ECOSYSTEM DYNAMICS OF AN OZARK CAVE

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By

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ABSTRACT

Ozark cave ecosystems have important natural resources, ecological processes that are poorly understood, and are experiencing multiple environmental stressors. This study, performed in Cave Springs Cave (CSC), Arkansas, 1997 - 1999, describes the ecosystem dynamics of a cave stream using environmental quality monitoring, organic matter budgeting, microbiological techniques, stable isotope analyses (SIA), and faunal inventories. The CSC ecosystem is oligotrophic (mean TOC - 1.3 mg /l), and receives about 2% of the energy input that the surface ecosystem above receives. Ninety-five percent of organic matter (OM) input is dissolved (4,730 g DOM/m^2/y). Gray bat (*Myotis grisescens*) guano input (10.4 g/m^2/y) represents only 1% of OM input, but this population (3,000 bats) is only 10% of historic abundance. Cave sediment is the major standing crop of OM (1,212 g/m^2), and SIA indicates that particulate OM is its source, emphasizing the importance of allochthonous inputs. CSC has an ecosystem efficiency of only 4%, benthic OM turnover time of 6 years, and community respiration rate one-half that of surface streams, indicating that much of OM input is exported and under-utilized. The microbial community appears to be nutrient-limited, having growth rates and peak biomasses less than surface stream communities -- addition of nutrients into CSC is expected to increase microbial activity. Water sampling upstream of bat roosts indicates that guano is not the origin of most coliforms -- SIA implicates septic and agricultural wastes as sources, with most transport occurring during storm flows and autumn. SIA suggest three trophic levels and two food chains exist: Ozark cavefish (*Amblyopsis rosae*) are the top predator, eating mainly isopods, which eat imported organics, including sewage; crayfish eat benthic leaf detritus, and seasonally, guano.

Nutrient pollutants appear to augment the trophic base of CSC, but may also have extirpated the rare Ozark cave amphipod. Nutrients, metals, and coliforms have repeatedly exceeded Arkansas water-quality standards -- metals are accumulating in the cave sediments and biota, and statistics suggest that surface pollutants were the origin. Reduction of septic and agricultural waste inputs and rehabilitation of bat populations may be necessary to restore ecosystem dynamics in this cave ecosystem.

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INTRODUCTION

THE IMPORTANCE OF GROUND-WATER ECOSYSTEMS

The vast majority of the world's freshwater resource lies within one of the most poorly understood environments – the subterranean. Approximately 25% of the world's human population depends upon freshwater from karst aquifers (Ford *et al.*, 1988), where geomorphic processes are based upon the chemical dissolution of bedrock, usually carbonates (White, 1988; White *et al.*, 1995). Humans are dependent upon the karst milieu for other needs as well, including mineral, gas, and oil reserves (Ford *et al.*, 1988; Maire and Pomel, 1994). Chemical processes in karst form a significant carbon dioxide sink that may play an important role in buffering atmospheric and climatic change (White *et al.*, 1995). Furthermore, acid rain is neutralized by flowing through karst units (Maire and Pomel, 1994). Karst environments serve both as a recorder and as an indicator of ecosystem status by trapping particulates from the surface environment; however, sediment trapping in karst may lead to the concentration of toxic substances (Maire and Pomel, 1994). The ground water of the world is being diminished in quality and quantity. Ground water is being mined at rates greater than aquifer recharge rates (Margat, 1994). Furthermore, degradation processes may be slow and residence times long in ground-water environments, making them vulnerable to pollution and difficult to rehabilitate (Notenboom *et al.*, 1994). Many aquifers contain toxic wastes, and ground-water contamination is a major problem at most of the priority hazardous ('Superfund') cleanup sites (Job and Simons, 1994).

Ground-water ecosystems contain some of the most unique and some of the most endangered fauna. In the United States, cave-limited fauna (troglobites) and ground-water limited fauna (stygobites) represent more than half of the imperiled (G1-G2) species listed in the Natural Heritage Program, yet less than 4% have federal protection status (Culver *et al.*, 2000). Elliot (2000) posits that at least 10 troglobitic and stygobitic species are extinct because of anthropogenic disturbance in the United States.

RESEARCH NEEDS AND STUDY FOCUS

Despite the importance of ground-water ecosystems, they have received very little study by the scientific community (Memon and Prohic, 1989; Cullimore, 1993; Job and Simons, 1994). Community compositions are not known for most ground-water systems, and little is known about the distribution of species in ground-water habitats and their limiting factors (Gounot, 1994; Strayer, 1994). Very few food webs have been described for ground-water communities (Culver, 1994). The nutrient dynamics and fluxes within subterranean communities and through the ground-water/surface-water interface are poorly understood (Gibert *et al.*, 1994). This lack of knowledge is exacerbated by the fact that man-sized conduits (caves) represent only a fraction of the entire active karst drainage systems (White, 1993), and only about one-tenth of cave complexes have openings to the surface (Curl, 1958). Yet, subterranean habitats are considered excellent ecosystems for ecological studies because the habitat is quite stable, autotrophic production is negligible, species numbers are low, the gene pool is restricted, and natural replication is plentiful (Culver, 1982). Caves are a model study system for detritus-based ecosystems (Poulson, 1976), which are important because, in general, the majority of

plant biomass production escapes the grazing food chain and must enter food webs through detrital chains (Polis and Strong, 1996). The most important reason to study cave streams is their link with, and their dependence upon, the surface watersheds that they drain -- a ground-water ecosystem cannot flourish below an abused or unprotected watershed, nor can a polluted aquifer meet the water needs of communities or industry above. If ground-water systems, such as the Springfield Plateau aquifer of the Ozark Plateaus Province, are to be managed properly, we must gain a better understanding of the flux of organics through the ecosystem and how they are utilized by biota. This study has provide some, but not all, of this needed information about energy flux and trophic interactions in an Ozark cave stream ecosystem, an environment that is poorly understood, experiencing multiple stressors, and yet vital for human populations as well as rare cave fauna.

BACKGROUND

ORGANIC MATTER DYNAMICS

Energy Flow in Cave Ecosystems

Like most headwater streams, subterranean streams are heterotrophic systems that rely almost entirely upon allochthonous sources of energy (Fisher and Likens, 1973; Vannote *et al.*, 1980, Culver, 1982). Organic matter that is present is usually of low food quality, and as such, most cave systems are severely nutrient limited, or oligotrophic (Brown *et al.*, 1994; Streever, 1996). Cave stream ecosystems with diffuse recharge (lacking discrete inputs such as sinkholes and swallets) have even less organic matter input (Poulson, 1976). Aley and Aley (1979) estimated that caves in the Ozarks receive less than $1/1{,}000^{th}$ of the energy received by the surface above them. In cave ecosystems, photosynthesis is absent and leaf litter inputs are greatly reduced. The majority of energy input is in the form of dissolved organic matter (DOM) in the cave stream (Brown *et al.*, 1994), except for cave systems with significant fecal inputs (bat guano, insect frass, mammal scat) (Harris, 1970). Floods import most of the organic matter in cave streams, and in some caves, flood/drought cycles regulate the life cycles of cave biota (Hawes, 1939). Utilization of DOM in stream ecosystems remains poorly understood (Cushing *et al.*, 1993; Hall and Meyer, 1998), and virtually nothing is known about DOM dynamics in cave ecosystems.

One of the only studies of organic matter fluxes in cave streams is Brown *et al.*'s (1994) study of the carbon dynamics in Logan Cave, Benton County, Arkansas. Figure 1 shows a summary of the carbon flux within this cave complex. Dissolved organic carbon

(DOC) accounted for the vast majority of carbon inputs in this cave complex, even though a significant gray bat (*Myotis grisescens*) population (*circa* 10,000 bats) exists. Neither the composition nor the fate of organic matter inputs into Ozark cave stream ecosystems is known, nor has the relative importance of these sources to the biota been determined. It is not known, for example, whether the food web is based fundamentally on bat guano, as hypothesized (Poulson, 1972; Willis, 1984), or based upon dissolved organic matter originating from the recharge zone. Novel, anthropogenic nutrient sources (septic and agricultural wastes) add complexity to these organic matter fluxes.

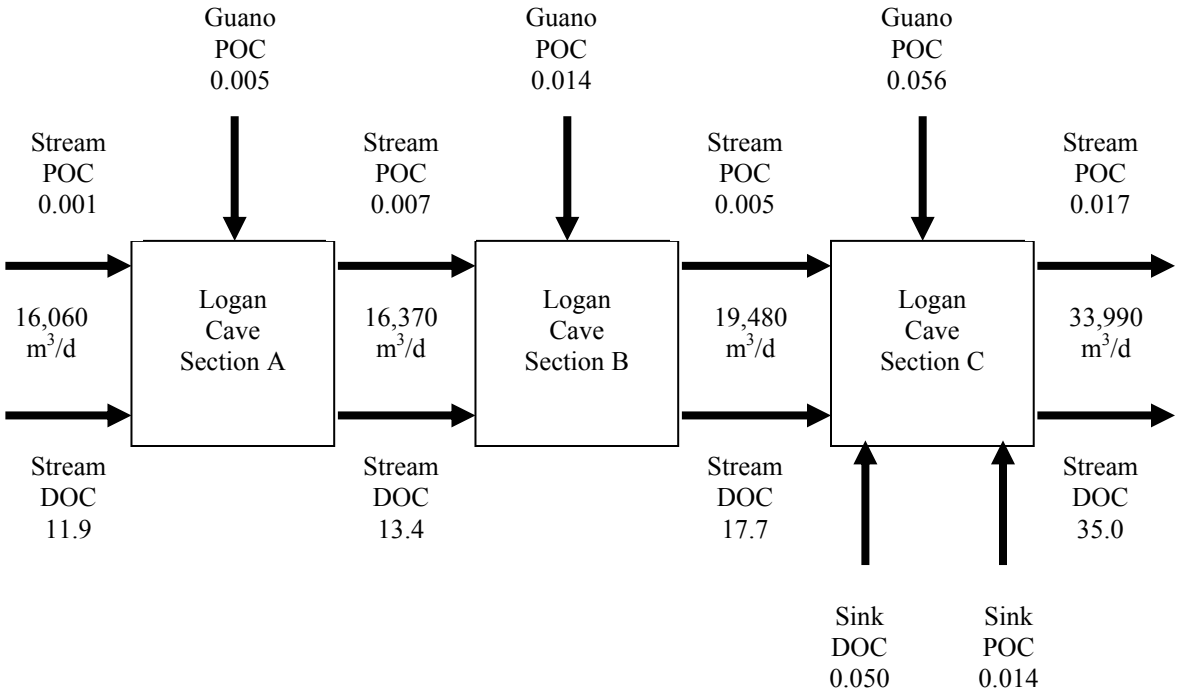

Figure 1. Organic carbon dynamics in three sections of Logan Cave, Arkansas (after Brown *et al.*, 1994). Components include discharge (m^3/d), stream and sinkhole dissolved organic carbon (DOC) loadings (kg/d), stream and sinkhole fine (< 0.5 mm) particulate organic carbon (POC) loadings (kg/d), and bat guano coarse particulate organic carbon (POC) loading (kg/d).

Bioavailability

Measuring water chemistry parameters is not adequate to assess the impact of pollution in aquatic ecosystems (Loeb, 1994). Even the measurement of organic matter quantities in an aquatic system is not sufficient because it does not describe the availability of these organics to the biota (Leff and Meyer, 1991; Morita, 1993; Meyer, 1994). Many of the organic compounds in natural environments are recalcitrant, and labile compounds are often complexed or are adsorbed to clay minerals (Morita, 1993). A measure of the bioavailability of animal waste constituents is urgently needed in the agri-sciences because standard nutrient analyses do not properly describe a waste's value as a nutrient source or its environmental impact (Sims, 1995). Thus, measurements of assimilability are needed to determine the relative importance of multiple food sources in aquatic food webs (Rosenfeld and Roff, 1992; Ward and Johnson, 1996). Few studies have measured the metabolic response of microbes to different DOM sources (Ward and Johnson, 1996). Lability has been estimated by comparing community respiration to carbon utilization (see review by Pierson, 1997). The approach used by many stream ecologists is the measurement of assimilable organic carbon (AOC), which is the peak microbial biomass that can be produced by the substrate in question, divided by the original organic content (mg/l of TOC) of the substrate (Leff and Meyer, 1991; Ward and Johnson, 1996). In this study, AOC was measured to estimate the bioavailability of the possible organic matter resources in a cave ecosystem. The AOC technique consists of incubating a water sample or leachate and measuring the increase in bacterial biomass (μg/l of carbon) per mass of substrate (mg/l of DOC). This assay cannot only evaluate differing trophic resources, but can also indicate the effect a pollutant may have upon the ecosystem. Yet,

measuring microbial biomass production does not fully describe the trophic dynamics of these ecosystems because there is no indication of which organics are incorporated nor are higher trophic transfers described. The analysis of the natural abundance of stable isotopes (described later) in DOM, microbes, and other biota can provide such needed information in cave ecosystems.

Trophic Status and Community Structure

Oligotrophy appears to structure cave communities by competition for food (Culver, 1982). Food payoff/risk ratio controls community complexity; high payoff (copious calories/gram/time/area) and high risk (high variability and low predictability of food renewal/time/area) favor simple communities where a few short-lived opportunists dominate. Low payoff/low risk resources are associated with complex communities having species of long-lived, efficiency experts (Connell and Orias, 1964; Poulson, 1976). Anthropogenic inputs tend to be in the high payoff/high risk category, and thus favor a few opportunistic species (Poulson, 1976). Eutrophication in cave systems could favor surface-dwelling (epigean) species, which are physically stronger, more active, and have higher fecundities than subsurface-dwelling (hypogean) species. Such enrichment could increase the food supply and thus the payoff for epigean species, such as sculpin (Cottidae) and crayfish (*Orconectes*), which may invade such disturbed caves and increase the risk of predation of troglobites (Poulson, 1976; Brown *et al.*, 1994). As early as 1976, Poulson suggested performing enrichment studies to test theories that related productivity to community structure.

Caves with significant guano resources add complexity and diversity to cave food webs and add environmental variability to caves by changing their thermal and humidity regimes and their gas composition (Harris, 1970). Bats have been described as "primary producers" in some caves (Horst, 1972), and bat guano can supply sufficient food to cave ecosystems to relax the selective pressure of oligotrophy, with resulting changes in community structure, including the presence of species without troglomorphic (cave adapted) characteristics (Culver, 1982). It has long been assumed that bat guano fuels cave food webs (Poulson, 1972; Willis, 1984), although few studies have tested this hypothesis, and available evidence is contradictory. Brown (1996) found no difference in total organic carbon (TOC) concentration of the water below and above bat colonies in Logan Cave, suggesting that guano was not a significant input into the food web even though thousands of bats occupy the cave in summer months. If guano is the primary food source for many caves, then the recent decline of many bat populations (Harvey, 1996) may result in the loss of a crucial trophic input. Concurrent with the decline of this natural nutrient input is the increase in anthropogenic nutrient inputs, which has been hypothesized to augment these cave food webs (Stewart, 1984; Sket, 1999).

Effects of Nutrient Pollution

Eutrophication is commonly defined as a process that increases nutrients, especially nitrogen and phosphorous forms, in an aquatic ecosystem with a corresponding increase in algae populations and a decrease in diversity (Morris, 1992). Nutrient pollutants alter the oligotrophic nature of ground-water ecosystems and severely alter ground-water food webs (Notenboom *et al.*, 1994). The introduction of organic pollution can extirpate the

indigenous fauna or completely replace the community with epigean fauna (Notenboom *et al.*, 1994). In the Cedars karst system, Virginia, for example, organic pollution extirpated the stygobitic isopods (*Caecidotea recurvata* and *Lirceus usdagalun*) and amphipods (*Crangonyx antenatus*) (Culver *et al.*, 1992).

In general, moderate pollution by sewage-derived organic matter (SDOM) results in a loss of biodiversity (especially intolerant species) and an increase in the standing crop of tolerant species (Sinton, 1984). The SDOM, via microbes, supports a denser macroinvertebrate community, which may assimilate up to 20% of the caloric value of the sewage (Sinton, 1984). In 1966, Holsinger described the effects of septic waste pollution on the ecosystem in Banners Corner Cave, Virginia. Compared to other pristine, central Appalachian caves, the polluted cave had significantly larger densities of invertebrates. Banners Corner Cave had cave isopod (*Caecidotea recurvata*) densities of 35 – 61 isopods/m^2, whereas the nearby and relatively pristine Chadwell's Cave, Tennessee, had an isopod density of only 6 isopods/m^2. Troglophilic flat worms (*Phagocata subterranea*) and oligochaetes (*Tubifex tubifex*) were also abundant in Banners Corner Cave, but only troglobitic flatworms (*Sphalloplana* sp.) were found in Chadwell's Cave (Holsinger, 1966). In 1997, Simon and Buikema did a follow up study in Banners Corner Cave, which continued receiving sewage inputs. Stygobitic amphipods (*Stygobromus mackini*) were absent from all polluted pools, while *Caecidotea recurvata* populations increased in moderately polluted pools, but were extirpated from heavily polluted pools.

Nutrient enrichment has other negative effects upon ecosystems. Pathogens in ground water increase as organics in soil increase (Gerba and Bitton, 1984). Excess organic loadings create a biological oxygen demand that can quickly deprive fauna of dissolved oxygen. Eutrophication thus threatens cave biota, many of which are endangered species (Brown *et al.*, 1994). Organic matter budgets can describe the current state of an ecosystem, identify organic sources and sinks, and provide a baseline from which a future enrichment trend could be detected (Minshall, 1993).

MICROBES IN CAVE ECOSYSTEM DYNAMICS

The Microbial Community

Until recently, the subsurface environment was thought to be virtually sterile (Gounot, 1994). To the contrary, the microbial community may support the entire food web of many ground-water systems (Stanford and Gibert, 1994). Subterranean food webs are fundamentally detrital, relying upon microbes and invertebrates to assimilate and enhance the few organics present (Culver, 1982). The majority of these imported organics are in the dissolved form, and the microbial community transforms this DOM into biomass that is consumed by higher trophic levels (Marxsen, 1988; Psenner, 1993; Ward and Johnson, 1996). The ground-water environment dictates that the microbial community will form biofilms on submerged surfaces (McCarty *et al.*, 1984), and in general, it is upon these surfaces that most carbon turnover occurs in oligotrophic, aquatic ecosystems (Morgan and Dow, 1985). Because of the oligotrophic nature of many aquatic systems and because of the rapid assimilation of any organics present by microbes, the microbial community is normally in the starvation/resting mode (Morita,

1993). Often, less than 30% of the total microbial population is metabolically active (Morita, 1993; Ward and Johnson, 1996). Microbial densities range from 10^2 to 10^4 cells/ml of free water and from 10^4 to 10^8 cells/g of dry sediment in caves (Gounot, 1994). A grab sample of water from springs (at base flow) in Benton and Washington counties revealed similar densities of viable bacteria in seston, and is summarized in Table 1. In contrast, typical abundances in surface streams range from 10^4 to 10^7 cells /ml for suspended (seston) microbes and from 10^{10} to 10^{12} cells/m^2 for microbes attached to submerged surfaces (Ward and Johnson, 1996).

Table 1. Density of microbes viable in water samples of springs in Benton and Washington Counties, Arkansas, at base flow, November 1997. Viable cell densities were determined by epifluorescence microscopy (Graening, unpublished data).

Viable Cell Density	Site	Location
20,000 cells/ml	Elm Spring	Elm Springs, Washington County
20,000 cells/ml	Cave Springs	Cave Springs, Benton County
4,000 cells/ml	German Spring	Savoy, Washington County
200,000 cells/ml	Langle Spring	Savoy, Washington County
30,000 cells/ml	Stillhouse Spring	Highfill, Benton County
5,000 cells/ml	Wilson Spring	Fayetteville, Washington County

Enumeration of Microbes using Epifluorescence Microscopy

The use of traditional culturing methods for the enumeration of aquatic bacteria is complicated by the fact that ordinary media do not support the growth of all bacteria present, and because colony counts do not distinguish between single cells and microcolonies (Costerton and Colwell, 1979). Because of the difficulty of culturing aquatic microbes, and because of the high sensitivity of fluorochromes, epifluorescence microscopy (EFM) has become the most accepted technique for the enumeration of

aquatic microbes (Ward and Johnson, 1996) and to estimate microbial biomass (Psenner, 1993). EFM was first used in aquatic studies in the early 1970's (Francisco *et al.*, 1973), and since then, has been refined substantially with the advent of highly specific fluorochrome stains. This microscopy technique works by stimulating a fluorochrome with ultraviolet or visible light, causing the stain to fluoresce in the visible spectrum and directing this emitted light through filters and into ocular lenses (Sherr *et al.*, 1993). The fluorochrome *4,'6-diamidino-phenylindole* (DAPI) is highly specific for DNA, and the DAPI-DNA complex of microbes (and eukaryotes) fluoresces bright blue, whereas unbound DAPI and DAPI associated with detritus fluoresces yellow (Sherr *et al.*, 1993). DAPI allows the investigator to discern between intact cells, lysed cells, and detritus, making this method the best estimator of viable microbes (King and Parker, 1988). Distinguishing between alive and dead bacteria is facilitated by the fact that expiring bacteria tend to lyse because of cell wall amidases (Costerton and Colwell, 1979). Thus, DAPI and similar dyes equate presence of intact plasma membrane with viability (Haugland, 1996). Direct measurements of bacterial productivity in ground water are scarce in the literature (Strayer, 1994), yet microbial biomass may be a good indicator of the trophic state of an entire aquatic ecosystem (Psenner, 1993).

