JOURNAL OF CAVE AND KARST STUDIES

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EDITORIAL

Production Changes for Future Publication of the *Journal of Cave and Karst Studies*

Scott Engel
Production Editor

The *Journal of Cave and Karst Studies* has experienced budget shortfalls for the last several years for a multitude of reasons that include, but are not limited to, increased cost of paper, increased costs of shipping through the United State Postal Service, increased submissions, and stagnant funding from the National Speleological Society (NSS). The cost to produce the *Journal* has increased 5 to 20 percent per year for the last five years, yet the budget for the *Journal* has remained unchanged. To offset rising costs, the *Journal* has implemented numerous changes over recent years to streamline the production and printing process. However, the increasing production costs, combined with the increasing rate of good-quality submissions, has resulted in the number of accepted manuscripts by the *Journal* growing faster than the acquisition of funding to print those manuscripts. As a result, the *Journal* is experiencing an unprecedented backlog of manuscripts to be published, resulting in long delays (up to one year) between acceptance of a manuscript and its printing. In order to clear our backlog and stay within budget, changes have to be made. *Journal* staff and the NSS Board of Governors have been evaluating numerous options for more than a year to find the best approach to maintain the *Journal* as the premier peer-reviewed journal for karst studies while keeping it within the budget constraints of the modern economy.

At the recommendation of the *Journal* Editor, the NSS Board of Governors voted in July 2011 to begin moving to an electronic publishing process to reduce production and printing costs (Act 81-854). Accordingly, beginning with the December 2011 issue, printed copies of the *Journal* will be automatically distributed to paid subscribers, institutions, and only those NSS members with active Life and Sustaining level memberships. The remainder of the NSS membership will be able to view the *Journal* electronically online but will not automatically receive a printed copy. Full content of each issue of the *Journal* will be available for viewing and downloading in PDF format at no cost from the *Journal* website www.caves.org/pub/journal.

Anyone wishing to receive a printed copy of the *Journal* will be able to subscribe for an additional cost separate from normal NSS dues. The cost and subscription process were still being determined at the time of this printing. Once determined, the subscription information will be posted on the *Journal* website. We anticipate that in the future, subscription fees will be included as an optional payment along with annual NSS dues renewal or can be made separately online or through the NSS office. We hope to reduce the financial burden on individuals by continuing to allow free access online to all.

While this is not an ideal solution for many current recipients of the *Journal*, it is consistent with the operation and distribution of many scientific journals around the world. The *Journal* has grown to become the most recognized karst science publication in the world and is the cornerstone of science education outreach in the NSS. We believe that the change to electronic publication will serve to preserve the *Journal* in a sustainable manner and will allow it to continue as a key resource for the Society's mission to advance the study and science of speleology.

IN SITU NITROGEN FIXATION BY CYANOBACTERIA AT THE ANDRAGULLA CAVE, SPAIN

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Abstract: Andragulla Cave is 2 m high, 6 m wide, and 2 m long (deep). Its lack of depth means that it is not isolated from external influences, and the microclimate is very similar to that experienced externally. The common stress factors on the growth of cyanobacterial communities on walls inside of Andragulla Cave include excess light, dryness, lack of nutrients, and cold temperatures. Nitrogen fixation, photon flux, relative humidity, and temperature in Andragulla Cave were measured hourly over 24 hours in winter. Nitrogen fixation by the reddish-brown mat formed by both cyanobacteria *Scytonema mirabile* and *Gloeocapsa sanguinea* in cave-like environments was measured in situ for the first time by acetylene reduction. The mat-specific rates (1.6 to 7.5 nmol C_2H_4 m^{-2} s^{-1}) were lower compared to published values from laboratory measurements of rehydrated samples from different environments. Daytime fixation was slightly higher than nighttime fixation, where nitrogen fixation by *Gloeocapsa sanguinea* played an important role. The most influential parameters for environmental nitrogen fixation in Andragulla Cave were photon flux for daytime fixation, temperature for nighttime fixation, and relative humidity for both. Nitrogen fixation by cyanobacteria may be significant in these N-poor ecosystems.

Introduction

Atmospheric nitrogen fixation ability is limited exclusively to prokaryotes. Cyanobacteria are the only prokaryotes capable of both nitrogen fixation and oxygenic photosynthesis. The coexistence in a single organism of oxygenic photosynthesis and nitrogen fixation appears to be paradoxical, because nitrogen fixation is an intrinsically anaerobic process. The key enzyme involved, nitrogenase, is rapidly and irreversibly inactivated in vivo when exposed to even low oxygen partial pressures.

In heterocysted cyanobacteria, a spatial separation takes place between the incompatible nitrogen fixation and oxygenic photosynthesis processes. Even though heterocysts retain photosystem activity I, they lack photosystem II and activity of ribulose bisphosphate carboxylase, a fundamental enzyme in the Calvin cycle. Consequently, heterocysts are neither able to fix CO_2 nor able to produce O_2. They also have a special cellular casing that restricts oxygen from entering (Walsby, 1985). For this reason, heterocysts are practically anoxic inside and are nitrogen fixation sites (Fay, 1992).

It is likely that all heterocysted cyanobacteria are able to fix molecular nitrogen (Gallon, 1980). When other compounds of nitrogen are limited, using atmospheric nitrogen gives a competitive advantage over those organisms lacking nitrogenase (Fogg et al., 1973). This capacity enables cyanobacteria to play a very important role in hostile environments (Housman et al., 2006; Zielke et al., 2005).

In general, studies into an organism's atmospheric nitrogen fixation capacity have been done in a laboratory setting. Therefore, very little information is available about the in situ fixing behavior of organisms (Aranibar et al., 2003; Grimm and Petrone, 1997; Sivonen et al., 2007), and such information in cave settings has been found only in the study of Griffiths et al. (1987), for the genera *Nostoc* and *Gloeothece*.

Because certain factors affecting nitrogen fixation are sometimes irreproducible in the laboratory and there is hardly any information available about nitrogen sources in such adverse environments as cave settings, prompted us to undertake this study. This work is the first to present in situ nitrogen fixation data in the *Scytonema* mat communities growing in caves.

Study Area

Andragulla Cave (UTM 30SWH848264) is situated at an elevation of 1,200 m in the municipality of Moratalla, in the Region of Murcia in southeastern Spain. The climate of this region is Mediterranean, with certain continental characteristics and frequent frosts in winter. The mean annual rainfall is 451 mm, and the average temperature is 16.3 °C. The area is made up of finely stratified limestone with overlaying layers of differently colored loams.

The cave entrance (Fig. 1), faces northwest and measures 2 m high, 6 m wide, and 2 m long (deep). Its lack of depth means that it is not so isolated from external influences, and the microclimate in the cave is very similar

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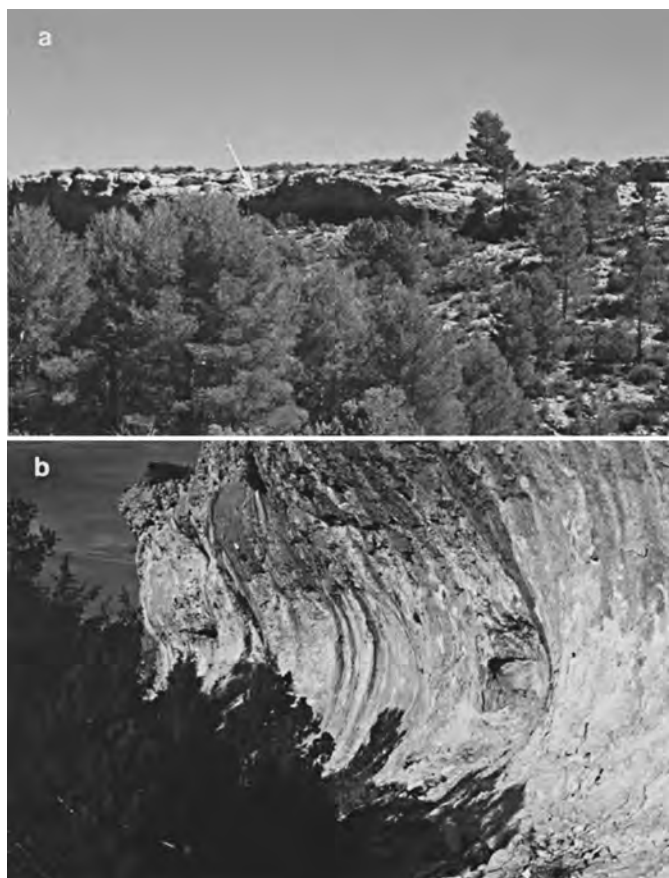
Figure 1. (a) Panoramic view of Andragulla cave in the Region of Murcia, southeastern Spain. (b) Walls of Andragulla Cave showing the cyanobacterial mats.

Figure 2. Light micrographs [scale bar 10 μm]. (a) *Scytonema mirabile*. (b) *Gloeocapsa sanguinea*.

to that experienced externally. Inside the cave there are schematic-style cave paintings characterized by simple figures dating from the end of the Neolithic to the Iron Age (Beltrán-Martínez, 1983).

Material and Methods

So as to not damage the cave paintings on surrounding surfaces, great care was taken to collect material on the parts of the Andragulla cave wall where the *Scytonema* community grew (Asencio and Aboal, 2001). Samples were not hydrated, but were incubated in situ in triplicate in transparent and opaque vials to reproduce light and dark environments, respectively. Then 10% of the gas in these vials was replaced with acetylene using syringes inserted into the rubber stoppers (Hardy et al., 1973). Gas samples were taken at hourly intervals from sunrise to sunset on one day in winter and stored for laboratory analysis. Throughout the incubation period, the photon flux (Photosynthetically Active Radiation, PAR), air temperature, and relative humidity values were recorded hourly. An LI-1400 data-logger model (LICOR) with an LI-192 sensor and a Delta Ohm HD 8501 H thermohygrometer

were used. Electrodes were placed on the rock surface adjacent to the mats.

Nitrogen fixation in gaseous samples was quantified by acetylene/ethylene reduction assays (Peterson and Burris, 1976) analyzed in a Shimadzu GC-14A gas chromatograph.

Results

Nitrogen fixation was detected for the first time in a community formed by *Scytonema mirabile* (Fig. 2a) and *Gloeocapsa sanguinea* (Fig. 2b) growing as a brownish-reddish mat in Andragulla Cave (Asencio and Aboal, 1996).

Nitrogenase activity during the day varied from 1.6 to 7.5 nmol C_2H_4 m^{-2} s^{-1} (Fig. 3). The nitrogenase activity remained low and constant in the morning from 8:00 a.m. to 11:00 a.m., at which time it began to increase, and it doubled by 1:00 p.m. to values of 3.5 nmol C_2H_4 m^{-2} s^{-1}. This activity continued to increase until a maximum value of 7.5 nmol C_2H_4 m^{-2} s^{-1} was reached at 2:00 p.m., and then it started to drop, reaching a value of around 1 nmol C_2H_4 m^{-2} s^{-1} at 3:00 p.m.

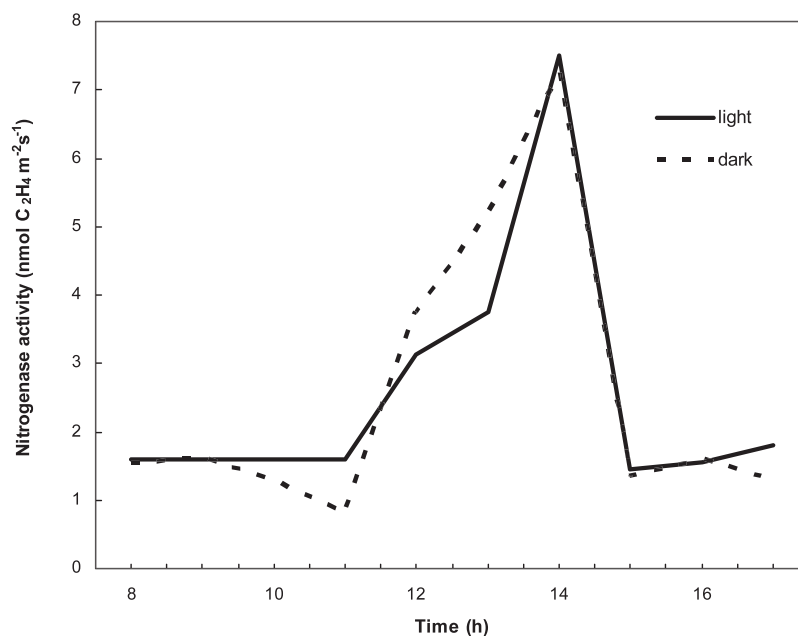**Figure 3. Nitrogenase activity by *Scytonema mirabile* (light) and *Gloeocapsa sanguinea* (dark) in Andragulla cave.**

The photosynthetically active radiation in the Andragulla cave ranged between 0.5 and 582.7 $\mu E \ m^{-2} s^{-1}$ (Fig. 4). From 8:00 a.m. to 4:00 p.m., the values rose from 0.5 to 138.8 $\mu E \ m^{-2} \ s^{-1}$, increasing to 582.7 $\mu E \ m^{-2} \ s^{-1}$ at 5:00 p.m., and dropped later.

The maximum relative humidity value (Fig. 4) was 79.9%, recorded at 8:00 a.m. and scarcely varying until noon, followed by a drop to the minimum value of 24.0% at 4:00 p.m. Afterwards, it began to rise once more.

The lowest temperature in Andragulla Cave was 1.5 °C, which was recorded in the morning at 8:00 a.m. After this time, it started to rise until it reached 4.7 °C, but started dropping again to reach 1.5 °C at 11:00 a.m. Later, it rose, and the highest temperature of 20.3 °C was recorded at 4:00 p.m., after which it began to fall again (Fig. 4).

Nighttime nitrogen fixation varied from 0.8 to 7.3 nmol $C_2H_4 \ m^{-2} \ s^{-1}$ (Fig. 3). At 8:00 a.m., a value of 1.53 nmol $C_2H_4 \ m^{-2} \ s^{-1}$ was recorded, which rose to 1.61 nmol C_2H_4 $m^{-2} \ s^{-1}$ at 9:00 a.m. From that time, it started to drop and reached its lowest value of 0.8 nmol $C_2H_4 \ m^{-2} \ s^{-1}$, recorded at 11:00 a.m. Afterwards, it rose to reach the highest value of 7.3 nmol $C_2H_4 \ m^{-2} \ s^{-1}$ at 2:00 p.m., when it started to drop again.

DISCUSSION

The nitrogenase activity recorded for the community formed by *Scytonema mirabile* and *Gloeocapsa sanguinea* in Andragulla Cave during the day ranged from 1.4 to 7.5 nmol $C_2H_4 \ m^{-2} \ s^{-1}$. These values are very similar to those recorded in Cathole Cave (USA) for *Nostoc* sp., which reached a maximum value of 8.3 nmol $C_2H_4 \ m^{-2} \ s^{-1}$ (Griffiths et al., 1987).

