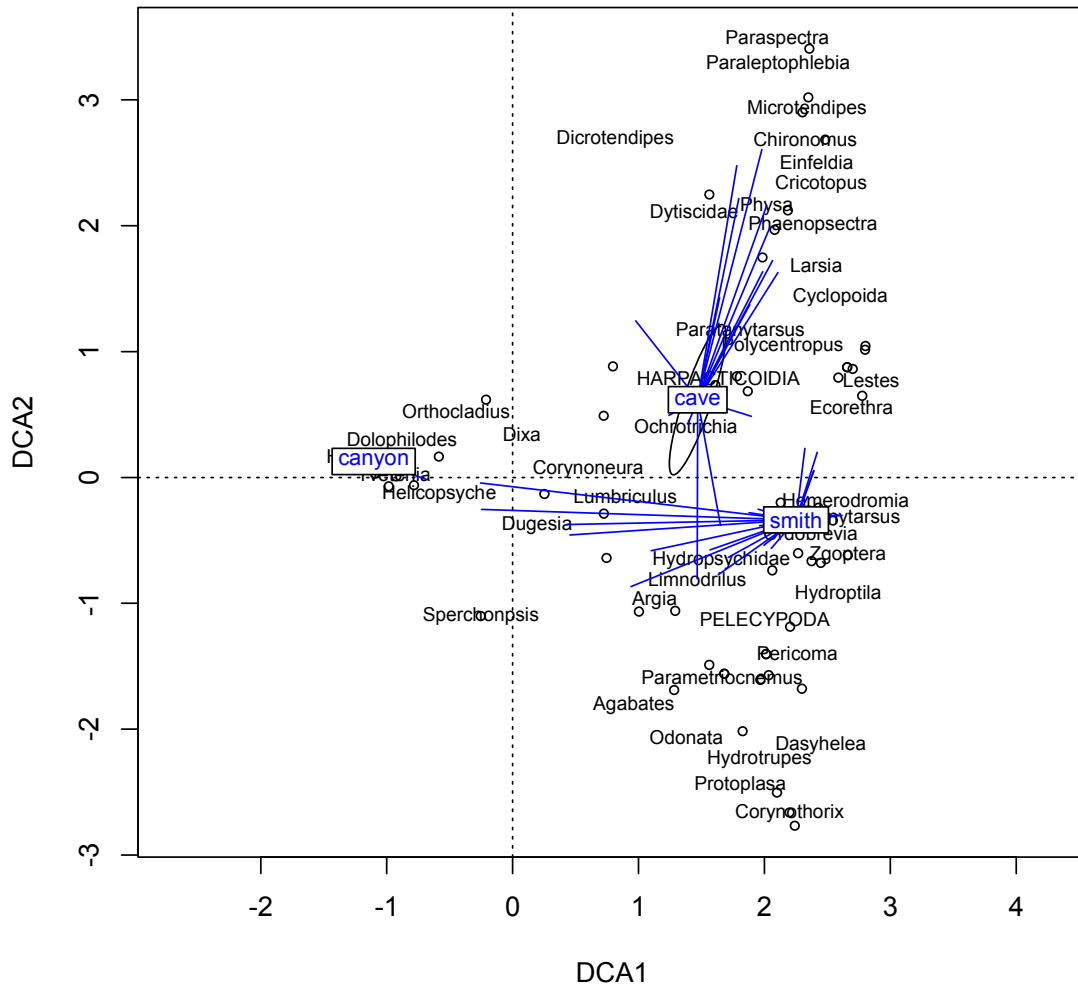


Figure 11. Detrended correspondence analysis depicting which taxa were associated with a particular spring.

Table 20. Sorenson's species similarity indices between lower, upper, and combined sites between the 1995 and 2011/2012 collections of Cave Spring and Smith Spring.

Cave Spring 2011/2012	Cave & Spring 1995	Smith Spring 2011/2012	Smith & Spring 1995
Lower	0.292	Lower	0.419
Upper	0.320	Upper	0.222
Combined	0.301	Combined	0.303

## Discussion

Spring waters usually maintain a constant temperature year round (van der Kamp 1995) as well as remain near the average air temperature for the region (Hynes 1970). Canyon Spring and Smith Spring had annual water temperatures of 19.1°C and 17.9°C respectively. This coincided with the annual air temperature recorded for Oklahoma in 2011 at 16.39°C (Oklahoma Climatological Survey 2013).

Sixteen of the 26 dissolved oxygen readings were below the standard dissolved oxygen saturation level of 5 mg/L. This would suggest that a low diversity count should be observed in those sample areas. This, however, was not the case. Samples from Canyon Spring head, for example, had the lowest dissolved oxygen levels, ranging from 1.4 mg/L to 1.6 mg/L, but had some of the highest numbers of individual organisms seen throughout the study. A study conducted by Nebeker et al. (1992) showed that *Hyalella azteca* complex had a 96-hour and 30-day LC50s of <0.3 mg/L with a lowest-no-adverse-effect concentration of >1.2mg/L. This suggests that *H. azteca* has adapted to surviving in low dissolved oxygen levels. This pattern of having lower dissolved oxygen levels at the emergence sites and higher levels downstream, a process known as re-aeration, is common (Hynes 1970). Values of pH seen throughout the collection fell within the typical range, 6.0 and 9.0, that best support aquatic life (Water Research Center 2013).

Unlike the other physiochemical data recorded, alkalinity levels were reported from the head only. Cave Spring's lowest reading, 289 mg/L, was measured during January 2011 and the highest reading was observed during April 2011. Smith Spring had the overall lowest alkalinity reading during the study at 248 mg/L

during January 2012. The highest overall reading, 334 mg/L, was found in Canyon Spring both during July and October 2011. This resulted in the free carbon dioxide readings being calculated from the average pH and the only alkalinity reading per sample.

According to the Pennsylvania Department of Conservation and Natural Resources (2009), an alkalinity value between 20 mg/L and 200 mg/L is typically found in freshwater ecosystems. Although most readings are between 20 mg/L and 200 mg/L, alkalinity levels rarely exceed 500 mg/L (British Columbia Resources Information Standards Committee 1998), suggesting the alkalinity readings seen throughout this collection, which ranged from a low of 248 mg/L in Smith Spring to a high of 334 mg/L in Canyon Spring, are capable of supporting aquatic life.

