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Trophic Dynamics and Pollution Effects in Cave Springs Cave, Arkansas

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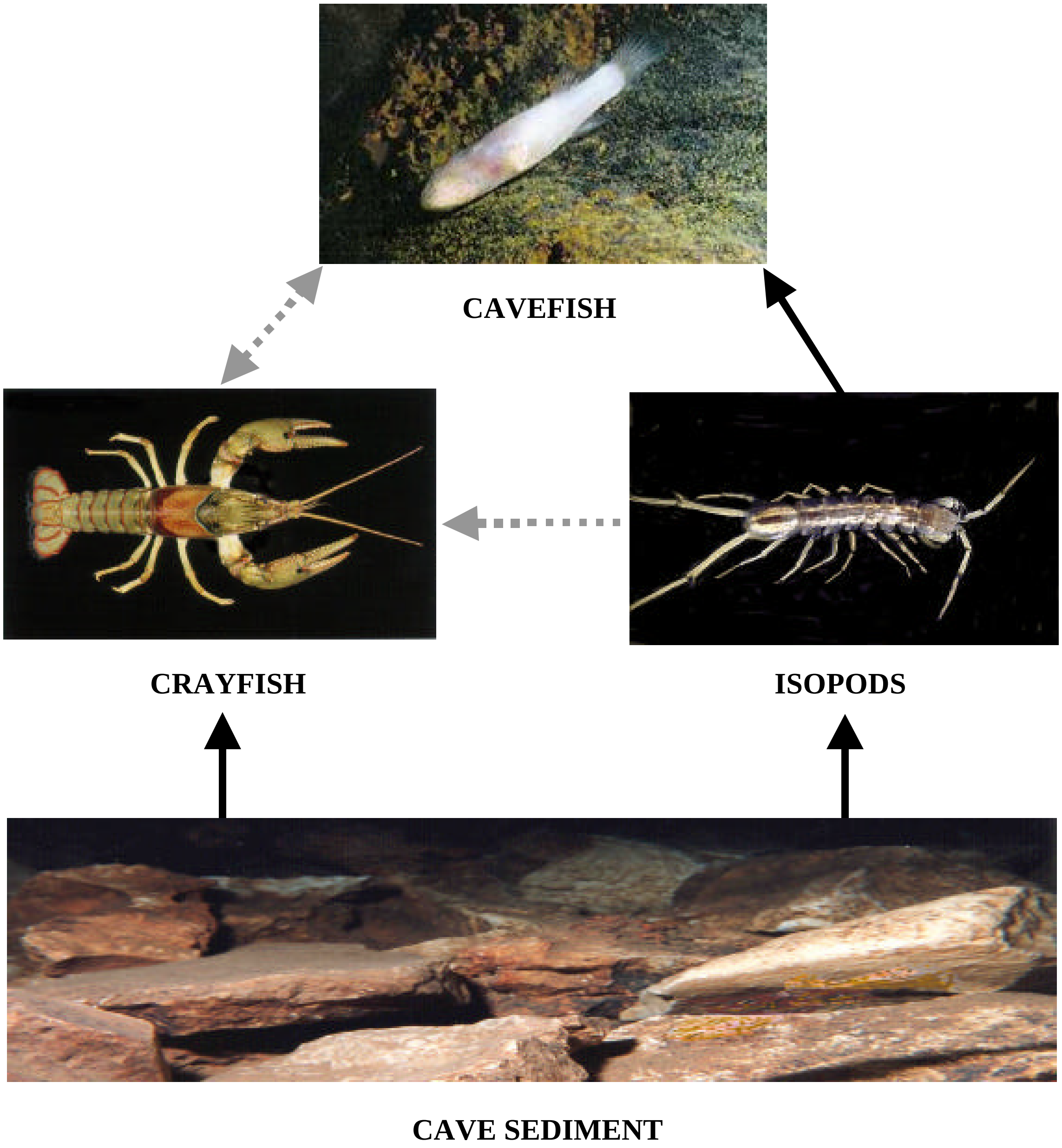

G. O. GRAENING AND A. V. BROWN

TROPHIC DYNAMICS AND POLLUTION EFFECTS IN CAVE SPRINGS CAVE, ARKANSAS

A Final Report Submitted to the

ARKANSAS NATURAL HERITAGE COMMISSION

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Department of Biological Sciences

ARKANSAS WATER RESOURCES CENTER

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EXECUTIVE SUMMARY

Cave Springs Cave, Benton County, Arkansas, is a refuge for several globally imperiled species. It harbors the largest population of threatened Ozark cavefish (*Amblyopsis rosae*), and it is a Primary Recovery Cave for the endangered gray bat (*Myotis grisescens*). With support from the cave's steward, the Arkansas Natural Heritage Commission, the authors have accumulated a considerable body of information during the past three years that describes the water quality, the status of the cave animals, and other aspects of the ecology of this cave ecosystem. It is hoped that this comprehensive case study of cave life, food web dynamics, and pollution effects will serve as a template for the management and conservation of other Ozark cave ecosystems.

The results of this and previous studies indicate that the Cave Springs Cave ecosystem is under stress from habitat degradation. The water quality has steadily declined in the past two decades and is often not fit for human consumption or even for primary contact recreation (swimming, *etc*.). Many water quality parameters continue to exceed Arkansas Regulation 2 water standards. Microbes, and particularly fecal bacteria, are at unnaturally high densities in the cave stream, especially during rainstorms. Concentrations of nutrients are also elevated in this cave stream water, and heavy metals are present at toxic levels. These metals are also accumulating in the cave sediments and in tissues of the cave organisms. While the effects of these pollutants are not certain, the cave fauna appear to be stressed. The gray bat population has decreased substantially, and the Ozark cave amphipod, an Arkansas Species of Concern, is no longer found in the cave. Other studies have documented the disappearance of cave amphipods from polluted caves. The abundance of cavefish appears to fluctuate from year to year, possibly due to mortality from pollution. The guano (feces) of gray bats is not the major source of the elevated amounts of nutrients, toxic metals, or bacteria. Rather, the source of these contaminants is from the cave stream's recharge zone, a 15 square mile area that captures polluted surface water from several drainage basins and focuses the water into the cave spring by many underground channels. This recharge area has many pollution sources, especially from applications of confined animal wastes and municipal sewage sludge to pastures, and the increasing numbers and decreasing effectiveness of septic systems. An increased level of protection will be required to conserve this unique ecosystem and its unique and endangered organisms, and this report includes a list of management recommendations that we think are essential for protecting Cave Springs Cave.

BACKGROUND

This is the third in a series of reports of studies of the status of the environmental quality and biota in Cave Springs Cave (CSC), Benton County, Arkansas (Brown *et al*., 1998; Graening and Brown, 1999), which has been commissioned by the Arkansas Natural Heritage Commission (ANHC). This cave provides refuge for several formally recognized imperiled species. It harbors the largest population of threatened Ozark cavefish (*Amblyopsis rosae*), it is a Primary Recovery Cave for the endangered gay bats (*Myotis grisescens*), and it is an historic habitat for two State Species of Concern, the Ozark cave amphipod (*Stygobromus ozarkensis*) and a cave isopod (*Caecidotea stiladactyla*). These imperiled species exist only as components of larger communities, and conservation of these species requires knowledgeable management of these entire ecosystems. Thus, the focus of these studies continues to be the provision of information about the CSC ecosystem, which will allow the most knowledgeable decisions to be made to protect it. The CSC ecosystem includes not only the cave, but also the stream flowing through it, and all of the land that is tributary to this stream. Protection of the water quality and biota of the CSC ecosystem requires protection of this entire tributary area, known as the recharge zone. This fifteen square miles of recharge area spans several drainage basins, and this zone is experiencing rapid human population growth and intensive land use practices. Land use in this area ranges from rural agriculture, with many confined animal feeding operations contributing nutrient and bacterial loads, to urban, with many subdivisions altering surface hydrology and many septic systems contributing nutrient and bacterial loads. This latest report documents the impact of these land uses upon the CSC ecosystem.

Status of Ground Water Ecosystems

The U.S. Environmental Protection Agency (1998a) reported that agricultural activity is the leading source of pollution that threatens 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 manure (Klugh and Abbe, 1994). Over 15 metric tons per hectare of confined animal waste are 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 are increasing (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). Furthermore, high nitrite levels can cause anemia and tissue damage in fishes (Eddy and Williams, 1987). Pasquarell and Boyer (1995) found a direct correlation between the mean nitrate concentration in karst springs (Greenbrier, West Virginia) and the percent of their recharge zones that were under agricultural use. Studies have shown that land uses such as manure 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 Boone-St. Joe 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. Cave Springs Cave ground water has a yearly average of over 5 mg/l NO_3-N.

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). In karst aquifers, fecal bacteria can be transported over several kilometers (Hallberg *et al.*, 1985; Green *et al.*, 1990). Karst terrains allow livestock-related bacteria to be transported by water from the surface, through the aquifer, and back out through resurgent springs (Pasquarell and Boyer, 1996). While 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 (coliform) bacteria 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). While Williams (1991) lists bat guano as a possible fecal coliform source, he discounted it because surrounding streams that recharge the CSC stream had similarly high levels of coliforms. Water samples taken in Logan Cave (Benton County, Arkansas) and Cave Springs Cave 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 in 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 food web 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 Status and Community Structure

Oligotrophy appears to structure cave communities by the competition for food (Culver, 1982). A 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, 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 the 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 indicate a 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).