If we compare the nitrogenase activity registered for *Scytonema mirabile* and *Gloeocapsa sanguinea* in Andragulla Cave with the mean value of 28.2 nmol C_2H_4 $m^{-2} \ s^{-1}$ recorded on the crusts of *Scytonema* sp. in semiarid areas of the USA (Jeffries et al., 1992), we find an important difference, basically owing to the fact that the latter samples had been previously rehydrated, as opposed to the limited natural moisture in the Andragulla Cave samples. This fact, along with the various experiments carried out by Renaut et al. (1975), who recorded higher nitrogenase activity values if samples had been moistened prior to nitrogen fixation, confirmed the importance of a high relative humidity in the nitrogen-fixation process.

Here we can observe how nitrogenase activity increased as the light intensity in the study area grew, which is in agreement with Dodds (1989). It grew to such an extent that irradiance was so high that it inhibited the activity of this enzyme. Its activity resumed afterwards, however, when light intensity diminished at sunset.

Nighttime ethylene production differed only slightly from that achieved in the daytime, with values between 0.8 and 7.3 nmol $C_2H_4 \ m^{-2} \ s^{-1}$, which are very similar to those recorded in Cathole Cave (USA) for *Gloeothece* sp., which reached a maximum of 7.9 nmol $C_2H_4 \ m^{-2} \ s^{-1}$ (Griffiths et al., 1987). This finding could indicate the capacity of *Gloeocapsa sanguinea* to fix nitrogen aerobically and in the dark, as shown in other species of this genus (Zhou and Chen, 1991).

The protection mechanisms that *Gloeocapsa sanguinea* may have against the effect of oxygen remain unknown. The temporal distributions of oxygenic photosynthesis and nitrogen fixation could be one of the mechanisms that
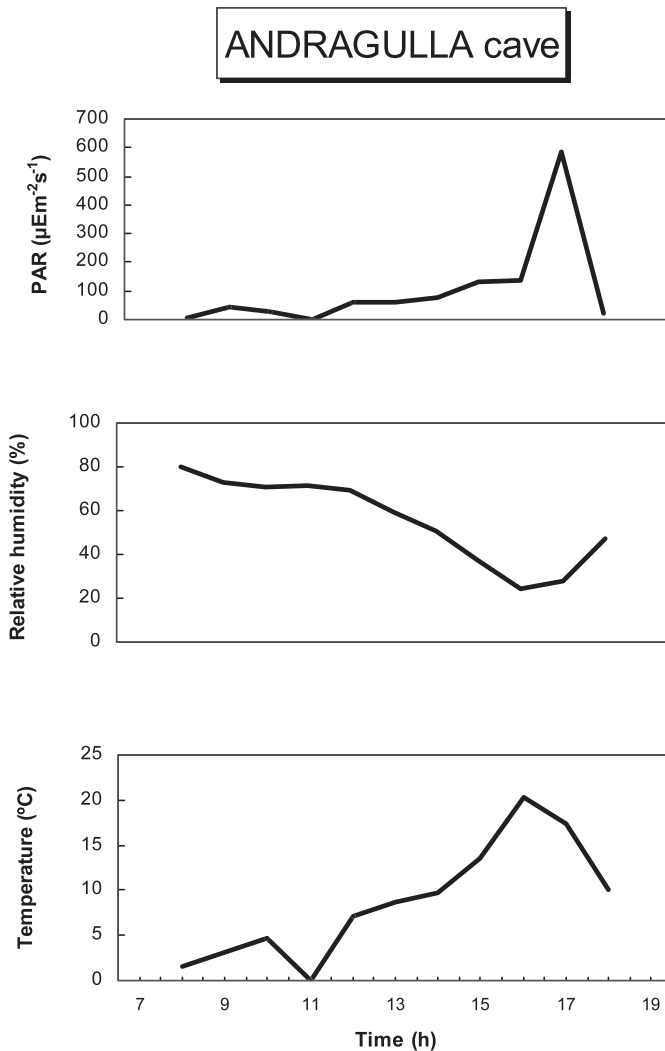
ANDRAGULLA cave

Figure 4. Photosynthetically active radiation (PAR), temperature, and relative humidity from sunrise to sunset in Andragulla cave at a cyanobacterial mat of *Scytonema mirabile* and *Gloeocapsa sanguinea*.

Gloeocapsa sanguinea uses to protect the nitrogenase enzyme from inactivation by oxygen. Indeed, during the nighttime, when the photosynthetic oxygen concentration decreases, the atmospheric nitrogen activity fed by aerobic respiration takes place, thus allowing nitrogenase activity to occur exclusively during the dark period. Another strategy could be the existence of a so-called pod-like structure that surrounds each cell and the cenobiums that may act as a physical barrier to prevent the entrance of atmospheric gases, as in *Gloeothece* (Meeks et al., 1978).

Minimum temperature values inhibited nitrogenase activity in the dark, which was clearly shown with *Gloeocapsa sanguinea* as ethylene production abruptly lowered with a drop in temperature, but increased when higher temperatures were recorded. This pattern coincides with the work of Griffiths et al. (1987) conducted in

Cathole Cave (USA), where nitrogenase activity was completely blocked in the coldest winter months.

The environmental parameters involved in nitrogen fixation studied in Andragulla Cave acted differently depending on whether daytime fixation or nighttime fixation was underway. From this, we may deduce that light intensity actively influences daytime fixation, temperature affects night-time fixation, and relative humidity acts with the same intensity on both.

Both the daytime and nighttime fixation values recorded for the first time in the community made up of *Scytonema mirabile* and *Gloeocapsa sanguinea* in Andragulla Cave are similar. We believe the presence of heterocysts is not an essential requirement for nitrogen fixation, and that non-heterocystic aerobic fixing organisms may contribute considerably to the overall nitrogen cycle in harsh environments such as cave entrances and other lighted areas.

Acknowledgements

References

Asencio, A.D., and Aboal, M., 1996, Cyanophytes from Andragulla abrigo (Murcia, SE Spain) and their environmental conditions: Archiv für Hydrobiology/Algological Studies, v. 83, p. 55–72.

Asencio, A.D., and Aboal, M., 2001, Biodeterioration of wall paintings in caves of Murcia (SE Spain) by epilithic and chasmoendolithic microalgae: Archiv für Hydrobiology/Algological Studies, v. 103, p. 131–142.

Aranibar, J.N., Anderson, I.C., Ringrose, S., and Macko, S.A., 2003, Importance of nitrogen fixation in soil crusts of southern African arid ecosystems: acetylene reduction and stable isotope studies: Journal of Arid Environments, v. 54, p. 345–358.

Beltrán-Martínez, A., 1983, El arte esquemático en la Península Ibérica: orígenes e interrelación: Bases para un debate: Zéphyrus, v. 36, p. 37–41.

Dodds, W.K., 1989, Microscale vertical profiles of N_2 fixation, photosynthesis, O_2, chlorophyll a and light in a cyanobacterial assemblage: Applied Environmental Microbiology, v. 55, p. 882–886.

Fay, P., 1992, Oxygen relations of nitrogen fixation in cyanobacteria: Microbiological Reviews, v. 56, p. 340–373.

Fogg, G.E., Stewart, W.D.P., Fay, P., and Walsby, A.E., 1973, The Blue-Green Algae, London, Academic Press, 459 p.

Gallon, J.R., 1980, Nitrogen fixation by photoautotrophs, *in* Stewart, W.D.P., and Gallon, J.R., eds., Nitrogen Fixation, New York, Academic Press, p. 197–238.

Griffiths, M.S.H., Gallon, J.R., and Chaplin, A.E., 1987, The diurnal pattern of dinitrogen fixation by cyanobacteria *in situ*: New Phytologist, v. 107, p. 649–657.

Grimm, N.B., and Petrone, K.C., 1997, Nitrogen fixation in a desert stream ecosystem: Biogeochemistry, v. 37, p. 33–61.

Hardy, R.W.F., Burns, R.C., and Holsten, R.D., 1973, Applications of the acetylene-ethylene assay for measurement of nitrogen fixation: Soil Biology and Biochemistry, v. 5, p. 47–81.

Housman, D.C., Powers, H.H., Collins, A.D., and Belnap, J., 2006, Carbon and nitrogen fixation differ between successional stages of biological soil crusts in the Colorado Plateau and Chihuahuan Desert: Journal of Arid Environments, v. 66, p. 620–634.

Jeffries, D.L., Klopatek, J.M., Link, S.O., and Bolton, H. Jr., 1992, Acetylene reduction by criptogamic crusts from a blackbrush

community as related to resaturation and dehydration: Soil Biology and Biochemistry, v. 24, p. 1101–1105.

Meeks, J.C., Wolk, C.P., Lockau, W., Schilling, N., Shaffer, P.W., and Chien, W.-S., 1978, Pathways of assimilation of ($[^{13}N]N_2$ and $^{13}NH_4^+$) by cyanobacteria with and without heterocysts: Journal of Bacteriology, v. 134, p. 125–130.

Peterson, R.B., and Burris, R.H., 1976, Conversion of acetylene reduction rates to nitrogen fixation rates in natural populations of blue-green algae: Analytical Biochemistry, v. 73, p. 404–410.

Renaut, J., Sasson, A., Pearson, W., and Stewart, W.D.P., 1975, Nitrogen-fixing algae in Morocco, in Stewart, W.D.P., ed., Nitrogen Fixation by Free-living Micro-organisms (IBP Handbook no. 6), Cambridge, Cambridge University Press, p. 229–246.

Sivonen, K., Halinen, K., Sihvonen, L.M., Koskenniemi, K., Sinkko, H., Rantasärkkä, K., Moisander, P.H., and Lyra, C., 2007, Bacterial diversity and function in the Baltic Sea with an emphasis on cyanobacteria: Ambio, v. 36, no. 2–3, p. 180–185.

Walsby, A.E., 1985, The permeability of heterocysts to the gases nitrogen and oxygen: Proceedings of the Royal Society B, v. 226, p. 345–366.

Zhou, H., and Chen, T., 1991, The isolation, purification and efficiency of nitrogen fixation for unicellular cyanobacteria Gloeocapsa spp: Acta Microbiologica Sinica, v. 31, p. 405–409.

Zielke, M., Solheim, B., Spjelkavik, S., and Olsen, R.A., 2005, Nitrogen fixation in the high Arctic: role of vegetation and environmental conditions: Arctic, Antarctic and Alpine Research, v. 37, no. 3, p. 372–378.

SPATIAL AND TEMPORAL DISTRIBUTION OF PROTOZOA AT CUEVA DE LOS RISCOS, QUERÉTARO, MÉXICO

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Abstract: Protozoa are important members of ecosystems, but protozoa that inhabit caves are poorly known worldwide. In this work, we present data on the record and distribution of thirteen protozoa species in four underground biotopes (water, soil, bat guano, and moss), at Cueva de Los Riscos. The samples were taken in six different months over more than a year. Protozoa species were ciliates (eight species), flagellates (three species), amoeboid (one species), and heliozoan (one species). Five of these species are reported for the first time inside cave systems anywhere, and an additional three species are new records for Mexican caves. *Colpoda* was the ciliate genera found in all cave zones sampled, and it inhabited the four biotopes together with *Vorticella*. The biotopes with the highest specific richness were the moss, sampled near the main cave entrance, and the temporary or permanent water bodies, with ten species each. The greatest number of species was observed in April 2006 (dry season). With the exception of water, all biotopes are studied for the first time.

INTRODUCTION

A great extent of Mexican territory is formed by sedimentary rocks that permit the formation of caves, but the number, location, and biodiversity of Mexican caves is only partially known. Records of subterranean protozoan taxa in Mexico are scarce, and refer only to caves in San Luis Potosí and Guerrero states (Osorio-Tafall, 1943; Hoffmann et al., 1986, p. 206–207), in which thirty species of sarcodines and ciliates have been reported.

There are records of nine protozoa species from caves in North America. In Bermuda, Hill et al. (1986) recorded *Euplotes iliffei* in subterranean anchialine habitats and Small et al. (1986) recorded *Glauconema bermudense* in marine caves. Holsinger (1966) found *Paramecium multimicronucleatum* and *Spirostomum ambiguum* in Virginia, and Barr (1968) reported *Phacus* sp., *Paramecium* sp., *Halteria* sp., *Difflugia* sp., and *Peranema* sp. as free-living inhabitants of the Mammoth Cave system in Kentucky.

Protozoa are cosmopolitan and tolerate a wide range of physicochemical factors, including pH, temperature, oxygen concentration, and salinity. They are not randomly distributed, but live in microhabitats, small regions that may be as tiny as a few cubic centimeters, within a body of water or a moist environment such as soil, vegetation, or the bodies of plants and animals (Bamforth, 1985). They occupy many different biotopes, in the sense of Olenin and Ducrotoy (2006).

According to Hoffmann et al. (1986, chap. III), roots, leaf and animal debris, and the guano of bats provide the primary energy sources in open system caves. Indeed, guano is considered the most important biotope, but water, soil, and moss also play important roles as sustainable habitats for diverse communities of microorganisms and metazoan taxa.

The protozoan trophozoite or cyst phase enters caves in water flow or infiltration through soil, in air currents, and by troglophile fauna present in the cave (Golemansky and Bonnet, 1994) and accidental or trogloxene organisms. The aim of this work is to record and analyze protozoan spatial and temporal distribution associated with different biotopes over more than a year at Cueva de Los Riscos.

MATERIALS AND METHODS

Cueva de Los Riscos is located in Jalpan de Serra, Querétaro, Mexico, at 1122 m asl, 21°11′38″N, 99°30′50″W. It is a mixed underground system (horizontal and vertical) with a length of 440 m (Lazcano-Sahagún, 1986a, p. 32; 1986b, p. 77–79), with four zones (A–D) and four entrances (Fig. 1); a detailed cave description is in Espino del Castillo et al. (2009).

Six visits were made to Cueva de Los Riscos from November 2005 to June 2007 (Table 1). Samples, including water, soil, bat guano, and moss, were collected where available in four large zones in the cave, A, B, C, and D (Fig. 1); other areas in narrow tunnels were not sampled. Access to the cave was done without special equipment, but for biosafety we wore face masks with filters (Milter disposable 3M 8210 N95).

