Temporal Variation in the Emergence Flights of the Bat, Myotis velifer, from Caves in Southern Arizona

Debbie C. Buecher
University of Arizona

Abstract

It has generally been shown that for many temperate insectivorous bats, the time of evening emergence from their day roosts is approximately parallel to sunset. Although this emergence time may vary between species, within species the bats are known to have similar activity patterns. A maternity colony of *Myotis velifer* (cave bat) in southern Arizona was monitored for four years and an interesting deviation from the emergence pattern was observed. These bats appeared to emerge sooner in the early spring and autumn than in late spring and summer. Besides this variation in the time of emergence, the character of the outflight also changed through the summer period. This roost was then compared to a roost of *M. velifer* approximately 20 miles south of the maternity colony. The differences and similarities are discussed and possible justification for the pattern variations are proposed. A potential explanation for the different activity patterns could be the reproductive condition of the females at the maternity colony. Other factors such as ambient temperature may also play a role. Probably no one element is the trigger for emergence, but rather a combination of factors may impact bat activity patterns.

An Incidental Take Permit for Endangered Karst Invertebrates in Bexar County, Texas

Steven W. Carothers, Ph.D
Kemble White
Casey Berkhouse
SWCA, Inc., Environmental Consultants
1712 Rio Grande Suite C, Austin, Texas, 78701
Phone: 512 476-0891

Abstract

Nine species of cave invertebrates presently known only from karst topography in north and northwest Bexar County, Texas, were listed as endangered on December 26, 2000. Species listed include: two troglobitic ground beetles, *Rhadine infernalis* and *R. exilis,* a mold beetle, *Batrisodes venyivi,* an eyeless harvestman, *Texella cokendolpheri,* and five eyeless spiders, *Cicurina baronia, C. madla, C. Venii, C. vespera,* and *Neoleptoneta microps.* A local landowner with three small caves, all occupied by one or two of the listed species, has recently applied for a Section 10(a) incidental take permit to close one of the caves and preserve, in perpetuity, each of the other two caves in small (one-acre) preserves. The applicant and the authors worked with the United States Fish and Wildlife Service (Austin, Texas, Ecological Service Field Office) to establish guidelines for evaluating the specifics of incidental take for the project, as well as establishing mitigation criteria and long-term protection guidelines for designated mitigation preserves. The preserves that will be established include nine caves, on 179 acres, each occupied by at least two and up to five of the listed species. This presentation will provide details of preserve establishment, maintenance and monitoring and comments on the distribution and demographic characteristics of some of the listed species.

Introduction

Section 9 of the Endangered Species Act, as amended, prohibits the "take" of listed wildlife species. Take, as defined by the Act, means "to harass, harm, pursue, hunt, shoot, wound, kill, trap, capture, collect, or to attempt to engage in any such conduct" (Endangered Species Act, 16 U.S.C. 1531 *et seq*.). Amendments to the Endangered Species Act in 1982 provided provisions in Section 10 that allow for the "incidental take" of endangered species, by non-federal entities, as long as the take is incidental to "otherwise lawful activities." Section 10(a)(2)(A) of the Act requires that an applicant for an incidental take permit detail in a "conservation plan" the impacts that are likely to result from the taking and the measures that will be taken to minimize and mitigate for such impacts. The administration of the Endangered Species Act and responsibility for issuing take permits for non-marine wildlife species is the responsibility of the United States Fish and Wildlife Service.

This paper provides a brief description of an incidental take permit (Permit No. TE044512-1) and supporting habitat conservation plan for three species of listed karst invertebrates. The activity requiring the permit is the commercial development (La Cantera) of approximately 1,000 acres in Bexar County, Texas, just northwest of the City of San Antonio. On December 26, 2000, the U.S. Fish and Wildlife Service published a final rule and determined nine cave-dwelling invertebrates from Bexar County, Texas, to be endangered species under the authority of the Endangered Species Act. These invertebrates are all endemic, obligate troglobites of local distribution in karst terrain in Bexar County. The species listed are: *Rhadine exilis* (no common name) and *Rhadine infernalis* (no common name), small, eyeless ground beetles; *Batrisodes venyivi* (Helotes mold beetle) a small, eyeless beetle; *Texella cokendolpheri* (Robber Baron Cave harvestman) a small, eyeless harvestman; *Cicurina baronia* (Robber Baron cave spider), *Cicurina madla* (Madlas cave spider), *Cicurina venii* (no common name), *Cicurina vespera* (Government Canyon Bat Cave spider), and *Neoleptoneta microps* (Government Canyon cave spider), all small, eyeless or essentially eyeless spiders (USFWS, 2000a).

Background

The life history and taxonomy of the Bexar County listed invertebrates is not represented by definitive studies. In 1993, the Service contracted two studies to summarize the known information on these species. One study focused on the overall karst geography in the San Antonio region and the potential geological and geographical barriers to karst invertebrate movement and limits to their distribution (Veni and Associates, 1994). The other study summarized the distribution of the nine invertebrates as understood at that time (Reddell, 1993).

The karst geography report (Veni and Associates, 1994) delineates six karst areas or karst regions within Bexar County. These regions are as follows: Stone Oak, University of Texas at San Antonio, Helotes, Government Canyon, Culebra Anticline, and Alamo Heights. The boundaries of these karst regions are geologic or geographic features that are thought to represent obstructions to invertebrate movement and which have resulted in the present-day distribution of invertebrates. Whether or not these karst region boundaries are truly barriers (past or present) to invertebrate distribution is presently uncertain. Additional studies are required before the relationship of invertebrate distribution and karst regions is fully understood.

The La Cantera property is located within the University of Texas at San Antonio karst region, which is bounded by Helotes Creek to the west, Leon Creek to the east, and the limits of exposure of the Edwards Group and Glenrose Limestone Formation to the north and south. The 1993 studies determined that only two of the nine listed species were present in the University of Texas region, *Rhadine exilis* and *Rhadine infernalis*. Subsequent studies have also documented occurrence of *Cicurina madla* in the region outside the La Cantera property (USFWS, 2000a). Biota surveys conducted by SWCA in 1994, 1995, and 2000 in three La Cantera caves resulted in discovery of eyeless *Cicurina* spiders and *Rhadine exilis*, but no *Rhadine infernalis*. Based on the best available scientific information, the *Cicurina* spider found on the La Cantera property is most likely the listed *Cicurina madla*. It is possible that this spider is an undescribed species of *Cicurina* (Cokendolpher, pers comm). Although an adult La Cantera eyeless spider sufficient for positive identification has not been collected, based on the fact that *Cicurina madla* has been verified as occurring in two caves within two to three miles of La Cantera, and no other eyeless *Cicurina* are known from the University of Texas karst area, this spider was assumed, for purposes of the incidental take permit, to be the federally listed species *Cicurina madla* (USFWS, 2001).

La Cantera Caves

Quality of caves on La Cantera

Over 400 potential karst features have been evaluated on the property. Three primary geological assessments have been performed in the past, and their combined scope has included the entire property (Raba-Kistner, 1993a and 1993b; SWCA, 2000; Horizon Environmental Services Inc., 2000).

During extensive karst surveys beginning in 1993 three caves (La Cantera Caves #1, #2, and #3) containing habitat for the listed karst invertebrates were found on the La Cantera property. Two of these caves (La Cantera Caves #1 and #2) are known to contain *Rhadine exilis* and *Cicurina madla.* The entrances to both caves lie within 200 feet of the west-bound frontage road of Loop 1604, a heavily traveled highway. Both caves are immediately south (approximately 100 feet) of a two-lane road designed to serve traffic to and from the commercial development. The entrance to La Cantera Cave #3, which contains *Cicurina madla,* lies within 100 feet of another internal thoroughfare. Because of the existing disturbances, none of the La Cantera caves is considered high-quality habitat for the invertebrates under consideration (USFWS, 2000b). The U.S. Fish and Wildlife Service has determined that all three La Cantera caves were of medium-quality.

None of the listed endangered invertebrates is known from other karst features present on the La Cantera property. However, the occurrence of *Rhadine exilis*, *Rhadine infernalis*, and/or *Cicurina madla* (the only known endangered karst species within the University of Texas karst region), or any of the other listed invertebrates elsewhere on the property cannot conclusively be ruled out given the potential for these species to occur in subsurface voids lacking obvious surface expression (Veni and Associates, 1994).

Karst Invertebrate Preserve Guidelines

In an effort to provide guidelines for the protection of endangered karst invertebrates, the U.S. Fish and Wildlife Service has determined that the minimum total area needed to protect caves or cave clusters containing karst invertebrates is 69 to 99 acres (USFWS, 2000b). Further, the agency suggests that an area within that area a minimum 100- to 200-meter (328- to 656-foot) radius from all karst features containing listed invertebrates should be preserved. This includes a core area encompassing the minimum 50-meter (164-foot) cave cricket foraging range and an additional buffer against edge effects. Also, since roads may hinder movement of several species of invertebrates and small mammals, no internal roads or other permanent habitat fragmentation should occur within the protected area. It is the current policy of the U.S. Fish and Wildlife Service that disturbances that approach closer than the standards detailed above, are likely to constitute take.

La Cantera Habitat Conservation Plan

On-site and Off-site Preserves.

As part of the habitat conservation plan's development, La Cantera will assure that seven karst preserves totaling approximately 181 acres will be protected in perpetuity by appropriate legal mechanisms (conservation easements, deed restrictions) before clearing or construction begins on undeveloped portions of the property. The karst preserves include one-acre on-site preserves for La Cantera Caves #1 and #2, and five off-site preserves totaling approximately 179 acres. These off-site preserves include: an approximately five-acre area encompassing Madlas Cave; an approximately four-acre area encompassing John Wagner Ranch Cave #3; approximately 70 acres encompassing Hills and Dales Pit; approximately 25 acres encompassing Helotes Hilltop and Helotes Blowhole Caves; and approximately 75 acres encompassing Scenic Overlook, Canyon Ranch Pit, and Fat Mans Nightmare Caves. All of the off-site caves within the proposed karst preserves contain endangered karst invertebrate species as well as other cave-adapted species. A summary of endangered invertebrate species known from each of the proposed on- and off-site preserve caves is provided in Table 1.

The U.S. Fish and Wildlife Service considered the La Cantera caves to be of medium quality with regard to habitat for listed invertebrates. For each of these caves, the habitat conservation plan provides for mitigation by preserving caves of similar or higher quality. For each La Cantera cave, the following mitigation has been provided: La Cantera Cave #1 – Hills & Dales Pit (approximately 70 acres, four listed species, one high-quality cave); La Cantera Cave #2 – Helotes Hilltop, Helotes Blowhole, Madlas Cave, and John Wagner Ranch Cave #3 (approximately 34 acres, five listed species, four medium-quality caves); La Cantera Cave #3 – Canyon Ranch Pit, (approximately 75 acres, five listed species, three high-quality caves).