Effects of Nutrient Pollution

Eutrophication is commonly defined as a process that increases the nutrients, especially nitrogen and phosphorous forms, in an aquatic ecosystem, with a corresponding increase in algae populations and a decrease in diversity (Morris, 1992). In other ecosystems such as grasslands, nutrient additions lead frequently to declines in plant species diversity (Foster and Gross, 1998). 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 cave-adapted (troglobitic) 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, who 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 substantially larger densities of invertebrates. Banners Corner Cave had isopod (*Caecidotea recurvata*) densities of 35 – 61 isopods/m^2, while the nearby and relatively pristine Chadwell's Cave, Tennessee, had an isopod density of only 6 isopods/m^2. Troglophilic (*i.e.*, not cave-adapted) 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 apparently was still receiving sewage inputs. Troglobitic amphipods (*Stygobromus mackini*) were absent from all polluted pools, while *C. 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 rob the fauna of dissolved oxygen. These case studies suggest that the eutrophication of Ozark cave streams will have significant impacts upon community structure, and ultimately, ecosystem processes in these habitats.

Use of Stable Isotope Analyses in Aquatic Ecology

One of the most interesting 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 researchers have used stable isotope techniques 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), while stable isotope analyses (SIA) provide 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 assays are now widely used in food web studies of freshwaters (Gu *et al.*, 1997). Furthermore, SIA 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.*, Atwell *et al.*, 1998; Kwak and Zedler, 1997). 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 methods 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 assays. 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. Because SIA can monitor watershed influences, such as inputs of terrestrial organic materials, on aquatic trophic dynamics (Fry, 1999), SIA was used in this study to describe the trophic web of CSC and to determine if organic pollutants from the recharge zone were contributing to this web.

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*"), and is called delta (δ) notation (McKinney *et al.*, 1950): $\delta\ (^{o}/_{oo}) = (R_{sa} / R_{std} - 1) \times 1{,}000$. The primary standards are the Peedee belemnite (PDB) marine limestone fossil for carbon ($^{13}C/^{12}C$) and atmospheric air for nitrogen ($^{15}N/^{14}N$) (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 stable nitrogen 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; Peterson and Fry, 1987).

OBJECTIVES

1) Assess the environmental quality of Cave Springs Cave, with emphasis on stressors identified in previous studies.
    - Analyze an array of water quality parameters at base flow during fall and spring at the mouth of the cave and deep in the cave upstream of bat roosts, for a total of four sampling sets.
    - Sample four storm events with at least one in early fall and one in early spring, measuring the same parameters as above, with four samples per storm, for a total of 16 sampling sets.

2) Determine the trophic structure of Cave Springs Cave and any contributions made by nutrient pollution.
    - Complete stable isotope analyses of the cave stream food web.

3) Update the status of the Ozark cavefish population in Cave Springs Cave.
    - Count cavefish visible in the cave using the same methods used in all previous surveys.

4) Continue the analysis of the Cave Springs Cave recharge area to aid in management of the Natural Area, and specifically to identify pollution inputs.
    - Refine the ArcView project and acquire relevant themes.

SITE DESCRIPTION

The Geologic Setting

The Cave Springs Cave Natural Area, owned by the Arkansas Natural Heritage Commission, 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 the less soluble, chert-filled Boone formation and the passageways dissolved from the purer limestone of the St. Joe formation. 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 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 ^oC, varying only by approximately 1 ^oC annually. The water hardness is approximately 150 mg/L as $CaCO_3$, with calcium being the dominant cation and bicarbonate being the dominant anion.

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*). In 1935, Indiana bats (*Myotis sodalis*, federally listed) were observed in Cave Springs Cave (Sealander and Young, 1955), but none are found today (pers. observ. of authors; M. Harvey, pers. comm., 1999). A maternity colony of approximately 3,000 gray bats (*M. grisescens*, federally listed) inhabits this cave during summer months (Michael Harvey and Ron Redman, unpublished data, 1999), but is dramatically reduced from its previous abundance, as shown in Figure 1 (Harvey, 1991). This cave also contains the largest known population of Ozark cavefish (*Amblyopsis rosae*), with a maximum observed population size of 166 individuals and a calculated density of 0.2 fish/m^2 (Graening and Brown, 1999). 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 during this study. The grotto salamander (*Typhlotriton spelaeus*) has also been reported from this cave (Brown *et al.*, 1994), but has not been seen in the last 3 years. Other vertebrates that inhabit the cave mouth include the eastern phoebe (*Sayornis phoebe*) and the eastern woodrat (*Neotoma floridana*). The invertebrate community includes the camel cave cricket (*Ceuthophilus sp.*), harvestmen (Opiliones), copepods, ostracods, and two Arkansas Species of Concern, *Caecidotea stiladactyla* and *Stygobromus ozarkensis*. The cave isopod *C. stiladactyla*, first found by Flemming in 1972, was abundant in the riffles with an estimated density of five individuals/m^2 during this study. The Ozark cave amphipod (*S. ozarkensis*) was reported from CSC (Holsinger, 1972), but has not been seen recently in the cave stream (Brown and Willis, 1984; pers. observ. of authors). The epigean Spot Handed crayfish *(Orconectes punctimanus*), which curiously never reaches more than about 6 cm in size in this habitat, was found in CSC at a density of 0.5 individuals/m^2 in the cave pools and 10 individuals/m^2 in the pool at the cave mouth during this study. 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.

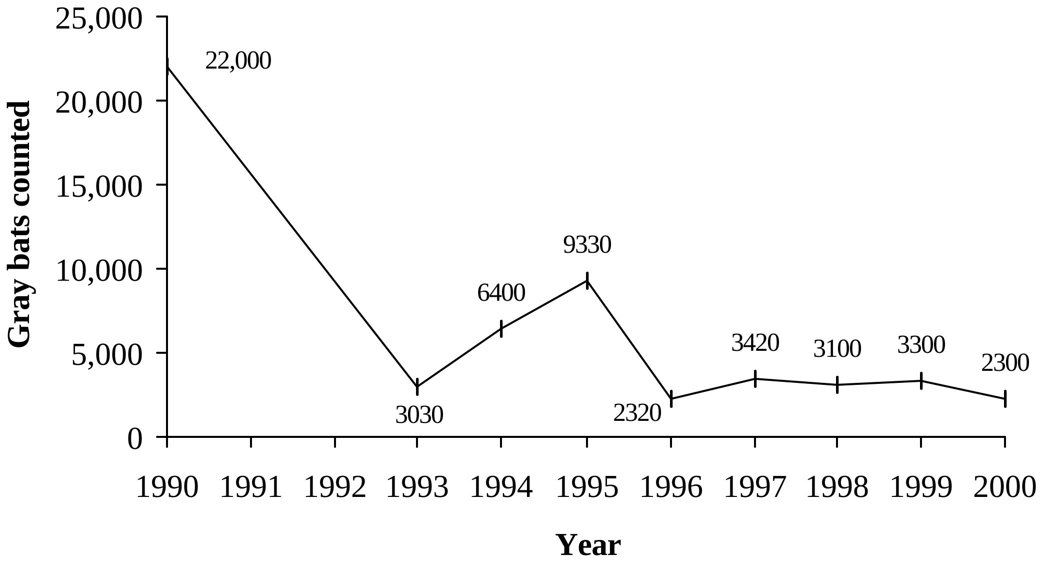

Figure 1. All known population counts of gray bats (*Myotis grisescens)* in Cave Springs Cave, Arkansas. (Harvey, 1991; Michael Harvey and Ron Redman's unpublished data, 2000).

