MONITORING CAVEFISH POPULATION AND ENVIRONMENTAL QUALITY IN CAVE SPRINGS CAVE, ARKANSAS

A Final Report Submitted to the

ARKANSAS NATURAL HERITAGE COMMISSION

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Monitoring Cavefish Population and Environmental Quality

in Cave Springs Cave, Arkansas

a final report submitted to the

Arkansas Natural Heritage Commission

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EXECUTIVE SUMMARY

Cave Springs Cave, Benton County, Arkansas, was monitored from October 1997 to June 1998 to determine the chemical and physical environmental quality and the status of the population of threatened Ozark cavefish, *Amblyopsis rosae*. The majority of the chemical parameters measured were indicative of adequate environmental quality in the Cave Springs Cave ecosystem. However, several significant problems were revealed. A trend analysis of known water quality studies of this cave complex suggests that many organic and inorganic chemicals have increased in concentration in the last 14 years. This ecologically sensitive water body did not meet Arkansas water quality regulations for fecal coliform densities, and copper, selenium, and lead concentrations exceeded limits for exposure to aquatic life. The geometric mean total coliform count for base flows was 500 MPN/100ml, and during the March storm event, coliform densities exceeded 20,000 MPN/100ml. When compared to the national primary drinking water regulations, this spring water exceeds the maximum contaminant levels (MCL) for turbidity, nitrite, total coliforms, and *Escherichia coli*, and approaches the MCL's for copper and zinc. During the March storm event, *Escherichia coli* densities exceeded 5,000 MPN/100ml. During the June storm event, nitrite levels reached 2 mg/L, twice the MCL for national drinking water standards. Nitrite toxicity is known to cause severe anemia in fishes and damage their tissues. One semi-volatile organic, Di (2-ethylhexyl) phthalate (DEHP), was found in significant concentration (500 ug/kg) in resident crayfish tissue. DEHP is known to bioaccumulate in fish tissue, and cause reproductive damage and reduced fertility in fish. A visual survey was performed on January 25, 1998, and 106 cavefish were sighted. This survey indicated a 30% decline in the Cave Springs Cave population. A comparison of base-flow sampling results at two different locations -- upstream and downstream of bat rookeries -- indicates that the majority of coliform bacteria are not attributed to bat guano. These findings suggest that bacteria are being imported into the cave stream from the recharge zone. The high nitrite, total coliform, and *E. coli* counts suggest that septic system leakage or the land application of animal waste is involved. Continued water quality monitoring and surveys of the Ozark cavefish population are recommended. Future monitoring should focus on storm events and parameters that measure pollutants originating from the recharge zone and their effect on the cave ecosystem. As well, investigation into the nature of the pollutants from the recharge zone is suggested.

INTRODUCTION

Ozark cavefish (*Amblyopsis rosae*) have been of considerable interest to biologists and others since their discovery (Garman, 1889) and formal description (Eigenmann, 1898) about a century ago. Dr. Arthur V. Brown and his students first became interested in cavefish and their potentially endangered status in 1976. They initially performed extensive surveys of caves and similar habitats throughout their range in Missouri, Arkansas, and Oklahoma (Brown and Brown, 1981; Brown *et al*., 1982; Brown and Willis, 1984; Willis, 1984; Willis and Brown, 1985). Dr. Brown proposed the fish for consideration by the U.S. Fish and Wildlife Service in September 1982 and it was formally recognized as "Threatened" in the Federal Register on November 1, 1984 (49 FR 43965). Dr. Lawrence D. Willis, the student that performed most of the work with Dr. Brown, was contracted by the Service to write the Ozark Cavefish Recovery Plan (Willis, 1985, 1989). Since the initial surveys, Dr. Brown and his students have performed periodic status surveys for Ozark cavefish (Brown and Todd, 1987; Brown, 1991). In April 1995, Dr. Willis, Mr. Stan Todd, and Dr. Brown surveyed the population of cavefish in Cave Springs Cave when requested to do so, and reported 153 cavefish sighted, the most ever seen by them.

Ozark cavefish are predators and do not exist alone in their cave habitats. Although caves are generally depauperate, cave ecosystems which support Ozark cavefish generally have communities which include several species each of bats, crayfish, isopods, amphipods, dipterans (flies), beetles, crickets, salamanders, and other fish (Barr, 1967; Black, 1971; Poulson, 1976; Culver, 1982; Willis and Brown, 1985). Attempted management of cavefish without careful consideration of the rather fragile communities and ecosystems (including water quality) of which they are a part would be futile. During cave surveys Dr. Brown and his students kept extensive field notes about cave habitats and organisms other than the cavefish.

Cave communities are almost totally dependent on allochthonous (imported) organic matter since there is no light for photosynthesis. Chemosynthesis rarely supplies much trophic support in well-aerated habitats such as limestone solution caves. The allochthonous matter is usually in the form of dissolved and fine particulate organic carbon that is transported hydrologically, leaves that are blown into sink holes, and guano deposited by bats and other animals (e.g., rodents and crickets, Brussock *et al*., 1988; Brown *et al*., 1994). This material is of low food quality, calorically and otherwise (Brown and Fitzpatrick, 1978; Valett and Stanford, 1987) and nearly always is low in quantity as well. Brown *et al*., (1994) proposed that caves are "low payoff-low risk" environments and that troglobites (obligate cave dwellers) are efficiency strategists. They suggested that troglobites are able to escape predation by being efficiency experts that allows them to live in habitats that have too little food to support less efficient species. If the food supply in the cave is further decreased, there may be too little to support even the efficient troglobites. On the other hand, if the "payoff" is increased by enriching the food supply in caves, then the "risk" of predation for the troglobites may also be increased because less efficient predators can invade the cave environment. Additions of organic carbon from septic tanks or surface application of confined animal wastes to pastures could subsidize troglophilic (cave opportunists) predators and jeopardize troglobitic species. In other words, if caves have enriched environments, surface crayfish and sculpins can make a living in them and occasionally eat cave crayfish and cavefish (Brown *et al*., 1994).

Enrichment has other effects. 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. Self-purification is possible if the indigenous bacteria have enough oxygen to metabolize the organic pollutants; yet the necessary conditions -- high dissolved oxygen levels, the absence of inhibiting chemicals, and dilute pollutants -- are rarely fulfilled (Maire and Pomel, 1994).

Much of the interest in cave-adapted species is due to the fascinating suite of characteristics that they have evolved to enable them to live in caves. Most of these traits are adaptations which enable them to live in an environment which is severely food-resource limited. Not only are they blind and without skin pigments, they lack fright response, have reduced metabolic rates and lower activities, much longer life spans, are of less robust body form, reproduce less frequently, and allocate much less energy to reproduction than their surface dwelling relatives (Woods and Inger, 1957; Poulson, 1963; Cooper and Cooper, 1976; Culver, 1982; Holsinger, 1988). The same traits that make them interesting also make them vulnerable to changes in their environment and subject to possible extinction.

It is well known that the best form of management for species, endangered or otherwise, is to protect their preferred habitat. This is even truer for cave species because they are so dependent on specific, unique, rare, insular (island-like) habitats. For this reason, Brown and others in very early investigations proposed that Logan Cave and Cave Springs Cave be purchased by public agencies for their protection. They also recommended purchase, or some kind of cooperative protection agreements, for several other habitats to protect cave species (e.g., see Brown and Willis, 1984; Willis, 1984).

The population of Ozark cavefish in Cave Springs Cave is the largest known for this species (Willis and Brown, 1985; Brown and Todd, 1987; Brown, 1991). Dr. Brown and others have monitored this population since 1980 and this deme had appeared to be steadily increasing. During most of this 15-year period, access has been strictly limited by the owners and management agencies, and the population has been allowed to recover, as planned (Willis, 1985, 1989).

OBJECTIVES

I) Determine the environmental quality at Cave Springs Cave
- A. Determine current water quality status
- B. Determine past water quality status
  - 1. Perform literature review
  - 2. Compare past data to current data and determine if trend(s) exist
- C. Perform microbiological assays
  - 1. Quantify microbial populations
  - 2. Determine source of microbes
- D. Determine storm response
  - 1. Determine how water chemistry variables respond
  - 2. Determine how microbial populations respond
- E. Determine if toxins exist in ecosystem
  - 1. Analysis of crayfish tissue for pesticides
  - 2. Analysis of crayfish tissue for volatile organic chemicals

II) Perform a census of the Ozark cavefish population

This survey of Cave Springs Cave is in response to a request from Mr. John Beneke, Arkansas Natural Heritage Commission, and was accompanied by selected tests of the physical and chemical water quality during the same period. Mr. Beneke has realized that real estate developments and other activities have increased in the aquifer recharge zone at this location, creating a need for closer monitoring. Another objective of this study was to compile all available information for this cave system that is relevant to this project. The compiled data in conjunction with results of our chemical analyses will then constitute a baseline of information for comparison of continued monitoring of water quality and cavefish in Cave Springs Cave. Troglophilic crayfish appear to have increased in Cave Springs Cave during our 15-year survey period. Invasion by troglophiles could threaten troglobites and may be due to organic enrichment. Thus, dissolved and fine particulate organic carbon assays were included in the chemical analyses in this study. Troglophilic crayfish were collected from the cave for tissue analysis for chlorinated hydrocarbons and pesticides. This is important for several reasons. Cavefish are long-lived top predators and are therefore subject to greater harm due to biological magnification of these substances. The troglophilic crayfish are preyed upon by cavefish and are an excellent choice for these tests because they are abundant and large enough to provide adequate sample size.