In addition to providing 181 acres of cave preserves, the La Cantera habitat conservation plan also provides for participation with the U.S. Fish and Wildlife Service in the development of an outreach program, and provides for a $20,000 grant to support DNA research of *Cicurina* taxonomy. The outreach program has the goal of raising awareness, understanding, and appreciation for Bexar County endangered karst invertebrates. Under this program information materials will be produced by public relations professionals and will be designed to reach the broadest possible audience (including school children, landowners, and the public at large). The intent of these materials will be to impress upon the audience the importance of preserving the threatened karst resources and their invertebrate inhabitants. These materials will be designed to render technical information relating to karst habitats and their inhabitants in non-technical terms and graphics.

U.S. Fish and Wildlife Service Assessment of Development Impacts to Listed Species

It is the U.S. Fish and Wildlife Service's opinion that take of *Rhadine exilis* will occur in La Cantera Caves #1 and #2, and take of *Cicurina madla* will occur in all three La Cantera caves, as a result of the development and occupation of the La Cantera property. Although the Fish and Wildlife Service recognizes that the existing quality of endangered species habitat presently provided by the three La Cantera caves is not optimal, development of the property would likely reduce the amount of such habitat present in the project region. Take of endangered karst invertebrates could also occur elsewhere on the property in the event previously undiscovered habitat is encountered. Although no endangered karst invertebrates are known to occur on the property in areas outside of the three La Cantera caves, potential exists for listed species to be present in subsurface void spaces lacking obvious surface expression. Such spaces could be destroyed or significantly disturbed by construction activities. As all portions of the property outside of the two proposed on-site karst preserves (at La Cantera Caves #1 and #2) are expected to be developed, any endangered karst invertebrates occurring on the property outside of these preserves are expected to be taken by completion of the development; however, such take will be fully mitigated for through the conditions detailed in the habitat conservation plan. Due to the extensive karst surveys of the property, the likelihood of discovering previously undetected habitat is considered low.

Protecting La Cantera Caves #1 and #2 within one-acre preserves will significantly reduce the risk of disturbing karst invertebrate habitat during construction. The U.S. Fish and Wildlife Service, however, believes that reduction of native vegetation to one-acre patches surrounding these caves will reduce the amount of nutrients entering these features, the amount of organic material available to be washed into the features, and the amount of habitat supporting cave crickets and other trogloxene species. According to the U.S. Fish and Wildlife Service, increased intensity of fire ant infestations within the karst preserves and/or introduction of other exotic species that could be detrimental to the karst ecosystem may also result from clearing, construction, and development activities. Due to cave depth (roughly 60 to 115 feet) and existing edge along the nearby Loop 1604 right-of-way, potential preserve edge effects (such as increased drying of woodland, with concomitant drying of cave habitat, and increased temperature fluctuations) are expected to be negligible. While proposed development may not result in elimination of *Rhadine exilis* and *Cicurina madla* from these two caves, it is anticipated that numbers of these two species within these caves will be reduced over time. (To put the existing density of invertebrates in perspective, the authors have visited Caves #1 and #2 approximately four times in nine years searching for karst invertebrates for a period of two hours per visit and have found an approximate total of five to six *R. exilis* and 20 to 30 eyeless *Cicurina*.) A monitoring program included in the habitat conservation plan will provide long-term data on the accuracy of these predictions.

The U.S. Fish and Wildlife Service believes that the overall impact to *Rhadine exilis* and *Cicurina madla* resulting from development of the La Cantera property will neither prevent nor seriously impact the long-term conservation of each species within the University of Texas at San Antonio karst region. The U.S. Fish and Wildlife Service desires that a minimum of three karst preserves for each species within each karst region be set aside to provide for long-term conservation of karst invertebrates (USFWS, 1994). Assuming development of the property will preclude on-site survival of the two species (which is not certain), sufficient habitat will likely remain within the University of Texas karst region to provide necessary conservation. Within the University of Texas karst region, two suitable preserves are now inhabited by *Cicurina madla*. Future exploration of Mastodon Pit (less than 0.5 mile south of the property) will probably also yield this species. Moreover, extensive conservation of known, occupied *Cicurina madla* habitat is provided outside the University of Texas karst region. The U.S. Fish and Wildlife Service believes that strict adherence to the "three occupied caves per species" rule may not be biologically required to ensure conservation of a species where the species' range includes several karst regions. Such is the case for *Cicurina madla.* One of the present anomalies of the karst region configuration as currently proposed (Veni and Associates, 1994) is the fact that *Cicurina madla* occurs in four of the six karst regions. The presence of this single taxa in multiple karst regions may call into question the hypothesis of geologic or geographic features obstructing invertebrate movement between karst regions.

Within the University of Texas at San Antonio karst region, at least five caves are known to be inhabited by *Rhadine exilis*. For *Cicurina madla*, positive identifications have been made in two large cave preserves (Hills and Dales Pit and Robbers Cave), and another four caves have produced eyeless *Cicurina* thought to be *Cicurina madla*, though positive identification requires further study.

Other University of Texas at San Antonio karst region caves known to have eyeless *Cicurina* spiders that are most likely *Cicurina madla* include: Mastodon Pit, Kamakazi Cricket, John Wagner Ranch Cave #3, and Three-fingers Cave. Outside the University of Texas at San Antonio karst region, *Cicurina madla* is known to occur in Christmas Cave, Madlas Cave, Madlas Drop Cave, and Helotes Blowhole Cave in the Helotes karst region; Lost Pothole Cave in the Government Canyon karst region; and Headquarters Cave in the Stone Oak karst region. Of these known localities, at least four sites are either in preserves now (Lost Pothole Cave, Headquarters Cave) or will be preserves as a result of the La Cantera habitat conservation plan (Madlas Cave, Helotes Blowhole Cave). Thus, actions effected as a result of the La Cantera permit are not likely to preclude the long-term conservation of either *Rhadine exilis* or *Cicurina madla.*

Because the habitat conservation plan would protect approximately 181 acres of on- and off-site land, the U.S. Fish and Wildlife Service has determined that the project is expected to provide an overall benefit to Bexar

County endangered karst invertebrates. The identification of species, evaluation of take, and design and configuration of the karst preserves are based on the best scientific information available. Protecting off-site karst ecosystems as provided in the habitat conservation plan would represent a major recovery action for other listed species besides *Rhadine exilis*, and *Cicurina madla*, particularly *Rhadine infernalis*, *Batrisodesvenyivi*, and *Texella cokendolpheri*, and the undescribed *Texella* new species and *Neoleptoneta* new species.

Summary and Conclusion

This document has summarized the conditions of the first incidental take permit involving the nine listed Bexar County karst invertebrates. We anticipate that many more will follow, and that the La Cantera permit will serve as a model for future permits. We believe that the La Cantera habitat conservation plan will provide significant conservation opportunities for the subject invertebrates. We are concerned, however, that the existing U.S. Fish and Wildlife Service standard of requiring 69 to 99 acres of habitat per cave or cave cluster could prove to be counterproductive to efforts to preserve cave habitat. We believe there are presently insufficient data to validate the need for these relatively large preserves.

While it is the responsibility of the U.S. Fish and Wildlife Service to err on the side of the species, smaller preserves may, in fact, provide

Table 1. Summary of Endangered Species Known to Occur in the La Cantera On-site and Off-site Preserve Caves.

Preserve Cave	Karst Region	**Endangered Species Present**	**Other Rare Karst Species Present**
La Cantera Cave #1	University of Texas at San Antonio	*Rhadine exilis*	eyless *Cicurina* sp. (probably *C. madla*)
La Canter Cave #2	University of Texas at San Antonio	*Rhadine exilis*	eyless *Cicurina* sp. (probably *C. madla*)
Hills and Dales Pit	University of Texas at San Antonio	*Rhadine exilis* *Cicurina madla*	*Neoleptoneta* new sp. *Texella* sp. (possibly *T. cokendolpheri*)
John Wagner Ranch Cave #3	University of Texas at San Antonio	*Rhadine exilis* (type location) *Rhadine infernalis* *Texella cokendolpheri*	*Neoleptoneta* new sp. eyless *Cicurina* sp. (probably *C. madla*)
Helotes Blowhole Cave	Helotes	*Rhadine exilis* *Rhadine infernalis* *Cicurina madla*	
Helotes Hilltop Cave	Helotes	*Rhadine exilis* *Batrisodes venyivi* (type location)	eyless *Cicurina* sp. (probably *C. madla*)
Madlas Cave	Helotes	*Rhadine infernalis* (type location) *Cicurina madla*	
Canyon Ranch Pit	Government Canyon	*Rhadine infernalis*	eyless *Cicurina* sp. (probably *C. madla*)
Fat Mans Nightmare Cave	Government Canyon	*Rhadine infernalis*	eyless *Cicurina* sp. (probably *C. madla*) *Texella* sp. (possibly *T. cokendolpheri*)
Scenic Over Look Cave	Government Canyon	*Rhadine infernalis* *Batrisodes venyivi* (third known location)	eyless *Cicurina* sp. (probably *C. madla*) *Texella* sp. (possibly *T. cokendolpheri*)

the same measure of protection for these troglobitic organisms. It is important, therefore, that relevant research be focused on this issue as soon as possible. Landowners may be far more willing to provide a five- to ten-acre buffer around significant karst features and our fear is that the 69- to 99-acre requirement will result indestruction of the vary resource we are trying to protect.

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Reasons Why We Should be Mindful of Microbes When We Consider Karst Systems: Impacts on Karst Development

Tricia Coakley
Shannon Wright
Larry Elliott
Chris Groves, Ph.D
Western Kentucky University

Abstract

A 1998 study of interstitial fluid geochemistry within Charonís Cascade in the Echo River/River Styx area of the Mammoth Cave System found carbon dioxide pressures higher than that of the fluids of the cave stream itself. This was confirmed by a limestone weight loss experiment in which samples dissolved at various levels below the streambed despite the low fluid velocities. The high CO_2 pressures appear to influence both conduit dissolution rates and geometry and presumably result from the microbial degradation of organics within the sediments. To explore the relationship between the geochemical environment of fluids and microbial ecology, additional samples were collected from the same location. Eight *Coliform* bacteria were identified to species level and inoculated in 65 milliliters of thioglycollate broth along with a calcite crystal of known weight and incubated at 12°C for 92 days. In the presence of five of the bacterial species, calcite dissolved more than the control, ranging up to 18.1 milligrams per square centimeter per year for *Escherichia coli*. Preliminary results suggest that in typical southeastern U.S. cave environments, bacteria within cave sediments may influence limestone dissolution. Further experiments are underway to better understand the relationships between microbial ecology and limestone dissolution kinetics.