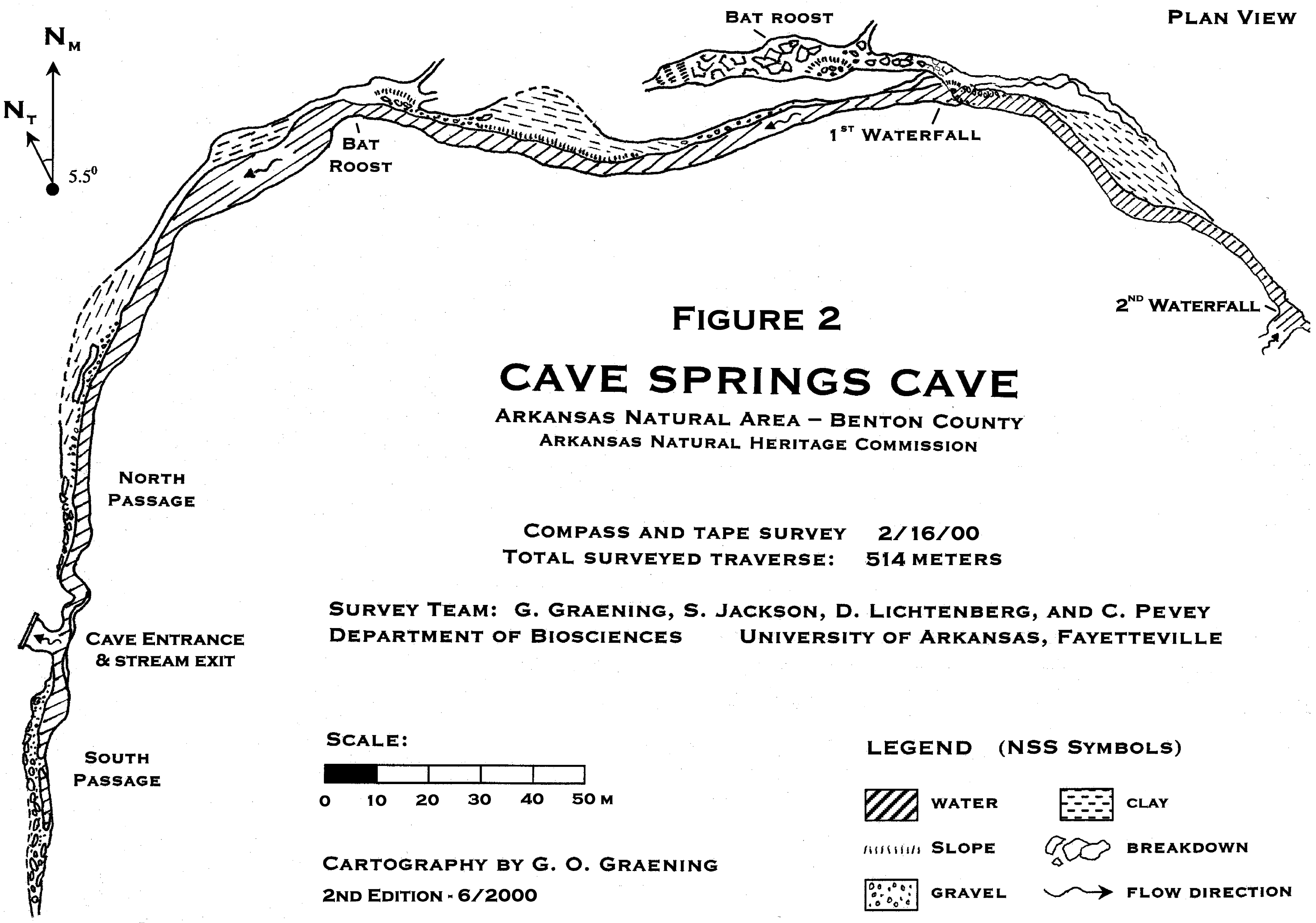

FIGURE 2

CAVE SPRINGS CAVE

ARKANSAS NATURAL AREA – BENTON COUNTY
ARKANSAS NATURAL HERITAGE COMMISSION

COMPASS AND TAPE SURVEY 2/16/00
TOTAL SURVEYED TRAVERSE: 514 METERS

SURVEY TEAM: G. GRAENING, S. JACKSON, D. LICHTENBERG, AND C. PEVEY
DEPARTMENT OF BIOSCIENCES UNIVERSITY OF ARKANSAS, FAYETTEVILLE

CARTOGRAPHY BY G. O. GRAENING
2ND EDITION - 6/2000

LEGEND (NSS SYMBOLS)

▨ WATER
CLAY
SLOPE
BREAKDOWN
GRAVEL
FLOW DIRECTION

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 the 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 SIA and heavy metals analyses with special permission from the U.S. Fish and Wildlife Service.

Environmental Quality Sampling

Meteorological data, including air temperature, barometric pressure, and rain accumulation, were taken 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 was computed from the relationship based upon USGS hydrological data (Brown *et al*., 1998): discharge (m^3/min) = 35.79 x (m) – 109.99. Stage and discharge measurements were measured every time water samples or other measurements were taken. Base flow samples were collected at the spring orifice in the sluice leading to the water wheel, from June 1999 through February 2000, and downstream of all bat rookeries (see the cave map in Figure 2). Base flow samples were also collected once at the waterfall at the very end of the accessible cave, approximately 0.5 k from the cave mouth, and upstream of all bat rookeries. Storm flow samples were collected at the spring orifice during each of four different storm events before, during, and after the peak discharge.

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 ^{TM} Dissolved Oxygen Meter, an Orbeco-Hellige Model 966 ^{TM} portable turbidimeter, and a portable pH meter. Water samples were taken at the downstream and/or the upstream station, and all water samples were held on ice and processed within 48 hours. The samples were analyzed for some or all of the following: total coliform, *Escherichia coli*, and total viable cell densities, nitrate, nitrite, ortho-phosphate, total phosphate, total Kjeldahl nitrogen, total organic carbon, dissolved organic carbon, sulphate, chloride, and dissolved metals. Analytical procedures followed approved USEPA methods, and appropriate quality assurance and quality control measures were taken. Depending upon the parameter, the water samples were analyzed by the authors at the Department of Biological Sciences, University of Arkansas at Fayetteville (UAF), at the Water Quality Laboratory (Arkansas Water Resources Center, UAF), Central Analytical Laboratory (Center for Excellence in Poultry Science, UAF) or at the Environmental Chemistry

Laboratory (Arkansas Department of Environmental Quality, Little Rock, Arkansas). Dissolved organic carbon samples were prepared by filtering water samples through pre-combusted 0.45 µm Whatman ^{TM} GF/C filters, and TOC and DOC samples were put into glass vials with Teflon ^{TM} 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 ^{TM} polycarbonate filters into glass vials with Teflon seals and acidified with nitric acid. For the metals analyses of whole spot-handed crayfish, 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 in a drying oven at 60 ^{0}C, pulverized, and analyzed at Central Analytical Laboratory. The sample of sewage sludge was collected directly from the belt press at the Springdale Sewage Treatment Plant, Benton County, Arkansas.

Stable Isotope Assays (SIA)

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 show 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 then scraping the residue off into clean glass vials. Spot-handed crayfish tissue samples were processed by the procedure of France (1996): the 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 size 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 Departments of Poultry Science and Animal Science (UAF). The mean($^{+}$/- SE) isotope ratios of the combination of layer feces, layer litter, and broiler litter samples from that study was $\delta^{13}C = -19.2\ ^{+}/\text{-}\ 1.9\ ^{o}/_{oo}$, $\delta^{15}N = 4.9\ ^{+}/\text{-}0.7\ ^{o}/_{oo}$. Septic system leachate and biosolids were collected from two different 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.

Statistics

Excel 2000 ^{TM} (Microsoft Corp.), SAS 8 for Windows ^{TM} and JMP ^{TM} (S.A.S., Inc.) were used for statistical analyses. Water quality parameters that were below detectible limits were analyzed as values of zero. A significance level (α) of at most 0.05 was used for all statistics. 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 the upstream and the downstream stations. To determine if bat guano contributed to bacterial densities, a paired (one-sample, one-sided) t-test was used to test whether the difference in mean bacterial densities between the upstream station and the downstream station equaled zero. To determine if differences existed between 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 statistics were quite large, indicating that the distribution was not Poisson. A negative binomial regression model (Type III) was then fitted to the data, and yielded much lower deviances and overdispersion parameters of less than one. The interaction terms were not significant, so they were 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 the metal concentrations of cave ecosystem components (sediment, biofilm, isopods, crayfish, cavefish), weighted least squares was used after the method of 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 the metal profiles of ecosystem components.

Ozark Cavefish Population Census

The visual survey was performed by the same method as previous surveys and included at least two of the people used in a previous survey (Willis and Brown, 1985; Brown and Todd, 1987). Using helmet lights as well as powerful diving lights underwater, three people moved slowly upstream and counted cavefish as they were sighted. Pearson *et al.* (1995) reported that the use of powerful dive lights underwater increased substantially the number of fishes observed over typical dry caving lights. During this study, length and location of cavefishes were also recorded. After sighting each cavefish, the fish length was visually estimated and put into one of three classes: 1) small – less than 2.5 cm; 2) medium – between 2.5 and 5 cm 3) large – greater than 5 cm. Michael Slay (UAF) and C. Stanley Todd and Brian Wagner (both of the Arkansas Game and Fish Commission), assisted with the census.

Recharge Zone Analyses

The cave map, published in Graening and Brown (1999), was revised on February 16, 2000, and extended 33 m to the second waterfall where it became too tight to proceed further, using the same equipment – a Suunto ^{TM} compass and 100 m fiberglass tape. The U. S. Geological Survey station water level gauge at the cave mouth was used as the zero datum. The cartographic methods followed the National Speleological Society's standards (Dasher, 1994) at a scale of 1 cm: 10 m. Figure 2 shows a reproduction of this cave map.

A geographic information system was created using ArcView 3.2 and Spatial Analyst and Image Analysis Extensions (ESRI), under the direction of Alex Johnson of Arête Systems, Inc., and Guy Graening of HSI-Geotrans (a TetraTech company). Aerial photographs of the recharge zone were furnished by Dr. John Harris of the Arkansas State Highway and Transportation Department. Aerial photographs were also purchased from the U. S. Geological Survey and from a private contractor, who took new color aerial photographs in the fall of 1999. The recharge zone boundary, water table contours and photo-lineaments/fracture traces were redrawn from Williams (1991) onto the Bentonville South and Springdale quads and digitized. The digital elevation model and topographic maps were acquired from the U. S. Geological Survey. A Garman III Plus global positioning system handheld unit was used to register specific locations (such as sinkholes) in the recharge zone during ground-truthing.

RESULTS

Environmental Quality Assessment

The results of the environmental quality sampling are shown in Tables 1 through 7, following. Total coliform densities and heavy metal concentrations continue to exceed Arkansas State water quality standards (Regulation 2) maximum contaminant levels (Arkansas Pollution Control and Ecology Commission, 1998). Most water quality variables, especially total coliforms and *Escherichia coli* densities, and heavy metal concentrations, were significantly correlated to discharge, and the results of pair-wise correlations are shown in the Appendix. However, because most of this year's sampling occurred during record low flows, many water quality parameter values were lower than in previous years.

Metals Analyses

Most dissolved metals (aluminum, barium, beryllium, calcium, chloride, copper, iron, magnesium, manganese, and lead) were 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 8. For example, the cave isopod metal profile can 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.

Table 1. Summary of water quality data at the downstream station (cave mouth) and at the upstream station (waterfall) at Cave Springs Cave during base flow 1999-2000.