Microbes and Land Use

Karst conduits can modify transport of surface pollutants to ground water such that non-point source pollution is concentrated, and behaves more like point source pollution (Pasquarell and Boyer, 1996). Karst terrains allow bacteria to be transported by water from the surface, through the aquifer, and back out through resurgent springs (Pasquarell

and Boyer, 1996). In karst aquifers, fecal bacteria can be transported over several kilometers (Hallberg *et al.*, 1985; Green *et al.*, 1990). Whereas fecal bacteria may only survive days in natural waters (McFeters and Stuart, 1972; McFeters *et al.*, 1974), bacteria can survive for months in aerobic soils (Gerba and Bitton, 1984).

It is difficult to determine the source of the fecal bacteria in cave streams because the intestinal coliforms of humans, bats, and livestock are quite similar. No proven and widely accepted method exists for distinguishing human and animal sources of bacteria (Pasquarell and Boyer, 1995). Methods such as measuring the ratio of fecal coliforms to fecal streptococci densities are not rigorous (Clesceri *et al.*, 1989). Bat guano is not necessarily the dominant source of fecal bacteria in Ozark caves. Whereas Williams (1991) listed bat guano as a possible fecal coliform source, he discounted it because surrounding streams that recharge the Cave Springs Cave (CSC) stream had similarly high levels of coliforms. Water samples taken in Logan Cave and CSC upstream of bat colony roosts often have higher coliform densities than samples taken downstream of the bat colonies (Means, 1993; Brown *et al.*, 1998; Graening and Brown, 1999). It is especially unlikely that guano input is significant during the winter season because the majority of gray bats do not overwinter in CSC, and in general, the reduced metabolism of bats during hibernation results in negligible guano inputs into the cave during winter (Harvey, 1992). Septic system leachate, livestock manures, and sewage sludge are other possible sources of the high fecal coliform loads in these ground-water ecosystems.

TROPHIC DYNAMICS AND STABLE ISOTOPE ANALYSES

Use of Stable Isotope Analyses in Aquatic Ecology

One of the most useful tools in ecological sciences is the use of mass spectrometry to measure the natural abundance of stable isotopes in organic matter. Stable isotopes are radiogenic isotopes, which are the stable product of natural radioactive decay processes (Fetter, 1994). Metabolic processes of organisms tend to fractionate, or distribute unevenly, stable isotopes of elements such as carbon, nitrogen and sulfur, resulting in unique ratios, or signatures, that may be used to determine the sources of organics that were incorporated into the organism. Many investigators have used stable isotope analyses (SIA) to determine time-integrated information about trophic relationships in aquatic food webs (Vander Zanden *et al.*, 1998). Gut content analyses provide specific feeding information over a brief period (ingestion), whereas SIA provides general feeding information over a long time (assimilation) (Vander Zanden *et al.*, 1998). Most importantly, this technique allows researchers to gather data on material fluxes from ecosystems rather unobtrusively, and without the need to introduce radioactive substances or manipulate the habitat or biota.

Stable isotope techniques are now widely used in food web studies of freshwaters (Fry, 1999). Furthermore, stable isotopes analyses have been a powerful tool in describing carbon sources and cycling (*e.g.*, Gearing, 1991) and elucidating the impact of the introduction of pollutants into aquatic ecosystems (*e.g.*, Kwak and Zedler, 1997; Atwell *et al.*, 1998). Geologists have been using the natural isotopic variation in oxygen and hydrogen to discern the sources and flow paths of ground water for decades (Anderson and Arthur, 1983; Fetter, 1994). Carbon isotopes have been used to reconstruct the

paleodiets of ancient hominids and animals in caves (Bocherens *et al.*, 1995; Nelson *et al.*, 1998). Isotopic techniques have also deciphered paleoclimatological data from cave sediments as well as differentiated agricultural pollution from diagenesis (Mitzutani *et al.*, 1992a; Bottrell, 1996). Sarbu *et al.* (1996) used multiple stable isotopes to describe a chemoautotrophic cave food web (Movile Cave, Romania). In an arctic marine food web, Atwell *et al.* (1998) found a significant correlation between mercury bioaccumulation and trophic level determined by nitrogen stable isotope analyses. Voss and Struck (1997) used stable carbon and nitrogen isotopes to document the eutrophication of the Pomeranian Bight (Baltic Sea) by industrial and agricultural activities over the last 100 years.

Isotope Ratio Theory and Approach

Stable isotopes are measured using an isotope ratio mass spectrophotometer. The ratio of heavy and light isotopes in a sample (R_{sa}) are compared to the ratios in a standard (R_{std}), and the difference is calculated on a parts per thousand basis ($^{o}/_{oo}$, or "*per mil"*), called delta (δ) notation (McKinney *et al.*, 1950): $\delta\ (^{o}/_{oo}) = (\ R_{sa}\ /\ R_{std}\ -\ 1\ )\ \ X\ 1000$. The primary standards are: the Pee Dee belemnite (PDB) marine limestone fossil for carbon ($^{13}C/^{12}C$); atmospheric air for nitrogen ($^{15}N\ /^{14}N$); the troilite standard of the Canyon Diablo meteorite ($^{34}S/^{32}S$); and for hydrogen and oxygen, the Vienna Standard Mean Ocean Water, ($^{2}H/^{1}H$) and ($^{18}O/^{16}O$) (Lajtha and Michener, 1994).

Carbon isotopic compositions of animals reflect those of their diets within about 1 $^{o}/_{oo}$, with a slight enrichment of ^{13}C occurring overall (Peterson and Fry, 1987; Michener and

Schell, 1994). Enrichment may occur because of preferential uptake of ^{13}C compounds during digestion, preferential loss of $^{12}CO_2$ during respiration, or metabolic fractionation (Michener and Schell, 1994). Nitrogen isotopic compositions of consumers are enriched by 2 to 5 $^{o}/_{oo}$ compared to their dietary nitrogen, and nitrogen stable isotopes can describe trophic structure and food chain length (number of trophic levels) by the consistent enrichment of the isotope ratio ($^{15}N/^{14}N$) by a mean 3.4 $^{o}/_{oo}$ at each trophic level (DeNiro and Epstein, 1981; Minagawa and Wada, 1984; Peterson and Fry, 1987; Kwak and Zedler, 1997). Nitrogen isotopes can also be used to determine the pollution source in ground water. Ninety percent of inorganic fertilizers have $\delta^{15}N$ values of less than 3 $^{o}/_{oo}$, whereas decomposing animal wastes (including septic) have nitrate $\delta^{15}N$ values between 9 and 22 $^{o}/_{oo}$ (Wells and Krothe, 1989). Hydrologists have taken advantage of this large difference between nitrogen sources to determine whether cultivation practices or animal wastes are polluting ground water with nitrates (see review by Wells and Krothe, 1989).

Stable isotope analyses are not universally applicable, especially in systems that are trophically complex (*i.e.*, having many organic matter inputs and trophic linkages). Stable isotope techniques have been effective in discerning energy sources in food webs of simpler systems, such as arctic ecosystems (Michener and Schell, 1994). In some lotic studies, however, carbon isotopes alone have failed to discern differences between various carbon sources (France, 1996). Multiple stable isotopes have proven more successful in discerning between organic matter sources where single isotope analyses have not (*e.g.*, Gearing, 1991). Stable isotope data are strengthened by other techniques such as gut content analyses, which can confirm trophic inferences (Mihuc and Toetz,

1993; Whitledge and Rabeni, 1997). Gut content analysis and visual observation are the traditional methods for determining food web structure, but these methods do not determine the exact sources of energy for organisms (Hershey and Peterson, 1996; Mihuc and Toetz, 1993). Stable isotope analyses determine which organics are actually *assimilated* into the organism, not just ingested (Rosenfeld and Roff, 1992). Combined, the two techniques are quite powerful in trophic studies (Hershey and Peterson, 1996).

STATUS OF OZARK GROUND-WATER ECOSYSTEMS

The U.S. Environmental Protection Agency (USEPA) (1998a) reported that agricultural activity is the leading source of pollution threatening the water quality of United States rivers and lakes. However, agriculture is not the only human activity affecting ground water -- an estimated 3 billion cubic meters of sewage and wastewater are discharged to the subsurface every year in the U.S. (Novotny and Olem, 1994). Even more sewage pollution is anticipated because most septic systems installed in the period from 1950 to 1980 have exceeded their design lives of 10 - 15 years (Novotny and Olem, 1994). The decline in America's water quality is serious because contaminated and inadequately treated ground water is responsible for an estimated one-half of all waterborne disease in the United States (Craun, 1979; Moore *et al.*, 1994).

The fractured and dissolved carbonate terrain (karst) of northwest Arkansas is highly susceptible to pollution from land application of animal wastes and other waste disposal practices (MacDonald *et al.*, 1976). Arkansas leads the nation in poultry production with over 1 billion birds grown per year, which results in 1 trillion kg (dry weight) of poultry

wastes (Klugh and Abbe, 1994). Over 15 metric tons per hectare of confined animal waste have been applied to pasture in the Illinois River basin every year (Soil Conservation Service, 1988). Bacterial contamination, especially from septic system leachate, is considered the most serious threat to Ozark ground-water quality (MacDonald *et al.*, 1974; MacDonald *et al.*, 1976; Steele, 1985). An estimated seventy-eight percent of wells and 90% of springs in northwest Arkansas are contaminated with coliform bacteria (Ogden, 1979; Steele, 1985). The spring issuing out of CSC has an exceptionally high fecal pollutant load, with average fecal coliform counts in the thousands (MPN/100ml) and peak storm flow counts approaching one hundred thousand (MPN/100ml) (Graening and Brown, 1999).

Nitrate (NO_3) is the most ubiquitous chemical contaminant in the world's aquifers, and the levels of contamination have increased over time (Spalding and Exner, 1993). Nitrate is not usually considered a direct toxicant, but nitrate can be reduced to nitrite by humans and aquatic organisms in their gastrointestinal tracts (USEPA, 1998b). Nitrite exposure can cause anoxemia, which causes tissue damage or even death, and nitrite is implicated as a cause of stomach cancer and birth defects (see review by Smith and Steele, 1990; USEPA, 1998b). High nitrite levels can also cause anemia and tissue damage in fishes (Eddy and Williams, 1987). Studies have shown that land uses such as confined-animal feeding operation waste application, grazing, and septic system treatment have directly contaminated the Springfield Plateau aquifer with nitrate (see review by Smith and Steele, 1990), and nitrates do not occur naturally in the rocks of northwest Arkansas (Willis, 1978). Ogden (1979) implicated septic leachate and animal waste in the

pollution of the Springfield aquifer by nitrates, sulfates, phosphates, and chlorides. Steele and Adamski (1987) confirmed these fecal wastes as pollution sources in an Arkansas study that compared water quality in wells near septic systems and confined-animal operations and those far from these land uses. Smith and Steele (1990) attributed unusually high nitrate concentrations of ground water to faulty septic systems, and they found an average of 2.6 mg/l and 1.8 mg/l of NO_3-N in the Springfield Plateau aquifer of Benton County in wet and dry seasons, respectively.

OBJECTIVES

A review of the scientific literature revealed that Ozark caves have important natural resources, that their ecosystem processes are poorly understood, that they are experiencing multiple environmental stressors, and that many questions remain unanswered. What is the trophic structure of Ozark cave streams? Is bat guano the dominant organic matter resource? How do cave stream microbial populations utilize these organics? Are nutrient pollutants augmenting these trophic webs? What effect will eutrophication have upon these oligotrophic ecosystems? This study has attempted to describe the ecosystem dynamics of an Ozark cave stream ecosystem using a multi-scalar approach involving environmental quality sampling, organic matter budgeting, microbiological techniques, stable isotope analyses, and faunal inventories. This study was conducted in Cave Springs Cave, Arkansas, from November 1997 to December 1999, but environmental quality monitoring and research continue in this cave complex.

This study had the following objectives, and was driven by several hypotheses:

Objective 1: Calculate the flux of organic matter through the cave stream ecosystem.

Objective 2: Assess the microbial assimilation of various organic matter inputs.

Hypothesis: The organic matter inputs into the cave food web are not equal in quality (defined by assimilable organic carbon).

Objective 3: Describe the trophic structure of the cave ecosystem and the organic matter resources available to the biota.

Hypothesis: The major organic matter resource for cave biota is bat guano.

Objective 4: Determine the extent of nutrient pollution and metals contamination in this cave habitat.

Hypothesis: The source of organic pollutants in the cave stream is confined-animal feeding operation waste.

Hypothesis: Concentrations of nutrients (TOC, nitrate, and total phosphorous) in cave stream water have increased since first records were kept (1984).

Hypothesis: Microbial densities reflect trophic status (*i.e.*, nutrient parameters are positively correlated to microbial cell densities).

SITE DESCRIPTION

THE GEOLOGIC SETTING

The Cave Springs Cave Natural Area, owned by the Arkansas Natural Heritage Commission (ANHC), is located in the city of Cave Springs, Benton County, Arkansas. The cave mouth has an entrance elevation of 329 m (Fanning, 1994) and a surveyed length of 514 m (see Figure 2). The cave complex is primarily a phreatic conduit system with extensive bedding plane dissolution, but some passages are joint-controlled and have some vadose development. This cave system is formed in Mississippian-aged limestones with the ceiling composed of the less soluble, chert-filled Boone Formation and the passageways dissolved from the purer limestone of the St. Joe Formation (Fanning, 1994). The complex is part of the Springfield Plateau of the larger Ozark Plateaus Province that lies on the Ozark Dome, an asymmetrical dome comprised of Paleozoic strata that dip radially away from the Precambrian center (Woods and Inger, 1957; Fanning, 1994).

The Cave Springs Cave resurgence is a rheocrene that is tributary to Osage Creek, which lies within the Illinois River watershed. Ground-water movement in the study area is concentrated in the Boone/St. Joe (or Springfield Plateau) aquifer, an unconfined aquifer with the potentiometric surface generally reflecting the surface topography (Fanning, 1994). The meteoric infiltration of local precipitation recharges this aquifer, and gravity springs discharge it relatively quickly. The cave complex has a diffuse recharge with an estimated spring basin area of 38 km^2, based upon the recharge area boundary delineation by Williams (1991). The total fall between the general location of the recharge area and

the ground-water high to the cave spring is approximately 55 m over 4.8 km (Williams, 1991). The mean annual discharge is 100 l/s, and the average annual water temperature is 14.4 ^{o}C, varying only by approximately 1 ^{o}C annually. Water hardness is approximately 150 mg/l as $CaCO_3$, with calcium being the dominant cation and bicarbonate being the dominant anion. The cave stream was partitioned into a study reach of 269 m, with two sampling stations: the downstream station was the cave orifice where the stream resurges, and the upstream station was the first waterfall (a chert ledge) deep in the cave, upstream of all bat roosts (see Figure 2). The total fall between the upstream station and the downstream station is approximately 1 m over 269 m, which corresponds to a gradient of 4 m/km.

THE BIOTIC COMMUNITY

Cave Springs Cave has a diverse biotic community with several globally rare species. The cave has approximately 100 eastern pipistrelles (*Pipistrellus subflavus*) (Graening, unpublished data). In 1935, Indiana bats (*Myotis sodalis*, federally listed as endangered) were observed in Cave Springs Cave (Sealander and Young, 1955), but none are found today (pers. observ.; M. Harvey, pers. comm., 1999). A maternity colony of 2,000 - 3,000 gray bats (federally listed as endangered) inhabits this cave during summer months (Michael Harvey and Ron Redman, unpublished data, 2000), but is substantially reduced from its previous abundance (Harvey, 1991), as shown in Figure 3. This cave also contains the largest known population of Ozark cavefish (*Amblyopsis rosae*), with a maximum observed population size of 166 individuals and an estimated density of 0.2 fish/m^2 (Graening and Brown, 1999); Figure 4 summarizes historical cavefish population counts. Other vertebrates include the cave salamander (*Eurycea lucifuga*), and the dark-sided salamander (*Eurycea longicauda melanopleura*) with up to 69 and 12 individuals, respectively, counted (Graening and Brown, 2000). The grotto salamander (*Typhlotriton spelaeus*) has also been reported from this cave (Brown *et al.*, 1994), but has not been seen since 1994. Other vertebrates that inhabit the cave mouth include the eastern phoebe (*Sayornis phoebe*) and the eastern woodrat (*Neotoma floridana*). The invertebrate community consists mainly of crayfish and isopods. The epigean spot-handed crayfish *(Orconectes punctimanus*), which curiously never reaches more than about 6 cm in length in this habitat, has an estimated density of 0.5 individuals/m^2 in the cave pools and 10 individuals/m^2 at the cave mouth in 1999 (Graening, unpublished data). A cave isopod (*Caecidotea stiladactyla*, Arkansas Species of Concern), first found

by Flemming in 1972, was abundant in riffles with an estimated density of 5 individuals/m^2 in 1999 (Graening, unpublished data). The Ozark cave amphipod (*Stygobromus ozarkensis*, Arkansas Species of Concern), was reported from CSC (Holsinger, 1972), but has not recently been found in the cave stream (Brown and Willis, 1984; pers. observ. of author). The cave amphipod *Stygobromus onondagaensis* has also been recorded from this cave (John Holsinger, pers. comm., 1999), but was not found during this study. Other invertebrates that inhabit the cave, especially near the cave mouth, include camel cave crickets (*Ceuthophilus* sp.), harvestmen (Opiliones), wolf spiders (*Lycosa* sp.), and water striders (Gerridae). Meiofauna collected in planktonic samples at CSC resurgence include bdelloid and monogonat (*Cephalodella* sp.) rotifers; cladocera (*Alona* sp., *Bosmina* sp., and *Simocephalus* sp.); cyclopoid and harpacticoid copepods; Collembola; ostracods; chironomids; oligochaetes; tardigrades; water mites (Acarina); and an ephemeropteran. Protists include a suctorian and a chlorophyte (*Scenedesmus*). Outside the cave in the surface stream, banded sculpins (*Cottus carolinae*), ringed crayfish (*Orconectes neglectus neglectus*), amphipods (*Gammarus* sp.), and water striders (Gerridae) were found

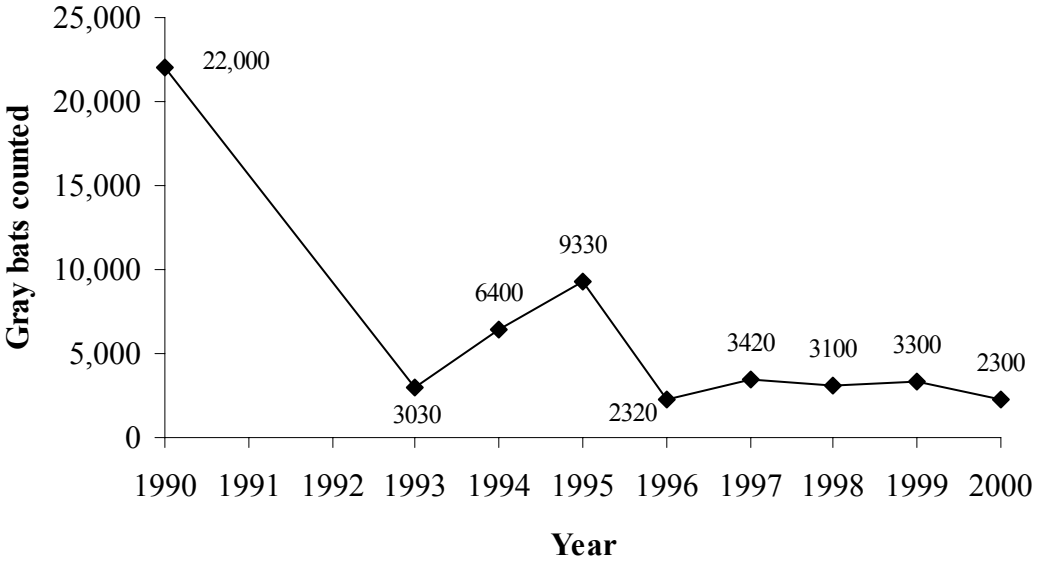

Figure 3. All known visual censuses of the maternity colony of gray bats (*Myotis grisescens*) in Cave Springs Cave (Harvey, 1991; M. Harvey and R. Redman, pers. comm., 2000). Linear regression indicates a significant ($p = 0.018$, $r^2 = 0.58$) decline in the population.