The Missouri Cave Life Survey

William R. Elliott, Ph.D.
Lawrence Ireland
Missouri Department of Conservation

Abstract

The purpose of this project is to assess the status of common cave-dwelling animals in Missouri. The Missouri Department of Conservation began systematic surveys of cave life in 1978. James E. and Treva Gardner visited 436 caves and 10 springs, where they collected specimens for identification, recorded observation, and counted vertebrates. The invertebrate data were published by James E. Gardner (1986). The vertebrate count data are the focus of the current study. We incorporated Gardner's records on 483 species into the Missouri Biospeleological Database from which we produced candidate lists of caves to visit in all seasons and from a wide geographic area. We obtained a "Partnerships in Wildlife" grant from the U. S. Fish and Wildlife Service to conduct follow-up surveys of 40 caves, utilizing volunteers from the Missouri Caves and Karst Conservancy, Missouri Western State College, and the University of Missouri/Columbia. Dozens of cavers were trained to identify and record species and other observations in the caves using a pictorial guide, data forms, rulers, and digital thermometers. A water sampling program is being led by Dr Robert Lerch. Samples are analyzed for typical parameters and selected contaminants. Prelilminary data will be presented, and at project's end, we will provide a summary report on the status of eastern pipistrelle bats, grotto salamanders, pickerel frogs, and other species. The results will be used for making land management decisions regarding cave communities.

Introduction

The purpose of this project is to assess the status of common cave-dwelling animals in Missouri. This study is an example of the Missouri Department of Conservation's mission to monitor the status of wildlife populations in the state. The Missouri Department of Conservation began systematic surveys of cave life in 1978. James E. Gardner and Treva Gardner visited 436 caves and ten springs, where they collected specimens for identification, recorded observations, and counted vertebrates. The invertebrate data were published by James E. Gardner (1986). An important baseline study on cave bats was begun by LaVal and LaVal (1980). Gardner's vertebrate data were not published, and are the focus of the current study. In this study we also record observations of invertebrates.

Materials and Methods

We incorporated Gardner's published and unpublished records into the "Missouri Biospeleological Database," which now contains information on 843 species and more than 800 caves. We produced candidate lists of caves to revisit. More than 200 caves had count data for at least one species. Caves were prioritized for higher counts, multiple species counts, and species of special interest (such as the grotto salamander, *Typhlotriton spelaeus*). A semifinal list of 81 caves was then evaluated by a committee of biologists and cavers to obtain a final selection of 40 caves with a representative geographic and seasonal spread.

We obtained a "Partnerships for Wildlife" grant from the U.S. Fish and Wildlife Service to conduct follow-up surveys of these 40 caves, utilizing volunteers from the Missouri Caves and Karst Conservancy, Missouri Western State College, and the University of Missouri/Columbia. This type of grant requires a sponsoring agency (Missouri Department of Conservation) and volunteers, who contribute time and expenses to carry out a wildlife study. The hours and travel expenses are carefully recorded to meet or exceed the minimum contribution required to obtain the grant. In this grant $20,000 worth of work will be contributed by Missouri Department of Conservation, Missouri Caves and Karst Conservancy, and two researchers, part of which is used to pay a part-time salary for the Project Leader (Lawrence Ireland), who schedules and leads the trips, quality-controls the field work and manages data. William R. Elliott, cave biologist for Missouri Department of Conservation, is the Project Director and designer.

The study began in July 2001, and will end in June 2002. Training sessions were held on two weekends in July and September 2001, at Reis Biological Station, operated by Saint Louis University, near Steelville, Missouri. Forty-five cavers were trained by the authors and David C. Ashley, Missouri Western State College, to identify and record species and other observations in the caves.

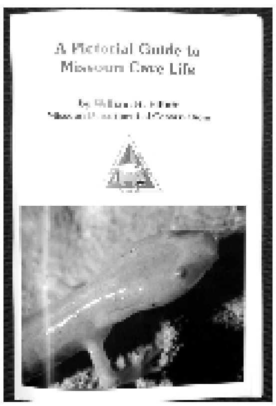

Figure 1. *This identification guide was desktop published for team members to use in the field. The Grotto salamander,* Typhlotriton spelaeus, *is on the cover.*

Training included slide lectures to acquaint cavers with 66 recognizable species and subspecies, their ecology, and methods of identifying roosting bats without touching them. However, more than 800 different species have been recorded from Missouri caves, so it is not feasible for the volunteers to accurately identify most species. Trips were quality-controlled by experienced naturalists who led the teams. Team members did not handle or collect fauna, but the leaders were authorized to collect small invertebrates when needed for identification.

We provided a desktop-published pictorial guide to the species for field use (Figure 1). Images and text from this guide may be seen in the Biospeleology web site under "Missouri Cave Life," at: http://www.utexas.edu/depts/tnhc/.www/biospeleology.

Rulers were provided so that teams could measure animals without handling them. In addition, high-resolution digital cameras were used to document some of the species and the survey work. We captured interesting and potentially valuable macrophotographs of color variation in some amphibians. The digital photos were shared via e-mail with biologists who identified or confirmed identifications of the species.

Students were taught how to use a field-tested data form (Attachment 1 and 2), which tied the cave life survey to numbers placed on a cave map, thus pinpointing locations of observations. The form has fields to record the cave's name, time in and out, and directions to and location of the cave. For purposes of satisfying the terms of the grant, team members recorded their names and the time and mileage contributed for that trip. The team collected trash in the cave and counted it up at the end of the trip. At the end of the trip the team evaluated the cave for six types of use and abuse, comparing to the many caves they have visited (Attachment 1).

The back of the form (Attachment 2) is a spreadsheet in which each row is a new observation or a water sample, or a continuation of the previous row if space is needed for tallying or for notes. A record number is marked on the cave map in the cave for each different species' occurrence, but teams may pool data within a 50-meter reach of the cave. There are columns for the place in the cave, distance from the entrance, type of habitat, temperature, number observed, and the initials of the observer or collector.

We purchased four Taylor digital pocket thermometers for the study. We calibrated the thermometers in a freezing water bath to within 0.1°F of each other, and they were periodically checked against each other in water to see if they still agree (Figure 2). In November 2001, we added a wet-dry bulb psychrometer to the study to record relative humidity because a long-term drought was affecting the humidity in many caves.

Figure 2. *Jeff Briggler uses a Taylor digital pocket thermometer.*

Since many bats and amphibians use caves seasonally, we revisited each cave within two weeks before or after the original date that it was visited. The original surveys were carefully recorded by Gardner and we tried to match the time and effort that were spent in each cave.

Typically each team had a leader with a camera; a data recorder; "spotters," who traveled abreast to find fauna on left and right walls, ceiling, and floor; and members who were responsible for the trash bag and a rugged container that had a digital pocket thermometer, rulers, and small items. The roles were sometimes swapped to allow team members to learn different aspects of the study (Figure 3).

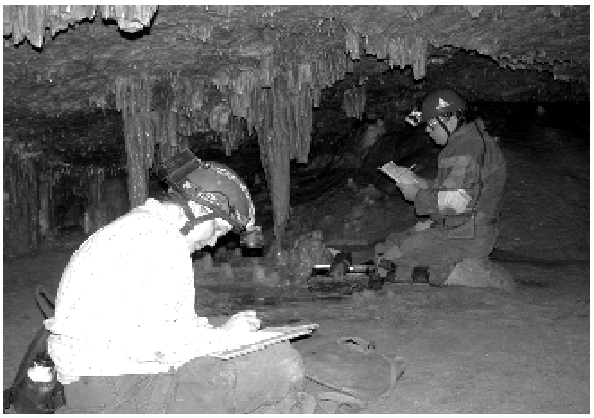

Figure 3. *Sally Kula and Bill Elliott collecting data.*

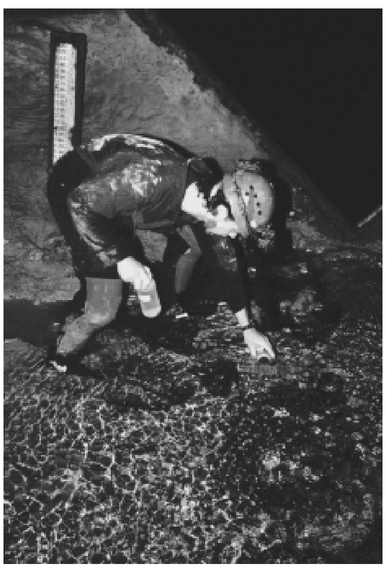

Figure 4. *Bob Lerch takes a water sample for analysis.*

Robert Lerch, U.S. Department of Agriculture and the University of Missouri/Columbia, led a water sampling program in conjunction with our study. Teams were issued prenumbered, analytically clean water sample bottles (Figure 4). Samples were sent on ice to Dr Lerch's laboratory, where they are being analyzed for typical water-quality parameters and selected contaminants. Those results are not yet available.

Results

The 14 caves studied to date in 2001 are given in Table 1. Volunteers contributed a total of 377 man-hours and 3,570 miles to carry out the 14 surveys we have done, for a mean of 27 man-hours and 255 miles per cave trip. These figures do not include paid time and mileage contributed by the agencies and universities involved. About one-third of the trainees have participated in trips so far. Reimbursements to volunteers for their work are all contributed to the Missouri Caves and Karst Conservancy for future cave conservation projects.

Table 1. Caves studied to date.

County	Cave	Date
Camden	Moles Cave	09/07
Carter	Blue Spring Cave	10/10
Carter	Lower Camp Yarn Cave	07/10
Carter	Secesh Cave	07/23
Christian	Math Branch Cave	08/09
Crawford	Jagged Canyon Cave	09/22
Crawford	Mud River Cave	09/22
Madison	Marsh Creek Cave #1	08/12
Oregon	Bockman Spring Cave	10/06
Oregon	Willow Tree Cave	10/06
Pulaski	Ryden Cave	08/10
Shannon	Marvel Cave	08/30
St. Louis	Woods Cave	07/17
Wright	Bill Dyer Lead Mine Cave	07/28

Preliminary data from 14 caves are presented, involving 17 common species and subspecies: cave salamander (*Eurycea lucifuga*, Figure 5), dark-sided salamander *(Eurycea longicauda melanopleura*), western slimy salamander (*Plethodon glutinosus or albagula*), Ozark salamander (*Plethodon angusticlavius*), southern redback salamander (*Plethodon serratus*), grotto salamander (*Typhlotriton spelaeus*), pickerel frog (*Rana palustris*, Figure 6), green frog (*Rana clamitans*), dwarf American toad (*Bufo americanus charlesmithi*), eastern American toad (*Bufo americanus americanus*), eastern pipistrelle bat (*Pipistrellus subflavus*, Figure 7), big brown bat (*Eptesicus fuscus*, Figure 8), little brown bat (*Myotis lucifugus*), northern long-eared bat (*Myotis septentrionalis*), gray bat (*Myotis grisescens*, Figure 9), Indiana bat (*Myotis sodalis*), and eastern phoebe (*Sayornis phoebe*). Other species, such as the herald moth (*Scoliopteryx libatrix*, Figure 10), will be evaluated in the final report.