Water samples were collected by using sterile pipettes; guano and soil samples were obtained with sterilized metallic

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[3] Acarología & Bioespeleología, Departamento de Biología Comparada, Facultad de Ciencias, Universidad Nacional Autónoma de México, Coyoacán 04510, D.F., México
[4] Additional Address: Unidad Multidisciplinaria de Docencia e Investigación, Facultad de Ciencias, Universidad Nacional Autónoma de México, Campus Juriquilla, Boulevard Juriquilla 3001, C.P. 76230, Querétaro, Querétaro, México

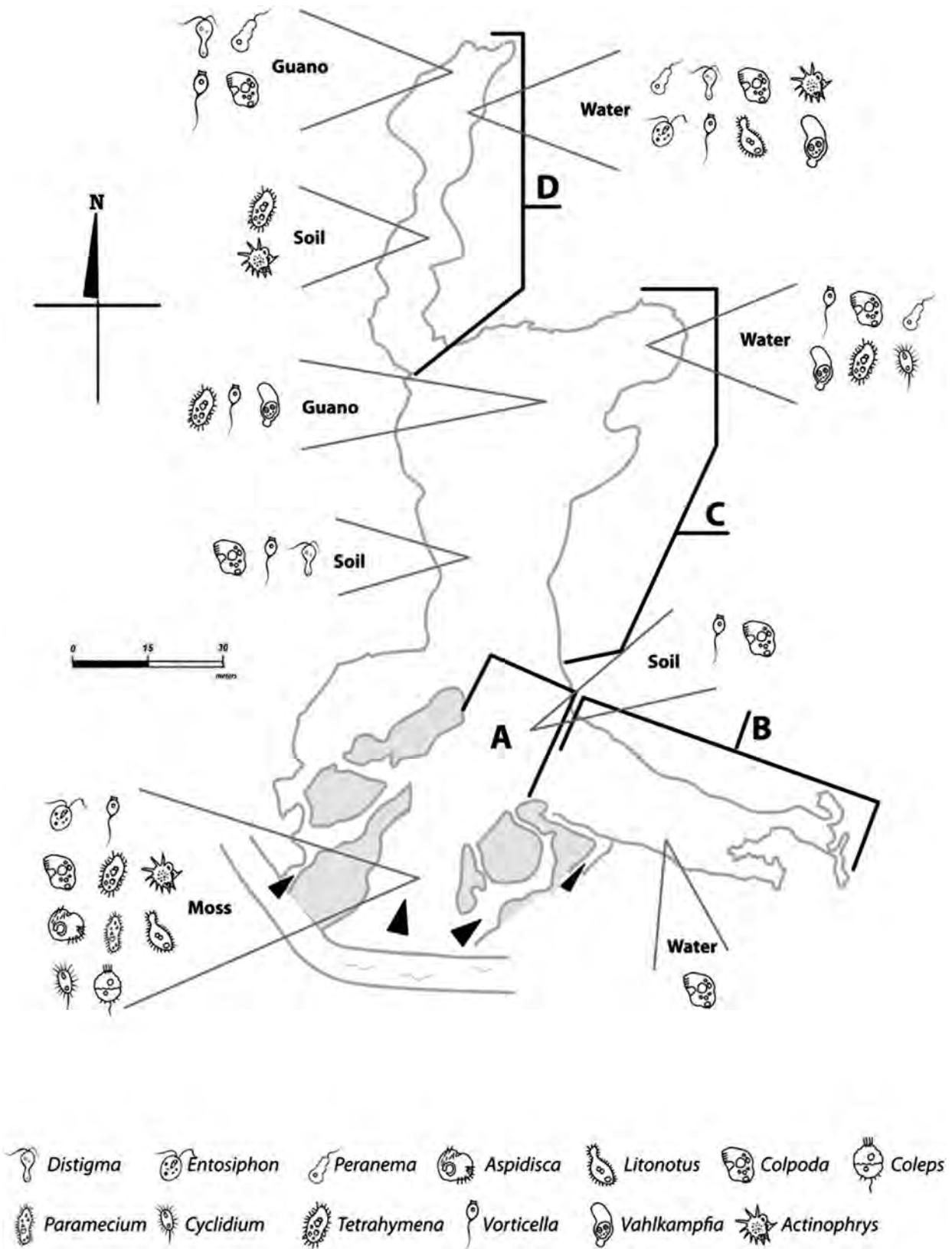**Figure 1. Cueva de Los Riscos map drawn by D. McKenzie of the Association for Mexican Cave Studies in 1966 (Lazcano Sahagún, 1986b, p. 79), modified by A. Espino del Castillo, M. Hernández, J.B. Morales-Malacara, and L. González of the Universidad Nacional Autónoma de México in March 2007, showing collecting places and species biodiversity at each biotope. Black arrow heads represent cave entrances.**

Table 1. Distribution of protozoa genera recorded on each biotope during the study at Los Riscos Cave.

	Year											
	2005		**2006**						**2007**			
	Rainy Season		Dry Season		Rainy Season		Rainy Season		Dry Season		Rainy Season	
	November		April		June		October		March		June	
Zone	Genus	Biotopes	Genus	Biotopes	Genus	Biotopes	Genus	Biotopes	Genus	Biotopes	Genus	Biotopes
A	NC	NC	Entosiphon	Moss	…	…	…	…	…	…	…	…
A	NC	NC	Vorticella	Moss	…	…	…	…	Entosiphon	Moss	…	…
A	NC	NC	Colpoda	Moss	Entosiphon	Moss	…	…	Vorticella	Moss	Entosiphon	Moss
A	NC	NC	Tetrahymena	Moss	Vorticella	Moss	…	…	Colpoda	Moss	Vorticella	Moss
A	NC	NC	Aspidisca	Moss	Colpoda	Moss	Vorticella	Soil	Litonotus	Moss	Colpoda	Soil
A	NC	NC	Paramecium	Moss	Cyclidium	Moss	…	…	Aspidisca	Moss	Tetrahymena	Moss
A	NC	NC	Cyclidium	Moss	…	…	…	…	Paramecium	Moss	…	…
A	NC	NC	Coleps	Moss	…	…	…	…	Cyclidium	Moss	…	…
A	NC	NC	Actinophrys	Moss	…	…	…	…	…	…	…	…
B	NC	NC	NC	NC	Colpoda	Water	NC	NC	NC	NC	NC	NC
C	NC	NC	Distigma	Soil	…	…	Vahlkampfia	Water	Vahlkampfia	Guano	Peranema	Water
C	NC	NC	Vorticella	Soil, Water	Vorticella	Water	Vorticella	Water	Vorticella	Guano	Tetrahymena	Water
C	NC	NC	Colpoda	Soil	…	…	Colpoda	Water	Tetrahymena	Guano	Cyclidium	Water
C	NC	NC	…	…	…	…	Tetrahymena	Water	…	…	…	…
D	Peranema	Water, Guano	Vorticella	Water, Guano	Peranema	Guano	NA	NA	NA	NA	Vahlkampfia	Guano
D	Distigma	Water	Colpoda	Guano	Distigma	Guano	NA	NA	NA	NA	…	…
D	Entosiphon	Water, Guano	Tetrahymena	Soil	Vorticella	Water	NA	NA	NA	NA	…	…
D	Vorticella	Water, Guano	Actinophrys	Soil	…	…	NA	NA	NA	NA	…	…
D	Colpoda	Water	…	…	…	…	NA	NA	NA	NA	…	…
D	Litonotus	Water	…	…	…	…	NA	NA	NA	NA	…	…
D	Actinophrys	Water	…	…	…	…	NA	NA	NA	NA	…	…

Note: NC means no samples collected.
NA means no protozoa recorded.

Figure 2. Protozoa species biodiversity found at Cueva de Los Riscos. Data in parentheses correspond to length and width measurements in micrometers, except for *Actinophrys sol* which corresponds to diameter. A. *Distigma* sp. (8.0 × 2.5); B.*Entosiphon* sp. (20.0 × 12.0); C. *Peranema* sp. (17.0 × 4.0); D. *Aspidisca* sp. (40.0); E. *Coleps hirtus* (40.0 × 30); F. *Colpoda* sp. (36.0 × 30.0); G. *Litonotus* sp. (80.0 × 34.0); H. *Paramecium caudatum* (200.0 × 60.0); I. *Cyclidium* sp. (20.0 × 10.0); J. *Tetrahymena pyriformis* (35.0 × 24.0); K. *Vorticella* sp. (25.0 × 20.0); L. *Vahlkampfia* sp. (30.0 × 10.0); *Actinophrys sol* (25). Abbreviations: a = a×opodium; c = cytostome; cc = caudal cilium; ct = conical tubule; e = endoplasm; ec = ectoplasm; ep = ectoplasmatic plates; f = food vacuole; fc = frontoventral cirri; fl = flagellum; hm = higher adoral zone of membranelles; i = indentation; k = kinety; lm = lower adoral zone of membranelles; lp = lateral projection; m = macronucleus; me = membranelles; mi = micronucleus; n = nucleus; p = paroral membrane; s = spines; st = stalk; t = transversal cirri; tr = trichocysts; u = uroid; v = contractile vacuole.

spoons, and for moss we utilized forceps. Approximately 2 ml or 2 cc of each sample was placed in one sterilized Falcon tube containing 5 ml of nutritive sterile pea infusion (Patterson and Hedley, 1992, p.17–18). Additionally, 100 ml or 100 cc of each sample was collected in sterilized 100 ml glass jars. Moss samples were collected in plastic bags. All samples were transported in a cooler without ice, in order to maintain all samples in good condition.

All laboratory procedures started within 24 hours of collection. In all cases, to obtain polyxenic cultures (multiple species), 2 ml of each sample was added to natural infusions of straw, rice, wheat, pea, and corn (Lee et al. 1985; Jahn et al. 1979, p. 10–12) and chemical media (Chalkley, peptone, and RPMI-1640 Sigma) (Manwell, 1968, p. 559–572; Kudo, 1971, p. 848–852). The samples and cultures were maintained in the dark at 25 °C in an incubator, except for the moss samples, which were exposed to sunlight. To identify the protozoa and for a photomicrographic record, all the cultures were periodically examined by using phase-contrast, differential-interference-contrast and bright-field microscopes (Nikon Labophot-2 with Nikon Digital Sight DS-2Mv and Nikon FX-35DX incorporated camera; Zeiss Axioskop 2 plus with Zeiss AxioCam MRC system). Diagnostic characters were studied with staining and impregnation techniques such as Harris hematoxylin, Klein, protargol, and butanol-nigrosine (Borror, 1969; Kudo, 1971, p. 863; Lee et al., 1985; Silva-Neto, 2000).

We utilized the Jaccard index to estimate the degree of similarity between biotopes with regard to genera. The similarity values obtained were summarized by clustering, using the UPGMA method (program NTSYS pc. v. 2.2, Exeter Software, Setauket, New York).

Results

We identified thirteen protozoa species, with three flagellates (*Entosiphon* sp., *Distigma* sp., *Peranema* sp.), one naked amoebae (*Vahlkampfia* sp.), one heliozoan (*Actinophrys sol* Ehrenberg), and eight ciliates [*Aspidisca* sp., *Litonotus* sp., *Colpoda* sp., *Coleps hirtus* (O. F. Müller), *Paramecium caudatum* Ehrenberg, *Cyclidium* sp., *Tetrahymena pyriformis* (Ehrenberg), and *Vorticella* sp.] (Table 1, Fig. 2). The protozoan distribution in relation to the cave zones and biotopes from all seasons is shown in Figure 1.

The species *Entosiphon* sp., *Distigma* sp., *Aspidisca* sp., *Litonotus* sp., and *Tetrahymena pyriformis* have not been recorded in caves anywhere in the world. The following species are new records for Mexican caves: *Actinophrys sol, Colpoda* sp., and *Paramecium caudatum* (Table 2).

The relative species richness among the cave biotopes, seasons, and zones, is shown in Figures 1 and 3. The most diverse biotopes were moss and water, and the soil was the least diverse (Table 1).

The greatest number of protozoan taxa was present in April 2006 (dry season), and the lowest was in October
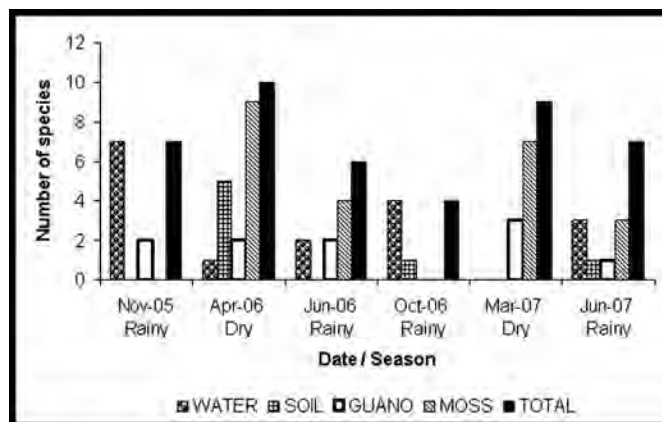

Figure 3. Number of protozoa species for each biotope and season at Cueva de Los Riscos. Note that some species appear in the totals for more than one biotope for each date.

(rainy season) of the same year, when flagellates were absent, with Zone A having the highest diversity

The genus *Colpoda* was found on all biotopes, zones, and periods. *Vorticella* sp. and *Tetrahymena pyriformis* were recorded in all biotopes and during all periods. The genus *Coleps* was recorded only in Zone A moss in April 2006.

Entosiphon was recorded in samples from five months (rainy and dry seasons), except for October 2006. The heliozoan *Actinophrys sol* was observed only in November 2005 (rainy season) and April 2006 (dry season). The amoeboid genus *Vahlkampfia* was recorded three times: October 2006 (rainy season), March 2007 (dry season), and June 2007 (rainy season).

In Zone B, we found only the genus *Colpoda*, which was observed in a draining water sample.

From the Jaccard similarity index two clusters were obtained; the first grouped the biotopes water, guano, and soil, and the second cluster included only the moss biotope. We obtained a similarity index of 0.6 between the water and guano biotopes, a similarity of 0.54 when both biotopes were compared with the soil, and a similarity of 0.38 when these three biotopes were compared with the moss.

Discussion

According to Corliss (2002), the protists are cosmopolitan in overall distribution, and, in particular, most protozoa play roles mainly as phagotrophs (particulate consumers). Free-living species have a very broad distribution as planktonic or benthic forms. Free-swimming flagellates and ciliates are the most important consumers of bacteria in aquatic and terrestrial ecosystems.

Considering the functional groups of protozoa in ecosystems proposed by Pratt and Cairns (1985), the species found in the biotopes at Cueva de Los Riscos

Table 2. Genera recorded in this study, with previous cave records, if any.

Europe	USA	México	References	Los Riscos Cave[a]	Record
...	*Peranema* sp.	...	Barr, 1986	*Peranema* sp.	P
...	...	*Peranema* sp.	Hoffmann et al., 1986		
...	...	*Vahlkampfia* sp.	Hoffmann et al., 1986	*Vahlkampfia* sp.	P
V. limax	...	...	Golemansky and Bonnet, 1994		
V. magna	...	...	Golemansky and Bonnet, 1994		
V. tachipodia	...	...	Golemansky and Bonnet, 1994		
Cyclidium oligotrichum	...	...	Carey et al., 2001	*Cyclidium* sp.	P
C. fuscum	...	...	Carey et al., 2001		
C. glaucoma	...	...	Golemansky and Bonnet, 1994		
...	...	*Cyclidium* sp.	Hoffmann et al., 1986		
...	...	...			
...	...	*Tetrahymena* sp.	Hoffmann et al., 1986	*Tetrahymena pyriformis*	P
...	...	*Vorticella* sp.	Hoffmann et al., 1986	*Vorticella* sp.	P
...	...	*Vorticella* sp. cf. *microstoma*	Osorio-Tafall, 1943		
Colpoda sp.	...	...	Barr, 1986	*Colpoda* sp.	M
C. steinii	...	...	Golemansky and Bonnet, 1994		
Paramecium caudatum	...	...	Golemansky and Bonnet, 1994	*Paramecium caudatum*	M
...	*Paramecium* sp.	...	Barr, 1986		
...	*P. micromultinucleatum*	...	Holsinger, 1966		
...	...	*P. aurelia*	Hoffmann et al., 1986		
Actinophrys sol	...	...	Golemansky and Bonnet, 1994	*Actinophrys sol*	M
A. vesiculata	...	...	Golemansky and Bonnet, 1994		
...	...	*Actinophrys* sp.	Osorio-Tafall, 1943		
...	...	*Coleps* sp.	Hoffmann et al., 1986	*Coleps hirtus*	W
...	...	*Coleps* sp. cf. *hirtus*	Osorio-Tafall, 1943		
...	...	...	...	*Entosiphon* sp.	W
...	...	...	...	*Distigma* sp.	W
...	...	...	...	*Aspidisca* sp.	W
...	...	...	...	*Litonotus* sp.	W

[a] This study.
W—New world and Mexican record for caves.
M—New Mexican record for caves.
P—Previously recorded in Mexican caves.

correspond to bactivores-detritivores (*Aspidisca* sp., *Colpoda* sp., *Coleps hirtus, Cyclidium* sp., *Litonotus* sp., *Paramecium caudatum, Tetrahymena pyriformis, Vorticella* sp., *Entosiphon* sp. and *Peranema* sp.), saprotophs (*Distigma* sp.), and non-selective omnivores (*Actinophrys sol*). Foissner and Berger (1996) reported some species of *Litonotus* as predators. The main functional role of substrate-associated protozoa is the processing of dead organic matter and its associated bacterial flora (Pratt and Cairns, 1985).

