

THE ECOLOGY OF HYPORHEIC INVERTEBRATES  
IN OKLAHOMA AND ARKANSAS STREAMS

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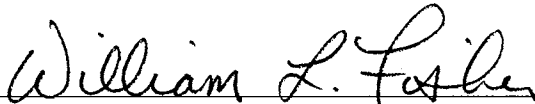
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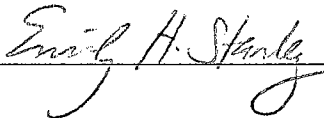
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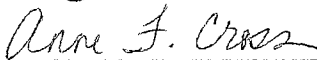
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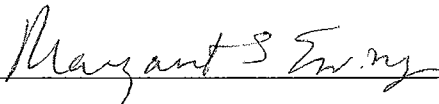
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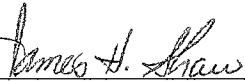


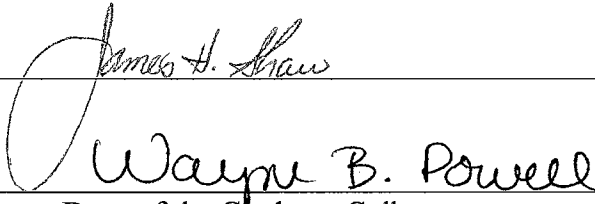
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## CHAPTER I

### INTRODUCTION

The hyporheic zone is the saturated subsurface region of streams and rivers which plays an important role in nutrient cycling and ecosystem metabolism and often harbors an unusual yet poorly studied invertebrate fauna. In the past 15 years, there has been a growing awareness of the key role of the hyporheic zone in strongly influencing ecological conditions in streams. However, research on these important habitats has been restricted to only a few locales. Most research has addressed ecosystem-level processes rather than focusing at the population-community level. Therefore there are significant gaps in our understanding of hyporheic ecology across geographically diverse regions, particularly concerning the unique fauna of these environments. These gaps exist despite the strong influence of hyporheic dynamics on many ecosystem processes, as well as the fact that hyporheic invertebrates may be potent indicators of local hydrologic conditions including pollution.

The following chapters address several of these concerns. Chapter II addresses the influence that environment has on the composition and abundance of the hyporheic fauna in 16 streams distributed over a large geographical area, i.e., the state of Oklahoma. The spatial and temporal distribution of hyporheic fauna in 5 of the Oklahoma streams is described in Chapter III. An assessment of the impact of treated sewage wastewaters on 4

Arkansas streams is reported in Chapter IV. In Chapter V are presented the results of an evaluation of alternatives to the sampling methods used in the above studies.

The following chapters were written in the format required for manuscript submittal for publication in Archives fur Hydrobiologie (Chapters II and III), the Journal of the North American Benthological Society (Chapter IV) and the Canadian Journal of Aquatic Sciences and Fisheries (Chapter V).



CHAPTER II

ENVIRONMENTAL FACTORS INFLUENCING THE COMPOSITION  
AND DISTRIBUTION OF HYPORHEIC FAUNA  
IN OKLAHOMA STREAMS

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**Abstract:** The hyporheic zones within 16 streams representing five ecoregions of Oklahoma were sampled. These included gravel-bottomed streams located in the Hot Continental and Subtropical Ecoregions in the east and streams with sand substrates in the central and western areas of the state (Prairie, Tropical/Subtropical Steppe and Temperate Steppe Ecoregions). Samples were collected using the Bou-Rouch method at well depths of 30 cm, 60 cm, and 100 cm below the stream bottom at midstream and at the same depths below the water level within instream bars and on lateral banks. Taxon richness and total abundances were greatest in eastern streams and decreased in streams located in the central and western portions of the state. Cyclopoid and harpacticoid copepods dominated the hyporheos in the eastern streams, whereas Chironomidae and Nematoda were often the only components in some western streams. Major factors influencing the composition and abundance of the hyporheos in Oklahoma streams as indicated by ordination and supported by Spearman rank correlations include longitude, substrate characteristics, and dissolved oxygen concentrations. Ongoing studies will provide a greater understanding of both spatial and temporal distributional patterns as related to environmental conditions.

## **Introduction**

Recent investigations have revealed a diverse assemblage of invertebrates inhabiting the hyporheic zone within North American streams. Initial studies include those of gravel-bottomed streams in Canada (Williams & Hynes 1974, Godbout & Hynes 1982) and Montana (Stanford & Gaufin 1974) and of sandy substrates in Minnesota (Urban 1971) and in Texas (Whitman and Clark 1984). More recent investigations include those in Colorado (Pennak and Ward 1986), Virginia (Palmer 1990, Strommer & Smock 1989), New York (Strayer 1988), Arizona (Boulton et al. 1992, Boulton & Stanley 1995), California (McElravy & Resh 1991), and Kansas (Rensner & Distler 1992, Turner & Distler 1995). The hyporheic zone includes the interstitial waters within sediments in the stream bed and adjacent shores (McElravy & Resh 1991). The fauna inhabiting these waters, identified as the hyporheos, consists of organisms from both the surface water, i.e., epigean (usually larval forms) and those found only in the interstitial waters, i.e., hypogean, and include protozoans, turbellarians, nematodes, rotifers, tardigrades, oligochaetes, insects, water mites, and crustaceans (Strayer 1994). The composition, abundance, and distribution of the hyporheos are dependent on many factors. Ward and Palmer (1994) suggested these factors include physical characteristics of the alluvium, exchange characteristics between groundwater and surface water, availability of food resources, biotic interactions, disturbance, hypogean affinity, reproductive patterns, and age distribution. They also suggested that geomorphic and hydrogeological features and interactions are the major determinants that structure the patterns of the hyporheos across

a range of spatial scales. Others have suggested that sediment grain characteristics and the availability of oxygen and organic matter are the most important determinants of composition and abundance of the hyporheos (Danielopol 1991, Boulton et al. 1992, Strayer 1994, Strayer et al. 1997). Composition has also been shown to be correlated with the glacial history (Strayer et al. 1995).

With the exception of Strayer et al. (1997), the hyporheic studies in North America have been limited to a single stream or streams with similar characteristics. The objective of the present study was to investigate the variation in composition and abundance of the hyporheos inhabiting streams across a large and diverse geographical area with no glacial history (Oklahoma, USA) in terms of variation of environmental factors. We describe spatial patterns in hyporheic assemblages and their relationships to physical and chemical habitat attributes.

### **Study Area**

Oklahoma covers a relatively large geographical area, i.e., 182,100 km<sup>2</sup> stretching 1,210 km from east to west and 613 km from north to south. Drainage is generally eastward; the northern two-thirds of the state is contained within the Arkansas River drainage and the southern third lies within the Red River drainage. Elevations range from almost 1525 m above sea level in the northwestern corner of the state to less than 92 m above sea level in the southeastern corner. Annual rainfall varies considerably, from less than 40 cm in the northwest to 127 cm or more in the east (Pettyjohn et al. 1983).

Vegetation in the state includes oak-hickory forests in the northeast, oak-pine forests in the southeast, tall grass prairies in the central and west, and short grass prairies in the northwest. The wide range of ecological habitats available within the state is reflected by the occurrences of five ecoregions within the state (Bailey 1996).

During the summers (June-August) of 1996 and 1997, we conducted a survey of the hyporheos among the diverse geographical areas of Oklahoma. Although a larger number of streams was visited, hyporheic samples were successfully obtained from 16 streams. The location of these streams within the five ecoregions of the state are presented in Fig. 1. Physical descriptive data are summarized in Table 1.

The northeastern portion of the state lies within the Hot Continental Ecoregion (HCE), including the western portion of the Ozark Plateau, and is located within the Arkansas River drainage. Streams within this region are fed by springs and have deeply incised channels with bed sediments consisting of chert and limestone gravels. Five streams within this region are included in this study: Baron Fork River, and Peacheater, Saline, Snake and Summerfield creeks.

The Subtropical Ecoregion (STE) in the southeastern corner is occupied by the Ouachita Mountains which rise to 610 m above sea level. Bedrock forms the bottom of most streams within this region, although gravel beds occur sporadically. The gravel beds of the Glover River, a tributary to the Red River, are included in this investigation.

Much of the central portion of the state is included within the Prairie Ecoregion (PE). This region consists of flat to rolling plains generally used for pasture and cropland.

Four PE streams are included in this investigation. Wild Hog Creek, located in the Tall Grass Preserve in the north and within the Arkansas River drainage, is characterized by pools separated by riffle areas consisting of silt, gravel, and cobbles. Brier, Rock, and Wolf creeks, all located in the southern PE and within the Red River drainage, are deeply entrenched streams draining relatively flat pasture and croplands. The sediments in Brier Creek consist of silt, sand, and fine gravel, whereas the sediments in both Wolf and Rock creeks are composed of medium to coarse sands.

The Tropical/Subtropical Steppe Ecoregion (TSSE) extends into the southwest corner of the state. Terrain is relatively flat, again with land used predominantly for pasture and cropland. This region is represented by two relatively large tributaries to the Red River: the Salt and Elm Forks. Bed sediments in both streams consist of shifting sands, although the sediments in the Elm Fork are coarser and locally contain small gravel.

The Temperate Steppe Ecoregion (TSE) encompasses the northwestern quarter of the state including the panhandle. Surface terrain ranges from rolling pastureland in the east to mesas approaching 1525 m above sea level in the west. Three streams were sampled during our survey. The Cimarron River, a major tributary to the Arkansas River, was sampled at two locations. Cimarron 1 is located at its eastern end where the stream meanders within a broad alluvial channel consisting of fine to coarse sand sediments. Cimarron 2 is located in the far northwestern corner of the state where it is known as the Dry Cimarron River. Sediments include coarse sand and gravel. According to the local landowner, Cimarron 2 had been dry for several months prior to our sampling. Flow had

returned only after recent rainfall. The other two streams were tributaries to the Cimarron. Turkey Creek, located near Cimarron 1, is a deeply entrenched stream draining pasture and cropland. Bed sediments consist of silt, sand, and small gravel. North Carrizo Creek, a tributary to the Dry Cimarron, also appeared to be an ephemeral stream. Bed sediments include silt, sand, and cobbles.

## **Methods**

Hyporheic samples were collected using the Bou-Rouch method of suctioning water from narrow wells (Bou 1974). At each stream site, hyporheic water was collected by installing wells of 2.5 cm ID PVC tubing. Wells were installed immediately prior to sampling at all streams except at Wild Hog Creek. The wells at this stream had been installed several months prior to the sampling results reported here. The tubing, perforated with 6.0 mm holes along the lower 15 cm, was driven into the stream's substratum by inserting a steel T-shaped driving rod into the well and driving the rod and well so that the top of the perforated section reached the desired depth. The T-bar was then removed, leaving the well in place.

Hyporheic samples, ranging in volume from 1-10 L, were collected from the hyporheic zone at three depth intervals where possible: 30-45 cm, 60-75 cm, and 100-115 cm below the stream bottom at mid-channel stations or below the water table at stations located on instream bars or on lateral banks. All samples were concentrated with a 63  $\mu\text{m}$  mesh sieve and preserved in 5% formalin. Organisms were identified to recognizable

taxonomic units using a dissecting microscope. Since many of the organisms belonged to taxonomically difficult groups and many of them were immature, most were identified to genus, family, or higher level. Crustacea, with the exception of the ostracods, were identified to the genus and/or species level. Although the copepod nauplii were counted, these data are not included herein. Sorting of the invertebrates from sample debris was aided by the addition of rose bengal stain.

Water samples were collected at each depth for dissolved oxygen (DO), conductivity, pH, alkalinity, nitrate-N ( $\text{NO}_3\text{-N}$ ), soluble reactive phosphate (SRP), sulfate ( $\text{SO}_4^{2-}$ ), and chloride ( $\text{Cl}^-$ ). DO was determined by Winkler titration, and alkalinity by titration with 0.2 N  $\text{H}_2\text{SO}_4$  (Wetzel and Likens 1991). Conductivity and pH of unfiltered samples were measured in the laboratory with Orion meters. Following filtration through a 0.7  $\mu\text{m}$  glass fiber filter,  $\text{Cl}^-$ ,  $\text{NO}_3\text{-N}$ , and  $\text{SO}_4^{2-}$  were measured with a Dionix DX-100 ion chromatograph and SRP was analyzed using the molybdate blue method (Murphy and Riley 1962). Temperature was measured in situ at each depth by lowering a thermometer into the well. To provide information on substrate characteristics, one to three representative sediment samples were collected from surface deposits at each site. In the laboratory, the sediments were dried at 100°C for 24 hours and grain-size distribution was measured by dry-sieving samples through a sieve series (6.3 mm, 2 mm, 0.5 mm, and 0.25 mm mesh). Throughout this report the sorted grain size classes will be referred to as follows: > 6.3 mm = gravel, 2-6.3 mm = very coarse sand, 0.5-2 mm = coarse sand, 0.25-0.5 mm = medium sand, < 0.25 mm = fine sand.



The relationship between physical and chemical environmental factors and the abundances of the hyporheos were investigated using two statistical approaches. The distribution and composition of hyporheic assemblages were related to environmental variables by canonical correspondence analysis (CCA) (ter Braak 1986) using the program CANOCO for Windows 4.0 (ter Braak and Smilauer 1997). This ordination technique directly relates community attributes (taxon abundances) to environmental variables for evaluation of community-environmental relationships (ter Braak 1987). This technique can also be used to summarize faunal assemblages and to reveal relationships among streams based on their biota. Thus, within a CCA ordination diagram, sites with similar taxonomic composition occur most closely together and each species is located at the centroid of the site locations in which it occurs. CCA extracts from the measured environmental and biotic variables, synthetic gradients (ordination axes) that maximize the niche separation among species. Environmental gradients are represented on the ordination diagram by arrows pointing in the direction of maximum change for each associated variable and with the relative length of each arrow indicating the variable's relative importance. Individual taxa are related directly to these axes under the assumption of a unimodal species response to the environmental variables. The location of site scores relative to the arrows indicate the environmental preferences of each species. Additional information regarding the interpretation of CCA ordination diagrams is provided by ter Braak and Verdonschot (1995). Eigenvalues are calculated that indicate the degree of correlation between species and sites. An eigenvalue near 1 indicates a high

degree of correlation and an eigenvalue near 0 indicated little correspondence. Taxa counts were square root transformed and abundances for rare taxa were down weighted in order to prevent extremely abundant or extremely rare taxa from having unique influence on the ordination (Gauch 1982). In addition, Spearman rank correlations were computed between the taxon abundances (number of organisms/L) and physical and chemical variables.

## **Results**

### *Physical and Chemical Data*

Selected physical and chemical data and sediment size distribution data are summarized in Tables 2 and 3, respectively. Streams located in the HCE were characterized by relatively low temperatures (17.7 to 25.6 °C) and conductivity (163 to 367  $\mu\text{S}/\text{cm}$ ) and high  $\text{NO}_3\text{-N}$  (0.41 to 2.70 ppm) and DO concentrations (4.8 to 8.9 ppm). SRP concentrations varied considerably (0.007 to 0.242 ppm). The Glover River located in the STE exhibited relatively low conductivities and nutrient concentrations and intermediate DO levels. Streams in both the HCE and STE were characterized by gravel substrates with 65-92 % of sediments exceeding 6.3 mm.

With the exception of Wild Hog Creek, streams within the PE, TSSE, and TSE exhibited smaller sediments (39 % or less of sediments exceeded 6.3 mm) and relatively low dissolved DO (0.21 to 1.1 ppm). These streams, including Wild Hog Creek, also exhibited relatively high conductivities (420 to 2666  $\mu\text{S}/\text{cm}$ ) and low SRP levels (0.014 to

0.073 ppm).

### *Faunal Composition*

A total of 95 samples were collected from the 16 streams. Total invertebrate density averaged 23 organisms/L for all samples. A total of 41 taxa was identified from the 16 streams (Table 4).

Cyclopoid copepods were the most abundant group comprising 50 % of all organisms and represented by at least 11 species (Fig. 2). These included at least two undescribed species of Diacyclops and one or more undescribed species each of Acanthocyclops, Microcyclops, and Paracyclops (Janet Reid, Smithsonian Museum, Washington, D.C., pers. comm.). Members of the genus Diacyclops, predominately D. yeatmani, comprised more than 95 % of all cyclopoids. Two other common cyclopoids included Eucyclops agilis and Macrocyclops albidus.

Harpacticoid copepods were the next most abundant component of the hyporheos, representing 16.4 % of all organisms. This group also included several undescribed species. More than 73 % of all harpacticoids were members of the family Canthocamptidae. Representative genera included Attheyella, Bryocamptus, Elaphoidella, and Moraria. Several species of Parastenocaris (26.2 % of harpacticoids) were also encountered. One representative of the family Ameiridae, i.e., Nitocra lacustris, also was collected.

Other less abundant crustaceans included Ostracoda, Chydoridae, Isopoda, and

Amphipoda. The chydorids were dominated by three species: Alona circumfimbriata, Alonella excisa, and Pleuroxus denticulatus. The Isopoda and Amphipoda were represented by the genera Caecidotea and Stygonectes, respectively.

Nematoda was the most abundant non-crustacean group comprising 9 % of all organisms. These were followed by the Insecta (7 orders representing 8 % of all organisms), Oligochaeta (2.4 %), and Acari (1.3 %). No other group comprised more than 1 % of the hyporheos. The Chironomidae and Plecoptera composed 43 % and 40 %, respectively, of all insects encountered. Other insects collected infrequently included the Ceratopogonidae, Trichoptera, Coleoptera, and Ephemeroptera. The insects were represented by, in almost every instance, early instars of the larval stages.

### *Geographical Distribution*

Streams located in the eastern portion of the state (HCE and STE) exhibited both greater densities (Fig. 3) and number of taxa (Fig. 4). The highest mean densities for the three depths sampled, i.e., 30, 60 and 100 cm, was 276/L in Saline Creek. An average of 11 taxa was identified for all samples collected from Saline Creek. Other streams exhibiting relatively high densities included Summerfield Creek (71/L), the Glover River (57/L), Peacheater Creek (57/L), and Wild Hog Creek (49/L). The lowest densities were observed (3/L or less) in the Cimarron River (both stations), Turkey Creek and the Salt Fork. Taxa richness was also generally lower in these streams.

The three most abundant invertebrate groups characteristic of each stream are

summarized in Table 5. Cyclopoid copepods were a dominant group in all HCE streams, the Glover River, and 3 of the PE streams (Wild Hog, Rock, and Brier creeks). However, the cyclopoids were rare or absent in the western regions (TSSE and TSE) of the state.

Harpacticoid copepods were the next most abundant invertebrate group, encountered in 9 of the 16 streams investigated, including all five HCE streams where they comprised from 13.1 to 33.1 % of the hyporheos. In addition, they composed 20.2, 12.9, and 2.4 % of the hyporheos in Wild Hog Creek, the Elm Fork, and the Glover River, respectively, the only other streams where they were encountered during the summer survey.

Streams located in the central and western portions of the state, i.e., Turkey, Brier, Wolf, and North Carrizo creeks, the Elm and Salt forks, and both Cimarron River locations (PE, TSSE, and TSE) were more often characterized by a hyporheos dominated by insects, predominantly Chironomidae, and/or nematodes. Ostracods were also a major component in the downstream Cimarron River, Elm Fork, and Summerfield Creek.

### *Community Ordination*

Variables that contributed most to the explanation of relative abundances of taxa were chosen from the habitat attributes listed in Tables 1 and 2 and the environmental variables listed in Table 3 by the stepwise analysis (forward selection procedure) in CANOCO, with a 5 % significance level as a cutoff. These included longitude, latitude, depth, all grain sizes except fine sand, temperature, alkalinity,  $\text{Cl}^-$ ,  $\text{NO}_3\text{-N}$ , SRP, and DO.

Eigenvalues for the first four CCA axes account for 28 % of the variation observed in the species data (Table 6) with the first 2 axes accounting for most (19 %) of the variation. The distribution of sampling sites grouped by ecoregion relative to the first 2 axes are diagramed in Fig. 5. Site scores located closer together indicates greater similarity in community composition. With one exception, Fig. 5 illustrates the similarities of sites within each ecoregion. The faunal composition at Wild Hog Creek, located in the northern portion of the Prairie Ecoregion, differs considerably from the more southern prairie streams. A biplot of taxa and environmental gradients (indicated by arrows) relative to these axes is presented in Fig. 6. In general, increases in  $\text{Cl}^-$ , medium and coarse sand, and longitude were associated with decreases in DO, gravel, and latitude along the first axis. The second axis is positively related to temperature and to a lesser degree, negatively related to depth, nutrient concentrations, and very coarse sand.

Taxa strongly associated with longitude, chloride, and the intermediate grain sizes included Nematoda, Ceratopogonidae, Chironomidae and the harpacticoids Nitocra lacustris and Bryocamptus morrisoni, both of which were encountered exclusively in the Elm Fork. Taxa whose presence was more highly correlated with higher DO included most crustaceans, Hydra, and Tardigrada.

The abundances of several groups, predominantly microcrustaceans, were highly correlated with temperature. These included Ephemeroptera, , Hydra, chydorids, Moraria cristata, and the cyclopoids Tropocyclops, Paracyclops, Macrocyclus, Eucyclops agilis, and Acanthocyclops. Taxa negatively related to temperature and positively related to

depth, very coarse sand, and nutrient level included Amphipoda, Isopoda, Ostracoda, Tardigrada, and the copepods Diacyclops, Attheyella, and Parastenocaris.

### *Spearman Rank Correlations*

Spearman rank correlation analyses supported the trends indicated by CCA. Total invertebrate abundances were positively correlated with latitude, precipitation, DO concentration, and the larger sediments (> 6.3 mm); and were negatively correlated with longitude, drainage basin area, conductivity,  $\text{Cl}^-$ ,  $\text{SO}_4^{2-}$  and smaller sediments (Tables 7, 8, and 9). This was generally true for most groups, including the cyclopoid and harpacticoid copepods, Ostracoda, Isopoda, Chydoridae, Acari, and the Oligochaeta. An exception, the harpacticoid Nitocra lacustris, collected only in the Elm Fork, was positively correlated with longitude, temperature, conductivity, and  $\text{SO}_4^{2-}$ , and negatively correlated with precipitation. Members of this genus have been referred to as brackish water forms (Borutskii 1964).

### **Discussion**

Hyporheic communities of Oklahoma streams varied widely throughout the 5 ecoregions included within the state and appeared to be strongly influenced by the types of hyporheic habitats that are available. Habitats included within this investigation range from spring fed streams with gravel substrates in the northeastern portion of the state (HCE) to ephemeral streams in the far northwestern corner (TSE).

The majority of the organisms collected can be considered as permanent hyporheos (Gibert et al. 1994). These include nematodes, oligochaetes, mites, copepods, ostracods, chydorids, and tardigrades. The majority of the insects are classified as occasional hyporheos since only the early instars occur in the hyporheic zone. The only obligatory hyporheos encountered during this study include Caecidotea, Stygonectes, and Parastenocaris.

Taxon richness and total abundances were greatest in eastern streams and decreased in streams located in the central and western portions of the state. Cyclopoid and harpacticoid copepods dominated the hyporheos in the eastern streams, whereas Chironomidae and Nematoda were often the only components in some western streams, a finding consistent with previous findings (Bass & Walker 1992). The two most abundant cyclopoids, D. yeatmani and A. pennaki, have been previously reported from subsurface environments in Tennessee and Illinois (Yeatman 1964) and Colorado (Reid 1992a), respectively. The finding of several previously undescribed cyclopoid copepods in the Oklahoma streams is comparable with other hyporheic studies in North America (Reid 1992b). Noticeably absent from all samples collected were the Syncaridae, although several specimens have been collected during previous sampling in the alluvium just upstream of the Cimarron River 2 site (unpublished data). Also of note was the rareness of the Acari in this study, a group which has been found in relatively large numbers in hyporheic waters over a broad range of geographical settings, e.g., Arizona (Boulton et al. 1992) and New York (Strayer 1988).



Major factors influencing the composition and abundance of the hyporheos in Oklahoma streams include longitude, sediment grain size, and DO concentrations. Although annual rainfall varies across the state, ranging from more than 127 cm in the east to less than 40 cm in the far west, this factor was not statistically significant in the ordination of the streams. This is not surprising, however, because with the exception of North Carrizo Creek and the upstream station on the Cimarron River (Cimarron 2), the streams included within this investigation were perennial and their hyporheos would be minimally affected except during severe drought.

The results of this investigation suggest that the most important factor determining the composition and abundance of invertebrates within the hyporheic zone of permanent streams across Oklahoma is the composition of the substrate. Substrate grain sizes generally decrease with increasing longitude (from east to west), depending on surficial geology and land use. Taxon richness and total abundances both decreased as substrate size decreased. Smaller grain size limits the movement of invertebrates which they must be small or burrowers (Fleeger & Decho 1987). In addition, smaller interstitial spaces between smaller grains reduce hydrologic exchange between hyporheic and surface waters thus limiting the replenishment of DO. Smaller sediments are also more susceptible to disturbance due to scour during spates.

Sediment grain sizes less than 0.2 mm have been reported to restrict colonization by interstitial invertebrates (Wieser 1959, McLachlan 1978, Fleeger & Decho 1987). The Oklahoma streams with fine to coarse sand substrates (<0.2 mm) were characterized by a

hyporheos dominated by Nematoda and Chironomidae, a finding in agreement with investigations in similar habitats (Whitman & Clark 1984, Strommer & Smock 1989). However, these groups were encountered in low numbers in most streams included in this study. Their dominance in the coarse grained substrates was because copepods were rare or absent.

The larger the proportion of smaller grains within a substrate, the smaller the interstitial spaces available, thus retarding the rate of exchange with surface waters and limiting the replenishment of DO. Typically, streams with smaller sediments exhibit decreasing invertebrate densities with increasing depth, directly related to decreasing DO levels (Whitman & Clark 1984, Strommer & Smock 1989, Strayer et al. 1997). In only a few centimeters, anoxic conditions can develop in such sediments if organic matter is present. The organic content of sediments was not addressed in this investigation.

Sand substrates are also highly susceptible to scour during spates (Whitman & Clark 1984, Resh et al. 1988, Palmer et al. 1992). Environments subject to disturbance are characterized by opportunistic and rapid colonizers which results in communities of simple structure and low diversity (Giller 1984). Thus, sand substrates typically exhibit lower diversity and abundances, consistent with our findings.

Streams located in the eastern portion of the state exhibited higher taxa richness and abundances, dominated by cyclopoid and harpacticoid copepods. The diversity of copepods, including several undescribed species, agrees with the findings of Strayer et al. (1995) in unglaciated regions in the southeastern portion of North America. The eastern

streams were characterized by gravel substrates of high hydraulic conductivity which allowed a greater rate of exchange with oxygen-rich surface waters. Gravel is also more resistant to movement during spates than sand thus reducing the frequency of disturbance, which may in turn foster greater diversity (Giller 1984).

The results of this investigation indicate the importance of substrate type and DO concentrations on the composition and abundance of invertebrates inhabiting the hyporheic zone within Oklahoma streams and are consistent with the results of previous investigations (Strayer 1994 & 1997, Ward & Palmer 1994, Ward et al. 1994). These results suggest that additional research should address those activities that may adversely affect these attributes in streams that currently support a rich hyporheic fauna.

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Table 1. Location and physical characteristics of 16 Oklahoma streams.

Stream	Location		Channel Width (m)	Annual Precipitation (m)	Drainage Area (km <sup>2</sup> )
	Latitude	Longitude			
<u>Hot Continental</u>					
Baron Fork	35°55'	95°51'	41	1.22	795
Peacheater	35°58'	94°41'	13	1.22	65
Saline	36°21'	94°58'	20	1.27	111
Snake	36°09'	95°08'	11	1.17	104
Summerfield	35°27'	95°00'	20	1.22	29
<u>Subtropical</u>					
Glover River	34°05'	94°54'	87	1.32	816
<u>Prairie</u>					
Wild Hog	37°51'	96°21'	26	0.91	13
Rock	34°35'	96°58'	26	0.91	114
Brier	33°55'	97°55'	10	0.97	54
Wolf	34°11'	96°56'	13	0.97	23
<u>Tropical/Subtropical Steppe</u>					
Elm Fork	34°55'	99°31'	20	0.61	2,170
Salt Fork	34°37'	99°25'	20	0.61	4,533
<u>Temperate Steppe</u>					
Turkey	35°59'	97°56'	26	0.76	1,100
Cimarron 1	35°58'	97°55'	120	0.76	40,697
Cimarron 2	36°53'	102°51'	20	0.36	2,865
North Carrizo	36°55'	102°58'	5	0.36	1,088

Table 2. Water chemistry data for 16 Oklahoma streams ( $\bar{x}$  = mean; SD = standard deviation; n = number of samples).

Stream	Temperature (°C)			Dissolved Oxygen (mg/L)			pH			Total Alkalinity (mgCaCO <sub>3</sub> /L)		
	$\bar{x}$	SD	n	$\bar{x}$	SD	n	$\bar{x}$	SD	n	$\bar{x}$	SD	n
<u>Hot Continental</u>												
Baron Fork	25.6	1.65	9	4.9	1.52	8	7.28	0.10	9	153.8	37.70	8
Peacheater	23.3	0.79	6	7.3	0.45	6	7.19	0.07	6	120.0	20.00	6
Saline	19.5	0.58	4	8.9	0.55	4	7.43	0.05	4	103.0	19.80	4
Snake	18.7	0.58	3	4.8	0.44	3	7.05	0.05	3	111.7	11.15	3
Summerfield	17.7	1.15	3	6.8	1.57	3	6.80	0.00	3	96.0	15.10	3
<u>Subtropical</u>												
Glover River	25.3	0.50	4	3.7	3.00	4	6.66	0.29	4	31.0	3.83	4
<u>Prairie</u>												
Wild Hog	29.7	2.57	12	5.2	2.99	12	7.90	0.17	12	174.3	5.84	12
Rock	27.0	1.22	6	1.1	1.41	6	7.36	0.12	6	211.0	20.00	6
Brier	25.3	0.65	4	1.1	nd	1	7.08	0.09	4	305.3	25.20	3
Wolf	25.5	1.02	6	nd	nd	0	7.21	0.25	6	432.7	91.93	6
<u>Tropical/Subtropical Steppe</u>												
Elm Fork	27.4	0.51	18	0.6	0.51	18	7.46	0.06	18	127.9	17.28	18
Salt Fork	26.0	0.25	9	0.7	0.94	7	7.48	0.13	9	118.0	17.64	9
<u>Temperate Steppe</u>												
Turkey	24.4	1.29	3	0.2	0.18	3	7.10	0.00	3	428.7	72.29	3
Cimarron 1	25.7	1.28	5	0.4	0.31	5	7.46	0.18	5	248.8	45.00	5
Cimarron 2	24.8	0.45	5	0.3	0.23	5	7.34	0.13	5	262.0	22.27	5
North Carrizo	24.0	0.00	2	0.6	0.42	2	7.40	0.00	2	244.0	22.63	2

nd = No data

Table 2. Continued.

Stream	Conductivity ( $\mu\text{S}/\text{cm}$ )			$\text{Cl}^-$ (mg/L)*			$\text{SO}_4^{2-}$ (mg/L)			$\text{NO}_3\text{-N}$ (mg/L)**			SRP (mg/L)		
	$\bar{x}$	SD	n	$\bar{x}$	SD	n	$\bar{x}$	SD	n	$\bar{x}$	SD	n	$\bar{x}$	SD	n
<u>Hot Continental</u>															
Baron Fork	192	2.56	9	5.7	1.01	9	6.0	0.08	9	0.93	0.03 <sup>+</sup>	9	0.007	0.010	9
Peacheater	163	1.94	6	7.6	0.86	6	4.4	0.18	6	2.70	0.10	6	0.108	0.182	6
Saline	367	2.63	4	15.4	1.25	3	6.0	0.73	3	0.91	0.05	3	0.030	0.007	4
Snake	228	2.08	3	10.2	10.2	3	5.0	0.30	3	0.41	0.09	3	0.242	0.259	3
Summerfield	332	12.58	3	11.0	11.0	2	5.1	0.98	2	0.73	0.08	3	0.026	0.005	3
<u>Subtropical</u>															
Glover River	107	9.50	4	4.3	0.77	4	4.5	0.30	4	0.02	0.01 <sup>+</sup>	4	0.090	0.009	4
<u>Prairie</u>															
Wild Hog	420	11.7	12	2.2	0.27	12	10.7	0.23	12	0.04	0.02 <sup>+</sup>	12	0.032	0.009	12
Rock	423	30.14	6	21.8	12.83	6	11.5	6.77	6	0.04	0.04	2	0.026	0.017	6
Brier	493	25.44	4	19.9	11.37	4	38.4	50.12	4	BDL		4	0.014	0.006	4
Wolf	658	89.59	6	24.7	7.47	5	30.0	26.30	5	BDL		6	0.009	0.002	6
<u>Tropical/Subtropical Steppe</u>															
Elm Fork	2666	236.4	18	ADL***		13	1299.5	142.59	14	0.47	0.37	13	0.025	0.006	18
Salt Fork	1472	290.2	9	215.5	68.73	9	1296.5	498.41	9	BDL		9	0.033	0.014	9
<u>Temperate Steppe</u>															
Turkey	1650	108.2	3	ADL		3	124.3	0.00	1	0.50	0.00	3	0.019	0.003	3
Cimarron 1	2434	20.44	5	ADL		5	91.4	27.31	3	1.82	3.09	5	0.073	0.043	5
Cimarron 2	1048	154.2	5	25.8	0.79	3	197.4	16.18	3	BDL		3	0.050	0.021	5
North Carrizo	929	61.52	2	24.9	0.42	2	129.8	1.46	2	BDL		2	0.039	0.009	2

ADL=Above detectable limit

BDL=Below detectable limit.