Figure 5. *The Cave salamander,* Eurycea lucifuga, *is commonly seen in wet Missouri caves.*

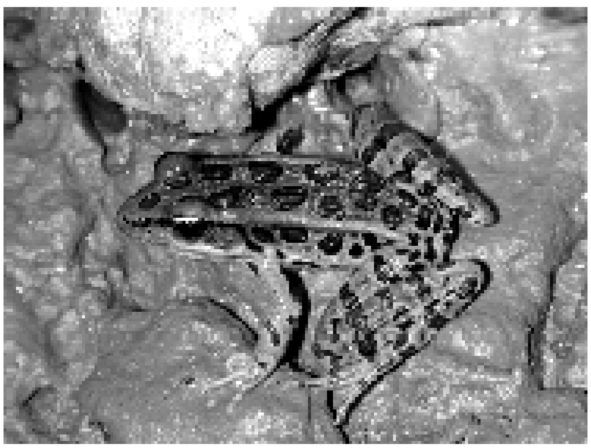

Figure 6. *The Pickerel frog,* Rana palustris, *takes refuge in Ozark caves during winter and drought.*

Figure 7. *The Eastern pipistrelle bat,* Pipistrellus subflavus, *is tolerant of humans, but we surveyed it to see if heavy traffic has reduced its use of caves.*

Figure 8. *The Big brown bat,* Eptesicus fuscus, *is a typical winter resident in chilly entrance areas.*

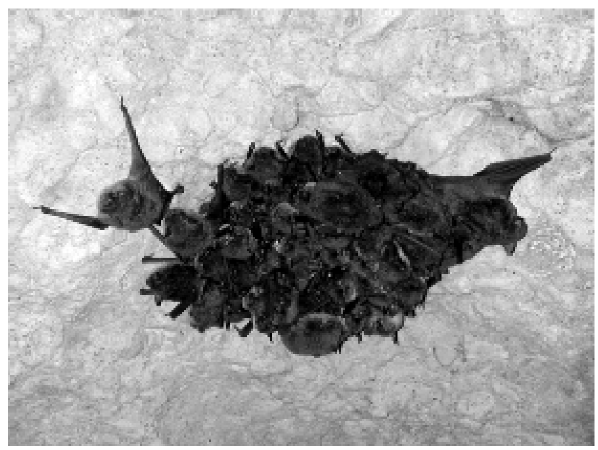

Figure 9. *A small, late summer cluster of Gray bats,* Myotis grisescens. *This endangered species is recovering in many caves where they are protected well, but may never reach its former numbers again.*

A bar graph (Figure 11) depicts the pooled count data for the above species from the first 14 caves visited. Black bars represent the initial surveys done around 1980, and hatched bars represent the current study. These are only preliminary data, which probably are not sufficient to warrant the statistical analysis that we plan to do at the conclusion of the study.

In general, however, since 1980 there has been a noticeable reduction in counts for many species. This is particularly true for grotto salamander, big brown bat, little brown bat, Indiana bat, and eastern phoebe. Gray bats are not graphed because the data would have greatly

Figure 10. Scoliopteryx libatrix, *the Herald Moth, overwinters in eastern U.S. caves.*

changed the Y-axis of the overall graph. Thirty-seven gray bats were observed in the first 14 caves in the earlier study, but we found about 2,747 gray bats in the current study, mostly from discovering an undocumented maternity colony in one cave. The latter discovery is good news for this species, which is slowly recovering in sites where it is well-protected (Elliott and Clawson, 1999).

Discounting gray bats, whose counts would obscure trends in the other data, total counts were down 34% (262 versus 172), amphibians were down 23% (165 versus 127), and bats were down 54% (93 versus 43). However, counts of pickerel frogs, which take refuge in wet caves in large numbers during drought and winter, held steady. Counts for a key species, the stygobitic grotto salamander, were down 67% (24 versus 8). Eastern pipistrelles, the most commonly seen bat, were up 233% (15 versus 35), while big brown bats were down 93% (54 versus 4).

Discussion

We emphasize that these are preliminary results only. Some species are not accurately represented in this data set because of their seasonal use of caves, for example big brown bats, which hibernate in caves but are not usually found there during the July 10 through October 10 time period of this data set. We expect that some of the "trends" will disappear or reverse after data for a full year are collected.

In one species with less seasonality, however, we see a suggestion of a downward trend that may be the result of three years of drought in the Ozark Region. Because of the drought, stygobitic grotto salamanders may have burrowed into moist, inaccessible microhabitats where we could not observe them, or they could have declined. Many cave streams are at extreme lows as we write this paper. Of the five caves where we counted grotto salamanders, three counts were down, one was up, and the species was found for the first time in one cave.

That drought may have affected some of the cave fauna is suggested by the apparent trend in two frogs, which take refuge in wet caves

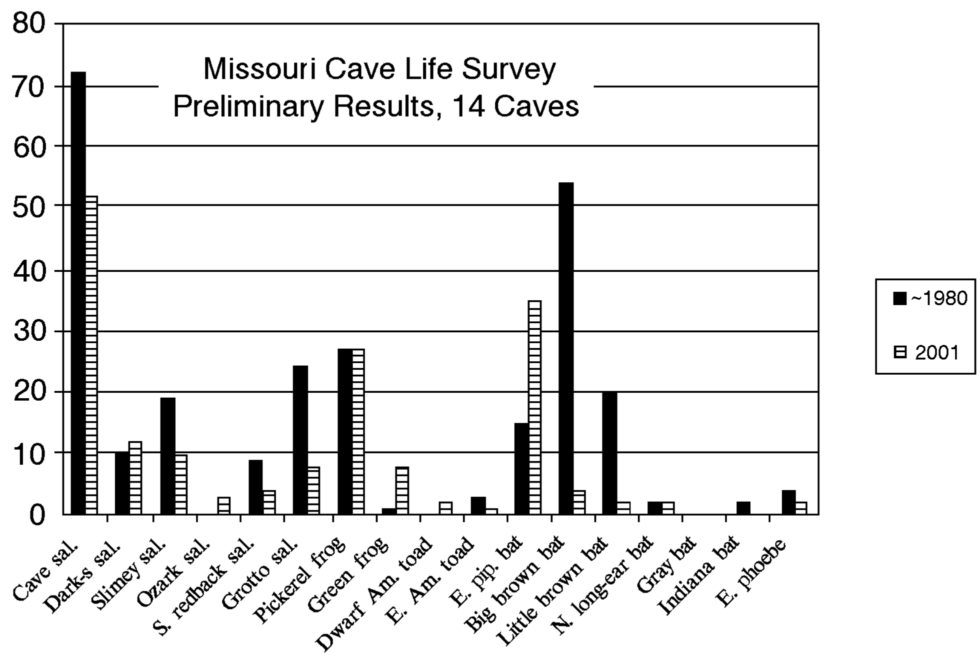

Figure 11. Graph of preliminary results from 14 caves and 17 species. Grey bats are omitted (See Results paragraphs).

during drought and winter. Pickerel frogs held steady (27 and 27), and green frogs increased (1 versus 8). For these frogs, relatively dry caves would still be wetter than dry, epigean habitat.

Conclusions

We are concerned that a key species, the grotto salamander, may have declined severely in Missouri, possibly as much as 67%. At the end of our study we may have sufficient data to confirm if this decline is true and to determine if drought, overuse of some caves, or both have contributed to such a decline. The grotto salamander formerly was a species of concern in Missouri, but it was removed from the state list in 1999.

Caves are not just habitat for troglobites and stygobites. Many trogloxenic and troglophilic species utilize caves for refuge, mating, or nesting. If common species have declined in caves, it would be important to identify if humans have caused the declines and to restore habitat. This study may not determine all the causes of declines, but it may provide direction for further study of certain species or land management activities that could restore wildlife populations in caves.

Other benefits of this study are the knowledge and resources gained by cavers and the Missouri Caves and Karst Conservancy for future projects. The booklet, data form, and procedures will be used in other projects. We probably will add new caves to the study to increase our baseline information for the future.

Acknowledgments

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A DNA Fingerprinting Technique to Survey Microbial Diversity in Caves

Rick Fowler
LuAnn Breeding
Jerry Ovesen
Chris Groves, Ph.D.
Shivendra Sahi
Biotechnology Center and the Hoffman Environmental Research Institute
Western Kentucky University

Abstract

A comprehensive survey of microbial species from cave sediments and karst aquifers is needed in order to appreciate their role in cavern formation, aquifer evolution, and cave ecology. The time consuming practice of culturing organisms from the environment has had limited success for only a few species, and those organisms that cannot be grown in the lab are omitted. We extract DNA directly from cave sediments and amplify bacterial, fungal, or algal 16S rDNA using the polymerase chain reaction and selected primers labeled with fluorescent dyes. Genetic libraries of bacterial 16S rDNA have been generated from cave sediments at selected sites in Mammoth Cave, and hundreds of cloned 16S rDNA sequences from cave bacteria have been analyzed. Species are being identified or taxonomically classified by phylogenetic sequence analysis and comparison to electronic nucleic acid databases, and characteristic fragment lengths have been tabulated for cloned or cultured cave bacterial 16S rDNA and standards. The 16S rDNA sequence and fragment database constitutes a reference to which DNA profiles of cave sediment bacterial communities can be compared.

Introduction

Mammoth Cave in Kentucky, with over 500 kilometers of surveyed passages, is the longest known cave system in the world. It has been the focus of much research into the formation and evolution of limestone caves and karst aquifers and it harbors a unique subterranean ecosytem. Earlier studies in our laboratories have examined the rate of limestone dissolution in stream sediments at the lowest level of Mammoth Cave where carbon dioxide partial pressures are an order of magnitude higher than in the stream itself. The higher levels of carbon dioxide presumably result from the action on organic materials by microorganisms in the sediment. Some of the microorganisms may be producing other acids that accelerate limestone dissolution and thus contribute to cavern enlargement and aquifer evolution (Vaughn, 1998; Vaughn *et al.*,1998)

Before the impact of microbial action on cave formation and cave ecology can be assessed, a thorough census of microorganisms of caves and karst aquifers is required. Some attempts have been made to survey and identify bacteria associated with Mammoth Cave sediments by selective culturing and morphologic characterization; but, of the strains that could be isolated and grown on a dish in the laboratory, the majority could not be identified (Rusterholz and Mallory, 1994). Current efforts in our group are addressing bacterial involvement in limestone dissolution by growing cave bacteria in liquid culture (Elliott *et al.*, 2000).