		Cave Mouth					Waterfall
	Date	6/7/99	7/26/99	9/8/99	10/11/99	2/16/00	2/16/00
Physical							
Water Temperature	Celsius	13.9	---	---	---	14.7	---
Water Stage	m	3.353	3.377	3.2	3.158	3.091	n.a.
Discharge	m^3/min	10	11	5	3	1	n.a.
Specific Conductivity	μS/cm	326	---	385	---	350	350
Turbidity	NTU	---	---	---	---	0.7	1.3
Nutrients							
TOC	mg/l	0.2	1.46	0.46	< 0.17	3.1	4.23
Ammonia	μg/l as N	---	---	---	---	17	6
Nitrate-N	mg/l as N	---	5.776	5.11	4.848	4.999	4.934
Total Phosphate	μg/l as P	---	---	---	---	30	25
Ortho-Phosphate	μg/l as P	---	---	---	---	19	15
Sulfate	mg/l	---	---	---	4.91	2.84	---
Microbial							
Escherichia coli	MPN/100ml	31	31	53	94.5	< 10	< 10
Total Coliforms	MPN/100ml	597	2710	6590	2880	53	10

Table 2. Summary of water quality data at the cave mouth of Cave Springs Cave during a storm event (9/7/99 to 9/8/99) with 3 cm rain accumulation.

	Date	9/8/99	9/8/99	9/9/99
Physical				
Rain Accumulation	cm	3	0	0
Water Stage	m	3.2	3.2	3.203
Discharge	m^3/min	5	5	5
Specific Conductivity	μS/cm	385	350	390
Nutrients				
TOC	mg/l	0.46	0.18	0.42
Nitrate-N	mg/l as N	5.11	5.38	5.42
Microbial				
Escherichia coli	MPN/100ml	53	42	64
Total Coliforms	MPN/100ml	6590	5040	5310

Table 3. Summary of water quality data at the cave mouth of Cave Springs Cave during a storm event (10/29/99 to 11/2/99) with 6.7 cm rain accumulation.

	Date	10/30/99	10/31/99	11/1/99	11/2/99
Physical					
Rain Accumulation	cm	3	1.5	1.2	1
Air Temperature	Celsius	14	16	15	7
Water Temperature	Celsius	14.6	14.4	14.4	14.2
Water Stage	m	3.133	3.133	3.133	3.136
Discharge	m^3/min	2	2	2	2
Specific Conductivity	µS/cm	340	330	330	330
pH		6.5	6.6	6.6	6.8
Turbidity	NTU	0.8	0.7	1	1.1
Nutrients					
TOC	mg/l	0.98	0.42	0.36	0.3
Nitrite-N	µg/l as N	< 1	< 1	< 1	< 1
Nitrate-N	mg/l as N	5.029	5.043	5.032	5.009
Microbial					
Escherichia coli	MPN/100ml	111	271	1652	2153
Total Coliforms	MPN/100ml	4060	1298	20050	9450

Table 4. Summary of water quality data at the cave mouth of Cave Springs Cave during a storm event (12/2/99 to 12/6/99) with 1.5 cm rain accumulation on 12/2/99 and 4.3 cm on 12/4/99.

	Date	12/4/99	12/5/99	12/6/99
Physical				
Rain Accumulation	cm	4.3	0	0
Air Temperature	Celsius	14	7	6
Water Temperature	Celsius	14.7	14.6	14.6
Water Stage	m	3.136	3.139	3.118
Discharge	m^3/min	2	2	2
Specific Conductivity	µS/cm	315	320	340
Dissolved Oxygen	mg/l	9.3	9.6	8.4
Nutrients				
TOC	mg/l	0.63	0.6	< 0.17
Nitrate-N	mg/l as N	5.311	5.083	5.037
Microbial				
Escherichia coli	MPN/100ml	178	624	453
Total Coliforms	MPN/100ml	200.5	> 200.5	5910

Table 5. Summary of water quality data at the cave mouth of Cave Springs Cave during a storm event (4/11/00 to 4/12/00) with 1.7 cm rain accumulation.

	Date	4/11/00	4/12/00	4/13/00
Physical				
Rain Accumulation	cm	1.7	0	0
Water Stage	m	3.091	3.094	3.097
Discharge	m^3/min	1	1	1
Specific Conductivity	µS/cm	320	330	320
Turbidity	NTU	0.8	1.2	0.8
Nutrients				
TOC	mg/l	0.5	0.4	0.5
Nitrate-N	mg/l as N	5.064	5.107	5.063
Sulfate	mg/l	2.9	3.31	2.99
Microbial				
Escherichia coli	MPN/100ml	885	429	420
Total Coliforms	MPN/100ml	3440	2880	2380

Table 6. Dissolved metal concentrations during sampling of base flow (at both sampling stations) and during one storm event at Cave Springs Cave. “n.d.” signifies parameter is below detectable limits.

		--- Base flow ---		--- Storm Event ---		
		Cave mouth	Waterfall			
	Date	2/16/00	2/16/00	4/11/00	4/12/00	4/13/00
Aluminum	µg/l	n.d.	n.d.	n.d.	n.d.	n.d.
Antimony	µg/l	n.d.	n.d.	n.d.	n.d.	n.d.
Arsenic	µg/l	n.d.	n.d.	n.d.	n.d.	n.d.
Barium	µg/l	240	200	40	40	40
Beryllium	µg/l	n.d.	n.d.	n.d.	n.d.	n.d.
Boron	µg/l	40	10	n.d.	n.d.	n.d.
Cadmium	µg/l	n.d.	n.d.	n.d.	n.d.	n.d.
Calcium	mg/l	61.9	61.0	61.5	62.8	59.9
Chloride	mg/l	7.026	7.18	9.6	9.8	9.8
Chromium	µg/l	n.d.	n.d.	n.d.	n.d.	n.d.
Cobalt	µg/l	n.d.	n.d.	n.d.	n.d.	n.d.
Copper	µg/l	10	n.d.	10	10	10
Fluoride	µg/l	50	0.06	40	60	40
Iron	µg/l	10	n.d.	60	40	40
Lead	µg/l	20	30	40	20	30
Magnesium	mg/l	1.64	1.63	1.68	1.71	1.64
Manganese	µg/l	10	10	0.03	0.02	0.02
Molybdenum	µg/l	10	n.d.	n.d.	n.d.	n.d.
Nickel	µg/l	n.d.	n.d.	n.d.	n.d.	n.d.
Selenium	µg/l	n.d.	20	n.d.	n.d.	n.d.
Vanadium	µg/l	n.d.	n.d.	10	n.d.	n.d.
Zinc	µg/l	10	n.d.	n.d.	n.d.	n.d.

Table 7. Metal concentrations (mg/kg, dry basis) in ecosystem components of Cave Springs Cave and of pollutant sources in the recharge zone. "n.d." signifies parameter is below detectable limits.

	Aluminum	Antimony	Arsenic	Barium	Beryllium	Boron	Cadmium	Calcium	Chromium	Cobalt
Sewage Sludge	7574	n.d.	1.95	237	0.07	21.5	1.09	22900	23.1	3.74
Cave Sediment	42616	n.d.	20.3	161.95	5.4	n.d.	5	15000	39.1	18.2
Cave Sediment	---	n.d.	6.6	72.5	0.9	1	n.d.	---	20.3	4.8
Cave Biofilm	21651	n.d.	10.1	---	1.9	n.d.	0.98	144000	24.6	9.2
Cave Biofilm	---	n.d.	2.7	28.8	0.5	3.8	0.2	---	23	4.3
Myotis Guano	2294	n.d.	1.4	163.7	0.31	1	9.4	3652	5	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	n.d.	n.d.	47.6	114206	27	6
Surface Crayfish	---	0.1	n.d.	13.3	0.1	n.d.	0.3	---	4.7	0.1
Surface Crayfish	772.5	n.d.	n.d.	129.8	n.d.	n.d.	1.67	148600	1.88	0.61
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
Cave Sediment	34.4	32977	45	2805	---	0.69	86.5	3.4	138	384.7
Cave Sediment	17	---	9.3	---	---	0.4	27.7	n.d.	116.3	114
Cave Biofilm	18	16400	17.3	1870	---	0.38	27.1	2.6	74	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
Myotis Guano	501	---	20	---	---	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	---	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	19730	48.4	2146	539	4.7	28.2	n.d.	122	539
Surface Crayfish	64.2	---	n.d.	---	---	0.9	7.6	n.d.	1.8	91.6
Surface Crayfish	118	339.8	2.37	1413	51.2	n.d.	2.27	3.39	1.79	100.5
Ozark Cavefish	14.4	---	n.d.	---	---	1.5	1.7	n.d.	n.d.	69.2

Table 8. 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 contributes to the cave component metal profile in the Cave Springs Cave ecosystem.

	Pollution Sources						
Cave Component	**Sewage Sludge**	**Septic Waste**	**Cow Manure**	**Poultry Litter**	**Bat Guano**	**Total**	**R^2**
Cave Sediment #1	0.6302	0.3698	0	0	0	1	0.916
Cave Sediment #2	0.1415	0.7337	0.1248	0	0	1	0.981
Cave Biofilm #1	0.5563	0.4337	0	0	0	1	0.935
Cave Biofilm #2	0.6381	0.2962	0	0	0	0.934	0.872
Cave Isopod	0.3313	0.2127	0.4560	0	0	1	0.978
Crayfish #1	0.1853	0	0.1556	0.4145	0.2445	1	0.997
Crayfish #2	0	0	0	0.3579	0.6421	1	0.998
Cavefish	0	0	1	0	1	1	0.943

Microbial Dynamics

A summary of microbial densities from 1997 to 1999 during base flow and storm flow is shown in Table 9, and includes data from previous studies (Brown *et al*., 1998; Graening and Brown, 1999). All measures of bacterial densities were significantly higher during storm events than during base flows (two sample t-test results: *E. coli*, p = 0.002; total coliforms, p = 0.001). Total coliforms and *E. coli* cell densities were significantly correlated to each other and to discharge. Total coliform and *E. coli* densities were also both positively correlated to TOC, nitrate, TKN, total phosphorous, ortho-phosphate (see Appendix for a statistical summary of correlations).