\*  $\text{Cl}^-$ , maximum detection=250mg/L\*\*  $\text{NO}_3\text{-N}$ , minimum detection=0.04mg/L, except for sample indicated by <sup>+</sup> where detection limit=0.002mg/L\*\*\*  $\text{Cl}^-$  average concentrations collected on subsequent dates (Dec. 1996, 1165 mg/L, March 1997, 370 mg/L, June 1997, 396 mg/L)

Table 3. Sediment size distribution (% composition by weight) for 16 Oklahoma streams.

Stream	Grain Size (mm)				
	Fine Sand	Medium Sand	Coarse Sand	Very Coarse Sand	Gravel
<u>Hot Continental</u>					
Baron Fork	3.3	3.5	12.7	15.5	65.0
Peacheater	0.6	1.3	8.6	16.0	73.4
Saline	0.0	0.1	0.6	7.4	92.0
Snake	2.5	1.8	8.6	14.6	72.6
Summerfield	0.0	0.1	1.1	15.7	83.1
<u>Subtropical</u>					
Glover River	0.7	1.3	8.8	12.2	77.1
<u>Prairie</u>					
Wild Hog	1.2	1.5	3.3	6.5	87.5
Rock	5.5	18.1	60.1	14.8	1.6
Brier	10.3	28.2	13.8	13.9	33.9
Wolf	32.0	42.6	18.5	6.2	0.7
<u>Tropical/Subtropical Steppe</u>					
Elm Fork	2.5	14.6	31.6	12.5	38.8
Salt Fork	39.6	50.6	9.3	0.4	0.2
<u>Temperate Steppe</u>					
Turkey	29.2	41.6	27.0	2.1	0.2
Cimarron 1	13.2	27.0	46.1	7.8	5.8
Cimarron 2	0.7	2.0	5.4	11.2	81.0
Carrizo	4.1	16.1	34.0	15.6	30.2

Table 4. List of hyporheic invertebrates collected Oklahoma stream survey

Taxa	Ecoregion				
	Hot Continental	Subtropical	Prairie	Tropical/ Subtropical Steppe	Temperate Steppe
Cnidaria					
<u>Hydra</u>	x		x		
Turbellaria			x		
Nematoda	x	x	x	x	x
Tardigrada	x				
Oligochaeta	x	x	x	x	x
Acari	x	x	x		
Insecta					
Coleoptera	x		x		
Collembola			x		
Diptera					
Ceratopogonidae	x		x	x	x
Chironomidae	x	x	x	x	x
Ephemeroptera	x	x	x		
Odonata					x
Plecoptera	x	x			x
Trichoptera		x			
Crustacea					
Amphipoda					
<u>Stygonectes sp.</u>	x		x		
Chydoridae					
<u>Alona circumfimbriata</u>			x		
<u>Alonella excisa</u>	x				
<u>Chydorus sphaericus</u>	x		x		
<u>Pleuroxus denticulatus</u>	x		x		
Isopoda					
<u>Caecidotea sp.</u>	x	x	x		
Ostracoda	x	x	x	x	x

Table 4. Continued.

Taxa	Ecoregion				
	Hot Continental	Subtropical	Prairie	Tropical/ Subtropical Steppe	Temperate Steppe
Crustacea (cont.)					
Copepoda					
Cyclopoida					
<u>Acanthocyclops</u> spp.	x		x		
<u>Diacyclops</u> spp.	x	x	x		
<u>Eucyclops agilis</u>	x		x		
<u>Macrocyclus albidus</u>			x	x	
<u>Microcyclus</u> sp.	x	x	x		
<u>Paracyclops</u> sp.			x		
<u>Tropocyclops</u> sp.			x		
Harpacticoida					
Ameiridae					
<u>Nitocra lacustris</u>					x
Camptocamptidae					
<u>Attheyella nordenskioldii</u>	x				
<u>Attheyella pilosa</u>	x				
<u>Bryocamptus hiemalis</u>	x				
<u>Bryocamptus morrisoni</u>			x		
<u>Elaphoidella</u> spp.	x	x	x		
<u>Moraria cristata</u>			x		
Unidentified sp.	x	x			
Parastenocaridae					
<u>Parastenocaris</u> spp	x	x	x		x

Table 5. The three most abundant taxonomic groups found in the hyporheos in Oklahoma streams.

Stream	1st Dominant	%	2nd Dominant	%	3rd Dominant	%
<u>Hot Continental</u>						
Baron Fork	Cyclopoida	61.2	Harpacticoida	13.1	Insecta	8.4
Peacheater	Cyclopoida	50.6	Harpacticoida	28.3	Insecta	6.3
Saline	Cyclopoida	59.6	Harpacticoida	14.7	Ostracoda	10.7
Snake	Cyclopoida	50.9	Harpacticoida	25.5	Nematoda	9.4
Summerfield	Harpacticoida	33.1	Ostracoda	33.1	Cyclopoida	23.4
<u>Subtropical</u>						
Glover River	Insecta	39.5	Cyclopoida	38.8	Nematoda	12.1
<u>Prairie</u>						
Wild Hog	Cyclopoida	45.1	Harpacticoida	20.2	Isopoda	10.7
Rock	Cyclopoida	75.4	Insecta	14.4	Acari	3.0
Brier	Nematoda	44.3	Cyclopoida	36.0	Ostracoda	8.3
Wolf	Nematoda	84.0	Insecta	10.1	Acari	5.4
<u>Tropical/Subtropical Steppe</u>						
Elm Fork	Nematoda	55.4	Ostracoda	16.1	Insecta	11.2
Salt Fork	Insecta	77.8	Oligochaeta	16.7	Nematoda	8.3
<u>Temperate Steppe</u>						
Turkey	Insecta	43.6	Nematoda	31.6	Oligochaeta	18.8
Cimarron 1	Ostracoda	37.5	Nematoda	37.5	Insecta	25.0
Cimarron 2	Nematoda	100.0				
North Carrizo	Nematoda	67.4	Oligochaeta	18.0	Insecta	14.6

Table 6. Canonical correspondence analysis. Eigenvalues for the first four axes.

	Axis				Total
	1	2	3	4	
Eigenvalue	0.394	0.273	0.150	0.139	3.412
Cumulative % variance of species data	11.5	19.5	23.9	28.0	



Table 7. Correlation between invertebrate abundances and physical factors. Spearman correlation values (r) significant at  $p \leq 0.01$ .

Taxa	Latitude	Longitude	Precipitation	Depth	Channel Drainage	
					Width	Basin
Total Invertebrates	0.30	-0.50	0.50			-0.62
Nematoda					-0.34	
Oligochaeta	0.27	-0.39	0.28			-0.35
Acari		-0.49	0.47			-0.29
Total Insects		-0.34	0.35	-0.28		
Chironomidae			0.26			
Amphipoda						
Isopoda	0.29	-0.49	0.44			-0.35
Ostracoda		-0.30	0.32			
Chydoridae	0.37	-0.43	0.33			-0.42
Ameiridae		0.30	-0.26			
Parastenocaridae		-0.44	0.45			
Canthocamptidae	0.38	-0.69	0.61			-0.52
Cyclopoida		-0.76				-0.59

Table 8. Correlation between invertebrate abundances and water chemistry factors. Spearman correlation values (r) significant at  $p \leq 0.01$ .

Taxa	Temperature	DO	pH	Alkalinity
Total Invertebrates		0.57		
Nematoda			-0.26	
Oligochaeta	-0.28	0.45		
Acari	-0.23	0.32		
Total Insects		0.36		
Chironomidae		0.29		
Amphipoda	-0.30			
Isopoda	-0.29	0.41		
Ostracoda		0.36		
Chydoridae	0.35	0.56		
Ameiridae	-0.27			0.63
Parastenocaridae	-0.36	0.45	-0.33	0.90
Canthocamptidae		0.76		
Cyclopoida				

Table 8. Continued.

Taxa	Conductivity	Cl <sup>-</sup>	SO <sub>4</sub> <sup>-2</sup>	NO <sub>3</sub> -N	SRP
Total Invertebrates	-0.50	-0.46	-0.51		
Nematoda					
Oligochaeta	-0.36	-0.39	-0.36		
Acari	-0.47		-0.50		
Total Insects	-0.31		-0.37		
Chironomidae					
Amphipoda					
Isopoda	-0.39	-0.31	-0.36		
Ostracoda					
Chydoridae	-0.38	-0.34	-0.35	-0.36	
Ameiridae	0.34		0.34		
Parastenocaridae	-0.43		-0.40		
Canthocamptidae	-0.61	-0.43	-0.57		
Cyclopoida	-0.73	-0.43	-0.65		

Table 9. Correlation between invertebrate abundances and grain size.  
Spearman correlation values (r) significant at  $p \leq 0.01$ .

Taxa	Grain Size				
	Fine Sand	Medium Sand	Coarse Sand	Very Coarse Sand	Gravel
Total Invertebrates	-0.53	-0.59	-0.46		0.59
Nematoda					
Oligochaeta	-0.36	-0.41	-0.39		0.38
Acari		-0.32			
Total Insects					
Chironomidae					
Amphipoda		-0.28			
Isopoda	-0.50	-0.54	-0.50		0.53
Ostracoda	-0.28	-0.32		0.30	0.27
Chydoridae	-0.33	-0.40	-0.42		0.42
Ameiridae					
Parastenocaridae	-0.29	-0.38		0.40	0.29
Canthocamptidae	-0.58	-0.69	-0.65		0.65
Cyclopoida	-0.51	-0.65	-0.52	0.28	0.58

## List of Figures

Fig. 1. Hyporheic sampling locations on Oklahoma streams.

BF-Baron Fork, BC-Brier Creek, CR1-Cimarron River 1, CR2-Cimarron River 2, EF-Elm Fork, GR-Glover River, NC-North Carrizo Creek, PC-Peacheater Creek, RC-Rock Creek, SF-Salt Creek, SaC-Saline Creek, SnC-Snake Creek, SuC-Summerfield Creek, TC-Turkey Creek, WC-Wolf Creek, WH-Wild Hog Creek.

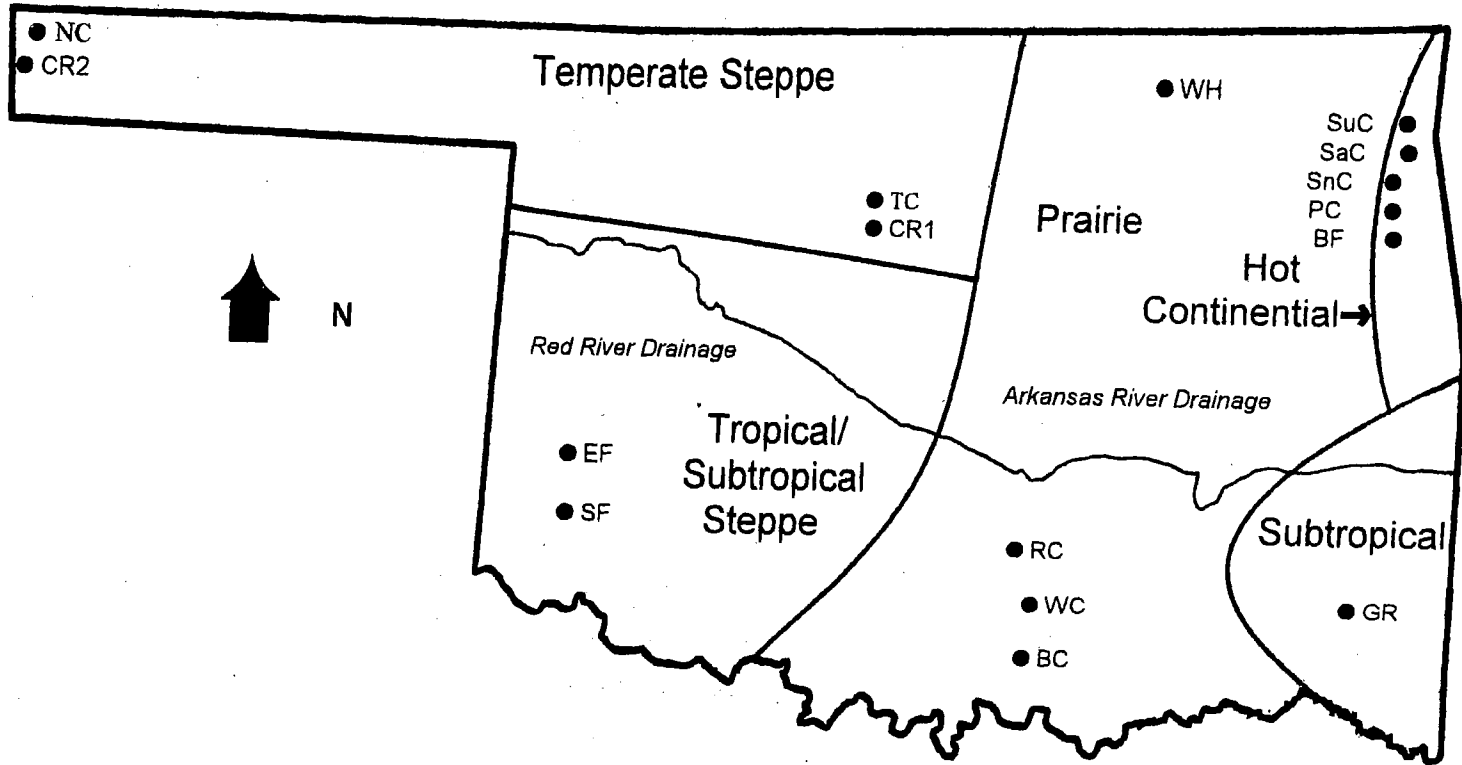
Fig. 2. Average densities (+ standard deviation) of major invertebrate taxa in the hyporheic zone within Oklahoma streams.

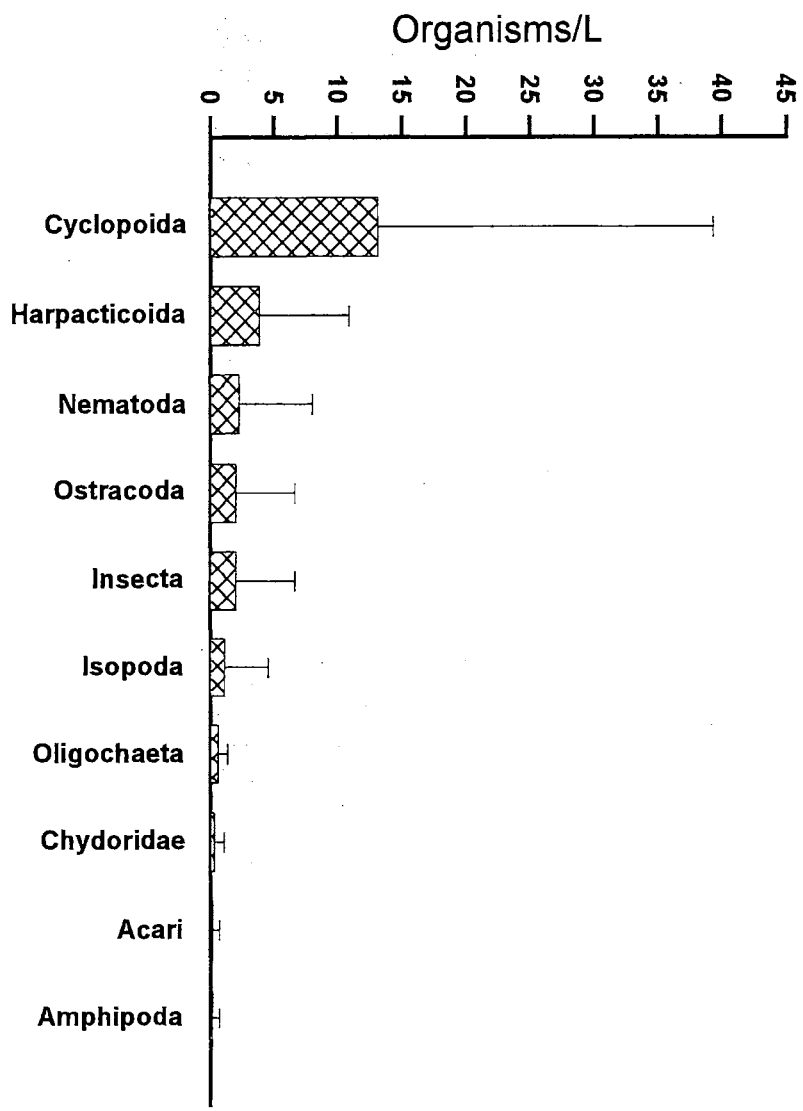
Fig. 3. Comparison of invertebrate abundances (total organisms) (+ standard deviation) in the hyporheic zone within Oklahoma streams. HCE = Hot Continental Ecoregion; STE = Subtropical Ecoregion; PE = Prairie Ecoregion; TSE = Temperate Steppe Ecoregion; TSSE = Tropical Steppe Ecoregion.

Fig. 4. Comparison of the number of taxa (richness) (+ standard deviation) in the hyporheic zone within Oklahoma streams. HCE = Hot Continental Ecoregion; STE = Subtropical Ecoregion; PE = Prairie Ecoregion; TSE = Temperate Steppe Ecoregion; TSSE = Tropical Steppe Ecoregion.

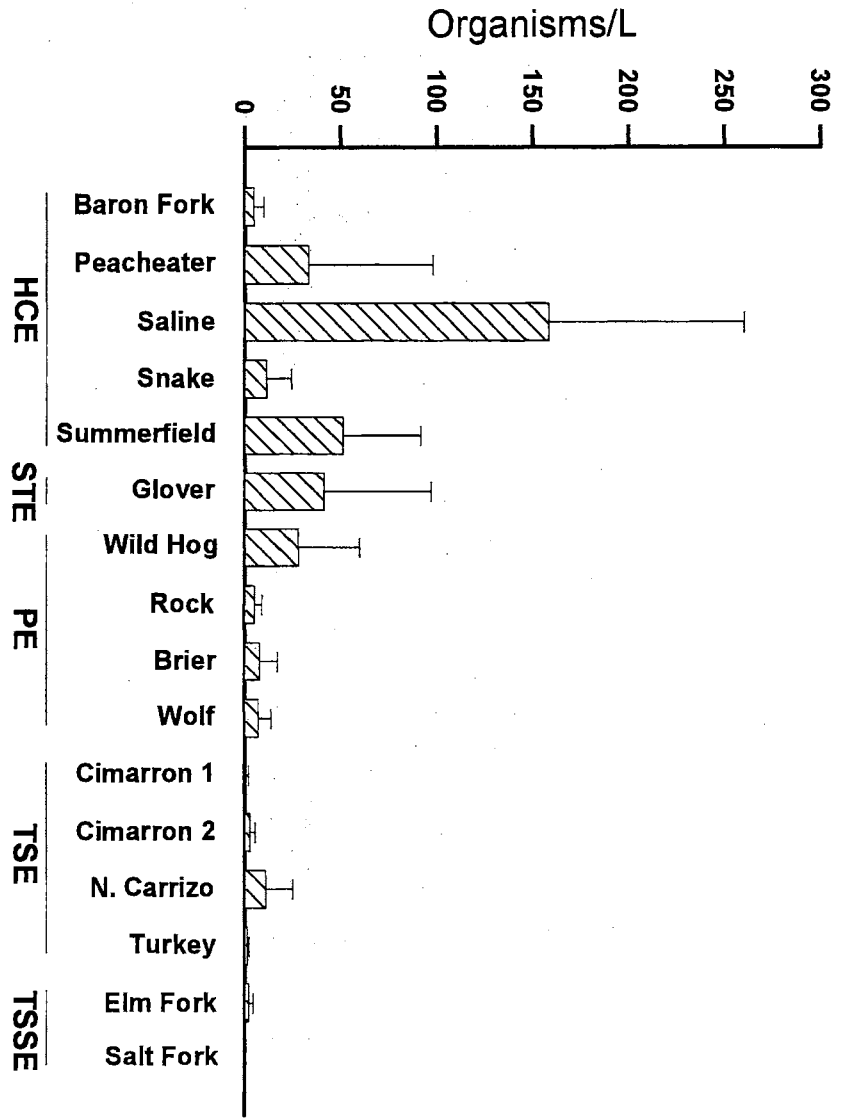
Fig. 5. Hyporheic sampling sites located relative to the first two CCA ordination axes. Sites are classified by ecoregion: Hot Continental Ecoregion (HCE) = □; Subtropical Ecoregion (STE) = Δ; Prairie Ecoregion (not including Wild Hog Creek) (PE) = ○; Prairie Ecoregion (Wild Hog Creek only) (PE)(WH) = ◊; Temperate Steppe Ecoregion (TSE) = □; Tropical Steppe Ecoregion (TSSE) = ◇.

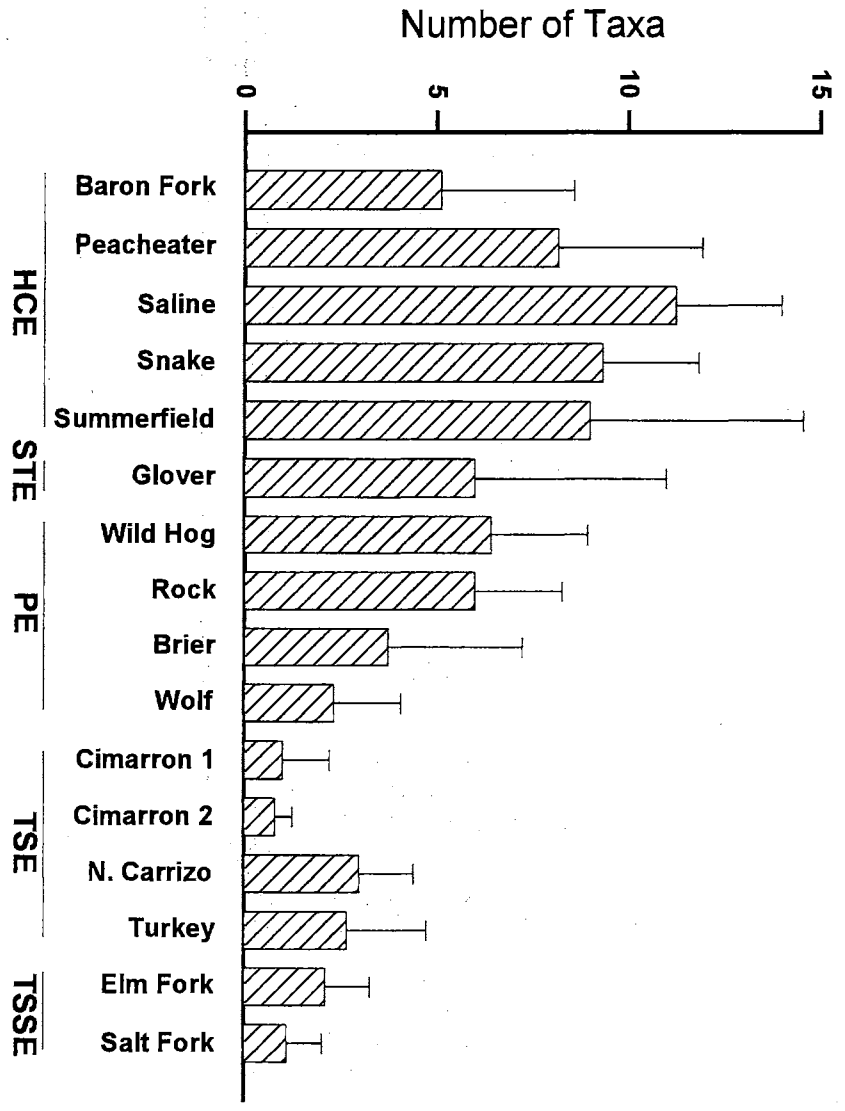
Fig. 6. Ordination diagram based on CCA of hyporheic invertebrate distribution with respect to environmental gradients (arrows) in the plane of the first 2 axes. Gravel, very coarse sand, and coarse sand refer, respectively, to the following grain sizes: >6.3 mm, 2-6.3mm, and <2mm. Abbreviations: Acan, Acanthocyclops; Alona cir, Alona circumfimbriata; Amph, Amphipoda; Att nor, Attheyella nordenskioldi; Att pil, A. pilosa; Bry hie, Bryocamptus hiemalis; Bry mor, B. morrisoni; Cerat, Ceratopogonidae; Chir, Chironomidae; Cole, Coleoptera; Coll, Collembola; Diacy, Diacyclops; Elap, Elaphoidella; Ephem, Ephemeroptera; Eucy, Eucyclops; Iso, Isopoda; Macrocy, Macrocyclus; Nemat, Nematoda; Odon, Odonata; Oligo, Oligochaeta; Ostra, Ostracoda; Paracy, Paracyclops; Parast, Parastenocaris; Plec, Plecoptera; Tard, Tardigrada; Trich, Trichoptera; Trop, Tropocyclops; Turb, Turbellaria.

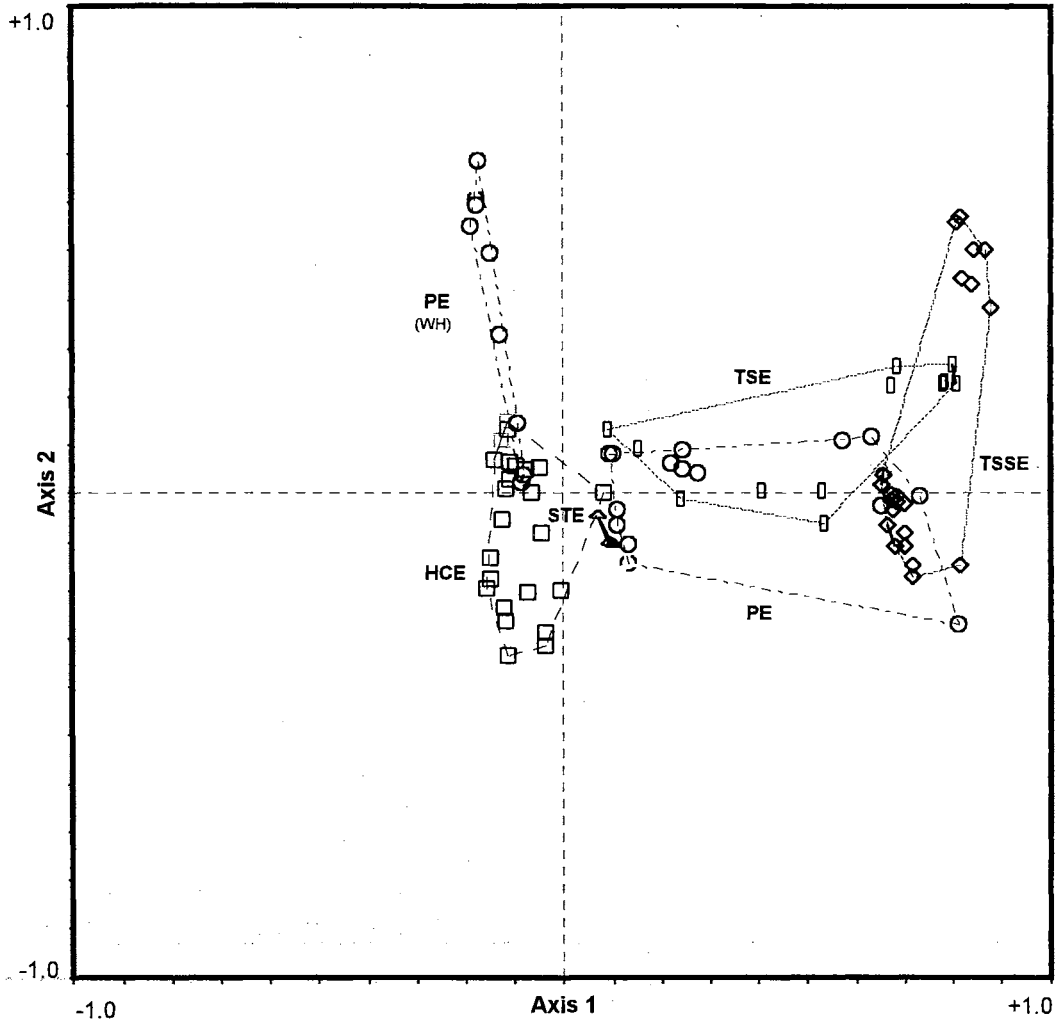


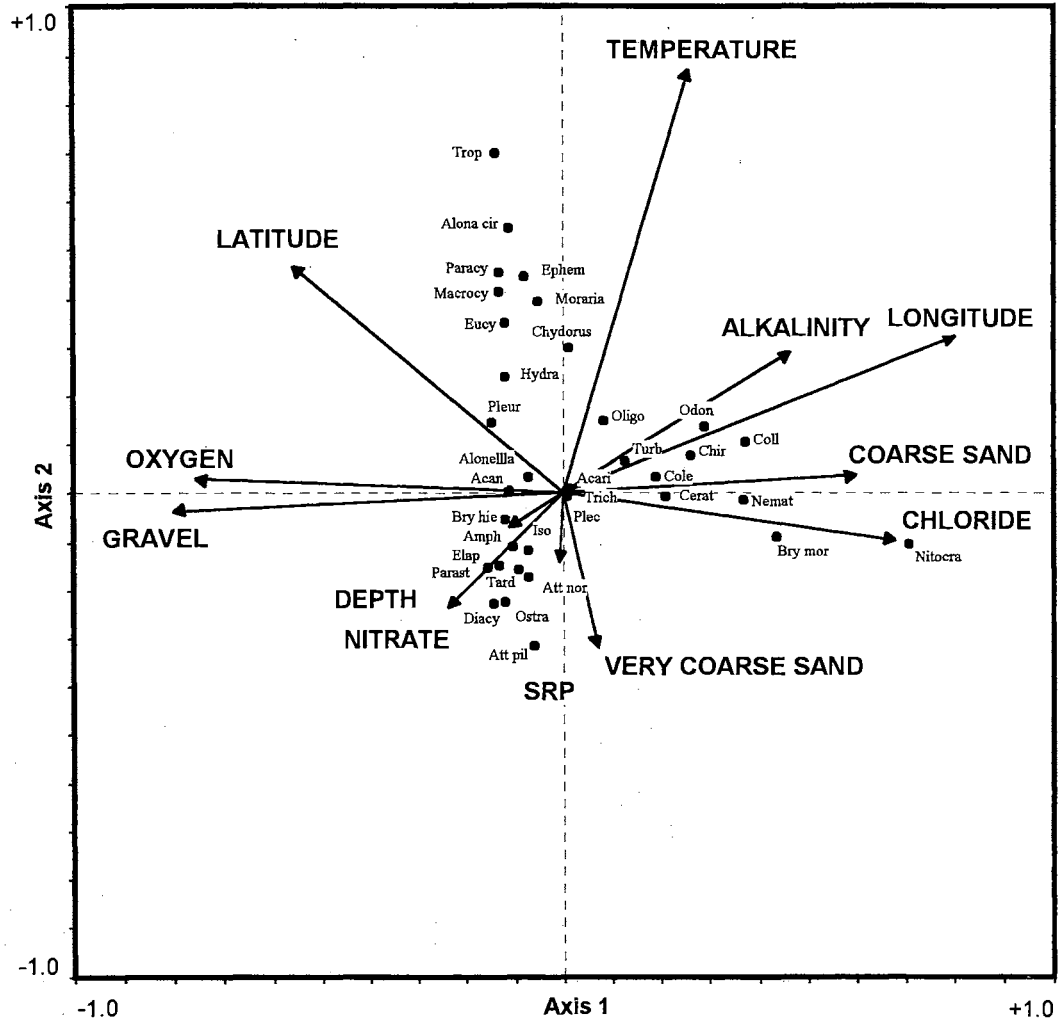












CHAPTER III

TEMPORAL AND SPATIAL DISTRIBUTION OF INVERTEBRATES  
WITHIN THE HYPORHEIC ZONE OF FIVE  
OKLAHOMA STREAMS

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**Abstract:** The seasonal distribution of fauna in the hyporheic zone has been investigated in few regions in the United States. To address this, hyporheic samples were collected seasonally from five streams representing the diverse geographical areas within unglaciated Oklahoma. Samples were collected using the Bou-Rouch method at well depths of 5-20, 30-45, 60-75, and 100-115 cm below the stream bottom. Copepods dominated the hyporheic fauna at most sites on most dates. The most widely distributed species included the cyclopoids Acanthocyclops exilis, Diacyclops yeatmani, and Eucyclops agilis, and the harpacticoid Attheyella nordenskioldi, the latter found in the encysted form during summer months. One or more previously undescribed species of Acanthocyclops, Diacyclops, Elaphoidella, Parastenocaris and Stygonitocrella were also collected. Species richness and invertebrate abundance were highest in the streams with gravel sediments and peaked in all streams during the spring. Densities were generally greatest at depths below 30 cm throughout the year. Important environmental factors influencing composition and abundance as determined by Canonical Correspondence Analysis and Spearman Rank Correlation included substrate size, dissolved oxygen, conductivity, and temperature. In terms of taxon richness and abundance, the hyporheos of Oklahoma streams are comparable to those reported in other unglaciated regions in the eastern United States.