Conductivity is defined as the capacity of water to conduct an electrical current based on specific types and quantities of dissolved substances found within the water (U.S. Geological Survey 2013; Radtke et al. 2005). Inland fresh waters have a range between 150  $\mu\text{mhos/cm}$  and 500  $\mu\text{mhos/cm}$ . Rivers of the United States have a range of 50  $\mu\text{mhos/cm}$  to 1500  $\mu\text{mhos/cm}$ . (Environmental Protection Agency 2013, British Columbia Resources Information Standards Committee 1998). Readings throughout the course of the study fall within typical conductivity ranges, with a low of 354  $\mu\text{mhos/cm}$  in Smith Spring and a high of 904  $\mu\text{mhos/cm}$  in Canyon Spring.

Ammonia levels seen throughout this study fall well below the natural levels seen in fresh water systems (Environmental Protection Agency 2013, British Columbia Resources Information Standards Committee 1998), ranging from a low of

0.093 in Canyon Spring to a high of 0.177 in Smith Spring. Typical nitrite levels in surface waters are <0.001 mg/L (British Columbia Resources Information Standards Committee 1998) and 1.0 mg/L for drinking water (Environmental Protection Agency 2013, British Columbia Resources Information Standards Committee 1998). All nitrate readings were below 10 mg/L, the federal standard for drinking water (Environmental Protection Agency 2013).

According to the North Carolina Water Quality Program (2006) orthophosphate levels of streams or flowing water not discharging into reservoirs or lakes should not exceed levels higher than 0.1 mg/L (EPA 1986). Of the 13 measured readings, four were recorded as under measuring range (UMR) while three were recorded as negative values. Six readings exceed the standard of 0.1 mg/L, occurring once in Cave Spring during January 2011 (0.109 mg/L), twice in Smith Spring during the months of January (0.147 mg/L) and October 2011 (0.204mg/L), and three times in Canyon Spring during the months of January (0.134mg/L), April (0.343mg/L), and October 2011 (0.164mg/L).

The overall water quality of each spring system, with a few exceptions, fell within ranges that allow aquatic life to exist. The low dissolved oxygen readings seen throughout Canyon Spring, and in various samples from Cave Spring and Smith Spring may be due partly to the decomposition of vegetation. According to the Minnesota Pollution Control Agency (2009), low dissolved oxygen levels may result from excessive algal growth when higher than normal phosphate levels are present. Canyon Spring had a high density of algal beds seen throughout the spring, corresponding with the orthophosphate readings seen. Two of the springs are

located on protected land and the other is on private property that is undeveloped and not used for agriculture. This lack of agriculture may explain why the water quality of each spring generally remains high.

Throughout the course of the study, a total of 127,048 individuals, representing 114 taxa were collected. One macroinvertebrate in particular was dominant throughout the study, the amphipod, *Hyaella azteca* complex. This one species represented 60.24% of all individuals collected during the study. The non-hexapods were the most numerous macroinvertebrates found, comprising 69% of individuals collected, whereas the hexapods represented 31% of individuals collected. The hexapods were more numerous and diverse in terms of taxa, with a total of 93, most of which are represented by members of the order Diptera. Similar findings are seen in other studies that investigate spring macroinvertebrate community composition (Rudisill and Bass 2005; Ilmonen *et al.* 2009; Gaskin and Bass 2000; Bass 2000).

Smith Spring was the only spring to be dominated by hexapods, specifically dipterans, with a total of 28,306 individuals. Cave Spring and Canyon Spring were each dominated by a different taxa of non-hexapod. Copepods were the most numerous taxa seen in Cave Spring, with a total of 1,861 individuals, whereas Canyon Spring was dominated by the amphipod *Hyaella azteca* complex with a total of 76,515 individuals. Each spring was dominated by different vegetation, which may have influenced the prominence, or absence, of specific macroinvertebrates. Smith Spring is characterized by a sandy, fine silt bottom, with various types of grasses and aquatic plants, as well as woody vegetation occurring along the spring

run. Cave Spring is characterized by a hard solid substrate, with numerous trees providing a leaf litter microhabitat within the spring, as well as various grasses along the spring run. Canyon Spring is characterized by a rocky and sandy substrate, with numerous algal beds within the spring run, and many large trees along one shoreline. Each spring overall represents a very distinct habitat in terms of substrate type and vegetation seen.

Cave Spring was the least species rich of the three springs, having species richness values of 6 (January 2011), 28 (April 2011), and 29 (January 2012), with an overall average of 21 taxa found throughout the study. This lower number is partly due to the drought that caused Cave Spring to cease flow and desiccate during much of the study period. In addition, the substrate of Cave Spring contained fewer microhabitats existing within the spring. Canyon Spring had the next highest species richness with values of 19 (January 2011), 37 (April 2011), 41 (July 2011), 31 (October 2011), and 34 (January 2012), with an overall average of 32.4 taxa, while Smith Spring had the highest overall species richness with values of 19 (January 2011), 53 (April 2011), 52 (July 2011), 56 (October 2011), and 44 (January 2012), with an overall average of 44.8 taxa. Although Smith Spring and Canyon Spring are fairly similar, the area surrounding each spring, as well as the chemical composition of the water, may have contributed to the species richness values seen throughout the study.

The April 2011 collections contained the largest number of individuals collected during the study, with a total of 34,368. October 2011 and July 2011 were the next most numerous in terms of individuals at 28,299 and 27,509, respectively.

The January 2012 collection had a total of 20,814 individuals while the January 2011 collection had a total of 16,059 individuals. The high individual counts seen from April to October 2011 may have been due in part to the emergence of vegetation within the area during the growing season, increasing both food resources and microhabitats. The addition of leaf debris during autumn would provide more microhabitats especially in the case of Cave Spring, which has a fairly dense tree canopy (this however was not to be observed, as Cave Spring was dry during July and October 2011). Similarity values between the months of each spring varied; Cave Spring and Canyon Spring had a high similarity value seen during April 2011, with value of 0.406 and 0.306 respectively while Smith Spring had a high similarity value measured during January 2012, with a value of 0.393. The species richness of each spring was the highest between the months of April and October 2011, except in the case of Cave Spring which could not have comparisons made due to drought, resulting in a lack of water.