Table 9. 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. Data are from Brown *et al*. (1998), Graening and Brown (1999), and this study.

	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
Storm flow					
Escherichia coli	(MPN/100ml)	42	15	2,337	20,050
Total Coliforms	(MPN/100ml)	42	165	10,790	83,100

The negative binomial regression results and parameter estimates are summarized in Table 10. The variable of season was significant for all three bacterial metrics. Year was significant for 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 lesser rain during the study period (1997-2000). Microbial densities were significantly lower in winter.

Table 10. Summary of 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. Data are from Brown *et al*. (1998), Graening and Brown (1999), and this study.

Parameter	**df**	**χ^2**	**p-value**
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

Stable Isotope Analyses Results

The results of the SIA are summarized in Table 11 and Figure 3. Some values are similar to published data. Sediment samples from a food web study of two Ozark streams in Arkansas yielded δ^{13}C values of –24.9 and –26.5 $^{o}/_{oo}$ and δ^{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* sp. guano has a mean δ^{13}C of –24 $^{o}/_{oo}$ and a mean δ^{15}N value of 7 $^{o}/_{oo}$ (Mitzutani *et al.*, 1992a), and the *M. grisescens* guano in CSC is equivalent in δ^{13}C value, but enriched in ^{15}N by 5 $^{o}/_{oo}$. Sewage-derived organic matter has a mean δ ^{13}C of -23 $^{o}/_{oo}$, but nitrogen isotope ratios are more variable with a mean δ^{15}N of 5 $^{o}/_{oo}$ (Van Dover *et al.*, 1992; Kwak and Zedler, 1997). The septic system biosolids and sewage sludge analyzed in this study are very similar to these mean literature carbon and nitrogen isotope values, but sewage sludge was enriched in ^{15}N by 8 $^{o}/_{oo}$ in this study. The δ ^{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. An Ozark stream study by Whitledge and Rabeni (1997) reported a mean δ^{13}C value –28 $^{o}/_{oo}$ and a mean δ^{15}N value of +6 $^{o}/_{oo}$ for *Orconectes punctimanus*. In this study, *O. punctimanus* had a mean δ^{13}C value –28.4 $^{o}/_{oo}$ and a mean δ^{15}N value of 10.6 $^{o}/_{oo}$. When looking at each replicate crayfish sample, however, seasonal diet shifting is evident (Figure 4). Crayfish samples cluster (n = 4, mean δ^{13}C value of –29.3 and a mean δ^{15}N value of +9.9) around a probable food resource of leaf litter and seasonally shift to a diet whose isotope signature is distinctly similar to *M. grisescens* guano.

Table 11. Stable isotope ratios of Cave Springs Cave ecosystem constituents (limestone, POM, guano, sediment, biofilm, invertebrates, vertebrates) and possible organic inputs (leaf litter, fescue, confined animal waste) from the recharge zone. 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**	**d ^{13}C**	**d ^{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		-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 (*C. stiladactyla*)	2	-22 $^+$/- 0.1	13.0 $^+$/- 1.8
Epigean crayfish (*O. 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 plant biosolids	1	-21.6	13.6
Poultry litter	1	-15.2	7.9
Cattle manure	1	-25.1	3.5

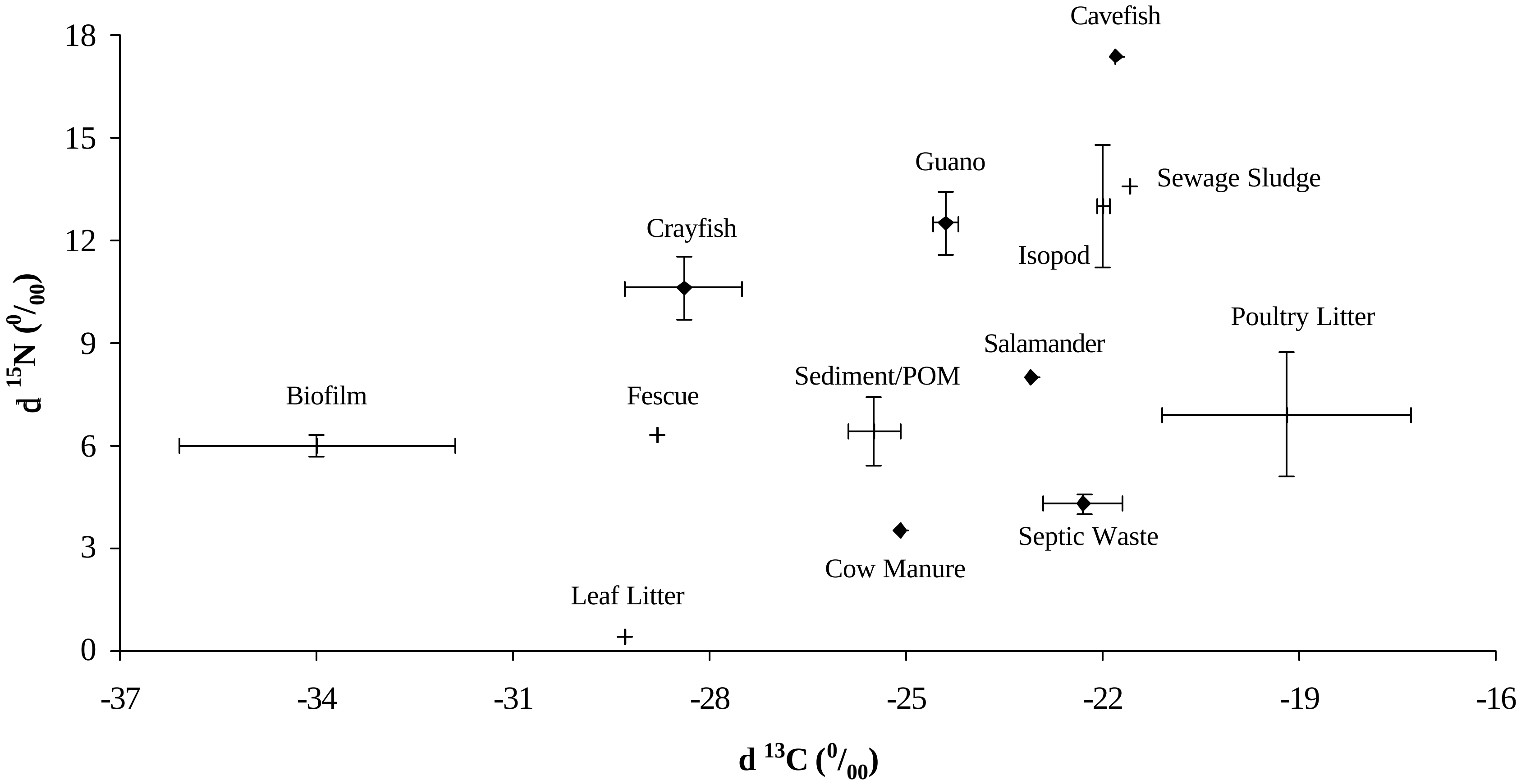

Figure 3. Dual-isotope crossplot of organic matter inputs (hardwood leaf litter, poultry litter, cow manure, municipal sewage biosolids, septic system biosolids, *M. grisescens* guano, fescue) and Cave Springs Cave ecosystem components (cave biofilm, cave sediment/POM, *O. punctimanus*, *C. stiladactyla*, *E. lucifuga*, and *A. rosae*). Data are mean carbon and nitrogen δ values ($^{o}/_{oo}$) with error bars (+/- 1 SE) for samples with replicates.