Members of genera *Coleps, Vorticella, Tetrahymena* and *Paramecium* are very common in many ecosystems and have been previously reported in some cave biotopes (Table 2). Previous records of Mexican cave protozoa include species that were observed only in water samples, in contrast to our findings from multiple biotopes. As shown in Figure 1, *Vorticella* sp., a detritivorous genus, was recorded in all biotopes of the cave; therefore we consider this species as having a broad distribution.

The flagellates are typically smaller, but much more abundant than ciliates and often mixotrophic in their nutrition; they occupy both planktonic and benthic levels (Corliss, 2002). We found the genera *Entosiphon, Distigma*, and *Peranema* as benthic organisms.

The samples collected during October 2006 (rainy season) had the lowest diversity, with only four genera, probably because of the excessive water flow in the cave, as compared with April and May, that probably washed out protozoan populations. However, some puddles remained as propitious microhabitats for the development of protozoan communities and other organisms like small metazoans.

For the water biotope, we observed the highest species richness during November 2005 and October 2006 (rainy season). For the soil biotope we found the highest number of species during the spring months; however samples were not obtained for all collections during this period. The bat guano biotope presented only slight differences in species richness among seasons (Figure 3). April 2006 and March 2007 (dry season) had the highest number of species inhabiting the moss substrate.

We conclude for this cave system that the spring months, corresponding to the dry season, have the highest protozoan diversity.

According to Finlay et al. (1998), the same ciliate species are found wherever their preferred habitat is found. Free-living ciliates may be ubiquitous, as they are continually being distributed by effective passive dispersal; these statements can be taken into account when we analyze the protozoa recorded previously in world caves.

According to Hausman and Hülsmann (1996), some species of *Colpoda* can resist lower temperatures, which favors its establishment in several habitats. However, caves are systems where microclimate conditions are almost constant through the year in dark zones, and this environmental stability could be a dominant factor for

some protozoa species found throughout the cave, such as *Colpoda* sp. and *Vorticella* sp.

Cyst production by protozoa is sometimes just part of the life-cycle, but often is a response to unfavorable environmental factors, such as desiccation, temperature, or starvation, and is triggered in response to these conditions. Cyst formation has been documented in species of the genera *Colpoda, Vahlkampfia, Actinophrys*, and *Paramecium* (Hausmann and Hülsmann, 1996, p. 154–157), which were found at Cueva de Los Riscos, and this capability facilitates the presence of these species in different seasons inside the cave.

The differences in specific richness in the cave zones can be explained as a function of the type of biotopes present. The greatest values were obtained in Zone A (the light zone), followed by Zone D (the darkest and deepest zone), and Zone C (the twilight and dark zone). The elevated specific richness of Zone A could be explained because of the presence of moss, a biotope that favors protozoan population growth. In Zone C, we found small bodies of water formed by temporary water sources, and also permanent gour pools, which provide a more suitable habitat for protozoan communities. Water facilitates oxygen uptake, contains food resources, has surface tension for movement and dispersion, and is a medium that also facilitates reproduction; this explains the record of seven protozoa genera. In Zone D, permanently dark and where vampire bats (*Desmodus rotundus* (Geoffroy) and *Diphylla ecaudata* Spix) were present in all seasons, the predominant biotope was the guano of the hematophagus bat, which had fewer species than the moss and water substrates, but provides nutrients such as nitrogen compounds for protozoa and is, according to Hoffmann et al. (1986, chap. III), a major energy source in caves, supporting five genera of heterotrophic protozoa. Zone B (twilight zone), had the lowest specific richness, but this can be due the small number of samples collected there because of its inaccessibility.

The Jaccard index demonstrated a similarity degree of 0.6 between the water and the bat guano biotopes, which shared mainly bactivore-detritivore species. Water and guano biotopes shared four species with the soil biotope, for a 0.54 similarity index. The biotopes guano, water, and soil were distributed into the darkest areas of the cave and proved to be suitable substrates for the protozoan colonization and establishment.

Soil is a microhabitat that could be frequently exposed to variable degrees of desiccation affecting the ciliates, flagellates and amoebaes. That could explain why we only found six species (three ciliates, one flagellate, and the heliozoan), as compared with other biotopes. However, a cave system usually exhibits high humidity, preventing desiccation. Nevertheless, protozoan diversity in soil was lowest, probably due to other factors, such as granulometric and physical characteristics that could prevent free movement and dispersion, among other functions.

The community assemblages of several taxa of protozo-ans in the same time and place could be explained by their trophic roles in relation to the type and availability of food resources. The protozoa have a wide spectrum of food requirements, and these were available in the different cave biotopes documented. Broad tolerances of the most common taxa suggest that some species should be found in nearly every natural system (Pratt and Cairns, 1985) and explain why most of the species recorded in the present work also have been reported in habitats other than caves worldwide. In respect to this particular cave ecosystem, previous data refer only to protozoa from water samples of caves. We found that the all the biotopes considered in this study were suitable for many protozoa, favoring reproduction and providing food sources, among others requirements.

CONCLUSIONS

We sampled four biotopes in Cueva de Los Riscos that were inhabited by thirteen protozoa species. Each biotope provided favorable conditions, but they harbored different species compositions and richness throughout the dry and rainy seasons. We recorded for the first time five protozoa species in caves worldwide; an additional three species are new records for Mexican caves. With these data we conclude that protozoa have a wide distribution in cave systems, but more studies are needed to complete the records in these ecosystems.

ACKNOWLEDGMENTS

REFERENCES

Bamforth, S.S., 1985, Ecology of protozoa, in Lee, J.J., Bovee, E.C., and Hutner, S.H., eds., An Illustrated Guide to the Protozoa: Lawrence, Kansas, Society of Protozoologists, p. 8–15.

Barr, T.C., 1968, Ecological studies in the Mammoth Cave System of Kentucky. I. The Biota. International Journal of Speleology, v. 3, no. 1–2, p. 147–204.

Borror, A.C., 1969, Application of the stain-fixative Nigrosin-HgCl₂-Formalin to fragile or contractile ciliates: Transactions of the American Microscopical Society, v. 88, no. 3, p. 454–458.

Carey, P.G., Sargent, A.J., Martínez Taberner, A., Ramón, G., and Moyà, G., 2001, Ecology of cavernicolous ciliates from the anchialine lagoons of Mallorca. Hydrobiologia, v. 448, p. 193–201.

Corliss, J.O., 2002, Biodiversity and biocomplexity of the protists and an overview of their significant roles in maintenance of our biosphere: Acta Protozoologica, v. 41, p. 199–219.

Espino del Castillo, A., Castaño-Meneses, G., Dávila-Montes, M.J., Miranda-Anaya, M., Morales-Malacara, J.B., and Paredes-León, R., 2009, Seasonal distribution and circadian activity in the troglophile long-footed robber frog, Eleutherodactylus longipes (Anura: Brachy-cephalidae) at Los Riscos Cave, Querétaro, Mexico: Field and laboratory studies: Journal of Cave and Karst Studies, v. 71, no. 1, p. 24–31.

Finlay, B.J., Esteban, G.F., and Fenchel, T., 1998, Protozoan Diversity: Converging estimates of the global number of free-living ciliate species: Protist, v. 149, p. 29–37.

Foissner, W., and Berger, H., 1996, A user-friendly guide to the ciliates (Protozoa, Ciliophora) commonly used by hydrobiologists as bioindi-cators in rivers, lakes, and waste waters, with notes on their ecology: Freshwater Biology, v. 35, p. 375–482.

Golemansky, V., and Bonnet, L., 1994, Protozoa, In: Juberthie, C., and V. Decu, V., eds., Encyclopaedia Bioespeologica: Société de Biospéolo-gie, p. 23–33.

Hausmann, K., and Hülsmann, N., 1996, Protozoology: Stuttgart, Georg Thieme Verlag, 338 p.

Hill, B.F., Small, E.B., and Iliffe, T.M., 1986, Euplotes iliffei n. sp.; a new species of Euplotes (Ciliophora, Hypotrichida) from the marine caves of Bermuda. Journal of the Washington Academy of Sciences, v. 76, no. 4, p. 244–249.

Hoffmann, A., Palacios-Vargas, J.G., and Morales-Malacara, J.B., 1986, Manual de Bioespeleología (con nuevas aportaciones de Morelos y Guerrero, Méx.): Universidad Nacional Autónoma de México, 274 p.

Holsinger, J.R., 1966, A preliminary study on the effects of organic pollution of Banners Corner Cave, Virginia. International Journal of Speleology, v. 2, no. 1–2, p. 75–89.

Jahn, T.L., Bovee, E.C., and Jahn, F.F., 1979, How to Know the Protozoa: Dubuque, Iowa, Wm. C. Brown, 279 p.

Kudo, R.R., 1971, Protozoology: Springfield, Illinois, Charles C. Thomas, 1174 p.

Lazcano-Sahagún, C., 1986a, Cavernas de la Sierra Gorda. v. I: Universidad Nacional Autónoma de Querétaro, Mexico, 181 p.

Lazcano-Sahagún, C., 1986b, Cavernas de la Sierra Gorda. v. II: Universidad Nacional Autónoma de Querétaro, Mexico, 206 p.

Lee, J.J., Small, E.B., Lynn, D.H., and Bovee, E.C., 1985, Some techniques for collecting, cultivating and observing Protozoa, in Lee, J.J., Bovee, E.C., and Hutner, S.H., eds., An Illustrated Guide to the Protozoa: Lawrence, Kansas, Society of Protozoologists, p. 1–7.

Manwell, R.D., 1968. Introduction to Protozoology, second edition. New York, Dover, 642 p.

Olenin, S., and Ducrotoy, J.P., 2006, The concept of biotope in marine ecology and coastal management. Marine Pollution Bulletin, v. 53, p. 20–29.

Osorio-Tafall, B.F., 1943, Observaciones sobre la fauna acuática de las cuevas de la región de Valles, San Luis Potosí (México): Revista de la Sociedad Mexicana de Historia Natural, v. 4, no. 1–2, p. 43–71.

Patterson, D.J., and Hedley, S., 1992, Free-Living Freshwater Protozoa: A Colour Guide: London, Wolfe Publishing, 223 p.

Pratt, J.R., and Cairns, J., 1985, Functional groups in the Protozoa: roles in differing ecosysrems: Journal of Protozoology, v. 32, no. 3, p. 415–423, doi: 10.1111/j.1550-7408.1985.tb04037.x.

Silva-Neto, I.D., 2000, Improvement of silver impregnation technique (Protargol) to obtain morphological features of protists ciliates, flagellates and opalinates: Revista Brasileira de Biologia, v. 60, no. 3, p. 451–459, doi: 10.1590/S0034-71082000000300010.

Small, E.B., Heisler, J., Sniezek, J., and Iliffe, T.M., 1986, Glauconema bermudense n. sp. (Scuticociliatida, Oligohymenophorea), a troglobitic ciliophoran from Bermudian marine caves. Stygologia, v. 2, no. 1–2, p. 167–179.

METHODOLOGY TO ASSESS WATER PRESENCE ON SPELEOTHEMS DURING PERIODS OF LOW PRECIPITATION, WITH IMPLICATIONS FOR RECHARGE SOURCES – KARTCHNER CAVERNS, ARIZONA

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Abstract: Beginning in January 2005, recharge processes and the presence of water on speleothems were monitored in Kartchner Caverns during a 44-month period when annual rainfall rates were 6 to 18 percent below the long-term mean. Electrical-resistance sensors designed to detect the presence of water were used to identify ephemeral streamflow in the channels overlying the cave as well as the movement of water within the cave system. Direct infiltration of precipitation through overhead rocks provided consistent inflow to the cave, but precipitation rates and subsequent infiltration rates were reduced during the comparatively dry years. Ephemeral stream-channel recharge through autogenic and allogenic processes, the predominant recharge mechanism during wetter periods, was limited to two low-volume events. From visual observations, it appeared that recharge from channel infiltration was equal to or less than recharge from overhead infiltration. Electrical-resistance sensors were able to detect thin films of water on speleothems, including stalactites, ribbons, and stalagmites. These films of water were directly attributed to overhead infiltration of precipitation. Periods of low precipitation resulted in decreased speleothem wetness.

INTRODUCTION

Kartchner Caverns, located in southeastern Arizona, USA, was opened to the public in November 1999 (Fig. 1). Prior to its opening, a baseline investigation of the cave's internal and external climate, geology, and hydrogeology was conducted to aid in identifying future impacts from development. The baseline investigation was conducted in the 1980s and early 1990s, a period that coincided with higher than average precipitation conditions. Results from the baseline investigation are documented in a special issue of the *Journal of Cave and Karst Studies* (vol. 61, no. 2, 1999). After the initial baseline investigation, observations of precipitation and groundwater levels continued. Concurrent to the opening of the cave to visitation was the onset of drier than average conditions (circa mid-1990s). During the late 1990s, moisture on the cave's formations noticeably declined. The decline was hypothesized to be a consequence of decreased precipitation causing decreased inflow to the cave, the introduction of drier air into the cave with visitors, or both (Rick Toomey, pers. comm.).

During the baseline investigation two primary mechanisms of inflow to the cave were identified as contributors of water to the cave: direct infiltration of overhead precipitation and infiltration of surface runoff in the stream channels that surround the cave (Graf, 1999). The direct infiltration of overhead precipitation has been defined by White (2003) as diffuse infiltration and categorized as autogenic recharge because the recharge water originates over the karst landscape and percolates in place. Infiltration of surface runoff through stream channels is a combination of discrete autogenic recharge (Lerch et al., 2005) and concentrated allogenic recharge (White, 1988, p. 281). In the former, precipitation over the karst landscape is concentrated into losing stream channels and infiltrates into the cave system. Allogenic recharge originates as precipitation over nonkarst areas upstream of the cave system and is concentrated into losing stream channels that are situated adjacent to the cave system. Groundwater inflow from adjacent aquifers was considered a possible source of cave water, but the initial investigators did not observe direct inflow from up-gradient aquifers, except in conjunction with streamflow in adjacent surface channels (Graf, 1999).