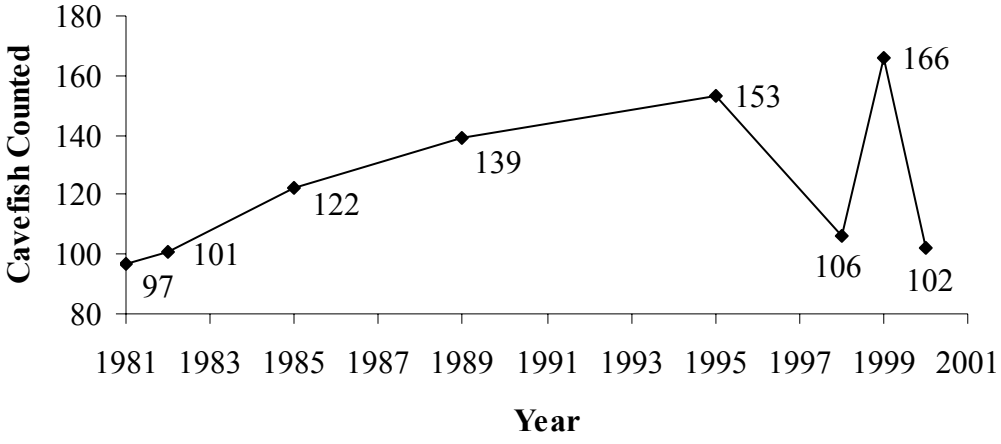

Figure 4. Summary of all visual censuses of Ozark cavefish in Cave Springs Cave performed by Brown and colleagues (Brown and Willis, 1984; Brown *et al*., 1998; Graening and Brown, 1999, 2000).

METHODS

PERMITS

This study was performed under the following permits: Federal Fish and Wildlife Service Permits No. PRT-834518, No. TE834518-2 and No. TE834518-1; ANHC Permit No. S-NHCC-99-005; and AGFC Educational Collecting Permit No. 1082. Impact was minimized by restricting visits into the cave to times when gray bats were not present and by avoiding wading in the cave stream whenever possible. One individual Ozark cavefish that was found severely wounded in the stream, possibly from inadvertent trampling during a population census, was collected and used for stable isotope and heavy metals analyses with special permission from the U.S. Fish and Wildlife Service.

ORGANIC MATTER BUDGET

Budget Design

Organic-matter budget methods followed Webster and Meyer (1997), including usage of the conversion factors of 2 g ash-free dry mass (AFDM)/g C and DOM/2 x DOC, and 5 kcal/g AFDM. The model structure is shown in Figure 5. Fresh guano, cave stream sediment, and cave stream biofilm from CSC were analyzed for percent dry matter, percent total carbon, percent TOC, and total calories by the Central Analytical Laboratory, University of Arkansas at Fayetteville (UAF). Percent total carbon and TOC were measured by the Dumas combustion method using an Elantech NA 2000 Carbon/Nitrogen Analyzer ™ (with a hydrochloric acid digestion for the TOC). Caloric value was measured by a PARR adiabatic bomb calorimeter. A photosynthetic efficiency

of 1.5 % was assumed for surface vegetation (Ricklefs and Miller, 1999). Average annual total horizontal insolation (British Thermal Units/ft^2/y) was estimated from Turner and Battle (1980) and converted to calories/m^2/y using a conversion factor of 252 calories/BTU.

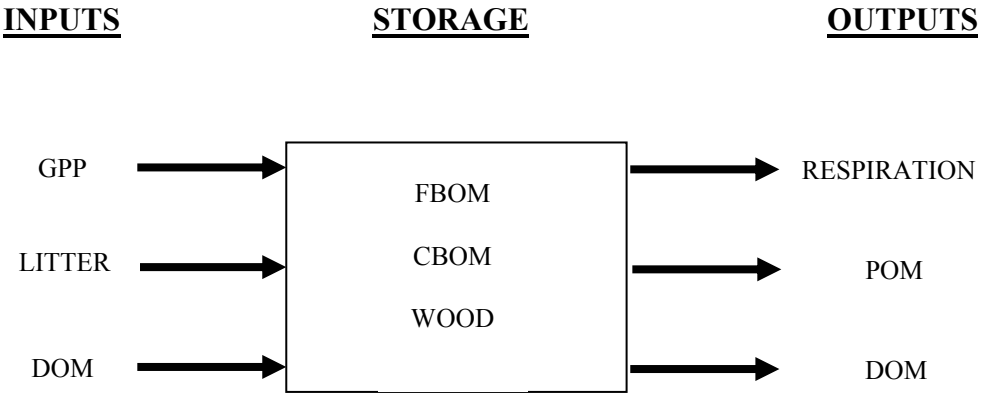

Figure 5. Diagram of organic matter budget model in this study (after Webster and Meyer, 1997). Components are gross primary production (GPP), litter inputs (both leaf fall and lateral movement), ground-water dissolved organic matter (DOM) inputs, standing crops of fine benthic organic matter (FBOM), coarse benthic organic matter (CBOM) and wood, and outputs of DOM, and particulate organic matter (POM).

Inputs

Because DOC concentrations (mg/l) at the upstream station did not significantly differ from concentrations at the downstream station over the 2-y period, upstream DOC concentrations inputs were set to the downstream values when data were not available because of cave closure for the gray bat maternity period (April 15th to September 15th of each year). DOC input loads (kg/d) were calculated by multiplying by the discharge (m^3/s) at the upstream station, and converted to DOM flux (g/m^2/y) using a study-reach, stream-bed area of 900 m^2 (calculated from the cave map). Guano inputs were estimated

following the plate capture method of Brown *et al.* (1994). Six plastic surgical trays (27 x 37 cm) were tethered and floated in the cave stream in the beginning of the North Passage, where the cave passage is restricted and the entire colony must fly through on their way out to forage (see cave map, Figure 2). Guano was collected four times between May and June 1999, and then oven-dried and weighed. Guano deposition rate ($g/m^2/d$) was calculated from this dry mass, the number of days collected, and the surface area of the plates (0.60 m^2). This rate was converted to an organic matter flux ($AFDM/m^2/y$) using a total guano deposition time of 150 days (the approximate length of stay of the maternity colony) and a measured TOC content of 41.3%, dry basis (d.b.).

Standing Crops

Fine benthic organic matter (FBOM, g/m^2) of cave sediments was estimated from the following: a sediment volume of 35 m^3 (mean depth of 50 cm and a sediment area of 700 m^2, calculated from the cave map), a mean density of 1.5 g/cm^3 (wet basis), and a TOC content of 4.7% (d.b.). For streambed areas not covered by sediment, the contribution of FBOM by biofilm was estimated using a mean biofilm density of 64 g/m^2 (d.b.) and TOC content of 1.7% (d.b.). The contribution of cave biota to standing crop was calculated from the total estimated population density of each species, individual mass of each animal (isopod, cavefish, or crayfish), and streambed area (900 m^2).

Outputs

Respiration associated with the cave benthic community was measured by the oxygen method in

recirculating chambers with four replicates (Bott *et al.*, 1978). Cave stream rocks, gravel and sediment were placed in 8-l plastic buckets and were buried in the cave streambed for 9 weeks to reestablish the epilithic community. The buckets were then sealed *in situ* with plastic lids fitted with a port for a dissolved oxygen probe (YSI Model 57 stirring oxygen meter). Dissolved oxygen was measured every 30 minutes for 6 hours. Community respiration was measured by the drop in oxygen concentration in these submerged chambers (Bott *et al.*, 1978). Data obtained in mg/l of oxygen were converted to mg/l of carbon by the following equation from Bott (1996): g C = g O_2 x RQ x 12/32, where RQ, the respiratory quotient (mol CO_2 released/mol O_2 consumed), was set at 0.85. Organic matter output by benthic respiration (g AFDM/m^2/y) was calculated using the mean respiration rate (g C/l/hr), the mean interstitial water volume (l), the bucket base area (0.0415 m^2), and a conversion factor of 2 g AFDM/g C. Respiration rate for seston was determined *in situ* using self-stirring oxygen probes in darkened BOD bottles (300 ml), with four replicates. Organic matter output from sestonic respiration was then calculated by the same method as for benthic respiration.

Estimates of DOM export were based upon 50 measurements of TOC or DOC at the downstream station over the 2-year period. Because no statistical difference existed between TOC and DOC concentrations ($t = 0.516$, $p = 0.309$, paired t-test), when only TOC was measured, DOC was calculated as 95% of TOC (mean ratio of DOC to TOC during the 2-year period). Daily export (kg/d) was determined by multiplying each DOC measurement (mg/l) by the discharge (l/s) at that sampling time (Harvey *et al.*, 1997). Graphs of DOC loading rate versus time were plotted for each season, and the total DOC

load (kg) for that season was estimated by integrating the area under the season curve. The summation of all seasons gave an estimate of yearly DOC export. Mean DOC loading over the 2-year study period was then used to calculate dissolved organic matter output ($g/m^2/y$) using a streambed area of 900 m^2. Particulate organic matter in transport was estimated using POC values (TOC – DOC), discharge, and the same integration procedure as above for DOC transport.

Comparison with Logan Cave

Logan Cave was chosen for the organic matter budget comparison because it is one of the only caves having budget data (Brown *et al*., 1994) and because it has many features in common with CSC, including similar biota, substrates, water chemistry, cave elevation length, and spring recharge area. Furthermore, both caves share the same watershed (Osage Creek), geologic formation (Boone), and aquifer (Springfield Plateau). Data from the carbon budget of Brown *et al.* (1994) (summarized in Figure 1), were converted using the following: a mean channel width of 2.7 m, streambed area of 2,470 m^2, and a mean annual discharge of 250 l/s (J. Brahana, US Geological Survey, written communication). The Logan Cave recharge area is 30.15 km^2 (Aley and Aley, 1987). A DOC input of 11.9 kg C/d was converted to 3,517 g AFDM/m^2/y, a DOC output of 35 kg C/d resulted in a dissolved transport of 25,550 kg AFDM/y, and a guano input of 0.075 kg/d was converted to 4.6 g AFDM/m^2/y. Using a mean ratio of 0.95 for DOC/TOC, ultra-fine particulate transport was estimated at 1,345 kg AFDM/y. Fine-particulate organic matter (FPOM) output was 12 kg/y (Brown *et al*., 1994). Note that POM transport in Logan

Cave did not include particles smaller than 0.55 mm. Logan Cave heterotrophic respiration was assumed to have a rate equal to that in CSC (200 g AFDM/m^2/y).

MICROBIAL ANALYSES

Estimation of Microbial Density and Biomass

Microbial density and biomass in water samples were estimated using direct counts of microbial cells by epifluorescence microscopy (Hoff, 1993). Water samples were collected in sterile, 120-ml containers and immediately preserved with Formalin (final sample concentration of 3.7% formaldehyde), which does not compromise the integrity of the cell membrane (Haugland, 1996). Samples were transported on ice, held at 4 ^{o}C at the lab, and processed within 48 hours. Subsamples, ranging from 0.001 - 30 ml depending upon microbial density, were diluted or directly pipetted into the funnel tower and stained with 1 ml of DAPI (1 mg/ml stock solution), then filtered through a 0.2-μm black, polycarbonate filter membrane (Nuclepore ^{TM}), backed by a polypropylene support filter, under low vacuum (Hoff, 1993). The polycarbonate filter was then mounted on a glass slide with a drop of immersion oil and covered with a cover slip. A Zeiss Standard microscope, outfitted with an ultra-violet light source (365 nm), filters, and 35-mm camera, was used. Quality control was ensured by performing cell counts on lab blanks of distilled water. For each subsample, at least 10 microscope fields (reticules) were counted with roughly 30 cells/reticule (Kirchman, 1993) at 1,000X. The bacteria on membrane filters appear to follow a Poisson distribution, and so the precision of the count depends upon total number of bacteria counted, with most researchers counting at least 100 cells/filter (Fry, 1990).

The cell counts were converted to cell density by the following equation, where the effective filter area = 201.06 mm^2, the reticule area = 0.0113 mm^2 at 1,000X:

$$\frac{\text{\# of cells}}{\text{reticule area (mm)}} \times \frac{\text{filter area (mm)}}{\text{sample volume (ml)}} \times \text{dilution factor} = \frac{\text{cells}}{\text{ml}}$$

Estimation of Bioavailability

To determine the utilization of allochthonous nutrient inputs by cave microflora, leachates were made and incubated with cave stream water as inoculum, and the peak microbial biomass determined. Cave stream water was used as a control. Leachates of leaf litter, poultry litter, myotis guano, septic biosolids, and cow manure were generated following the procedure of Ward and Johnson (1996): 10-100 g of organics (depending upon % TOC) were added to 1 l of deionized water, covered and stirred for 24 hours at 5 ^{o}C. The leachate was then strained through stacked sieves down to 43 μm, and then filtered through a 0.7-μm Whatman GF/F filter into sterile, opaque bottles.

For leachate incubation, a modified procedure of Coffin and Cifuentes (1993) was used. Filtered leachate samples of 1-l volume were inoculated with 50 ml of cave stream water, with three replicates. At this time, total viable cell density and DOC concentration were determined. The samples were incubated in the dark in sterile, 1-l Erlenmeyer flasks for at least 200 hours at 14 ^{o}C. Subsamples were taken every 24 hours for approximately 10 days, and total viable cell density determined each time. A growth curve was plotted for

each incubation, and growth rate was determined by linear regression of the exponential growth phase. Peak microbial biomass increase was determined by subtracting the maximum cell density by the starting cell density, and multiplying by 20 fg bacterial C/cell. The assimilable organic carbon (bioavailability) of each nutrient source was determined by comparing the increase in microbial mass to the DOC concentration for each substrate, following the method of Leff and Meyer (1991). Specifically, the peak microbial biomass (μg C) was divided by the initial DOC concentration (mg/l), to yield the assimilable organic carbon (AOC) in μg C/mg DOC of substrate.

TROPHIC ANALYSES

Stable Isotope Analyses

Samples were oven-dried, pulverized, acidified with 1 N HCl to remove inorganic carbon, re-dried, and passed through a No. 30-mesh screen. Samples were sent in glass vials with Teflon lids to the Stable Isotope Ratio Facility for Environmental Research, University of Utah at Salt Lake City, or to the UAF Stable Isotope Laboratory for natural abundance carbon and nitrogen isotope ratio analyses. Analytical variability was estimated to be 0.1 $^{o}/_{oo}$. Discriminant analysis dissimilarity plots were used to discern trophic interactions (Hershey and Peterson, 1996), and a univariate plot of ^{15}N values was used to estimate trophic position.

Particles in transport for POC/PON were collected as per Voss and Struck (1997) by filtering thousands of liters of cave water through an in-line filter which contained pre-combusted GF/F filters, then oven-drying the filters, and scraping the residue off into

clean glass vials. Crayfish (*Orconectes punctimanus*) tissue samples were processed by the procedure of France (1996): crayfish were collected by dip net, placed into clean glass vials, preserved in ice, and brought back to the lab where the abdominal muscles were excised, dried, and pulverized. Because the mean length of crayfish was only 46 mm, a composite sample of approximately 10 adult crayfish was used for each sample. Composite samples of whole cave isopods (*Caecidotea stiladactyla*) were dried and ground. Poultry litter (feces, rice hull bedding, feathers, and blood) and beef cattle manure were obtained from the Savoy Experimental Farm (UAF). To increase the sample size and accuracy of poultry litter stable isotope ratios, isotope values from this study were combined with similar data from an Ozark stream study by Kwak (1999), who obtained poultry wastes from the same source – the UAF Departments of Poultry Science and Animal Science. The mean ($^{+}$/- SE) isotope values of the combination of layer feces, layer litter, and broiler litter samples from that study was $\delta^{13}C = -19.2$ $^{+}$/- 1.9 $^{o}/_{oo}$, $\delta^{15}N =$ 4.9 $^{+}$/-0.7 $^{o}/_{oo}$. Septic system leachate and biosolids were collected from two residential septic systems near the study area. Sewage sludge (press-cake) was collected from the Springdale Sewage Treatment Plant, which has historically (1990 – 1996) applied these biosolids upon the CSC recharge area. Samples of soil, pasture grass (*Festuca arundinacea*) and leaf litter (*Quercus* spp., *Platanus occidentalis*, and *Celtis occidentalis*), were collected from the cave recharge zone no more than 500 m from the cave mouth.

Gut Content Analyses

Crayfish (*Orconectes punctimanus*) were captured and preserved in cold 10% formalin solution for stomach content analysis. Stomachs were excised in lab and contents examined using dissecting and compound microscopes. On-site observations of trophic interactions and other behavior were also recorded.

ENVIRONMENTAL QUALITY ANALYSES

Meteorological data, including air temperature, barometric pressure, and rain accumulation, were obtained from the Rogers Automatic Weather Observing / Reporting System (KROG), Rogers, Arkansas, and from Drake Field (KFYV), Fayetteville, Arkansas, at the following World-wide Web URL's:

http://tgsv7.nws.noaa.gov/weather/current/KROG.html

http://tgsv7.nws.noaa.gov/weather/current/KFYV.html

Stage (ft) was read on a USGS gauge *in situ* at the pool at the cave orifice, converted to meters (m), and discharge (m^3/min) was computed from the stage/discharge relationship based upon USGS hydrological data (1997, unpublished data): 35.79 x (m) – 109.99. Because of the difficulty of getting a flow meter into the back of the cave, discharge at the upstream station was determined using the method of Robins and Crawford (1954): average velocity (V) was estimated by choosing a uniform cross-section and channel length, and then timing a floating object (ping-pong ball injected with water) over a 5 meter stretch at ¼, ½, and ¾ intervals on the transect, and averaging the resulting velocities; finally, flow (R) is calculated using the following formula: R = W x D x A x V, where D is depth at midpoint of cross-section, W is the width of the stream channel,

and A is a bottom factor constant (0.8 for rough, 0.9 for smooth). Since the substrate of the study reach is predominately chert cobble, A = 0.8. To estimate the recharge area, the recharge area boundary (delineated by Williams, 1991) was digitized using a digitizing pad, then imported into a geographic information system (ArcView 3.2), and a recharge area of 41 km^2 (4100 ha) was calculated.

Specific conductivity (μSiemens/cm), turbidity (nephlometric turbidity unit), pH, temperature (^{0}C), and dissolved oxygen (mg/l) were measured *in situ* at the cave orifice using a YSI model 85 ™ Dissolved Oxygen Meter, an Orbeco-Hellige Model 966 ™ portable turbidimeter, and a portable pH meter. Water samples were taken at the downstream or the upstream station, and all water samples were held on ice and processed within 48 hours. Samples were analyzed for some or all of the following: total coliform and *Escherichia coli* densities (Most Probable Number/100ml); total viable cell density (cells/ml); nitrate, nitrite, and total kjeldahl nitrogen (mg/l as nitrogen); ortho-phosphate and total phosphate (mg/l as phosphorous); total organic carbon and dissolved organic carbon (mg C/l); sulfate (mg/l); chloride (mg/l); and dissolved metals (mg/l). Analytical procedures followed approved USEPA methods, and appropriate quality assurance and quality control measures were taken. Depending upon the parameter, samples were analyzed by the author at the Department of Biological Sciences (UAF), at the Water Quality Laboratory (Arkansas Water Resources Center, UAF), Central Analytical Laboratory (Center for Excellence in Poultry Science, UAF) or at the Water Chemistry Laboratory (Arkansas Department of Environmental Quality (DEQ), Little Rock, Arkansas). DOC samples were prepared by filtering water samples through pre-

combusted 0.45 μm Whatman ™ GF/C filters, and TOC and DOC samples were put into glass vials with Teflon ™ seals, then acidified (pH < 1) with HCl. TOC and DOC were measured at the Water Quality Lab using a Shimadzu TOC-500 Total Carbon Analyzer. For dissolved metals analyses, water samples were filtered through 0.45 μm Gelman Supor-450 ™ polycarbonate filters into glass vials with Teflon seals and acidified with nitric acid.

For the metals analyses of whole crayfish (*Orconectes punctimanus*), sewage sludge, cave sediments and cave biofilm, the samples were collected in pre-washed glass containers, stored in ice and immediately transferred back to UAF where they were then dried at 60 ^{0}C, pulverized, and analyzed at Central Analytical Laboratory (UAF). A SPECTRO ™ Flame Modula E inductively coupled plasma optical emission spectrometer was used to measure metal concentration. Solid samples were prepared by weighing, ashing, digestion with HCl, and centrifugation. The sample of sewage sludge was collected directly from the belt press at the Springdale Sewage Treatment Plant, Benton County, Arkansas.

STATISITICS

Excel 2000 ™ (Microsoft Co.), SAS 8 for Windows ™ and JMP ™ (S.A.S., Inc.) were used for statistical analyses. Water quality measurements that were below detectible limits were set to zero. A significance level (α) of at most 0.05 was used for all statistical tests. Pairwise correlations were used to explore relationships between water quality parameters. A two-sample t-test was used to determine if mean chemical and bacterial

parameters differed between storm and baseflows and between upstream and downstream stations. To determine if bat guano increased bacterial densities, a paired (one-sample, one-sided) t-test was used. To determine if differences existed among seasons in the mean bacterial densities, negative binomial linear regression was used. Initially, a Poisson regression model was fit to the data with year, season, and their interaction as predictors. However, the deviance statistic was quite large (18,334.85 on 12 df), indicating that the distribution was not Poisson. A negative binomial regression model (Type III) was then fitted to the data, and yielded much lower deviance (23.03) and an overdispersion parameter of 0.32. The interaction term was not significant ($p = 0.7213$), so it was excluded from the model. To determine if a relationship existed between metal concentrations of pollutants (sewage sludge, septic waste, cow manure, poultry litter, guano) and those of cave ecosystem components (sediment, biofilm, isopods, crayfish, cavefish), weighted least squares was used following Pasternack (1962). Metal profiles (antimony, arsenic, beryllium, boron, cadmium, chromium, cobalt, copper, lead, molybdenum, nickel, selenium, vanadium, and zinc), were converted into proportions, and weighted least squares (with the sum of estimates constrained between 0 and 1) was used to determine what fraction of metals in pollution sources contributed to metal profiles of ecosystem components.