Because of the significant impact of storm recharge on the ground water in caves, it is important to have storm-flow as well as base-flow water quality data. For this project, we obtained baseline data during peak storm flow and during base flow. The potential impact of the bat guano on the water chemistry requires that samples also be collected above and below the bat colony in order to represent water quality changes caused by the bat guano separately from those caused by conditions in the aquifer recharge zone. During this study we could not sample upstream of the bat colonies during high flows. This would require use of an automatic sampling device that we did not have, and would require more frequent access to the cave interior.

SITE DESCRIPTION

Cave Springs Cave is located in Cave Springs, Benton County, Arkansas, at the following coordinates: latitude = 36 15’ 40” and longitude = 94 13’ 37”, NE ¼, SE ¼, sec. 1, T.18 N., R.31 W., Benton County (007), HU: 11110103, Bentonville South Quadrangle. The Cave Springs Cave resurgence lies in the Osage Creek drainage basin and the larger Illinois River watershed. The cave complex lies between the St. Joe limestone geological formation and the Boone formation, which is a Mississippian age, chert-bearing limestone with many faults, joints, and fractures (Willis, 1984). In general, it is part of the Springfield Plateau of the Ozark Highlands, which lies in the western portion of a large karst area extending through the central United States (Woods and Inger, 1957).

The cave complex has a diffuse recharge with an estimated recharge area of 41 km^2 (15 mi^2), based upon the recharge area boundary delineation of 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 average annual temperature is 14.1 ^oC, with a seasonal variation of about 1 ^oC during the year. The mean annual discharge during the study period was 5.4 m^3/min(3.1 ft^3/s). Cave Springs Cave contains several rare and endangered species, including the Ozark cavefish (*Amblyopsis rosae)*, gray bats (*Myotis grisescens)*, and the grotto salamander (*Typhlotriton spelaeus*) (Brown *et al*., 1994; Williams, 1991).

According to Arkansas State Regulation No. 2., the Cave Springs Cave resurgence has 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 (Pollution Control and Ecology Commission, 1998). It has not, however, been given the designation, "extraordinary resource water," which would give it further protection. All the necessary permits for this study were obtained from the Arkansas Natural Heritage Commission, Arkansas Game and Fish Commission, and the U.S. Fish and Wildlife Service.

METHODS

Environmental Quality

Base-flow samples were collected at the spring orifice in the sluice leading to the water wheel, monthly from November 1997 through June 1998, and downstream of all bat rookeries. Base-flow samples were also collected twice, December 17, 1997, and January 25, 1998, at the waterfall at the very head of the accessible cave, approximately 0.5 k from the cave mouth, and upstream of all bat rookeries. Four storm-flow samples were collected at the spring orifice during two different storm events (March 5-11, 1998 and June 8-10, 1998), before, during, and after the peak discharge. All water samples were analyzed for the following parameters: temperature, conductance, pH, turbidity, ammonia + ammonium nitrogen, nitrate, nitrite, total Kjeldahl nitrogen (TKN), dissolved reactive phosphate (orthophosphate), total phosphorus, total organic carbon (TOC), dissolved organic carbon (DOC), dissolved metals (aluminum, arsenic, cadmium, copper, iron, lead, manganese, nickel, selenium, silver, zinc), total coliforms, and *Escherichia coli*. The data and information gained from these samples allowed preliminary interpretations concerning the state of the water quality and the potential of future contamination to the system. Analytical procedures followed approved U.S. Environmental Protection Agency methods and appropriate quality assurance and quality control measures. Please refer to the Quality Assurance Project Plan for specific methods, citations, and quality controls.

Stage and discharge measurements were added to the present water quality analyses, and such metrics will aid future studies. The stage/discharge relationship is especially useful in the flow-dependent computation of pollutant loading. Stage was read on a gauge *in situ* in the pool at the cave orifice, and discharge was computed from data from a previous USGS study of the site (Dr. Van Brahana, USGS and Department of Geology, U. of A., unpublished data). The Appendix has the stage/discharge relationship.

Cavefish Population Monitoring

The visual survey was performed by the same method as previous surveys and included at least two of the same people used in previous surveys. Using bright lights, three people moved slowly upstream and counted cavefish as they were sighted. This method can produce fairly reliable quantitative population information with minimal impact on the cave habitats and their inhabitants, endangered or otherwise. Despite their contrary interpretations, the data by Means (1993) and Means and Johnson (1995) for Logan Cave populations of Ozark cavefish indicate that the number of cavefish *observed* in a given cave does not vary much, even when the population is very small, and is therefore a good estimator of population status. In the larger Cave Springs Cave population, the method is probably even stronger because the percent effect of sighting or not sighting a few fish is much smaller. For example, if five fish are seen, or not seen, in a population where about 25 fish are normally seen (like Logan Cave) this represents an error of about 20%. In a population where 125 fish are normally seen a deviation of five fish represents an error of less that 5%, a level generally considered statistically acceptable. Important management decisions for fish and wildlife are often based on much less precise population data, for a variety of reasons. A change in survey results with these methods are more likely to be real representations of the population change, due possibly to habitat degradation from land use changes (e.g. development). Dr. Lawrence D. Willis, currently employed by the Virginia Water Quality Board, Mr. C. Stanley Todd and Mr. Brian Wagner, both of the Arkansas Game and Fish Commission, assisted with the surveys. They are both experienced with every aspect of this method and have performed previous surveys in Cave Springs Cave as well as the other cavefish habitats (Brown and Todd, 1987: Willis and Brown, 1985).

RESULTS

Current Environmental Quality Status at Cave Springs Cave

The results of the water quality analyses are shown in Tables 1 through 3, following. Dissolved oxygen, pH, conductivity, temperature, ortho-phosphate, total phosphorous, total and dissolved organic carbon, and the concentration of most of the dissolved metals met state and federal water quality standards. However, total coliform densities, copper, selenium, and lead concentrations did not meet Arkansas State Water Quality Standards. Turbidity, nitrite, total coliform and *Escherichia coli* densities did not meet National Drinking Water Standards. One volatile organic compound, Bis (2-ethylhexyl) phthalate, was found in significant concentration in resident crayfish tissue.

Arkansas State Regulation 2 requires that ecologically sensitive water bodies and primary contact waters, such as the resurgence at Cave Springs Cave, do not exceed fecal coliform counts of 200 MPN/100ml between April 1 and September 30 and never exceed 1,000 MPN/100ml (Pollution Control and Ecology Commission, 1998). During this study, two base-flow samples and both storm events exceeded this limit, and during the March storm event, exceeded 5,000 total coliform MPN/100ml. The geometric mean total coliform count for base flows was 500 total coliform MPN/100ml, and during the first storm event monitored, counts over 20,000 total coliform MPN/100ml were observed. Furthermore, concentrations of copper, selenium, and lead exceeded the acute and chronic exposure criteria for aquatic life (Pollution Control and Ecology Commission, 1998). The criteria were computed using a hardness value of 150 mg/L as $CaCO_3$ (USGS, 1996, unpublished data). The average base-flow concentrations of copper and selenium exceeded both the acute and chronic criteria, while lead concentrations once exceeded the chronic limit criteria for aquatic life. These standards are set for surface streams and their fauna, and little is known about ground-water toxicology and no standards exist for subterranean fauna. The Cave Springs Cave resurgence water met all other state criteria for the Ozark Highlands ecoregion, OH-1 (Pollution Control and Ecology Commission, 1998).

When compared to the national primary drinking water regulations, this spring water exceeds the maximum contaminant levels (MCL) for turbidity, nitrite, total coliforms, and *Escherichia coli*, and approaches the MCL's for zinc and copper (U.S. EPA, 1998a). During the second of two storms monitored (6/9/98 - 6/10/98), nitrite concentrations reached almost 2 mg/L as nitrogen, twice the limit set by the U. S. EPA for national drinking water standards (U.S. EPA, 1998a). While there does not appear to be any consistent aquatic life standards for nitrite-N at this time, nitrite toxicity in fishes is well documented. Nitrite toxicity causes severe anemia and other circulatory problems as well as tissue damage in fishes (Eddy and Williams, 1988; Mitchell, 1997).