The objectives of this study were to determine if the observed declines in water flow on speleothems could be attributed to climate fluctuation and to identify the relative contributions of sources of inflow to the cave system during drier-than-average conditions.

To meet these objectives, it was necessary to use a novel method for detecting the presence of water on speleothems. Electrical-resistance (ER) sensors have been used to determine the presence of flow in ephemeral stream channels based on the ability of water to conduct electricity (Blasch et al., 2002). ER sensors were constructed using modified TidbiT sensors (Onset Corporation, Bourne, Massachusetts) as described by Blasch et al. (2002). ER sensors have been successfully used to monitor streamflow timing in coarse-grained alluvial channels (Blasch et al., 2002) and bedrock channels (Adams et al., 2006) and on
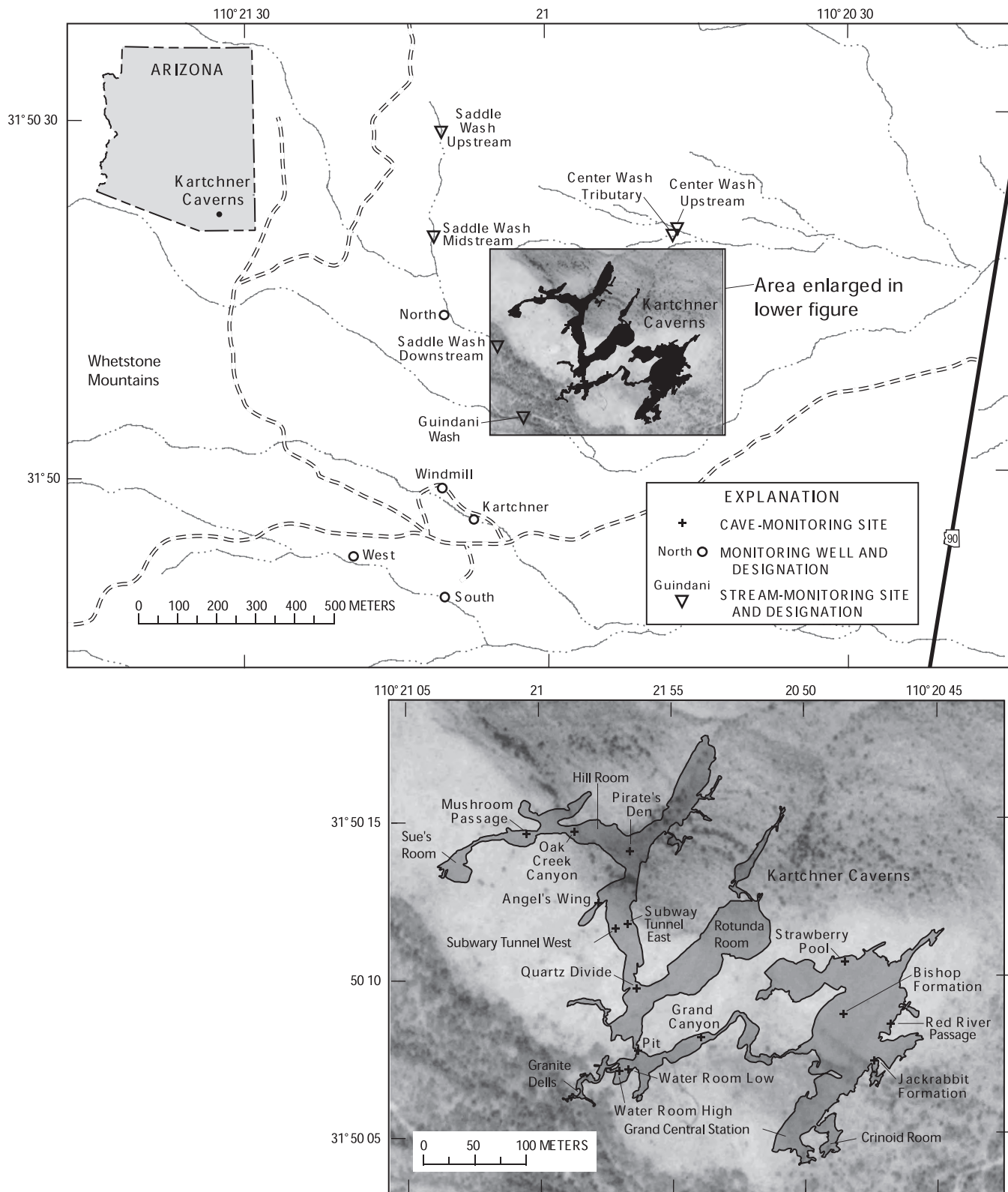
Figure 1. Map of Kartchner Caverns study area and monitoring sites.

bedrock walls. ER sensors should be useful for monitoring the presence of water within the cave environment, including cave walls, formations, pools, and drainage channels. An additional objective of the study was to evaluate the use of electrical resistance sensors for monitoring formation wetness and water flow within a cave.

Study Area

Kartchner Caverns is located approximately 75 km east of Tucson and 30 km west of Tombstone at the base of the Whetstone Mountains in southern Arizona. A full description of the hydrogeology of Kartchner Caverns State Park was presented by Graf (1999) as part of the Kartchner Caverns State Park Symposium special issue of the *Journal of Cave and Karst Studies* (vol. 61, no. 2, 1999). Regional geologic history and dating of speleothems and sediments indicates that the cave formed roughly 200 ka ago, with the main speleothem development occurring between then and 70 ka (Hill, 1999). Kartchner Caverns formed at the base of the Whetstone Mountains within a ridge of Mississippian-age Escabrosa Limestone. The ridge itself is at the surface of the Kartchner block that has been displaced downward from the Whetstone Mountains to the west, while the San Pedro alluvial basin to the east of the Kartchner block has subsided even farther.

A major fault on its western boundary separates the Kartchner block from the Precambrian-age Pinal Schist and the Whetstone Mountains. The upthrown side of the fault is Pinal Schist, which extends to a greater depth than the Escabrosa Limestone of the Kartchner block on the downthrown side of the fault. The fault is within 0.25 km of the known extent of Kartchner Caverns. The Pinal Schist is overlain by alluvial sediments, called the granite wash, of Illionian age (Melton, 1965) that in turn are overlain by the pediment surface called the Whetstone surface. Gray (1967) describes the granite wash as a combination of alluvial sediments, decomposed granite, mud flow, and alluvial fan sediments. The depth of the granite wash, as approximated by Graf (1999), is on the order of tens of meters. Using non-pumping water-level measurements, pump tests, and specific electrical conductivity measurements, Graf (1999) concluded that the groundwater yield in the granite wash is small and that connectivity in the formation is poor. Similarly, groundwater yields in the Pinal Schist are low.

The main basin-and-range fault separating the Kartchner block from the San Pedro alluvial basin to the east was mapped by Graf (1999) using gravity data from Lange et al. (1990). The basin-and-range fault is located less than 0.5 km from the known eastern boundary of Kartchner Caverns. The San Pedro alluvial basin immediately east of the fault is composed of a course alluvium overlain by granite wash. To the east, the coarse alluvium deposition is replaced by the finer-grained Pliocene and Pleistocene sediments of the St. David Formation. Groundwater levels measured within the St. David Formation indicate flow from the edge of the Whetstones eastward toward the San Pedro River.

Kartchner Caverns is in highly faulted limestone and formed under shallow-phreatic conditions (Graf, 1999). The regional water-table conditions that existed during its formation are no longer present. Depth to water ranges today from tens to hundreds of meters in the vicinity of the cave.

The channels of three ephemeral streams bound the cave (Fig. 1). Guindani Wash originates in the Whetstone Mountains and traverses the southern boundary of the cave from west to east. Center Wash originates on the northern slopes of the cave ridge and flows west to east. Center Wash empties into Guindani Wash northeast of the cave boundary. Saddle Wash originates northwest of the cave and runs along the western and southwestern edge of the cave, eventually merging into Guindani Wash. Both Saddle Wash and Guindani Wash overlie fault boundaries in proximity of the cave.

The explored regions of Kartchner Caverns range in elevation from about 1400 m near the Red River Passage at the eastern end of the cave to about 1425 m in Sue's Room at the western end of the cave (Fig. 1). Sediment depths within the rooms vary, so the actual elevations of the bedrock floors are unknown. The stream channels adjacent to the cave system are all above 1432 m (4700 ft) in elevation, allowing infiltrating water to travel vertically towards the cave system. Saddle Wash passes about 15 meters from the mapped boundaries of the Granite Dells and Guindani Wash passes about 90 meters from the mapped boundaries of Grand Central Station (Fig. 1).

The conceptual flow model for the cave system was originally developed during the baseline investigation of the cave. Components of the flow system were documented by Graf (1999), but important unpublished data still reside with the Kartchner Caverns Cave Resources Unit. As indicated, the primary sources of inflow to the cave originate as overhead precipitation and channel recharge. Graf (1999) noted that the water table is about 200 m below the known depth of the cave, and thus unsaturated conditions and processes are present.

Graf (1999) estimated that inflow from channel infiltration of ephemeral streamflow events accounted for the majority of the water entering the cave and that the remaining inflow was from direct overhead infiltration of precipitation. Buecher (1992, 1999) estimated about 230,000 L of water (7.6 mm over the surface area of the cave) enters the cave through overhead infiltration from precipitation by way of faults, fractures, Darcy flow through permeable beds, and flow down the surface of impermeable, dipping beds (Jagnow, 1999). Using drip studies, Buecher estimated that it took 4 to 12 days for water to percolate from the surface to the ceiling of the cave. This translated into an average groundwater flow

rate of 15 m/day through the overhead limestone and pediment.

Three ephemeral stream reaches were identified as contributing areas to the cave through observations, geophysics (Lange et al., 1990, and Lange, 1999), and dye tracers (Buecher, 1992). Fluorescein dye was used in September 1990 to confirm the connection between the ephemeral flow in Saddle Wash downstream from the North well and Sue's Room. Rhodamine WT dye was used in January 1991 to confirm the connection between the junction of Saddle and Guindani Washes and the Granite Dells. Flow from Guindani Wash upstream from the trail was also observed in the Crinoid Room.

Graf (1999) suggested the conditions generally necessary before recharge waters are observable in the cave. These conditions include surface flows in Guindani or Saddle Washes for more than one week and excess soil moisture exceeding 30.5 mm in one month or 38.1 mm over two consecutive months as computed by the Thornthwaite potential-evapotranspiration method.

Once water enters the cave, it tends to pond in place and slowly drain through the cave floor. If the flow rate into the cave is higher than the draining rate, ponding will increase until water spills over to neighboring rooms (Graf, 1999). For large flood events this process continues until flow is dispersed throughout most of the cave. Some rooms remain disconnected from the overall flow system due to impermeable units. In general, flow is from west to east, because the surface stream channels are on the western and southern borders of the cave and room elevations within the cave decrease from west to east. Eventually, water flowing through the cave drains through the Red River Passage, at 1400 m the lowest recorded point in the cave. Graf (1999) estimated an overall drainage rate based on the three largest flood events to be about 22 L/min, though drainage rates in the rooms vary. It is important to note that the structure of the cave below the mapped rooms (Fig. 1) is unknown. Thus constraints on draining and flow between rooms within the cave are not entirely understood.

METHOD

Hydrologic parameters were monitored inside and outside of the cave in order to describe the interaction between the surface and subsurface flow system and monitor speleothem wetness.

OUTSIDE THE CAVE
Monitoring of precipitation, streamflow, and groundwater levels outside the cave started well before this study. Precipitation has been measured about 1 kilometer southeast of the cave and recorded on an event basis using a 20.32-cm bucket rain gauge with an accuracy of 0.25 cm. A second bucket rain gauge with an accuracy of 0.50 cm was used to collect weekly measurements. The rain-gauge record started in January 1992. In order to quantify flow in an ephemeral stream, continuous monitoring (15-minute interval) in Guindani Wash (Fig. 1) began about January 2000.

For this study, ephemeral streamflow presence was monitored using TidbiT electrical-resistance (ER) sensors and TidbiT temperature sensors installed in stream channels surrounding the cave (Fig. 1). Monitoring began in December 2004, and recording intervals were set to 1 hour. Sensors were installed in perforated PVC housings and tethered to trees adjacent to the channels. Boulders were placed over the sensors to maintain their positions in the lowest portion of the cross-section and to shield the sensors from direct rainfall.

Three sets of ER and temperature sensors were installed in Saddle Wash. The upstream sensors were placed north of Kartchner Hill. The midstream sensors were installed about 275 meters downstream of the upstream sensors, and the downstream sensors were installed about 400 m downstream of the midstream sensors. The middle set of sensors was placed near a dye injection location used by Graf (1999) during an earlier surface channel recharge investigation.

One set of sensors was installed in Center Wash and an ER sensor was installed in a tributary wash to Center Wash. The unnamed tributary wash originates on the north side of Kartchner Caverns Hill and trends to the northeast into Center Wash.

Groundwater-level monitoring started during the baseline investigation. Monthly groundwater levels were measured in five wells (Fig. 1) during this investigation using a Solinst water level meter. Water temperatures were also measured in the North and West wells on a monthly basis.

INSIDE THE CAVE
ER sensors and temperature sensors were installed within the cave drainage channels (Fig. 1). To preserve the cave environment, only selected regions of the cave were accessible, and within these regions only prescribed footpaths could be used for sensor installation. Thus some lower-elevation drainage channels were not accessible for monitoring. The lowest-elevation locations in the rooms that could be reached from the foot paths were instrumented. Other placement considerations included consistency with previous dye-trace sites and records of water presence from the baseline investigation. Previous investigations observed entrainment of fines during periods of inflow to the cave. Consequently, sensors were installed about 1 cm from the sediment surface to avoid deposition of fines on the electrodes.

ER sensors were installed at the bottom of an area that collects flow referred to as the Strawberry Pool. The sensors were placed in a low section of the pool. Water enters the pool from low-flow features and dripping features. ER sensors were installed in the thalweg of a drainage channel called the Red River Passage. The Red

River Passage is a drain at the eastern edge of the cave. Flow rates in the cave are small enough that the sensors were not put into protective housings. Sensors were installed in the upper and lower regions of the Water Room and in Mushroom Passage exactly where the dye-trace receptors had been placed. Sensors were also installed in the Grand Canyon, the Pit, Quartz Divide, Angel's Wing, Subway Tunnel East, Subway Tunnel West, entrance to the Pirate's Den, and the Hill Room (the sensor location is called Oak Creek Canyon).

A second type of ER sensor that was installed on cave formations was the four-channel external HOBO sensors (Onset Corporation, Bourne, Massachusetts) with open leads. These data-loggers have four channels and sensor wires about 20 cm in length. The leads of the wires were exposed at the end by 1 cm and were mounted on the formations using flagging tape (Fig. 2). Each data-logger was housed in a sealed plastic bag containing desiccant.