Similarity indices were calculated for comparisons between springs. Comparisons between Cave Spring and Canyon Spring during January 2011 indicate a very low species similarity at the upper and lower collection sites, with a value <0.16. Smith Spring and Canyon Spring had a much greater similarity index, both the upper and lower collection sites having a value above 0.44. Each spring became slightly more similar during the April 2011; with Cave Spring and Smith Spring having a combined value (the upper and collections combined) of 0.426. Cave Spring and Canyon Spring showed an increased combined value of 0.352, and Smith Spring and Canyon Spring being the most similar with a combined value of 0.504. The

highest similarity value, 0.615, was observed during July 2011 between Smith Spring and Canyon Spring in the lower collection, indicating a very similar species composition. Each spring comparisons from the January 2012 collections all had relatively high similarity values, the lowest value, 0.318, being between Cave Spring and Smith Spring in the upper collection sites and the highest, 0.531, was between Smith Spring and Canyon Spring.

When examining the combined collections of each spring between the upper and lower collection sites an opposite trend is seen. The highest average similarity index for the entire study was between Cave Spring and Smith Spring at 0.548, which is a much higher index than when comparing each site per month. Cave Spring and Canyon Spring also show an increase in species similarity, 0.464, but the opposite occurs when comparing Smith Spring and Canyon Spring, which had a value of 0.425. This may be due to more data being used for calculations between Smith Spring and Canyon Spring, while Cave Spring only allowed for two comparisons between Cave Spring and Smith Spring and Cave Spring and Canyon Spring due to the absence of water.

Species diversity values comparing the upper, lower, and combined collection sites were calculated for each spring for each collection. All three upper site collections for Cave Spring were more diverse than the lower site collections, with values ranging from a low of 1.386 (January 2011) to a high of 1.856 (January 2012). This was surprising as the substrate type in the lower collections provided more microhabitat than the substrate in the upper collections. Four of the five upper collection sites from Smith Spring possessed higher diversity values throughout the



study, the lowest value, 0.594, was observed during April 2011. Although the habitat at the lower collection sites looked to have more microhabitats, the upper collection sites were more diverse, owing to the fact that more dipterans were found in the upper site. The opposite is seen in Canyon Spring with the diversity values being higher in the lower sites, with one exception during July 2011. This is not surprising, as the dissolved oxygen levels were much lower at the upper collection sites than the lower collection sites, which may have limited the presence of certain organisms.

Comparisons of species similarity made between the 1995 (Bass 2000) collection and the current collection showed slight similarities for each spring at each location. The upper collection sites of Cave Spring were slightly more similar (0.320) than the lower collection sites (0.292). The lower collection sites for Smith Spring were much more similar with a value of 0.419. Indices were also calculated to compare the upper and lower collection sites from the 1995 (Bass 2000) survey to the current survey. Cave Spring had a low similarity value of 0.286 as well as Smith Spring with a similarity value of 0.333. These differences between collections may be due to habitat changes from 1995 to 2012 as well as individual variations in sampling techniques.

With the exception of low dissolved oxygen concentrations seen in Canyon Spring, the differences seen in numbers of individuals, species richness, species diversity, and similarity may be attributed to life cycle patterns as well as emergence patterns. The largest influence on the macroinvertebrate communities may have been the vegetation changes seen throughout the seasons, providing

different microhabitats and food types for various organisms. An increase in vegetation, not only vegetation growing within and along the spring runs, but also allochthonous vegetation, such as leaf litter, allows populations to increase in number.

## Conclusion

This study was conducted to 1) determine macroinvertebrate community composition of Smith Spring, Cave Spring, and Canyon Spring, 2) compare the macroinvertebrate results of each spring to each other, 3) compare the results from this study to results from the investigation conducted by Bass (2000), and 4) determine overall water quality of the springs within, and near, Pontotoc Ridge Nature Preserve.

The overall water quality of each spring system, with a few exceptions, falls well within the standards that support and allow for aquatic life. The low dissolved oxygen levels seen in Canyon Spring may be due to the decomposition of the algal beds found within the spring, which resulted in increased the phosphate levels. Seeping of phosphate into the groundwater from surrounding agricultural areas may have also attributed to the higher phosphate levels seen in Canyon Spring. Although dissolved oxygen levels were below standards in Canyon Spring, through re-aeration, dissolved oxygen levels increased further down stream. The remaining nutrient concentrations fell well within the standards for drinking water, therefore seeming to have no impact on the faunistic compositions of the springs.

A total of 127,048 individuals, representing 114 taxa, were collected and identified throughout the course of this study. Compared to previously mentioned studies conducted in Oklahoma, these numbers are quite large. Gaskin and Bass (2000) sampled seven springs and only found a total of 59 species, while Rudisill and Bass (2005) sampled from three springs and found a total of 64 taxa (21,268 individuals). Although 60% of the individuals collected were *Hyalella azteca*

*complex*, the remaining 50,519 individuals still constitute a large number of macroinvertebrates collected from only three springs. Not only are the number of individuals higher than previous studies, so too were the number of taxa identified. The 114 taxa found over the course of this study, when compared to Gaskin and Bass (2000) and Rudisill and Bass (2005), is almost twice the number of taxa. Dipterans were the most diverse macroinvertebrates collected having a total of 44 taxa identified (38.6%), which is a very common occurrence in springs (Rudisill and Bass (2005), Illmonen et al. (2009), Mattson et al. (1995), Anderson and Anderson (1995)).

Differences in the number of individuals and dominant taxa were observed throughout the course of this study when comparing each spring. Canyon Spring had the largest number of individuals collected during the study with 84,339 individuals, followed by Smith Spring with 38,837 individuals, and Cave Spring with only 3,873 individuals. Smith Spring contained the greatest species richness with a total of 91 taxa collected and identified. Canyon Spring has the next highest richness value with a total of 58 taxa, followed by Cave Spring being the least species rich with a total of 44 taxa. Although Canyon Spring had the most macroinvertebrates collected throughout the study, the low dissolved oxygen levels, seen from both the head and downstream sections, may have influenced the presence and abundance of other organisms.