Table 1. Summary of Baseflow Water Quality Data at Cave Springs Cave, 1997-1998

	Location **Time** **Date**	mouth 11:00 11/24/97	mouth 17:00 12/17/97	deep cave 18:00 12/17/97	mouth 11:30 1/25/98	deep cave 14:00 1/25/98	mouth 13:00 2/18/98	mouth 20:00 3/5/1998	mouth 15:00 4/15/98	mouth 14:00 5/14/98	mouth 10:30 6/8/98
Physical											
Air Temp.	Celsius	---	---	---	---	---	10.6	3	25	27	18
Air Pressure	mm of Hg	---	---	---	---	---	758	760	752	761	760
Water Temp.	Celsius	13.9	13.4	13.4	14.5	14.5	14.2	14.1	13.9	14.6	14.1
Water Stage	m	3.109	3.115	n/a	3.252	n/a	3.243	3.255	3.377	3.252	3.203
Discharge	m^3/min	1	1	n/a	6	n/a	6	7	11	6	5
Spec.Cond.	uS/cm	310	320	325	290	300	320	290	300	240	345
Turbidity	N.T.U.	0.2	1	3.4	0.01	0.01	1.4	4	1	1	0.9
Ph, field		7.3	7.5	7.2	6.9	6.9	7.1	7.2	7.1	6.9	6.9
Diss. Oxygen	mg/l	9.4	9.5	9	10.3	8	9.4	10.6	9.3	9.3	9.5
Dissolved Metals											
Aluminum	ug/l	< 11	22	48	13	14	13	20	14	< 11	< 11
Arsenic	ug/l	< 12	< 12	< 12	< 12	< 12	< 12	< 12	< 12	< 12	< 12
Cadmium	ug/l	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
Chloride	mg/l	8.868	8.952	9.469	8.055	8.115	9.518	6.62	6.08	9.422	6.96
Copper	ug/l	12	27	29	24	34	28	20	47	16	< 6
Iron	ug/l	< 3	6	33	< 3	8	5	13	6	< 3	< 3
Lead	ug/l	< 11	< 11	< 11	< 11	14	12	< 11	< 11	< 11	< 11
Manganese	ug/l	< 0.6	0.6	2.9	0.6	1.5	1.7	0.7	1.2	< 0.6	< 0.6
Nickel	ug/l	< 24	< 24	< 24	< 24	< 24	< 24	< 24	< 24	< 24	< 24
Selenium	ug/l	32	< 15	< 15	< 15	< 15	< 15	15	< 15	< 15	< 15
Silver	ug/l	< 15	< 15	< 15	< 15	< 15	< 15	< 15	< 15	< 15	< 15
Sulfate	mg/l	---	7.13	7.319	3.6	4.67	3.762	3.705	3.465	2.585	3.17
Zinc	ug/l	< 6	18	18	19	29	31	25	83	13	8
Nutrients											
T.O.C.	mg/l	1.32	4.12	3.55	0.74	1.04	0.34	0.99	1.87	2.01	1.32
D.O.C.	mg/l	---	3.4	2.79	---	---	---	---	---	1.77	---
Ammonia-N.	ug/l as N	18	55	52	< 9	< 9	< 9	< 9	11	25	29
Nitrite-Nitrogen	ug/l as N	< 3	< 3	< 3	< 3	< 3	< 3	< 3	41	< 3	< 3
Nitrate-Nitrogen	mg/l as N	4.276	4.807	4.657	6.395	5.57	6.59	6.295	6.25	6.207	5.51
T.K.N.	mg/l as N	0.12	0.13	0.17	0.07	0.13	< 0.03	0.12	0.03	0.026	0.29
Total Phosph.	mg/l as P	0.027	0.029	0.027	0.023	0.023	0.089	0.075	0.027	0.043	0.017
Ortho-Phosp.	mg/l as P	0.026	0.029	0.028	0.034	0.031	0.05	0.044	0.021	0.025	0.019
Microbial											
E.coli , Colilert	MPN/100ml	5.3	36.5	27.1	3.1	2	7.5	15	271	129.8	207
Total Coliforms	MPN/100ml	222	1445	1184	344	34.4	129.8	165.2	4060	1652	5600

Table 2. Summary of Water Quality Data at Cave Springs Cave during a storm event (3/5/1998 to 3/11/98) with 7.62 cm rain accumulation.

	Date	3/5/98	3/7/98	3/8/98	3/9/98	3/11/98
Physical						
Air Temp.	Celsius	3	6	2.2	-6	-5
Air Pressure	mm of Hg	760	759	750	765	778
Water Temp.	Celsius	14.1	14.2	14	13.9	14
Water Stage	m	10.68	10.7	11	11.1	10.99
Discharge	m^3/min	4	4	6	7	6
Spec.Cond.	uS/cm	290	290	285	260	260
Turbidity	N.T.U.	4	1	3.2	8	1.4
Ph, field	---	7.2	7.2	6.8	6.8	6.8
Diss. Oxygen	mg/l	10.6	10.2	8.4	9.4	9.2
Dissolved Metals						
Aluminum	ug/l	20	< 11	< 11	< 11	< 11
Arsenic	ug/l	< 12	< 12	< 12	< 12	< 12
Cadmium	ug/l	< 1	< 1	< 1	< 1	< 1
Chloride	mg/l	6.62	7.735	7.145	6.575	7.44
Copper	ug/l	20	< 6	19	< 6	< 6
Iron	ug/l	13	< 3	7	40	8
Lead	ug/l	< 11	< 11	< 11	< 11	< 11
Manganese	ug/l	0.7	< 0.6	< 0.6	< 0.6	< 0.6
Nickel	ug/l	< 24	< 24	< 24	< 24	< 24
Selenium	ug/l	15	< 15	< 15	13.2	18.7
Silver	ug/l	0	0	0	0	0
Sulfate	mg/l	3.705	4.135	4.37	4.055	4.37
Zinc	ug/l	25	< 6	16.9	< 6	7
Nutrients						
T.O.C.	mg/l	0.99	0.68	1.18	3.14	2.02
D.O.C.	mg/l	---	0.58	---	2.46	1.79
Ammonia-N.	mg/l as N	< 0.009	< 0.009	0.017	< 0.009	0.011
Nitrite-Nitrogen	mg/l as N	< 0.003	< 0.003	0.003	0.003	0.001
Nitrate-Nitrogen	mg/l as N	6.295	5.895	6.17	7.2	6.96
T.K.N.	mg/l as N	0.12	0.15	1.3	0.22	0.06
Total Phosph.	mg/l as P	0.075	< 0.021	0.069	0.025	0.05
Ortho-Phosp.	mg/l as P	0.044	0.098	0.088	0.061	0.037
Microbial						
E.coli , Colilert	MPN/100ml	15	144.5	5040	1652	150
Total Coliform	MPN/100ml	165.2	831	>20050	7380	697

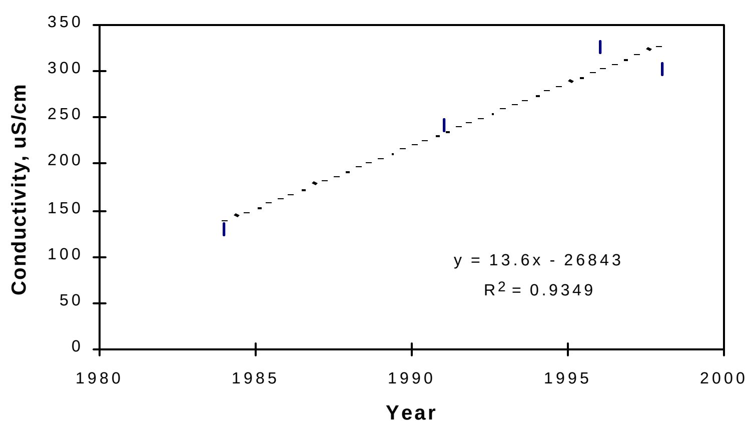
Table 3. Summary of Water Quality Data at Cave Springs Cave during a storm event (6/8/98 to 6/10/98) with 2 cm rain accumulation.