## **Introduction**

Hyporheic zones in North American streams often support a diverse and abundant fauna (Strayer 1988, Griffith & Perry 1993, Pennak & Ward 1986, Palmer 1990, Stanford & Ward 1988, Boulton et al. 1992) and discoveries of previously undescribed species are frequent (Whitman 1984; Pennak & Ward 1985a, 1985b; Reid 1991, 1992a, 1992b; Reid et al. 1991; Reid & Strayer 1994; Strayer 1988). However, with few exceptions (Whitman & Clark 1984, Pennak & Ward 1986, Strommer & Smock 1989, Palmer 1990, Ward & Voelz 1990), existing studies of hyporheic invertebrates provide little information on changes in abundance and composition as affected by season.

A statewide survey of the invertebrates inhabiting the hyporheic zone in Oklahoma streams identified a wide variety of hyporheic conditions and invertebrate assemblages (Chapter II). Taxon richness and abundance were influenced by various environmental factors including dissolved oxygen, sediment grain size, and temperature. The survey was limited, however, to the summer season. In order to better understand the composition and abundance of invertebrates inhabiting the hyporheic zone of Oklahoma streams, seasonal sampling was continued at a subset of streams representing the diverse geographical areas within the state. The objectives of this investigation are to expand our knowledge regarding the temporal and spatial distribution over a large and diverse geographical area and to identify those environmental gradients most important in determining community structure throughout the year.

## **Study Area**

Five streams representing three of the five ecoregions located within Oklahoma are included in this study (Fig. 1). Physical data for the streams are summarized in Table 1. Two streams (Baron Fork and Saline Creek) represent the Hot Continental Ecoregion (Bailey 1996) in northeastern Oklahoma. Both streams are spring fed with bed sediments consisting of chert and limestone gravels. However, the Baron Fork drainage is over 7x larger than Saline Creek's basin.

The Prairie Ecoregion is represented by two streams located at opposite ends of the state. Wild Hog Creek, in north-central Oklahoma, is the smallest of the streams included in this investigation and is characterized by pools separated by riffle areas consisting of silt, gravel, and cobbles. Rock Creek, located in southcentral Oklahoma, is spring fed and bed sediments are medium to very coarse sands.

The fifth stream, Elm Fork of the Red River, represents the Tropical/Subtropical Steppe Ecoregion, in the southwest corner of the state. Bed sediments are coarse sand and small gravel.

## **Methods**

Hyporheic samples were collected seasonally at each stream using the Bou-Rouch method of suctioning water from narrow wells (Bou 1974). At each stream site, wells, consisting of 2.5-cm ID PVC tubing, were installed immediately prior to sampling at all streams except Wild Hog Creek where permanent wells were installed during the initial



sampling. Each PVC well, perforated with 6.0-mm holes along the lower 15 cm, was driven into the stream's substratum by inserting a steel T-shaped driving rod into the tubing and driving the rod and tubing so that the top of the perforated section reached the desired depth. The T-bar was then removed, leaving the well in place.

During the initial sampling at Baron Fork, Elm Fork, and Rock Creek in summer 1996, single hyporheic samples, ranging in volume from 4-8 L, were collected from the hyporheic zone at three depth intervals where possible: 30-45 cm, 60-75 cm, and 100-115 cm below the stream bottom at mid-channel stations. All samples collected from Saline Creek and the samples collected at all streams after August 1996 were standardized at 1L. A parallel investigation (Chapter V) indicated that the initial 1-L sample collected by the Bou-Rouch method provides the most reliable density estimates. Also, after August, a fourth depth interval, i.e., 5-20 cm, was sampled at each sampling location. All samples were concentrated with a 63- $\mu$ m mesh sieve, preserved in 5% formalin, and stained with rose bengal to facilitate sorting. Organisms were identified to recognizable taxonomic units using a dissecting microscope. Since many of the organisms belonged to taxonomically difficult groups and were immature, most were identified to genus, family, or higher level. Crustacea, with the exception of the ostracods, were identified to genus or species level.

Water samples were collected at each depth for dissolved oxygen (DO), conductivity, pH, alkalinity, nitrate-N ( $\text{NO}_3\text{-N}$ ), ammonium-N ( $\text{NH}_4$ ), soluble reactive phosphate (SRP), sulfate ( $\text{SO}_4^{2-}$ ), and chloride ( $\text{Cl}^-$ ). DO was determined by Winkler

titration, and alkalinity by titration with 0.2 N H<sub>2</sub>SO<sub>4</sub> (Wetzel and Likens 1991). Conductivity and pH of unfiltered samples were measured in the laboratory with Orion meters. Following filtration through a 0.7- $\mu$ m glass fiber filter, Cl<sup>-</sup>, NO<sub>3</sub>-N, and SO<sub>4</sub><sup>2-</sup> were measured with a Dionix DX-100 ion chromatograph, SRP was analyzed using the molybdate blue method (Murphy and Riley 1962) and NH<sub>4</sub> was analyzed using the phenolhypochlorite method (Solorzano 1969). Temperature was measured in situ at each depth by lowering a thermometer into the well. Sediment analysis for the streams is described in Chapter 1. Gravel, very coarse sand, coarse sand, medium sand, and fine sand refer, respectively, to the following grain sizes: >6.3 mm, 2-6.3 mm, 0.5-2 mm, 0.25-0.5 mm, and <0.25 mm.

Relationships between environmental factors and taxa abundances were investigated using two statistical approaches. The distribution and composition of hyporheic assemblages were related to environmental variables by canonical correspondence analysis (CCA) (ter Braak 1986, 1987) using the program CANOCO for Windows 4.0 (ter Braak and Smilauer 1997). Information regarding the interpretation of CCA ordination diagrams is provided by ter Braak and Verdonschot (1995). Taxa counts were square root transformed and abundances for rare taxa were down weighted in order to prevent extremely abundant or extremely rare taxa from having unique influence on the ordination (Gauch 1982). Eigenvalues are calculated that indicate the degree of correlation between species and sites. In addition, Spearman rank correlations were computed between the taxon abundances (number of organisms/L) and physical and

chemical variables.

## **Results**

### *Overview*

#### Water chemistry

All sites can be characterized as neutral to alkaline, and in most instances were enriched with nitrogen relative to phosphorus, although nutrient concentrations ( $\text{NO}_3\text{-N}$ ,  $\text{NH}_4$ , and SRP) varied widely in all streams (see Appendix). Perhaps the most striking difference among the streams is the high conductivities and the related high concentrations of  $\text{Cl}^-$  and  $\text{SO}_4^{2-}$  of Elm Fork. The water chemistry of streams in southwestern Oklahoma such as the Elm Fork site are strongly affected by numerous saline springs contributing to their base flows (Oklahoma Water Resources Board 1980).

#### Assemblage composition

Annual invertebrate densities (organisms/L) ranged widely among the five streams (Fig. 2). Densities ( $\bar{x} \pm \text{SE}$ ) were greatest in the two Ozark streams (96  $\pm$  24 in Baron Fork and 105  $\pm$  17 in Saline Creek) and lowest in the streams with sandy substrates (5  $\pm$  1.5 in Rock Creek and 12  $\pm$  2.9 in Elm Fork). The higher densities in the Ozark streams reflected the abundances of cyclopoid (43-65/L) and harpacticoid (8-21/L) copepods and other crustaceans (13-17/L). Crustacean groups were also major components of the Wild Hog Creek fauna, although oligochaetes were the single most abundant group based on

the average for all dates. Insecta (1-6/L), predominantly chironomids, and nematodes (0.3-4.5/L) occurred in low numbers in all five streams.

A total of 51 taxa were collected from the 5 streams (Table 2). The greatest taxon richness occurred in Saline Creek (34 taxa), followed by Baron Fork (32), and Wild Hog Creek (33). The relatively high number of taxa in these streams reflected the large number of copepod species (13-15). The most widely distributed copepods included the cyclopoids Diacyclops yeatmani, Microcyclops, Eucyclops agilis, Paracyclops and Acanthocyclops exilis and the harpacticoids Attheyella nordenskioldi, Elaphoidella (several species), and Parastenocaris (several species). Although Microcyclops sp. was identified in all streams except Elm Fork, it was usually difficult to distinguish between this and smaller cyclopoid copepodid stages. Thus, little can be said regarding its relative abundance. Ostracods, predominantly early instars, were encountered in all streams.

Several crustaceans were abundant in only specific streams. Locally abundant copepods included an undescribed species of Diacyclops (sp. A) (Baron Fork and Wild Hog Creek), Attheyella pilosa (Saline Creek), Moraria cristata (Wild Hog Creek), Nitocra lacustris (Elm Fork), and Stygonitocrella sequoyahi (Baron Fork and Saline Creek). Interestingly, this last genus has not previously been reported from North America (Reid et al. In review). Several chydorid cladocerans, the amphipod Stygonectes, and the isopod Caecidotea were also collected, although usually in low densities and only from the three northeastern streams. The most common chydorids included Alona circumfimbriata, Alonella excisa, Chydorus sphaericus, and Pleuroxus denticulatus.

## *Seasonal and Spatial Distribution*

### Baron Fork

Only minor variations in temperature and DO were observed between hyporheic and surface waters of Baron Fork (Fig. 3a,b). DO was near saturation throughout the year, thus concentrations were higher in winter and spring and lower in summer and autumn.

Total invertebrate densities paralleled DO, with highest densities in the winter (120/L) and spring (216/L) (Fig. 3c). Taxon richness was also greatest in winter and spring; 19 taxa were recorded at the 30-45 cm and 5-20 cm depths, respectively. Lowest densities and taxon richness (with spring as an exception) occurred at the 5-20 cm depth (Fig. 3c,d). Densities recorded in the summer 1996 were perhaps underestimated as the densities were based on 4 L samples (see Chapter V).

Cyclopoids were the dominant component of the hyporheos in Baron Fork at all depths in all seasons (Fig. 4). Dominant species included *D.* sp. A (33 and 63% of cyclopoids in winter and spring, respectively) and *D. yeatmani* (20 and 23%) with the latter mostly confined to the 60-75 and 100-115 cm depths. *A. exilis* was relatively abundant in winter when it comprised 22% of all cyclopoids. Harpacticoid copepods and other crustaceans (i.e., ostracods and the chydorid *A. excisa*) were also most abundant in winter and spring. The most abundant harpacticoids included *Stygonitocrella* and *Parastenocaris*, both of which were almost entirely confined to the 60-75 and 100-115 cm depths, and *Elaphoidella*, which occurred at all depths. *A. nordenskioldi*, *A. illinoisensis*,

and Bryocamptus hiemalis were rare. Among the other crustaceans, small numbers of isopods were collected at the 60-75 and 100-115 cm depths in all seasons and amphipods were collected at these depths only in summer and autumn. Although found in all seasons, nematodes, oligochaetes, and insects (92% of insects consisted of early chironomid instars) were also most abundant in winter and spring with highest densities at intermediate depths.

### Saline Creek

Of the five streams investigated, temperature and DO were the most constant in Saline Creek, both spatially and seasonally (Fig. 5a,b). As in Baron Fork, DO was near saturation at all depths throughout the year. Taxon richness also remained relatively constant, ranging from 7 taxa at the 100-115 cm depth in autumn to 16 at the 5-20 cm depth in spring (Fig. 5d). Total invertebrate densities, however, fluctuated widely both spatially and seasonally with the highest average densities observed in summer 1996 (157/L) and spring 1997 (148/L) (Fig. 6c).

Cyclopoids were the most abundant invertebrate group in most seasons and at most depths, composing 24-60% of the total invertebrates (Fig 6). The cyclopoids were dominated by copepodids (50%), and D. yeatmani (41%), although adults of the latter were identified only in winter. Harpacticoids, composing 21% of all invertebrates, were most abundant at the 30-45 cm depth in winter and spring and at 100-115 cm depth in August 1996. Lowest densities occurred at the 60-75 cm depth. The more common

harpacticoids included Elaphoidella (all seasons), A. nordenskioldi (winter, spring and summer), B. hiemalis (spring), and A. pilosa (autumn). Parastenocaris and S. sequoyahi were rare. Also, low numbers of encysted A. nordenskioldi were collected in summer samples (1996 and 1997).

Among other crustaceans, ostracods were the most abundant group composing 11% of all invertebrates. Relatively high densities occurred in all seasons. They were more numerous in the shallower depths in winter and spring; however, greater numbers occurred below 60 cm in summer and autumn. Amphipods, also lumped with other crustaceans in Fig. 6, were collected only in autumn at the 5-20 and 60-75 cm depths. Nematodes and insects, again predominantly chironomids, were present throughout the year and usually were most numerous in the shallower depths. Oligochaetes were most abundant in winter and spring at the 5-20 cm depth (Fig. 6).

#### Wild Hog Creek.

Although temperatures in Wild Hog Creek fluctuated widely among seasons, only minor spatial variation was observed on any one date (Fig. 7). DO was near saturation above the 75 cm depth throughout the year, but near anoxic conditions were observed at 100-115 cm depth in all seasons except spring. Wide spatial and seasonal fluctuations were observed in both total invertebrate densities (8-243/L) and in taxon richness (3-20). Average invertebrate densities ranged from 5/L at the 5-20 cm depth in winter to 243/L at the same depth in spring. Oligochaetes were the major component (26%) due to high

spring densities (Fig. 8). Oligochaetes numbered 3/L or less in other seasons.

Harpacticoids were the most abundant crustacean group, representing 18% of all invertebrates. Seasonal densities ranged from less than 3/L in autumn to 16/L in summer (Fig. 8). M. cristata and A. nordenskioldi composed 69% and 18%, respectively, of the harpacticoids. Both species were concentrated in the shallower depths. Cyclopoids composed 11% of all invertebrates. Densities ranged from less than 2/L in winter to more than 10/L in spring. Densities also were generally higher at the shallower depths. Almost half of all cyclopoids consisted of early copepodid stages. The more abundant species included Paracyclops, D. yeatmani, D. sp. A, E. agilis and A. exilis, each comprising from 5 to 17% of all cyclopoids.

The chydorids, i.e., A. circumfimbriata, C. sphaericus, and P. denticulatus, were common in spring, summer and autumn at the three upper depths. Ostracods, common in spring and autumn, were most abundant at the 100-115 cm depth in the spring. Isopods were confined almost exclusively to the 100-115 cm depth with densities ranging from 11/L in summer to 38/L in the autumn.

Other invertebrates occurred only sporadically in the samples. Nematodes and rotifers were relatively common above the 100-115 cm depth in autumn with densities of 12-24/L in the upper three depths and a rotifer density of 18/L at the 30-45 cm depth (Fig. 8). Early instars of chironomids, plecopterans, and ephemeroptans were collected from the 5-20 and 30-45 cm depths in spring and summer.



## Rock Creek

Conditions in Rock Creek were considerably different from those observed in the streams previously discussed. Although temperature fluctuated only slightly among depths and seasons, DO exhibited very low concentrations within the hyporheic zone throughout the year (Fig. 9a,b). Consequently, the lowest invertebrate densities and taxon richness of the five streams investigated occurred in this stream (Fig. 9c,d). Densities were 8/L or less at all depths and in all seasons except June 1997 when densities ranged from 8 to 24/L at the 60-75 and 100-115 cm depths, respectively. The more abundant invertebrates included tardigrades, *D. yeatmani*, *Parastenocaris*, ostracods, oligochaetes, and chironomids. Densities were very low and lacked any consistent trends with respect to depth (Fig. 10). However, tardigrades were highly concentrated (18/L) at the 100-115 cm depth in the summer of 1997.

## Elm Fork

Wide seasonal fluctuations in temperature and spatial fluctuations in DO were observed in Elm Fork (Fig. 11). Seasonal temperature ranged from 5°C in winter to near 30°C in summer (1996). Temperatures of hyporheic and surface waters were nearly identical. The DO in hyporheic waters ranged from near saturation in winter and spring to near anoxia in summer. Invertebrate densities were consistently low in Elm Fork, ranging from an average of 2/L in the summer to 19/L or greater in both spring and autumn. Taxon richness was also low, with 8 or fewer taxa encountered in all samples. The mid-

channel of the stream could not be sampled during the summer 1997 because of high water. Consequently, hyporheic samples were collected from the submerged shoreline but contained no invertebrates. Hence, low densities reported for this date may not be representative of the hyporheic zone.

The invertebrate fauna consisted almost entirely of the harpacticoid copepods Parastenocaris and N. lacustris. No cyclopoids or chydorids were collected. Ostracods, oligochaetes, nematodes, and chironomids also occurred in low numbers throughout the year. No trends in vertical distribution were observed (Fig. 12).

### *Ordination*

The forward selection procedure in CANOCO was used to identify those environmental gradients that were most significant ( $p = 0.01$ ) in explaining the relative abundances of taxa. These included oxygen, temperature, depth, pH, grain size,  $\text{Cl}^-$ ,  $\text{SO}_4^{2-}$ , and conductivity. The arrows for the latter three variables were found to be parallel in preliminary analyses due to high ionic concentrations in the Elm Fork. Therefore, only conductivity was included in the ordination plots described below.

Approximately 30% of the variation observed in the species data is accounted for by the eigenvalues for the first four CCA axes (Table 3). A triplot of taxa, environmental gradients and sampling sites by stream (enclosed by envelopes) relative to the first 2 axes is presented in Fig. 13. Increases in conductivity and medium and coarse sand were associated with lower DO and less gravel. Increasing depth was associated with decreases

in temperature and pH.

Taxa strongly associated with increases in conductivity and medium to coarse sand sediments included the harpacticoids N. lacustris, Parastenocaris, and an unidentified canthocamptid species, nematodes, and the instars of several insect orders (i.e., Ceratopogonidae, Trichoptera, Coleoptera, Chironomidae, and Collembola) (Fig. 13).

Most taxa, including most crustaceans, were associated with increasing oxygen concentrations and predominantly gravel sediments. Taxa strongly associated with increasing depth and decreasing temperature included S. sequoyahi, Elaphoidella, A. rustata, A. excisa, Amphipoda, Acari, Tardigrada, and two or more species each of Acanthocyclops, Bryocamptus, and Diacyclops. Taxa most associated with increasing pH and temperature included the Ephemeroptera, Plecoptera, Turbellaria, and several crustaceans found almost exclusively in Wild Hog Creek. The most common of these included M. affinis and A. nordenskioldi.

### *Spearman Rank Correlations*

The abundances of total invertebrates and most groups were positively correlated with DO, gravel, and pH and negatively correlated with temperature, drainage basin area, sand substrates, conductivity,  $\text{Cl}^-$ ,  $\text{SO}_4^{-2}$  and alkalinity (Table 4). Both N. lacustris and Parastenocaris were positively correlated with sand substrates,  $\text{Cl}^-$  and  $\text{SO}_4^{-2}$ . Taxa positively correlated with  $\text{NO}_3\text{-N}$  included D. sp. A, D. yeatmani, Elaphoidella, Parastenocaris, Stygonitocrella and total insects including the chironomidae. E. agilis and

N. lacustris were both positively correlated with temperature and N. lacustris was positively correlated with conductivity. Only the isopods were positively correlated with depth, whereas oligochaetes, insects including chironomidae, and A. nordenskioldi were negatively correlated with depth.

## **Discussion**

The abundance and composition of hyporheos in the five study streams varied widely. Although low numbers of chironomids, nematodes, and oligochaetes occurred in all streams throughout the year, microcrustaceans dominated in most instances and were the only group addressed at the genus and/or species level. The greatest differences in abundances were related to substrate size and season, with greatest densities and taxon richness occurring in gravel-bed streams during the spring.

The invertebrate fauna inhabiting the hyporheic zone of the three gravel-bed streams (Baron Fork, Saline Creek, and Wild Hog Creek) was quite diverse (30+ taxa) and is comparable to the taxon richness reported in other North American streams (cf. Godbout and Hynes 1982, Pennak and Ward 1986, Boulton et al. 1992), although the level of taxonomic determination is not similar in all studies. In the Oklahoma streams, almost half of all taxa were copepods. The dominance of copepods agrees with several other studies that used similar sampling procedures (Pennak & Ward 1986, Boulton et al. 1992).

Microcrustaceans encountered were dominated by forms previously encountered in

the groundwater. Of the harpacticoids, both Parastenocaris and Stygonitocrella are classified as hypogean genera (Rouch 1986). A. pilosa and M. cristata were originally described from caves in Indiana and Kentucky and Indiana and Ohio, respectively (Borutsky 1964). A. nordenskioldi and A. illinoisensis have also been reported from the hyporheos of North American streams (Strayer 1988, Williams 1989). Although freshwater harpacticoids have been reported to encyst during summer months (Sarvala 1979), the finding of encysted A. nordenskioldi in the hyporheos of Saline Creek during the summer months is the first report for this species.

Of the cyclopoids, D. yeatmani was originally described from a cave in Tennessee (Yeatman 1964) and D. jeanneli putei was found in North Carolina wells (Wilson & Yeatman 1959). D. crassicaudus brachycerus, D. nearcticus, E. agilis, and M. albidus have also been identified as inhabitants of the hyporheos (Whitman & Clark 1984, Strayer 1988, Williams 1989, Ward et al. 1994). The occurrence of undescribed species is anticipated in unglaciated portions of North America (Strayer et al. 1995), including Oklahoma. The presence of N. lacustris in the saline waters of Elm Fork is not surprising as this harpacticoid is classified as euryhaline by Lang (1948) and has been collected in Texas (Wilson & Yeatman 1959). Members of the genus Parastenocaris, another harpacticoid collected in Elm Fork, also occurs in brackish groundwater (Remane & Schlieper 1971).

The cladocerans encountered in the streams were all chydorids, i.e., P. denticulatus, A. excisa, C. sphaericus, A. rustata, and L. leydigia, and all are common

inhabitants of the hyporheos (Dumont & Negrea 1996). Both the amphipod Stygonectes (Holsinger 1986) and the isopod Caecidotea (Henry et al. 1986) are also considered as hypogean genera.

Although this investigation addressed only the microcrustaceans at the genus and/or species level, each stream was characterized by a unique assemblage. Variations in assemblage composition have previously been found not only among different streams (Boulton et al. 1992, Williams 1989) but also between different habitat types within a single stream (Ward & Voelz 1990, Pennak & Ward 1986, Boulton et al. 1992, Ward et al. 1994, Williams 1989). Factors cited as influencing assemblage composition included site specific hydraulic conditions and substratum properties (Ward et al. 1994, Ward & Voelz 1990). Strayer et al. (1997) suggests that substratum properties are the most important factor. This study likewise indicates the importance of substrate characteristics on assemblage composition.

The seasonal distribution observed in the Oklahoma streams was similar to those for a Texas stream (Whitman & Clark 1984). Total densities were lowest in the summer and highest in the spring. However, our results differed somewhat from seasonal trends reported by Palmer (1990) who observed that microcrustacea in the hyporheic zone of a Virginia stream peaked in the autumn. The high densities in the Virginia stream were attributed to low flow conditions during this season. The adverse effect of high discharge on hyporheic invertebrates in the spring was also reported by Strommer & Smock (1989). The gravel bottomed streams included in our investigation may be more resistant to

erosion, thus allowing hyporheic populations to peak during the spring months.

The abundance of the hyporheic fauna below the 60 and 100 cm depths in the Oklahoma streams differs from that reported from other streams. Many investigators have reported greatest abundances in the upper 20 cm of bed sediments and declining abundances with increasing depth (Williams & Hynes 1974, Whitman & Clark 1984, Pugskey & Hynes 1986, Strommer & Smock 1989, McElravy & Resh 1991, Betschko 1992, Strayer et al. 1997). The inverse correlation between abundance and depth is typically attributed to decreasing DO availability (Whitman & Clark 1984, Strommer & Smock 1989, Strayer et al. 1997) and pore size (Pool & Stewart 1976). Insect larvae were a more important component of the hyporheos in these streams as compared to the Oklahoma streams. Boulton et al. (1992) reported two separate faunas inhabiting the hyporheos of Arizona streams. These included the shallow hyporheic assemblages, dominated by copepods, found within the upper 50 cm of the streambed, and phreatic assemblages, characterized by bathynellids and Parastenocaris, below 50 cm. Copepods were the most abundant component of the the hyporheos at most depths on most dates in the Oklahoma streams perhaps due to the presence of several taxa adapted to the hyporheic habitat as previously described.

The results of this investigation agree with a previous investigation of Oklahoma streams (Chapter II) and elsewhere (Strayer 19994, Strayer et al. 1997, Ward & Palmer 1994, Ward et al. 1994) indicating the importance of substrate type and DO concentrations on the composition and abundance of the hyporheic fauna. However,

additional factors can be important both locally and seasonally. These results illustrate the importance that season and/or temperature can have on composition and abundance. The presence and abundance of insect larvae in the hyporheos corresponds to adult emergence in midsummer followed by larval recruitment in the fall (Pool & Stewart 1976, Godbout & Hynes 1982, McElravy & Resh 1991). In addition, temperature has been reported as a major factor influencing the distribution of copepods (Williamson 1991), the major component of the hyporheos of Oklahoma streams.

The results of this survey along with the information presented in Chapter 1 indicate the abundance in terms of the number of taxa and in densities of invertebrates inhabiting the hyporheic zone of Oklahoma streams offering suitable substrates and oxygen supply. However, only single stations on five streams were included in this investigation. To better understand the hyporheos in Oklahoma streams, seasonal sampling should be extended to more streams, specifically in the southeastern portion of the state and in the broad alluvial plains commonly located adjacent to many of the state's streams. During this study, only the chydorids and copepods were identified to the genus and/or species level. Future studies should address other taxonomic groups, specifically the ostracods, oligochaetes, and chironomids.

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Illinois. - *J. Tennessee Acad. Sci.* 39: 95-98.

Table 1. Physical data for sampling locations in 5 Oklahoma streams.

Stream	Ecoregion <sup>a</sup>	Location		Channel	Precipitation (m)	Drainage	Grain Size (%)				
		Latitude	Longitude	Width (m)		Area (km <sup>2</sup> )	Fine Sand	Medium Sand	Coarse Sand	Very Coarse Sand	Gravel
Baron Fork	HCE	35°55'	95°51'	41	1.22	795	3.3	3.5	12.7	15.5	65.0
Saline	HCE	36°21'	94°58'	20	1.27	111	0.0	0.1	0.6	7.4	92.0
Wild Hog	PE	37°51'	96°21'	26	0.91	13	1.2	1.5	3.3	6.5	87.5
Rock	PE	34°35'	96°58'	26	0.91	114	5.5	18.1	60.1	14.8	1.6
Elm Fork	TSSE	34°55'	99°31'	20	0.61	2,170	2.5	14.6	31.6	12.5	38.8

<sup>a</sup> HCE, Hot Contential Ecoregion; PE, Prairie Ecoregion; TSSE, Tropical/Subtropical Steppe.



Table 2. List of hyporheic invertebrates collected in 5 Oklahoma streams.

	Stream				
	Baron Fork	Saline	Wild Hog	Rock	Elm Fork
Number of Taxa	32	34	33	18	11
<u>Taxa</u>					
Cnidaria					
Hydra	x	x	x		
Turbellaria	x		x	x	
Nematoda	x	x	x	x	x
Rotifera	x	x	x	x	
Tardigrada	x	x		x	x
Oligochaeta	x	x	x	x	x
Acari	x	x	x	x	
Insecta					
Coleoptera	x	x	x	x	x
Diptera					
Ceratopogonidae	x	x	x	x	x
Chironomidae	x	x	x	x	x
Culicidae	x				
Simulidae			x		
Tipulidae			x		x
Ephemeroptera	x	x	x	x	
Plecoptera	x	x	x	x	
Trichoptera		x			
Crustacea					
Amphipoda					
Stygonectes sp.	x	x	x		
Chydoridae					
Alona circumfimbriata		x	x		
A. rustata		x			
Alonella excisa	x	x			
Camptocercus					
oklahomensis			x		
Chydorus sphaericus	x		x		
Leydigia leydigia		x			
Pleuroxus denticulatus		x	x		
Isopoda					
Caecidotea sp.	x	x	x		
Ostracoda	x	x	x	x	x

Table 2. Continued.

Taxa	Stream				
	Baron Fork	Saline	Wild Hog	Rock	Elm Fork
Crustacea (cont.)					
Copepoda					
Cyclopoida					
<u>Acanthocyclops exilis</u>	x	x	x		
<u>A. sp. (vernalis group)</u>				x	
<u>A. sp. A</u>		x			
<u>Diacyclops crassicaudus</u>					
<u>brachycercus</u>		x			
<u>D. jeanneli putei</u>	x	x	x		
<u>D. nearcticus</u>				x	
<u>D. yeatmani</u>	x	x	x	x	
<u>D. sp. A</u>	x		x		
<u>Eucyclops agilis</u>		x	x	x	
<u>Macrocylops albidus</u>			x		
<u>Microcylops sp.</u>	x	x	x	x	
<u>Paracyclops sp.</u>	x	x	x		
<u>Tropocyclops sp.</u>	x		x		
Harpacticoida					
Ameiridae					
<u>Nitocra lacustris</u>					x
<u>Stygonitocrella sequoyahi</u>	x	x			
Camptocamptidae					
<u>Attheyella illinoisensis</u>	x		x		
<u>A. nordenskioldii</u>	x	x	x		
<u>A. pilosa</u>		x			
<u>Bryocamptus hiemalis</u>	x	x			
<u>B. morrisoni</u>					x
<u>B. vej dovskii</u> forma					
<u>minutiformis</u>	x				
<u>Elaphoidella spp.</u>	x	x	x		
<u>Moraria cristata</u>			x		
Unidentified canthocamptidae					x
Parastenocaridae					
<u>Parastenocaris spp.</u>	x	x		x	x

Table 3. Canonical correspondence analysis. Eigenvalues are for the first 4 axes.

	Axis				Total
	1	2	3	4	
Eigenvalue	0.445	0.352	0.291	0.262	4.574
Cumulative % variance of species data	9.7	17.4	23.8	29.5	

Table 4. Spearman correlation coefficients (r) for significant relationships ( $p \leq 0.01$ ) between invertebrate abundances and environmental factors in 5 Oklahoma streams.