RESULTS

ORGANIC MATTER BUDGET

The results of the organic matter budget for CSC are summarized in Table 2. The mean TOC concentration at the cave mouth during the 2-year period was 1.3 mg/l for base flow and 1.7 mg/l for storm flow, ranging from concentrations below detectable limits during low flow (1-3 m^3/min), to a maximum of 5.1 mg/l during record high flow (20 m^3/min, May 1999). A strong, positive correlation ($r^2 = 0.738$, $p < 0.0001$) was detected between TOC and discharge, yet no significant difference was found between mean storm flow and mean base flow TOC concentrations, (t-test: df = 48, t = -0.482, p = 0.316). DOC comprised between 78% and 100% of TOC (with a mean of 95%), but no significant difference exists between TOC and DOC concentrations (paired t-test: df = 6, t = -0.191, p = 0.428). Furthermore, no significant difference was found in TOC or DOC concentrations between the upstream station and the downstream station (paired t-test: df = 3; t = -0.226, p = 0.418). TOC was also correlated to conductivity, turbidity, total phosphorous, nitrate, lead, iron, calcium, aluminum, and manganese (statistical summary in Table 15, Appendix).

Because of the lack of insolation and the lack of any evidence of chemolithotrophy, primary production was assumed negligible. Direct and lateral inputs of organic matter such as leaf fall were also negligible because of the lack of karst windows such as collapsed dolines (sinkholes) or losing streams in this cave complex. From plate capture, the guano deposition rate in CSC was estimated to be 84 mg /m^2/d, or 10.4 g AFDM/m^2/y. The DOM input was estimated to be 4,730 g AFDM/m^2/y. No coarse

particulate matter such as wood or leaves was present, and the only CBOM was guano, which was estimated at 1 g AFDM/m^2. The FBOM of the cave sediment measured 1,209 g AFDM/m^2, and where there was no sediment, the epilithic biofilm contributed approximately 2 g AFDM/m^2. The density of fauna was low in this cave complex (0.2 fish/m^2, 0.5 crayfish/m^2, and 5 isopods/m^2), and the resulting standing crop was less than 1 g AFDM/m^2. The mean oxygen loss rate from benthic respiration was 0.2 mg O_2/l/hr, and the resulting organic matter export was 102 g AFDM/m^2/y. The mean oxygen loss rate from sestonic respiration was also 0.2 mg O_2/l/hr, and the resulting organic matter export was 98 g AFDM/m^2/y, yielding a total heterotrophic respiration output of 200 g AFDM/m^2/y. The mean yearly TOC transport was 2,803 kg C/y, and resulted in a DOM output of 5,330 kg AFDM/y and a POM output of 280 kg AFDM/y. The vast majority of particulate transport occurred during storm events.

Table 2. Summary of organic matter budget for Cave Springs Cave, Arkansas. All masses are expressed as ash-free dry mass.

Inputs	
Gross primary production (g/m^2/y)	0
Lateral leaf movement (g/m^2/y)	0
DOM (g/m^2/y)	4,730
Guano (g/m^2/y)	10.4
Standing Crops	
CBOM (g/m^2)	1
FBOM (g/m^2)	1,212
Wood (g/m^2)	0
Outputs	
Autotrophic respiration (g/m^2/y)	0
Heterotrophic respiration (g/m^2/y)	200
Particulate transport (kg/y)	280
Dissolved transport (kg/y)	5,330

MICROBIAL ANALYSES

A summary of microbial densities during base flow and storm flow is shown in Table 3. All measures of bacterial densities were significantly higher during storm events than during base flows (Table 3). Total coliforms, *E. coli*, and total viable cell densities were positively correlated to each other and all bacterial metrics were positively correlated to discharge (statistical summary in Table 16, Appendix). Total coliform and *E. coli* densities were both positively correlated to TOC, nitrate, TKN, total phosphorous, ortho-phosphate, whereas total viable cell densities were not significantly correlated to any nutrient parameters (Table 16, Appendix).

Table 3. Summary of microbial densities in Cave Springs Cave water samples taken at the cave mouth from November 1997 to December 1999 during base flow and storm flow. Second table shows results of comparison of base flow and storm flow densities of each microbial metric using a two-sample t-test.

	Unit	N	Minimum	Mean	Maximum
Base flow					
Escherichia coli	(MPN/100ml)	21	1	235	3,240
Total Coliforms	(MPN/100ml)	21	53	3,136	10,910
Total Viable Cells	(cells/100ml)	21	20,000	348,100	1,640,000
Storm flow					
Escherichia coli	(MPN/100ml)	42	15	2,337	20,050
Total Coliforms	(MPN/100ml)	42	165	10,790	83,100
Total Viable Cells	(cells/100ml)	30	50,000	2,991,700	32,100,000

	df	t	p
Escherichia coli, base vs. storm	45	2.323	0.012
Total Coliforms, base vs. storm	45	2.914	0.003
Total Viable Cells, base vs. storm	42	1.765	0.042

The negative binomial regression results and parameter estimates are summarized in Table 4. Year explained significant variance in total coliform and *E. coli* densities, although most contrasted years were not consistently and significantly different.

Significant interactions between years may reflect periods of greater and less rain during the study period. Season was significantly related to all three bacterial metrics, and microbial densities were significantly lower in winter (Table 4).

Table 4. Summary of parameter statistics for negative binomial regression of variables year and season and response, mean bacterial density, showing degrees of freedom, chi-square value, and probability value.

Parameter	df	χ^2	p
E. coli			
Year	3	14.14	0.0027
Season	3	23.69	< 0.0001
Total Coliforms			
Year	3	12.36	0.0062
Season	3	35.32	< 0.0001
Total Viable Cells			
Year	3	6.38	0.0945
Season	3	10.30	0.0162

Laboratory simulations of nutrient pulses into the cave ecosystem were measured by peak microbial biomass production and assimilable organic carbon (AOC), and these data are summarized in Table 5. This experiment demonstrated a significant ($n = 17$, $r^2 = 0.85$, $p < 0.0001$) positive relationship between DOC concentration of leachates and peak microbial biomass (μg of microbial carbon) that they produce (Figure 6). One replicate, the 3rd septic leachate incubation (16 mg/l, 7.8 μg C) was excluded because the extraordinarily high microbial biomass measured was not proportional to the concentration of DOC introduced. Laboratory additions of organic matter into cave

water increased microbial densities by up to three orders of magnitude over normal base flow densities supported by cave water DOC alone. Of the organics tested, septic waste produced the highest peak biomass (4×10^8 cells/ml, 7.8 μg C) and leaf litter, the lowest (9×10^7 cells/ml, 0.9), as shown in Figure 7. However, analysis of variance revealed that no significant difference in AOC existed between leaf litter, poultry litter, cow manure, guano, or septic waste (with or without the outlier included).

Table 5. Summary of incubations simulating organic inputs of varying type and dissolved organic carbon (DOC) concentration (mg/l) into cave stream water, the peak microbial biomass grown (μg of microbial carbon), and the resulting assimilable organic carbon (AOC, the peak biomass divided by original DOC concentration).

LEACHATE TYPE	DOC mg C / l	BIOMASS μg C	AOC μg C/mg DOC
Bat Guano	5.6	0.361	0.0644
	19.7	0.620	0.0135
	21.0	0.015	0.0007
Cow Manure	7.1	0.268	0.0377
	28.0	0.205	0.0075
	52.0	0.753	0.0145
Leaf Litter	85.4	1.443	0.0169
	170	0.997	0.0059
	243	1.773	0.0073
Poultry Litter	49.2	1.070	0.0218
	220	2.669	0.0121
	327	3.333	0.0102
Septic Biosolids	3.4	0.027	0.0080
	6.5	0.698	0.1081
	16.0	7.800	0.4887
Cave Water	0.1	0.0004	0.0034
	0.1	0.0005	0.0065
	5.0	0.0171	0.0034

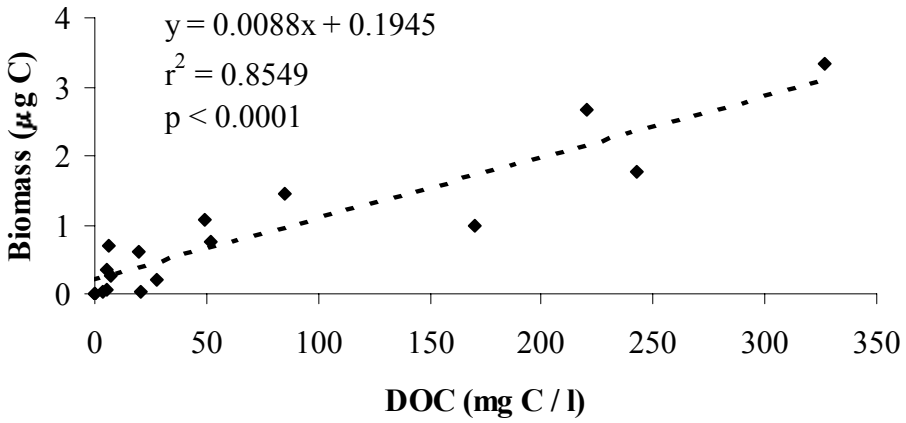

Figure 6. Plot showing significant relationship between dissolved organic carbon (DOC) concentration of leachates and peak microbial biomass (µg of microbial carbon) for the assimilable organic carbon incubations, which simulated nutrient enrichment of the cave ecosystem.

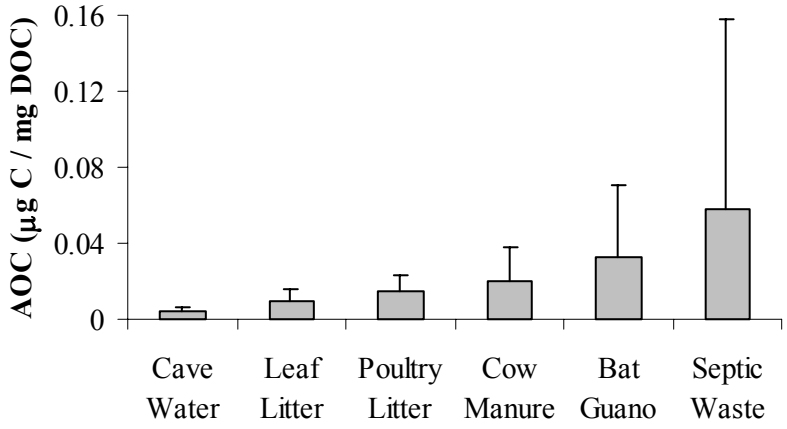

Figure 7. Comparison of the mean assimilable organic carbon values (µg C / mg DOC) for cave stream water and possible organic matter inputs (error bars = 2 SE). Analysis of variance revealed that means were not significantly different.

TROPHIC ANALYSES

Stable Isotope Analyses Results

The results of the SIA are summarized in Table 6 and Figure 8. Some CSC samples have similar isotopic ratios to published data. Sediment samples from a food web study of two Ozark streams in Arkansas yielded $\delta^{13}C$ values of –24.9 and –26.5 $^{o}/_{oo}$ and $\delta^{15}N$ values of +4.5 and +5.1 $^{o}/_{oo}$ (Kwak, 1999), which are within 1 $^{o}/_{oo}$ of the CSC stream-sediment signatures. *Myotis* spp. guano had a mean $\delta^{13}C$ of –24 $^{o}/_{oo}$ and a mean $\delta^{15}N$ value of +7 $^{o}/_{oo}$ (Mitzutani *et al.*, 1992a), and the gray bat guano in CSC was equivalent in $\delta^{13}C$ value, but enriched in ^{15}N by 5 $^{o}/_{oo}$. Sewage-derived organic matter had a mean $\delta^{13}C$ of -23 $^{o}/_{oo}$, but nitrogen isotope ratios were more variable with a mean $\delta^{15}N$ of +5 $^{o}/_{oo}$ (Van Dover *et al.*, 1992; Kwak and Zedler, 1997). Septic system biosolids and sewage sludge analyzed in this study were similar to mean literature carbon and nitrogen isotope values, but sewage sludge was enriched in ^{15}N by 8 $^{o}/_{oo}$ in this study. The $\delta^{13}C$ values of hardwood leaves and their leachates range from –28 to -31 $^{o}/_{oo}$ (Coffin *et al.*, 1989; McArthur and Moorhead, 1996), and the hardwood leaf litter of CSC fell within this range.

My nitrogen and carbon stable isotope analyses suggest that the CSC food web has three distinct trophic levels: a food base of biofilm and benthic detritus, a guild of invertebrate consumers (isopods and crayfish), and a secondary consumer, Ozark cavefish (Figures 9 and 10). An Ozark stream study by Whitledge and Rabeni (1997) reported a mean $\delta^{13}C$ value –28 $^{o}/_{oo}$ and a mean $\delta^{15}N$ value of +6 $^{o}/_{oo}$ for the spot-handed crayfish. In my study, the spot-handed crayfish had a mean $\delta^{13}C$ value –28.4 $^{o}/_{oo}$ and a mean $\delta^{15}N$ value

of +10.6 $^{o}/_{oo}$. These data suggest that the diet of spot-handed crayfish residing in CSC is primarily benthic sediments consisting of leaf litter. However, variability among crayfish samples taken during different seasons suggests seasonal diet shifting (Figure 11). Most crayfish samples (n = 4) appeared to cluster around a mean $\delta^{13}C$ value of –29.3 and a mean $\delta^{15}N$ value of +9.9, and leaf litter is distinctly similar in its carbon ratio, although depleted in nitrogen. Crayfish diet appeared to shift from this probable resource to a diet whose isotope signature is distinctly similar to gray bat guano.

Table 6. Stable isotope ratios of Cave Springs Cave, Arkansas, constituents (limestone, POM, guano, sediment, biofilm, invertebrates, vertebrates) and possible organic inputs (leaf litter, fescue, confined animal waste). Columns are sample size (N) and mean carbon and nitrogen δ values ($^{o}/_{oo}$) with one standard error for samples with replicates. Each sample may contain a composite of several individuals. No nitrogen detected in limestone sample.

Sample Description	N	$\delta^{13}C$	$\delta^{15}N$
Cave Ecosystem Components			
Cave stream limestone cobble	1	2.9	---
Cave stream POM	2	-25.3 +/- 0.2	+6.1 +/- 2.5
Cave stream sediment	2	-25.7 +/- 0.8	+6.8 +/- 0.2
Cave stream biofilm	2	-34.0 +/- 2.1	+6.0 +/- 0.3
Soil in cave recharge zone	1	-27.8 +/- 0.1	-1.0 +/- 0.8
Leaf litter (mixed hardwoods)	1	-29.3	+0.1
Pasture grass (*Festuca arundinacea*)	1	-28.8	+6.3
Cave Fauna			
Cave isopod (*Caecidotea stiladactyla*)	2	-22 +/- 0.1	+13.0 +/- 1.8
Spot-handed crayfish (*Orconectes punctimanus*)	5	-28.4 +/- 0.9	+10.6 +/- 0.9
Ozark cavefish (*Amblyopsis rosae*)	1	-21.8	+17.4
Cave salamander (*Eurycea lucifuga*)	1	-23.1	+8.0
Organic Inputs			
Bat guano (*Myotis grisescens*)	5	-24.4 +/- 0.2	+12.5 +/- 0.9
Septic system biosolids	3	-22.0 +/- 0.6	+4.3 +/- 0.3
Sewage treatment facility biosolids	1	-21.6	+13.6
Poultry litter	1	-15.2	+7.9
Cattle manure	1	-25.1	+3.5

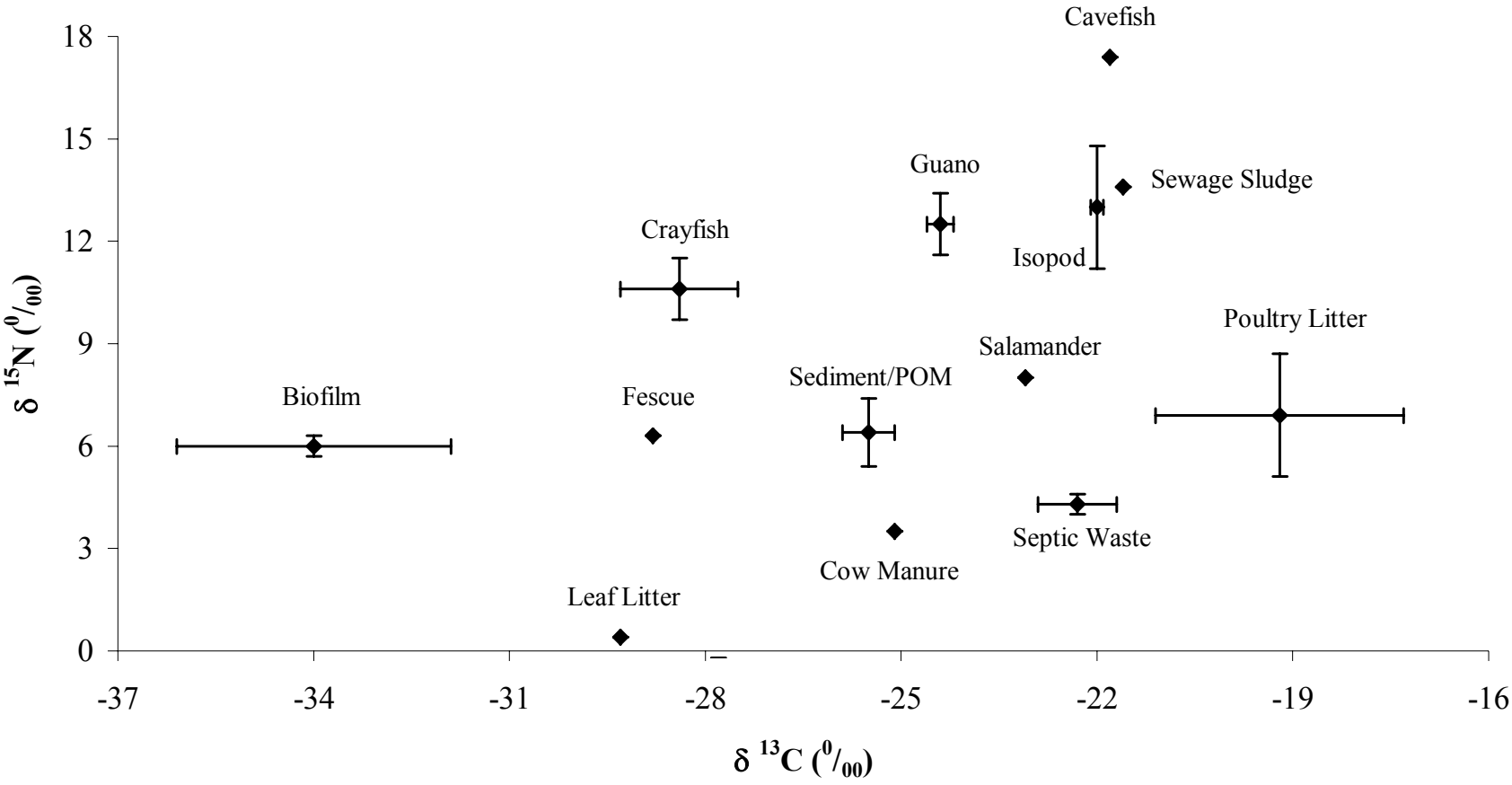

Figure 8. Dual-isotope plot of organic matter inputs (hardwood leaf litter, poultry litter, cow manure, municipal sewage biosolids, septic system biosolids, gray bat guano, fescue) and Cave Springs Cave ecosystem components (cave biofilm, cave sediment/POM, spot-handed crayfish, cave isopod, cave salamander, and Ozark cavefish). Data are mean carbon and nitrogen δ values ($^{o}/_{oo}$), with error bars (+/- 1 SE) for samples with replicates.