	Date	6/8/98	6/9/98	6/9/98	6/10/98	6/10/98
Physical						
Air Temp.	Celsius	18	26	29	27	27
Air Pressure	mm of Hg	760	762	761	762	760
Water Temp.	Celsius	14.1	14.3	14.5	14.7	14.8
Water Stage	m	3.2	3.2	3.2	3.2	3.2
Discharge	m^3/min	5	5	5	5	5
Spec.Cond.	uS/cm	345	350	350	350	350
Turbidity	N.T.U.	0.9	1	0.5	0.5	0.4
Ph, field	---	6.9	6.9	6.8	6.8	6.9
Diss. Oxygen	mg/l	9.5	9	9.6	10	10.5
Dissolved Metals						
Aluminum	ug/l	< 11	< 11	< 11	< 11	< 11
Arsenic	ug/l	< 12	< 12	< 12	< 12	< 12
Cadmium	ug/l	< 1	< 1	< 1	< 1	< 1
Chloride	mg/l	6.96	6.97	8.849	6.12	8.97
Copper	ug/l	< 6	21	7	< 6	< 6
Iron	ug/l	< 3	< 3	< 3	< 3	< 3
Lead	ug/l	< 11	< 11	< 11	< 11	< 11
Manganese	ug/l	< 0.6	< 0.6	< 0.6	< 0.6	< 0.6
Nickel	ug/l	< 24	< 24	< 24	< 24	< 24
Selenium	ug/l	< 15	< 15	< 15	< 15	< 15
Silver	ug/l	< 15	< 15	< 15	< 15	< 15
Sulfate	mg/l	3.17	2.69	2.397	2.79	2.63
Zinc	ug/l	8	13	7	< 6	< 6
Nutrients						
T.O.C.	mg/l	1.32	1.23	1.24	0.76	0.79
D.O.C.	mg/l	---	---	---	---	---
Ammonia-N.	mg/l as N	0.029	0.038	0.011	0.052	0.013
Nitrite-Nitrogen	mg/l as N	< 0.003	< 0.003	< 0.003	< 0.003	1.97
Nitrate-Nitrogen	mg/l as N	5.51	5.838	5.87	8.774	5.395
T.K.N.	mg/l as N	0.29	0.54	0.08	0.18	0.06
Total Phosph.	mg/l as P	0.017	0.023	0.03	< 0.021	< 0.021
Ortho-Phosp.	mg/l as P	0.019	0.026	0.023	0.025	0.017
Microbial						
E.coli , Colilert	MPN/100ml	207	306	384	222	207
Total Coliform	MPN/100ml	5600	6970	6970	6240	6590

Analysis of Past Data and Eutrophication Trend

A review of the literature produced three other known water quality studies of this site (Willis, 1984; Williams, 1991;USGS, unpublished data, 1996). When these data are averaged and compared to the averaged data from this study, a statistically significant upward trend was observed in many of the water quality parameters, including conductivity, nitrate, total phosphorous, ammonia, and some dissolved metals (see Table 4). Figures 1 through 5 show concentrations of selected parameters from all known studies and the corresponding linear regressions and R-squared values of the data sets. Eutrophication is commonly defined as a process that increases the nutrients, especially nitrogen and phosphorous, in an aquatic system, with a corresponding increase in algae populations and a decrease in diversity (Morris, 1992).

Figure 1. Comparison of conductivity between past and current studies



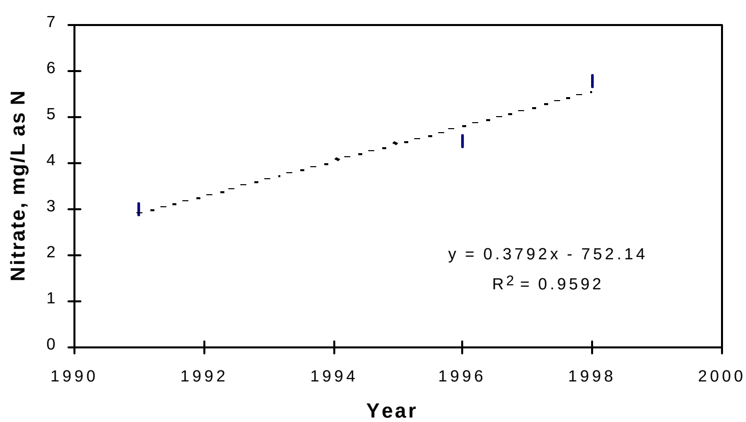


Figure 2. Comparison of nitrate concentrations between past and current studies

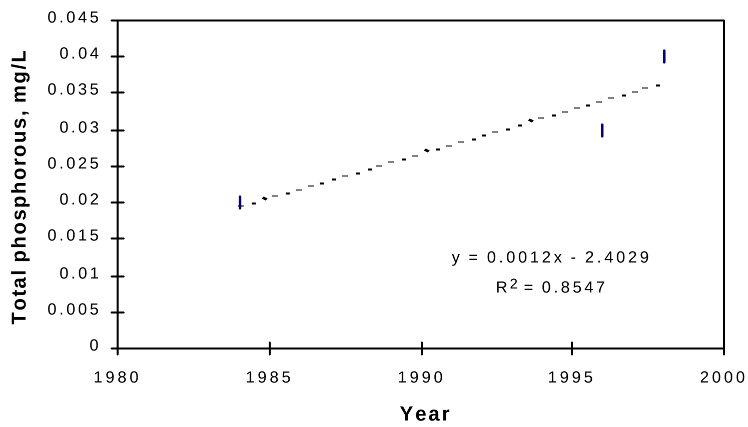


Figure 3. Comparison of total phosphorous concentrations between past and current studies

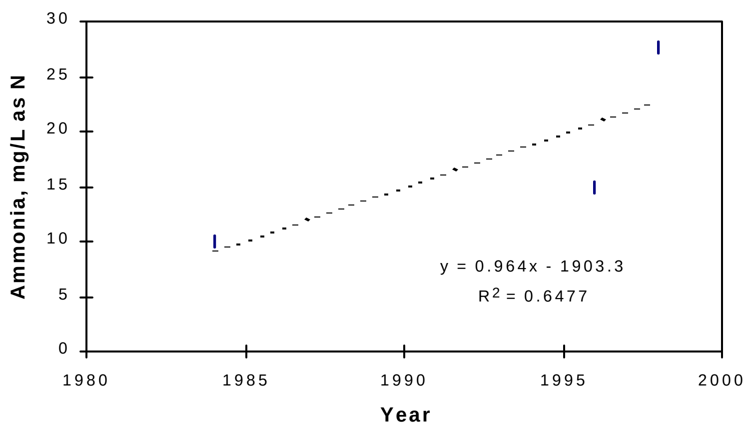


Figure 4. Comparison of ammonia concentrations between past and current studies

Figure 5. Comparison of copper and zinc concentrations between past and current studies

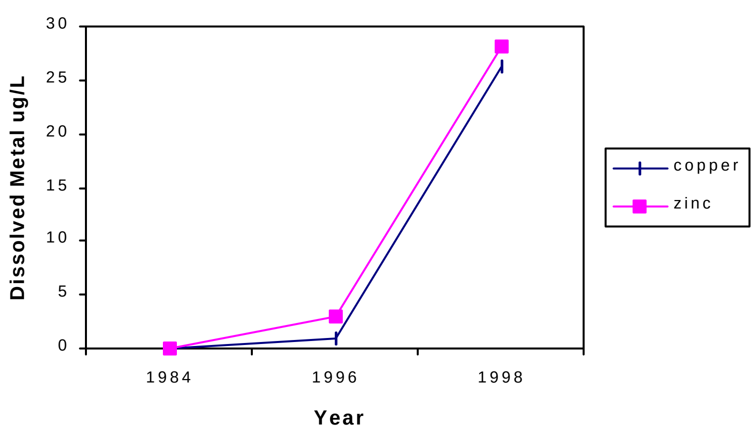


Table 4. Comparison of water quality data from previous and current studies at Cave Springs Cave, Arkansas

	SOURCE DATE	Willis,'84 5/1/1984	Williams, 1991 4/2/1991	 4/16/91	USGS '96 8/29/96	This study 11/97 to 6/98 Averaged	 Minimum	 Maximum
Physical								
Air Temperature	Celsius	---	---	---	25.8	---	---	---
Air Pressure	mm of Hg	---	---	---	742	---	---	---
Water Temperature	Celsius	14	---	---	14.3	14.1	13.4	14.6
Spec.Conductance	micro S/cm	130	280	205	327	302	240	345
Turbidity	N.T.U.	5.9	---	---	---	1.19	0.01	4
Ph, field		6	7.07	7.1	7.2	7.1	6.9	7.5
Dissolved oxygen	mg/l	6.8	---	---	7.3	9.66	8	10.6
Inorganic								
Alkalinity	mg/l as CaCO3	120	---	---	129	---	---	---
Bicarbonate	mg/l as HCO3	---	---	---	159	---	---	---
Carbonate	mg/l as CO3	---	---	---	0	---	---	---
Chloride	mg/l	---	5.8	5.5	7	8.06	6.08	9.52
Fluoride, dissolved	mg/l	---	---	---	0.1	---	---	---
Hardness, computed	mg/l as CaCO3	---	---	---	150	---	---	---
Potassium, diss.	mg/l	---	---	---	1.1	---	---	---
Sodium	mg/l	---	---	---	4	---	---	---
Silica, dissolved	mg/l as SiO2	---	---	---	8.9	---	---	---
Sulfate	mg/l as SO4	---	---	---	5.7	3.92	2.56	7.31
Total Diss. Solids	mg/l	---	175	30	188	---	---	---
Dissolved Metals								
Aluminum	ug/l		---	---	3	16.4	<11	48
Arsenic	ug/l	< 5	---	---	1	< 12	< 12	< 12
Cadmium	ug/l	< 0.5	---	---	1	< 1	< 1	< 1
Calcium	mg/l	---	---	---	59	---	---	---
Copper	ug/l	< 10	---	---	1	26.3	< 6	47
Iron	ug/l	---	---	---	3	7.5	< 3	33
Lead	ug/l	< 1	---	---	1	12	< 11	14
Magnesium	mg/l	---	---	---	1.5	---	---	---
Manganese	ug/l	---	---	---	1	1	< 0.6	2.9
Nickel	ug/l	---	---	---	2	< 24	< 24	< 24
Selenium	ug/l	---	---	---	1	23.5	< 15	32
Silver	ug/l	---	---	---	1	< 15	< 15	< 15
Zinc	ug/l	< 3	---	---	3	28.1	< 6	83
Nutrients								
Total Organic Carbon	mg/l	---	---	---	---	1.59	0.34	4.12
Diss. Organic Carbon	mg/l	---	---	---	---	2.59	1.77	3.4
Ammonia-Nitrogen	ug/l as N	10	< 10	< 10	15	27.6	< 9	55
Nitrite-Nitrogen	ug/l as N	---	20	10	10	41	< 3	41
Nitrate-Nitrogen	mg/l as N	---	5.27	0.68	4.49	5.79	4.276	6.59
Total Nitrogen, Kjdl.	mg/l as N	---	---	---	0.2	0.11	< 0.03	0.29
Total Phosphorous	mg/l as P	0.02	---	---	0.03	0.04	0.017	0.089
Ortho-Phosphate	mg/l as P	0.02	---	---	0.03	0.031	0.019	0.044
Microbial								
Fecal Coliform	cfu/100 ml	---	---	---	8100	---	---	---
E.coli	MPN/100ml	---	---	---	2400	84	2	207
Total Coliform	MPN/100ml	---	< 1	2200	---	1700	34.4	5600
Fecal Strep., KF ag.	cfu/100 ml	---	---	---	3000	---	---	---