There are difficulties in using direct culturing methods for the study of microbial ecology in environmental settings. Traditional methods rely on the ability to culture any bacterial species present under laboratory conditions using classical microbiological techniques. In natural environments bacteria do not live alone in isolated culture, but instead they form interdependent communities of bacterial species called biofilms. Environmental strains have unknown nutritional requirements and less than 1% of those actually present are ever isolated in the laboratory (Amann *et al.*, 1992; Siering, 1998). The unknown factors are magnified greatly when attempting to culture microorganisms from extreme environments such as hydrothermal springs or volcanic vents (Moyer *et al*., 1994; Hugenholz *et al*., 1998), deep-sea sediments (Vetriani *et al*., 1999), salt lake beds (Minz *et al*., 1999), and subterranean ecosystems (Rusterholtz and Mallory, 1994; Elliott *et al*., 2000).

However, modern DNA analysis techniques are revolutionizing our understanding of bacterial diversity in the environment and have been applied to extreme environments including particular caves known to harbor bacterial communities in isolated and unusual geochemical conditions. New genera of bacteria capable of expressing genes with medical and practical applications have been discovered and are now the focus of many cave microbial studies (Angert *et al*., 1998; Holmes *et al*., 2001; Northtup *et al*., 2000).

We have begun a survey of microorganisms inside Mammoth Cave using modern DNA analysis techniques for the first widespread inventory of its microbial communities. The method described below is suitable for a broad survey of bacterial communities throughout the vast cave system and is applicable to a large number of samples. Our technique relies upon comparison of bacterial DNA fingerprints to a cave bacterial database of detailed genetic information derived from cultured and cloned organisms from selected cave sediments. We invite collaborations with other caves nationwide to contribute to the growth of our cave biomarker database.

Description of the Technique

Genetic identification of environmental strains.

Using modern DNA technology, bacteria can be identified and classified according to the sequences of their genes encoding 16S ribosomal RNA (16S rDNA). Different species of bacteria possess characteristic 16S rDNA sequences. Bacterial 16S rDNA sequences may be selectively amplified from the mixture of DNA fragments extracted from the environment to create many copies for more detailed studies (Figure 1). With this technique, bacterial species can be identified and their genetic relationships can be determined without the need to culture individual strains in the laboratory. Furthermore, environmental bacteria that cannot be grown in the laboratory can still be detected by the presence of 16S rDNA (Siering, 1998; Angert *et al*., 1998; Holmes *et al*., 2001).

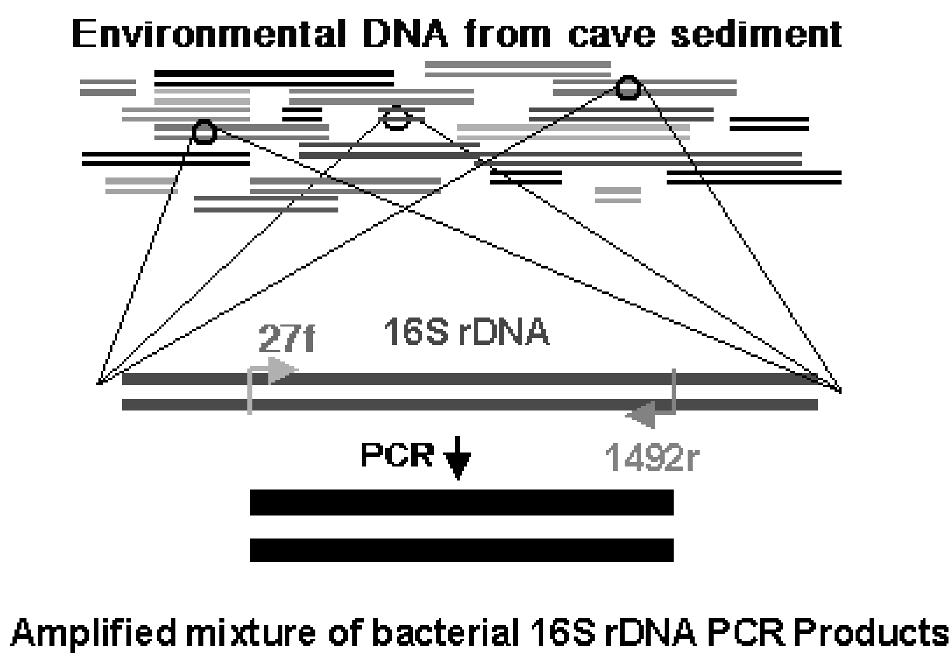

Figure 1. *Diagram showing how specific DNA sequences extracted from cave sediment can be targeted for analysis using the Polymerase Chain Reaction (polymerase chain reaction).* Some of the many different fragments of environmental DNA encode bacterial 16S rDNA (top). The 27f and 1492r short DNA sequences are conserved among the bacteria (middle), and they can be used as primers to amplify a mixture of bacterial 16S DNA sequences (bottom) while incorporating fluorescent dyes for analysis.

Sampling and DNA extraction

Sediment samples were collected from upstream, middle, and downstream points within Charons Cascade, along Echo River at the lowest level of Mammoth Cave. Sediment was scooped wearing latex gloves into sterile centrifuge tubes (Figure 2A) and kept on ice until DNA was extracted. DNA was extracted from one gram of cave sediment using a simplified procedure, and the mixed environmental nucleic acids were visualized by agarose gel electrophoresis (Figure 3). Cave sediment contains many microorganisms, including bacteria, fungi, protozoans, and even larger cave invertebrates (Figure 2B) with small particles of dead plant and animal material. All of these things contribute to the mixture of DNA fragments that can be extracted directly from cave sediment.

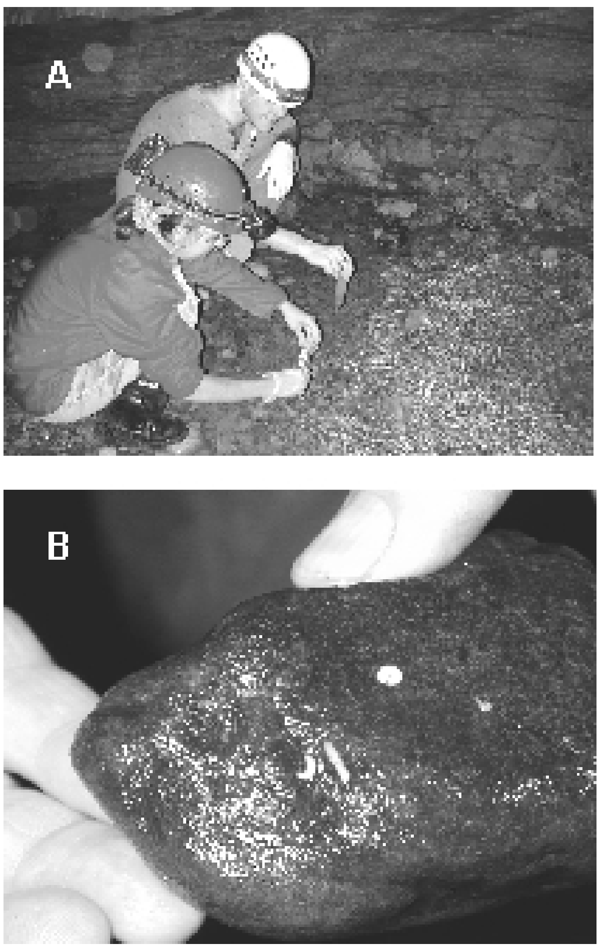

***Figure 2**. Sample collection in Mammoth Cave.* Sediment was scooped wearing latex gloves into sterile tubes (A) and kept on ice until DNA was extracted. In addition to bacteria, environmental DNA contains sequences from fungi, protozoans, and even cave invertebrates (B) with decomposed plant and animal material.

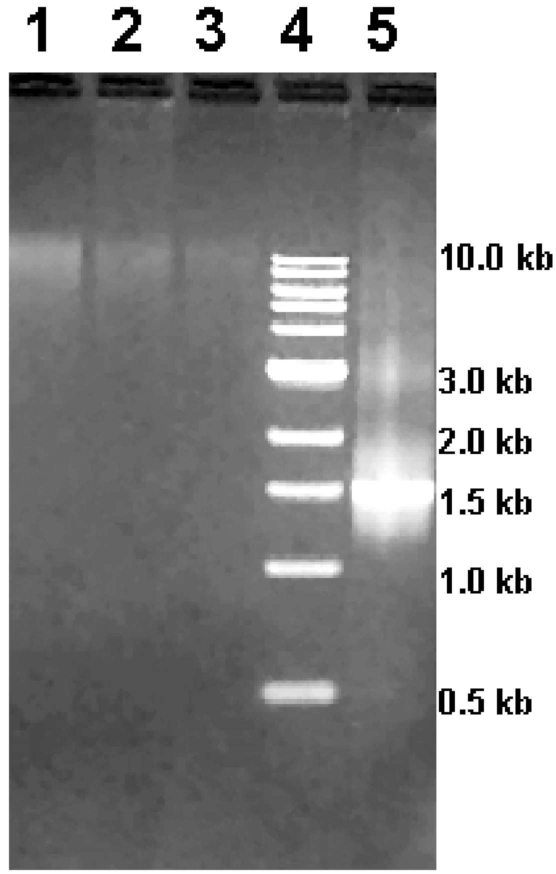

Figure 3. *Agarose gel showing cave sediment DNA and 16S rDNA polymerase chain reaction product.* DNA fragments at approximately 10.0 kb were extracted from 0.5 g sediment collected at upstream (lane 1), middle (lane 2), and downstream (lane 3) sites near Charons Cascade. DNA was amplified by polymerase chain reaction to

Amplification of 16S rDNA

In order to study the DNA of cave bacteria among all the DNA fragments present, specific DNA sequences were amplified out of the mixture using the polymerase chain reaction with specific bacterial primers. Our study focuses on the bacterial community in general thus we are using primers 27f and 1492r, short sequences that are conserved among a broad range of bacteria (Lane, 1991; Layton *et al*., 1994).