Temperature, resource and microhabitat availability, low dissolved oxygen levels, and high phosphates levels influenced overall species richness, diversity, and various similarity values calculated. Temperature effects can be clearly seen in Cave

Spring, which desiccated during the months of July and October 2011. Although a negative effect of temperature was seen in Cave Spring due to temperature, a positive relationship between temperature and vegetation was seen in Canyon Spring and Smith Spring. This growth of surrounding terrestrial vegetation during the spring months allowed for more resources and microhabitats, and increased macroinvertebrate densities. There was also a slight rise in the number of individuals and taxa during October 2011; this is thought to be due to the introduction of decomposing vegetation, allowing for a variety of new, or different, microhabitats and food resources to exist within the springs. The success of *Hyaella azteca complex* may be in part due to their ability to survive in environments with such low dissolved oxygen levels (Nebeker et al. 1992), such as those recorded in Canyon Spring.

Comparisons between the survey conducted by Bass (2000) and the current survey indicate a fairly high similarity value, 0.419, between the lower collections sites of Smith Spring. This would suggest that even after such a long time span between collections, the spring habitat and surrounding area has undergone little change, allowing the macroinvertebrate communities to also remain constant over time. Comparison between spring sites from the 1995 survey (Bass 2000) to the current survey indicates a much lower similarity, 0.286, between the collections made in Cave Spring. This spring was dry for several months, including two of the collection periods during the present investigation. According to Jona Tucker this happens quite often throughout the year. This reoccurring desiccation of Cave Spring may influence the macroinvertebrate fauna community structure, and over a

16-year period this pattern may have had a large impact. The similarity index of 0.333 between Smith Spring surveys is slightly higher. A 16-year period between surveys is a large amount of time to pass and other factors will have also had an influence on the macroinvertebrate compositions. Variations in similarity observed within and between each spring community throughout the current study may be primarily attributed to life cycle patterns as well as vegetation within and around each spring that provide various microhabitats.

The Pontotoc Ridge Nature Preserve is a very important ecosystem in southern Oklahoma. It serves both as a site for various types of research and as an educational resource to the public. The continual study of spring systems within Oklahoma is vital and the springs found within and around the Pontotoc Ridge Nature Preserve are considered as nearly pristine, based on the findings of this investigation. To keep the springs in this area and other areas throughout the state in this nearly pristine condition continued research is important. This research allows for identification and inventory of the macroinvertebrate community and water quality analysis indicating potential groundwater pollution.

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## Appendix 1.

### A. Water Temperature (°C)

Sample Dates	Cave Spring		Smith Spring		Canyon Spring	
	H	D	H	D	H	D
7-Jan-11	16.4	13.5	17.5	17.2	-	-
15-Jan-11	-	-	-	-	19.1	18.3
8-Apr-11	17.3	19.5	17.5	17.7	-	-
10-Apr-11	-	-	-	-	19.3	19.5
8-Jul-11	DRY		17.9	18.1	19.3	19.9
7-Oct-11	DRY		17.9	20.4	19.2	19.3
13-Jan-12	17.5	15.9	17.3	17.2	18.6	18.5
Average Water Temperature (°C)	17.1	16.3	17.6	18.1	19.1	19.1

### B. Dissolved Oxygen (mg/l)

Sample Dates	Cave Spring		Smith Spring		Canyon Spring	
	H	D	H	D	H	D
7-Jan-11	6.1	6.1	5.6	5.7	-	-
15-Jan-11	-	-	-	-	1.6	4.5
8-Apr-11	2.6	4.6	5.0	5.5	-	-
10-Apr-11	-	-	-	-	1.5	4.9
8-Jul-11	DRY		3.5	4.1	1.5	4.5
7-Oct-11	DRY		2.7	3.8	1.4	4.6
13-Jan-12	6.5	7.6	6.0	6.3	1.5	4.9
Average Dissolved Oxygen (mg/l)	5.1	6.1	4.6	5.1	7.5	4.7

### C. Percent Dissolved Oxygen Saturation

Sample Dates	Cave Spring		Smith Spring		Canyon Spring	
	H	D	H	D	H	D
7-Jan-11	60	57	57	57	-	-
15-Jan-11	-	-	-	-	17	45
8-Apr-11	26	47	50	56	-	-
10-Apr-11	-	-	-	-	15	57
8-Jul-11	DRY		34	41	15	48
7-Oct-11	DRY		28	41	15	49
13-Jan-12	67	75	62	64	14	47
Average % D.O. Saturation	51	59.7	46.2	51.8	15.2	49.2

Appendix 1 (continued).

D. Free Carbon Dioxide (mg/l)

Sample Dates	Cave Spring		Smith Spring		Canyon Spring	
	H	D	H	D	H	D
7-Jan-11	<10		28		-	-
15-Jan-11	-	-	-	-	25	
8-Apr-11	37		20		-	-
10-Apr-11	-	-	-	-	31	
8-Jul-11	DRY		29		38	
7-Oct-11	DRY		24		26	
13-Jan-12	<10		<10		16	
Average Free Carbon Dioxide (mg/l)	N/A		N/A		27.2	

E. pH

Sample Dates	Cave Spring		Smith Spring		Canyon Spring	
	H	D	H	D	H	D
7-Jan-11	7.7	-	7.9	-	-	-
15-Jan-11	-	-	-	-	7.4	-
8-Apr-11	7.3	7.3	7.4	7.4	-	-
10-Apr-11	-	-	-	-	7.1	7.4
8-Jul-11	DRY		7.3	7.3	7.2	7.4
7-Oct-11	DRY		7.4	7.7	7.3	7.5
13-Jan-12	7.7	8.0	7.6	7.7	7.5	7.6
Average pH	7.6	7.7	7.5	7.5	7.3	7.5

F. Alkalinity (mg/l)

Sample Dates	Cave Spring	Smith Spring	Canyon Spring
7-Jan-11	289	270	-
15-Jan-11	-	-	310
8-Apr-11	330	287	-
10-Apr-11	-	-	309
8-Jul-11	DRY	290	334
7-Oct-11	DRY	300	334
13-Jan-12	302	248	324
Average Alkalinity (mg/l)	307	279	322.2

Appendix 1 (continued).