Source of Microbes

A dramatic reduction in microbe densities was observed as the water progressed from the cave resurgence downstream through the trout pond to Lake Keith, as shown in Figure 6, with the data tabulated in Table 5. The reduction in bacterial numbers is due probably to sedimentation, dilution, and die-off. Most importantly, this comparison demonstrates the alarming microbial densities in the cave complex compared to nearby bodies of water.

A comparison of base-flow sampling results at two different locations -- at the cave mouth, downstream of all bat rookeries, and deep in the cave at the waterfall, upstream of bat rookeries -- indicates that the majority of bacteria, including coliforms, is not attributed to bat guano. Figures 7 and 8 show the bacterial counts at these two sampling locations. These findings suggest that bacteria are being imported into the cave stream from the recharge zone. The high coliform counts suggest that septic system leakage or the land application of animal waste is involved, although it is not possible at this time to distinguish between the two types of fecal contamination. The high nitrite level observed supports this conclusion, because fertilizers, human, and animal waste often contaminate water supplies with nitrite and nitrate (U.S. EPA, 1998a).

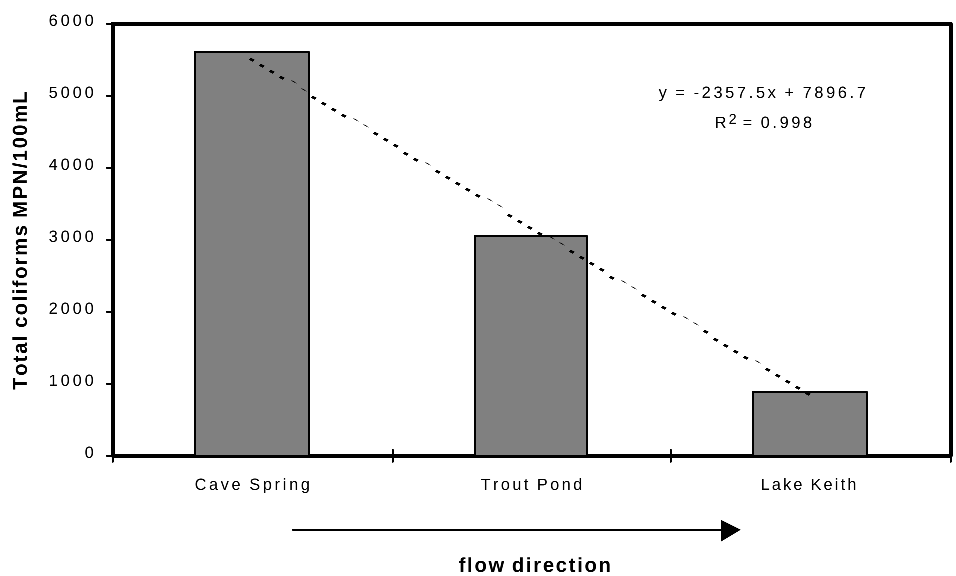


Figure 6. Comparison of microbial densities with respect to stream flow from the Cave Springs Cave resurgence, through the trout pond, and into Lake Keith

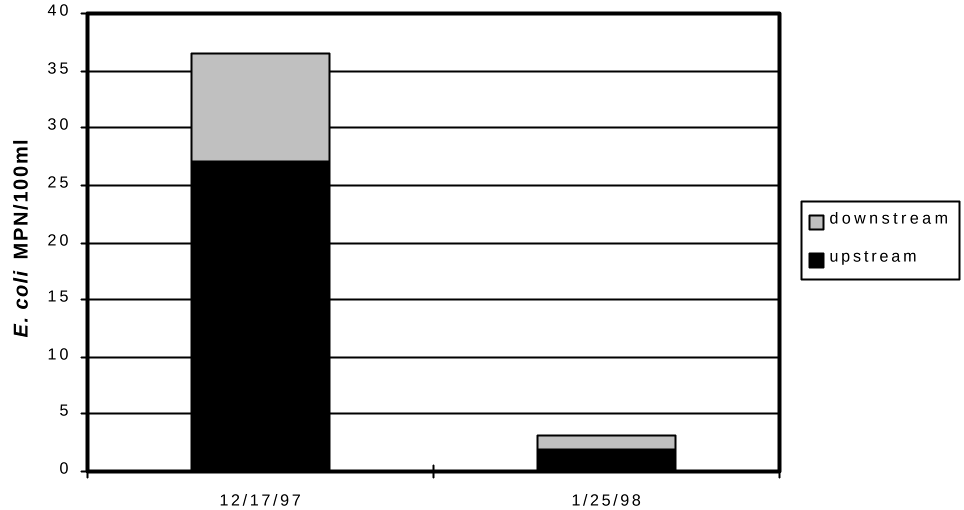


Figure 7. Comparison of *Escherichia coli* densities upstream and downstream of bat colonies during two different sampling events.

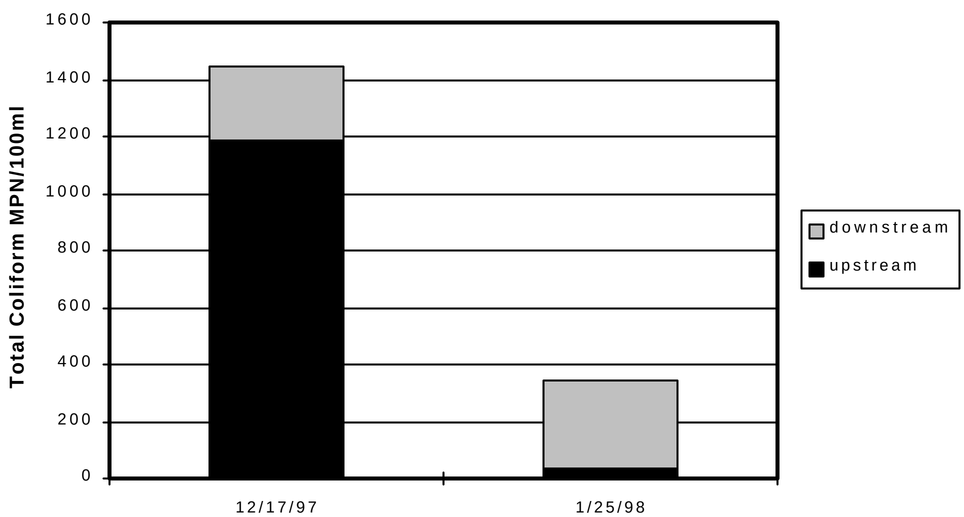


Figure 8. Comparison of total coliform densities upstream and downstream of bat colonies during two different sampling events.

Table 5. Summary of Known Water Quality Data at Lake Keith and the trout pond, downstream of Cave Springs Cave, Arkansas.