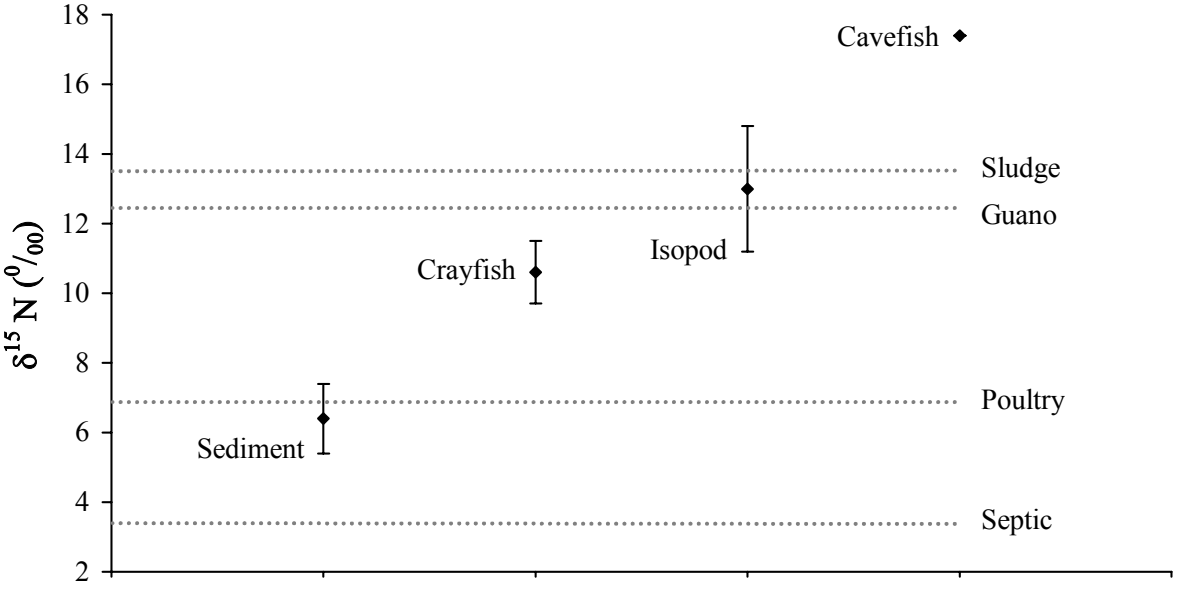

Figure 9. Univariate plot of $\delta^{15}N$ mean values ($^{o}/_{oo}$) +/- 1 SE for Cave Springs Cave sediment/POM and cave fauna (spot-handed crayfish, cave isopod, and Ozark cavefish) relative to those derived for organic matter inputs.

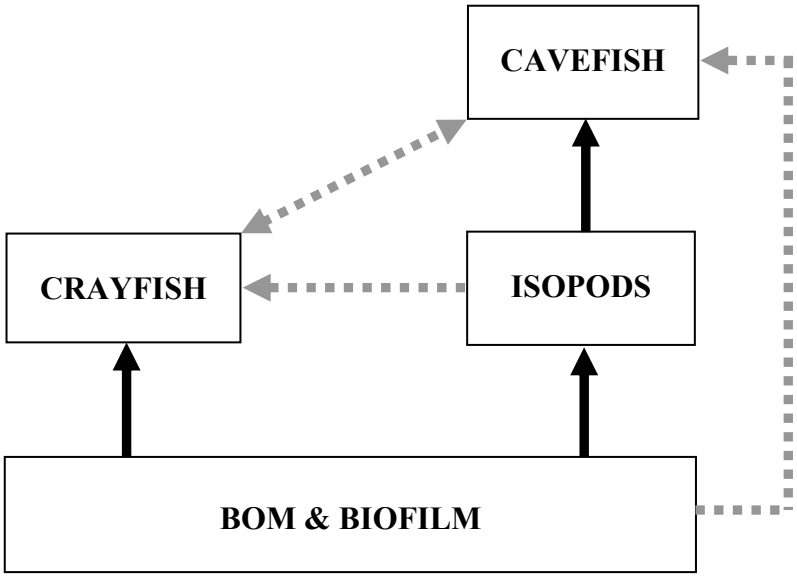

Figure 10. Food web model of Cave Springs Cave showing strong (heavy arrows) and weak (light arrows) trophic transfers. The food web appears to have two distinct food chains (detritus/crayfish and detritus/isopods/cavefish) and three trophic levels (detrital base, invertebrate consumers, and a vertebrate predator). Detrivory, omnivory and ontogenetic diet shifts add undocumented complexity to this food web.

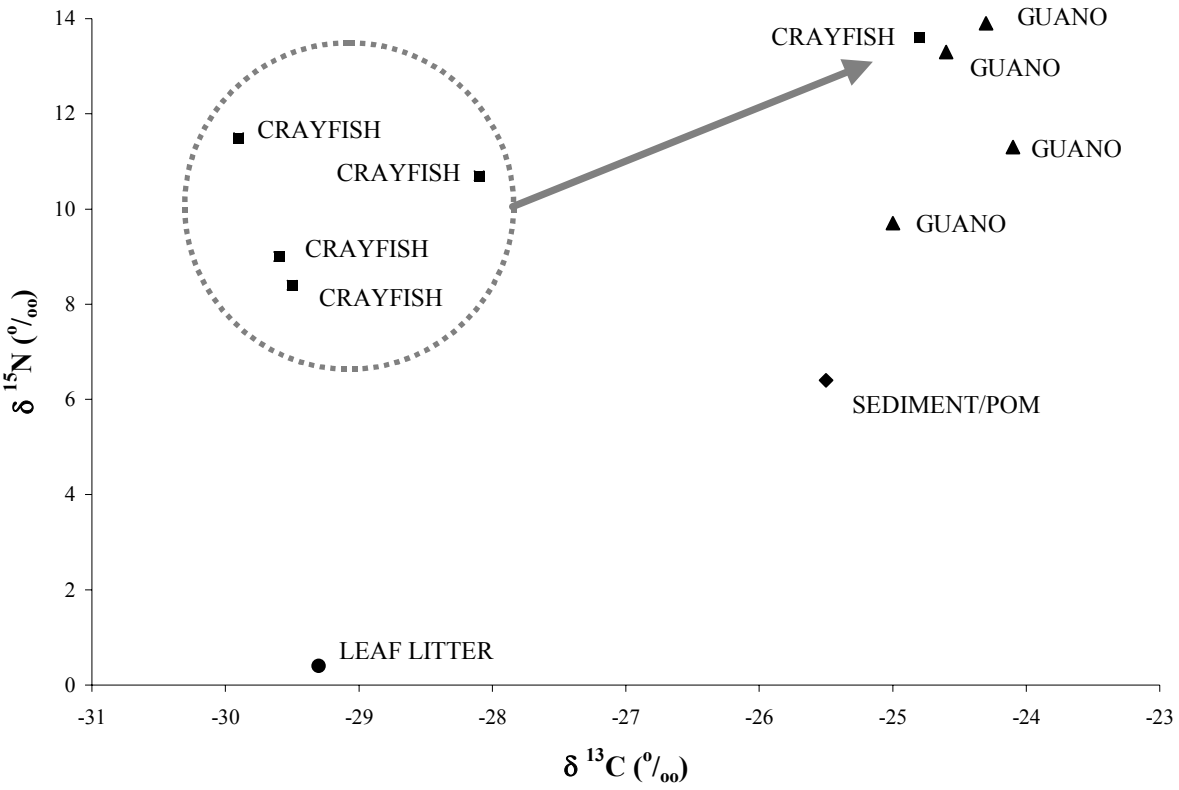

Figure 11. Dual-isotope plot of gray bat guano samples, crayfish samples (each replicate is a composite of spot-handed crayfish individuals), leaf litter, and cave sediment/POM, which illustrates that crayfish diet occasionally shifts from a food resource related to litter detritus (encircled, mean $\delta^{13}C$ value of –29.3 and a mean $\delta^{15}N$ value of +9.9) to a diet of bat guano.

Gut Content Analyses

Identification of stomach contents of the spot-handed crayfish from CSC was complicated by the fact that the average individual length was only 6 cm, and because most contents were pulverized by the gastric mill. The foregut contents that were identifiable included filamentous algae, diatoms, Lepidoptera scales, and short mammalian hair, probably from bats. There was no evidence of ingested vertebrates such as cavefish or salamanders.

ENVIRONMENTAL QUALITY ANALYSES

The complete results of environmental quality analyses in CSC are shown in Tables 9 through 14 in the Appendix. Certain environmental stressors were detected, including excessive microbial densities and nutrient concentrations, a significant trend of increase in specific conductivity and lead and zinc concentrations, the presence of heavy metals in the cave sediment and cave biota, and the presence of one semi-volatile organic compound in crayfish tissue. No organochlorine pesticides were detected in water samples in 1996, in crayfish tissue in 1998, or in cave water, crayfish tissue or bat guano in 1999.

Total coliforms in CSC continued to exceed Arkansas State Water Quality Standards, Regulation 2, (Arkansas Pollution Control and Ecology Commission, 1998) during the study period -- sometimes by a factor of 1,000. During two storm events, total phosphorous concentrations exceeded the Regulation 2 limit of 100 μg P/l, with the highest concentration detected being almost twice the state standard. Substantial concentrations of nitrate were also present (with a yearly average of over 5 mg NO_3-N/l). Chloride, nitrate, ortho-phosphate, and total phosphorous concentrations were positively correlated to discharge. In 1998, di (2-ethylhexyl) phthalate (DEHP) was detected in a crayfish tissue sample at a concentration of 500 ppb. In 1999, eight different phthalate compounds were detected in crayfish tissue, and four phthalates were found in each of a base flow water sample and a guano sample, yet none of these phthalates detected were substantially above the concentrations found in laboratory blanks. While the effects of

these phthalates upon aquatic organisms are unknown, the USEPA considers phthalates to be human carcinogens and hormone disrupters (USEPA, 1998c).

All known water quality data from the literature were reviewed to determine if any trends existed over time, and is summarized in Table 13, Appendix. Regression techniques did not detect a significant trend of increase in nutrients over the last two decades, but sample size was low. However, specific conductivity and lead and zinc concentrations have increased significantly in the past 15 years, as shown in Figure 12. During this current study, heavy metals were found not only in the cave water, but also in sediments and tissues of crayfish, isopods, and cavefish. Furthermore, beryllium, copper, lead, selenium, and zinc were present in concentrations in the cave water that exceeded the Regulation 2 standards for chronic, and sometimes acute, toxicity to aquatic life. Of the dissolved metals screened, aluminum, barium, beryllium, calcium, chloride, copper, iron, magnesium, manganese, and lead concentrations were all significantly correlated to discharge. Constrained least squares analyses of metal profiles (As, Be, B, Cd, Co, Cr, Cu, Mo, Ni, Pb, Sb, Se, V, Zn) of cave ecosystem components and potential pollution sources revealed possible inter-relationships, and the results are shown in Table 7. For example, the cave isopod metal profile could be constructed by taking 33% of the sewage sludge metal profile, 21% of the septic waste profile, and 46% of the cow manure profile, with a goodness of fit (R^2) of 0.928, among other possible combinations.

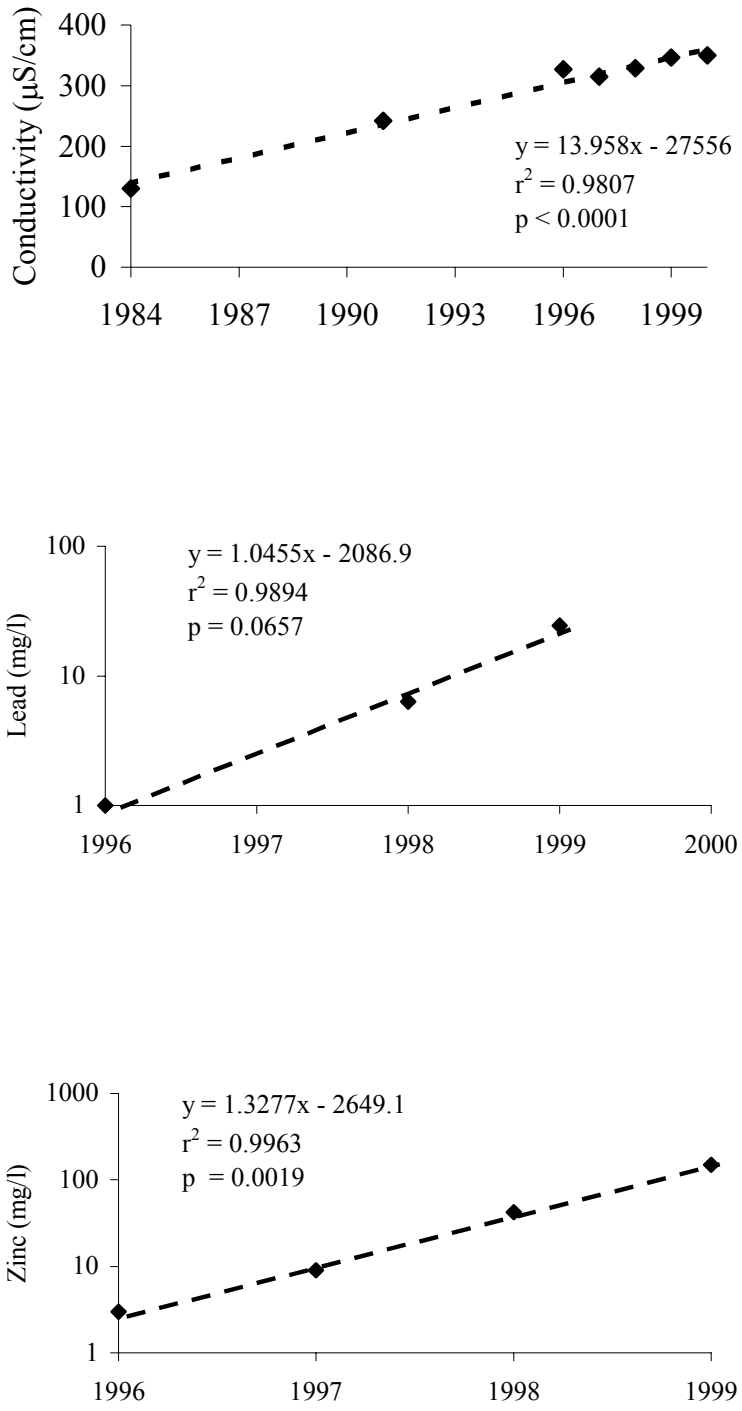

Figure 12. Graphs showing significant increase over time of specific conductivity, lead, and zinc concentrations in stream water during base flow at Cave Springs Cave, Arkansas, using all available data. Note that lead and zinc were not detected in water samples in 1984.

Table 7. Summary of constrained least squares analyses showing fraction of pollution source metal profile (As, Be, B, Cd, Co, Cr, Cu, Mo, Ni, Pb, Sb, Se, V, Zn) that contributed to the cave component metal profile.

	Pollution Sources						
Cave Component	**Sewage Sludge**	**Septic Waste**	**Cattle Manure**	**Poultry Litter**	**Bat Guano**	**Total**	**R^2**
Cave Sediment #1	0.630	0.370	0	0	0	1	0.916
Cave Sediment #2	0.142	0.734	0.125	0	0	1	0.981
Cave Biofilm #1	0.556	0.434	0	0	0	1	0.935
Cave Biofilm #2	0.638	0.296	0	0	0	0.934	0.872
Cave Isopod	0.331	0.213	0.456	0	0	1	0.978
Crayfish #1	0.185	0	0.156	0.415	0.245	1	0.997
Crayfish #2	0	0	0	0.358	0.642	1	0.998
Cavefish	0	0	1	0	0	1	0.943

DISCUSSION

ORGANIC MATTER DYNAMICS

The organic matter budget of CSC is not balanced (in surplus), probably because of unaccounted lateral ground-water fluxes. It is difficult to balance the hydrological budget in karst systems because caves and springs focus ground water from the recharge basin through immeasurable flow paths. Although TOC and discharge were highly correlated, no statistical difference was found between mean storm flow TOC and base flow TOC concentrations. However, most surface streams have a considerably higher concentration of DOC during high discharge, which is normally attributed to the greater importance of surface and shallow subsurface flow paths (Mulholland, 1997).

The organic carbon dynamics of CSC were compared to a study of Logan Cave (Brown *et al.*, 1994), and many similarities exist (see data summary in Table 8 and Figure 13). DOC accounted for the vast majority of carbon inputs in both cave complexes, and guano inputs were similar for both caves. Even though Logan Cave differs by having a collapsed doline that allows lateral inputs of organic matter, these inputs were insignificant compared to the DOC input. However, this sinkhole did contribute a standing crop of wood that was absent in CSC.

Although Logan Cave has a discharge two and a half times greater than that of CSC, the caves

had similar carbon fluxes when adjusted for discharge (*i.e.*, having equal mean DOC concentrations of 1 mg/l). As in CSC, DOC concentrations in Logan Cave did not differ

significantly between sampling stations or between base flows and storm flows.

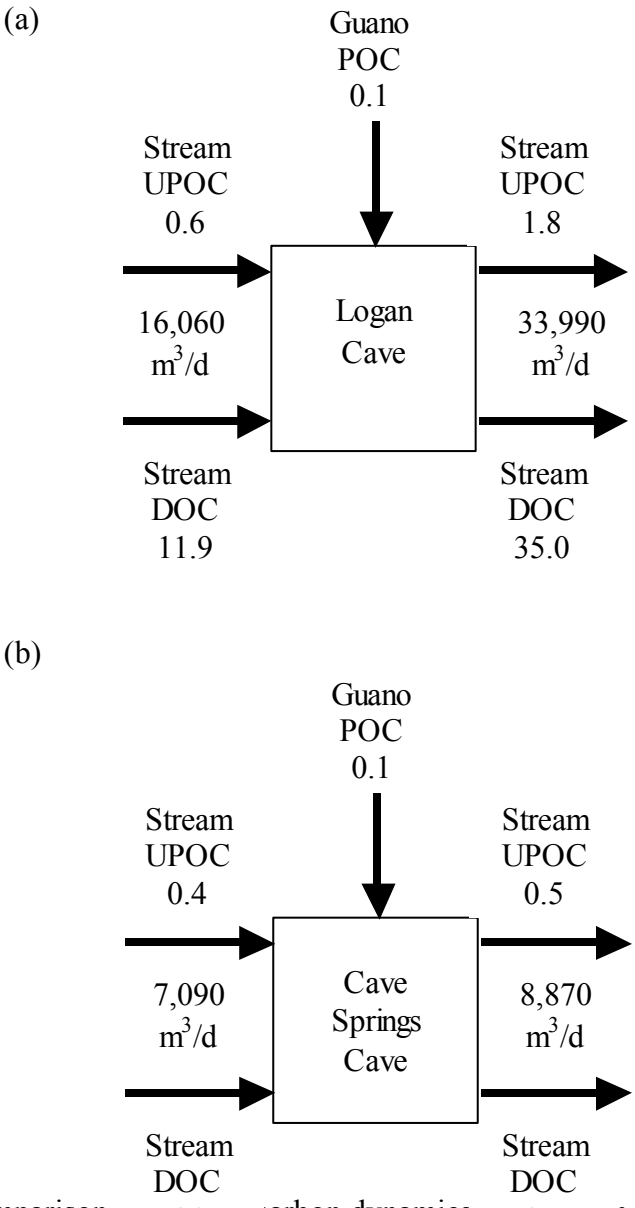

Figure 13. Comparison carbon dynamics n Cave stream and (b) Cave Springs Cave stream during the summer season. Components include discharge (m^3/d), and loading rates (kg/d) of dissolved organic carbon, ultra-fine particulate (between 0.45 μm and 0.5 mm) organic carbon, and bat guano coarse particulate organic carbon. Data for Logan Cave are from Brown *et al*. (1994).

Table 8. Comparison of organic matter budgets of CSC, Logan Cave (Brown *et al.*, 1994), and 34 surface streams from around the world (Webster and Meyer, 1997b). All masses are expressed as ash-free dry mass.

	Cave Springs	Logan Cave	Summary of 34 Surface Streams		
Physical Characteristics			**Minimum**	**Mean**	**Maximum**
Latitude	36	36	18	44	78
Stream Order	1	1	1	3	9
Watershead area (ha)	3770	3015	8	87,843	1,987,100
Streambed area (m^2)	900	2500	150	632,900	13,300,000
Gradient (m/km)	3.7	4.0	0.2	87.6	450.0
Mean annual water temp. (C)	14	14	1	9	22
Mean annual discharge (l/s)	100	250	2	17,765	466,100
Mean annual precipitation (cm)	114	114	10	139	438
Mean stream width (m)	3.6	2.7	0.3	26.1	400.0
Inputs					
Gross primary production ($g/m^2/y$)	0	0	0	542	5,400
Leaffall ($g/m^2/y$)	0	0	0	372	843
Lateral movement ($g/m^2/y$)	0	56	0	210	3,520
DOM input ($g/m^2/y$)	4,730	3,520	0	1,540	36,040
Guano deposition ($g/m^2/y$)	10.4	9.1	---	---	---
Standing Crops					
CBOM (g/m^2)	1	1	3	653	5,117
FBOM (g/m^2)	1,212	632	0	350	1,903
Wood (g/m^2)	0	25	0	5,286	28,993
Outputs					
Autotrophic respiration ($g/m^2/y$)	0	0	0	289	2,700
Heterotrophic respiration ($g/m^2/y$)	200	200	21	522	2,664
Particulate transport (kg/y)	280	1,357	37	679,942	17,800,000
Dissolved transport (kg/y)	5,330	25,550	50	7,470,260	170,000,000

Organic matter budgets for CSC and Logan cave streams were compared to a summary of 34 surface streams from North America, Australia, Antarctica, and Europe (data from Webster and Meyer, 1997b), and those data are summarized in Table 8. The relationship among the physical characteristics of these two cave streams (watershed size, mean annual discharge, stream order, and annual precipitation) is similar to the regression models of these 34 streams comparing the same physical characteristics (Webster and D'Angelo, 1997). These cave streams are markedly different than surface streams by the absence of autotrophic production and respiration, the reduction or elimination of leaf and wood inputs and standing crops, and the contribution of bat guano. Low BOM standing crops in Cave Springs and Logan Caves may be explained by the fact that BOM storage is directly related to the input rate of leaf litter into streams (Jones, 1997), and BOM storage is inversely proportional to mean annual stream temperature (Sinsabaugh, 1997). Benthic respiration rates in these cave streams were low, but benthic respiration has been found to be directly proportional to mean annual stream temperature, with a corresponding Q_{10} value of 8.9 (Sinsabaugh, 1997). In general, water temperature is one of the most important variables influencing organic processes in stream ecosystems (Webster and Meyer, 1997).