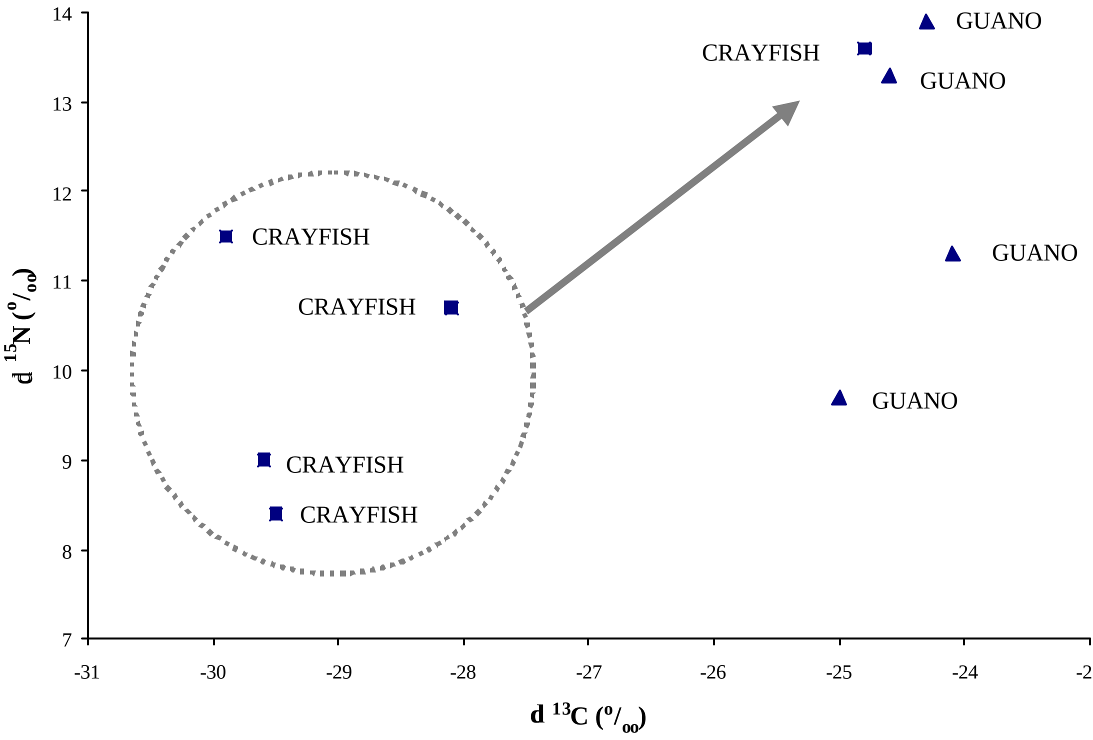

Figure 4. Dual-isotope crossplot of gray bat guano samples and crayfish samples (each replicate is a composite of spot-handed crayfish individuals) in Cave Springs Cave, which illustrates that crayfish cluster (encircled, mean δ^{13}C value of –29.3 and a mean δ^{15}N value of +9.9) around a probable food resource of leaf litter, and seasonally shift to a diet of bat guano.

Cavefish Population Monitoring

On February 16, 2000, a bioinventory of CSC was performed and 102 cavefish were counted. Approximate length of each individual and its position in the cave were also recorded (see Figure 6). Previous studies reported a significant trend of increase ($p = 0.004$, $r^2 = 0.95$) in the number of cavefish seen in Cave Springs Cave, and in 1999, the highest number ever reported was published (Brown *et al*., 1998; Graening and Brown, 1999). However, the low counts in 1998 and 2000 add variability to this trend that makes the linear regression non-significant ($p = 0.320$, $r^2 = 0.16$). The population censuses are summarized in Figure 5.

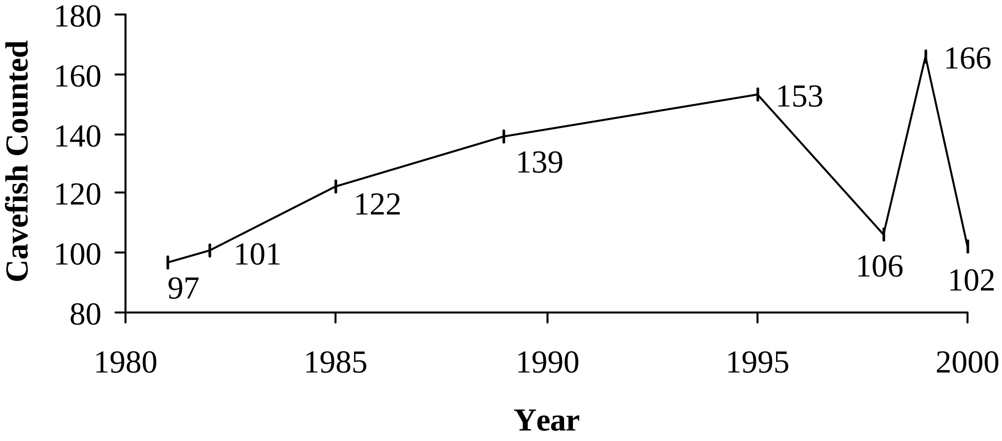

Figure 5. Summary of all visual surveys of the Ozark cavefish population in Cave Springs Cave, Arkansas, performed by the authors and colleagues (Brown and Willis, 1984; Brown *et al.*, 1998; Graening and Brown, 1999; this study).

Recharge Zone Geographical Information System

The ArcView 3.2 TM geographical information system that was created last year (Graening and Brown, 1999) has been refined and expanded, and is expected to be completed in 2001. Three different sets of aerial photographs have been acquired, digitized, and tiled, including black/white sets from the 1980's and 1990's, and a new color set that was taken in the fall of 1999. These three sets of aerial photographs are being analyzed for changes in land cover and land use over the last twenty years using image analysis (ArcView Image Analyst TM extension). Other themes (data layers) that have been acquired include: the digital elevation models and digital raster graphs (Bentonville South and Springdale 7.5 minute quadrangles); major road and water ways; the cave map; fracture traces/photolineaments, with and without buffers (50 and 500m); potentiometric surface (water table contours); point data (sinkholes, dye injections, sewage applications); and geological surfaces - depth to bedrock (Boone Formation), and depth to confining layer (Chattanooga shale).

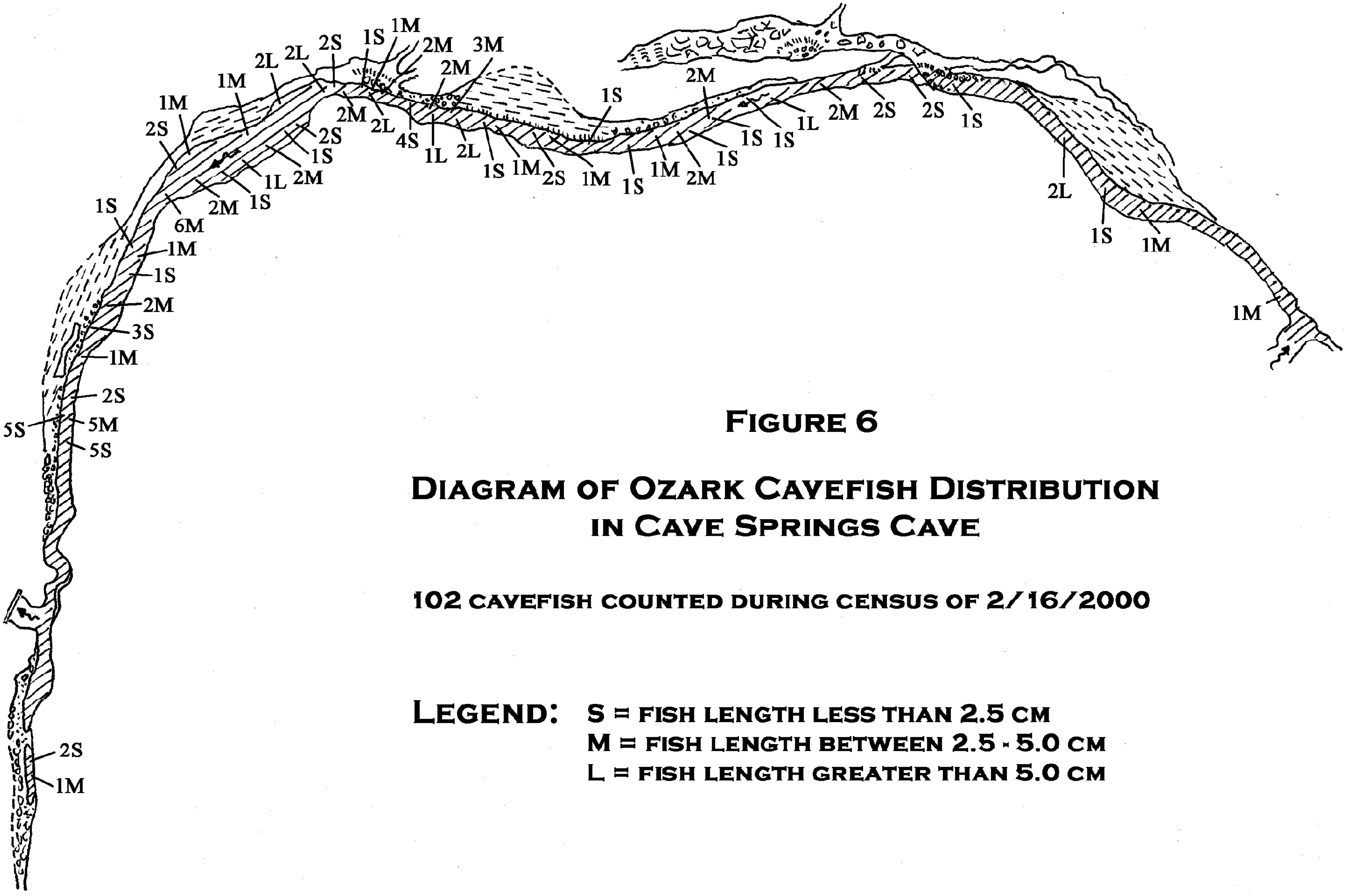

FIGURE 6

DIAGRAM OF OZARK CAVEFISH DISTRIBUTION IN CAVE SPRINGS CAVE

102 CAVEFISH COUNTED DURING CENSUS OF 2/16/2000

LEGEND:
S = FISH LENGTH LESS THAN 2.5 CM
M = FISH LENGTH BETWEEN 2.5 - 5.0 CM
L = FISH LENGTH GREATER THAN 5.0 CM

DISCUSSION

Pollution Effects

The Cave Stream Cave 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 some previous storm flow samples detected total phosphorous concentrations well above the State limit (Graening and Brown, 1999). 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. Mark Collier (pers. comm., 1999), landowner of Lake Keith, believes that the amount of algae in the lake has increased noticeably in the twenty years that his family has owned 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 this study and other monitoring efforts indicate that not only the Cave Springs Cave stream, but the entire Osage Creek drainage basin, was experiencing pollution enrichment and degradations in water quality. Because of the lack of sunlight in the cave ecosystem, the excessive growth of aquatic vegetation is not an issue. Nevertheless, organic pollution has negative effects on cave ecosystems, including alteration of the community assemblage, impoverishment of biodiversity, and increased risk of predation from surface animals. In the last three years, the authors have not seen the cave amphipods *Stygobromus ozarkensis* and *S. onondagaensis* in Cave Springs Cave, where they were formerly reported. Septic pollution has eradicated the invertebrates from other Ozark caves (*e.g.* Aley, 1976). However, troglobitic isopods (*Caecidotea* sp.) 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 troglobitic 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 cave 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 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. Further research is needed to determine the biological ramifications of augmenting the cave stream trophic web with anthropogenic nutrients.