	SOURCE	Williams, 1991		AWRC	AWRC
	LOCATION	Lake	Lake	Lake	TroutPond
	DATE	4/16/98	5/29/91	6/9/1998	6/9/1998
Physical					
Air Temp.	Celsius	---	---	26	29
Air Pressure	mm of Hg	---	---	762	760
Water Temp.	Celsius	---	---	17.5	15.9
Water Stage	m	---	---	---	---
Discharge	m^3/min	---	---	---	---
Spec.Cond.	uS/cm	205	275	360	370
Turbidity	N.T.U.	---	---	1.2	0.4
Ph, field	---	6.8	7.43	7.4	7.11
Diss. Oxygen	mg/l	---	---	11.4	13.1
Tot. Diss. Solids	mg/l	130	175	---	---
Dissolved Metals					
Aluminum	ug/l	---	---	< 11	< 11
Arsenic	ug/l	---	---	< 12	< 12
Cadmium	ug/l	---	---	< 1	< 1
Chloride	mg/l	5	6.6	6.93	6.7
Copper	ug/l	---	---	16	19
Iron	ug/l	---	---	< 3	< 3
Lead	ug/l	---	---	< 11	< 11
Manganese	ug/l	---	---	1.2	< 0.6
Nickel	ug/l	---	---	< 24	< 24
Selenium	ug/l	---	---	< 15	< 15
Silver	ug/l	---	---	< 15	< 15
Sulfate	mg/l	---	---	2.62	2.65
Zinc	ug/l	---	---	8	14
Nutrients					
T.O.C.	mg/l	---	---	2.08	8.91
D.O.C.	mg/l	---	---	---	7.9
Ammonia-N.	mg/l as N	0.07	0.03	0.059	< 0.009
Nitrite-Nitrogen	mg/l as N	0.02	0.04	0.23	< 0.003
Nitrate-Nitrogen	mg/l as N	5.63	3.77	5.4	5.835
T.K.N.	mg/l as N	---	---		
Total Phosph.	mg/l as P	---	---	0.027	< 0.021
Ortho-Phosp.	mg/l as P	---	---	0.002	0.026
Microbial					
E.coli , Colilert	MPN/100ml	---	---	30.6	222
Total Coliform	MPN/100ml	2750	30	885	3060

Storm Response

Water samples taken at the cave mouth during the March and June storm events show a dramatic response of many of the water quality parameters to the storm events. Parameters such as TOC, TKN, ammonia, nitrite, and ortho-phosphate showed a similar response to the storm events monitored. Figures 9 through 13 show their concentrations and stream discharge during the March storm. This response to a storm-flow event suggests that monitoring only during base-flow conditions may miss important, though ephemeral, changes in water chemistry during other flow regimes. Nitrite concentrations, for example, appear to be at low levels during monthly base-flow sampling, yet reached possibly toxic levels in the June storm event. Bacterial counts increased dramatically, as shown in Figures 14 and 15. It was not obvious if this increase in bacterial populations was due to the entrainment of bat guano deposits, the importation of fecal material from the recharge area, or a combination of both. To sort this out we will have to collect samples during rainstorms inside the cave, upstream of bat roosts.

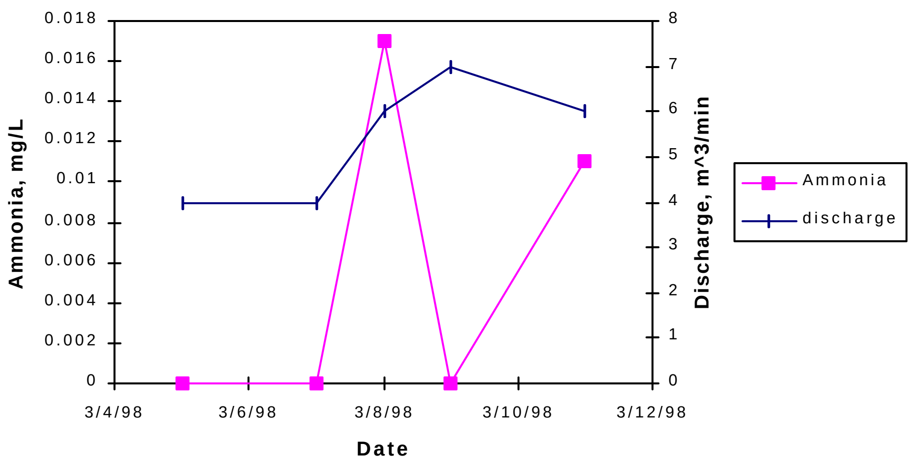


Figure 9. Ammonia concentration and spring discharge during the March storm event

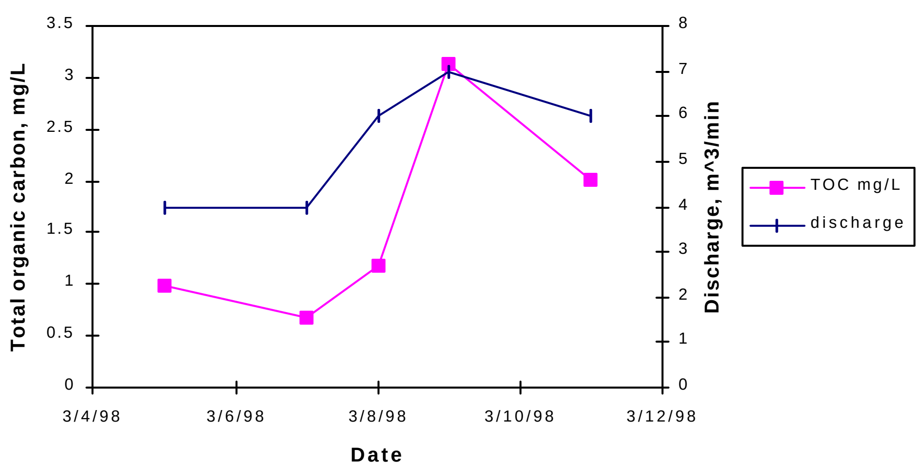


Figure 10. Total organic carbon concentration and spring discharge during the March storm event.

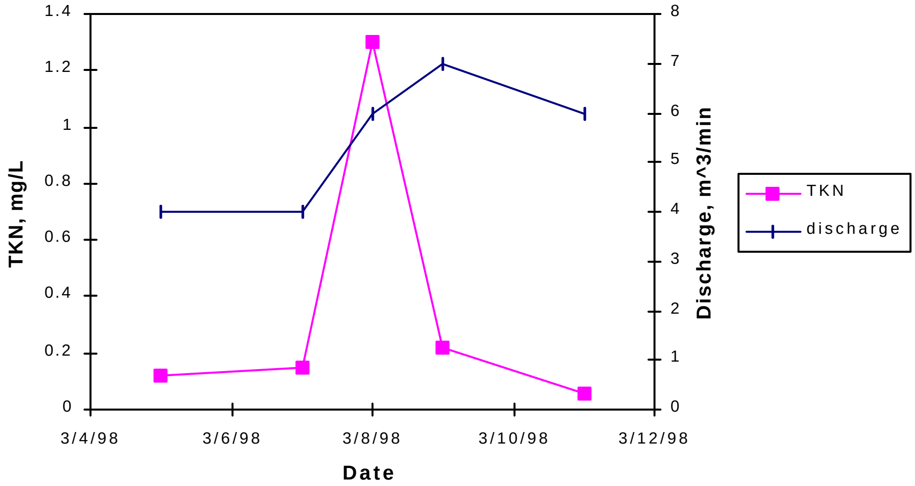


Figure 11. Total Kjeldahl nitrogen concentration and spring discharge during the March storm event.

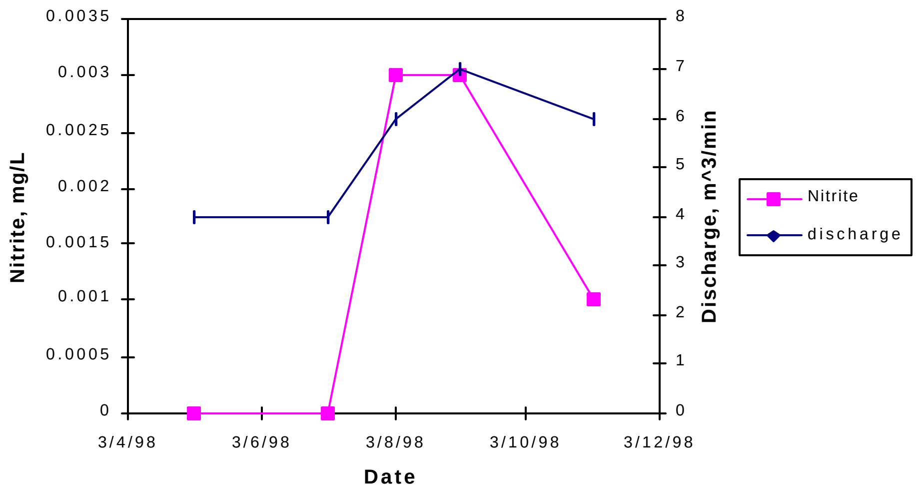
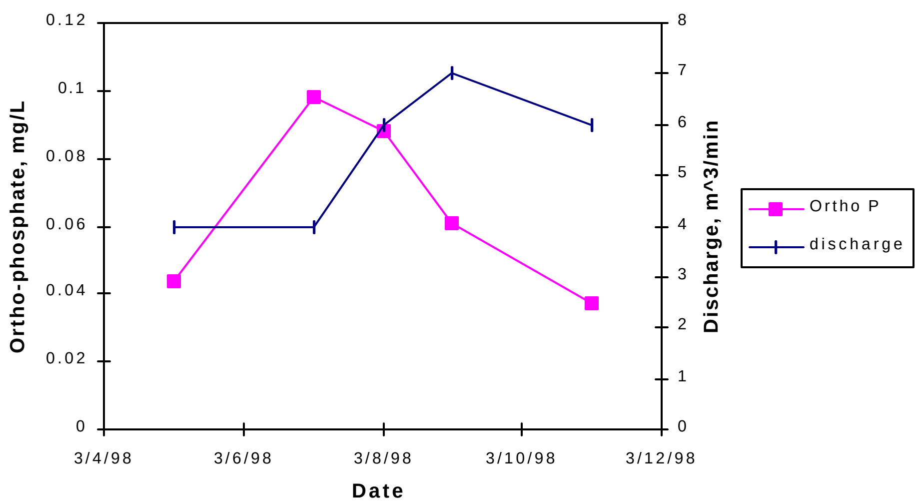


Figure 12.