Taxa	Temperature	DO	Depth	Channel Width	Drainage Basin	Grain Size				
						Fine Sand	Medium Sand	Coarse Sand	Very C. Sand	Gravel
Total Invertebrates	-0.38	0.69			-0.47	-0.54	-0.68	-0.68	-0.35	0.68
Nematoda		0.34				-0.30	-0.30	-0.30		0.30
Oligochaeta	-0.35	0.64	-0.25	-0.25	-0.50	-0.42	-0.47	-0.47	-0.46	0.47
Acari		0.30				-0.24	-0.34	-0.34		0.34
Total Insects	-0.27	0.46	-0.44			-0.24	-0.31	-0.31		0.31
Chironomidae	-0.28	0.45	-0.40			-0.24	-0.30	-0.30		0.30
Amphipoda										
Isopoda	-0.30	0.30	0.25		-0.37	-0.49	-0.58	-0.58	-0.28	0.58
Ostracoda	-0.43	0.60			-0.26	-0.39	-0.53	-0.53		0.53
Chydoridae		0.52			-0.43	-0.31	-0.47	-0.47	-0.27	0.47
Cyclopoida	-0.27	0.59			-0.44	-0.41	-0.63	-0.63		0.63
<u>Acanthocyclops exilis</u>	-0.23	0.31								
<u>Diacyclops sp. A</u>	-0.24	0.24		0.49		0.25			0.39	
<u>Diacyclops yeatmani</u>	-0.25			0.22			-0.26	-0.26		0.26
<u>Eucyclops agilis</u>	0.25				-0.31				-0.29	
<u>Paracyclops sp.</u>					-0.36		-0.22	-0.22	-0.31	0.22
Harpacticoida	-0.35	0.61		-0.30	-0.37	-0.55	-0.59	-0.59	-0.40	0.59
<u>Attheyella nordenskioldi</u>	-0.27	0.41	-0.25		-0.32	-0.37	-0.41		-0.28	0.41
<u>Elaphoidella sp.</u>	-0.38	0.43			-0.23	-0.46	-0.55			0.55
<u>Moraria cristata</u>		0.33		-0.35	-0.65	-0.33	-0.34		-0.65	0.34
<u>Nitocra lacustris</u>	0.27				0.37		0.22	0.22		-0.22
<u>Parastenocaris spp.</u>					0.38	0.23		0.33		
<u>Stygonitocrella sequoyahi</u>					0.30	0.29				0.34

Table 4. Continued

Taxa	Conductivity	Cl <sup>-</sup>	SO <sub>4</sub> <sup>-2</sup>	NO <sub>3</sub> -N	SRP	pH	Alkalinity
Total Invertebrates	-0.50	-0.31	-0.33			0.34	-0.26
Nematoda							-0.29
Oligochaeta		-0.39		-0.25		0.58	
Acari	-0.39		-0.34				
Total Insects	-0.26			0.26	0.27		-0.22
Chironomidae	-0.27			0.35	0.22		-0.29
Amphipoda			-0.24				
Isopoda	-0.43		-0.32				-0.22
Ostracoda	-0.51		-0.41				-0.30
Chydoridae	-0.48	-0.50	-0.31			0.32	
Cyclopoida	-0.72	-0.27	-0.54				
<u>Acanthocyclops exilis</u>							
<u>Diacyclops sp. A</u>	-0.37			0.36			-0.38
<u>Diacyclops yeatmani</u>	-0.49		-0.38	0.40		-0.35	-0.35
<u>Eucyclops agilis</u>		-0.24				0.34	
<u>Paracyclops sp.</u>		-0.45				0.31	
Harpacticoida	-0.28					0.35	-0.22
<u>Attheyella nordenskioldi</u>							
<u>Elaphoidella sp.</u>	-0.44		-0.33	0.28			-0.38
<u>Moraria cristata</u>		-0.64		-0.54		0.66	0.33
<u>Nitocra lacustris</u>	0.36	0.28	0.36				
<u>Parastenocaris spp.</u>		0.32		0.33			
<u>Stygonitocrella sequoyahi</u>				0.38			-0.36

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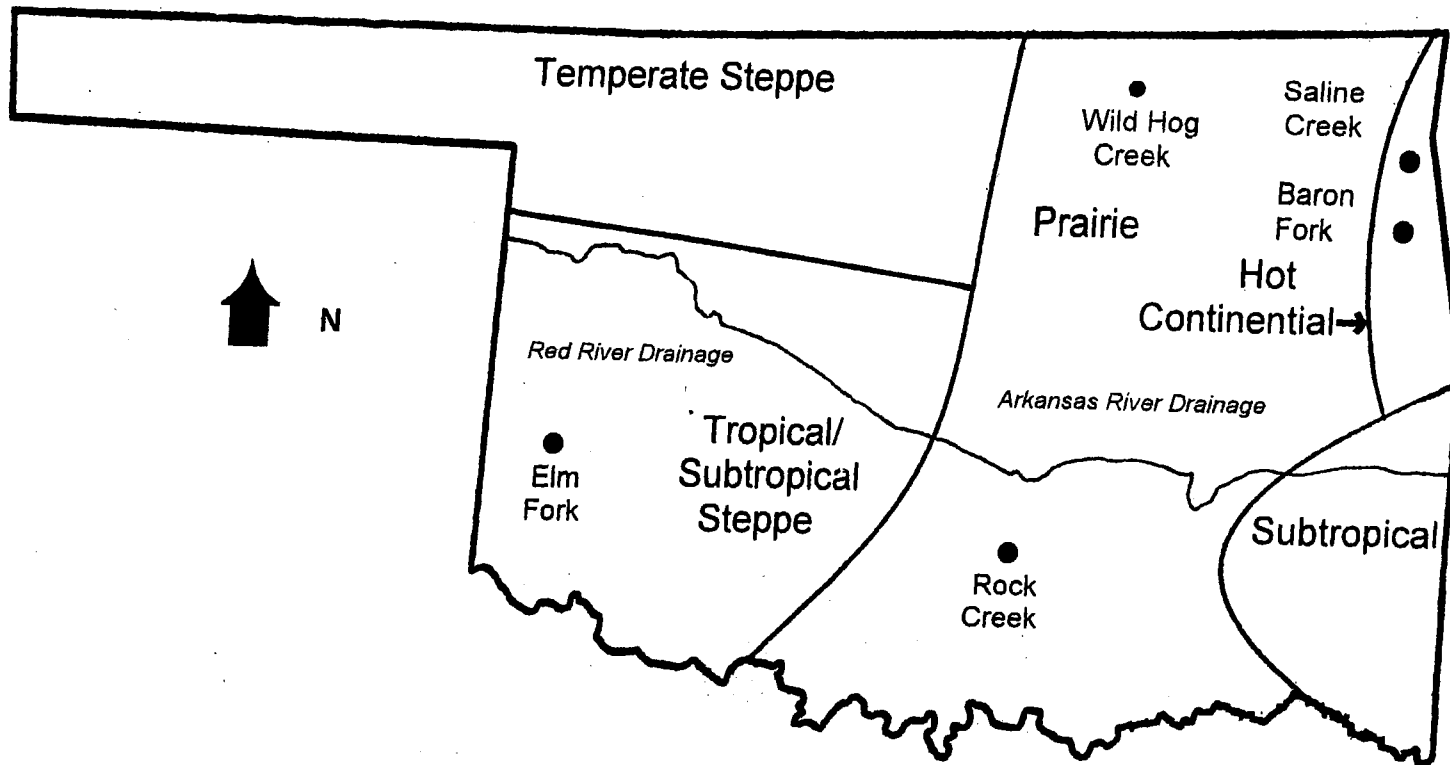
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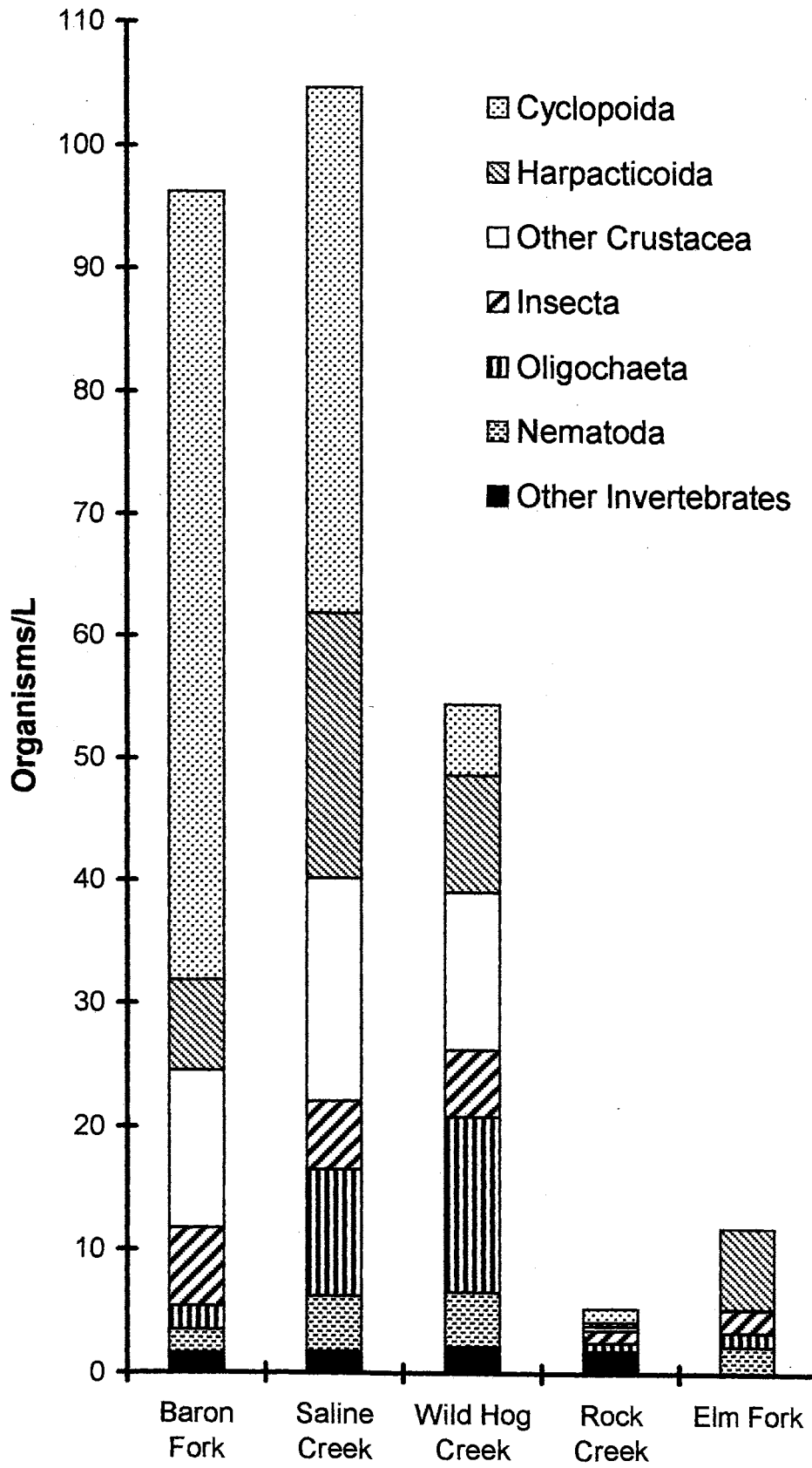
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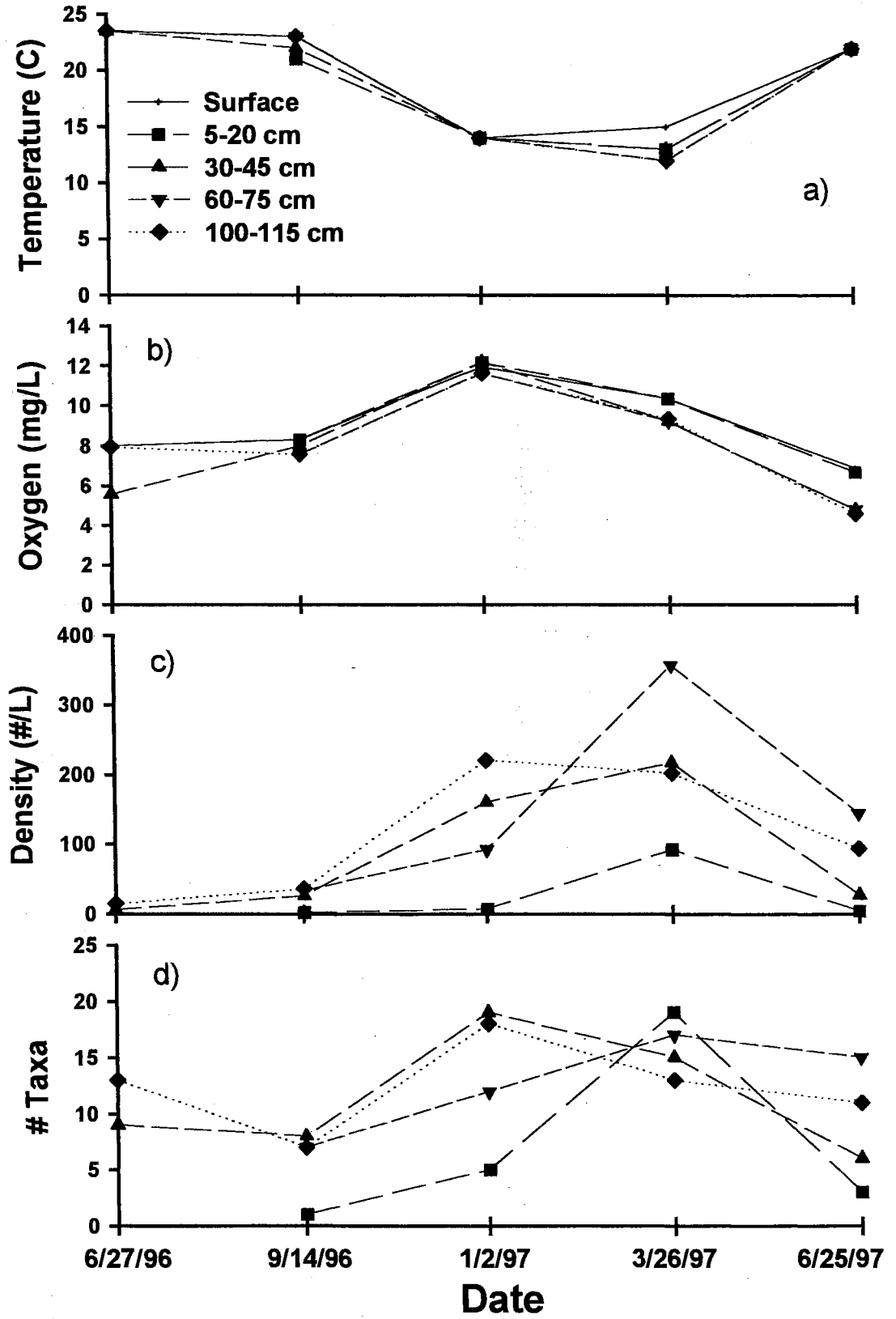
Fig. 15. Triplot based on CCA of hyporheic invertebrate distribution with respect to 5 streams and environmental gradients (arrows) in the plane of the first 2 axes. Axis 1 is horizontal, axis 2 is vertical. Sampling sites grouped by stream are enclosed by labeled polygons. Gravel, very coarse sand, coarse sand, medium sand, and fine sand refer, respectively, to the following grain sizes: >6.3 mm, 2-6.3mm, 0.5-2mm, 0.25-0.5mm, and <0.25mm. Abbreviations: Aca, Acanthocyclops sp.; Ace, A. exilis; Alc, Alona circumfimbriata; Alr, A. rustata; Ale, Alonella excisa; Amp, Amphipoda; Atc, Attheyella cyst; Ati, A. illinoisensis; Atn, A. nordenskioldi; Atp, A. pilosa; Brh, Bryocamptus hiemalis; Cam, Camptocercus oklahomensis; Can, Unidentified canthocamptidae; Cer, Ceratopogonidae; Chi, Chironomidae; Chy, Chydorus sphaericus; Cle, Coleoptera; Cll, Collembola; Dib, Diacyclops crassicaudus brachycercus; Dic, D. crassicaudus; Dij, D. jeanneli putei; Diy, D. yeatmani; DiA, D. sp. A; Ela, Elaphoidella; Eph, Ephemeroptera; Ecy, Eucyclops; Iso, Isopoda; Ley, Leydigia leydigia; Mcy, Macrocylops; Nem,

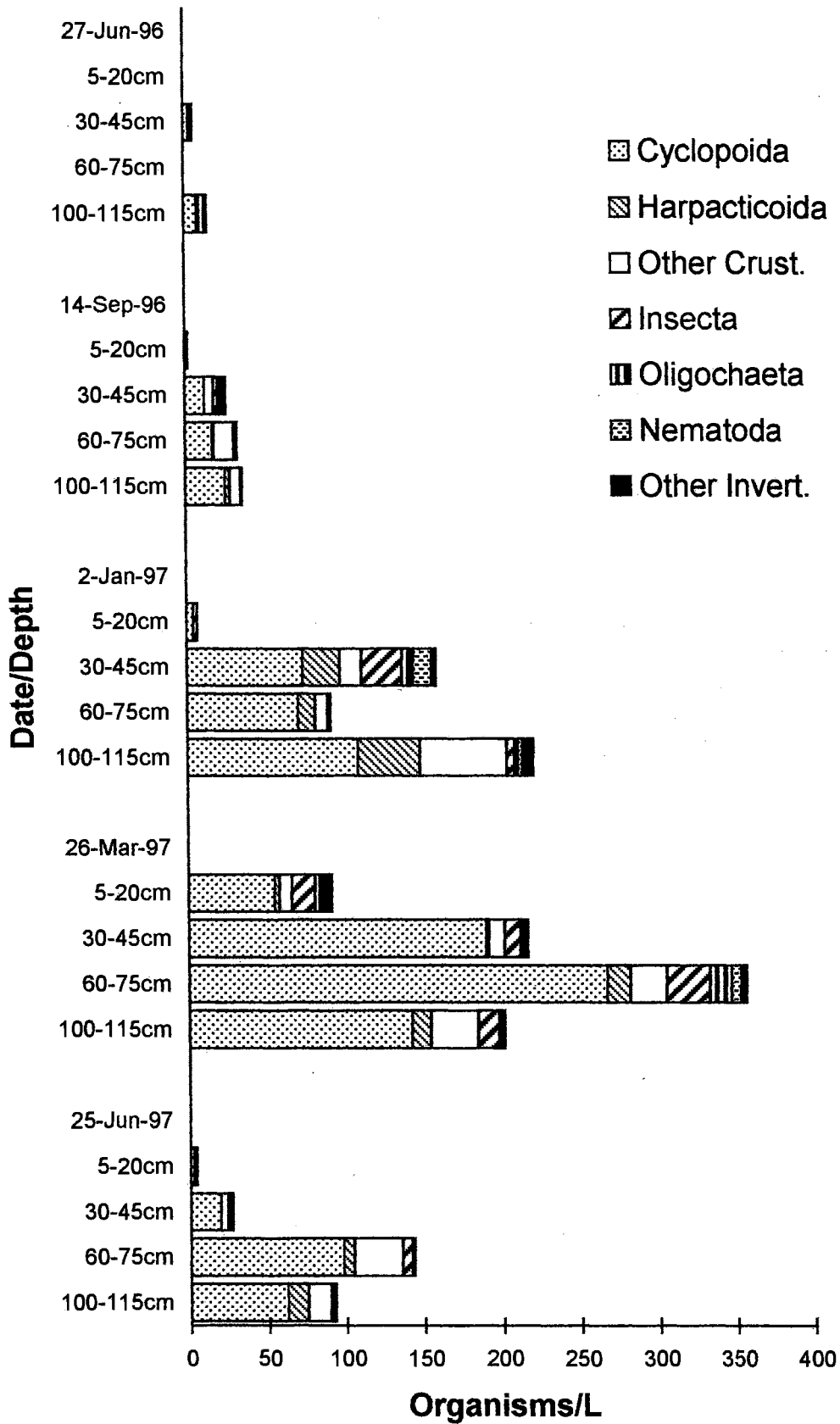
Nematoda; Odo, Odonata; Oli, Oligochaeta; Ost, Ostracoda; Pcy, Paracyclops; Par,  
Parastenocaris; Ple, Plecoptera; Plu, Pleuroxus denticulatus; Sty, Stygonitocrella; Tar,  
Tardigrada; Tri, Trichoptera; Tro, Tropocyclops; Tur, Turbellaria.