An initial group of sensors was installed on December 4, 2004, on the Bishop formation and on the Jackrabbit formation. The Bishop formation is a large column that is one of the monumental formations within the cave. The leads on the Bishop formation were installed on a stalactite and in wet and dry alcoves. The Jackrabbit formation is another significant formation mass with a considerable number of soda straws, stalactites, and ribbon features. Leads were installed on a soda straw and a ribbon tip and on the ground below the formation.

Finally, an In-Situ Level Troll 500 pressure transducer was installed in the Subway Tunnel East (Fig. 1), an area with significant ponding, to determine the rate at which water drained. The Subway Tunnel is at a lower elevation and receives flow from upstream rooms.

Results and Discussion

Electrical Resistance Sensor Performance
Electrical Resistance Sensor (TidbiT)

The Strawberry Room was one of the wettest areas monitored within the cave on the basis of sensor data and visual observations. The Strawberry Room is easily accessible within the cave, and therefore, more visual observations were obtained during the investigation. These visual observations were used to confirm the presence or absence of water as measured by the ER sensors.

Water was detected by the ER sensor in the summer of 2005 and summer of 2008 (Fig. 3). Four visual observations during the summer of 2005 and 2008 were in agreement with the ER sensor. Seven additional visual observations during dry periods were also confirmed. These data are consistent with successful detections in ephemeral channels previously documented (Blasch et al., 2002). The success of the ER sensors within the cave environment is attributed to the lack of physical hazards such as high streamflow velocities, debris, and scour or deposition processes.
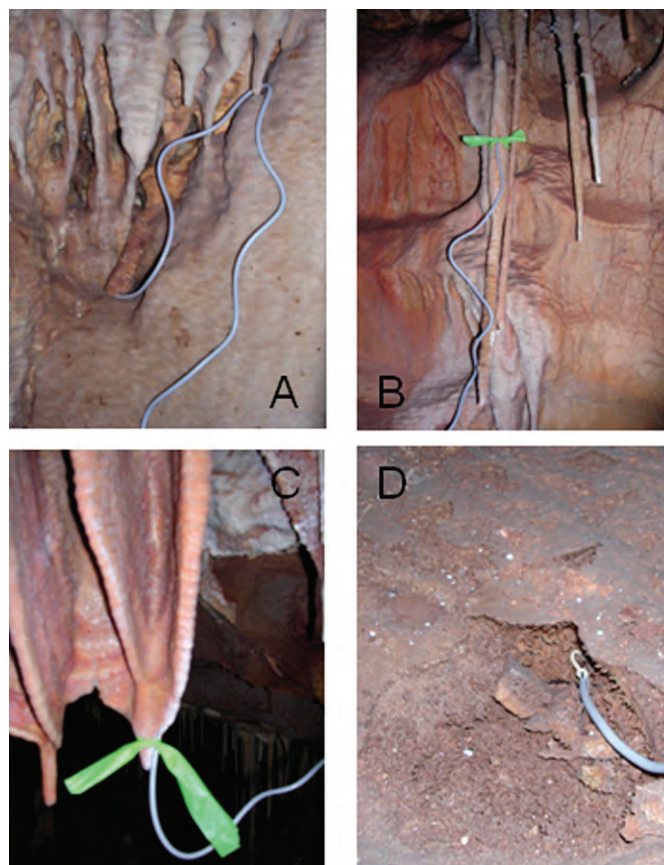

Figure 2. Placement of HOBO electrical resistance sensors on the Bishop Formation (A) and Jackrabbit Formation stalactite (B), ribbon (C), and (D) ground below.

Electrical Resistance Sensor (HOBO)

The HOBO sensors on the Jackrabbit formation recorded data for almost the entire period of study. The data reveals a more complex signature than the TidbiT ER sensors (Fig. 4). This may be attributed to the measurement of thin films of water on the formations or exposure of the circuitry to the humid environment. Voltages recorded before and after disconnecting the sensors from the data-logger, required for downloading, were noticeably different. During data analysis the recorded voltages were adjusted based on the voltage readings prior to the disconnection. With these corrections, the presence of water on the formations is indicated with lower voltage output, implying a higher conductivity.

During the winter of 2005, the spring and summer of 2007, and the summer of 2008 water drops were seen on the tip of the ribbon formation. The HOBO ER sensor recorded a lower voltage (higher conductivity) during these periods as indicated by the light gray points in Figure 4. The sensor on the floor recorded wet and dry conditions consistent with visual observations. The response recorded on the stalactite is not as conclusive (Fig. 4). The decrease in voltage from October 2006 to June 2007 cannot be
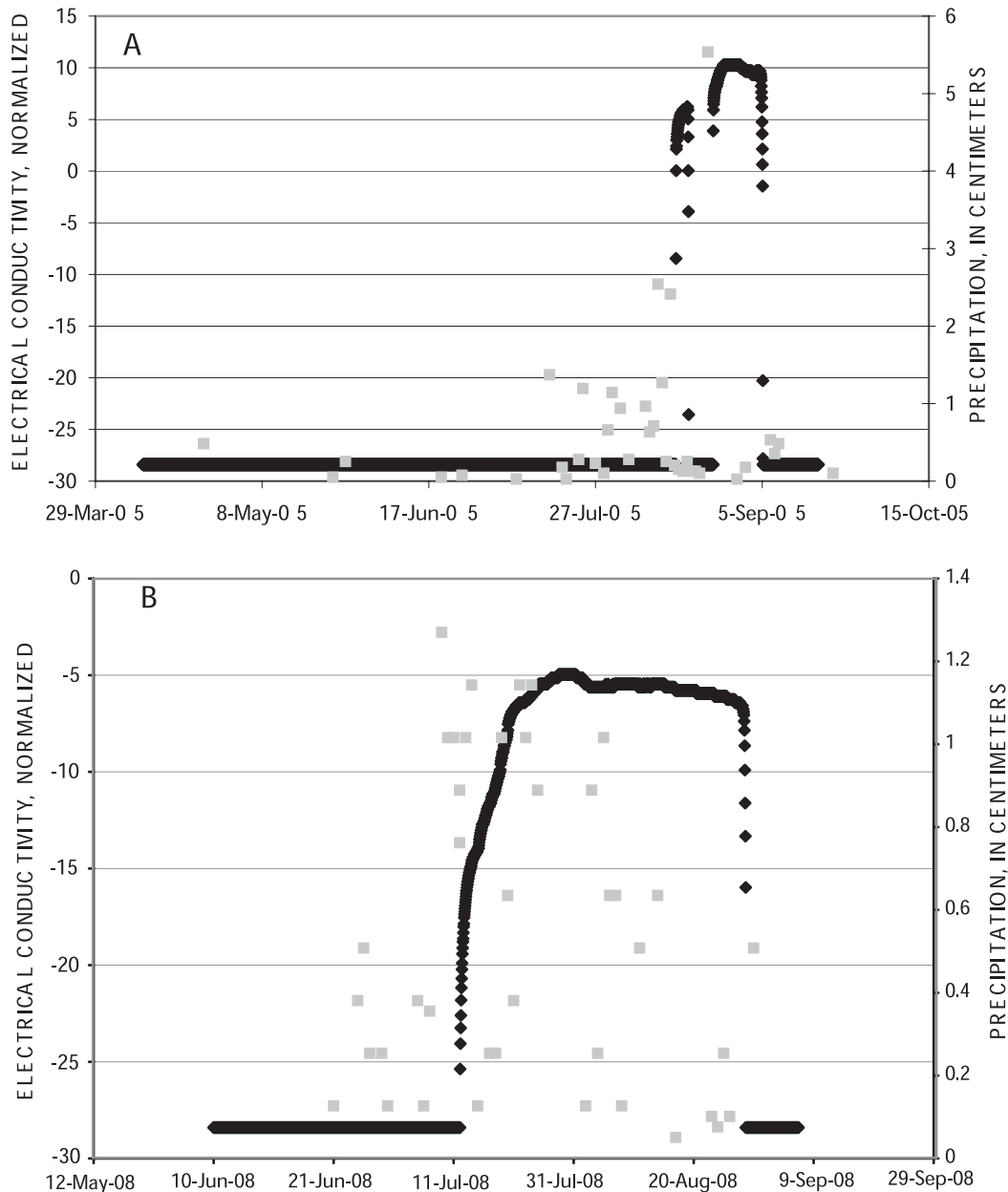
Figure 3. Measurements of normalized electrical conductivity in the pool of the Strawberry Room for (A) 2005 and (B) 2008. Daily precipitation represented by gray squares.

explained. During this time period visual observations indicated the formation was dry.

The sensors on the Bishop formation recorded wetness on the formation consistent with observations until the instruments failed in the humid environment. The last recorded day was April 9, 2005.

Within the cave, the success of the two types of ER sensors differed. The modified TidbiT sensors were well suited for cave channels, ponding areas, and formations with ponding surfaces. The short leads and the weight of the instruments prohibited them from being installed on delicate formations or small fractures. The flexibility of the

long leads from the 4-Channel HOBOs was well suited for installation on the delicate formations, but the high humidity of the cave environment was harmful to the HOBO's circuitry and made necessary frequent visits to change the desiccant packaged with the HOBO. One HOBO failed within the first year, likely owing to the high-humidity environment. Output from the HOBOs was also not as clear as output from the TidbiTs. The proprietary nature of HOBO and TidbiT circuitry prohibited a clear explanation for the difference in readings. For cave channels and ponding situations the TidbiTs performed well, but a combination of the TidbiT's ruggedness and
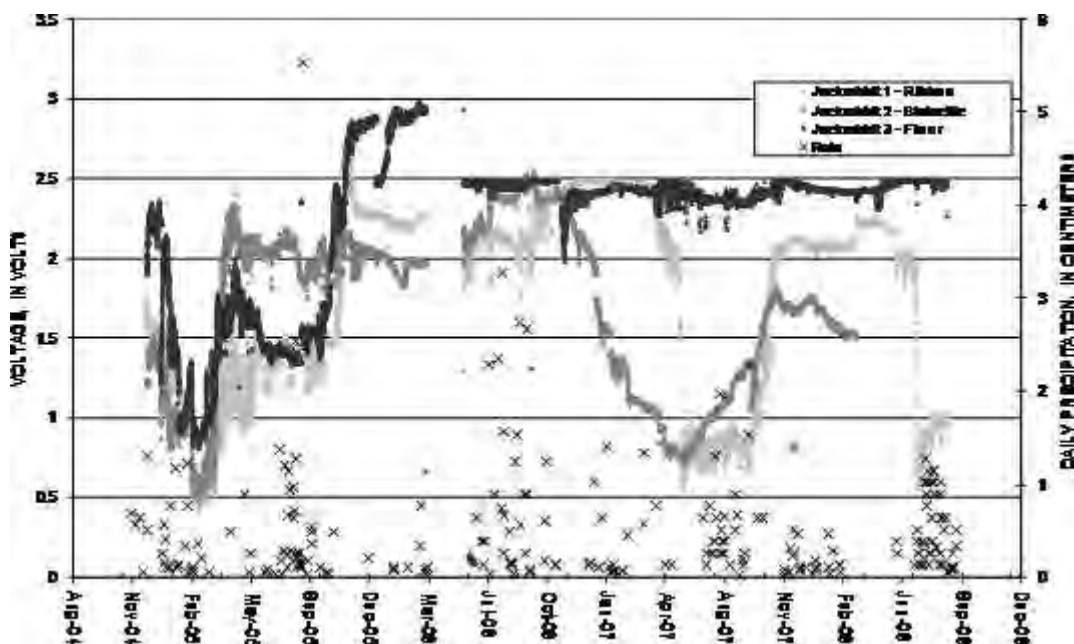
Figure 4. HOBO data for the presence of water on the Jackrabbit Formation. Daily precipitation represented by crosses.

output with the four-channel and long-lead capability of the HOBOs would be an optimal sensor for cave-formation monitoring.

WATER INFLOW AND MOVEMENT IN THE CAVE SYSTEM
External Cave Environment

Climate conditions at Kartchner Caverns were drier than the long-term mean. Mean annual precipitation monitored at Tombstone, Arizona, (National Climatic Data Center, 2008a) was 36 cm from 1900 to 2005 (Fig. 5).

Rainfall measured at Kartchner Caverns from 1992 to 2007 averaged 34 cm. During this study, annual rainfall values at Kartchner Caverns were 32, 31, and 28 cm per year, respectively. This translates into a reduction of 6 to 18 percent from mean annual rainfall. The Palmer Drought Severity Index (PDSI) for the southeast region of Arizona was used as an indicator of dryness and moisture storage (Fig. 6). PDSI values were obtained from the National Oceanic and Atmospheric Administration's National Climate Data Center (National Climatic Data Center,
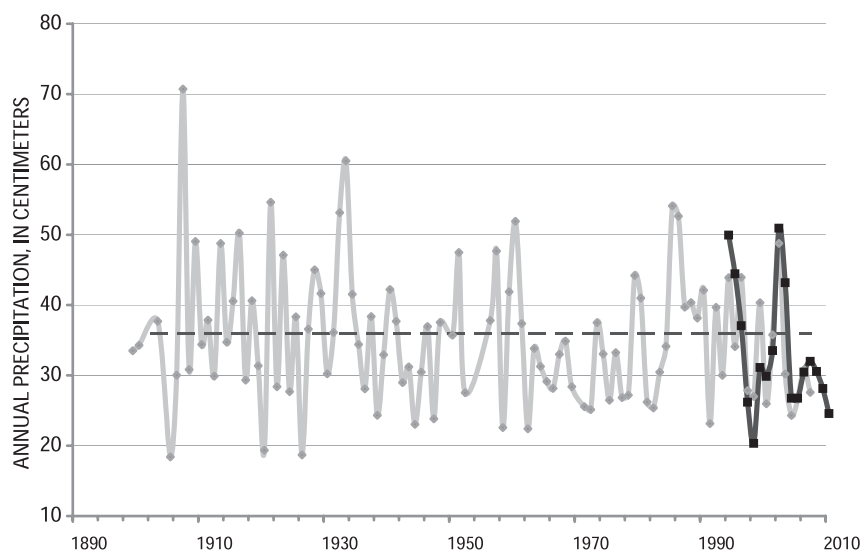

Figure 5. Annual precipitation for Tombstone, Arizona, (gray diamonds) and annual precipitation for Kartchner Caverns (black squares).
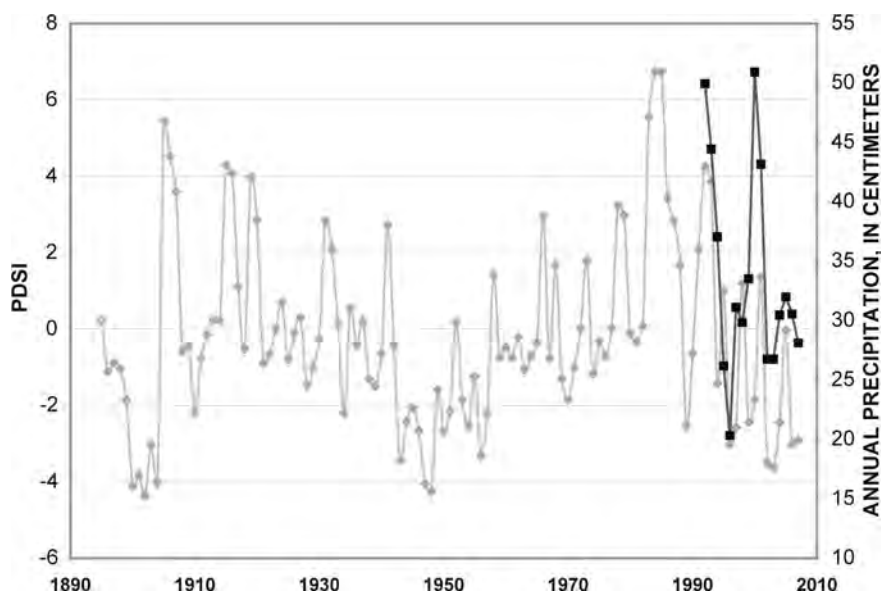
Figure 6. The Palmer Drought Severity Index (PDSI) for the southeast region of Arizona (National Oceanic and Atmospheric Administration's National Climate Data Center, 2008b) and annual precipitation for Kartchner Caverns (black squares).