G. Turbidity (JTU)

Sample Dates	Cave Spring	Smith Spring	Canyon Spring
7-Jan-11	95.2%T (0.02)	98.1%T (<0.02)	-
15-Jan-11	-	-	99.5%T (<0.01)
8-Apr-11	97.8%T (<0.02)	99.9%T (<0.01)	-
10-Apr-11	-	-	98.1%T (<0.02)
8-Jul-11	DRY	99%T (<0.01)	100%T (0)
7-Oct-11	DRY	100%T (0)	100%T (0)
13-Jan-12	99%T (<0.01)	99%T (<0.01)	100%T (0)
Average Turbidity (JTU)	97.3	99.2	99.5

H. Conductivity ( $\mu$ mhos/cm)

Sample Dates	Cave Spring	Smith Spring	Canyon Spring
7-Jan-11	417	354	-
15-Jan-11	-	-	580
8-Apr-11	539	455	-
10-Apr-11	-	-	732
8-Jul-11	DRY	418	906
7-Oct-11	DRY	410	701
13-Jan-12	400	328	568
Average Conductivity ( $\mu$ mhos/cm)	452	393	697.4

I. Ammonia (mg/l)

Sample Dates	Cave Spring	Smith Spring	Canyon Spring
7-Jan-11	0.097	0.094	-
15-Jan-11	-	-	0.093
8-Apr-11	0.127	0.112	-
10-Apr-11	-	-	0.122
8-Jul-11	DRY	0.142	0.135
7-Oct-11	DRY	0.160	0.163
13-Jan-12	0.161	0.177	0.168
Average Ammonia (mg/l)	0.128	0.137	0.136

Appendix 1 (continued).

J. Nitrites (mg/l)

Sample Dates	Cave Spring	Smith Spring	Canyon Spring
7-Jan-11	*0.015	*0.005	-
15-Jan-11	-	-	*0.010
8-Apr-11	*0.012	*0.012	-
10-Apr-11	-	-	-0.002
8-Jul-11	DRY	*0.012	*0.010
7-Oct-11	DRY	*0.012	*0.013
13-Jan-12	*0.011	*0.012	*0.011
Average Nitrites (mg/l)	N/A	N/A	N/A

K. Nitrates (mg/l)

Sample Dates	Cave Spring	Smith Spring	Canyon Spring
7-Jan-11	0.252	*0.107	-
15-Jan-11	-	-	0.422
8-Apr-11	0.335	0.280	-
10-Apr-11	-	-	0.520
8-Jul-11	DRY	0.320	0.558
7-Oct-11	DRY	0.281	0.500
13-Jan-12	0.747	0.779	0.364
Average Nitrates (mg/l)	0.445	N/A	0.473

L. Orthophosphates (mg/l)

Sample Dates	Cave Spring	Smith Spring	Canyon Spring
7-Jan-11	0.109	0.147	-
15-Jan-11	-	-	0.134
8-Apr-11	*0.126	*0.141	-
10-Apr-11	-	-	0.343
8-Jul-11	DRY	*1.95	*1.44
7-Oct-11	DRY	0.204	0.164
13-Jan-12	-0.017	-0.021	-0.022
Average Orthophosphates (mg/l)	N/A	N/A	N/A

Appendix 2A. Macroinvertebrates Collected January 2011.

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TAXA	Cave Spring								Smith								Canyon							
	1A	1B	1C	Q	1D	2A	2B	Q	4A	4B	4D	Q	4D	5A	5B	Q	6A	6B	6C	Q	6D	5C	5D	Q
<i>Agabates</i>															1									
<i>Aquarius</i>												*												
<i>Archilestes</i>												*												
<i>Argia</i>									3		1		3	2	10	*	13	16	25				1	
Astacidae					1																			
<i>Astacus</i>				*																				
<i>Atherix</i>									1															
<i>Beatis</i>											1		1				1							
<i>Caloparyphus</i>																			2		1			
<i>Calopteryx</i>					1					1		*				*								
<i>Culicoides</i>																	3							*
<i>Dixa</i>																				*	1			
<i>Dugesia</i>									1	4	3	*	10	1	2	*	83	33	54		56	50	24	
<i>Euparyphus</i>																	3							
<i>Helichus</i>																				*				
<i>Helicopsyche</i>			2						2	11	14	*	3	8	7	*	147	273	189				1	
<i>Hyalpella azteca</i>																	1043	583	1178		3663	4500	3830	
<i>Limnodrilus</i>						1			9						12		1					8	27	
<i>Lumbriculus</i>									1						3		1	11	12		3	5	2	
<i>Nematoda</i>																	1		2					
<i>Neurocordulia</i>																		2						
<i>Ordobrevia</i>													1											
<i>Orthocladus</i>																	13	1			18	2		
<i>Physa</i>		2	3	*	1				1			*				*								

Appendix 2A, continued.

TAXA	Cave Spring							Smith							Canyon									
	1A	1B	1C	Q	1D	2A	2B	Q	4A	4B	4D	Q	4D	5A	5B	Q	6A	6B	6C	Q	6D	5C	5D	Q
<i>Protoplasa fitchii</i>															1									
<i>Sperchonopsis verrucosa</i>											1													
<i>Sphaerium</i>														4	29		2	1	4					
<i>Stenonema femoratum</i>																	1							
<i>Tabanus</i>																	1							
<i>Tipula</i>														3										
<i>Trepobates</i>																				*				
<i>Tropisternus</i>												*			1									
Total Species	0	1	2		3	1	0		7	3	6		5	5	9		14	8	8		6	7	4	
Total Individuals	0	2	5		3	1	0		18	16	20		18	18	66		131		146		374	456	388	
Species Diversity			0.6		1.1				1.5	0.7	1.1		1.2	1.4	1.6			0.9						
	0	1	7		0	1	0		3	8	3		4	0	3		0.76	2	0.70		0.12	0.09	0.08	

\*Indicates presence in qualitative samples



Appendix 2B. Macroinvertebrates Collected April 2011.