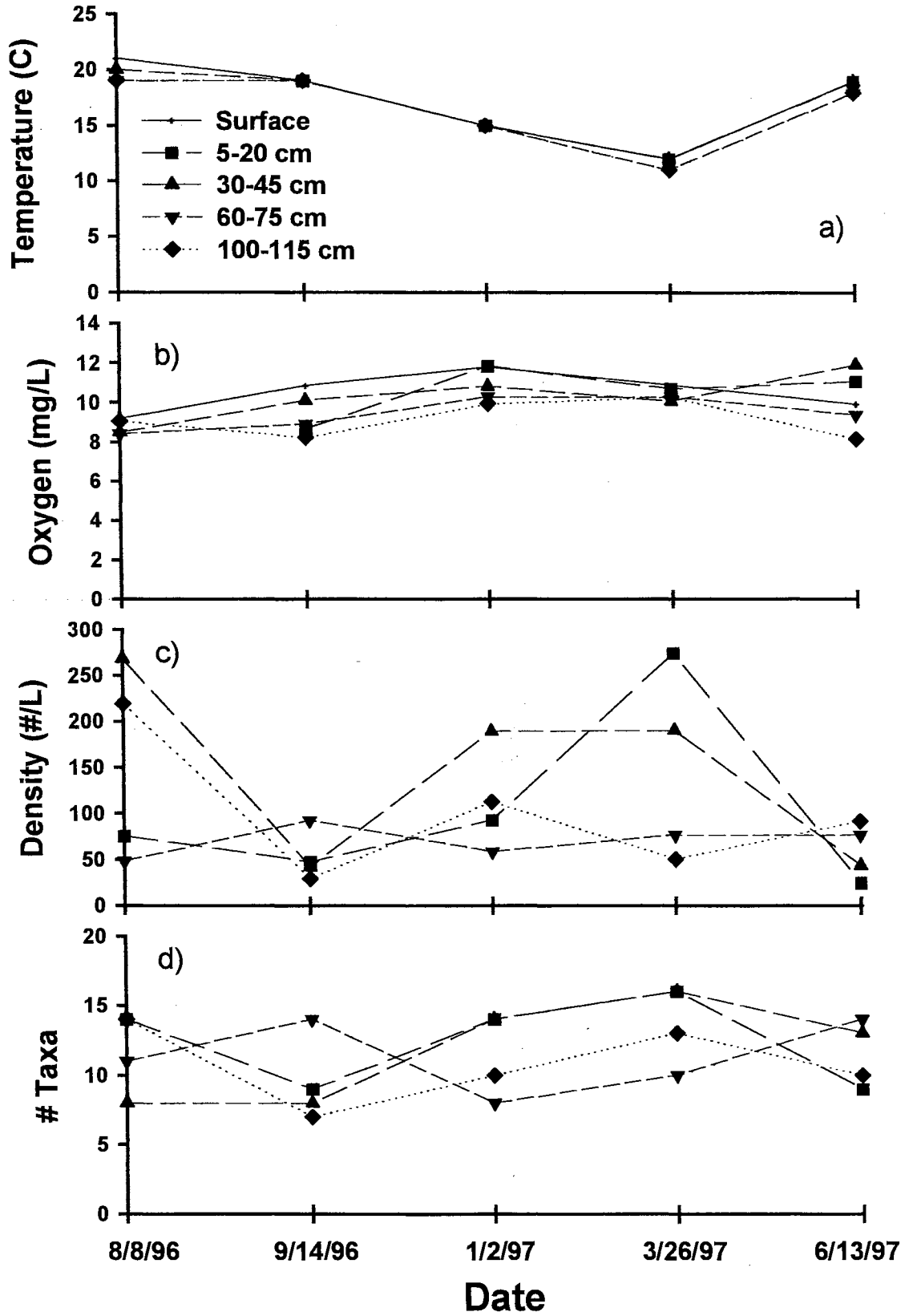


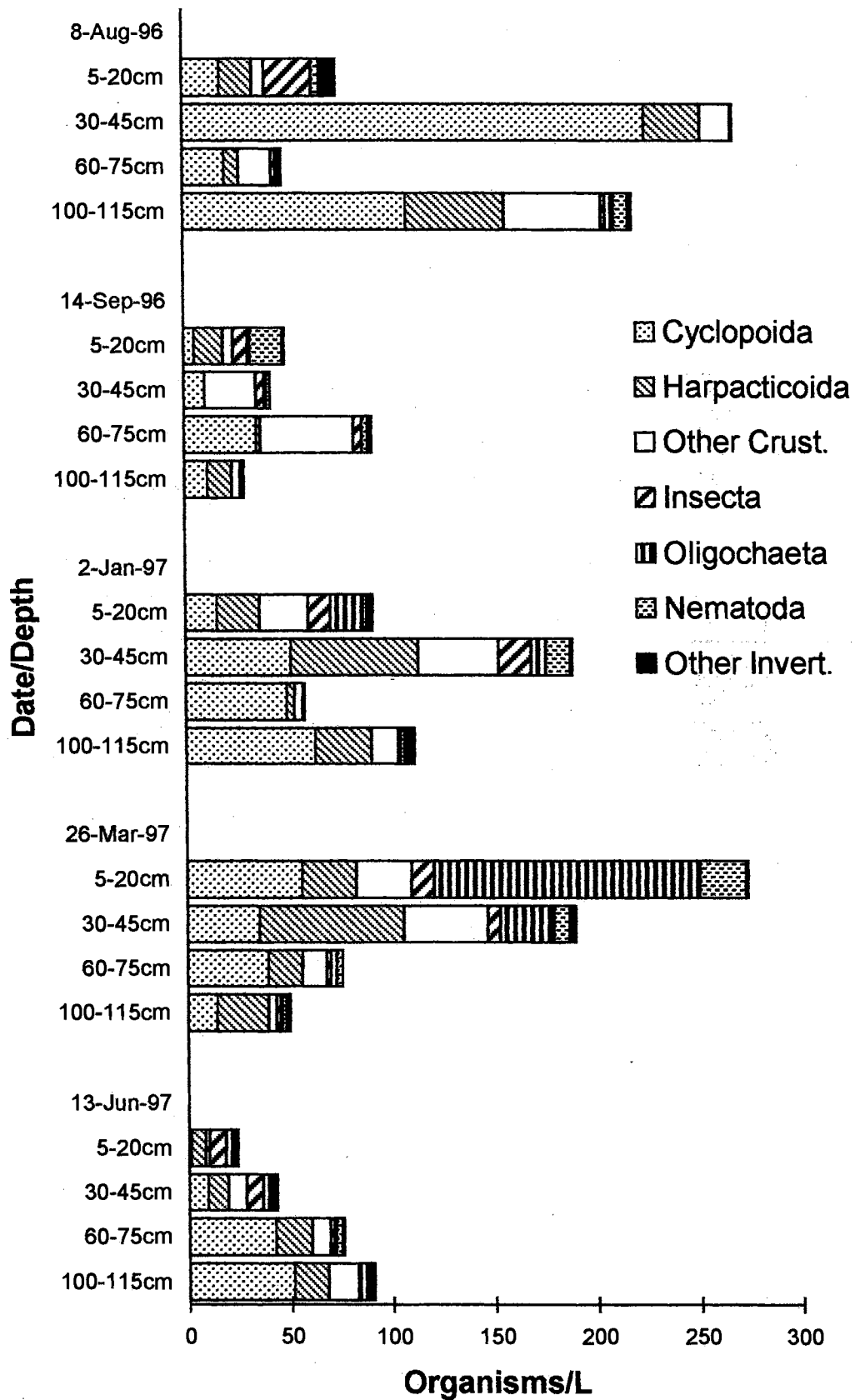


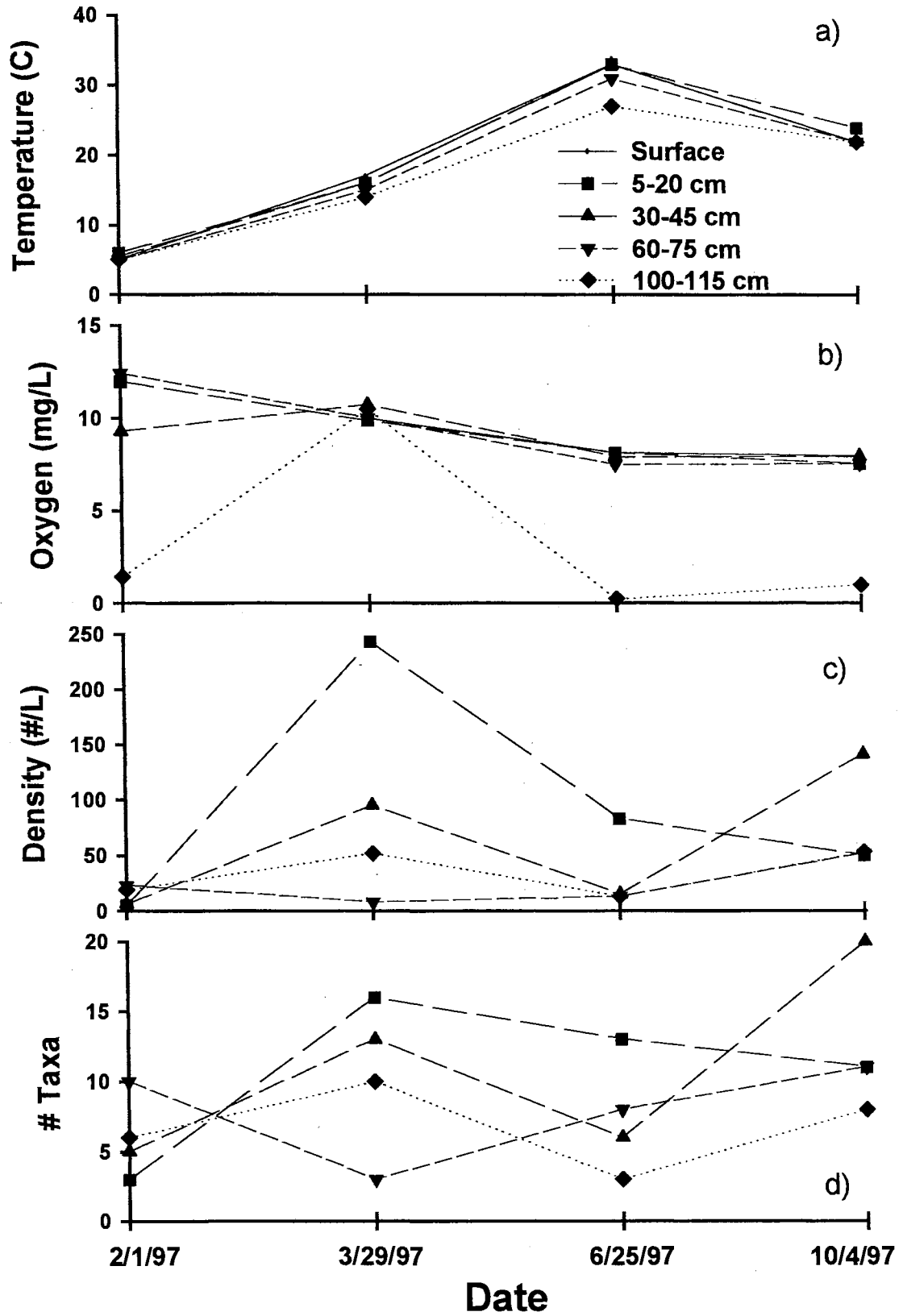


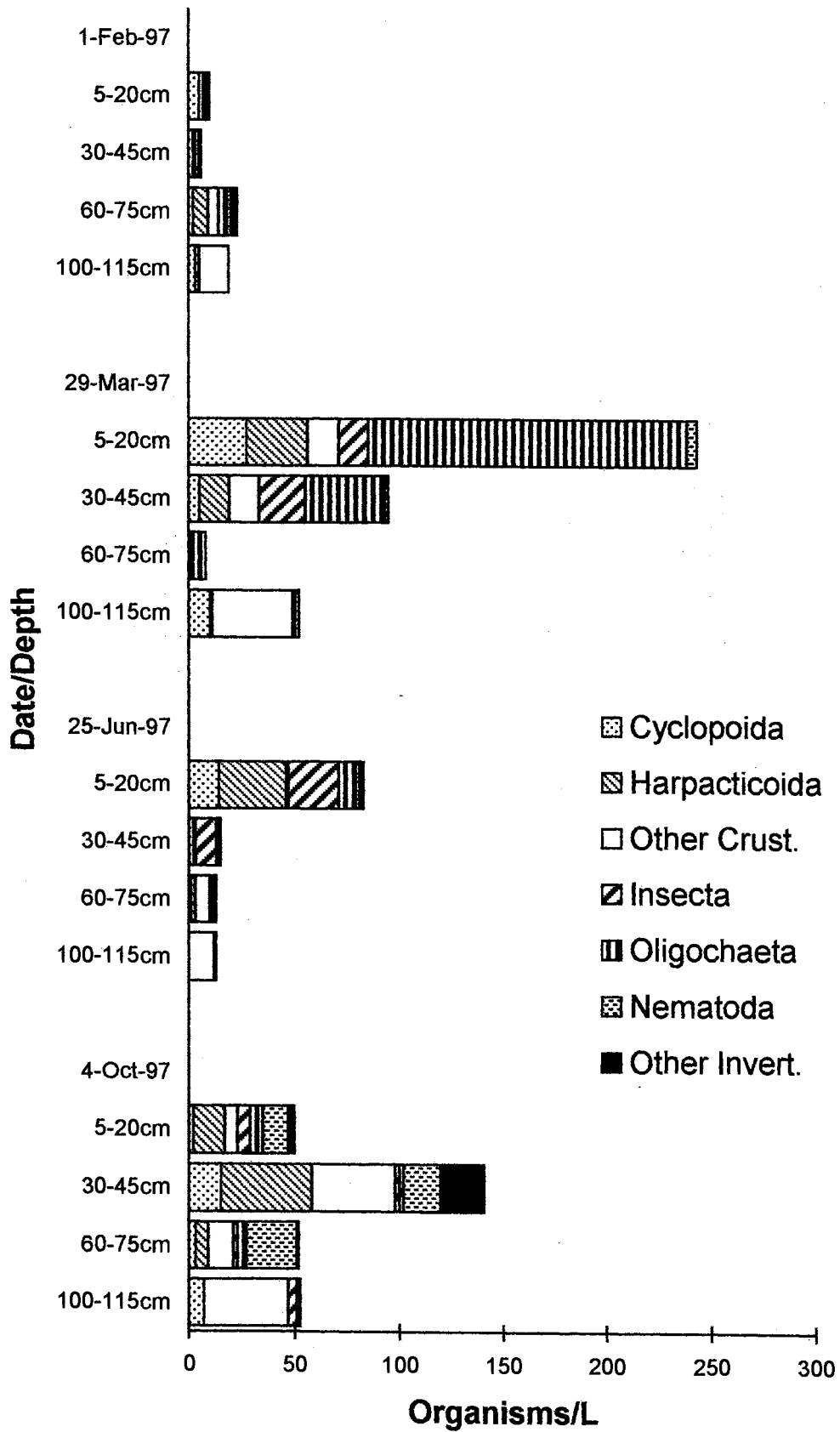




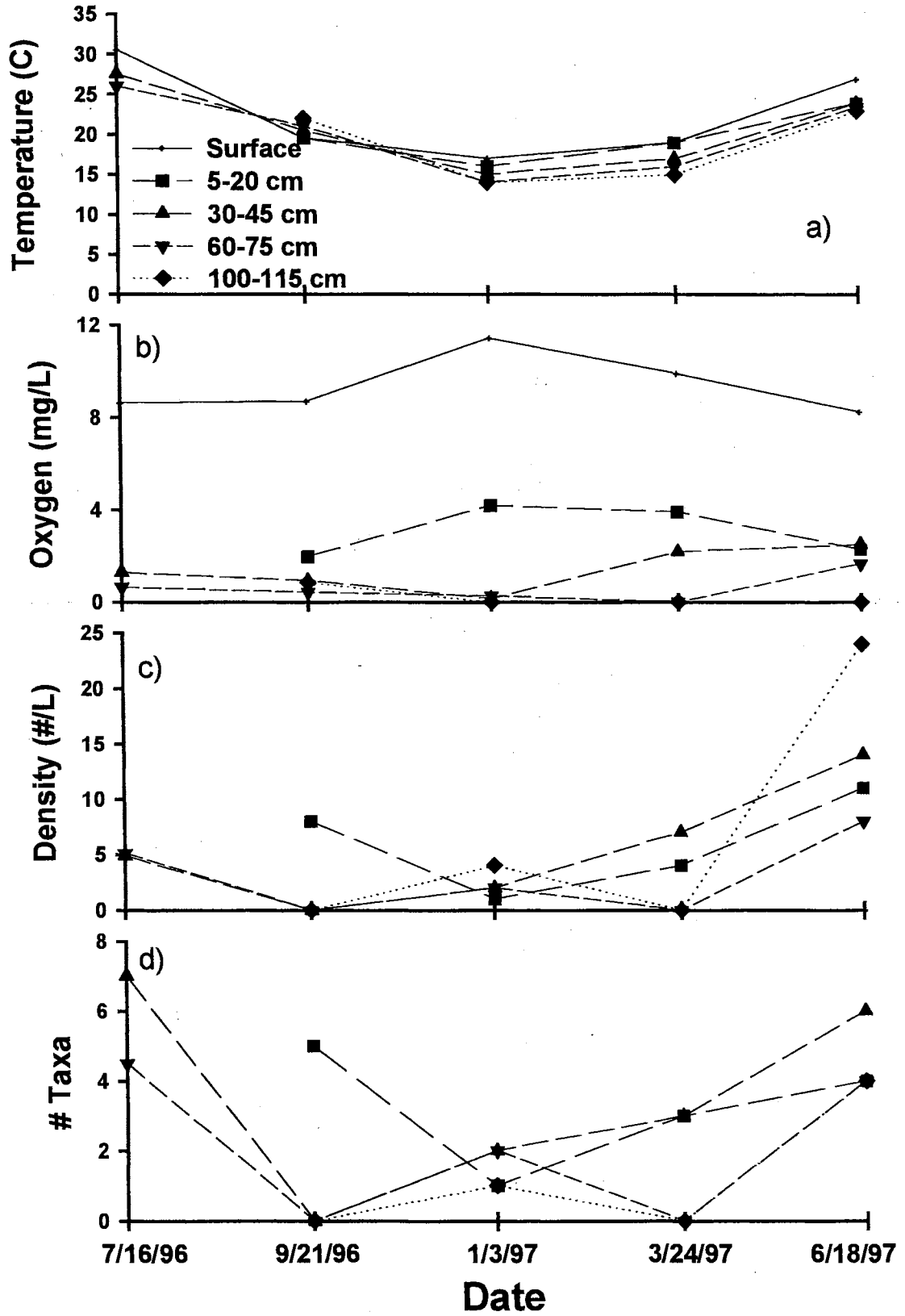


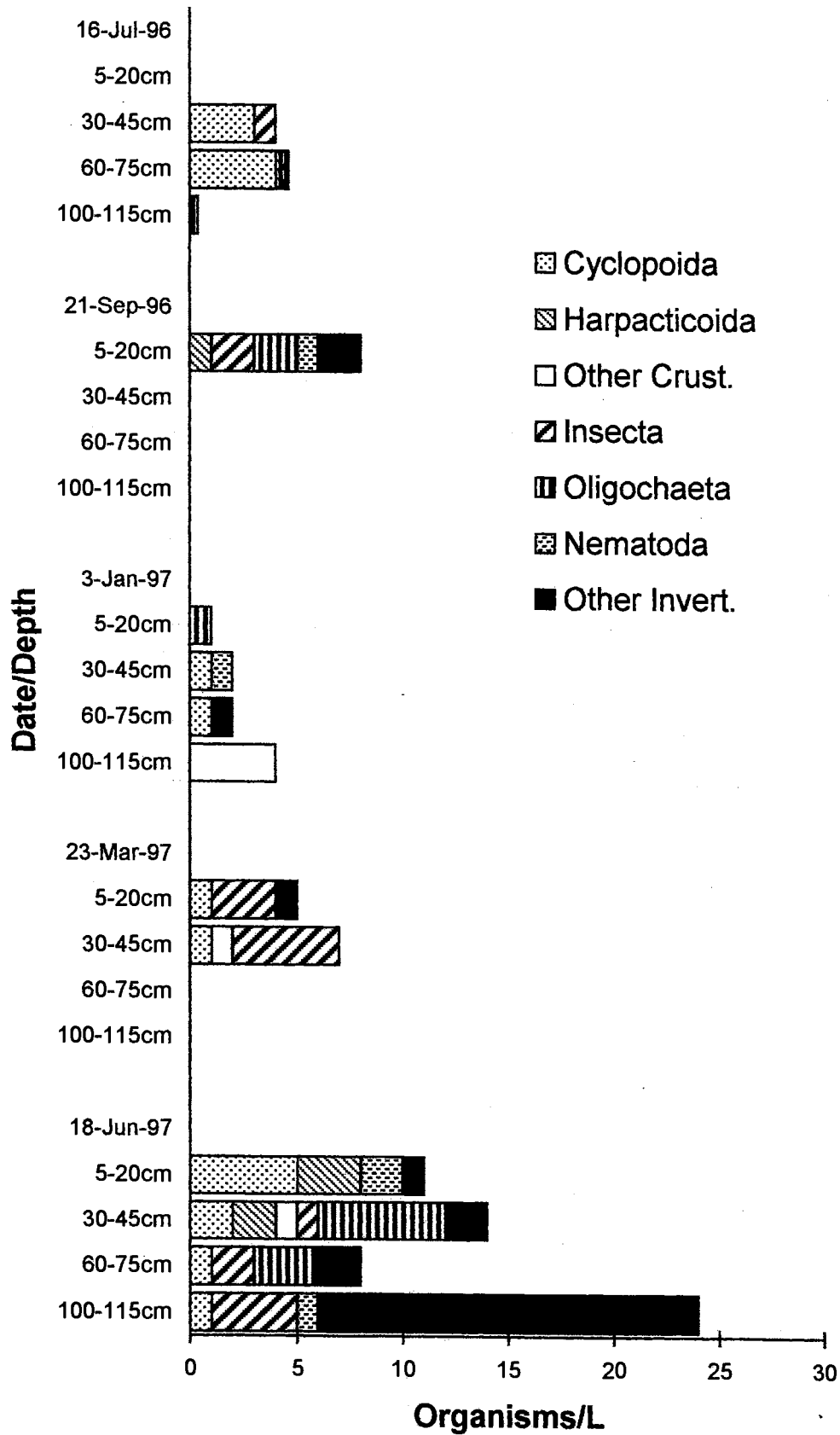


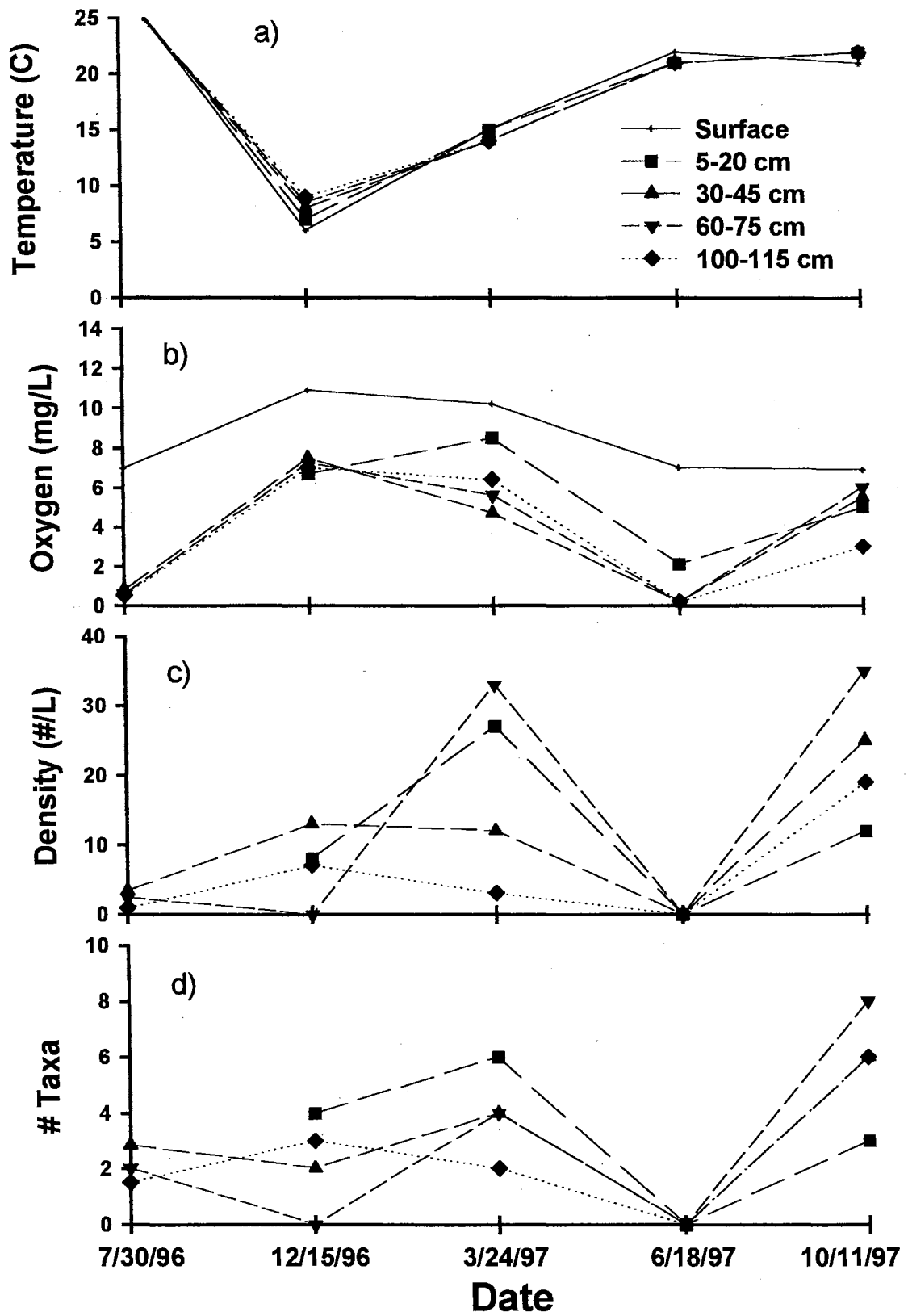


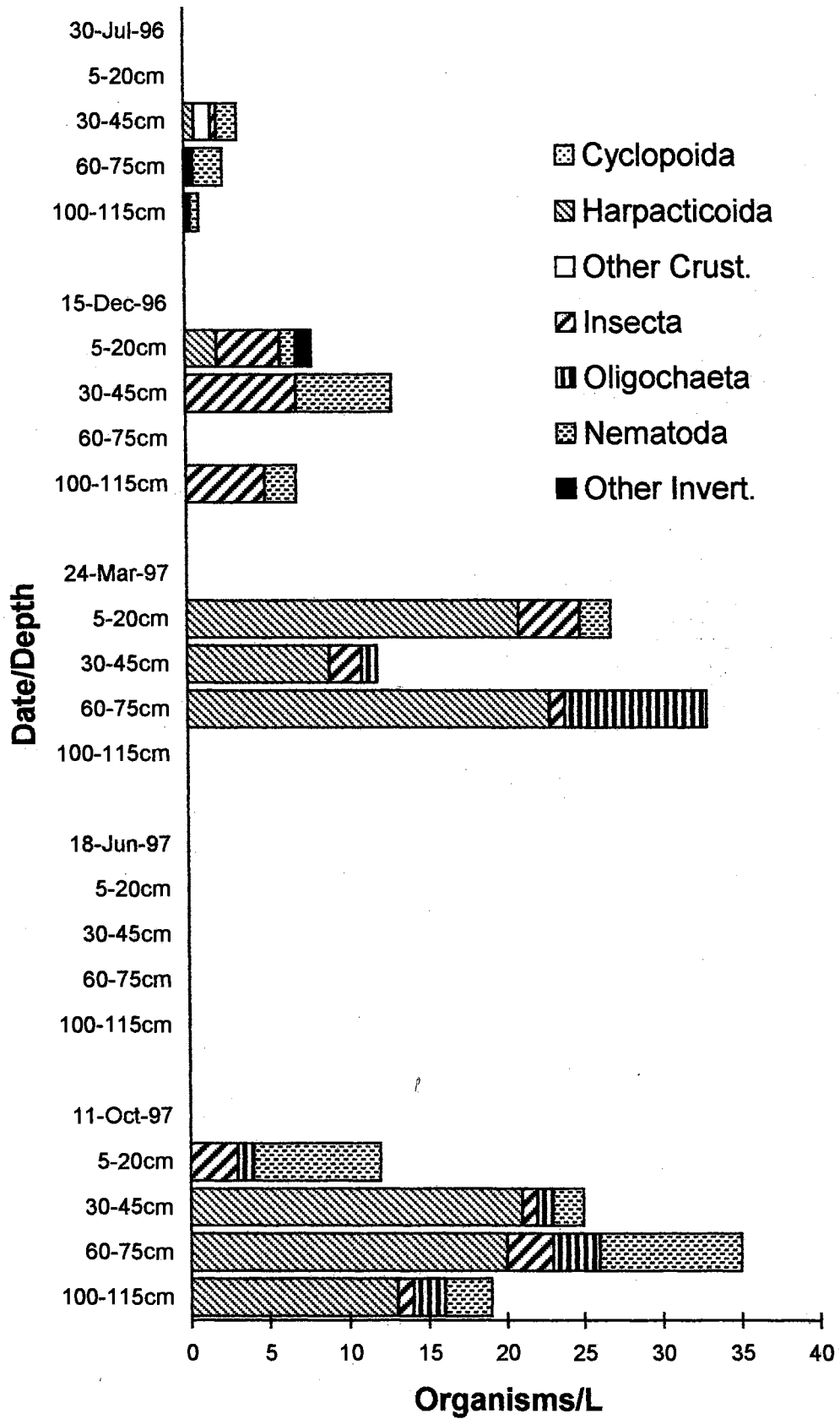


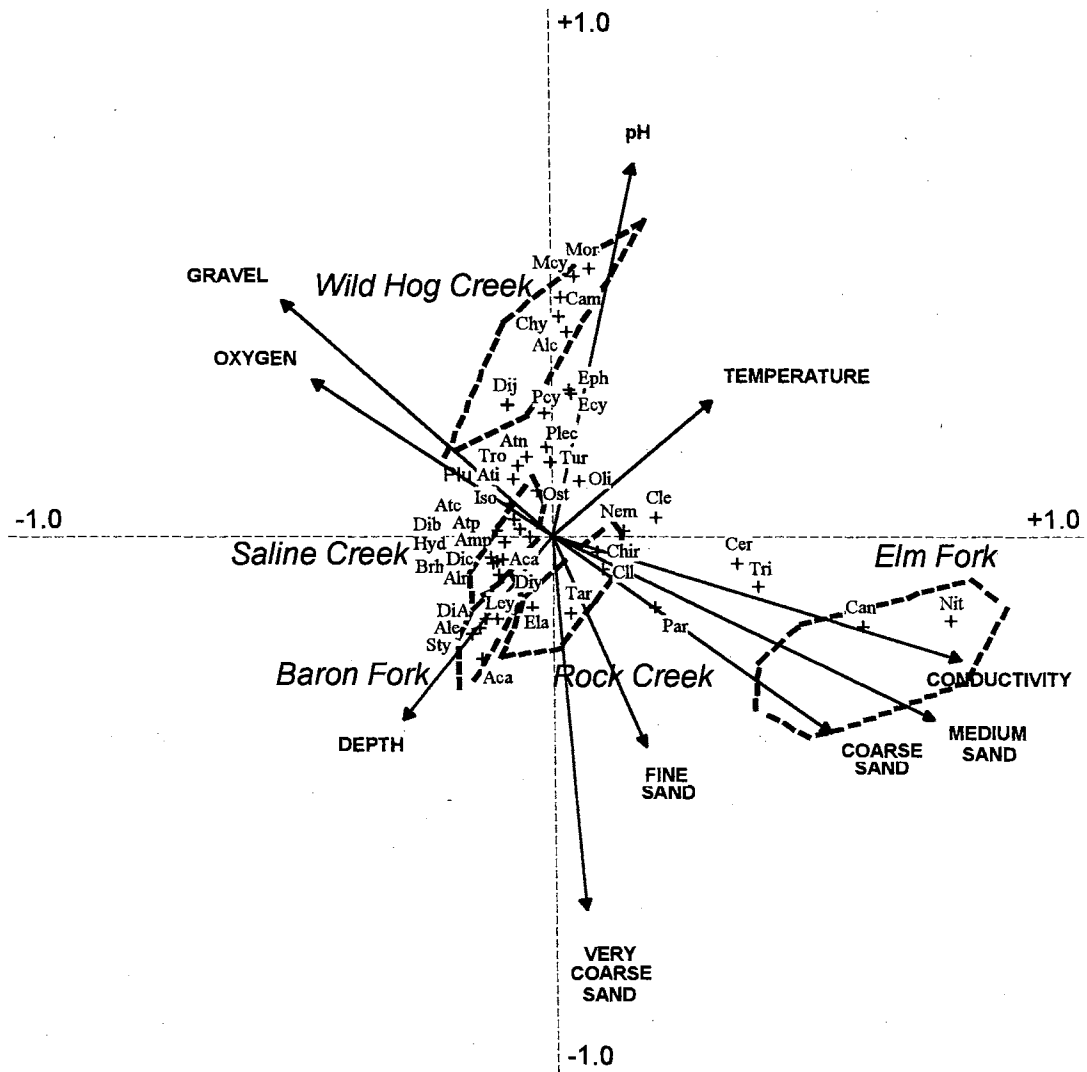












## Appendix

Appendix. Water chemistry data (annual means) for hyporheic samples collected from 5 Oklahoma streams (n=number of samples).

Stream	Temp °C	DO mg/L	DO Sat %	pH	Alkalinity mg/L	Cond μS/cm	Cl <sup>-</sup> mg/L	SO <sub>4</sub> <sup>-2</sup> mg/L	NO <sub>3</sub> -N mg/L	NH <sub>4</sub> mg/L	SRP mg/L
Baron Fork											
n	18	18	18	18	18	18	17	17	17	15	14
Mean	18.3	8.41	88	7.5	89	208	4.9	6.3	1.011	0.025	0.033
Min.	12.0	4.58	52	7.3	64	191	4.0	3.4	0.224	0.010	0.002
Max.	23.5	12.19	118	7.8	220	228	6.5	8.5	1.610	0.069	0.059
Saline Creek											
n	20	20	19	20	20	20	19	19	19	17	15
Mean	16.7	9.75	100	7.5	94	261	11.2	6.4	0.724	0.037	0.054
Min.	11.0	7.33	86	7.3	68	202	5.4	5.0	0.434	0.017	0.007
Max.	20.0	11.88	128	7.7	126	370	17.7	8.2	0.964	0.081	0.152
Wild Hog Creek											
n	44	44	44	44	44	44	44	44	44	43	44
Mean	18.0	7.50	76	7.9	189	434	2.6	16.0	0.025	0.035	0.032
Min.	5.0	0.20	2	7.4	170	337	1.4	8.8	0.000	0.001	0.005
Max.	33.0	12.43	113	8.3	212	505	5.0	25.4	0.070	0.110	0.194
Rock Creek											
n	18	18	18	18	18	18	18	18	17	18	18
Mean	19.8	1.29	14	7.5	252	575	12.7	15.7	0.076	0.133	0.048
Min.	14.0	0.00	0	7.0	148	342	6.0	6.3	0.005	0.031	0.002
Max.	27.5	4.19	42	7.9	392	894	24.0	45.1	0.237	0.433	0.157
Elm Fork											
n	16	15	15	16	16	16	13	16	16	13	16
Mean	18.8	3.94	39	7.4	187	5587	1169.0	1071.0	1.446	0.054	0.064
Min.	7.0	0.21	2	7.0	123	2545	190.0	160.0	0.057	0.021	0.010
Max.	27.5	8.51	84	7.8	358	9870	2069.0	1649.0	4.726	0.099	0.201

CHAPTER IV

THE EFFECT OF POINT SOURCE EFFLUENT ON  
HYPERHEIC COMMUNITIES IN  
FOUR OZARK STREAMS

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## **Abstract**

The impact of treated sewage on the hyporheos was investigated at 4 Ozark streams in northwestern Arkansas. Samples were collected on 3 dates during the period June 9-August 11, 1997, at 4 stations on each stream. One station was located above the discharge and 3 stations were located downstream of the discharge at various distances. Samples were collected using the Bou-Rouch method at well depths of 5-20, 30-45, 60-75 and 100-115 cm below the stream bottom. Invertebrate densities and taxon richness above and below the discharge points did not differ significantly. Ordination and Spearman rank correlations indicated that community composition in the four streams is strongly dependent on DO availability which decreased with depth in the hyporheic zone. Low DO levels in the hyporheic zone suggest that organic loading from nonpoint source runoff may be producing adverse impacts to the stream which have not been indicated during monitoring of benthic macroinvertebrates.

## Introduction

The composition, density and distribution patterns of hyporheic invertebrates can be affected by changes in the physical and chemical characteristics of water within the hyporheic zone (Williams 1984 and 1989, Bretschko and Leichtfried 1988, Danielopol 1989, Triska et al. 1989, Dole-Olivier and Marmonier 1992, Stanley and Boulton 1993). Two important factors include the quantity of organic matter and the oxygen content (Williams and Hynes 1974, Gibert et al. 1990, Dole-Olivier and Marmonier 1992). Strayer et al. (1997) suggested that oxygen is the most important factor and that organic matter is important only where ample oxygen is present. Because the hyporheos are sensitive to abiotic conditions (Danielopol 1991a, Rouch 1991, Dole-Olivier and Marmonier 1992) and the dynamic interaction between the hyporheic zone and contiguous surface waters has been well documented (reviewed by Jones and Holmes 1996), it has been suggested that hyporheic organisms may be suitable for monitoring environmental conditions (Danielopol 1991b, Essafi et al. 1992, Gibert 1991, Lafont et al. 1992, Malard et al. 1996). Thus, it follows that any pollutant which may affect the quality of surface water will also impact the hyporheic zone (Mestrov and Lattinger-Penko 1981). Once pollutants have entered the subsurface waters, sorption and degradation processes are generally low and residence times are long, making these waters especially vulnerable (Travis and Doty 1990).

Benthic macroinvertebrate responses to municipal wastewater treatment plant (WWTP) discharges are well documented (Hynes 1960, Welch 1992). However, few studies have addressed the impacts that these discharges have on the hyporheos (Ward et al. 1992, Sinton 1984). Due to the apparent sensitivity of the hyporheic fauna to dissolved

oxygen, it is anticipated that these organisms can be adversely affected by WWTP discharges. The objective of this investigation was to determine the impact of WWTP discharges on hyporheic communities of 4 streams located in northwestern Arkansas.

### **Study Area**

In recent years, the Ozark Plateau region in northwestern Arkansas has experienced rapid growth in human population as well as growth in the poultry industry. These have contributed significantly to point source water discharges in the region. Ozark streams are clear, fast flowing streams characterized by flat gravel bottoms with base flows provided by springs. A recent investigation (Chapter 1) indicated that the bed sediments of Ozark streams are occupied by a diverse group of hyporheic invertebrates. In order to determine the impact that point source discharges are having on hyporheic invertebrates, four streams (Columbia Hollow and Osage, Sager, and Spring Creeks) receiving WWTP discharges were investigated. Stream locations are presented in Figure 1. Descriptive information regarding the streams and discharges is summarized in Table 1. Wastewaters discharged into Sager, Osage and Spring Creeks receive tertiary treatment, whereas the discharge into Columbia Hollow receives only secondary treatment. Tertiary treatment reduces biochemical oxygen demand (BOD) concentrations in these discharge often to less than 2 mg/L and residual phosphorus to less than 0.1 mg/L (ADPCE 1995 & 1997). Secondary treatment is not nearly as efficient, reducing BOD to less than 30 mg/L (Welch 1992).

## Methods

During low flow conditions in 1997, hyporheic samples were collected within the 4 streams on 3 dates during the period June 9-August 11. One station was established immediately above each WWTP discharge and 3 additional stations were positioned at varying distances downstream. Site selection was dependent on the presence of suitable substrates and accessibility. Bed sediments at all stations consisted of medium to large gravel. Location of the stations relative to the WWTP discharges is provided in Table 2. All surface flow in Columbia Hollow flowed underground approximately 0.5 km downstream from Station 3 and remained underground for the next 5 km. Therefore, only 3 stations were sampled on this stream.

Hyporheic samples were collected using the Bou-Rouch method of suctioning water from narrow wells (Bou 1974). At midstream at each station, standpipe wells, consisting of 2.5 cm ID PVC tubing, were installed immediately prior to sampling at all sites. The tubing, perforated with 6.0 mm holes along the lower 15 cm, was driven into the stream's substratum by inserting a steel T-shaped driving rod into the well and driving the rod and well so that the top of the perforated section reached the desired depth. The T-bar was then removed, leaving the well in place.

Hyporheic samples (1 L) were collected from the hyporheic zone at 4 depth intervals where possible: 5-20 cm, 30-45 cm, 60-75 cm, and 100-115 cm, below the stream bottom. All samples were concentrated with a 63- $\mu$ m mesh sieve and preserved in 5% formalin. Organisms were sorted and identified to recognizable taxonomic units using a dissecting microscope. Since many of the organisms belonged to taxonomically difficult groups and many of them were immature, most taxonomic units used were above the

generic and family level. The taxonomic composition of the fauna will be addressed in a subsequent paper. Sorting of the invertebrates from sample debris was aided by the addition of rose bengal stain.

Water samples were collected from the surface and at each depth for dissolved oxygen (DO), conductivity, pH, nitrate-N ( $\text{NO}_3\text{-N}$ ), ammonium-N ( $\text{NH}_4\text{-N}$ ), soluble reactive phosphate (SRP), sulfate ( $\text{SO}_4^{2-}$ ), and chloride ( $\text{Cl}^-$ ). DO was determined by Winkler titration, and alkalinity by titration with 0.2 N  $\text{H}_2\text{SO}_4$  (Wetzel and Likens 1991). Conductivity and pH of unfiltered samples were measured in the laboratory with Orion meters. Following filtration through a 0.7- $\mu\text{m}$  glass fiber filter,  $\text{Cl}^-$ ,  $\text{NO}_3\text{-N}$ , and  $\text{SO}_4^{2-}$  were measured with a Dionix DX-100 ion chromatograph and SRP and  $\text{NH}_4\text{-N}$  were analyzed using the molybdate blue method (Murphy and Riley 1962) and phenol hypochlorite method (Solorzano 1969), respectively. Temperature was measured in situ at each depth by lowering a thermometer into the well.

Total invertebrate densities and taxon richness were compared among stations and dates for significant differences with a 2-way ANOVA with repeated measures using SigmaStat for Windows (Release 2.01, Jandel Software, San Rafael, California). Streams served as subjects and stations and dates as treatments. Station 4 and the 100-115 cm depth were not included in the analysis because of the large number of missing data points. The analyses were performed using  $\log_{10}(x+1)$  transformed data.

The relationships between physical and chemical environmental factors and the abundances of the hyporheos were investigated using two statistical approaches. The distribution and composition of hyporheic assemblages were related to environmental variables by canonical correspondence analysis (CCA) (ter Braak 1986) using the program

CANOCO for Windows (Release 4.0, Microcomputer Power, Ithaca, New York) (ter Braak and Smilauer 1997). This ordination technique directly relates community attributes (taxon abundances) to environmental variables for evaluation of community-environmental relationships (ter Braak 1987). This technique can also be used to summarize faunal assemblages and to reveal relationships among streams based on their biota. Thus, within a CCA ordination diagram, sites with similar taxonomic composition occur most closely together and each species point is located at the centroid of the site locations in which it occurs. CCA extracts from the measured environmental and biotic variables synthetic gradients (ordination axes) that maximize the niche separation among species. Environmental gradients are represented on the ordination diagram by arrows pointing in the direction of maximum change for each associated variable and with the relative length of each arrow indicating the variable's relative importance. Individual taxa are related directly to these axes under the assumption of a unimodal species response to the environmental variables. The location of site scores relative to the arrows indicate the environmental preferences of each species. Information regarding the interpretation of CCA ordination diagrams is provided by ter Braak and Verdonschot (1995). Eigenvalues are calculated that indicate the degree of correlation between species and sites. An eigenvalue near 1 indicates a high degree of correlation and an eigenvalue near 0 indicates little correspondence. Taxa counts were square root transformed and abundances for rare taxa were down weighted in order to prevent extremely abundant or extremely rare taxa from having unique influence on the ordination (Gauch 1982).

In addition, Spearman rank correlations were computed between the taxon richness and specific taxon abundances (number of organisms/L) and physical and

chemical variables.