Several indices of stream ecosystem function were calculated for these two caves as per Webster and Meyer (1997b). One measure is ecosystem efficiency (Fisher and Likens, 1973), which is calculated as total respiration divided by inputs. CSC has an ecosystem efficiency of only 4%, and Logan Cave, only 6%. Ecosystem efficiencies for the 34-river study ranged from 1 to 473% with a median of 36% (Webster and Meyer, 1997b).

Nutrient spiraling, the coupling of nutrient cycling and downstream transport, can be quantified as spiral length, which is composed of uptake length (average distance a molecule travels in the dissolved, inorganic form before being utilized biologically) and turnover length (average distance a molecule travels in the organic form before being mineralized) (Webster and Meyer, 1997b). Whereas uptake length is difficult to measure, turnover length is quantified by dividing the downstream flux of organic matter per unit stream width by total stream respiration (Newbold *et al.*, 1982). CSC has a calculated turnover length of 8 km, and Logan Cave, 50 km. Turnover length in the 34-river study ranged from 0.3 to 43,700 km (Webster and Meyer, 1997b), and greater turnover lengths imply lesser efficiencies. Neither cave complex is as long as its turnover length, implying that nutrient cycles are not completed before nutrients are exported out of these cave ecosystems (Newbold *et al*., 1982). Benthic organic matter biological turnover time is measured as BOM divided by total inputs (Webster and Meyer, 1997b) or BOM divided by heterotrophic respiration (Sinsabaugh, 1997). By the method of Webster and Meyer (1997b), CSC has a BOM turnover time of 13 weeks, and Logan Cave, 9 weeks, and the turnover time for the 34-river study ranged from 2 weeks to 33 years. By the method of Sinsabaugh (1997), CSC has a BOM turnover time of 6 years, and Logan Cave, 3 years, and the 34-river study ranged from 0.1 to 425 years. These three metrics emphasize different aspects of energy flow in these cave streams. The cave community (benthic and sestonic) appears to process new inputs relatively quickly (months), but appears to processes the BOM standing crop relatively slowly (years). Furthermore, these cave ecosystems appear to be energetically inefficient in the

transformation and retention of nutrient inputs, with a substantial portion of the organics being transported downstream and out of the cave complexes.

A biomass pyramid of Cave Springs Cave, based on empirical estimates, was constructed to describe the relative proportions of the food base (allochthonous organics), primary consumers (invertebrates), and secondary consumers (cavefish), and is shown in Figure 14. The extremely large 1st tier of the biomass pyramid implies a food base of low nutritional quality. Deposit feeders that feed upon refractory POM mixed with sediments must 'bulk feed', processing one to many times their body mass in BOM per day with low assimilation efficiencies (Golladay *et al.*, 1983; Allan, 1995). Because of these low assimilation efficiencies, much of the organic material that accumulates in streams is exported (Golladay, 1997), and this is apparently the case in CSC.

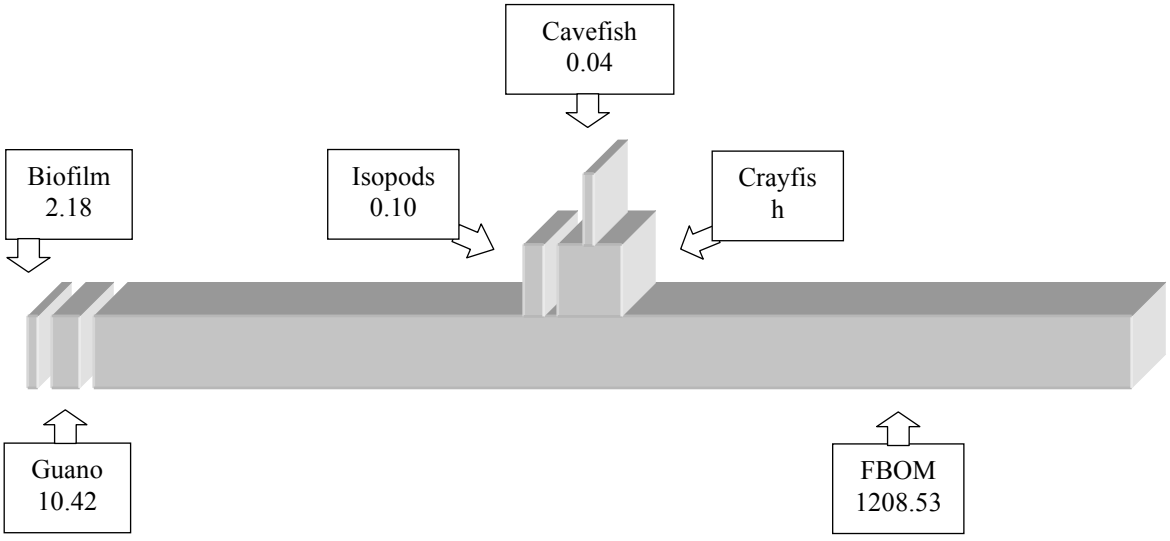

Figure 14. Pyramid of biomass (g AFDM/m^2) for the Cave Springs Cave community based upon bioinventories and the organic matter budget estimates in Table 7.

Converting the CSC organic matter budget into an energy budget, the energy flow in this subterranean ecosystem was compared to the surface ecosystem above it, as shown in

Figure 15. On an area basis (kcal/m^2/y), CSC receives approximately 2% of the energy that the surface above the cave receives. On an ecosystem-wide basis (kcal/y), the CSC complex receives about 0.0003% of the energy that the CSC recharge zone receives. These approximate calculations demonstrate that this cave ecosystem has drastically reduced energy input compared to ecosystems that receive sunlight, and coupled with its low, overall ecosystem efficiency and nutrient turnover, indicate a severe limitation upon the biota that can be sustained by this ecosystem.

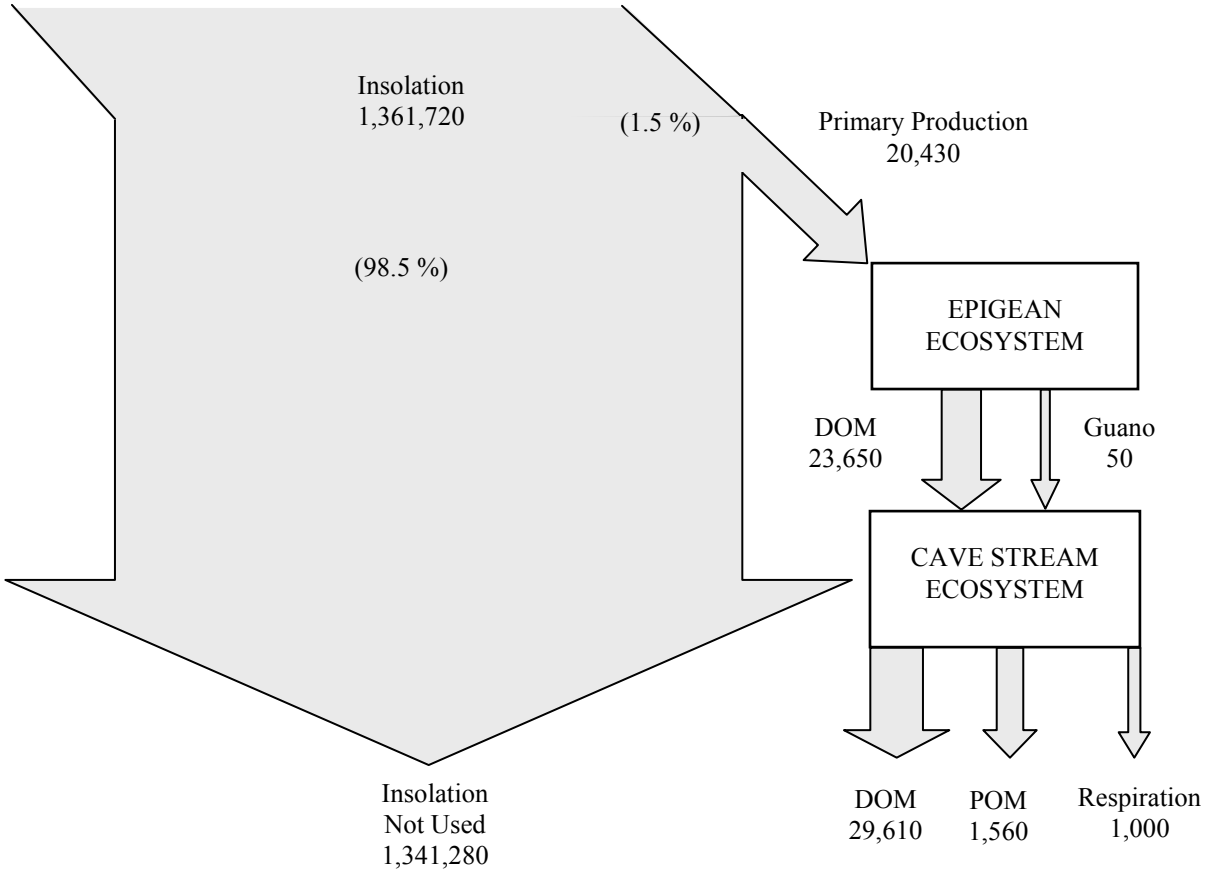

Figure 15. An energy flow diagram of Cave Springs Cave, where energy flow and storage is expressed as kcal/m^2/y. For the energy budget, a conversion factor of 5 kcal/g AFDM was used. A photosynthetic efficiency of 1.5 % for surface vegetation primary productivity was assumed. Average annual total horizontal insulation for northwest Arkansas calculated from Turner and Battle (1980).

MICROBIAL DYNAMICS

Data collected from two years of monitoring in CSC indicate the importance of surface anthropogenic inputs upon the microbial dynamics of this cave ecosystem. Much of the microbial transport in CSC appears to occur during storm events -- the mean annual storm flow microbial biomass (60 pg C) was 8 times greater than base flow biomass (7 pg C). Guano does not appear to be the primary source of microbes because no significant difference was detected between mean bacterial counts at the upstream station (upstream of bat roosts) and the downstream station (downstream of bat roosts). Total coliform densities were significantly correlated with turbidity, ortho-phosphate, nitrate, and stream discharge in this system. Such correlations strengthen the conclusion that fecal coliforms originate from the surface and are being flushed in during storm events.

Furthermore, bacterial densities (total coliforms, *E. coli*, and total viable cells) show a significant and distinct seasonal pattern, with winter/early spring having the lowest densities and summer/early autumn having the highest densities (Figure 16). Pasquarell and Boyer (1995) noted a distinct pattern in fecal coliform densities over a 2-year study of ground water in the karst area of Greenbrier, West Virginia, which was impacted by cattle-grazing: a recession period beginning in August and continuing until mid-November, where coliform densities decreased; then a recovery period lasting until mid/late winter where coliform densities increased; and then a level period from mid/late winter to spring/summer where coliform densities remained constant. This pattern was attributed to seasonal changes in hydrology *(i.e.*, summer drought and winter recharge), but not to timing of when cattle were grazing. A direct correlation was found between

the mean nitrate concentration in these springs and the percent of their recharge zones that were under agricultural use (Boyer and Pasquarell, 1995). However, the median fecal coliform densities for these spring sites were not correlated to the percent land use in agriculture within the corresponding basins (Pasquarell and Boyer, 1995). Pasquarell and Boyer (1995) evoked four factors to explain the seasonal variation of fecal coliform densities: presence/absence of cattle, amount of soil water available to transport bacteria to ground water, storage of bacteria in the soil zone, and the rate of bacterial die-off in ground water. These factors have not yet been studied as they relate to Ozark cave ecosystems, and such research is greatly needed for the protection and management of ground-water habitats.

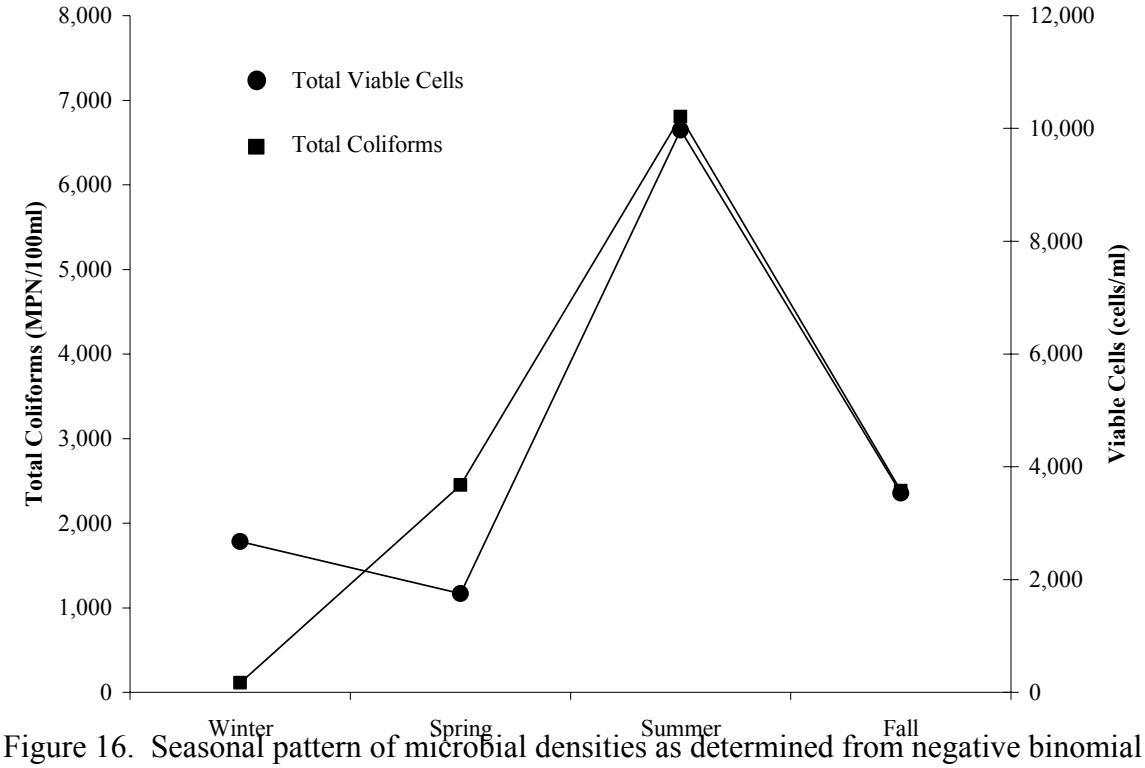

Figure 16. Seasonal pattern of microbial densities as determined from negative binomial regression. Total coliform and total viable cells densities were lowest in winter and highest in summer.

The significant relationship between DOC and bacterial biomass in laboratory incubations simulating nutrient pulses confirms the intuitive conclusion that increases in

DOC concentrations lead to corresponding increases in microbial biomass. Water quality monitoring in CSC confirmed that TOC is significantly correlated with total coliforms and, specifically, *E. coli*. However, water quality studies in Logan Cave, Benton County, revealed no relationship between coliform densities and total organic carbon concentrations (Means and Johnson, 1995; Brown, 1996). Surprisingly, my laboratory experiments revealed no significant difference between organic matter types. Apparently, the quantity of organics available to stream microbes is more important than the quality of organics. Furthermore, peak biomasses (and resulting AOC values) were low compared to other studies. Leff and Meyer (1991), for example, reported AOC values an order of magnitude higher than this study for native bacteria grown *in vitro* on ample nutrients and three orders of magnitude higher for the bacterial community in the Ogeechee River, a blackwater river in Georgia. The low AOC values reported for CSC suggest that factors besides nutrient concentrations limit the bacterial biomass in CSC. One explanation may be that the indigenous bacterial community of CSC is adapted to oligotrophy, because nutrient-limited bacteria have slow population growth rates (Neidhardt *et al.*, 1990). Slow growth rates may also be explained by the relatively low mean annual water temperature of CSC (14.4 ^{o}C), because lower temperatures slow enzymatic action and thus, metabolism (the Q_{10} effect). In general, microbiological activities in subsurface habitats proceed at rates that are orders of magnitude slower than those in surface habitats (Kieft and Phelps, 1997). These experiments suggest that microbial densities will increase if nutrient input into CSC increases, or if temperature increases (*i.e.*, climate change).

TROPHIC DYNAMICS

Omnivory adds to the stability of simple food webs (Holyoak and Sachdev, 1998), but omnivory also obscures discrete trophic level transfers (Polis and Strong, 1996). In a food web study of Movile Cave, Romania, a chemoautotrophic cave ecosystem, variation from the expected isotope ratios was explained by generalist feeding behavior or by the incorporation of ^{13}C depleted invertebrate chitin by predators (Sarbu *et al.*, 1996). Although omnivory is common in cave ecosystems, my nitrogen and carbon stable isotope analyses suggest that the CSC food web has three distinct trophic levels (Figures 9 and 10). The SIA/food web study of Movile Cave revealed three trophic levels as well, with producers (chemoautotrophic microbial mats), invertebrate grazers, and invertebrate predators (Sarbu *et al.*, 1996). A trophic study of an anchialine cave ecosystem (Mexico) reported 3 to 3.5 trophic levels, with producers (algae, bacteria, detritus), invertebrate consumers, and invertebrate predators/scavengers (Pohlman *et al.*, 1997). Pohlman *et al.* (1997) also concluded that soil POM and algal POM supplied the majority of organic matter to the food web, and that benthic POM equaled soil POM in the cave.

Of note is the high nitrogen stable isotope ratio of the Ozark cavefish ($\delta^{15}N = 17.4\ ^{o}/_{oo}$), which suggests that Ozark cavefish is the top predator in this food web (Figure 9). This high ratio could also be explained by poor body condition, because starvation produces tissues enriched in ^{15}N (Hobson *et al.*, 1993). Hobson *et al.* (1993) explained that as lean body mass in birds decreased, starving animals showed a progressive increase in the $^{15}N/^{14}N$ ratio of their tissues as "lighter" nitrogen is excreted. It is well known that cavefish are food limited, with females having an average reproductions per lifetime ratio of only 0.6 (Culver, 1982). Another explanation for a high nitrogen isotope ratio

resulting from nitrogen recycling is cannibalism (Poulson, 1963). Stable isotope results from my study also suggest that the primary diet of Ozark cavefish is cave isopods, a prey item reported by several researchers. Eigenmann (1909) reported Ozark cavefish gut contents of juvenile crayfish, juvenile cavefish, crickets, and numerous isopods. Gut content analyses of 21 Ozark cavefish from Cave Springs Cave revealed 183 whole copepods (2 spp.), 11 isopods, 9 gammarids, 3 cladocerans, 2 crayfish, 2 dytiscid larvae, 1 plecopteran nymph and considerable insect chitin from bat guano (Poulson, 1961). Stomach analyses of cavefishes by Poulson (1963) indicated that copepods made up 70-90% v/v of the diet, with the remainder being primarily small salamanders and crayfish, isopods, amphipods, and young of their own species. The isotopic ratios of cave isopods suggest that organic matter sources other than guano constitute their diet -- septic system waste and sewage sludge are likely because of their proximity to isopods in the dual isotope crossplot (Figure 8).

Carbon and nitrogen stable isotope analyses of spot-handed crayfish samples suggests that the diet of crayfish residing in the cave is primarily benthic sediments. Whitledge and Rabeni (1997) did a comprehensive trophic study of the golden (*Orconectes luteus*) and spot-handed crayfishes in an Ozark stream (Missouri) using stable isotope analyses and gut content analyses. According to their gut content analyses, 89% of the diet of spot-handed crayfish was detritus, while less than 5% was animal matter. Even after correction for differential assimilation of dietary components, terrestrial plant detritus made up 63% of the food resource of this crayfish. While restricted in organic matter content, subterranean benthic sediments contain some bacteria, fungi, and actinomycetes,

which are an important food source for cave crustaceans (Dickson, 1975). A stable isotope study of a thermomineral cave ecosystem in Italy with chemoautotrophic microbial mats revealed that bacterial contribution to the food chain varied among species from 0 to 100% (Southward *et al.*, 1996). However, it is thought that the detritus itself must furnish a substantial portion of the energetic needs of detrivores because microbial biomass, while of high quality, is not a substantial portion of the stream detritus (see review by Allan, 1995). Cave sediment or benthos in CSC appears to derive from stream POM because of the similarity in both nitrogen and carbon isotopic signatures (a difference in means of $\delta^{13}C = 0.2\ ^{o}/_{oo}$, $\delta^{15}N = 0.7\ ^{o}/_{oo}$). In general, POM is an important food source for interstitial fauna (Mathieu *et al.*, 1991). The source of organic matter that makes up stream POM in CSC was not obvious from isotopic analyses, but leaf litter, septic waste, and cow manure were possible sources. As with isopods, crayfish do not appear to be relying exclusively upon the guano resource. Brown (1996) found no difference in total organic carbon concentration of the water below and above bat colonies in Logan Cave, suggesting that guano DOM was not a significant input into the food web even though thousands of bats occupy the cave in summer months. However, my isotopic analyses suggest that myotis guano cannot be ruled out as a food source for the Ozark cavefish, which is known to ingest guano (Poulson, 1961). Also of note is that the mean $\delta^{13}C$ value for bat guano in this study was $-24.5\ ^{o}/_{oo}$, implying that the contribution of C_3 plants to the food chain leading to bats is larger than that of C_4 plants. Mitzutani *et al.* (1992b) reached a similar conclusion in their study of cores of guano of Mexican free-tailed bats (*Tadarida brasiliensis*).