TAXA	Cave								Smith							Canyon								
	4A	4B	4C	Q	4D	5A	5B	Q	1A	1B	1C	Q	1D	2A	2B	Q	5C	5D	6A	Q	6B	6C	6D	Q
<i>Ablabesmyia</i>	1																							
<i>Agabates</i>																								
<i>Agabus</i>	2						1							1	5									
<i>Aquarius</i>												*												
<i>Argia</i>									1	7	5	*	49	8	10	*		9	34	*	3	1		*
<i>Atherix</i>											1			1										
<i>Beatis</i>		4			4				13	39	2		57	89	21	*	2	2	2					
<i>Buena</i>												*												
Caecidotea					1									1							1	1		
<i>Caloparyphus</i>																*		2						
Cambaridae										1														
Cambarinae						6	2	*																
<i>Cardiocladius</i>																		1						
<i>Chironomus</i>	6	1	2		2	5	7																	
<i>Corynoneura</i>					1				7	5	1		6	7			8	163	165		1			
<i>Cricotopus</i>	1	1																						
<i>Cryptochironomus</i>																	11	2						
<i>Culicoides</i>									1	1				20	31			5	5					
Cyclopoida					8	5	11				2			7	19		1							
<i>Dasyhelea</i>									1															
Decopoda						7														*				*
<i>Dicrotendipes</i>	15	28	1		21	5	3			4					5		9	4	1					
<i>Dixa</i>																								
<i>Dugesia</i>									24	41	22		46	56	108	*	45	229	235		39	55	4	

Appendix 2B, continued.

TAXA	Cave								Smith								Canyon							
	4A	4B	4C	Q	4D	5A	5B	Q	1A	1B	1C	Q	1D	2A	2B	Q	5C	5D	6A	Q	6B	6C	6D	Q
<i>Dytiscidae</i>		1																						
<i>Einfeldia</i>					4					1				1	2									
<i>Eukiefferiella</i>													1					18	46		1			
<i>Euparyphus</i>																		3	1		2			
<i>Graptocorixa</i>										1														
Harpacticoida															4									
<i>Heleniella</i>													2	1										
<i>Helicopsyche</i>									6	47	52		111	60	19		190	355	1056	*	14	2		
<i>Heterina</i>														1										
<i>Hyallela azteca</i>																	1285	3037	3013	*	4816	3538	1962	*
<i>Hygrotus</i>	1		1																					
Hydropsychidae													2	23	13			8	27					
<i>Hydroptila</i>														10	27									
<i>Hydrotrupes</i>													1											
<i>Laccobus</i>																								*
<i>Larsia</i>	17	12	2		11	4	5		11	9				3	1			2	1		1			
<i>Limnodrilus</i>	6				1	6			140	136	1				4		3		1				29	
<i>Lumbriculus</i>	1								9	22	1			3	12		1	3	1			1	15	
<i>Microtendipes</i>	16	17	3		3	7	1																	
<i>Myxosargus</i>													1											
<i>Nectopsyche</i>																		3						
Nematoda	2	2							4	8	1		3	11	29		2	3	5		1			
<i>Neurocordulia</i>																								

Appendix 2B, continued.

TAXA	Cave								Smith							Canyon								
	4A	4B	4C	Q	4D	5A	5B	Q	1A	1B	1C	Q	1D	2A	2B	Q	5C	5D	6A	Q	6B	6C	6D	Q
<i>Ochrotrichia</i>													2	7				7	22					
<i>Ordobrevia</i>										4	2		3	6	2			1						
<i>Orthocladus</i>			1			2	4						8	28	7		2	54	80		54	10		
<i>Paraleptophlebia</i>		9			12			*																
<i>Parametriocnemus</i>							2							2			6	18	16		2			
<i>Paraspectra</i>		8																						
<i>Paratanytarsus</i>	1	1				1			1	5			6	9	12		1	1						
<i>Paratendipes</i>										1														
<i>Paratrichocladius</i>																			26		15			
<i>Pelecypoda</i>									2						2									
<i>Peltodytes</i>										1	1			1										
<i>Phaenopsectra</i>	16	3				8			1	6														
<i>Physa</i>	4	1	2	*	1	1	4	*		5	2	*				*								
<i>Polycentropes</i>													3											
<i>Polypedilum</i>									1	1									1					
<i>Procladius</i>									1															
<i>Probezzia</i>										18	3		14	68	22				1					
<i>Rhagovelia</i>												*								*				*
<i>Rheotanytarsus</i>																	1	3	2					
Sphaeridea										19	1	*	1											
<i>Sphaerium</i>														1			10	3						
<i>Sublettea</i>																								
<i>Tabanus</i>																								

Appendix 2B, continued.

TAXA	Cave							Smith							Canyon									
	4A	4B	4C	Q	4D	5A	5B	Q	1A	1B	1C	Q	1D	2A	2B	Q	5C	5D	6A	Q	6B	6C	6D	Q
<i>Tanytarsus</i>	17	1	3		1	1	2		334	328	317		580	5320	3841	*	6	2	1		3			
<i>Thienemannimyia</i>														1					1					
<i>Tipula</i>												*		7	1									
<i>Trepobates</i>												*												
<i>Triacanthagyna</i>												*												
<i>Tricorythodes</i>																	2	2	1					
<i>Tropisternus</i>															2									
<i>Tvetenia</i>																		66	552					
Total Species	15	14	8		13	13	11		17	25	15		19	29	24		18	30	25		14	7	4	
Total Individuals	106	89	15		70	58	42		557	712	412		896	5753	4199		1585	4008	5295		4953	3608	2010	
Species Diversity	2.25	2.04	1.99		2.07	2.39	2.15		1.26	1.91	0.89		1.33	0.46	0.51		0.75	1.01	0.39		0.17	0.01	0.13	

\*Indicates presence in qualitative samples

Appendix 2C. Macroinvertebrates Collected July 2011.

TAXA	Smith								Canyon							
	1A	1C	5A	Q	5B	5C	5D	Q	2C	2D	3A	Q	3B	3C	3D	Q
<i>Agabus</i>														1		
<i>Aquarius</i>																*
<i>Argia</i>	26	30	9	*	182	141	37		2	6	7	*				
<i>Atherix</i>					10	2				1						
<i>Beatis</i>					1	11	1		6	5	4					
<i>Caecidotea</i>														4		
<i>Caloparyphus</i>					1	1	1				1				1	
<i>Corynoneura</i>					75	85	5		22	11	47		2			
<i>Cryptochirnomus</i>	1	1					2		3	1	1					
<i>Chrysops</i>						2										
<i>Culicoides</i>	3	1	3		5	24	22		21	5	4		22	52	25	
Cyclopoida	60	36	16		1	2	2						1			
<i>Dicrotendipes</i>									3							
<i>Dugesia</i>	38	36	10	*	77	138	107		19	18	68		6	33	36	
Dytiscidae													1			
<i>Ecorethra</i>			1													
Ephemeroptera	53	3														
<i>Eukiefferiella</i>									8	4	9			1	1	
<i>Gyraulus</i>												*				
<i>Harpacticoidia</i>	2					1										
<i>Heleniella</i>		1	2		4	55	36									
<i>Helichus</i>																
<i>Helicopsyche</i>	12		3		20	54	41		144	162	137	*		1		*
<i>Hemerodromia</i>	14	10			6	25	12									

Appendix 2C, continued.