2008b). Since 1994 the average annual PDSI has risen above normal only three years (1995, 1998, and 2001). The initial baseline investigations were conducted after six years of above normal values (1982–1987). During the baseline investigation from 1988 to 1992, the years 1989 and 1990 were below normal.

Ephemeral streamflow events, previously identified as the predominant source of recharge to the cave, are produced by high precipitation rates. There were no data on streamflow events collected during the baseline study in the early 1990s to compare. Streamflow events were detected in the ephemeral streams about nine times per year in Saddle Wash and about five times per year in Central Wash (Fig. 7). With the exception of the event on December 1, 2007, in upstream Saddle Wash, the remaining flow events occurred during the wet season (July–September) or during the fall (October–November). The wet season is a period of above-average precipitation (24 cm for the three-month period) due to high-intensity, convective rain storms. Average streamflow event duration for Saddle Wash (midstream) was 79 hours and the median event duration was 8 hours. Average event duration for Center Wash (main stem) was 27 hours and the median event duration was 5 hours.

Ephemeral streamflow events in Guindani Wash were rare. Streamflow events did not occur during 2003, 2004, 2006, or 2007. There were seven streamflow events in 2000 and two events in 2001. There was insufficient data for 2002. One flow event was detected in 2005 from 14–31 August, 2005 (Fig. 7). This event was associated with a measured rainfall of 2.4 cm on August 14, 2005, and 5.5 cm on August 23, 2005. Flow was observed during the July 22,

2008 event, but the gauge sustained damage during this high-flow event and the data were lost.

On the basis of rainfall and streamflow data, a rainfall intensity of 1.2 cm d^{-1} at Kartchner Caverns is generally required to generate runoff in the surface channels. Lower intensity rainfall events of 0.6 cm d^{-1} generated runoff if the event succeeded several days of rainfall. Winter storm intensities generally were not high enough to produce runoff during this period of study, though Graf (1999) observed earlier winter streamflow events.

Based on Graf's (1999) personal communication with Robert Buecher, one of the requirements for observed flow in the cave was at least one continuous week of ephemeral streamflow within Guindani or Saddle Wash. From December 2004 through September 2008 there was only one event in Saddle and Center Washes that was more than a week in duration. The 2008 wet-season event that started on July 22 caused flow for 36 days in Saddle Wash. Guindani Wash had flow that exceeded one week during this same time period, as well as a 14-day event during September 2005.

The full extent of the cave both laterally and vertically is unknown. Recent geophysical exploration supports the existence of large voids the size of the Rotunda Room to the west of the explored cave perimeter (Dale Rucker, personal communication, October 2007). Existence of these voids and their connection to Kartchner Caverns has not been verified. Voids between the known cave system and the adjacent washes could be important for the transmission of water from the washes into the known cave system. The size and location of voids affects the storage capacity and can reduce the volume and rate of flow into the
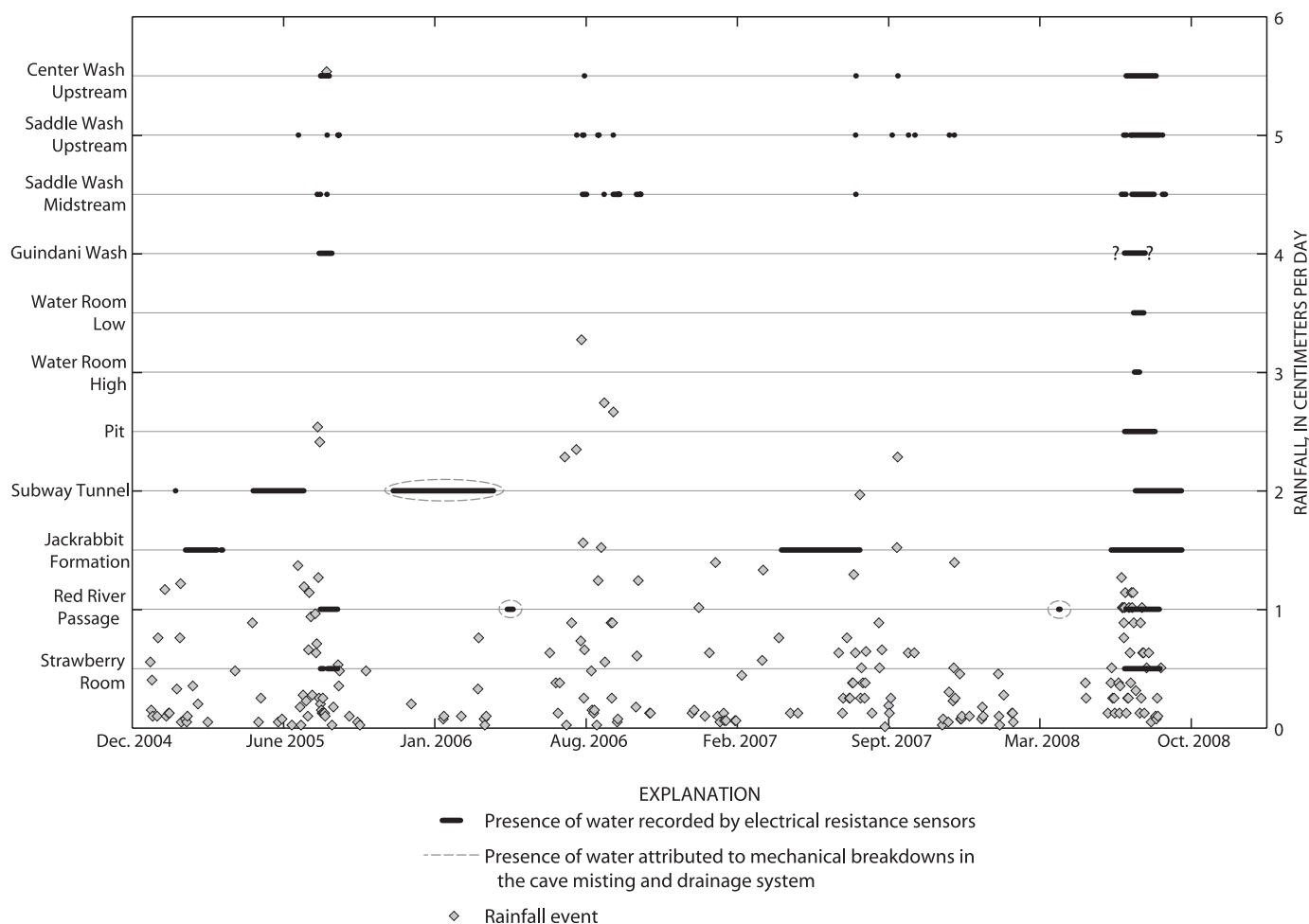
Figure 7. Water occurrence within the ephemeral stream channels outside the cave and formations and rooms within the cave, Kartchner Caverns, Arizona. Only monitoring sites with a nearly full period of record are shown. Daily precipitation totals represented by gray diamonds.

currently mapped and monitored portions of the cave system.

Groundwater levels in the adjacent granite wash and Pinal schist aquifers declined during the period of this investigation, but water levels remained higher than the lowest cave elevations monitored during the study (Fig. 8). Water levels were about 1 to 10 meters lower than historic water levels measured in 1995 and 2000. Graf (1999) analyzed historic water levels and concluded that groundwater inflow to the cave from the adjacent aquifer was negligible with the exception of infiltration over the fault boundaries underlying Saddle and Guindani Washes. Groundwater inflow to the cave was not observed during this investigation.

Internal Cave Environment

Measurements of humidity and temperature obtained from the Kartchner Cave Resources Unit shows a nearly undetectable increase in temperature and humidity for the Strawberry Room from September 2000 to September 2008

(Fig. 9). A general analysis of trend shows temperature increased from 21.44 °C to 21.67 °C and humidity increased from 97.9 percent to 98.2 percent. Over the same time period, measurements at Red River Passage showed an increase in temperature from 21.28 °C to 21.44 °C and almost no change in humidity when outliers are removed.

Water was not detected in the channel observation sites within the Mushroom Passage, Oak Creek Canyon, intersection with the Pirate's Den, Angel's Wing, or the Grand Canyon for the duration of the investigation. Water often observed in footprints along the paths within some of these rooms probably came from infiltration of rainfall and dripping from the ceiling. The ponding was not significant enough in these rooms to cause runoff to the drainage channels being monitored.

The Strawberry Room is on the northeastern edge of the cave and is the farthest monitoring site from the surface stream channels. Water was noticeable in the summer of 2005 and the winter and summer of 2008. Pooling in this room due to rainfall occurred within one day of a rainfall
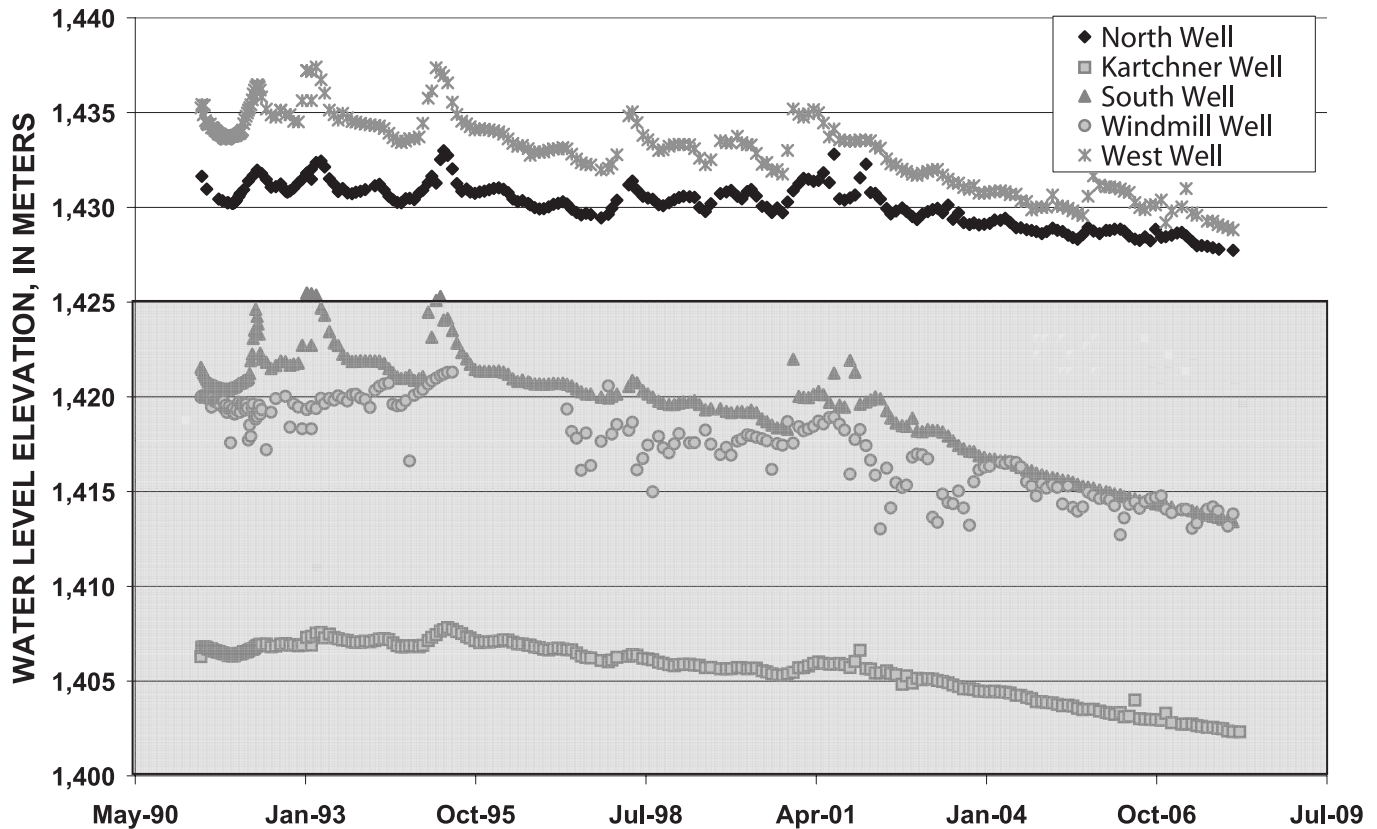
Figure 8. Groundwater levels (elevations) for wells adjacent to Kartchner Caverns. The patterned box indicates known depths of the cave system.

event. On August 15, 2005, ponded water was detected. This followed seven days with rain and a wet July. In particular, August 11 and August 14 both had rainfalls of about 2.5 cm. On August 18 the ponded water was no longer present. However, a rainfall event on August 23 produced about 5.5 cm over the cave system. Within one day (August 24), another pulse of water pooled in the Strawberry Room (Fig. 3A). The longest recorded ponding occurred from July 12, 2008, to August 28, 2008 (Fig. 3B).

Ponded water was recorded in the Red River Passage in September 2005 and April 2006. During the winter of 2007/2008 there was evidence of water, but the ER sensor had failed. Flow also was recorded in July and August of 2008 for a period of 44 days. Water detected at the Red River Passage during September 2005 and the September 2008 heavy-rainfall events was probably a combination of rainfall and infiltration from Guindani Wash (Fig. 7). The Red River Passage showed periods of wetting during April 2006 and April 2008 when there was no rain or streamflow activity. Personnel at Kartchner Caverns suspect that a floor sump near the Red Passage overflowed, discharging water into the Red River Passage. The floor sump drains water that is introduced through a plumbing system to clean foreign contamination from the paved trails.

The summer wet season of 2008 was the only time water was recorded by the Water Room High and Water Room Low sensors and in the Pit. Given these rooms' proximities to Saddle Wash, this water is likely to have originated as infiltration from this steam during the long period of flow.