## **Results**

### Physicochemical Conditions

Stream surface waters below the discharges, i.e., Stations 2-4, exhibited increased levels of conductivity,  $\text{Cl}^-$ ,  $\text{NO}_3\text{-N}$ , and  $\text{SO}_4^{2-}$ , and SRP when compared with Station 1 above the discharges (Table 3). Hyporheic waters generally exhibited similar levels to overlying surface waters. Elevated  $\text{NH}_4\text{-N}$  occurred only downstream of the Decatur discharge as is typical of discharges of effluent receiving only secondary treatment (USGS 1995).

Temperature also increased downstream of the discharge points. Although wastewater effluents can affect stream temperatures throughout much of the year, the increases we observed most likely reflect the time of day that measurements were taken. Station 1 was sampled in early morning with downstream stations visited in numerical order. Station 4 was usually sampled in early afternoon. Thus, the surface water temperatures increased during the day paralleling the daily increase in air temperatures. Hyporheic waters at each station generally exhibited very similar temperatures to the overlying surface waters indicating rapid exchange between surface and hyporheic waters. DO saturation was less than 100% in most samples, with higher values most often occurring in downstream stations. Higher downstream values probably were the result of increasing water temperatures as described above. DO saturation values decreased with increasing depth.

### Community Composition

The compositions of invertebrate populations in the Ozark streams were comparable in Osage, Sager and Spring Creeks with several taxonomic groups relatively abundant (Table 4). The microcrustaceans, i.e., Cyclopoida, Harpacticoida and Ostracoda, were among the most abundant groups in these streams. Other abundant groups included Insecta larvae (predominantly Chironomidae), Oligochaeta and Nematoda. Isopoda, Amphipoda and Ephemeroptera also were important groups within these streams. The hyporheos in Columbia Hollow, in contrast, was dominated by Oligochaeta (73%), with Nematoda the next most abundant group. No Isopoda, Amphipoda nor Ephemeroptera were collected from Stations 1-3 on this stream.

### Comparison of Stations

Average total invertebrate densities, i.e., sum of all depths for each station, are presented in Fig. 2. Densities were much greater in Sager Creek ( $\geq 390/L$ ) at all stations than in either of the other streams. Densities in the other streams generally averaged below 200/L, although a higher average density, i.e., 313/L was observed at Station 3 in Spring Creek. A comparison of relative vertical distribution by stations and stream (Fig. 3) indicates considerable variability among stations within each stream. In both Columbia Hollow and Osage Creek, invertebrate densities were greater at the 5-20 and 30-45 cm depths. However, no samples were collected at the 100-115 cm depth at Stations 2-3 in Columbia Hollow. In Sager Creek, invertebrates were most concentrated above 45 cm at Stations 1-2, but were more abundant at the lower depths at Stations 3-4. In Spring Creek, just the opposite condition occurred.



Although average densities were greatest at downstream stations in 3 of the streams, comparison of stations by the 2-way ANOVA with repeated measures indicated that the total invertebrate densities at Stations 1-3 (sum of densities at the 5-20, 30-45 and 60-75 cm depths) did not differ significantly ( $P>0.05$ ) from one another (Table 5).

Average taxon richness also was greatest at the Sager Creek stations (Fig. 4). The lowest richness was exhibited in Columbia Hollow. Although taxon richness was slightly higher at Station 2 in three of the streams, i.e., Columbia Hollow and Osage and Sager Creeks, statistical comparisons (Table 5) indicated that taxon richness among Stations 1-3 (taxa occurring in the upper 3 depths) did not differ significantly ( $p=0.05$ ).

Composition of the hyporheos by station and stream is presented in Fig. 5. As previously stated, the richness within Columbia Hollow was considerably less than in the other 3 streams with Oligochaeta the dominant group. With few exceptions, relative composition was remarkably similar among stations within each stream.

### Ordination

Variables that contributed most to the explanation of relative abundances of taxa were chosen by the stepwise analysis (forward selection procedure) in CANOCO, with a 5 % significance level. Significant variables included depth, temperature, DO, and  $\text{SO}_4^{2-}$ .

Eigenvalues for the first four CCA axes accounted for 20.7 % of the variation observed in the species data (Table 6) with the first 2 axes accounting for most (14.9%) of the variation. A biplot of taxa and environmental gradients (indicated by arrows) relative to these axes is presented in Fig. 6. The first axis is primarily related to depth and DO with increases in depth associated with decreases in DO. The second axis is most strongly

related to  $\text{SO}_4^{2-}$ ,  $\text{Cl}^-$  and temperature with increasing temperature and  $\text{Cl}^-$  associated with decreases in  $\text{SO}_4^{2-}$ .

Taxa strongly associated with DO included the insects (i.e., Chironomidae, Coleoptera, Ephemeroptera, and Trichoptera), Oligochaeta, Turbellaria, and Chydoridae. Taxa whose presence was more highly correlated with increasing depth included Amphipoda, Isopoda and Cyclopoida. Organisms positively related to temperature included Oligochaeta, Chydoridae and Hydra. Those negatively correlated to temperature included Ostracoda and Tardigrada.

The distribution of sampling sites relative to the first 2 axes classified by stations and depth are diagrammed in Figs. 7 and 8, respectively. Site scores located closer together indicate greater similarity in community composition. Site scores based on station location (Fig. 7) suggest little differentiation among the stations. Site scores classified by depth (Fig. 8) indicate the importance of this factor in affecting community composition.

#### Spearman Rank Correlations

Spearman rank correlation analyses supported the trends indicated by CCA. Both taxa richness and total abundance exhibited a positive correlation with DO and negative correlations with depth and  $\text{NH}_4\text{-N}$  concentrations. The abundances of several major groups including Oligochaeta, Total Insecta, Chydoridae, and Harpacticoida showed strong positive correlations with DO. Conversely, all groups with the exception of Acari and most crustaceans, i.e., Amphipoda, Cyclopoida, Harpacticoida, and Ostracoda, were negatively correlated with depth. Of the major groups, only the copepods were negatively correlated with  $\text{NH}_4\text{-N}$ . With few exceptions, few other significant positive or

negative correlations were observed.

### **Discussion**

The results of this investigation indicated there were no significant differences in hyporheos abundance or taxon richness located above and below treated sewage effluent discharges from municipal WWTPs into 4 Ozark streams. These results are comparable to previous investigations on macroinvertebrates inhabiting the benthos of these streams (ADPCE 1995 & 1997). Macroinvertebrate communities in Osage, Sager and Spring Creeks downstream of the discharge points included in their investigation showed no significant or only slight impairment compared with communities located above the discharge points. Furthermore, the benthic communities in the three streams did not differ significantly from communities in nearby non-impacted reference Ozark streams (ADPCE 1995 & 1997).

The hyporheos of the streams receiving effluent from tertiary treatment were similar to the communities in Saline Creek, a relatively pristine Ozark stream located in northeastern Oklahoma (Chapter II and III). Densities in June and August samples in this stream ranged from 100-200/L, similar to the findings in the Arkansas Ozark streams. However, the hyporheos in Saline Creek were relatively abundant at the 60-75 and 100-115 cm depths reflecting high DO at those depths. Also, copepods composed a larger component of the hyporheos in Saline Creek and ostracods and oligochaetes were not as abundant as observed in the Ozark. The latter two groups have been found in streams receiving treated sewage. Ward et al. (1992) reported both groups to be much more abundant downstream than upstream of treated sewage discharges in two Colorado

streams. In addition, ostracods have been reported to be resistant to pollution (Schmidt et al. 1991) and low DO (Creuzê des Châtelliers et al. 1992). In the present study, ostracods were most abundant at downstream stations, i.e., Stations 2 & 3 in Sager Creek and Station 3 in Spring Creek.

Ordination and rank correlations suggest that the abundance of the hyporheos in the Ozark streams is strongly dependent on depth and DO availability, a finding consistent with previous findings in Chapter II and III and by others, i.e., Strayer (1994), Strayer et al. (1997), Ward and Palmer (1994), Ward et al. (1994). DO availability within the hyporheic zone is dependent on depth and the rate of exchange between surface and hyporheic waters. This exchange is, in turn, dependent on surface flow and the porosity of the bed sediments. In general, hyporheos abundances are greatest in the upper 20 cm of bed sediments and decline with increasing depth due to decreasing DO (Williams and Hynes 1974, Whitman and Clark 1984, Pugsley and Hynes 1986, Strommer and Smock 1989, McElravy and Resh 1991, Betschko 1992, Strayer et al. 1997). Abundances in the Ozark streams were greatest above the 45-cm depth.

Although invertebrate abundances and taxon richness in the streams included in this study do not appear to be adversely affected by WWTP discharges receiving tertiary treatment, the hyporheic zones appear to be DO stressed when compared with the relatively pristine Saline Creek. The headwaters of Columbia Hollow and Osage, Sager and Spring creeks drain primarily urban areas. Thus, the water quality of the streams may be adversely affected by nonpoint runoff. Urban stormwater runoff contains a much higher organic load than runoff from pristine areas (Welch 1992) and can be comparable to that of untreated sewage. Such intermittent organic loading can adversely affect DO

concentrations within the hyporheic zone that may not be reflected by the benthic macroinvertebrate populations. Thus, the hyporheos may provide greater promise in biomonitoring than the benthic macroinvertebrates under some stream conditions. Additional comparisons of the responses of benthic macroinvertebrates and the hyporheos to low to moderately polluted stream systems should be performed.

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Table 1. Descriptive information for 4 Ozark streams.

	Stream			
	Sager Creek	Spring Creek	Osage Creek	Columbia Hollow
Point source	Siloam Springs WWTP	Springdale WWTP	Rogers WWTP	Decatur WWTP
Distance from headwaters (km)	10	7	10	5
Drainage area above point source (km <sup>2</sup> )	35	21	98	10
Stream discharge at point source (L/sec)	343	456	895	50
Point source discharge (L/sec) <sup>a</sup>	150	445	173	108

<sup>a</sup> Arkansas Department of Pollution Control and Ecology

Table 2. Location of sampling stations on 4 Ozark streams receiving WWTP discharges. Locations for stations 2-4 are the distances (km) downstream of the discharge point. ns = not sampled.

	Stream			
	Sager Creek	Spring Creek	Osage Creek	Columbia Hollow
Station 1	-0.1	-0.2	-0.4	-0.1
WWTP	0.0	0.0	0.0	0.0
Station 2	2.4	0.9	3.8	2.6
Station 3	4.7	2.5	5.2	3.2
Station 4	6.3	6.6	6.7	ns

Table 3. Summary of mean water chemistry data ( $\pm$ SE) collected in 4 Ozark streams receiving treated municipal sewage effluent. Means are for 3 sampling dates between June 9 and August 8, 1997. Upstream includes Station 1. Downstream includes Stations 2-4 for Osage, Sager and Spring creeks and Station 2-3 for Columbia Hollow. SRP = soluble reactive P. nd = indicates that no data were collected. a = mean is based on <3 samples.

Parameter	Depth	Columbia Hollow		Osage Creek		Sager Creek		Spring Creek	
		Upstream	Downstream	Upstream	Downstream	Upstream	Downstream	Upstream	Downstream
Temperature (° C)	Surface	18.3 (1.12)	21.5 (1.04)	17.3 (0.76)	21.5 (0.95)	20.8 (0.28)	21.8 (0.41)	17.2 (0.46)	23.4 (0.57)
	5-20cm	20.0 (1.00)	21.7 (1.61)	17.3 (1.20)	21.2 (1.30)	21.2 (0.17)	21.7 (0.56)	17.2 (0.44)	22.0 (0.94)
	30-45cm	20.0 (1.00)	21.0 (1.45)	17.7 (1.45)	21.0 (1.25)	21.3 (0.33)	21.6 (0.53)	17.2 (0.44)	19.9 (1.68)
	60-75cm	19.8 (0.73)	20.8 (2.20)	17.7 (1.45)	19.8 (0.92)	21.5 (0.76)	21.3 (0.49)	16.8 (0.60)	18.9 (2.50)
	100-115cm	20.5 (1.04)	nd nd	18.0 a	20.0 (1.26)	nd nd	20.8 (0.57)	17.0 (0.58)	19.3 (1.03)
Dissolved oxygen (% sat)	Surface	78.7 (6.5)	60.3 (7.4)	68.5 (3.4)	90.1 (4.0)	55.2 (3.9)	70.8 (2.4)	77.7 (2.6)	89.1 (3.6)
	5-20cm	84.5 (0.5)	62.0 (8.5)	66.0 (3.1)	77.1 (9.2)	51.3 (8.4)	66.9 (6.0)	78.7 (3.8)	78.1 (7.5)
	30-45cm	75.5 (1.5)	42.2 (14.6)	41.0 (9.6)	33.3 (12.9)	33.7 (0.7)	51.1 (8.1)	62.3 (6.6)	46.7 (13.2)
	60-75cm	29.7 (13.8)	3.0 (1.5)	11.3 (4.7)	16.4 (7.6)	3.0 (1.0)	30.7 (9.0)	56.7 (7.2)	18.0 (15.0)
	100-115cm	13.3 (9.9)	nd nd	7.0 a	12.6 (8.1)	nd nd	30.3 (9.2)	30.7 (15.3)	27.3 (10.0)

Table 3. Continued.

Parameter	Depth	Columbia Hollow		Osage Creek		Sager Creek		Spring Creek	
		Upstream	Downstream	Upstream	Downstream	Upstream	Downstream	Upstream	Downstream
Conductivity ( $\mu\text{S}/\text{cm}$ )	Surface	341 (19.0)	704 (39.5)	391 (28.7)	452 (17.2)	339 (5.7)	494 (13.6)	402 (18.1)	589 (12.8)
	5-20cm	354 (33.0)	689 (54.7)	390 (45.2)	447 (24.3)	329 (15.4)	497 (20.1)	405 (27.6)	577 (22.8)
	30-45cm	352 (38.0)	668 (44.3)	385 (45.8)	459 (23.4)	330 (15.4)	501 (20.2)	407 (24.0)	668 (38.8)
	60-75cm	345 (19.4)	672 (59.0)	377 (44.3)	466 (26.0)	345 (14.3)	499 (19.2)	410 (22.0)	655 (45.3)
	100-115cm	337 (21.2)	nd nd	385 a	453 (36.9)	nd nd	501 (16.6)	409 (21.5)	555 (29.3)
pH	Surface	7.6 (0.06)	7.5 (0.09)	7.2 (0.03)	7.8 (0.06)	7.6 (0.06)	7.6 (0.04)	7.3 (0.03)	7.9 (0.08)
	5-20cm	7.7 (0.00)	7.6 (0.10)	7.1 (0.03)	7.7 (0.10)	7.4 (0.09)	7.6 (0.07)	7.4 (0.09)	7.8 (0.13)
	30-45cm	7.7 (0.05)	7.4 (0.12)	7.2 (0.04)	7.5 (0.12)	7.3 (0.06)	7.5 (0.07)	7.4 (0.02)	7.4 (0.10)
	60-75cm	7.3 (0.07)	7.2 (0.10)	7.1 (0.03)	7.5 (0.10)	7.3 (0.09)	7.3 (0.08)	7.4 (0.06)	7.2 (0.07)
	100-115cm	7.3 (0.09)	nd nd	7.3 a	7.4 (0.14)	nd nd	7.3 (0.06)	7.3 (0.06)	7.1 (0.09)

Table 3. Continued.

Parameter	Depth	Columbia Hollow		Osage Creek		Sager Creek		Spring Creek	
		Upstream	Downstream	Upstream	Downstream	Upstream	Downstream	Upstream	Downstream
Cl <sup>-</sup> (mg/L)	Surface	9.15 (0.78)	47.65 (4.23)	11.07 (1.68)	29.38 (2.34)	16.19 (0.47)	45.19 (3.51)	10.67 (0.46)	40.77 (1.96)
	5-20cm	9.90 (0.55)	46.37 (6.04)	9.98 (1.28)	29.02 (3.35)	15.90 (1.06)	44.88 (5.21)	12.04 (0.79)	38.77 (3.20)
	30-45cm	9.79 (1.27)	49.59 (7.20)	9.18 (1.43)	29.33 (2.99)	16.23 (1.28)	45.07 (5.20)	11.43 (0.67)	49.06 (6.11)
	60-75cm	12.34 (1.39)	47.81 (10.28)	9.32 (0.22)	27.60 (2.89)	16.70 (0.65)	44.71 (5.18)	11.83 (0.73)	46.94 (7.32)
	100-115cm	11.13 (1.10)	nd nd	9.22 a	29.02 (2.44)	nd nd	44.42 (4.87)	10.53 (0.65)	27.39 (5.21)
SO <sub>4</sub> <sup>2-</sup> (mg/L)	Surface	5.07 (0.61)	23.04 (0.61)	3.99 (0.33)	10.57 (0.54)	7.27 (0.18)	13.57 (0.66)	8.26 (0.54)	55.08 (2.39)
	5-20cm	6.48 (0.69)	23.98 (0.79)	4.40 (0.34)	10.74 (0.84)	7.33 (0.31)	13.66 (0.91)	8.73 (1.02)	47.89 (4.24)
	30-45cm	5.71 (1.11)	23.11 (0.84)	4.42 (0.47)	11.22 (1.08)	7.27 (0.10)	13.46 (0.91)	8.99 (0.94)	22.15 (1.18)
	60-75cm	6.50 (0.61)	23.80 (0.55)	5.16 (0.88)	11.17 (1.44)	7.69 (0.19)	13.42 (0.96)	9.07 (0.72)	21.99 (1.85)
	100-115cm	6.47 (0.57)	nd nd	5.32 a	12.20 (1.19)	nd nd	13.19 (0.79)	7.66 (1.48)	31.40 (9.14)

Table 3. Continued.

Parameter	Depth	Columbia Hollow		Osage Creek		Sager Creek		Spring Creek	
		Upstream	Downstream	Upstream	Downstream	Upstream	Downstream	Upstream	Downstream
NO <sub>3</sub> -N (mg/L)	Surface	2.75 (0.34)	10.61 (0.97)	2.63 (0.10)	2.97 (0.09)	1.37 (0.09)	6.22 (0.39)	1.93 (0.13)	2.77 (0.22)
	5-20cm	2.98 (0.69)	10.84 (1.45)	2.73 (0.19)	2.71 (0.17)	1.28 (0.17)	6.09 (0.57)	1.93 (0.20)	3.00 (0.41)
	30-45cm	3.00 (0.65)	10.53 (1.26)	2.53 (0.06)	1.95 (0.28)	1.38 (0.15)	5.86 (0.49)	1.95 (0.24)	9.93 (1.22)
	60-75cm	2.61 (1.13)	10.71 (2.44)	1.90 (0.09)	1.66 (0.40)	0.37 (0.02)	5.73 (0.54)	2.01 (0.18)	9.57 (2.07)
	100-115cm	2.71 (1.01)	nd nd	2.02 a	1.95 (0.52)	nd nd	5.54 (0.48)	2.03 (0.20)	4.71 (0.75)
NH <sub>4</sub> -N (mg/L)	Surface	0.118 (0.050)	0.254 (0.075)	0.096 (0.030)	0.068 (0.010)	0.054 (0.006)	0.033 (0.006)	0.057 (0.016)	0.070 (0.006)
	5-20cm	0.160 a	0.219 (0.092)	0.029 (0.010)	0.105 (0.040)	0.044 (0.009)	0.039 (0.010)	0.057 (0.027)	0.083 (0.009)
	30-45cm	0.219 a	0.123 (0.065)	0.026 (0.000)	0.208 (0.140)	0.034 (0.018)	0.039 (0.016)	0.026 (0.015)	0.102 (0.055)
	60-75cm	0.133 (0.007)	0.767 (0.007)	0.033 (0.010)	0.288 (0.220)	0.041 (0.006)	0.024 (0.007)	0.056 (0.034)	0.521 (0.246)
	100-115cm	0.029 (0.029)	nd nd	0.357 a	0.067 (0.040)	nd nd	0.076 (0.048)	0.041 (0.018)	0.039 (0.007)



Table 3. Continued.

Parameter	Depth	Columbia Hollow		Osage Creek		Sager Creek		Spring Creek	
		Upstream	Downstream	Upstream	Downstream	Upstream	Downstream	Upstream	Downstream
SRP (mg/L)	Surface	0.196	6.478	0.102	0.788	0.120	0.935	0.083	3.085
		(0.040)	(0.462)	(0.010)	(0.090)	(0.015)	(0.092)	(0.029)	(0.414)
	5-20cm	0.274	6.546	0.239	0.717	0.137	0.959	0.176	2.641
		(0.160)	(0.649)	(0.090)	(0.120)	(0.045)	(0.124)	(0.050)	(0.515)
	30-45cm	0.226	6.453	0.210	0.668	0.152	1.110	0.234	6.036
		(0.120)	(0.846)	(0.010)	(0.130)	(0.022)	(0.210)	(0.085)	(0.827)
	60-75cm	0.580	6.641	0.366	0.540	0.270	0.911	0.201	5.967
		(0.180)	(0.770)	(0.140)	(0.170)	(0.066)	(0.113)	(0.058)	(0.866)
	100-115cm	0.723	nd	0.357	0.457	nd	0.867	0.147	1.330
		(0.130)	nd	a	(0.240)	nd	(0.118)	(0.022)	(0.499)

Table 4. Composition (%) of major invertebrate taxa collected in the hyporheos of 4 Ozark streams. L = larval form.

Taxon	Stream			
	Columbia Hollow	Osage Creek	Sager Creek	Spring Creek
Hydra	0.0	0.2	0.4	0.1
Turbellaria	1.8	0.2	0.4	0.2
Nematoda	9.8	9.8	3.7	6.2
Rotifera	0.6	0.6	0.1	0.8
Tardigrada	3.1	0.2	0.0	0.5
Oligochaeta	73.2	7.3	20.2	15.0
Acari	0.4	1.3	0.9	0.6
Total Insecta	0.8	16.6	12.9	9.5
Chironomidae (L)	0.4	8.4	8.4	7.4
Coleoptera (L)	0.0	1.7	1.1	0.2
Ephemeroptera (L)	0.0	4.0	2.9	1.4
Plecoptera (L)	0.0	0.7	0.0	0.3
Trichoptera (L)	0.0	1.7	0.4	0.2
Amphipoda	0.0	1.0	0.6	1.0
Chydoridae	2.5	0.3	6.4	8.3
Cyclopoida	4.4	18.1	22.2	22.5
Harpacticoida	1.0	12.9	19.1	17.9
Isopoda	0.0	5.7	3.7	4.8
Ostracoda	2.5	23.4	4.1	10.6

Table 5. Summary of 2-way ANOVA with repeated measures comparing total invertebrate densities and taxon richness [ $\log_{10}(x+1)$ ] among streams, stations, and dates. Invertebrate densities are the sum for the 5-20, 30-45 and 60-75 cm depths. Taxon richness includes all taxa collected at the 5-20, 30-45 and 60-75 cm depths. Stations 1-3 are included in the analysis.

Effect	SS	df	MS	F-ratio	P-value
Total organisms					
Stream	2.350	3	0.7832		
Station	0.219	2	0.1096	0.907	0.453
Station $\times$ stream	0.725	6	0.1209		
Date	0.169	2	0.0846	0.988	0.426
Date $\times$ stream	0.514	6	0.0857		
Station $\times$ date	0.717	4	0.1794	1.342	0.311
Residual	1.604	12	0.1336		
Total	6.298	35	0.1800		
Total taxa					
Stream	1.384	3	0.4612		
Station	0.036	2	0.0179	0.774	0.502
Station $\times$ stream	0.139	6	0.0231		
Date	0.070	2	0.0350	3.427	0.102
Date $\times$ stream	0.061	6	0.0102		
Station $\times$ date	0.051	4	0.0128	0.510	0.730
Residual	0.302	12	0.0252		
Total	2.043	35	0.0584		

Table 6. Canonical correspondence analysis. Eigenvalues for the first 4 axes.

	Axis				Total
	1	2	3	4	
Eigenvalue	0.090	0.051	0.031	0.024	0.947
Cumulative % variance of species data	9.5	14.9	18.1	20.7	

Table 7. Spearman correlation coefficients (r) for significant relationships ( $p \leq 0.01$ ) between invertebrate richness and abundances and selected environmental factors in 4 Ozark streams receiving treated municipal wastewater discharge.

	Date	Station	Depth	Temp	DO	pH	Cond	Cl <sup>-</sup>	SO <sub>4</sub> <sup>-2</sup>	NO <sub>3</sub> -N	NH <sub>4</sub>	SRP
Taxon richness				-0.27		0.27						-0.32
Total invertebrates			-0.29		0.25						-0.25	
Rotifera			-0.20									
Nematoda			-0.21	0.22								
Oligochaeta			-0.38		0.36	0.22			0.26			
Acari									-0.27			
Insecta (total)			-0.50		0.36							
Chironomidae			-0.51		0.32							
Coleoptera (L)			-0.26									
Ephemeroptera (L)			-0.44		0.27	0.22						
Plecoptera (L)			-0.21		0.24							
Amphipoda												
Chydoridae	-0.23		-0.29	0.30	0.23			0.25	0.23			0.20
Cyclopoida											-0.35	
Harpacticoida					0.21						-0.34	
Isopoda			0.22									-0.38
Ostracoda												