Depleted carbon isotope ratios (mean $\delta^{13}C = 34.0\ ^{o}/_{oo}\ ^{+}/_{-}\ 2.1$) were found in the CSC biofilm and may indicate fractionation by microbial processes. The $\delta^{13}C$ values for POM in an anchialine cave system (Blue Hole Cenote, Mexico) averaged $-35.3^{o}/_{oo}$, and the assimilation of isotopically light biogenic CO_2 by photosynthesizers was evoked (Pohlman *et al*, 1997). Kelley *et al.* (1998) found depleted carbon isotope values ($-33^{o}/_{oo}$) for bacteria in the Gulf of Mexico, and concluded that neither phytoplankton production nor terrestrial organic matter could account for the values -- other sources included the incorporation of carbon derived from light hydrocarbons of seep areas and the chemoautotrophic processes of methane oxidation and nitrification. In freshwater wetlands, for example, carbon isotope ratios of methane from anaerobic processes (methanogenesis) range from -65 to $-55\ ^{o}/_{oo}$ (Boutton, 1991). Yet, no anaerobic processes or methane sources are evident in the CSC ecosystem. Macko and Estep (1984) studied the microbial alteration of stable isotope compositions of various organic matter substrates, and found that large fractionations of the substrates occurred when one microbial population dominated or one particular compound in the substrate dominated. Fractionation of carbon in the organic substrate ranged from -5.5 to $+11.1\ ^{o}/_{oo}$, and nitrogen fractionation ranged from -12.9 to $+22.3\ ^{o}/_{oo}$. However, Macko and Estep (1984) posited that in natural environments with heterogeneous substrates and microbial populations, these large fractionations could cancel each other and not be detected. Thus, it is possible that the microbial biofilm in CSC is dominated by a particular population or that the DOM substrate is dominated by a particular compound.

POLLUTION EFFECTS

Environmental quality monitoring indicates that the CSC stream ecosystem has become contaminated with excess nutrients, fecal bacteria, and toxic metals. The mean base flow concentrations of nitrate and ortho-phosphate are greater than background concentrations for the nation's ground water (USGS, 1999), and several storm flow samples detected total phosphorous levels well above the State limit. Lake Keith, the receiving body for the CSC resurgence, is eutrophied, with copious algae covering all submerged surfaces and a reoccurring nuisance odor of decaying algae coming from the lake. A study conducted by the Arkansas Department of Pollution Control and Ecology (1984) determined that the Springdale Sewage Treatment Plant's discharges were nutrifying Osage Creek and compromising the water quality, impacting the invertebrate community, and degrading the entire ecosystem of Spring Creek. Since that study, remedial measures were taken and Williams (1991) reported improved water quality in Spring Creek. The data from my study and other monitoring efforts indicate that not only the Cave Springs Cave stream, but the entire Osage Creek drainage basin, is experiencing pollution enrichment and degradations in water quality. Because of the lack of sunlight in the cave ecosystem, the growth of aquatic vegetation is not an issue. Nevertheless, organic pollution has negative effects on cave ecosystems, including the alteration of the community assemblage, the impoverishment of biodiversity, and the increased risk of predation by surface animals. During the course of this study, we have not seen either cave amphipod species (*Stygobromus onondagaensis* and *S. ozarkensis*), in Cave Springs Cave, where they were formerly reported. Septic pollution has eradicated the invertebrates from other Ozark caves (*e.g.*, Aley, 1976). However, stygobitic isopods are

abundant in Cave Springs Cave, which parallels the findings of Simon and Buikema (1997) in a cave system polluted with septic waste (Banners Corner Cave, Virginia). Simon and Buikema (1997) found that stygobitic isopods (*Caecidotea recurvata*) could use sewage-fed bacteria as a food source and that population densities were higher in cave pools with moderate sewage enrichment, while amphipods (*Stygobromus mackini*) were very sensitive to sewage pollution and were absent from Banners Corner Cave. The similar dearth of amphipods and abundance of isopods and organic pollution in CSC may implicate the many septic systems in the recharge zone of this cave stream in the alteration of the community assemblage. The impact of nutrification upon oligotrophic, subterranean ecosystems is not well documented, however. Stewart (1984), in recommending listing the Ozark cavefish as 'threatened" in the Federal Register, suggested that land-applied animal waste was not a threat to the amblyopsid habitat, but may in fact augment the food supply. Sket (1977) reported that stygobites were abundant in a cave stream enriched with organic pollution, and suggested that moderate organic pollution may benefit stygobites in oligotrophic habitats provided that epigean fauna do not invade (1999). Further research is needed to determine the biological ramifications of augmenting the cave stream trophic web with agricultural or septic wastes.

Lead and zinc concentrations in water at the CSC resurgence have increased exponentially since the 1980's. Furthermore, cave isopods, crayfish, cavefish, and sediment had significantly higher concentrations of heavy metals than in water samples. However, there was no statistically significant trend of increasing metal concentration in the biota with increasing trophic level. These data suggest that metals are accumulating

in cave sediments and the tissues of cave organisms, but not necessarily biomagnifying in the food web. Constrained least-squares analyses of metal profiles suggest that anthropogenic inputs, especially human and animal waste, contribute substantially to the metals contamination of this ecosystem. This contamination of CSC is of concern because some of the heavy metals are present in concentrations of acute toxicity to aquatic organisms. Even heavy metals in low concentrations may be bioaccumulated to lethal concentrations because of the longevity of cave-adapted organisms (Dickson *et al*., 1979), and this study detected metals in the tissues of cave-adapted isopods and Ozark cavefish.

CONCLUSION

SUMMARY OF FINDINGS

Organic Matter Dynamics

- The CSC stream ecosystem is oligotrophic, with a mean annual total organic carbon concentration of only 1 mg/l. This cave habitat receives less than 2% of the energy input that the surface habitat above it receives, on an area basis.

- Total organic carbon does not significantly increase downstream of bat roosts or during storm events.

- The majority of the organic matter flux is in the dissolved form (4,730 $g/m^2/y$ input and 5,330 $g/m^2/y$ output), with DOC comprising about 95% of TOC.

- Gray bat guano (10.4 g $AFDM/m^2/y$) represents less than 1% of the organic matter input into CSC. The reduction of the current gray bat population (3,000 bats) by 90% of its historic abundance may have lessened the guano input, and thus its significance in the organic matter dynamics of this ecosystem.

- Fine benthic organic matter is the major standing crop of organic matter. The bulk of the benthic organic matter resource appears to be refractory, with a proportionally large mass of organic matter (1,212 g $AFDM/m^2$) supporting a very small biotic community (1 g $AFDM/m^2$). Stable isotope analyses suggest that POM in transport is the source of benthic sediments, which emphasizes the importance of allochthonous inputs.

- The respiration rate of the cave benthos is approximately equal to the respiration rate of the cave seston (*circa* 100 g $AFDM/m^2/y$), and the total community respiration rate less than 50% of the surface stream average.

- CSC has an ecosystem efficiency of only 4%, a BOM turnover length of 8 km, and a BOM turnover time of 6 years, indicating that much of the organic matter input is exported without being utilized.

- The organic matter budget and fluxes of CSC are similar to another Ozark cave (Logan Cave), suggesting that these findings may have wider applicability for caves in the Ozark Plateaus Province.

Microbial Dynamics

- Gray bat guano is not the probable origin of the majority of the yearly import of coliform bacteria.

- Microbial densities were significantly higher during storm flows and during the late summer/early autumn season.

- No significant difference was found in the growth response of indigenous bacteria to different nutrient pollutants (septic waste, sewage sludge, cow manure, poultry litter, and bat guano), suggesting that the quantity of organic matter available to the microbial community is more important than the quality (or type) of organic matter.

- The cave microbial community appears to be nutrient-limited, with growth rates and peak biomasses less than surface stream communities. It is possible that the cave microbial community may be adapted to oligotrophy (and thus, not adapted for efficient use of nutrients at higher concentrations).

- Addition of nutrients into the CSC ecosystem is expected to increase microbial activity and biomass.

Trophic Dynamics and Pollution Effects

- There appear to be three trophic levels in CSC: a detrital food base of dissolved organics, guano and benthic sediments, a guild of invertebrate consumers, and a secondary consumer.

- Cavefish are the top predator, and cavefish diet consisted mainly of isopods, and secondarily guano. Cavefish body condition may be poor, indicating starvation, which is documented in the literature.

- Stable isotope analyses suggest that crayfish diet consists mainly of benthic detritus and guano. Guano is an important food resource seasonally for the crayfish food chain, but not necessarily for the cavefish food chain. Isopods do not appear to be utilizing guano, but other imported organics, probably septic and sewage sludge.

- Nutrient pollutants appear to be augmenting the trophic base of CSC.

- Nutrients and heavy metals are the major pollutants in this ecosystem. Concentrations of total phosphorous, nitrate, and heavy metals (Be, Cu, Pb, Se, Zn) and total coliform densities have repeatedly exceeded state and federal water quality criteria. Concentrations of nutrients and metals were greater than regional and national background levels.

- Lead and zinc concentrations and specific conductivity have increased significantly in the last 15 years.

- Metals have accumulated in the cave sediments and in the tissues of cave biota. Metal profiles of pollutant sources (human and animal wastes) showed a significant relationship to metal profiles of cave ecosystem components (sediment, isopods, cavefish, *et cetera*), suggesting that surface pollutants may be the origin of these heavy metals.

- A possible consequence of nutrient pollution is the disappearance (or extirpation) of the Ozark cave amphipod from this habitat, because other studies have documented the cave amphipods' sensitivity to nutrient pollution.

RECOMMENDATIONS AND FUTURE DIRECTIONS

Environmental quality monitoring in CSC is continuing with funding from the ANHC. A recharge zone analysis using a geographical information system has begun that will attempt to assess the current and future impacts of land-use activities and inputs upon the cave ecosystem. With funding from the US Fish and Wildlife Service, the research techniques used in this study will be expanded into three similar cave complexes in Benton County, Arkansas, to determine if the ecosystem processes and stressors reported here are unique to CSC, or if they have a widespread applicability to other caves of the Springfield Plateau.

Little is known about the metabolic activities of stream microbial communities, especially in the subterranean realm. The stable isotope analyses of this study revealed depleted carbon isotope values for the biofilm, suggesting that cave microbes may be fractionating organic substrates by some undocumented mechanism. Furthermore, the dynamics of microbial activity and energy flux in cave sediments are poorly documented,

and the presence of heavy metals invites research in sediment ecotoxicology. On a larger scale, research in organic matter budgets of streams needs to inform the nutrient management of watersheds, especially in light of the recent total maximum daily load approach to meeting water quality criteria. The rehabilitation of gray bat and Indiana bat populations is crucial for not only the continuation of these endangered species, but also to restore historic ecosystem processes in Ozark caves. The reduction of nutrient input via human and animal waste will also repair ecosystem dynamics, and may be necessary to restore endemic cave crustacean assemblages. It is recommended that the application of sewage sludge and agricultural waste be reduced or ceased, especially on lands adjacent to intermittent streams, photo-lineaments, or other karst features. Increased protection for sensitive karst areas and ground-water habitats of high biodiversity is also recommended. According to Regulation 2, the Cave Springs Cave resurgence meets the criteria for the designation, "ecologically sensitive," because it contains threatened and endangered species, and has the designated use, "primary contact," because its watershed is greater than 10 square miles (Arkansas Pollution Control and Ecology Commission, 1998). Under this same regulation, the resurgence at Cave Springs Cave also meets the criteria of a "fisheries water body" because it sustains a significant fish and invertebrate community. It has not, however, formally been given any of these designations. Formal declaration is recommended, and designating this cave stream an "extraordinary resource water" would afford it further protection under Regulation 2, which sets more strict water quality standards for these water body designations. Ultimately, protection will only occur if the State water quality regulations are enforced.

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APPENDIX

Table 9. Base-flow water-quality data at the Cave Springs Cave resurgence. Parameters are: water temperature (Celsius); discharge (m^3/min); specific conductivity (μSiemens/cm); turbidity (nephlometric turbidity unit); field pH; dissolved oxygen (mg/l); sulfate (mg/l); total and dissolved organic carbon (mg/l); ammonia, nitrate, and nitrite (μg/l as N); total kjeldahl nitrogen (mg/l as N); total phosphorous and ortho-phosphate (μg/l as P); *Escherichia coli* and total coliform densities (MPN/100ml), and total viable cell density (cells/ml). No datum indicated by "---".

Date	Temp. Celsius	Disch. m^3/min	Cond. μS/cm	Turb. NTU	pH pH unit	DO mg/l	SO_4 mg/l	TOC mg/l	DOC mg/l	NH3 μg/l N	NO_2 μg/l N	NO_3 mg/l N	TKN mg/l N	Tot. P mg/l P	PO_4 mg/l P	*E. coli* MPN	T. Coli. MPN	Viable cell/ml
11/24/97	13.9	1	310	0.2	7.3	9.4	---	1.3	---	18	< 3	4.276	0.12	0.027	0.026	5	222	8000
12/17/97	13.4	1	320	1.0	7.5	9.5	7.13	4.1	3.4	55	< 3	4.807	0.13	0.029	0.029	37	1445	3000
01/25/98	14.5	6	290	0.0	6.9	10.3	3.60	0.7	---	< 9	< 3	6.395	0.07	0.023	0.034	3.1	344	1000
02/18/98	14.2	6	320	1.4	7.1	9.4	3.76	0.3	---	< 9	< 3	6.590	< 0.03	0.089	0.050	8	130	1000
03/05/98	14.1	7	290	4.0	7.2	10.6	3.71	1.0	---	< 9	< 3	6.295	0.12	0.075	0.044	15	165	500
04/15/98	13.9	11	300	1.0	7.1	9.3	3.47	1.9	---	11	41	6.250	0.03	0.027	0.021	271	4060	500
05/14/98	14.6	6	240	1.0	6.9	9.3	2.59	2.0	1.77	25	< 3	6.207	0.03	0.043	0.025	130	1652	1000
06/08/98	14.1	5	345	0.9	6.9	9.5	3.17	1.3	---	29	< 3	5.510	0.29	0.017	0.019	207	5600	2000
07/16/98	14.8	3	390	0.7	6.8	11.6	---	1.6	1.98	< 9	---	5.280	0.15	0.040	0.029	192	10130	3000
08/17/98	15.0	2	370	1.1	6.5	11.4	---	1.6	1.4	19	< 3	4.975	0.05	0.033	0.026	164	10910	16400
09/13/98	14.8	2	395	0.7	---	10.8	---	1.0	1.46	34	---	4.686	0.21	0.047	0.018	178	5310	3000
10/05/98	15.2	2	352	0.3	---	9.2	---	0.8	1.48	< 9	< 3	4.843	0.05	0.070	0.020	3240	8850	200
11/13/98	---	2	---	0.3	---	---	---	0.9	0.77	< 9	< 1	5.193	0.11	0.043	0.022	32	1013	500
12/15/98	---	2	---	---	---	---	---	1.9	---	---	---	---	---	---	---	78	1184	4500
01/26/99	---	1	---	---	---	---	---	---	---	---	---	---	---	---	---	1	101	13000
02/24/99	---	2	330	---	---	---	---	---	---	---	---	6.290	---	---	---	11	53	3000
03/08/99	14.5	2	340	---	7.1	8.9	---	---	---	---	---	---	---	---	---	---	---	3500
05/03/99	14.4	7	350	0.9	---	8.3	---	---	---	---	< 1	7.445	---	---	0.025	150	1920	2000
06/07/99	13.9	10	326	---	---	---	---	0.2	---	---	---	---	---	---	---	31	597	2000
07/26/99	---	11	---	---	---	---	---	1.5	---	---	---	5.776	---	---	---	31	2710	---
09/08/99	---	5	385	---	---	---	---	0.5	---	---	---	5.110	---	---	---	53	6590	3000
10/11/99	---	3	---	1.0	---	---	4.91	< 0.2	---	---	---	4.848	---	---	---	95	2880	2000

Table 10. Base-flow water-quality data at the Cave Springs Cave resurgence. Parameters are dissolved metals in parts per million (mg/l) or parts per billion (µg/l): aluminum (Al), antimony (Sb), arsenic (As), barium (Ba), beryllium (Be), cadmium (Cd), calcium (Ca), chloride (Cl), chromium (Cr), cobalt (Co), iron (Fe), lead (Pb), magnesium (Mg), manganese (Mn), nickel (Ni), selenium (Se), and zinc (Zn). No datum indicated by "---", concentrations below detectable limits indicated by "n.d."

	Al	Sb	As	Ba	Be	Cd	Ca	Cl	Cr	Co	Cu	Fe	Pb	Mg	Mn	Ni	Se	Zn
Date	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	mg/l	mg/l	µg/l	µg/l	µg/l	µg/l	µg/l	mg/l	µg/l	µg/l	µg/l	µg/l
11/24/97	< 11	---	< 12	---	---	< 1	---	8.868	---	---	12	< 3	< 11	---	< 0.6	< 24	32	< 6
12/17/97	22	---	< 12	---	---	< 1	---	8.952	---	---	27	6	< 11	---	0.6	< 24	< 15	18
01/25/98	13	---	< 12	---	---	< 1	---	8.055	---	---	24	< 3	< 11	---	0.6	< 24	< 15	19
02/18/98	13	---	< 12	---	---	< 1	---	9.518	---	---	28	5	12	---	1.7	< 24	< 15	31
03/05/98	20	---	< 12	---	---	< 1	---	6.620	---	---	20	13	< 11	---	0.7	< 24	15	25
04/15/98	14	---	< 12	---	---	< 1	---	6.080	---	---	47	6	< 11	---	1.2	< 24	< 15	83
05/14/98	< 11	---	< 12	---	---	< 1	---	9.422	---	---	16	< 3	< 11	---	< 0.6	< 24	< 15	13
06/08/98	< 11	---	< 12	---	---	< 1	---	6.960	---	---	< 6	< 3	< 11	---	< 0.6	< 24	< 15	8
10/05/98	505	6	5	51	1	n.d.	---	---	4	2	16	221	32	0.153	6	n.d.	6	113
03/08/99	25	n.d.	2	54	1	n.d.	64	---	n.d.	1	n.d.	10	10	1.740	2	n.d.	13	285
05/03/99	n.d.	2	n.d.	61	n.d.	1	54.6	---	n.d.	2	28	n.d.	39	1.890	14	n.d.	14	15

Table 11. Storm-flow water-quality data at the Cave Springs Cave resurgence. Parameters are: water temperature (Celsius); discharge (m^3/min); specific conductivity (μSiemens/cm); turbidity (nephlometric turbidity unit); field pH; dissolved oxygen (mg/l); sulfate (mg/l); total and dissolved organic carbon (mg/l); ammonia, nitrate, and nitrite (μg/l as N); total kjeldahl nitrogen (mg/l as N); total phosphorous and ortho-phosphate (μg/l as P); *Escherichia coli* and total coliform densities (MPN/100ml), and total viable cell density (cells/ml). No datum indicated by "---".