A polymerase chain reaction product from cave sediment representing the cave bacterial community was seen by agarose gel electrophoresis with the expected size of about 1.5kb (Figure 3). The environmental polymerase chain reaction product consists of a mixture of 16S rDNA from all bacterial species that have in common

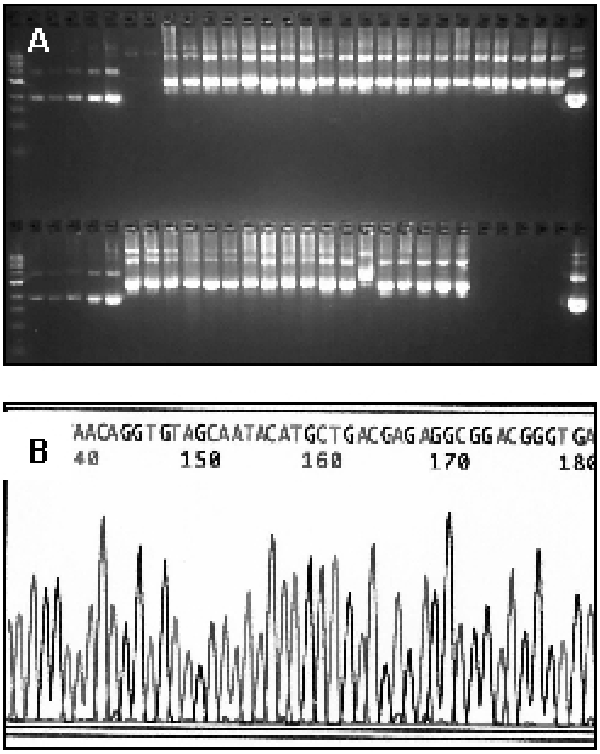

Figure 4. *Agarose gel (A) showing plasmid DNAs from a cave clone library and automated DNA sequencing data (B).* Each of the cloned plasmid DNA molecules shown on the gel carries one kind of bacterial 16S rDNA from the cave sediment (A). The DNA sequence of each cloned 16S rDNA was determined using automated fluorescent DNA sequencing with capillary electrophoresis (B) to generate data that was compared to online genetic databases. Table 1 shows a summary of the taxonomic groupsof the nearest genetic relatives using the Ribosomal Database Project online (http://rdp.cme.msu.edu).

the 27f and 1492r primer sequences. In order to differentiate among the many types of bacteria in the community, we must sort the amplified genes by molecular cloning and DNA sequencing or distinguish them by their terminal restriction fragment lengths.

Cloning and Sequencing

The amplified 16S rDNA was spliced into a cloning and sequencing vector plasmid DNA. The circular recombinant plasmid molecules thus produced were used to transform *E. coli* for studies of individual copies of the environmental genes. A cave clone library of *E. coli* host cells carrying cave DNA sequences was created and plasmid DNA was purified from each clone. Each clone harbors just one type of recombinant plasmid DNA representing one bacterial 16S rDNA sequence originating from the cave sediment (Figure 4A).

Table 1. Nearest genetic relatives within clone library of bacteria from Mammoth Cave.

Taxon:	No. of clones (%)
Nitrospina sub dv.	8 (18%)
Proteobacteria	
Alpha	6 (14%)
Beta	8 (18%)
Gamma	1 (2%)
Delta	3 (7%)
Gram-positive	4 (9%)
Environ. clone WCHB1-31 grp.	4 (9%)
Unclassified/Unaligned	4 (9%)
Planctomyces and relatives	3 (7%)
Environ. clone PAD1 grp.	1 (2%)
Green non-sulphur and relatives	1 (2%)
Flexibacter/Cytophaga/Batteroides	1 (2%)

Nucleotide sequences of bacterial 16S rRNA genes from the clone library and cultured bacteria have been determined (Figure 4B) and compared to DNA sequence databases to find the taxonomic classification of the nearest genetic relative (Table 1). Four subgroups of Proteobacteria representing a high degree of diversity corresponded to 41% of the clones sequenced. It is noteworthy that 18% of the clones were closely related to the Nitrospina subdivision with few species previously known. Nitrospina may contribute to cave geochemistry and acid production through nitrification reactions that accumulate nitrate, particularly in the absence of plants. Other clones were related to Gram positive species, Planctomycetes, and various uncharacterized bacteria commonly found in soil. Some of the matches raised ecological red flags by indicating the presence of bacteria that derive energy through biodegradation of petroleum, creosote, heavy metals, or sewage.

Fragment Analysis

Rather than commit to cloning and sequencing from every cave sample examined, a snapshot of bacterial diversity can be generated easily and quickly for a larger number of samples by terminal restriction fragment length polymorphism (TRFLP) analysis. Snapshots from environmental samples depict multiple types of bacteria within the community in a given sediment sample, and the profile generated is a "fingerprint" with information about the types of bacteria present and their relative abundance.

Environmental DNA from cave sediments, plasmid DNA from the cave clone library, and

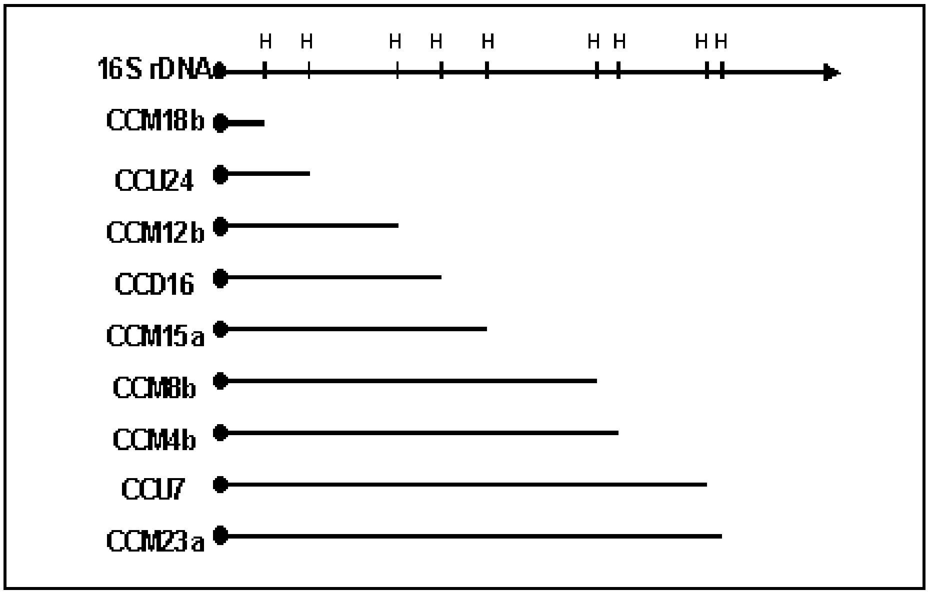

Figure 5. *Diagram showing fluorescent fragments that can be used as biomarkers for bacteria.* Fluorescent 27f primer was used to produce a mixture of labeled bacterial 16S rDNA sequences from cave sediment (top). Depending upon their individual DNA sequences, the fragments are cleaved by the enzyme HhaI (H) at some specific distance from the fluorescent terminus. A profile of the fragments derived from cave sediment is called a fingerprint. Fragment lengths with DNA sequence data from cloned and cultured bacterial 16S rDNA can be used to interpret the fingerprints.

Environmental DNA profiles are interpreted with the aid of the tabulated fragment data. DNA fingerprints of cave bacterial communities are labeled blue with 27f primer, while cloned or cultured standards are labeled green or yellow. Digestion of both environmental and cultured or plasmid DNA with HhaI followed by simultaneous capillary electrophoresis allows the corresponding peaks in the environmental profile to be directly superimposed with the 16S rDNA data from cloned and cultured bacteria (Figure 6).

genomic DNA from cultured organisms was amplified with fluorescent-labeled primer 27f and non-labeled 1492r. We obtained copies of 16S rDNA labeled at the 5' end of the 27f primer sequence with blue, green, or yellow fluorescent dyes. The end-labeled fluorescent polymerase chain reaction products were then digested with the restriction enzyme HhaI to generate fragments which were analyzed on a fluorescent genetic analyzer. Only the fragment from the fluorescent terminus up to the most proximal HhaI site is labeled and therefore observed by the fluorescence detector.

When TRFLP analysis is applied to the purified plasmid DNA samples in the clone library, each clone yields a single peak in the electropherogram with a characteristic defined fragment length determined by that particular DNA sequence. A total of 103 bacterial 16S rRNA genes have been analyzed by TRFLP including 87 from Charons Cascade in Mammoth Cave, along with nine cultured organisms from Mammoth Cave, four cultured from Lost River Cave, and three ATCC standard cultures. Their fragment sizes have been averaged over multiple determinations and tabulated in a database along with the corresponding DNA sequences and phylogenetic data.

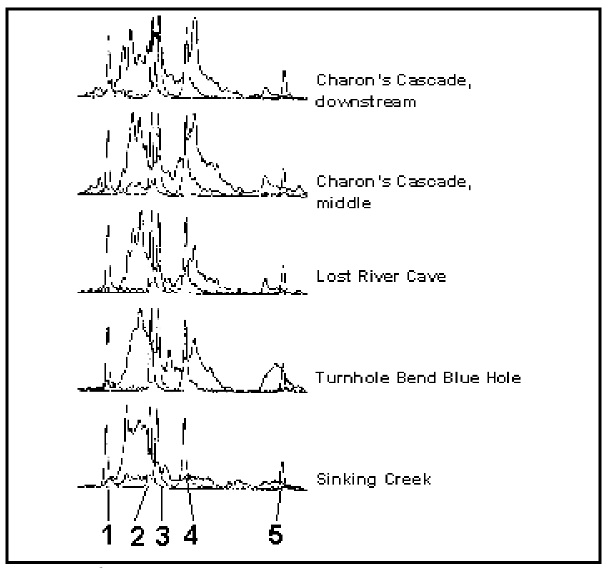

Figure 6. *Bacterial DNAfragment profiles from various cave and karst sediments superimposed with DNA fragments from the cave bacterial database. Bacterial 16S rDNA fingerprints labeled with blue fluorescent dye were mixed with yellow and green 16S rDNA fragments from cloned and cultured bacteria in the database. Standards are (1) MCNP clone CCU10, (2) Pseudomonas env. str. MCNP-CCCO12, (3) Pseudomonas env. str. MCNP-CCCO8, (4) MCNP clone CCU8, and (5) Staphylococcus aureus from a* standard depository (ATCC).

Summary

Our technique, summarized in Figure 7, allows many different bacterial types to be surveyed in a single DNA test that can be applied to a larger number of cave sites. Of particular interest are those sites known to be undergoing limestone dissolution and cavern enlargement and where geochemical and hydrological data are being collected. The growing database of DNA sequence and phylogenetic information along with fragment sizes from the cave clone database provides a means for recognizing and monitoring bacterial species in cave sediments, without the need to isolate and culture the organisms.