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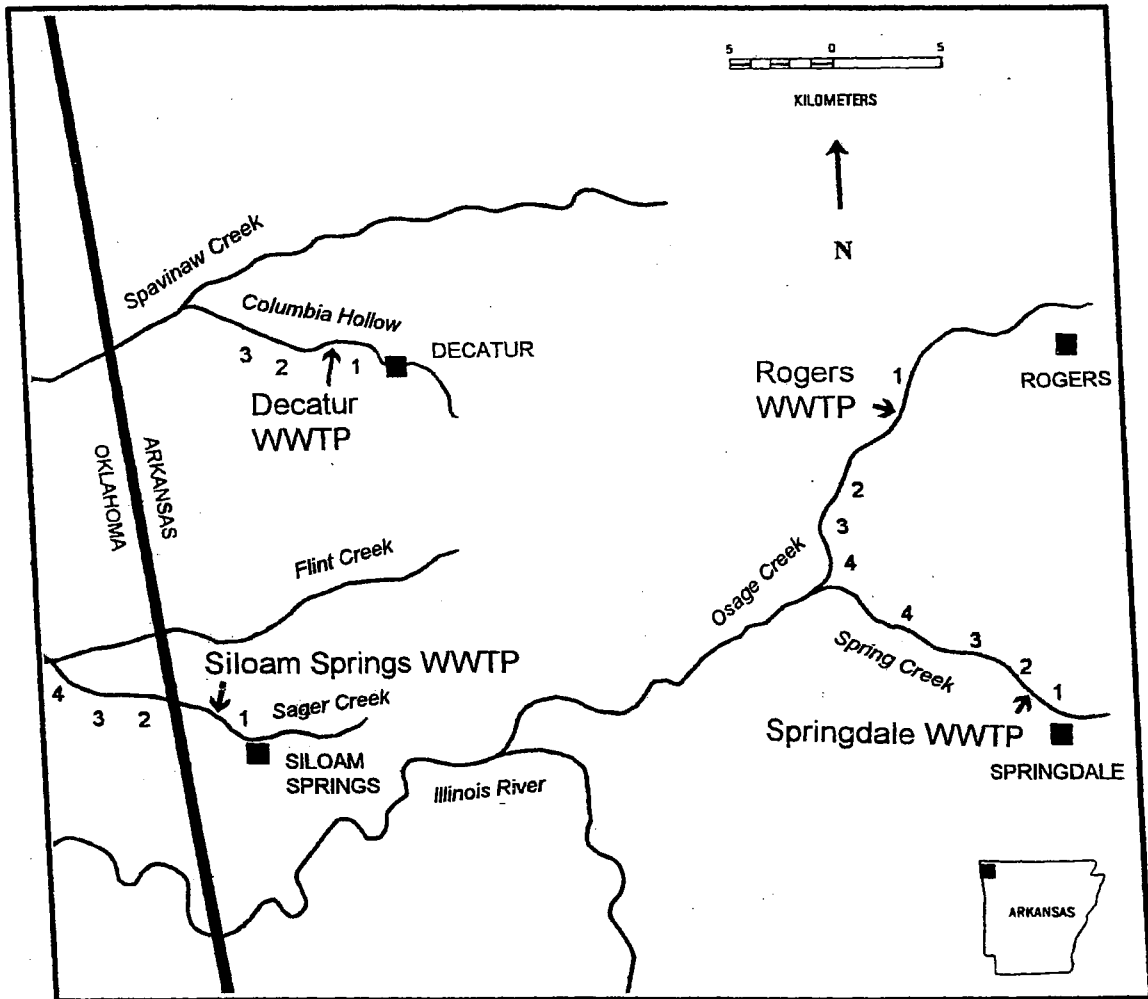
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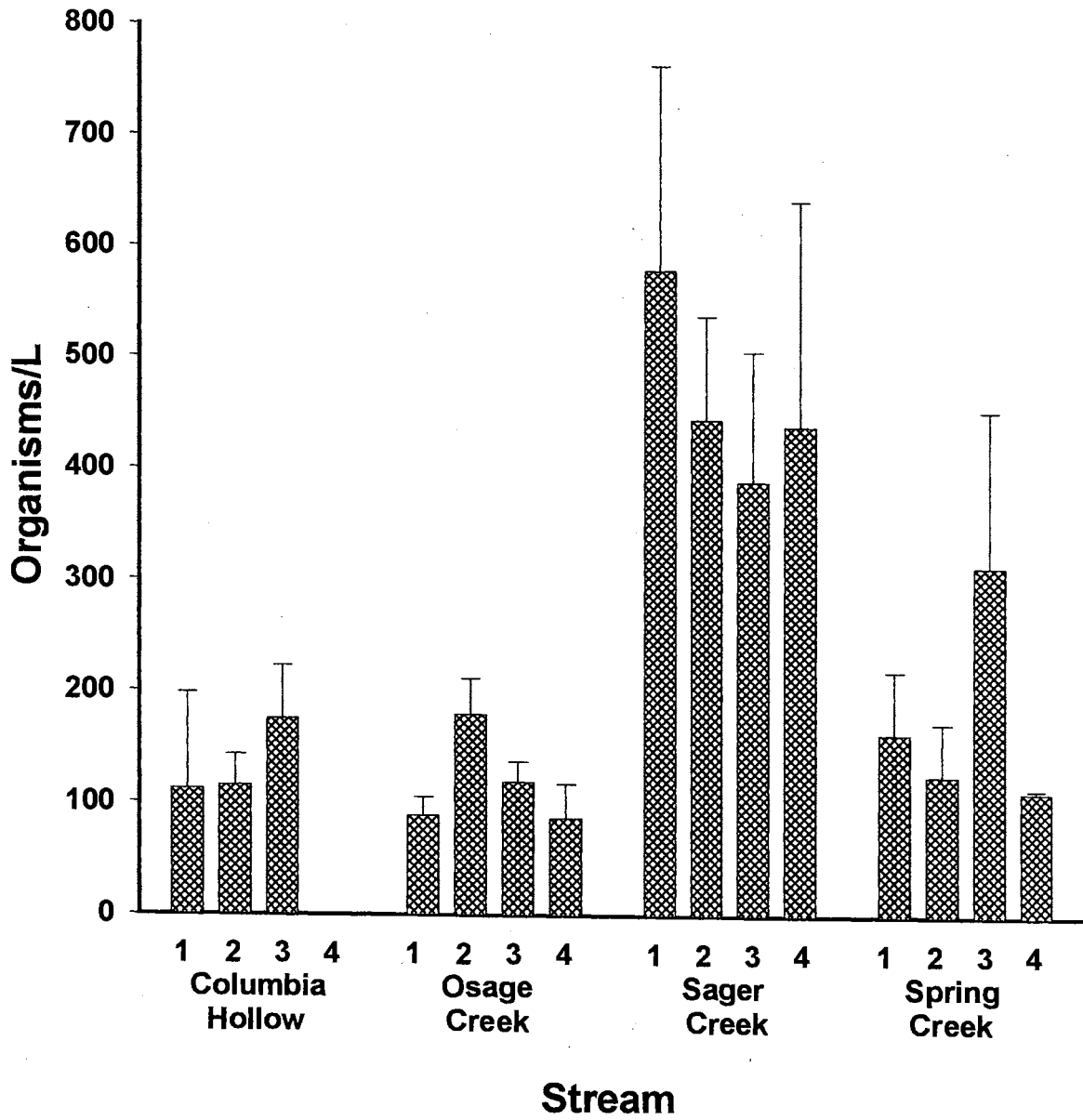
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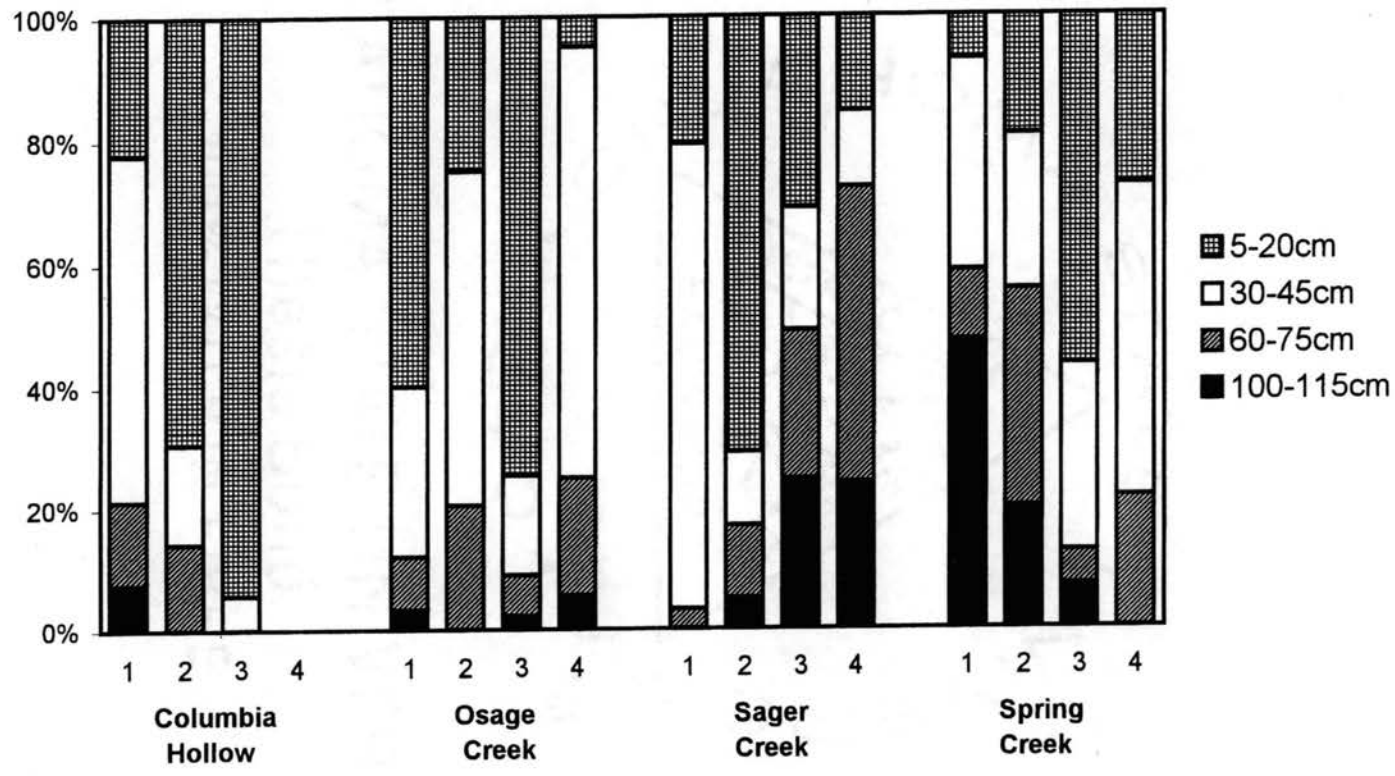
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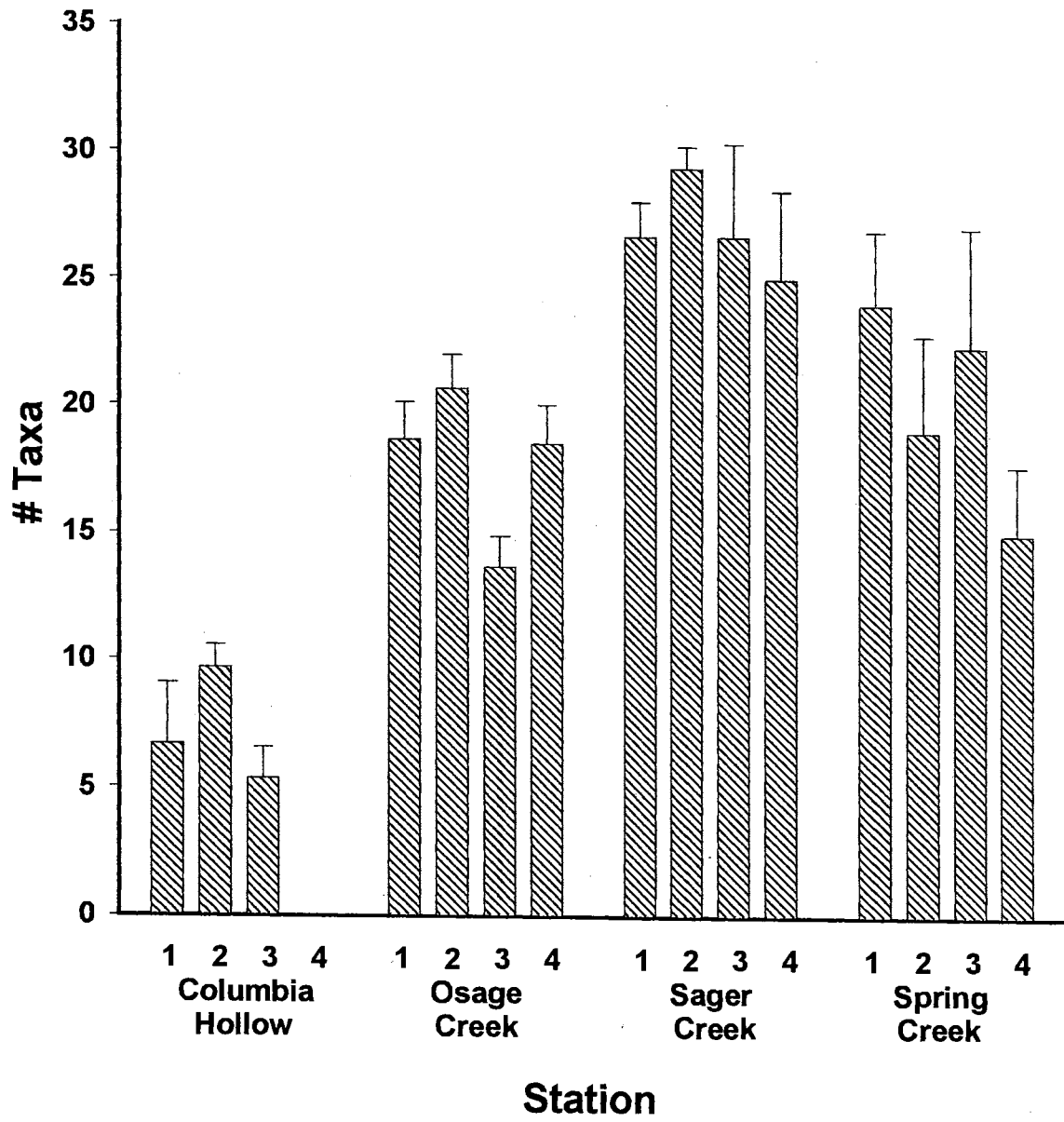
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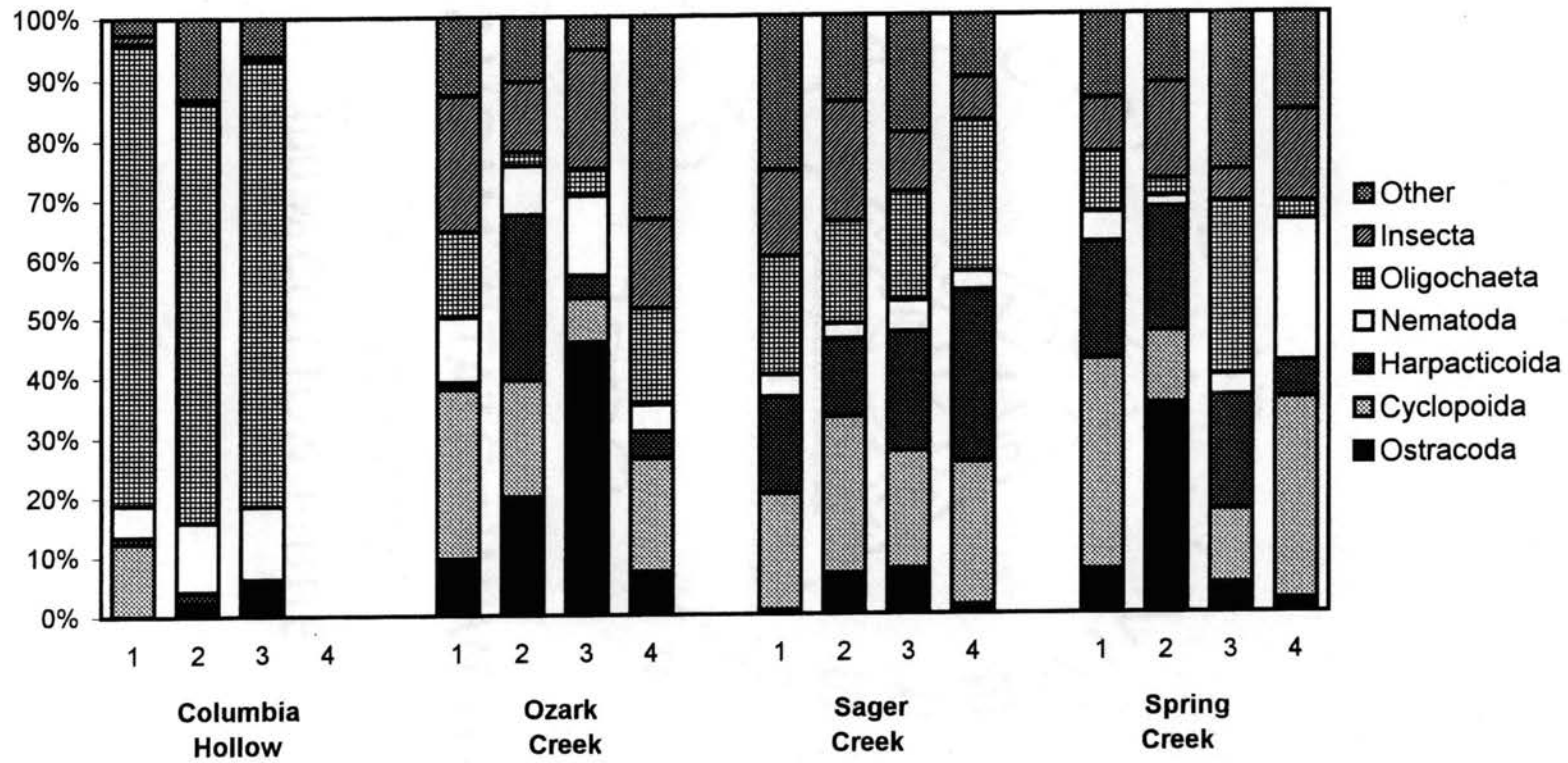


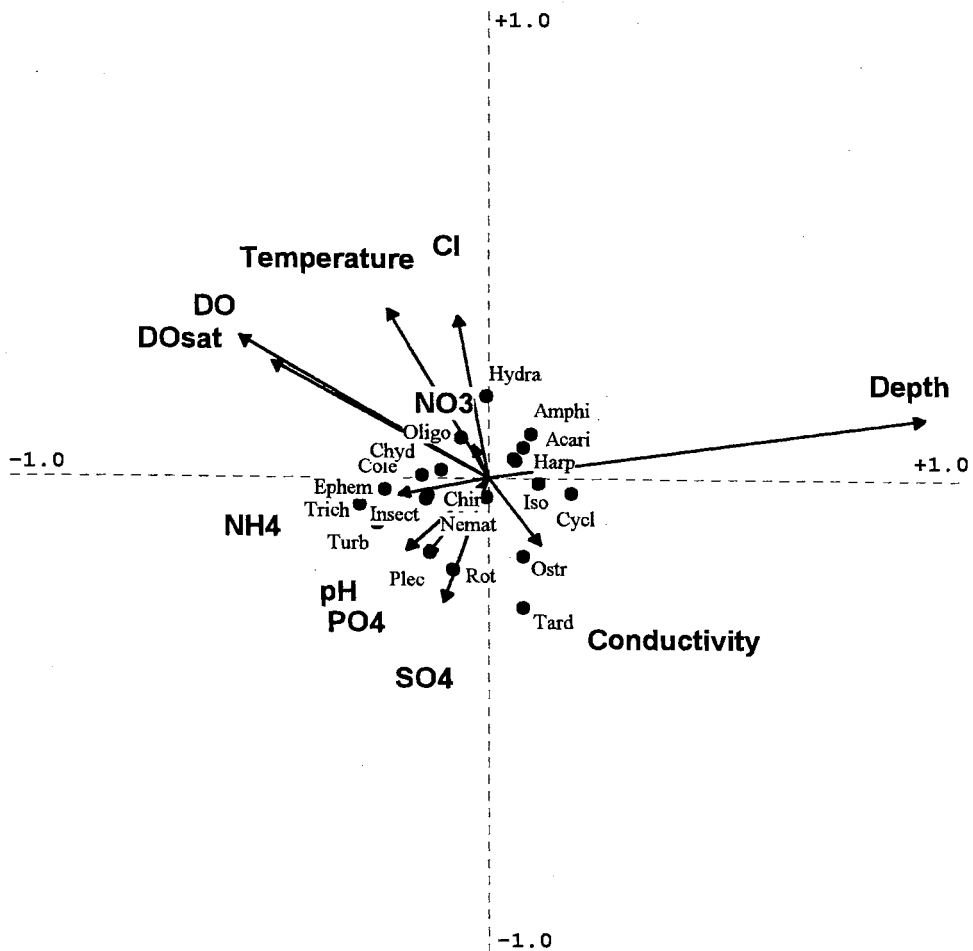


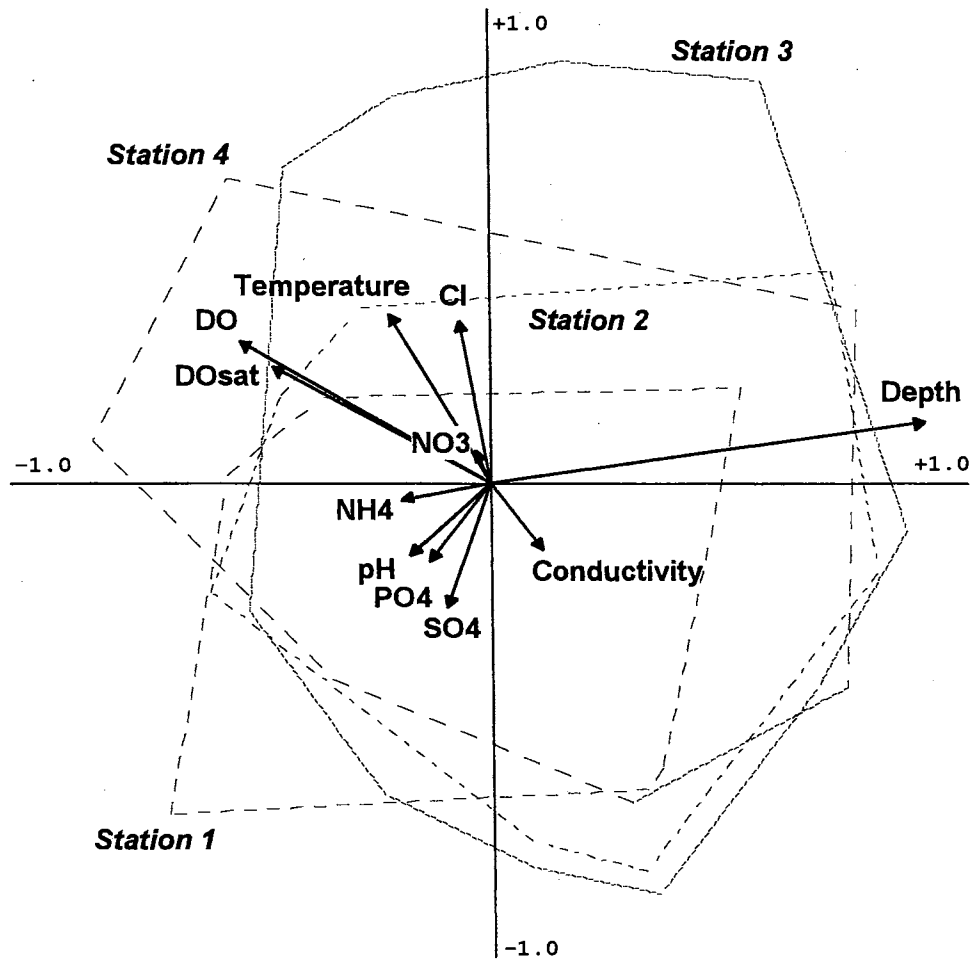


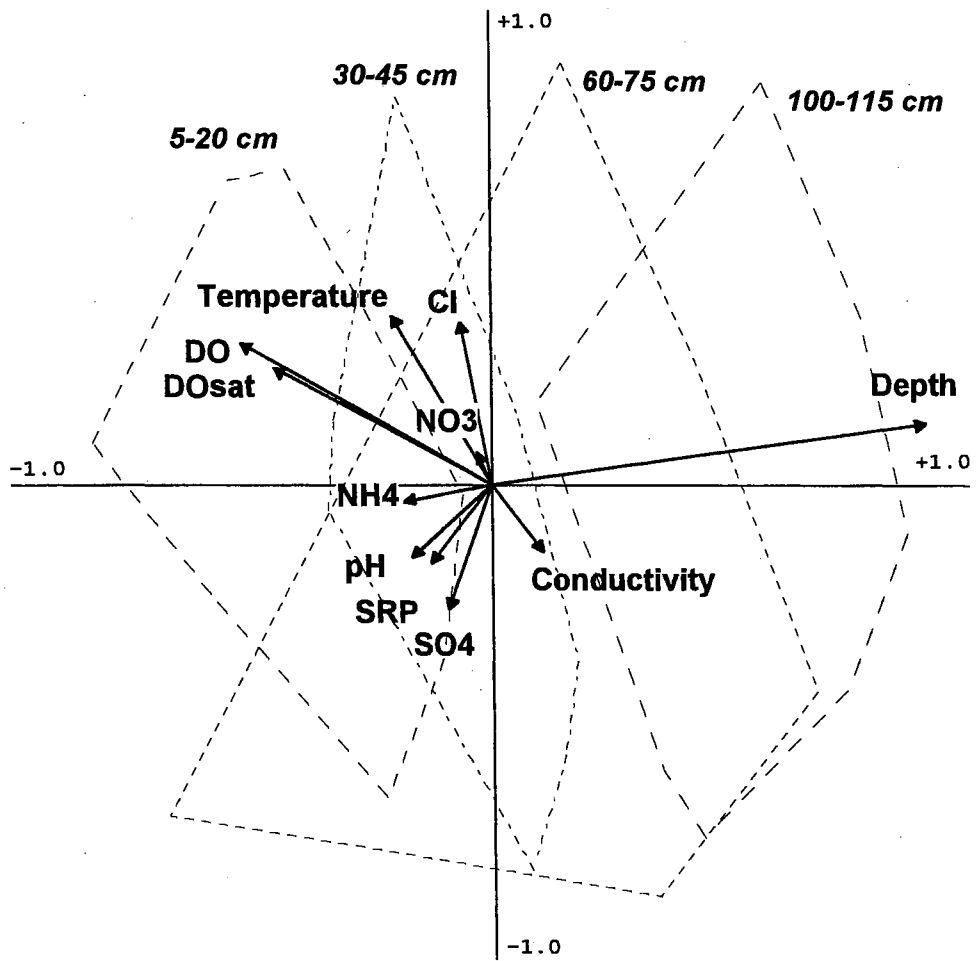












CHAPTER V

AN EVALUATION OF ALTERNATIVE HYPORHEIC  
SAMPLING PROCEDURES

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## **Abstract**

Although the Bou-Rouch method is widely used in sampling the hyporheic fauna, several concerns have been raised as to the accuracy of data collected. The objectives of this investigation was to investigate the influence that well design, pumping rate and sample volume size have on population estimates in 3 streams with differing bed sediment characteristics ranging from course sand to larger gravel. A comparison of 5 well designs, i.e., temporary wells with 4-, 6- and 8-mm pores and with no pores drilled along the lower 15 cm and permanent wells with 6-mm pores, indicated no significant difference in either total densities or taxon richness. A comparison of 2 pumping rates, i.e., 1.5 and 4 L/min, indicated significantly higher densities (2 streams) and taxon richness (1 stream) were estimated using the faster pumping rate. A comparison of 5 sample volume sizes, i.e., 0.5, 1.0, 1.5, 2.0 and 2.5 L, indicated that smaller sample volumes produced significantly higher estimates of density and taxon richness. These results provide greater evidence of the importance of maintaining consistent pumping rates and sample volume sizes during the performance of hyporheic investigations.

## **Introduction**

Recent advances in understanding the role of the hyporheic zone have added significantly to stream ecology (Dahm and Valett 1996). However, a number of problems persist in obtaining quantitative and qualitative data on the organisms inhabiting this habitat (Fraser and Williams 1997). Perhaps the simplest method involves excavating a pit on the lateral shore or instream bar and filtering inflowing water (i.e., the Karaman-Chappuis method), a method first described by Chappuis 1942. However, this method is not quantitative, it lacks vertical resolution, and it cannot be used in areas where surface water is present (i.e., it is restricted to banks and bars). The collection of core samples is the most quantitative method (Williams and Hynes 1974). Unfortunately, this method is limited in depth and to sand sediments (Palmer and Strayer 1996). Other methods including freeze coring (Stocker and Williams 1972, Hynes 1974, Bretschko 1985) and the use of artificial substrates (Tabacchi 1990). However, these methods can be time consuming, labor intensive, and expensive.

The method perhaps most often used consists of pumping hyporheic waters from specific depths in the stream bed sediments from either temporary or permanently installed standpipe wells (Palmer and Strayer 1996). This method, originally described by Bou and Rouch (1967) and referred to as the Bou-Rouch method, requires relatively little labor and inexpensive equipment. However, specific concerns have been raised as to the accuracy of data collected by this method. Standpipe wells may be selective for specific groups of organisms, depending on the volume sampled (Danielopol 1976) and the filtering effect of the substrate (Williams 1984). Fraser and Williams (1997) reported that this method underestimates larger animals, i.e., insect larvae, that are able to grasp on to substrates

particles and thus resist suction of the pump. Well installation and its diameter and shape may modify the substrate texture in the well's immediate vicinity, thus affecting sampling success (Tillman et al. 1996). The exact region of the stream bed that is sampled also cannot be guaranteed (Palmer and Strayer 1996) because of possible heterogeneity of the sediments.

The permanency of the well can also affect sampling results. Hakenkamp and Palmer (1992) demonstrated that organisms colonizing a permanent well differed significantly, both in composition and abundance, from those animals sampled from a newly installed well. They also reported that samples taken sequentially from a well cannot be used as replicates and that a 48-h period between sequential samples from the same well did not allow adequate time for recovery by the fauna in the immediate vicinity of the well. Other sources of bias in samples from colonization or permanent wells include the trapping action of such wells and the possible attraction of predators and/or scavengers (Bretschko and Klemens 1986).

Recent experience by the author in using the Bou-Rouch method in a wide variety of habitats (Chapters 1, 2 and 3) raised several specific concerns regarding sources of variability in sampling results. The objective of this investigation is to address these concerns by evaluating various aspects of the Bou-Rouch method in streams with varying types of bed sediments. Four specific hypotheses were tested: Sampling results obtained by the Bou-Rouch method are influenced by:

Hypothesis 1: Well design.

Hypothesis 2: Pumping rate.

Hypothesis 3: Sample volume.

Hakenkamp and Palmer (In press) have pointed out the need to evaluate the efficiency of the Bou-Rouch method and other methods in bed sediments of varying grain sizes. This study will, in part, address these concerns and allow the development of recommendations that will improve the reliability of data collected by the Bou-Rouch method.

### **Sampling Locations**

Samples were collected in June and July 1998 from three streams with different substrate types. Rock Creek, in Murray Co., Okla., is a spring-fed stream with fine to coarse sand bed sediments. Baron Fork is a larger Ozark stream in Cherokee Co., Okla., and has bed sediments consisting of loosely sorted gravels and smaller cobbles. Sager Creek, a smaller Ozark stream in Delaware Co., Okla., has bed sediments consisting of small gravel. Physical data describing these streams is presented in Table 1. Detailed information on water chemistry is presented in Chapters 2 and 3 of this dissertation.

### **Methods**

#### Well design and pumping rate

Standpipe wells used in this investigation consisted of 2.5 cm ID PVC tubing. The tubing was driven into the stream's substratum by inserting a steel T-shaped driving rod into the well and driving the rod and well to the desired depth. The T-bar was then removed, leaving the well in place. Five well designs were evaluated. These included 4 types of temporary wells (i.e., wells with no pores, wells with 4-, 6-, or 8-mm pores drilled along the lower 15 cm of the tubing) and 1 type of permanent well (6 mm pores). The

perforated areas included 4 rows evenly spaced along the 15 cm length with 8 holes in each row. The perforated wells were installed such that the perforated section was located between 30 and 45 cm below the bottom of the stream. The non-perforated wells were installed such that the open end was located at 37.5 cm below the substrate surface. The intake of the suction hose was positioned at the top of the perforated section of the perforated wells or above the open end of the non-perforated wells. Samples were collected from each temporary well on the date of well installation. Four weeks prior to installation and sampling of the temporary wells, permanent wells were installed in each of the streams. Permanent wells were capped after first simulating sampling by pumping 2.5 L from each well on the day of installation. Samples were then collected from the permanent wells in each stream on the same day that the temporary wells were installed and sampled. Ten replicates of each well design were installed in each stream.

To investigate the effect that pumping rate may have on sampling results, two types of hand pump, varying in pumping rate (1.5 and 4 L/minute) were used in the collection of the samples described above. The smaller pump was a Nalgene hand operated vacuum pump (Hach Company) and the larger pump was a “Guzzler” diaphragm hand pump (Cole-Palmer Instrument Co.). For each set of 10 replicates described above, 5 replicates were sampled by each of the 2 pumps. In Rock Creek, the pumping rate was often adjusted downward to reduce the amount of sand particles sucked up with sample water.

In each stream the wells were positioned randomly in 5 blocks, each block containing all combinations of well design and pumping rate. Thus, each block would include 2 wells of each design, with one well of each design sampled at the slow pumping

rate and the second at the faster pumping rate. The 5 blocks of wells in each stream were installed in adjacent segments of the stream exhibiting similar substrates, depth and current velocity. Wells within each block were installed randomly, with wells separated by a minimum of 0.7 m. The numbers of wells of each design and sampled by each pumping rate are summarized for each stream in Table 2. Non-perforated wells could not be sampled properly in Rock Creek due to the quantity of sand sucked up with the sample water. In addition, although installed, all permanent wells in Baron Fork and 3 in Rock Creek were washed out by high storm waters prior to sampling.

#### Sample volume

In order to investigate the influence that volume size has on density estimates provided by the Bou-Rouch method, a 2.5-L hyporheic sample was pumped from each well in sequential 0.5-L aliquots. Each 0.5-L aliquot was concentrated with a sieve, placed in a separate labeled jar and preserved in 5% formalin. Samples collected from Baron Fork and Rock Creek were concentrated with a 63- $\mu\text{m}$  mesh sieve. Due to frequent high turbidity of the Sager Creek hyporheic waters, samples from this stream were concentrated with a 120- $\mu\text{m}$  mesh sieve. Organisms were sorted by major group using a dissecting microscope. Sorting was aided by the addition of rose bengal stain.

#### Statistical analyses

Comparisons within each stream of density and taxon richness collected from the 5 well designs (permanent wells with 6-mm pores and temporary wells with no pores and 4-, 6- and 8-mm pores) and 2 pumping rates were performed using a 2-way ANOVA with

repeated measures. Locational effects (block effects) were tested in addition to all combination of effects due to well design and pumping rate. Differences between density (#/L) and accumulated taxon richness based on incremental increases in sample volume size, i.e., 0.5, 1.0, 1.5, 2.0, and 2.5 L, were tested for significance using a one-way ANOVA with repeated measures. All tests were performed on  $\log_{10}(x+1)$  transformed data. The analysis did not include the 7 remaining permanent wells in Rock Creek which, although sampled, were not deemed adequate to include in the statistical analysis.

## **Results**

### **Composition**

The hyporheic fauna composition of the three streams varied considerably (Table 3). Average densities (based on all samples) ranged from 49 to 96 organisms/2.5-L sample in the Baron Fork and Sager Creek, respectively. Four groups, i.e., Ostracoda, Cyclopoida, Insecta (predominantly Chironomidae), and Oligochaeta, together composed 75% of the all organisms collected in the Baron Fork. Percentage composition is derived from average densities based on all samples for each stream. The 4 most abundant groups in Rock Creek (83%) included Insecta, Cyclopoida, Oligochaeta, and Harpacticoida. Diversity was greatest in Sager Creek, where Crustacea were the predominant taxonomic group (74%); Cyclopoida, Harpacticoida, and Isopoda together composed 60% of the organisms collected in this stream. Although a larger mesh size was used at Sager Creek, this had no noticeable effect on the results of this investigation.

### Influence of well design

Invertebrate densities and taxon richness (mean  $\pm$  1SE) collected from the 5 well designs and 2 pumping rates are presented in Figs. 1 and 2, respectively. The difference in the mean values among the different well designs was not significant in any of the streams based on statistical analysis with the 2-way ANOVA with repeated measures (Tables 4 and 5). Both densities and taxon richness in samples collected at the 2 pumping rates were significantly different in both Rock and Sager Creeks and taxon richness was significantly different only in Rock Creek (Tables 4 and 5).

### Influence of volume size

On average, 50-55% of all organisms collected in 2.5-L samples were collected in the first 0.5-L aliquot (Fig. 3) at each of the 3 streams. The percentage decreased with consecutive 0.5-L aliquots. Mean densities (#/L) ( $\pm$ 1SE) were calculated for the five sample volumes, i.e., 0.5 L, 1.5 L, 2.0 L, & 2.5 L for each of the streams (Fig. 4). Density estimates for the total invertebrates and major groups decreased with increasing volume. However, taxonomic composition remained relatively constant regardless of sample volume (Table 6). Density estimates based on different sample volumes were significantly different in all 3 streams (Table 7). Multiple comparisons using the Student-Neuman-Keuls Method (Fig. 4) indicated that for each stream, densities estimated by smaller volume sizes were significantly greater than estimated by increasing volume size, i.e., 0.5 L > 1.0 L > 1.5 L > 2.0 L > 2.5 L.

Estimates of taxon richness did not vary as much as density (Fig. 5). However, differences were statistically significant in all 3 streams (Table 8). Multiple comparisons



(Fig. 5) indicated that in several of the comparisons, larger sample volumes provided significantly greater estimates of taxon richness.

## **Discussion**

The results of this investigation indicate the importance of maintaining consistent methodologies in the sampling of the hyporheic fauna using the Bou-Rouch method.

Sample size and pumping rate were both found to significantly affect density estimates regardless of sediment type.

Sample size has been identified as an important factor in obtaining quantitative hyporheic samples by Danielopol (1976). This author suggested that the first 10-L sample from a well is the most productive and perhaps most representative for organisms living in close proximity to sediments including nematodes, oligochaetes, and harpacticoids. Pospisil (1992) recognized the importance of the origin of hyporheic water and the selective nature of pumping from varying types of bed sediments. He suggested that limiting the sample volume guarantees that it is taken from a well defined zone. However, in some cases it has been suggested that the first 0.2-0.5 L of water removed from a well using the Bou-Rouch method should be discarded to avoid the risk of contamination with surface water and its biota (Boulton et al. 1992). Similarly, permanent wells may serve as traps selectively accumulating specific taxonomic groups, hence the first few liters may not be representative of the hyporheic fauna and should be discarded (Pospisil 1992).

However, our findings suggest that the first 0.5 L removed from a temporary well may be the only truly quantitatively representative sample that can be obtained by the Bou-Rouch method, further supporting Pospisil's observation regarding the importance of limiting the

volume of the sample collected by this method. The size of the sample collected from temporary wells is a critical factor that must be given greater emphasis in quantitative studies using the Bou-Rouch method.