TAXA	Smith								Canyon							
	1A	1C	5A	Q	5B	5C	5D	Q	2C	2D	3A	Q	3B	3C	3D	Q
<i>Heterina</i>		1					1									
<i>Hyallega azteca</i>						1	3		662	482	1156	*	930	7029	4111	*
Hydropsychidae	21	6	3	*	47	97	36		1	2	4					
Isotomidae					1											
<i>Larsia</i>	89	124	19		7	18	48		2	2	2					
Leptohyphidae		7	5		5	8			15	9	12					
<i>Lestes</i>	4	2														
<i>Limnodrilus</i>	149		17		5		101		1				1	8	8	
<i>Lumbriculus</i>	14	33				23				1	1			2		
<i>Microtendipes</i>	1	5														
<i>Myxosargus</i>					1											
Nematoda	35	6	12	*	85	156	407		8	2	4			2		
<i>Ochrotrichia</i>	2	1	3		13	19	13		1	3	4					
<i>Ordobrevia</i>	84	6	32		21	30	22			1						
<i>Orthocladus</i>	2								19	1	3		1	9		
<i>Parametriocnemus</i>		1			137	139	37		7	2	16					
<i>Paratanytarsus</i>						5	11		2							
<i>Paratendipes</i>	1	4	1		1	4			3							1
<i>Paratrachocladus</i>									17	5	1		2	17	4	
Pelecypoda	2	8					35			1						
<i>Peltodytes</i>						1	1									
<i>Pericoma</i>						2										

Appendix 2C, continued.

TAXA	Smith								Canyon							
	1A	1C	5A	Q	5B	5C	5D	Q	2C	2D	3A	Q	3B	3C	3D	Q
<i>Phaenopsectra</i>		6	4		7	19	3									
<i>Polycentropes</i>						1										
<i>Polypedilum</i>					5	3	2			1						
<i>Probezzia</i>	13	6	3		11	37	27				1				1	
<i>Protoplasa fitchii</i>					2											
<i>Rhagovelia</i>												*				*
<i>Rheotanytarsus</i>					1				5	11	3					
<i>Silvus</i>														1		
<i>Sphaeridea</i>			2						4							
<i>Sphaerium</i>					1	6	2									
<i>Spinactalets</i>					8											
<i>Stenochirnomus</i>	3		1			10										
<i>Tanytarsus</i>	1860	555	192		738	2953	1490		1		3					
<i>Thienemannimyia</i>					1	4	5		2	1					1	
<i>Tipula</i>							2									
<i>Trepobates</i>	1	2														
<i>Tvetenia</i>									18	16	43					
<i>Zygoptera</i>						127										
Total Species	25	25	20		31	35	50		26	25	23		9	13	10	
Total Individuals	2515	916	358		1510	4239	2562		996	753	1531		966	7160	4189	
Species Diversity	1.176	1.509	1.757		1.881	1.432	1.607		1.395	1.285	1.061		0.208	0.123	0.811	

\*Indicates presence in qualitative samples

Appendix 2D. Macroinvertebrates Collected October 2011.

TAXA	Smith						Canyon					
	3A	3B	3C	3D	4A	4B	4C	4D	6A	6B	6C	6D
<i>Anisoptera</i>						1						
<i>Aquarius</i>												*
<i>Archilestes</i>			1									
<i>Argia</i>	19	21	9	59	80	10	*	3	6	28	*	1
<i>Atherix</i>										1		
Baetidae										3		
<i>Beatis</i>				1	25					1		
<i>Caecidotea</i>					1						2	
<i>Caloparyphus</i>										5		1
<i>Chironomus</i>			1									
Coenagrionidae						2						
<i>Cordulegaster</i>	1											
Corixidae		1										
<i>Corynoneura</i>			3	177	110	12	2	8	39		1	
<i>Cryptochironomus</i>	2	6	4	1	9	15						
<i>Culicoides</i>	1	1	6	17	14	13					3	
Cyclopoida	127	151	126	5	13	4	1					
<i>Dicrotendipes</i>					2							
<i>Dixa</i>		3			1							3
<i>Dugesia</i>	21	17	41	155	268	41	4	24	239	6	41	7
Empididae					3	2						
Ephemeroptera		131	80	2	6							
<i>Ergasilus</i>				1								
<i>Eukiefferiella</i>									5			



Appendix 2D, continued.

TAXA	Smith						Canyon							
	3A	3B	3C	3D	4A	4B	4C	4D	6A	6B	6C	6D		
<i>Glaenocoris</i>			1											
Harpacticoidia	4		2	14	38	9								
<i>Heleniella</i>	9			44	56	2								
<i>Helicopsyche</i>	6	5	3	*	20	20	9	6	52	201	6	1		
<i>Hemerodromia</i>		1			2									
<i>Hetaerina</i>			1					1						
<i>Hyallela azteca</i>	2	1		1		1	452	1026	5646	*	3391	3330	3832	*
Hydropsychidae			4	20	49	1			8					
Hydroptila			1		7	3								
Isotomidae					1									
Laccophilinae			1											
<i>Larsia</i>	36	34	7	15	33	33			1					
Leptohyphidae	307	61	152		8				12					
<i>Limnodrilus</i>	21	13	14	295	585	387				6	15	56		
<i>Lumbriculus</i>		31		19	56	28			2	2	2	2		
Nematoda	16	16	15	76	325	102	2			1				
Odonata			1	9										
<i>Ochrotrichia</i>		3	3	26	27									
<i>Ordobrevia</i>	27	30	22	34	91	6			1					
<i>Orthocladus</i>			2		1	1					24			
<i>Parametriocnemus</i>		1		192	816	3			3					
<i>Paratanytarsus</i>		1			6									

Appendix 2D, continued.