Lead and zinc concentrations in water at the CSC resurgence have increased exponentially since the 1980's (Graening and Brown, 1999). Furthermore, cave isopods, crayfish, cavefish, and sediment have substantially higher concentrations of toxic metals than water samples. However, there was no 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 toxic metals in low concentrations may be bioaccumulated to lethal concentrations because of the longevity of cave-adapted organisms (Dickson *et al.*, 1979), and metals were detected in the tissues of cave-adapted isopods and one Ozark cavefish in CSC. The principal lead and zinc mining districts are found in Boone, Baxter, Lawrence, Marion, Newton, and Searcy counties (Adams, 1904; McKnight, 1935). The major zinc and lead deposits occur in the Everton and Boone formations, and are found north of the Boston Mountains in outcrops along the Eureka Springs escarpment (McKnight, 1935). While low-grade deposits do occur in the Boone Formation in Benton County, none of them has been commercially profitable (McKnight, 1935), so it is unlikely that the source of these metals is from natural deposits in the recharge area. The stable isotope analyses of the CSC food web supports the conclusion that surface pollutants, especially sewage waste, are influencing the Cave Springs Cave ecosystem.

Microbial Dynamics

The data collected from three years of monitoring in CSC indicates 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. Guano must not be the primary source of microbes because no significant difference existed between mean bacterial counts at the upstream station (upstream of bat roosts) and the downstream station (downstream of bat roosts). Total coliform densities were positively correlated to 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 into subsurface conduits during storm events. Furthermore, bacterial densities show a significant and distinct seasonal pattern, with winter/early spring having the lowest densities and summer/early fall having the highest densities (see Figure 7). 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), and not to timing of when cattle were grazing. A positive 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 these ground water habitats.

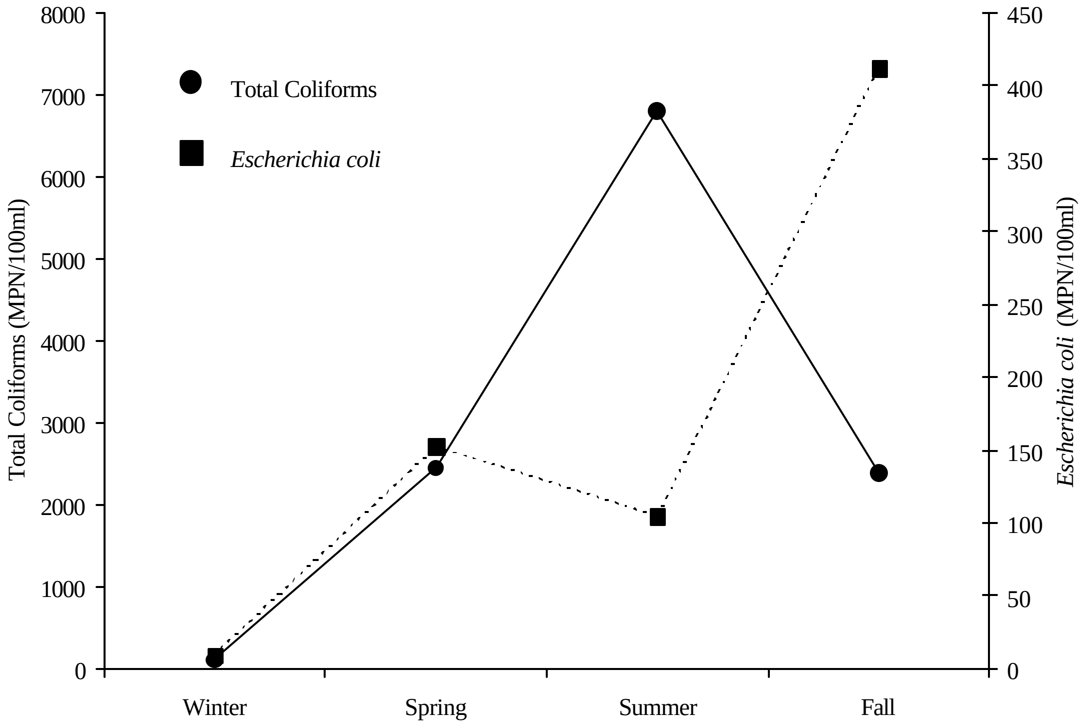

Figure 7. The seasonal pattern of microbial densities in Cave Springs Cave stream water is shown as determined from the negative binomial regression model fitted to the data from 1997 to 2000 (Brown *et al.*, 1998; Graening and Brown, 1999; this study). Total coliform cell densities were found to be lowest in winter and highest in summer. *Escherichia coli* cell densities were found to be lowest in winter and highest in the fall.

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, variation from the expected isotope ratios was explained by generalist feeding behavior and/or by the incorporation of ^{13}C depleted invertebrate chitin by predators (Sarbu *et al.*, 1996). Although omnivory is common in cave ecosystems, our 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 (see Figures 8 and 9). 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) reports 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.

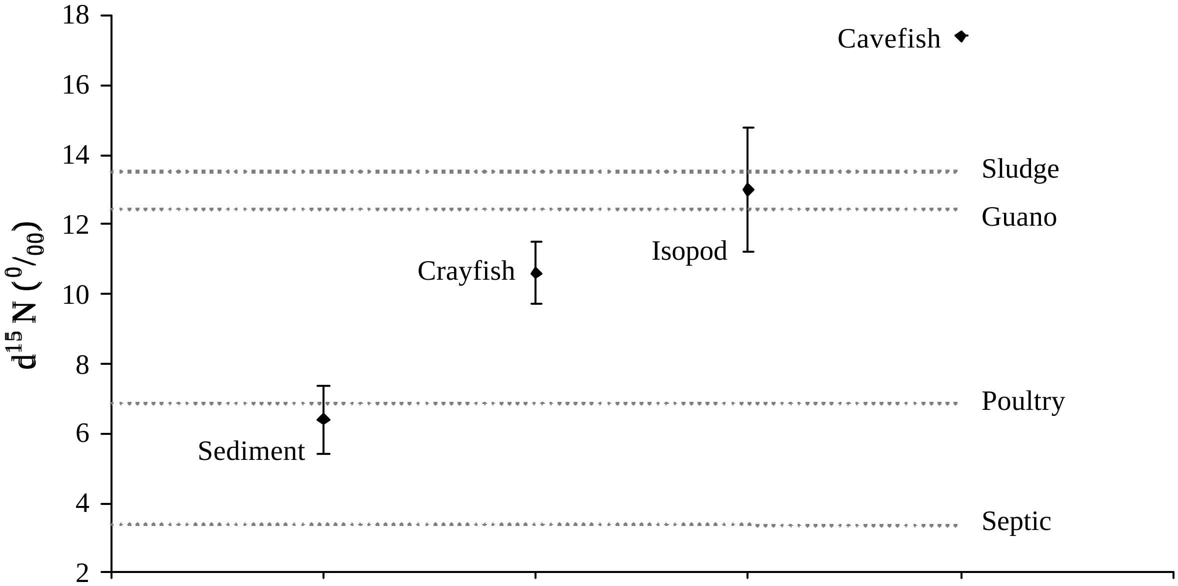

Figure 8. Univariate plot of δ^{15}N mean values ($^{o}/_{oo}$) +/- 1 SE for cave sediment/POM and cave fauna (*O. punctimanus, C. stiladactyla,* and *A. rosae*) in Cave Springs Cave relative to those derived for organic matter inputs from the cave recharge area.

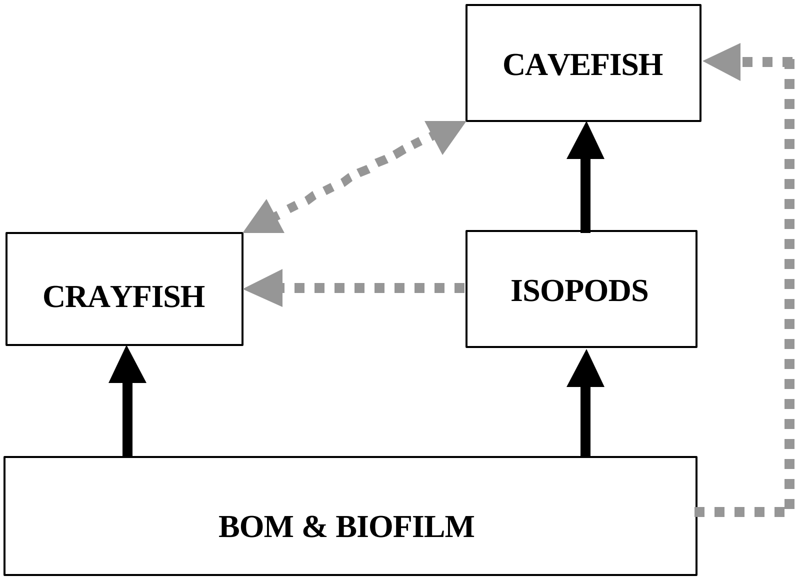

Figure 9. Food web model of Cave Springs Cave showing strong (heavy arrows) and weak (light arrows) trophic transfers. The food web has two distinct food chains (detritus to crayfish and detritus to isopods to cavefish) and three trophic levels (detrital base, invertebrate consumers, and a vertebrate predator). Detrivory, omnivory and ontogenetic diet shifts add undocumented complexity to this food web.