Pumping rate can also influence the quality of hyporheic samples. Different taxonomic groups exhibit varying mobility and behavior, with some organisms better able to avoid capture. Danielopol (1976) reported that organisms less dependent on the substrate, i.e., cyclopoids, ostracods, isopods, and amphipods, are more readily captured by pumping. However, his finding was based on larger sample volumes than we collected. Composition of our samples (based on 1-L samples) was not affected by pumping rate. As demonstrated in this investigation, increasing the pumping rate from 1.5 to 4 L/min more than doubled density estimates at all three streams. In streams with smaller sediments, such as Rock Creek, pumping rate may need to be adjusted to minimize the quantity of sediments sucked into the sample. The optimal pumping rate obviously will vary with bed sediment type; however, perhaps the optimum rate is the maximum rate that can be used.

We are unaware of any studies that have considered the influence that well design may have on sampling of the hyporheic fauna. Typically, perforations measuring 5 mm (e.g., Danielopol 1976) and 6 mm in diameter (e.g., Pennak and Ward 1986) have been used, although use of unperforated wells is also widespread (e.g., Emily Stanley, University of Wisconsin at Madison, personal communication). Increasing the surface area of the bed sediments exposed to the influence of the pump's suction would appear to reduce the screening action of the sediments and to produce a more representative sample at least for smaller sample volumes. However, the differences in total number of

organisms collected in 2.5 L from wells of varying design, including permanent colonization wells, were not significantly different.

In conclusion, we suggest the use of higher pumping rates to collect small volumes of water for quantitative sampling of hyporheic invertebrates for a variety of well designs. Future analysis of hyporheic sampling should also address mesh size for concentrating invertebrates and the number of replicates required to provide a statistically reliable estimate of abundance.

### **Acknowledgments**

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Table 1. Descriptive information for 3 streams.

	Baron Fork	Rock Creek	Sager Creek
Location			
Latitude	35° 55'	34° 35'	36° 12'
Longitude	95° 51'	96° 58'	94° 35'
Sampling date	7-18-97	7-5-97	6-27-97
Stream depth (cm)	21-30	4-20	8-13
Stream width (m)	36	8	20
Water Temperature (°C)	25	21-26	25-27
Bed Sediment Grain Size (%)			
Fine sand (<0.25 mm)	3.3	5.5	3.5
Medium sand (0.25-0.5 mm)	3.5	18.1	1.8
Coarse sand (0.5-2 mm)	12.7	60.1	5.0
Very coarse sand (2-6.3 mm)	15.5	14.8	13.4
Gravel (>6.3 mm)	65.0	1.6	76.3

Table 2. Total number of wells of 5 designs sampled in 3 streams. Size of pores (diameter in mm) in distal end of well: no pores, 4-mm, 6-mm and 8-mm. Temporary wells sampled on day of installation. Permanent wells installed 4 weeks prior to sampling. An equal number of wells of each design were sampled at 2 pumping rates. Non-perforated wells could not be sampled properly in Rock Creek due to the quantity of sand sucked up with the sample water. Although installed, all permanent wells in Baron Fork and 3 in Rock Creek were washed out by high storm waters prior to sampling.

	Location					
	Baron Fork		Rock Creek		Saline Creek	
Pumping rate (L/min)	1.5	4.0	1.5	4.0	1.5	4.0
Temporary wells						
No pores	5	5	0	0	5	5
4 mm	5	5	5	5	5	5
6 mm	5	5	5	5	5	5
8 mm	5	5	5	5	5	5
Permanent wells						
6 mm	0	0	4	3	5	5

Table 3. Composition of the hyporheic fauna collected at three streams. %=percentage of the mean invertebrate density/2.5L,  $\bar{x}$  =mean density/2.5L, SE=standard error.

Taxon	Baron Fork			Rock Creek			Sager Creek		
	%	$\bar{x}$	$\pm$ SE	%	$\bar{x}$	$\pm$ SE	%	$\bar{x}$	$\pm$ SE
Total Invertebrates	100.0	48.8	9.27	100.0	62.6	7.73	100.0	96.0	18.49
Crustacea	57.1	27.8	5.17	45.6	28.5	5.10	73.9	70.5	11.58
Cyclopoida	20.1	9.8	1.88	25.2	15.8	2.76	26.7	25.6	5.28
Harpacticoida	3.8	1.9	0.57	11.2	7.0	1.83	17.5	16.5	3.35
Copepod nauplii	4.5	2.2	0.63	5.7	3.6	1.20	0.6	0.6	0.35
Ostracoda	21.0	10.2	2.44	3.3	2.1	0.60	8.3	8.0	1.46
Chydoridae	5.1	2.5	0.85	0.2	0.1	0.07	3.6	3.4	1.11
Isopoda	1.8	0.9	0.34	0.0	0.0	0.00	16.3	15.7	4.61
Amphipoda	0.8	0.4	0.28	0.0	0.0	0.00	0.9	0.8	0.17
Insecta	18.6	9.1	2.20	28.5	17.8	2.28	2.7	2.6	0.55
Ceratopogonidae	1.0	0.5	0.23	0.4	0.2	0.12	0.0	0.0	0.00
Chironomidae	8.1	3.9	0.96	23.6	14.8	2.01	1.0	1.0	0.24
Coleoptera	2.3	1.1	0.30	0.0	0.0	0.00	1.1	1.1	0.28
Ephemeroptera	5.7	2.8	0.76	2.8	1.8	0.31	0.4	0.4	0.10
Plecoptera	0.5	0.3	0.14	0.0	0.0	0.03	0.0	0.0	0.02
Trichoptera	1.0	0.5	0.15	0.4	0.3	0.10	0.0	0.0	0.03
Acari	3.8	1.9	0.53	3.7	2.3	0.38	1.7	1.7	0.63
Turbullaria	0.9	0.5	0.23	2.3	1.5	0.37	0.1	0.1	0.05
Nematoda	1.5	0.7	0.30	0.4	0.3	0.08	7.6	7.4	2.56
Rotifera	2.1	1.0	0.38	0.0	0.0	0.03	0.0	0.0	0.02
Oligochaeta	15.4	7.5	2.04	18.3	11.4	3.23	13.9	13.5	7.74
Tardigrada	0.3	0.1	0.10	0.9	0.6	0.22	0.0	0.0	0.00



Table 4. Comparison (2-way ANOVA with repeated measures) of total organisms (#/2.5L) collected from varying well designs at 2 pumping rates. Baron Fork includes temporary wells with no pores and 4-, 6- and 8-mm pores. Rock Creek includes temporary wells with 4-, 6- and 8-mm pores. Sager Creek includes permanent wells with 6-mm pores and temporary wells with no pores and 4-, 6- and 8-mm pores.

Stream	Source of Variation	Sum of Squares	df	Mean Square	F	P
Baron Fork	Block	1.684	4	0.421		
	Pump	1.881	1	1.881	1.696	0.263
	Pump x block	4.436	4	1.109		
	Well type	0.510	3	0.170	0.651	0.598
	Well type x block	3.135	12	0.261		
	Pump x well type	0.224	3	0.075	0.463	0.713
	Residual	1.933	12	0.161		
	Total	13.803	39	0.354		
Rock Creek	Block	0.826	4	0.207		
	Pump	0.723	1	0.723	11.89	0.026
	Pump x block	0.243	4	0.061		
	Well type	0.201	2	0.101	2.04	0.192
	Well type x block	0.394	8	0.049		
	Pump x well type	0.240	2	0.120	1.41	0.299
	Residual	0.681	8	0.085		
	Total	3.308	29	0.114		
Sager Creek	Block	4.882	4	1.221		
	Pump	1.965	1	1.966	22.043	0.009
	Pump x block	0.357	4	0.089		
	Well type	0.238	4	0.060	0.422	0.790
	Well type x block	2.258	16	0.141		
	Pump x well type	0.941	4	0.235	1.845	0.170
	Residual	2.041	16	0.128		
	Total	12.683	49	0.259		

Table 5. Comparison (2-way ANOVA with repeated measures) of taxon richness (#/2.5L) collected from varying well designs at 2 pumping rates. Baron Fork includes temporary wells with no pores and 4-, 6- and 8-mm pores. Rock Creek includes temporary wells with 4-, 6- and 8-mm pores. Sager Creek includes permanent wells with 6-mm pores and temporary wells with no pores and 4-, 6- and 8-mm pores.

Stream	Source of Variation	Sum of Squares	df	Mean Square	F	P
Baron Fork	Block	0.276	4	0.069		
	Pump	0.182	1	0.182	0.94	0.388
	Pump x block	0.775	4	0.194		
	Well type	0.116	3	0.039	0.71	0.567
	Well type x block	0.656	12	0.055		
	Pump x well type	0.049	3	0.016	0.43	0.733
	Residual	0.455	12	0.038		
	Total	2.509	39	0.064		
Rock Creek	Block	0.206	4	0.052		
	Pump	0.062	1	0.062	31.09	0.005
	Pump x block	0.008	4	0.002		
	Well type	0.092	2	0.046	2.44	0.149
	Well type x block	0.151	8	0.019		
	Pump x well type	0.058	2	0.029	1.55	0.270
	Residual	0.149	8	0.019		
	Total	0.727	29	0.025		
Sager Creek	Block	0.687	4	0.172		
	Pump	0.023	1	0.023	0.78	0.428
	Pump x block	0.120	4	0.030		
	Well type	0.169	4	0.042	1.47	0.259
	Well type x block	0.461	16	0.029		
	Pump x well type	0.114	4	0.028	0.90	0.486
	Residual	0.504	16	0.032		
	Total	2.077	49	0.042		

Table 6. Composition (%) of density estimates based on 5 sample volume sizes in 3 streams.

Sample Size (L)	Stream														
	Baron Fork					Rock Creek					Sager Creek				
	0.5	1.0	1.5	2.0	2.5	0.5	1.0	1.5	2.0	2.5	0.5	1.0	1.5	2.0	2.5
<u>Taxon</u>															
Cyclopoida	21	20	20	20	20	17	19	22	22	23	22	23	24	24	25
Harpacticoida	5	4	4	4	4	7	9	10	10	11	23	22	22	21	21
Ostracoda	14	18	20	21	21	5	5	5	4	4	5	6	6	7	8
Isopoda	2	2	2	2	2	0	0	0	0	0	13	14	15	15	15
Insecta	20	19	19	18	19	51	44	40	41	36	3	3	3	3	3
Oligochaeta	19	17	16	16	15	7	11	12	11	15	18	16	15	14	13
Nematoda	2	2	2	2	1	1	0	0	0	0	10	10	9	9	9
Other	17	17	18	18	18	12	12	12	11	11	6	6	6	7	7

Table 7. Comparison (1-way ANOVA with repeated measures) of total densities of hyporheic organisms in 3 streams estimated by 5 sample volumes (0.5, 1.0, 1.5, 2.0 and 2.5 L).

Stream	Source of variation	Sum of squares	df	Mean square	F	P
Baron Fork	Between samples	70.94	39	1.819	58.1	< 0.001
	Between volumes	2.63	4	0.658		
	Residual	1.77	156	0.011		
	Total	75.35	199			
Rock Creek	Between samples	13.736	29	0.474	258.2	< 0.001
	Between volumes	3.007	4	0.768		
	Residual	0.345	116	0.003		
	Total	17.155	149			
Sager Creek	Between samples	56.578	39	1.451	155.7	< 0.001
	Between volumes	3.903	4	0.976		
	Residual	0.978	156	0.006		
	Total	61.458	199			

Table 8. Comparison (1-way ANOVA with repeated measures) of taxon richness in 3 streams estimated by 5 sample volumes (0.5, 1.0, 1.5, 2.0 and 2.5 L).

Stream	Source of variation	Sum of squares	df	Mean square	F	P
Baron Fork	Samples	16.917	39	0.434	23.4	< 0.001
	Volumes	0.516	4	0.129		
	Residual	0.862	156	0.006		
	Total	18.296	199			
Rock Creek	Samples	3.524	29	0.122	36.9	< 0.001
	Volumes	0.123	4	0.031		
	Residual	0.097	116	0.001		
	Total	3.744	149			
Sager Creek	Samples	8.644	39	0.222	22.0	< 0.001
	Volumes	0.165	4	0.041		
	Residual	0.292	156	0.002		
	Total	9.100	199			

## List of Figures

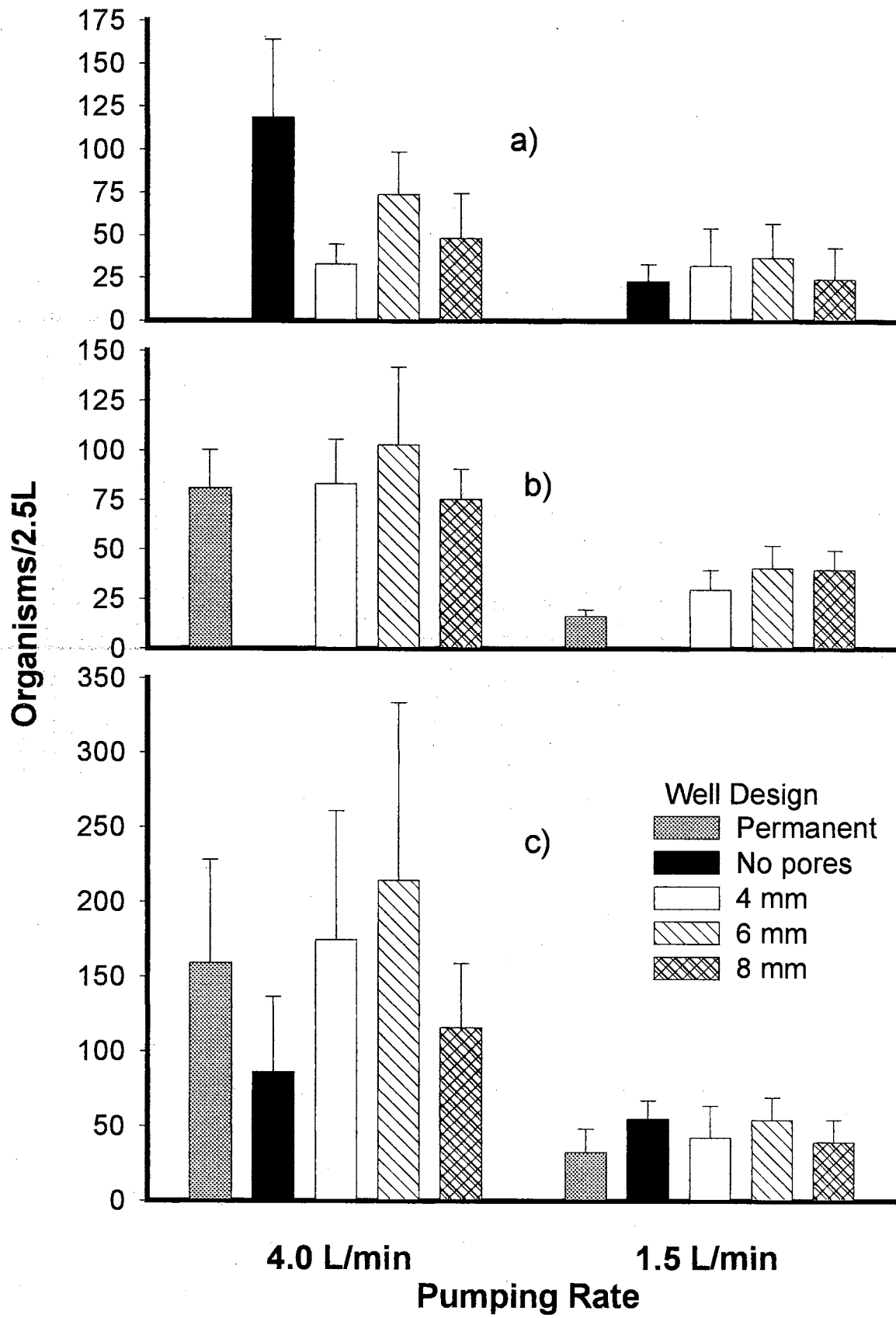
Fig. 1. Comparison of total organisms (#/2.5 L) collected from varying well designs at 2 pumping rates: a. Baron Fork, b. Rock Creek, c. Sager Creek. Mean  $\pm$ 1SE. Although densities for permanent wells from Rock Creek are included in this figure, they were not included in the statistical analysis because of the loss of 3 of 10 replicate wells.

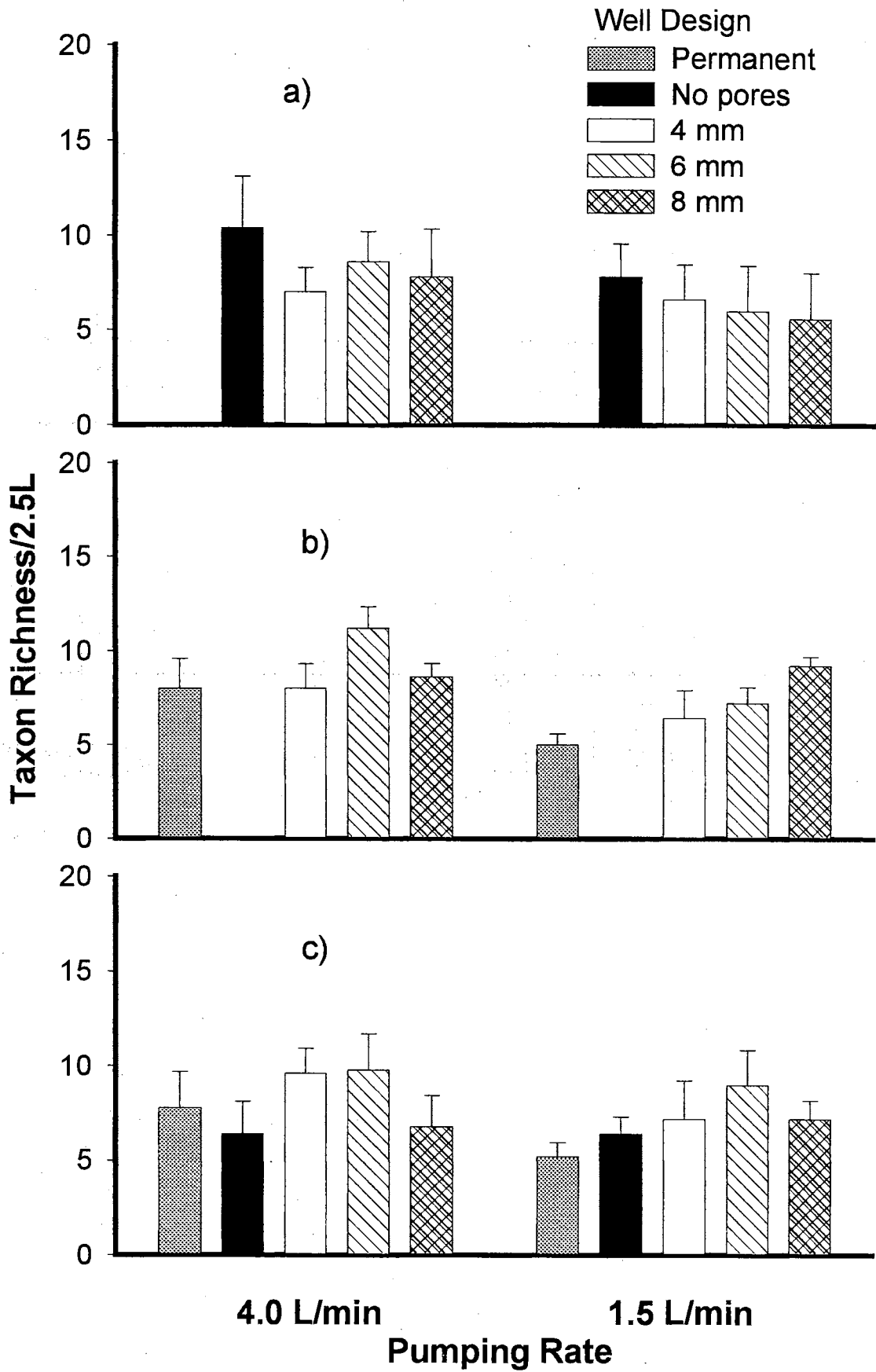
Fig. 2. Comparison of taxon richness (2.5-L samples) collected from varying well designs at 2 pumping rates: a. Baron Fork, b. Rock Creek, c. Sager Creek. Mean  $\pm$ 1SE. Although richness for permanent wells from Rock Creek are included in this figure, they were not included in the statistical analysis because of the loss of 3 of 10 replicate wells.

Fig. 3. Average proportion ( $\pm$ 1SE) of the hyporheic fauna collected in consecutive 0.5-L samples at 3 streams. Samples collected in 5 sequential 0.5-ml aliquots.

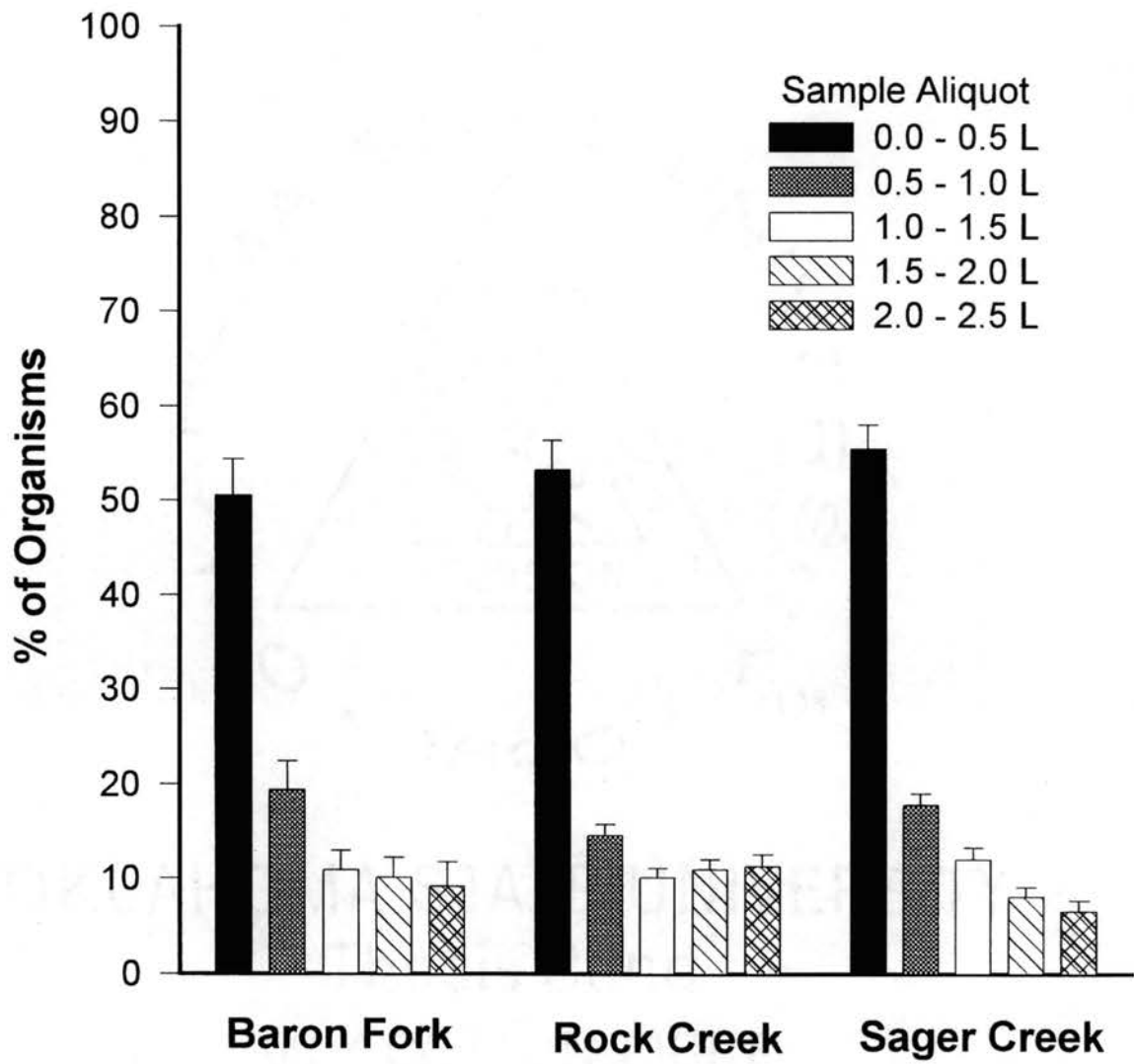
Fig. 4. Comparison of mean density ( $\pm$ 1SE) of the hyporheic fauna in 3 streams estimated by 5 sample volumes. Horizontal lines above bars indicate statistically significant differences between density estimates.

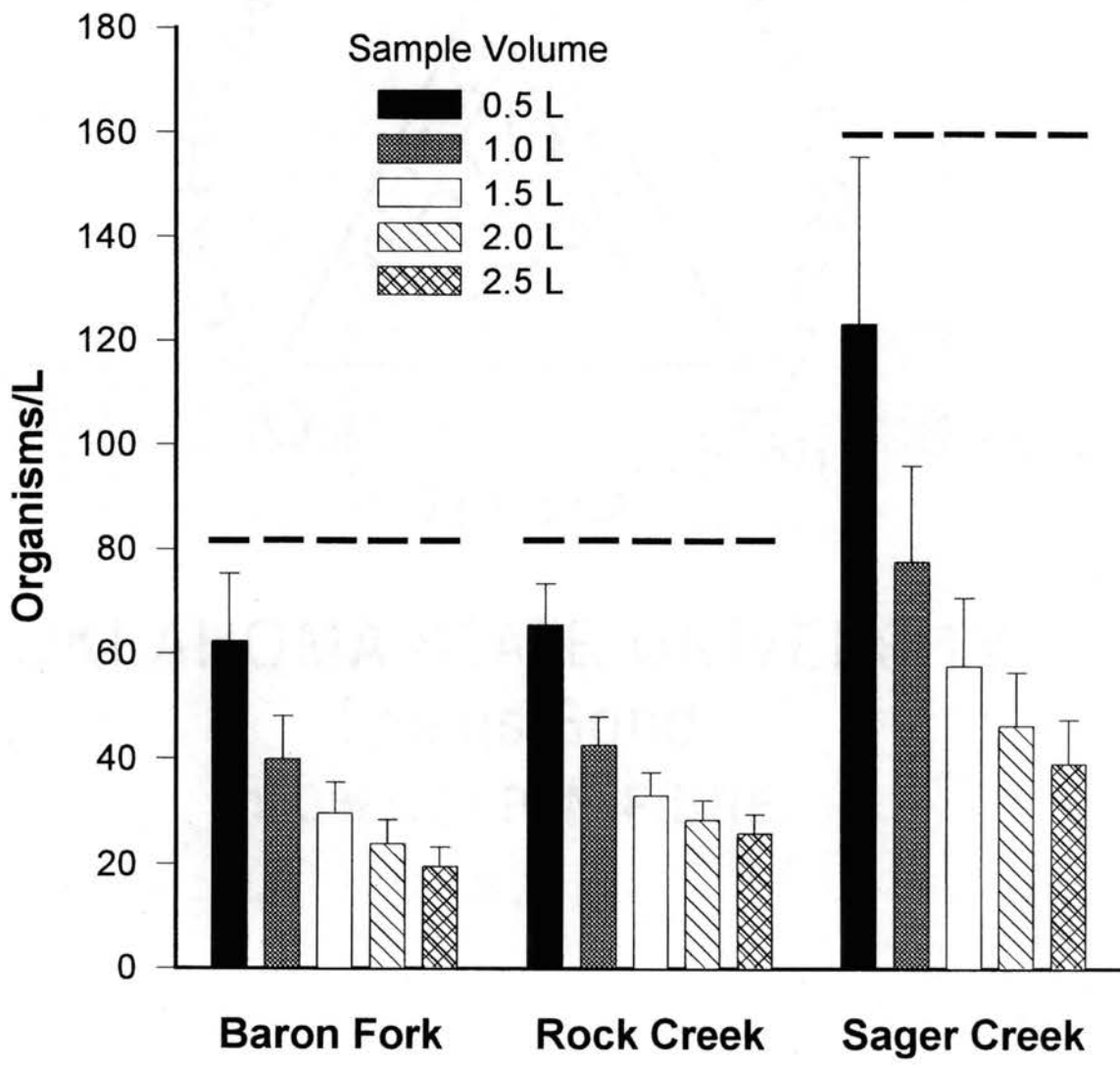
Fig. 5. Comparison of mean taxon richness ( $\pm$ 1SE) of the hyporheic fauna in 3 streams estimated by 5 sample volumes. Horizontal lines above bars indicate statistically significant differences between density estimates.

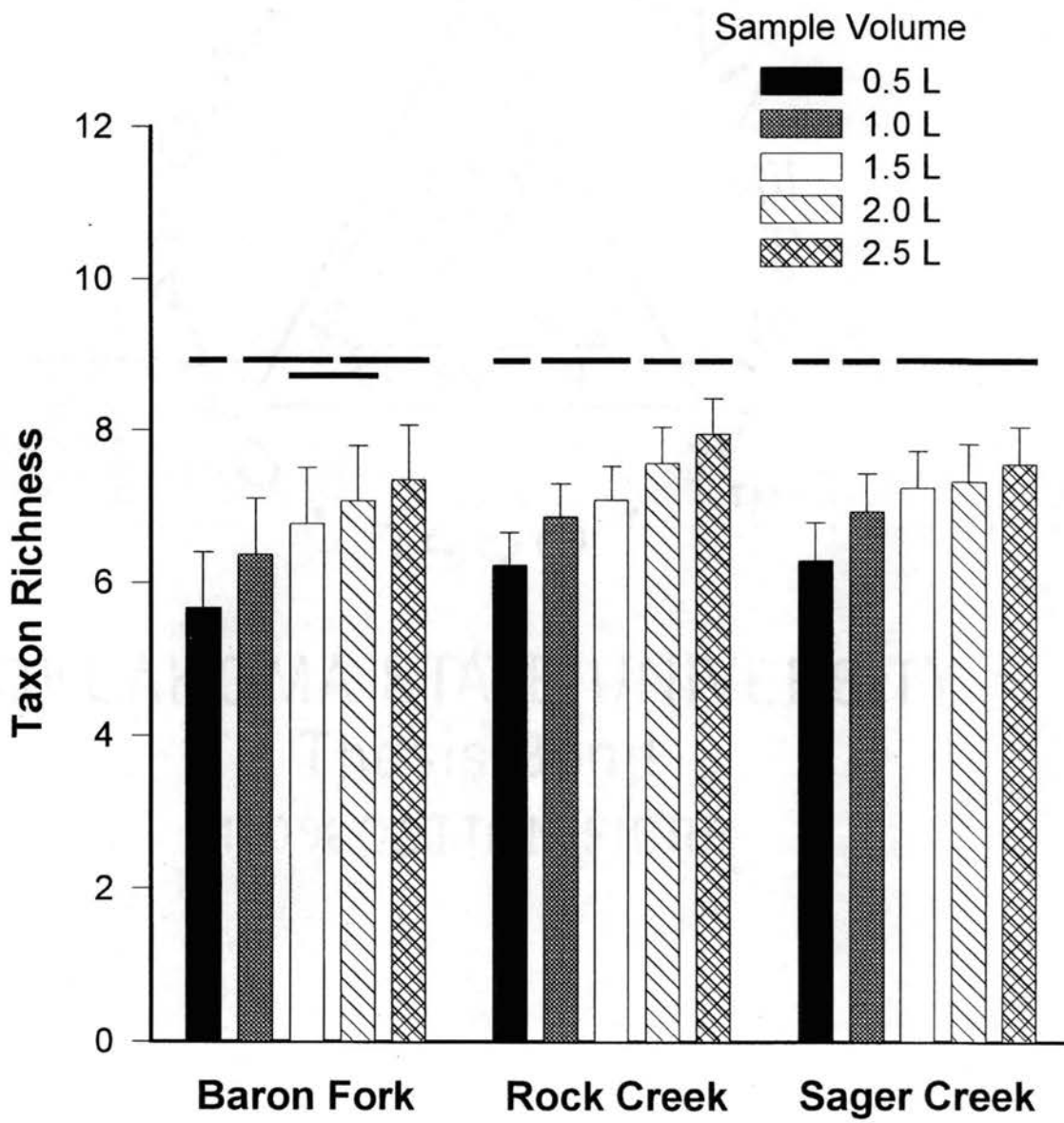












VITA

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Doctor of Philosophy

Thesis: THE ECOLOGY OF HYPORHEIC INVERTEBRATES IN OKLAHOMA AND ARKANSAS STREAMS

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