Acknowledgments

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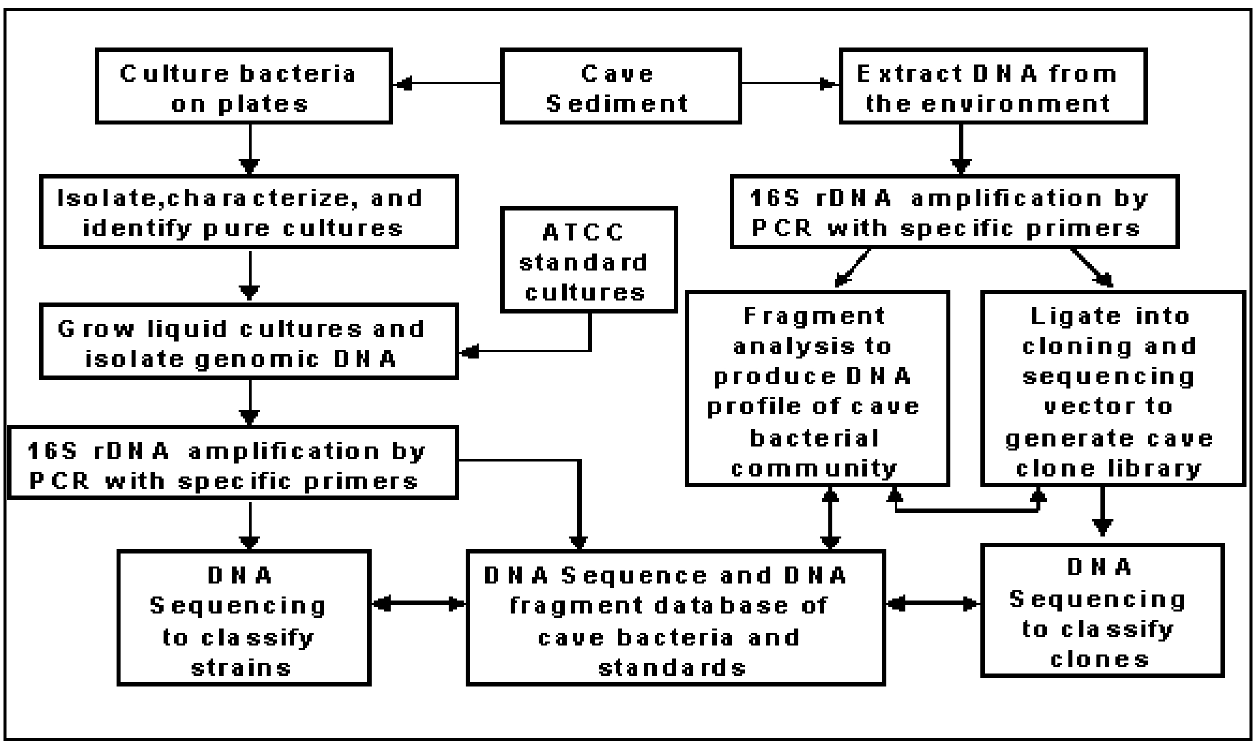

Figure 7. *Flow chart of information leading to the creation and growth of the database of genetic markers for cave bacteria.* On the left, cave sediment is the source for bacteria cultured and identified on plates. Isolated bacteria from the environment and from standard collections can be grown in liquid culture to yield DNA that can then be analyzed by DNA sequencing and fragment analysis to contribute to the database.On the right, environmental DNA can be extracted directly from cave sediment and subjected to polymerase chain reaction with specific bacterial primers. The amplified mixture of polymerase chain reaction products can be individually sorted and identified by cloning and sequencing the cave bacterial 16S rDNA. To survey a larger number of samples, the amplified polymerase chain reaction mixture is labeled with fluorescent dye and subjected to fragment analysis to produce a profile that is interpreted with the aid of the database.

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Caves and Mine Adits as Wildlife Resources in the Sonoran Desert Region

Kenneth J. Kingsley
SWCA, Inc., Environmental Consultants, Tucson, Arizona
kkingsley@swca.com

Thomas R. Strong
E. Linwood Smith
Environmental Planning Group, Tucson, Arizona
tstrong@epgaz.com

Tim K. Snow
Arizona Game and Fish Department, Tucson, Arizona

Presented as a Poster

Abstract

Natural caves are rare in the Sonoran Desert region and anthropogenic mine adits are abundant and similar to caves in many respects. Both caves and mines are important resources for several wildlife species that live in the Sonoran Desert region. Wildlife uses include short-term shelter from variable ambient temperature and humidity and long-term uses such as maternity roosts, den sites, and nest substrates. Some predatory species also use mines and caves as hunting sites. Animals that use these resources include mammals (several species of bats, bighorn sheep, collared peccaries, ringtails, foxes, packrats, mice, mountain lions, and others), birds (turkey vultures, rock wrens, Say's phoebes, barn owls, and others), herpetofauna (several species of rattlesnakes, toads, lizards), and invertebrates. Many of these sites are also used by people for recreational or economic uses. The wildlife values of these sites have prompted their inclusion as protected sites in the Sonoran Desert Conservation Plan, a county government plan for the long-term protection of biodiversity in the Tucson area and 5,000,000-acre Pima County. This paper will describe wildlife uses of caves and mines in this area, list species known to us to use caves and mine adits, give examples of especially important sites, discuss management approaches, and review the process of including them in the Conservation Plan.

Introduction

Natural caves are very rare in the Sonoran Desert region. Mine adits are abundant and similar to caves in many respects. Pima County, Arizona, an area of over 5,000,000 acres has only a handful of natural caves but hundreds of mines. The State of Arizona estimates some 100,000 inactive mines statewide. More often than not, mines are found in areas devoid of natural caves, under geological conditions that do not foster cave development. Mines may be the only accessible subterranean features under these circumstances and they provide important resources for native wildlife.

Very few species of true troglobites have been described from Arizona and none are also known from mines. Many species of trogloxenes are known to use both caves and mines. In some instances, simplified ecosystems resembling those found in natural caves in other parts of the world have developed in mines in the Sonoran Desert.

Because of their rarity and locations on public lands, most Sonoran Desert caves are protected. The wildlife values of mines warrant consideration for protection also. The wildlife values of caves and mines in the Sonoran Desert sites have prompted their inclusion as protected sites in the Sonoran Desert Conservation Plan, a Pima County government plan for the long-term protection of biodiversity in the Tucson area and the 5,000,000+-acre county.

Wildlife Uses of Caves and Mines

Both caves and mines are important resources for several wildlife species that live in the Sonoran Desert region. They provide shelter from hot, dry conditions and from predators. There are few currently known species of troglobites in the Sonoran Desert region. Most wildlife species use caves and mines as short-term shelter, occupying a site for only a few hours or days during adverse weather conditions. Several species of bats use caves and mines as maternity roosts, day roosts, night roosts, or courtship areas. Some species of birds use caves and mines as shelters in which they build nests. Several mammal species use caves and mines as den sites. Table 1 lists the wildlife taxa using caves or mines observed by one or more of the authors.

Table 1. Wildlife Species Known to Use Caves and Mines in the Sonoran Desert Region

Invertebrates
- camel crickets
- daddy longlegs
- flies
- mosquitoes
- springtails

true troglobites– *few described, not known from mines*

Amphibians
- red-spotted toad
- barking frog
- lowland leopard frog
- tiger salamander

Reptiles
- tree lizard
- side-blotched lizard
- eastern fence lizard
- Clark's spiny lizard
- desert spiny lizard
- alligator lizard
- Gila monster
- desert tortoise
- western diamondback rattlesnake
- Mojave rattlesnake
- tiger rattlesnake
- black-tailed rattlesnake
- rock rattlesnake
- speckled rattlesnake

Birds
- great horned owl
- barn owl
- white-throated swift
- cliff swallow
- violet-green swallow
- Say's phoebe
- canyon wren
- rock wren
- house wren
- turkey vulture
- black-throated sparrow

Mammals
- Townsend's big-eared bat
- Allen's big-eared bat
- spotted bat
- pallid bat
- cave myotis
- southwestern myotis
- small-footed myotis
- fringed myotis
- California myotis
- Yuma myotis
- western pipistrelle
- big brown bat
- California leaf-nosed bat
- lesser long-nosed bat
- Mexican long-tongued bat
- Mexican free-tailed bat
- western mastiff bat
- cactus mouse
- canyon mouse
- brush mouse
- white-throated woodrat
- desert woodrat
- Mexican woodrat
- porcupine
- rock squirrel
- black bear
- ringtail
- bobcat
- mountain lion
- gray fox
- kit fox
- coati
- spotted skunk
- striped skunk
- hognosed skunk
- mule deer
- bighorn sheep
- collared peccary

Biological Exploration of Caves and Mines in the Sonoran Desert

Few of the known caves have been well studied over a period of years. Unique species have been found. Other caves have had very little, if any, biological exploration. Some caves receive some level of recreational use. One known cave is strictly protected and only accessible to researchers.

Most mines have never been examined by biologists. Of those that have, about one in ten (on average) are used by bats as day roosts and about four in ten are used as night roosts. In one recent survey of 21 adits four had no evident use by wildlife, five were used by bats, eight were used by other mammals, eight were used by birds, 11 had rattlesnakes of three species, and nine had evidence of vandalism, including beer cans, shotgun and cartridge shells, and other trash.

The wildlife values of inactive mines are so important that they should be studied and protected. Most government agencies that manage land with inactive mines now require surveys before any officially sanctioned disturbance occurs. Few efforts have been made to protect mines from vandalism.

Exploring inactive mines can be much more dangerous than cave exploration. Walls and ceilings are unstable, support timbers may be rotten, hazardous materials (including explosives) may have been left behind, booby traps may have been set, and some wildlife species may react defensively to human intruders.

Caves, Mines, and The Sonoran Desert Conservation Plan

The Sonoran Desert Conservation Plan is an ongoing process being developed by Pima County to guide future development and management of land while ensuring continued high biological value and protecting the biodiversity of the County. The process includes designation of Conservation Lands within the County, including all known caves as well as mines that are known to be used by bats. Several species of cave and mine roosting bats are included as species covered by the plan process. Known roosts of these bats are included in the planning process as constraints, to be protected under all alternatives for the plan. Protective measures will be developed for each as appropriate and necessary.

How Much Surface Habitat is Enough? Preserve Design and Application for Cave-Limited Species

Jean Krejca
U.S. Fish and Wildlife Service

Lisa O'Donnell
Kathryn Kennedy
Shannon M. Knapp
Alisa Shull
Charmaine Delmatier

Abstract

Central Texas supports some of the world's most biologically rich and diverse cave ecosystems. The rapid pace of urban expansion threatens many of these ecosystems and has led to the federal listing of 16 cave-limited invertebrates as endangered. Due to their rarity and endemicity, destroying even a very few caves means certain extinction for many cave species, as these environments cannot be recreated. To avoid this outcome and assist developers in complying with the Endangered Species Act, we have developed preserve design recommendations to promote the species' survival in perpetuity. Historically, conservation efforts have focused solely on protecting cave entrances and drainage basins. Here, we take a broader perspective and consider population viability requirements of the surface plants and animals that are intricately intertwined with the life support system of each cave. We conclude that long-term protection entails a minimum preserve size of 69 to 99 acres (0.27923 to 0.40064 square kilometers) around a given cave or cave cluster, as well as maintenance and adaptive management to ameliorate other insidious threats, such as infestations of red, imported fire ants (*Solenopsis invicta*). Problems associated with setting these preserve standards in rapidly developing areas include inflated land values, public response, limited data on the species of interest, and the improbability of re-populating a cave once the species is extirpated.