Of note is the high nitrogen stable isotope ratio of the cavefish ($\delta^{15}N = 17.4\ ^{o}/_{oo}$), which suggests that the Ozark cavefish is the top predator in this food web (see Figure 8). 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 decreased, starving animals showed a progressive increase in the $^{15}N/^{14}N$ ratio of their tissues as "lighter" ^{14}N 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). Stable isotope results also suggest that the primary diet of Ozark cavefish is cave isopods, a prey item reported by several researchers. Eigenmann (1909) reported *A. rosae* gut contents of juvenile crayfish, juvenile cavefish, crickets, and numerous isopods. Gut content analyses of 21 *A. rosae* 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 amblyopsids by Poulson (1963) indicate that copepods make 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 suspect because of their proximity to isopods in the dual isotope crossplot (Figure 3).

Carbon and nitrogen stable isotope assays 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 *O. luteus* and spot-handed crayfishes in an Ozark stream (Missouri) using stable isotope assays 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 with 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 Allen, 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 suspect.

As with isopods, crayfish do not appear to be relying primarily 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 dissolved organic matter (DOM) was not a significant input into the food web even though thousands of bats occupy the cave in summer months. However, these 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 values (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.

Ozark Cavefish Population Dynamics

The comparatively low count of Ozark cavefish obtained in 1998 and again this year (see Figures 5 and 6) are not explainable with any certainty. There are several possible reasons: 1) the method of counting is imprecise and the counts do not indicate real fluctuations in the population, 2) the cavefish migrate in and out of the cave passageway to which we have access, 3) the cave population has experienced at least two die-off events with subsequent replenishment between 1998 and 1999 censuses, perhaps by immigration into the cave from areas inaccessible to humans.

If the census technique is highly variable, or large migrations of cavefish occur, then the five population counts during the period 1981-1995 were remarkably consistent by chance alone. There was comparatively little variability in these counts. A correlation analysis of these data produced a statistically significant trend ($p = 0.004$, $r^2 = 0.95$). Reasons 1 and 2 cannot be ruled out even though they seem unlikely.

The third possible explanation is quite feasible. Our informal observation after conducting the 1995 census was that a large portion of the population consisted of large individuals. The size-frequency distribution of cavefish and other hypogean fauna are often skewed towards the larger size classes, and is indicative of k-selected traits in energy-limited habitats (Poulson, 1963; Cooper, 1975), although exceptions exist (Culver and Ehlinger, 1980; Streever, 1996). During subsequent censuses, we noticed an apparent decrease in the number of larger cavefish. Therefore, this year we recorded the apparent sizes of the cavefish as they were being censused. The size distribution was skewed toward smaller fish (see Figures 6 and 10). Poulson (1963) recorded five “year” classes of Ozark cavefish in this cave in 1955, 1957, 1959, and 1962 and found them to be consistently skewed towards the fourth (large) year class, as shown in Figure 11. The possibility that the cavefish population has experienced some large die-offs cannot be ruled out. There could have been some illegal collections of them, they could have died because of pollution events, or they could have been flushed out of the cave during storm events (documented in Graening and Brown, 1999). Our data confirm an increasing trend of eutrophication, accumulation of toxic concentrations of metals, and the presence of toxic, volatile organic compounds (Brown *et al*., 1998; Graening and Brown, 1999), suggesting that mortality due to pollutants is a distinct possibility.

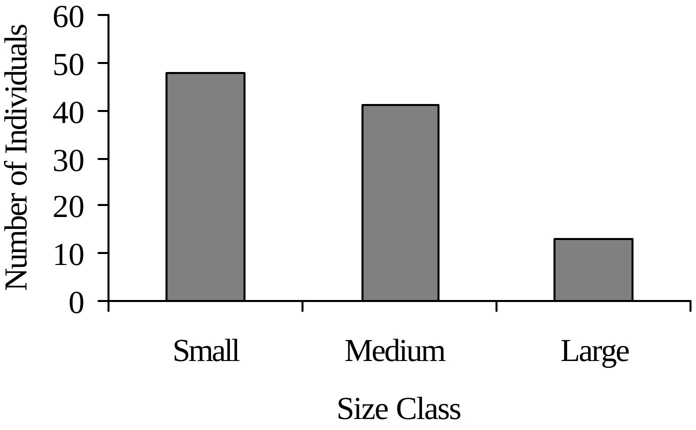

Figure 10. Population structure of the Ozark Cavefish population in Cave Springs Cave, Arkansas, based upon data from the 2000 census. Sizes were defined as: small – less than 2.5 cm; medium – between 2.5 – 5.0 cm; large – greater than 5.0 cm.

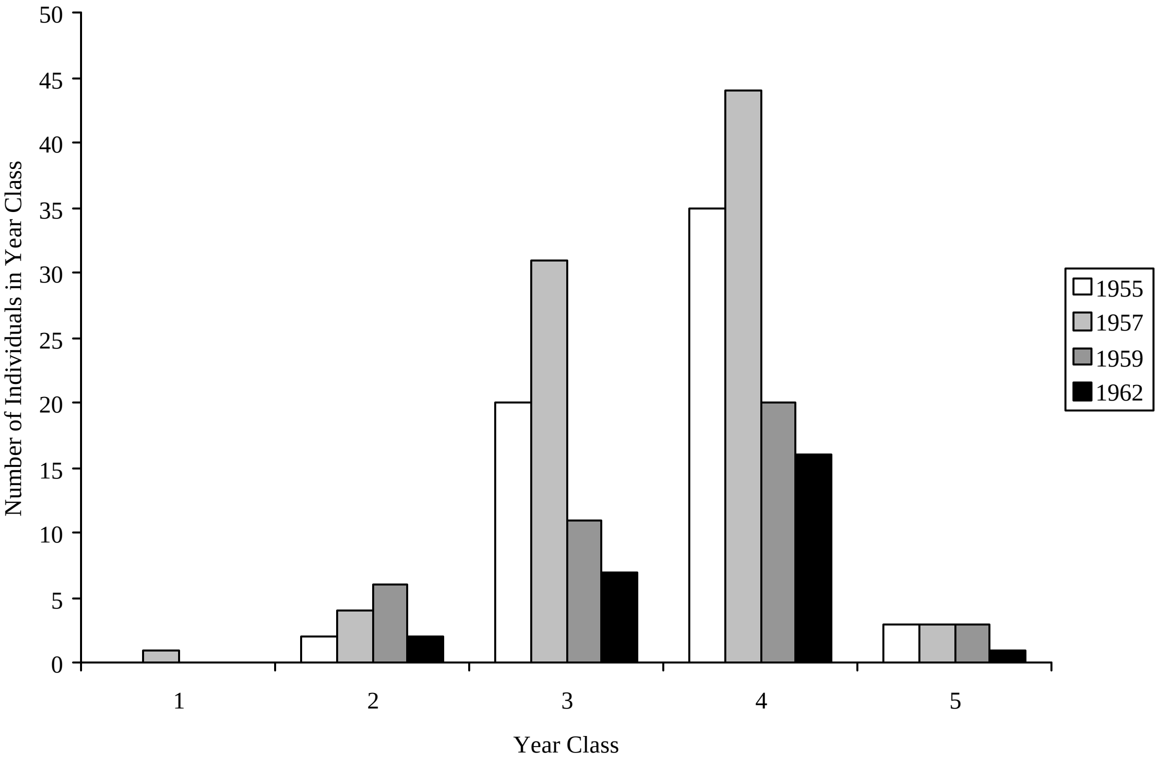

Figure 11. Graph of age structure (year class) of Ozark cavefish in Cave Springs Cave, Arkansas, for the years of 1955, 1957, 1959, and 1962, showing that the age structure of the deme is shifted towards older individuals. Data are from Poulson (1963).

MANAGEMENT RECOMMENDATIONS

1) Enforce the Arkansas Regulation 2 water quality standards for the streams in the recharge area (Cave Springs, Puppy Creek, Osage Creek, Cross Creek, and Spring Creek).

2) Formally designate the cave stream an “Ecologically Sensitive Water Body” because it has federal and state listed endangered species.

3) Upgrade the water body status to “Extraordinary Resource Water Body.”

4) Phase out the application of confined animal waste in the cave’s recharge zone, especially in the sensitive areas indicated on the map on the accompanying compact disc.

5) Revoke any existing permits and deny future permit applications to apply biosolids from municipal sewage treatment plants onto the recharge area.

6) Apply the most stringent requirements for new septic systems in the recharge zone, and require the rehabilitation or upgrading of existing septic systems.

7) Afford the cave entrance more protection from unauthorized visitors. Specifically, refurbish the fence in front of the cave mouth and install electronic surveillance equipment.

8) Investigate the ecotoxicological effects of the cave sediments that are contaminated with toxic metals.

9) Continue monitoring the environmental quality in the cave complex, especially during storm events.

10) Complete the identification of potential pollution sources and sensitive areas in the recharge zone.

11) Acquire cooperative agreements, titles, or conservation easements of lands in sensitive areas in the cave recharge zone.

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APPENDIX

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
Iron	Aluminum	0.7758	34	0.0000
Iron	Discharge	0.4778	34	0.0043
Iron	Ph	-0.6932	23	0.0002

Variable	By Variable	r	Count	p
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
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

Variable	**By Variable**	**r**	**Count**	**p**
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