Date	Temp. Celsius	Disch. m^3/min	Cond. μS/cm	Turb. NTU	pH pH unit	DO mg/l	SO_4 mg/l	TOC mg/l	DOC mg/l	NH3 μg/l N	NO_2 μg/l N	NO_3 mg/l N	TKN mg/l N	Tot. P mg/l P	PO_4 mg/l P	*E. coli* MPN	T. Coli. MPN	Viable cell/ml
03/05/98	14.1	7	290	4.0	7.2	10.6	3.705	1.0		0	0	6.295	0.12	0.075	0.044	15	165	500
03/07/98	14.2	7	290	1.0	7.2	10.2	4.135	0.7	0.58	0	0	5.895	0.15	0.000	0.098	145	831	500
03/08/98	14.0	10	285	3.2	6.8	8.4	4.370	1.2		17	3	6.170	1.30	0.069	0.088	5040	20050	7000
03/09/98	13.9	11	260	8.0	6.8	9.4	4.055	3.1	2.46	9	3	7.200	0.22	0.025	0.061	1652	7380	3000
03/11/98	14.0	10	260	1.4	6.8	9.2	4.370	2.0	1.79	11	1	6.960	0.06	0.050	0.037	150	697	500
06/08/98	14.1	5	345	0.9	6.9	9.5	3.170	1.3	---	29	0	5.510	0.29	0.017	0.019	207	5600	2000
06/09/98	14.3	5	350	1.0	6.9	9	2.690	1.2	---	38	0	5.838	0.54	0.023	0.026	306	6970	4000
06/09/98	14.5	5	350	0.5	6.8	9.6	2.397	1.2	---	11	0	5.870	0.08	0.030	0.023	384	6970	3000
06/10/98	14.7	5	350	0.5	6.8	10	2.790	0.8	---	52	0	8.774	0.18	0.000	0.025	222	6240	2000
06/10/98	14.8	5	350	0.4	6.9	10.5	2.630	0.8	---	13	2	5.395	0.06	0.000	0.017	207	6590	3000
09/13/98	14.8	2	395	0.7	---	10.8	---	1.0	1.46	34	---	4.686	0.21	0.047	0.018	178	5310	3000
09/14/98	---	2	400	0.8	---	---	---	---	---	---	5	---	---	0.024	---	288	5040	6000
09/14/98	14.8	2	400	0.9	6.8	10.2	---	---	---	---	5	---	---	0.024	---	178	8850	3500
03/08/99	14.5	2	340	0.8	7.1	8.9	---	---	---	6870	2	5.700	0.08	0.193	0.025	74	271	3500
03/08/99	14.5	2	340	12.3	6.8	9.1	---	---	---	3527	4	5.865	---	0.088	0.028	1445	5910	247000
03/09/99	14.4	3		1.9	6.8	9.1	---	---	---	3437	1	6.150	---	0.091	0.020	1091	3840	10500
03/10/99	14.4	2	350	1.9	6.8	9.1	---	---	---	323	104	5.955	---	0.057	0.020	1091	5910	2500
03/13/99	---	6	310	12.1	---	---	---	---	---	35	7	8.175	---	0.057	---	5040	20000	321000
03/14/99	---	8	310	6.5	---	---	---	---	---	0	3	8.172	---	0.057	---	1110	20000	69000
03/15/99	---	9	310	5.1	---	---	---	---	---	0	2	8.256	---	0.057	---	2880	20000	---
03/16/99	---	10	310	---	---	---	---	---	---	13	1	7.600	---	0.052	0.024	201	201	44000
03/18/99	14.5	10	320	---	---	10	---	---	---	0	0	8.740	---	0.041	0.025	406	1652	23000
03/20/99	14.5	8.0	---	---	---	---	---	---	---	0	0	7.460	---	---	0.020	101	1184	5500

Table 11, cont. Storm-flow water-quality data at the Cave Springs Cave resurgence. Parameters are water temperature (Celsius), discharge (m^3/min), specific conductivity (μSiemens/cm), turbidity (nephlometric turbidity unit), field pH, dissolved oxygen (mg/l), sulfate (mg/l), total organic carbon (mg/l), dissolved organic carbon (mg/l), ammonia (μg/l as nitrogen), total kjeldahl nitrogen (μg/l as N), nitrite (μg/l as N), nitrate (μg/l as N), total phosphorous (μg/l), ortho-phosphate (μg/l as phosphorus), *Escherichia coli* density (MPN/100ml), total coliforms density (MPN/100ml), and total viable cell density (cells/ml). No datum indicated by "---".

Date	Temp. Celsius	Disch. m^3/min	Cond. μS/cm	Turb. NTU	pH pH unit	DO mg/l	SO_4 mg/l	TOC mg/l	DOC mg/l	NH3 μg/l N	NO_2 μg/l N	NO_3 mg/l N	TKN mg/l N	Tot. P mg/l P	PO_4 mg/l P	*E. coli* MPN	T. Coli. MPN	Viable cell/ml
04/03/99	---	9	330	19.3	---	---	---	3.4	---	---	10	6.740	---	---	0.054	4060	20050	---
04/03/99	14.3	13	300	20.0	6.2	9.5	---	3.7	---	---	12	8.021	---	---	0.054	10910	20050	---
04/04/99	14.4	14	295	14.2	6.6	9.9	---	3.8	---	---	1	7.374	---	---	0.062	20050	20050	---
04/05/99	14.5	12	305	5.9	6.6	10.0	---	2.5	---	---	3	8.235	---	---	0.042	2220	5910	---
05/03/99	14.4	7	350	0.9	---	8.3	---	2.7	---	17	0	7.445	---	0.026	0.025	150	1920	---
05/04/99	14.4	8	360	1.2	---	8.9	---	2.7	---	0	0	6.140	---	0.027	0.025	782	5310	---
05/04/99	14.0	16	250	48.0	---	9.0	---	5.1	---	0	26	8.245	---	0.149	0.057	5300	38400	---
05/05/99	14.4	20	280	18.1	---	8.8	---	4.7	---	0	13	6.218	---	0.174	0.103	16520	40600	---
05/06/99	14.3	17	280	8.1	---	8.7	---	4.6	---	0	0	7.595	---	0.123	0.096	10130	83100	---
09/08/99	---	5	385	---	---	---	---	0.5	---	---	---	5.110	---	---	---	53	6590	3000
09/08/99	---	5	350	---	---	---	---	0.2	---	---	---	5.380	---	---	---	42	5040	2000
09/09/99	---	5	390	---	---	---	---	0.4	---	---	---	5.420	---	---	---	64	5310	16500
10/30/99	14.6	2	340	0.8	6.5	---	---	1.0	---	---	0	5.029	---	---	---	111	4060	---
10/31/99	14.4	2	330	0.7	6.6	---	---	0.4	---	---	0	5.043	---	---	---	271	1298	---
11/01/99	14.4	2	330	1.0	6.6	---	---	0.4	---	---	0	5.032	---	---	---	1652	20050	---
11/02/99	14.2	2	330	1.1	6.8	---	---	0.3	---	---	0	5.009	---	---	---	2153	9450	---
12/04/99	14.7	2	315	---	---	9.3	---	0.6	---	---	---	5.311	---	---	---	178	201	---
12/05/99	14.6	2	320	---	---	9.6	---	0.6	---	---	---	5.083	---	---	---	624	201	---
12/06/99	14.6	2	340	---	---	8.4	---	< 0.2	---	---	---	5.037	---	---	---	453	5910	---

Table 12. Storm-flow water-quality data at the Cave Springs Cave resurgence. Parameters are dissolved metals in parts per million (mg/l) or parts per billion (µg/l): aluminum (Al), arsenic (As), barium (Ba), beryllium (Be), calcium (Ca), chloride (Cl), cobalt (Co), iron (Fe), lead (Pb), magnesium (Mg), manganese (Mn), nickel (Ni), selenium (Se), and zinc (Zn). No datum indicated by "---".

Date	Al µg/l	As µg/l	Ba µg/l	Be µg/l	Ca mg/l	Cl mg/l	Co µg/l	Cu µg/l	Fe µg/l	Pb µg/l	Mg mg/l	Mn µg/l	Ni µg/l	Se µg/l	Zn µg/l
03/05/98	20	0	---	---	---	6.620	---	20	13	0	---	0.7	0	15	25
03/07/98	0	0	---	---	---	7.735	---	0	0	0	---	0	0	0	0
03/08/98	0	0	---	---	---	7.145	---	19	7	0	---	0	0	0	17
03/09/98	0	0	---	---	---	6.575	---	0	40	0	---	0	0	13	0
03/11/98	0	0	---	---	---	7.440	---	0	8	0	---	0	0	19	7
06/08/98	0	0	---	---	---	6.960	---	0	0	0	---	0	0	0	8
06/09/98	0	0	---	---	---	6.970	---	21	0	0	---	0	0	0	13
06/09/98	0	0	---	---	---	8.849	---	7	0	0	---	0	0	0	7
06/10/98	0	0	---	---	---	6.120	---	0	0	0	---	0	0	0	0
06/10/98	0	0	---	---	---	8.970	---	0	0	0	---	0	0	0	0
03/08/99	25	2	54	1	64.0	---	1	0	10	10	1.74	2	0	13	285
03/08/99	64	5	55	0	62.1	---	1	0	57	10	1.78	6	0	16	288
03/09/99	26	4	54	1	62.9	---	1	0	11	11	1.80	0	0	7	159
03/10/99	58	4	56	1	61.0	---	0	0	64	9	1.86	18	0	20	2
03/13/99	113	3	67	1	51.5	---	2	10	83	2	2.32	13	0	14	7
03/14/99	136	5	67	1	49.2	---	1	0	88	1	2.35	5	0	12	0
03/15/99	49	5	64	1	50.0	---	1	7	39	5	2.33	4	0	6	0
03/18/99	99	4	62	1	50.5	---	1	42	93	5	2.32	4	0	17	12
03/20/99	105	7	62	1	53.7	---	1	33	61	26	2.21	0	0	17	0
04/03/99	150	2	71	0	53.4	---	1	23	510	22	2.13	29	0	8	12
04/03/99	270	12	77	0	46.3	---	0	10	640	20	2.33	31	0	15	17
04/04/99	220	12	71	0	45.0	---	0	7	370	12	2.29	22	0	18	13
04/05/99	100	14	67	0	46.7	---	0	6	150	29	2.29	16	0	12	11
05/03/99	0	0	61	0	54.6	---	2	28	0	39	1.89	14	0	14	15
05/04/99	39	0	62	0	54.3	---	2	11	0	38	1.91	14	0	17	6
05/04/99	275	0	95	0	30.1	---	2	31	156	15	2.53	45	1	17	20
05/05/99	220	0	66	0	39.1	---	2	230	147	36	1.96	24	3	18	133
05/06/99	201	6	67	0	38.1	---	2	27	156	28	2.08	19	0	16	19

Table 13. Summary of past base-flow water-quality data (Willis, 1984; Williams, 1991; USGS, 1996, unpublished data, respectively) and the mean data from this study for 1997 through 1999 at the Cave Springs Cave resurgence. No datum indicated by "---".

	Date	5/1/1984	4/2/1991	4/16/91	8/29/96	1997	1998	1999
Physical								
Specific Conductivity	µS/cm	130	280	205	327	315	329	337
Dissolved Oxygen	mg/l	6.8	---	---	7.3	9.5	10.1	8.6
Dissolved Metals								
Aluminum	µg/l	---	---	---	3	11	81	13
Arsenic	µg/l	< 5	---	---	1	0	0.7	1
Cadmium	µg/l	< 0.5	---	---	1	0	0	0.5
Chloride	mg/l	---	5.8	5.5	7	8.9	7.8	8.1
Copper	µg/l	< 10	---	---	1	20	22	14
Lead	µg/l	< 1	---	---	1	0	6	25
Manganese	µg/l	---	---	---	1	0.3	1.5	8
Nickel	µg/l	---	---	---	2	0	0	0
Selenium	µg/l	---	---	---	1	16	3	14
Zinc	µg/l	< 3	---	---	3	9	42	150
Nutrients								
Ammonia-N	µg/l as N	10	< 10	< 10	15	36.5	10.7	---
Nitrite-N	µg/l as N	---	20	10	10	0	4.6	0
Nitrate-N	mg/l as N	4.30	5.27	0.68	4.49	4.54	5.70	5.90
T.K.N.	mg/l as N	---	---	---	0.20	0.13	0.11	---
Total Phophorous	µg/l as P	20	---	---	30	28	46	---
Ortho-phosphate	µg/l as P	20	---	---	30	28	28	25
Sulfate	mg/l	---	---	---	5.7	7.1	3.4	4.9
Microbial								
Escherichia coli	MPN/100ml	---	---	---	2400	21	377	54
Total Coliforms	MPN/100ml	---	1100	---	---	834	4112	691

Table 14. Concentrations (mg/kg, d.b.) of metals in ecosystem components of Cave Springs Cave and of nutrient sources.

	Aluminum	Antimony	Arsenic	Barium	Beryllium	Boron	Cadmium	Calcium	Chromium	Cobalt
Sewage Sludge	7574	n.d.	2.0	237.0	0.1	21.5	1.1	22900	23.1	3.7
Cave Sediment	42616	n.d.	20.3	162.0	5.4	n.d.	5	15000	39.1	18.2
Cave Sediment	---	n.d.	6.6	72.5	0.9	1.0	n.d.	---	20.3	4.8
Cave Biofilm	21651	n.d.	10.1	---	1.9	n.d.	1.0	144000	24.6	9.2
Cave Biofilm	---	n.d.	2.7	28.8	0.5	3.8	0.2	---	23.0	4.3
Myotis Guano	2294	n.d.	1.4	163.7	0.3	1.0	9.4	3652	5.0	6.7
Myotis Guano	---	n.d.	1.3	159.2	0.1	n.d.	9.6	---	0.6	6.7
Septic Biosolids	---	n.d.	6.4	146.9	1.5	n.d.	n.d.	---	12.7	15.8
Cow Manure	---	n.d.	n.d.	94.8	0.1	n.d.	0.1	---	1.1	0.8
Poultry Litter	---	n.d.	18.5	31.9	0.1	11.5	0.3	---	3.2	0.8
Cave Isopod	24785	n.d.	n.d.	451.0	n.d.	n.d.	47.6	114206	27.0	6
Surface Crayfish	---	0.1	n.d.	13.3	0.1	n.d.	0.3	---	4.7	0.1
Surface Crayfish	773	n.d.	n.d.	129.8	n.d.	n.d.	1.7	148600	1.9	0.6
Ozark Cavefish	---	1.1	n.d.	54.6	0.1	n.d.	0.3	---	2.3	n.d.

	Copper	Iron	Lead	Magnesium	Manganese	Molybdenum	Nickel	Selenium	Vanadium	Zinc
Sewage Sludge	190.3	6995	25.5	8400	192.5	10.4	57.6	4.27	20.4	454.0
Cave Sediment	34.4	32977	45.0	2805	---	0.7	86.5	3.40	138.0	384.7
Cave Sediment	17.0	---	9.3	---	---	0.4	27.7	n.d.	116.3	114.0
Cave Biofilm	18.0	16400	17.3	1870	---	0.4	27.1	2.60	74.0	162.3
Cave Biofilm	10.1	---	10.3	---	---	0.4	24.7	0.12	24.2	87.5
Myotis Guano	434.9	5044	19.9	2055	112.9	8.3	18.2	n.d.	27.2	904.0
Myotis Guano	501.0	---	20.0	---	---	14.7	6.1	n.d.	23.1	n.d.
Septic Biosolids	10.5	---	17.6	---	---	n.d.	30.5	n.d.	149.6	71.3
Cow Manure	22.0	---	n.d.	---	---	1.5	1.5	n.d.	2.1	47.8
Poultry Litter	330.4	---	n.d.	---	---	4.7	13.9	n.d.	6.1	607.5
Cave Isopod	174.0	19730	48.4	2146	539.0	4.7	28.2	n.d.	122.0	539.0
Surface Crayfish	64.2	---	n.d.	---	---	0.9	7.6	n.d.	1.8	91.6
Surface Crayfish	118.0	340	2.4	1413	51.2	n.d.	2.3	3.39	1.8	100.5
Ozark Cavefish	14.4	---	n.d.	---	---	1.5	1.7	n.d.	n.d.	69.2

Table 15. Results of significant ($\alpha = 0.01$) pairwise correlation analysis of all physical, chemical, and microbiological parameters for water samples (base flow and storm flow combined) at Cave Springs Cave, Arkansas, from November 1997 to December 1999. Pearson's correlation coefficient = r.

Variable	By Variable	r	Count	p
Aluminum	Discharge	0.7031	34	0.0000
Aluminum	Ph	-0.6565	23	0.0007
Aluminum	Turbidity	0.8288	32	0.0000
Ammonia	Zinc	0.8873	30	0.0000
Arsenic	Aluminum	0.5803	34	0.0003
Arsenic	Ph	-0.6784	23	0.0004
Barium	Aluminum	0.85154	18	0.0000
Barium	Conductivity	-0.7765	16	0.0004
Barium	Discharge	0.6812	18	0.0019
Barium	Ph	-0.8824	7	0.0085
Barium	Turbidity	0.8883	16	0.0000
Boron	Arsenic	0.7106	18	0.0009
Calcium	Aluminum	-0.8222	18	0.0000
Calcium	Barium	-0.8418	18	0.0000
Calcium	Conductivity	0.9174	16	0.0000
Calcium	Discharge	-0.9310	18	0.0000
Calcium	Turbidity	-0.7036	16	0.0024
Cobalt	Arsenic	-0.6247	19	0.0042
Cobalt	Diss. Oxygen	-0.7236	14	0.0034
Conductivity	Discharge	-0.6099	51	0.0000
Copper	Discharge	0.5369	34	0.0011
DOC	TOC	0.8398	11	0.0012
E. coli	Aluminum	0.7709	34	0.0000
E. coli	Arsenic	0.4780	34	0.0042
E. coli	Calcium	-0.5925	18	0.0096
E. coli	Conductivity	-0.3632	50	0.0095
E. coli	Copper	0.4723	34	0.0048
E. coli	Discharge	0.6644	57	0.0000
E. coli	Iron	0.6481	34	0.0000
E. coli	Manganese	0.6327	34	0.0001
E. coli	Ortho-P	0.6375	37	0.0000
E. coli	TKN	0.7304	22	0.0001
E. coli	TOC	0.6451	43	0.0000
E. coli	Total P	0.6179	37	0.0000
E. coli	Turbidity	0.5579	44	0.0001

Variable	By Variable	r	Count	p
Iron	Aluminum	0.7758	34	0.0000
Iron	Discharge	0.4778	34	0.0043
Iron	Ph	-0.6932	23	0.0002
Iron	Turbidity	0.5915	32	0.0004
Lead	Aluminum	0.4946	34	0.0029
Lead	Beryllium	-0.6660	18	0.0025
Magnesium	Aluminum	0.6093	18	0.0073
Magnesium	Barium	0.7670	18	0.0020
Magnesium	Calcium	-0.6902	18	0.0015
Magnesium	Conductivity	-0.6779	16	0.0039
Manganese	Aluminum	0.8625	34	0.0000
Manganese	Barium	0.8327	18	0.0000
Manganese	Beryllium	-0.6777	18	0.0020
Manganese	Calcium	-0.6918	18	0.0015
Manganese	Discharge	0.6055	34	0.0001
Manganese	Iron	0.7270	34	0.0000
Manganese	Lead	0.5970	34	0.0002
Manganese	Ph	-0.6670	23	0.0005
Manganese	Turbidity	0.8553	32	0.0000
Nitrate	Aluminum	0.4980	34	0.0027
Nitrate	Arsenic	0.4768	34	0.0044
Nitrate	Conductivity	-0.4536	48	0.0012
Nitrate	Discharge	0.6669	53	0.0000
Nitrate	Magnesium	0.8969	18	0.0000
Nitrate	TOC	0.5767	42	0.0001
Nitrate	Turbidity	0.4986	42	0.0008
Ortho-P	Aluminum	0.4636	31	0.0086
Ortho-P	Calcium	-0.7668	15	0.0009
Ortho-P	Conductivity	-0.5621	34	0.0005
Ortho-P	Discharge	0.6925	37	0.0000
Ph	Turbidity	-0.4826	30	0.0069
Selenium	Aluminum	0.4543	34	0.0070
Silicon	Iron	0.9075	18	0.0000
Silicon	Ph	-0.8954	7	0.0064
TOC	Aluminum	0.7982	25	0.0000
TOC	Calcium	-0.8836	9	0.0016
TOC	Conductivity	-0.4746	39	0.0023
TOC	Discharge	0.7381	43	0.0000
TOC	Iron	0.5478	25	0.0046

Variable	By Variable	r	Count	p

TOC	Lead	0.5786	25	0.0024
TOC	Manganese	0.7865	25	0.0000
TOC	Turbidity	0.7137	35	0.0000
Total Coliforms	Aluminum	0.6895	34	0.0000
Total Coliforms	Calcium	-0.7300	18	0.0006
Total Coliforms	Discharge	0.6142	57	0.0000
Total Coliforms	*E. coli*	0.6716	57	0.0000
Total Coliforms	Iron	0.3894	34	0.0028
Total Coliforms	Manganese	0.5878	34	0.0003
Total Coliforms	Ortho-P	0.6528	37	0.0000
Total Coliforms	Ph	-0.6226	30	0.0002
Total Coliforms	TKN	0.7078	22	0.0002
Total Coliforms	TOC	0.6231	43	0.0000
Total Coliforms	Total P	0.4942	37	0.0019
Total Coliforms	Turbidity	0.5377	44	0.0002
Total Phosphor.	Aluminum	0.6463	29	0.0002
Total Phosphor.	Ammonia	0.5442	35	0.0007
Total Phosphor.	Iron	0.6032	29	0.0005
Total Phosphor.	Manganese	0.5393	29	0.0025
Total Phosphor.	TOC	0.6091	26	0.0010
Total Phosphor.	Turbidity	0.5486	35	0.0006
Total Phosphor.	Zinc	0.6208	29	0.0003
Turbidity	Conductivity	-0.4322	41	0.0048
Turbidity	Discharge	0.6317	44	0.0000
Zinc	Magnesium	-0.6663	18	0.0025