Managing Endangered Species: Charting the Course of the Illinois Cave Amphipod with Non-lethal Censusing

Julian J. Lewis, Ph.D.
Salisa T. Rafail
J. Lewis & Associates, Biological Consulting

Diane Tecic
Illinois Department of Natural Resources

Abstract

In 1940 the Illinois Cave Amphipod, *Gammarus acherondytes*, was described as a new species. The only obligate subterranean amphipod of the genus *Gammarus* in North America, this unique crustacean was described from two caves in southwestern Illinois. By 1988, cave bioinventories had revealed *Gammarus acherondytes* in a total of six caves just southeast of metropolitan Saint Louis. Over time, groundwater quality deteriorated in the area as land use changed. In 1995 *Gammarus acherondytes* could not be found in two previous sites and was barely present in two others. The amphipod was listed as a federally endangered species in 1998. In 1999 bioinventory by The Nature Conservancy revealed six additional cave populations, two in groundwater basins where the amphipod was previously unknown. As an endangered species, *Gammarus acherondytes* presented a censusing dilemma. There was no way known to monitor the 12 cave populations of *Gammarus acherondytes* without killing the amphipods to count them. In 2000 a project was initiated to see if it would be possible to measure the population sizes without killing the tiny endangered animal. Experimental census transects were established in several caves. To eliminate sampling prejudice, quadrats were randomly placed within the transects. Using a hand-held 15X microscope it was possible to separate *Gammarus acherondytes* from three other species of co-occurring cave amphipods. All animals were identified, measured, and released immediately back into the stream. The method was painstaking and labor intensive, but successful. Full-scale censusing of the endangered species commenced in 2001.

Introduction

The subterranean amphipod, *Gammarus acherondytes,* was described by Hubricht and Mackin (1940) from specimens collected by Leslie Hubricht from Morrisons Cave (Illinois Caverns) and Stemler Cave in the karst of southwestern Illinois. Bousfield (1958) redescribed the species but added no new localities. Based on collections in the mid-1960s, Peck and Lewis (1978) added Fogelpole, Krueger/Dry Run, and Pautler Caves to the list of localities from which this amphipod was known. In 1976, Lewis visited Illinois Caverns and Stemler Cave to evaluate the sites for the Illinois Natural Areas Inventory. The cave communities were inspected and appeared intact at that time, but no collections were made. However, over the next 20 years the land use of the area began to change from primarily agricultural and second growth forest into a region with an increasing suburban component. Webb (1995) reported that *G. acherondytes* could no longer be found in Stemler Cave and only small numbers of the amphipods were present in the other sites (Pautler Cave was reportedly closed by the owner). Fueled by the growing interest in *G. acherondytes*, The Nature Conservancy conducted a bioinventory of caves in Monroe and Saint Clair Counties (Lewis, Moss, and Tecic, 1999). This project resulted in the report of six additional caves with populations of *G. Acherondytes*. During that same year, *Gammarus acherondytes* was added to the U.S. Endangered Species List.

During the bioinventory by The Nature Conservancy it became apparent that little had been done to provide a basis by which the populations of *Gammarus acherondytes* could be measured. Webb (1995) had collected amphipods in various parts of several caves using a biased sampling technique that considered only the amphipod subset of the total community. Lewis, *et al*. (1999) collected samples in a similar manner to produce results that could be compared to what had already been done. These samplings of the populations provided little data that could be duplicated to determine the ongoing situation with *G. acherondytes*, while killing the endangered animals that were purportedly being "saved."

Thus was born the *raison d'etre* for developing a non-lethal method for estimating populations: to provide a yardstick by which the status of *Gammarus acherondytes* could be measured in the future to see if the situation was getting better, getting worse, or staying the same. The best method for censusing anything is to count the entire population. This is obviously not possible with a cavernicolous invertebrate, therefore leading to the alternative of examining a subset of the population. Many methods are known for preparing population estimates. We have chosen to use one that was suggested to Julian J. Lewis by cave ecologist Thomas L. Poulson for population biology studies in the aquatic communities of the Flint-Mammoth Cave System of central Kentucky.

Population Estimate Methodology

In general, the method consists of counting and measuring all species present (not just a target organism of interest) in multiple, randomly-selected quadrats along a series of transects. Analyzing the entire community, rather than merely a population within it, provides a much more complete picture of what is happening in the ecosystem. Concerning measurement of the animals, many stygobitic organisms have populations that are skewed toward older (larger) individuals with fewer juveniles (smaller) or ovigerous females. Although it might be impossible to glean the exact size of an amphipod, an estimate of six millimeters for an amphipod places the animal in a subadult cohort that obviously differs from a two-millimeter brood release or an 18-millimeter adult. This provides important information when the entire community is measured.

The fauna

Aquatic cave communities are usually relatively simple, comprising a handful of species that can frequently be identified easily, even in the field by the naked eye. Unfortunately this is not the case in western Illinois cave streams in which there are four species of amphipods that are of approximately the same size and shape. Non-lethal identification of the amphipods was the most challenging part of the project.

Cave stream communities in the western Illinois karst of Monroe and Saint Clair Counties typically comprise an assemblage of species: the flatworm *Sphalloplana hubrichti* (stygobite); snails *Fontigens antroecetes* (stygobite); *Physella* sp. (stygobite or stygophile); isopods *Caecidotea packardi* (stygobite); *Caecidotea brevicauda* (stygophile); and amphipods *Gammarus acherondytes* (stygobite), *Gammarus troglophilus* (stygophile), *Bactrurus brachycaudus* (stygobite), *Crangonyx forbesi* (stygophile). Detailed analysis of the identification of these animals was presented by Lewis (2000).

Census transects

The first priority in the establishment of transects was the presence of a landmark felt to be of an enduring nature, such that a researcher desiring to repeat the census a century from now would have an excellent chance of finding the same spot again. For each riffle transect, when facing upstream the census start point was the point at which the riffle ended and pool habitat started on the right-hand side of the riffle. A square foot (30 by 30 centimeters) Surber sampler was used to collect samples. Randomization of the sample sites was done by selecting each sample site with a number taken from a random numbers chart (available in most statistics books). The starting spot in the random numbers chart was selected first by random selection on the chart. From the point selected, the numbers were read down the column and the first two digits used to select the sample spot. A flexible plastic tape measure was stretched down the right hand side (facing upstream) of the riffle. Using the random number, the first digit was used to select the number of feet up the tape for the first quadrat. The second digit was the percentage across the stream from the right hand bank. For example, if the first number was 4268, and the stream was 10 feet wide, the first quadrat would be placed four feet up the riffle and 20% (two feet) across the stream from the right bank. After the Surber sampler was placed, a ruler was placed in the shallowest and deepest part of the quadrat and the depth recorded. If the water depth was less than about 2.5 centimeters the animals present were censused *in situ*. If the water was deeper than 2.5 centimeters the gravel was dislodged and animals allowed to wash into the sampler. All rocks were visually inspected for animals clinging on them.

Several large plastic beverage cups were carried into the cave and used to wash any animals or other material clinging onto the net of the sampler into the plastic container on the bottom of the sampler. Usually 8 to 12 washings were adequate. The contents of the sampler were released at streamside into a plastic bowl. On the first day of censusing a four-inch square bowl was used and was immediately recognized as inadequate in size. That evening an 8- by 12-inch Rubbermaid plastic bowl was purchased and was found to be an ideal size for carrying into the cave as well as containing the samples. All animals except amphipods were identified immediately visually, measured with a millimeter grid placed in the bottom of the bowl, and released back to the stream. Amphipods were placed in a dish with a millimeter grid prepared by photocopying graph paper (five grids per centimeter) onto 8½ by 11 inch 3M Transparency Film. Initially an 8X Loop was utilized for identification of the amphipods, but the 15X magnification provided by a Waltex hand microscope was found to give better viewing of animals less than six millimeters in length. A 2.5X Optivisor was found to be ideal for identification of amphipods greater than ten millimeters and the other aquatic invertebrates present in the samples. Immediately after identification all invertebrates were released back into the stream.

As-noted habitat was characterized by measuring the water depth in centimeters and giving an approximate description of the composition. Small particle size was characterized as clay if it was smooth when rubbed between the thumb and forefinger, and sand if it was gritty to the touch. Gravel was anything larger than sand up to three centimeters in size, cobbles were larger than three centimeters. Pieces of breakdown present were measured and noted.

It was noted that some animals, particularly flatworms and snails, occurred mostly under larger pieces of rock. Thus, in each transect it was decided to use a timed census rock count. This method consists of picking up larger rocks, identifying all of the fauna present on them, sight-estimating the size, then returning the rock and animals immediately to the stream. It was decided to do five-minute timed counts and to lift rocks larger than about ten centimeters throughout the transect. The number of rocks surveyed and an estimate of the size of the rocks were included in the census data.

Results

The raw data was recorded in the cave and then transcribed into a standardized spreadsheet format. On this datasheet is contained the name of the site, the location within the cave of the census area, date and personnel conducting the census, random numbers used to generate the quadrats, a description of the quadrat microhabitat, and the lengths of all animals found.

Population size of the Illinois Cave Amphipod can be estimated by extrapolating the area sampled to encompass the total area of the transect. Alternately, the relative proportions of the populations in different caves (or different parts of the same cave) can be compared by analyzing the mean number of amphipods per quadrat (square foot), which requires no extrapolation. For example, areas censused in Fogelpole Cave ranged from 0 amphipods per quadrat in the nature preserve entrance area to 1.7 amphipods/quadrat in the upstream part of the caves. The largest populations were found in Pautler Cave (up to 1.3 amphipods per quadrat), Fogelpole Cave (1.7 amphipods per quadrat) and Frog Cave (up to 3.3 amphipods per quadrat).

The data can be analyzed in a variety of other ways. For example, microhabitat preference of *Gammarus acherondytes* was examined as a function of substrate versus water depth, with the data indicating that the amphipod strongly prefers gravel/cobble substrates in shallow water.

These are just a few examples of results. Complete data was presented by Lewis (2000, 2001).

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Limiting Lamp Flora in Developed Passages Within Mammoth Cave

Rick Olson
Mammoth Cave National Park

Abstract

Lamp flora—a European term for the algae, mosses, and ferns that grow near electric lights—are a problem in nearly all show caves. These growths have been regarded as a "nuisance," but are actually a serious distortion to cave ecosystems. Control has been achieved largely via chemical treatments, which are indiscriminate killers. Ecological impact has been limited through careful application, but the safety of people doing this work remains as an issue. The idea of limiting lamp flora growth by wavelength selection is not new. This concept has arisen independently around the world over the past two decades. Though early tests were somewhat disappointing, recent advances in lighting technology, particularly Light Emitting Diode lamps, have made this approach feasible. Testing of yellow (595-nanometer) Light Emitting Diode lamps in Mammoth Cave has resulted in no re-growth of lamp flora in a former problem area over a 1.5-year period at an intensity of 4.6 foot-candles (49.5 Lux).