TAXA	Smith						Canyon					
	3A	3B	3C	3D	4A	4B	4C	4D	6A	6B	6C	6D
<i>Paratendipes</i>	30	146	66		5	20						
<i>Paratrichocladus</i>										1		
Pelecypoda	15	50	15	28	168	257	1					
<i>Peltodytes</i>		1		1								
<i>Pericoma</i>					3							
<i>Phaenopsectra</i>		17	4	4	15	5						
<i>Physa</i>	1			*								*
<i>Probezzia</i>	2			13	21	6	1					
<i>Procladius</i>			2			1						
<i>Rhagovelia</i>							1		*			*
<i>Rheotanytarsus</i>	4											
Sphaeridea				2								
<i>Sphaerium</i>		7	2	1	7	26						*
<i>Stenochironomus</i>	1	1	1	3								
<i>Tabanus</i>											1	
<i>Tanytarsus</i>	928	590	391	323	325	108						
<i>Thienemannimyia</i>					2							
<i>Tricorythodes</i>	1	9										
<i>Tvetenia</i>									3			
Total Species	22	28	31	29	37	30	10	6	17	8	9	7
Total Individuals	1581	1349	981	1558	3199	1108	473	1117	6198	3414	3419	3902
Species Diversity	1.448	2.048	2.048	2.351	2.444	2.134	0.269	0.373	0.429	0.052	0.002	0.104

\*Indicates presence in qualitative samples

Appendix 2E. Macroinvertebrates Collected January 2012.

TAXA	Cave								Smith								Canyon							
	6C	6B	6A	Q	1B	1D	2D	Q	8C	8B	8A	Q	7B	7A	8D	Q	13A	7D	7C	Q	13D	13C	13B	Q
<i>Agabus</i>		2			1		1				1													
<i>Aquarius</i>												*				*								
<i>Argia</i>									1	1			4	11	4		5	5	10	*			1	*
<i>Atherix</i>																*	1							
<i>Beatis</i>													1	4	5				7					
<i>Buenoa</i>																								
<i>Caecidotea</i>					1				1	2			3	2	3						1	1		
<i>Caloparyphus</i>																	2				1	1	1	
<i>Calopteryx</i>									1		1													
<i>Chironomus</i>									1					1										
<i>Corynoneura</i>		12	682							1	1		2	31	4		22		10		1		2	
<i>Corynothorix</i>															14									
<i>Cricotopus</i>									1				1		1									
<i>Cryptochironomus</i>									1		1		3					1						
<i>Culicoides</i>		1	3						3		1		1				23		3		4	6	8	
Cyclopoida	3	25	29						1															
<i>Dicrotendipes</i>		1															3		10					
<i>Dixa</i>																	1	1						*
<i>Dolophilodes</i>																	1							
<i>Dugesia</i>		1	24		1				11	4	3	*	26	86	35	*	131	11	53		23	29	63	*
Dytiscidae		6	4		1																			
Empididae											1													
Ephemeroptera		1							1		1								1	1				
<i>Eukiefferiella</i>														2			1		1					

Appendix 2E, continued.

TAXA	Cave						Smith						Canyon							
	6C	6B	6A	1B	1D	2D	8C	8B	8A	7B	7A	8D	13A	7D	7C	13D	13C	13B		
<i>Harpacticoidia</i>		25	1755																	
<i>Heleniella</i>							1			2										
<i>Helicopsyche</i>							3	8	3	57	51	46	37	8	91	*		1	*	
<i>Heterina</i>															1					
<i>Hyalpella azteca</i>	1		4										603	78	1053	*	3364	2813	4109	*
<i>Hygrotus</i>		3	13			*	1		3											
Hydropsychidae		1	52				2			2	13	4								
<i>Hydroptila</i>							5			3	8	10								
Isopoda												2								
<i>Larsia</i>		8				2	4			5		2			1					
Leptohyphidae													3		35					
<i>Limnodrilus</i>	1		151	2	9	2	3	13	17	114	81	31				59	9	3		
<i>Lumbriculus</i>			12				1	4		2		3	3		3	10				
Nematoda		5	126			1	7		4	29	30	10	1	3	1	2	1	4		
<i>Neurocordulia</i>																				
<i>Ochrotrichia</i>		2	370		1	1	2				6		1	1	1					
Odonata								1	1		19	59	4		3			4	2	
<i>Ordobrevia</i>							3			1	9	2								
<i>Orthocladus</i>	1	1					10		1	7	34		52		6					
<i>Parametriocnemus</i>		1	15		8					1	5	1	1	1	3	1				
<i>Paraspectra</i>																				
<i>Paratanytarsus</i>			2							2			2		2					

Appendix 2E, continued.

TAXA	Cave						Smith						Canyon					
	6C	6B	6A	1B	1D	2D	8C	8B	8A	7B	7A	8D	13A	7D	7C	13D	13C	13B
<i>Paratrichocladius</i>								1				1						
Pelecypoda		1						1	1	42	13			1	6		1	1
<i>Peltodytes</i>												7						
<i>Pericoma</i>							1				1							
<i>Phaenopsectra</i>			1															
<i>Physa</i>	6	54	15	*	5	*												
<i>Polycentropes</i>		1																
<i>Probezzia</i>			17						1	5	2							
<i>Pteronanyis</i>			1															
<i>Petronarcella</i>			2															
<i>Rhagovelia</i>															*			
<i>Rheotanytarsus</i>													1		2			
<i>Sphaerium</i>										18	7	3						
<i>Stenochironomus</i>												1						
<i>Tanytarsus</i>							1188	161	180	752	643	399			4		1	
<i>Thienemannimyia</i>													1					
<i>Trepobates</i>										*								
<i>Tricorythodes</i>													12		11			
<i>Tvetenia</i>			5				1						2		2			
Total Species	5	19	21	5	4	5	25	11	16	24	22	24	24	11	25	10	10	11
Total Individuals	12	151	3283	6	23	7	1254	197	220	1079	1062	649	937	111	1345	3466	2866	4195
Species Diversity	1.314	2.053	1.483	1.561	1.203	1.549	0.353	0.813	0.856	1.251	1.638	1.585	1.301	1.158	0.935	0.169	0.119	0.122

\*Indicates presence in qualitative sample