Ortho-phosphate concentration and spring discharge during the March storm event.
Figure 13. Nitrite concentration and spring discharge during the March storm event at Cave Springs Cave.

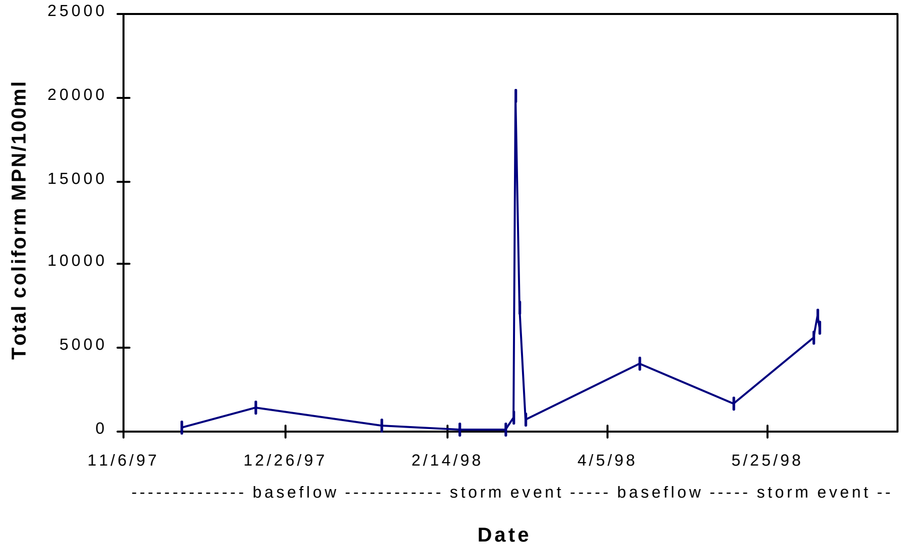


Figure 14. Total coliform densities during monthly base flows and two storm events at Cave Springs Cave.

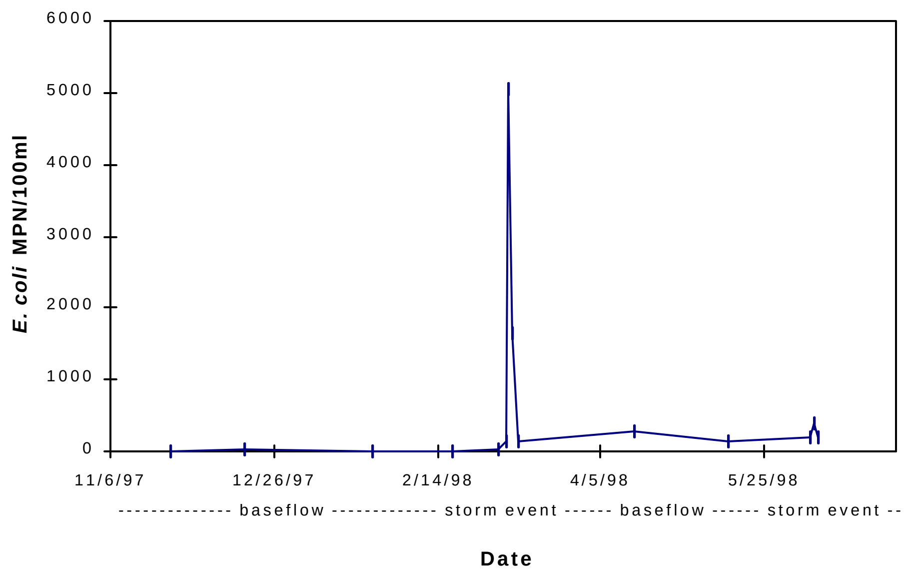


Figure 15. *Escherichia coli* densities during monthly base flows and two storm events at Cave Springs Cave.

Pesticide and Volatile Organic Compound Assays

The 1996 NAWQA USGS analysis of Cave Springs Cave water included extensive pesticide and volatile organic chemical testing and found no contaminants. In this study, the Water Quality Lab (Arkansas Water Resources Center) subcontracted West Coast Analytical Service, Inc. (WCAS) to analyze crayfish (*Orconectes* sp.) tissue for pesticide accumulation, specifically organochlorine pesticides (EPA method 8080) and semi-volatile organics (EPA method 625/8270). WCAS found the crayfish tissue to be free of all organochlorine pesticides. One semi-volatile organic, Bis (2-ethylhexyl) phthalate (better know as Di (2-ethylhexyl) phthalate or DEHP), was found in significant concentration, 500 ug/kg, (12 times the amount found in the lab blank). The federal primary drinking water standards stipulate an MCL limit of 6 ug/l for this volatile organic compound (U.S. EPA, 1998a). This phthalate is a plasticizer, used primarily in the production of poly-vinyl chloride (PVC). Its wide use and distribution, high volatility, and persistence, make DEHP a common contaminant of water bodies, sediments, and fish (U.S. EPA, 1998b). DEHP is a probable human carcinogen and teratogen, and is known to bioaccumulate in aquatic organisms, including fish (U.S. EPA, 1998b). DEHP has low acute toxicity to aquatic life, and long-term effects are unknown. Chronic toxic effects on fish include shortened lifespan, reproductive problems, lower fertility, and behavior changes (U.S. EPA, 1998b). Because the half-life of DEHP is only one to two weeks (U.S. EPA, 1998b), such bioaccumulation in crayfish tissue implies a reoccurring presence of DEHP in the cave complex.

Gut content analyses of the crayfish revealed no evident signs of *Amblyopsis rosae*. The contents included filamentous algae, diatoms, moth (Lepidoptera) scales, and hair (possibly from bats), in the foreguts of *Orconectes* sp. caught at the cave mouth.

Censuses of *Amblyopsis rosae*

A sight survey was performed on January 25, 1998 by Larry Willis, Stan Todd, and Gary Graening, and 106 cavefish were sighted. This survey indicated a 30% decline in the Cave Springs Cave surveyed population. Figure 16 below summarizes all of the surveys to date. One Ozark cavefish was sighted in April 1998, by Mark Collier, in the defunct trout pond below the cave orifice and weir, but could not be found by the authors on later inspection. Art Brown, Gary Graening, and Brian Wagner (Arkansas Game and Fish Commission) tried to repeat the survey of cavefish on April 14, 1998, but near the back of the cave they encountered the endangered gray bats (*Myotis grisescens*) roosting early and had to terminate the survey and vacate the cave. However, the survey up to the roosting area resulted in a count of only 71 fish, and in previous surveys, the majority of the cavefish were already accounted for up to this point in the cave. Thus, this partial survey supports the January 1997 survey finding of a reduced *Amblyopsis* population.

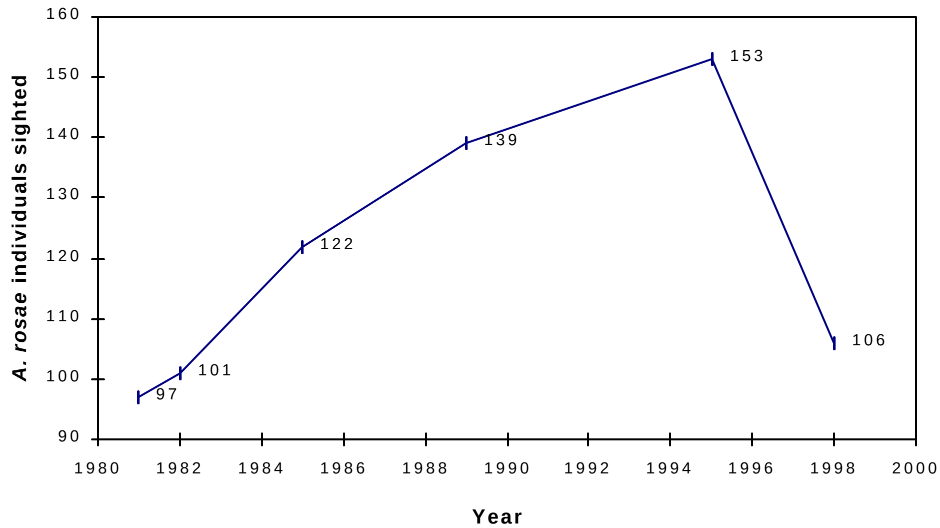


Figure 16. Summary of visual surveys of Ozark cavefish in Cave Springs Cave, Arkansas.

DISCUSSION

The Status of Environmental Quality at Cave Springs Cave

The majority of the chemical parameters measured fall within the limits of the state and federal water quality standards, and indicate typical, although far from pristine, environmental quality for Ozark streams (Petersen *et al*., 1998). Previous studies agree with these findings (Willis, 1984; Williams, 1991; USGS, 1996, unpublished data). This compilation of water quality analyses provide a detailed baseline, or current status, of present environmental quality at Cave Springs Cave, and can be used to detect future changes in ecosystem status. Results of water quality sampling during storm events and upstream of the bat roosts suggest that the environmental quality in this cave complex may be degrading. A trend analysis of past and current water quality data suggests that the concentration of inorganic and organic compounds have increased in the past 14 years. Bacterial counts are extremely high and probably originate from the surface recharge zone. Crayfish in the cave have large amounts of a toxic phthalate in their tissue, and a very high nitrite level was observed, which is known to be toxic to fish. Significant levels of selenium, copper and lead were also detected. The cavefish population seems to have decreased by about one-third since 1995. Furthermore, this study monitored only two storm events, and the data indicate that environmental quality was the worst during storm events. Thus, this study represents a conservative report of environmental quality in this cave complex.

In general, the carbonate terrain of northwest Arkansas is highly susceptible to pollution from land application of animal wastes and other waste disposal practices (MacDonald *et al*., 1976). Bacterial contamination is considered the most serious threat to Ozark ground-water quality (MacDonald *et al*., 1976; Steele, 1985). Seventy-eight percent of the wells and an estimated 90% of the springs in northwest Arkansas are contaminated with coliform bacteria (Steele, 1985). The resurgence of Cave Springs Cave is no exception. By state standards, the water is not even safe for wading, much less fishing, swimming, or drinking (Arkansas Pollution Control and Ecology Commission, 1998). The monitoring results indicate that fecal contamination from the recharge area is a major contributor to the high bacterial levels observed. The presence of significant amounts of nitrate, nitrite, ammonia, copper, and zinc support this finding. Marc Collier, owner of the land surrounding Cave Springs Cave, has observed that the mouth of the cave periodically reeks of animal manure. Furthermore, many organic constituents in the water have been increasing in concentration since 1984. While algae blooms, common in eutrophied lakes, should not threaten this cave complex that is devoid of light, eutrophication has other effects on subterranean habitats. Organic pollutants alter the oligotrophic nature of ground-water ecosystems and severely alter ground-water food webs (Notenboom *et al*., 1994). Pathogens in ground water increase as organics in soil increase, which could explain the increase in fecal coliforms at this site (Gerba and Bitton, 1984). The biological oxygen demand increases as organic concentrations increase (Maire and Pomel, 1994).

Organic loadings are only one of the threats to ground-water ecosystems such as the Cave Springs Cave complex. Toxins are another concern. Sediment trapping in the karst systems leads to the concentration of toxic substances (Maire and Pomel, 1994), and metabolic processes can concentrate toxins in a process known as bioaccumulation (U. S. EPA, 1988b). Chemical spills in a cave drainage basin are perhaps the single greatest threat to the cave ecosystem (Willis, 1984). Although U.S. Highway 71 was rerouted to minimize impact on the Cave Springs complex, U.S. Highway 71 still lies within the recharge zone of Cave Springs and highway drainage can enter the complex (Williams, 1991). U.S. Highway 71 has significant numbers of carriers of potential pollutants traveling it daily (Williams, 1991).

While no pesticides were found accumulating in resident crayfish during this study, one volatile organic compound of industrial origin, DEHP, did accumulate in collected crayfish. This chemical is known to bioaccumulate in fish and cause reproductive damage. Nitrite was detected in this study, and interferes with oxygen transport in fish. Significant levels of copper, selenium, and lead were also found in the cave water. Bioaccumulation and acute toxicity experiments using ground-water fauna are scarce (Notenboom *et al*., 1994). However, troglobites such as cave crayfish and cavefish are more susceptible to intoxication by bioaccumulation because of their increased longevity (Dickson *et al*., 1979). The distribution and abundance of ground-water organisms are good indicators of aquifer status (Job and Simons, 1994; Notenboom *et al*., 1994). If this is true, then a 30% reduction in the Amblyopsid population may indicate an aquifer of poor environmental quality.

Because of the fragile nature of the cave environment and because of the narrow range of its adaptive responses, the Ozark cavefish has lowered stability which increases its chance of extinction (see summary by Willis, 1984). In general, ground-water animals have extremely slow dispersal and colonization rates, indicating that recovery from anthropogenic disturbance might be too slow to avoid local extinctions of ground-water fauna (Strayer, 1994; Notenboom *et al*., 1994). Such disturbances would thus be irreversible (Strayer, 1994). *Amblyopsis rosae* reproduces and grows very slowly (Poulson, 1963; Means, 1993; Brown, 1996), and the presence of contaminants that attack reproductive and metabolic processes in freshwater fishes does not bode well for this unique and threatened species.

Increased activity in the aquifer recharge zone, including sale of land for a housing addition and construction of a regional jetport nearby, has increased the need for monitoring environmental quality at Cave Springs Cave. Monitoring the cavefish population yearly in this cave is needed for timely detection of negative impacts that might result from these disturbances, and is consistent with the objectives of the Ozark cavefish recovery plan (U. S. Fish and Wildlife Service, 1989). Furthermore, the recovery plan requests surveys of the historic and potential range of the Ozark cavefish every five to ten years (U. S. Fish and Wildlife Service, 1989). The last survey of the cavefish's range was in 1984

(Willis, 1984). It is important to survey the other Ozark cavefish habitats to determine if the other cavefish populations are declining as well.

New techniques in stream ecology could improve environmental monitoring in the Cave Springs Cave complex. Living organisms (and organic matter) have unique signatures of stable (non-radioactive) isotopes that can be used to determine food webs and the fate of pollutants (e.g. Kwak and Zedler, 1997). Stable isotope assays could be used in this ecosystem to determine the type of pollutants entering the cave complex. Furthermore, new assays in microbial ecology allow the quantification not only of fecal bacteria, but total bacterial populations and their metabolic state (e.g. King and Parker, 1988). These techniques using epifluorescence microscopy could determine the impact of organic pollutants and fecal bacteria on the cave ecosystem. Finally, the use of aerial reconnaissance, global positioning systems, and geographical information systems could determine the point and non-point sources of pollution in the recharge zone and can facilitate the management of Cave Springs Cave.

RECOMMENDADIONS

Findings of this study indicate environmental monitoring of Cave Springs Cave should continue. The cavefish population appears to have decreased after fifteen years of survey data that showed a steady increase. Water quality appears to be deteriorating as indicated by the analyses of chemical nutrients, metal ions, toxins (DEHP), and bacterial densities. We recommend the following.

1) Base-flow, broad scale sampling of a diverse array of water quality parameters should continue, but at a reduced frequency: two samples a year, in fall and spring, at the mouth of the cave and deep inside the cave upstream of bat rookeries, for a total of four sample sets. This should provide additional baseline data and reveal other problems, should they occur.

2) Storm event sampling of a specific array of water quality parameters should be increased to detect any severe reductions in water quality that may occur when materials are washed in by rainwater.

3) An automatic sampler should be obtained to collect water samples from deep inside the cave during storm events. This would help determine whether the recharge zone or the bat roost was the source of the chemicals and bacteria.

4) The cavefish population should be surveyed twice, once in the fall and again in early spring, using the same methods and personnel that have been used since 1980.

5) The presence of Di (2-ethylhexyl) phthalate, or DEHP, in this environment should be investigated more thoroughly by analyzing samples of water, bat guano, and isopod tissues, in addition to another sample of crayfish tissue. In addition, crayfish and/or isopod tissue should be analyzed for the presence of accumulated metals, especially copper, lead, and selenium.

6) Sources of organic carbon, nitrogen, and sulfur for the cave biota should be determined, if possible, using the technique of stable isotope ratio analyses. This might sort out whether problems originate from septic tanks, agricultural contaminants, or bat guano

7) The aquifer recharge zone should be studied and mapped using a variety of methods to identify potential problem areas. In addition, a cartographic survey of the cave complex would aid in monitoring efforts, enabling the computation of pollutant loads and the distribution of biota.

8) Other cavefish habitats in Arkansas, Missouri, and Oklahoma should be surveyed to count *Amblyopsis rosae* populations, and water quality should be evaluated in them as well.

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APPENDIX

Stage/Discharge Relationship for Cave Springs Cave Resurgence