The record of water detected in the Subway Tunnel was not consistent with the precipitation or streamflow records (Figs. 7 and 10). The source of the water was traced to leaking misters in the Rotunda Room. Several misters had been installed at the entrance of the cave to reduce foreign contamination such as lint from being deposited into the cave by visitors. Leaking mister sprayers were replaced December 14, 2005, January 21, 2005, and March 30, 2005. In each case the water level immediately declined in the Subway Tunnel after the replacements. These events provided the data to conclude that a flow of water into the Subway Tunnel from the Rotunda Room occurred through sediments, thus establishing a hydraulic connection between these rooms. Additionally, in order for water to travel from the Rotunda Room to the Subway Tunnel, the horizontal rate of travel had to be higher than the vertical drainage rate of the sediments within the Rotunda Room. The water level in the Subway Tunnel drops approximately 0.8 cm/d, according to the pressure transducer, when no water is flowing into it.

Climate Impacts and Future Considerations.

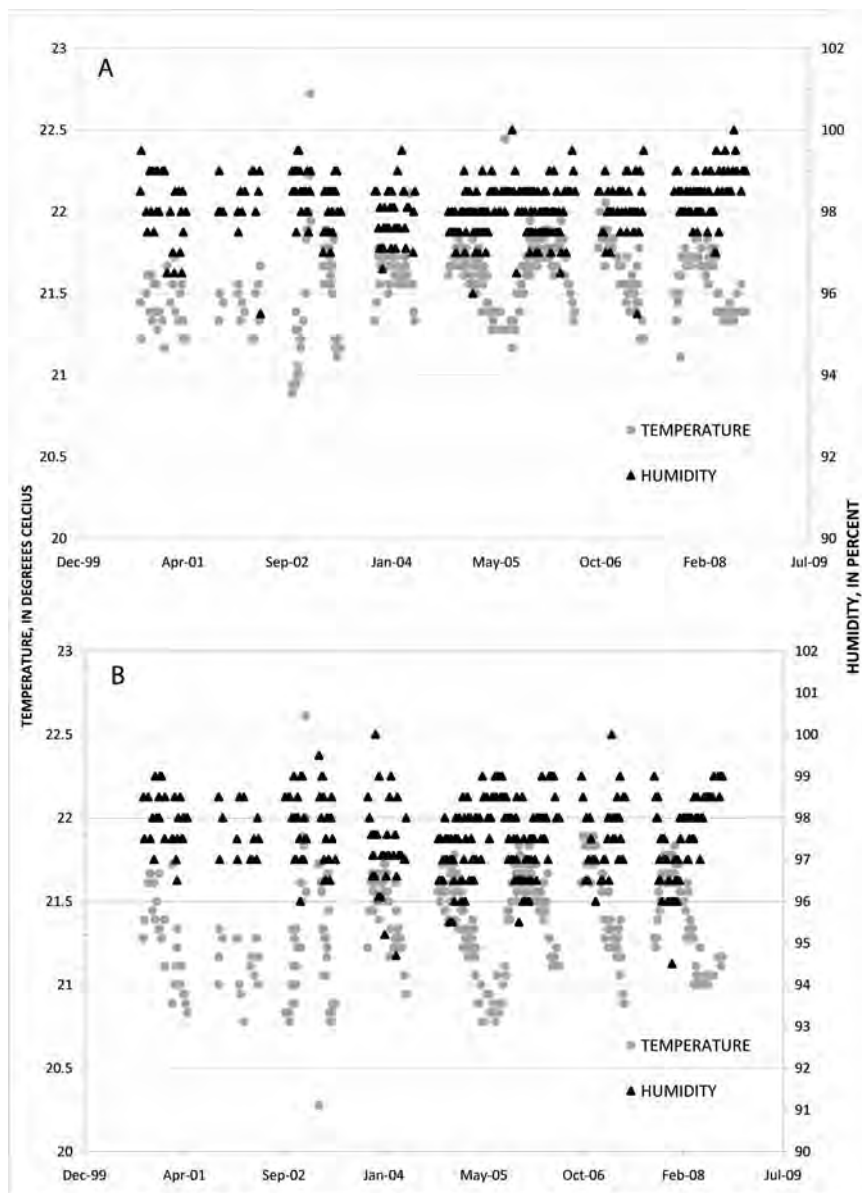Compared to the period of the baseline investigation, the magnitude of recharge observed within Kartchner

Figure 9. Humidity and temperature for the Strawberry Room (A) and the Red River Passage (B).

Caverns from both ephemeral streamflow infiltration (autogenic and allogenic recharge) and direct infiltration of precipitation (autogenic recharge) was less. There are not sufficient data to compare the total volume of water from each of these recharge mechanisms, but through visual observations the amount of water introduced through streamflow infiltration (two possible events over 44 months) was not noticeably higher than the amount introduced by direct infiltration. This would have to be verified with a more detailed study, but is in contrast to the results from the baseline period of above average rainfall.

During this investigation, autogenic recharge from direct infiltration occurred throughout the entire period of the investigation. One consideration for cave manage-

ment is to identify the higher and lower permeability fracture zones overlying the cave through the continued use of ER sensors and drip monitoring. To aid in this endeavor, precipitation measurements should be initiated above the cave. These data would be useful for producing a detailed assessment of vulnerability of formations to low-rainfall years.

Both climate fluctuations and the opening of the cave to visitors are likely contributing to changes in water and water vapor transport within the cave, but the data above show that decreased precipitation and overhead infiltration of water into the cave contributes directly to the reduction of water on the formations throughout the cave. Even if opening of the cave is increasing the inflow of dry air near the entrance, humidity measurements near the formations
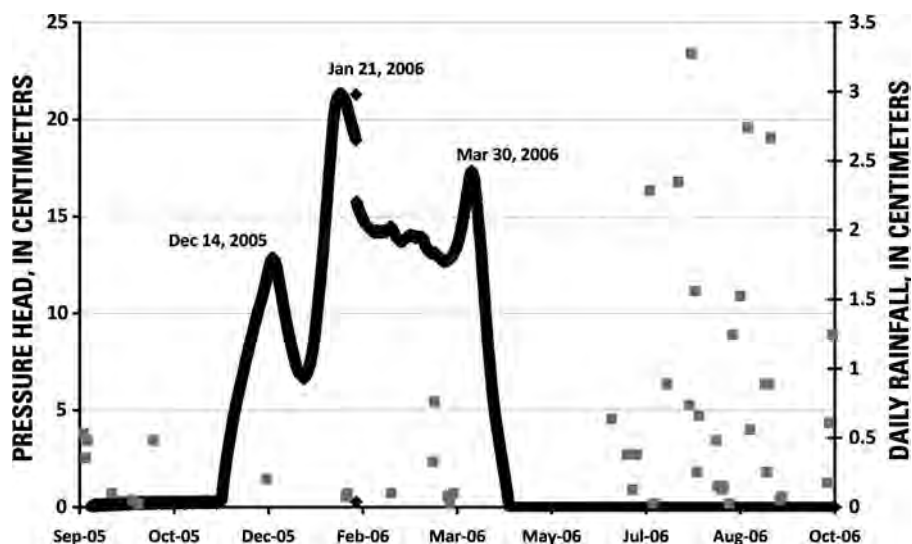
Figure 10. Pressure-head (depth) measurements of ponded water in the Subway Tunnel. Daily precipitation shown as gray squares.

show from no change to a slight increase. While more detailed air-inflow studies are in progress at the cave entrance, humidity measurements, speleothem drip-rate measurements, and ER measurements should continue to be conducted throughout the cave for assistance with future visitor management.

CONCLUSIONS

During this investigation, recharge of water to Kartchner Caverns from surface channel infiltration, overland infiltration from precipitation, and groundwater inflow was monitored using electrical-resistance sensors, temperature sensors, pressure transducers, and groundwater levels. The investigation coincided with a period of less-than-average rainfall, permitting the comparison of results from this study to the baseline investigations at Kartchner Caverns conducted during a period of higher-than-average rainfall. Monitoring of ephemeral streamflow in the surface channels overlying the cave and the presence of water in the cave itself indicated that the primary difference between wet and dry climate periods is the almost complete absence of any surface channel infiltration recharging the cave system during dry periods. Additionally, overhead autogenic recharge from infiltrating precipitation decreased during drier periods. Humidity measured in the cave at the monitoring points away from the cave entrance was consistent with wetter periods. This would indicate that, although the amount of water flowing on speleothems decreased during the drier period, existing drops on cave speleothems do not more readily evaporate. Finally, electrical-resistance sensors proved valuable for wetness detection on speleothems and within cave drainage channels. Encapsulated circuitry was necessary for instrument integrity within the humid cave environment.

REFERENCES

Adams, E., Monroe, S., Springer, A., Blasch, K.W., and Bills, D.J., 2006, Electrical resistance sensors record spring flow timing, Grand Canyon, Arizona: Ground Water, v. 44, no. 5, p. 630–641. doi: 10.1111/j.1745-6584.2006.00223.x.

Blasch, K., Ferré, P., Christensen, A., and Hoffmann, J.P., 2002, A new field method to determine streamflow timing using electrical resistance sensors: Vadose Zone Journal, v. 1, p. 289–299.

Buecher, R.H., 1992, Final report, environmental and geologic studies for Kartchner Caverns State Park, Arizona Conservation Projects, Inc., 328 p.

Buecher, R.H., 1999, Microclimate study of Kartchner Caverns, Arizona: Journal of Cave and Karst Studies, v. 61, no. 2, p. 108–120.

Graf, C.G., 1999, Hydrogeology of Kartchner Caverns State Park, Arizona: Journal of Cave and Karst Studies, v. 61, no. 2, p. 59–67.

Gray, R.S., 1967, Petrography of the Upper Cenozoic non-marine sediments in the San Pedro Valley, Arizona: Journal of Sedimentary Petrology, v. 37, p. 774–789.

Hill, C.A., 1999, Overview of Kartchner Caverns, Arizona: Journal of Cave and Karst Studies, v. 61, p. 41–43.

Jagnow, D.H., 1999, Geology of Kartchner Caverns State Park, Arizona: Journal of Cave and Karst Studies, v. 61, no. 2, p. 49–58.

Lange, A.L., 1999, Geophysical Studies at Kartchner Caverns State Park, Arizona: Journal of Cave and Karst Studies, v. 61, no. 2, p. 68–72.

Lange, A.L., Gustafson, E.P., and Walen, P.A., 1990, Geophysical field studies at Kartchner Caverns State Park, Arizona, Arizona Conservation Projects, Inc, 49 p.

Lerch, R.N., Wicks, C.M., and Moss, P.L., 2005, Hydrologic characterization of two karst recharge areas in Boone County, Missouri: Journal of Cave and Karst Studies, v. 67, no. 3, p. 158–173.

Melton, M.A., 1965, The geomorphic and paleoclimatic significance of alluvial deposits in southern Arizona: Journal of Geology, v. 73, p. 1–38. doi: 10.1086/627044.

National Climatic Data Center, 2008a, Climate Summaries, http://www.wrcc.dri.edu/summary/climsmaz.html, [accessed September 14, 2008].

National Climatic Data Center, 2008b, U.S. Temperature-Precipitation-Drought Index (DSI-9640), http://www7.ncdc.noaa.gov/CDO/CDODivisionalSelect.jsp#, [accessed September 14, 2008].

White, W.B., 1988, Geomorphology and Hydrology of Karst Terrains, New York, Oxford University Press, 464 p.

White, W.B., 2003, Conceptual models for karstic aquifers: Speleogenesis and Evolution of Karst Aquifers , v. 1, no. 1, 6 p., re-published from:, Palmer, A.N., Palmer, M.V., and Sasowsky, I.D., eds., 1999, Karst Modeling, Karst Waters Institute Special Publication 5, p. 11–16.

ESCHERICHIA COLI, OTHER COLIFORM, AND ENVIRONMENTAL CHEMOHETEROTROPHIC BACTERIA IN ISOLATED WATER POOLS FROM SIX CAVES IN NORTHERN ALABAMA AND NORTHWESTERN GEORGIA

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Abstract: *Escherichia coli* and other bacteria can be used as indicators of water quality within a cave ecosystem. However, bacterial species within caves have not been thoroughly documented, especially in the southeastern United States. Water from isolated pools was gathered along transects from six caves in northern Alabama and northwestern Georgia. We used cultivation techniques to isolate and characterize bacteria. Diversity of coliforms and some environmental genera were determined for each cave, and abundance was determined for *E. coli* and other coliforms. Distance from the entrance in most caves did not statistically correlate with abundance or species richness of bacteria. A total of fifty bacterial species and one fungal species were isolated from the six caves, with over half of these species considered potentially pathogenic in humans. Some species isolated, such as *Vibrio alginolyticus* and *V. fluvialis*, are considered primarily marine and are not expected isolates of cave waters. Most of the species we isolated have never been reported from limestone cave ecosystems. Overall, coliforms were found in all tested caves, indicating fecal contamination of all six caves.

INTRODUCTION

Microbial communities in caves are poorly understood (Groth and Saiz-Jimenez, 1999), but caves do support a complex microbial life (Northup and Lavoie, 2001; Caumartin, 1963). Caves contain unique populations that rely on both chemoautotrophic and heterotrophic interactions for survival. Many bacteria in caves are considered non-native species that have been transported into a cave via water, air, or animals (Northup and Lavoie, 2001), and how these bacteria interact and compete with endemic microflora is unknown. Alabama and Georgia are cave-rich states (Culver et al., 2006) that offer opportunities for microbial studies. Most caves in these two states are found in the Appalachian Plateaus and Interior Low Plateaus physiographic provinces and are formed in fossiliferous Paleozoic limestone. Despite its having a high density of caves, there is a scarcity of literature for this region concerning cave bacteria.

Most caves are considered to be nutrient (carbon) limited (Poulson and White, 1969; Culver, 1970), and cave-inhabiting organisms may be constrained by these limitations (Peck, 1976; Taylor et al., 2005). By breaking down organic matter and recycling nutrients, heterotrophic bacteria are important components of a cave ecosystem. Most bacterial communities in caves depend, like other heterotrophs, on allochthonous sources of organic matter (Laiz et al., 2000). However, large increases of bacteria in cave waters, associated with increased organic matter, can rob the waters of dissolved oxygen, which could cause massive changes in community structure of invertebrates and other organisms (Graening and Brown, 2003).

Coliform bacteria (Enterobacteriacae) are gram-negative, aerobic and facultatively anaerobic, rod-shaped bacteria that ferment lactose to produce acid. They produce β–galactosidase within 48 hours at 35 °C (Clesceri et al., 1998). Some coliforms are found in soil or vegetation, whereas fecal coliform are bacteria that usually live in the intestines of warm-blooded animals, making them a useful indicator of fecal contamination. Outside the intestines of warm-blooded animals, fecal coliform bacteria can survive for extended time periods. In karst aquifers, *E. coli* and other fecal coliform bacteria can remain viable for several months in water and stream sediments (Davis et al., 2005) and can be transported several kilometers in karst aquifers (Green et al., 1990). Lower temperatures found in cave environments reduce bacterial metabolism and increase their life span, allowing them to survive in these less-than-optimum conditions (Davis et al., 2005).

Coliform bacteria can be indicators for potential pathogens responsible for various waterborne diseases (Hunter et al., 2004) or be pathogens themselves, such as certain strains of *E. coli*. Coliform contamination can occur in caves (e.g. Lerch et al., 2001; Hunter et al., 2004; Kelly et al., 2009), with probable sources being the

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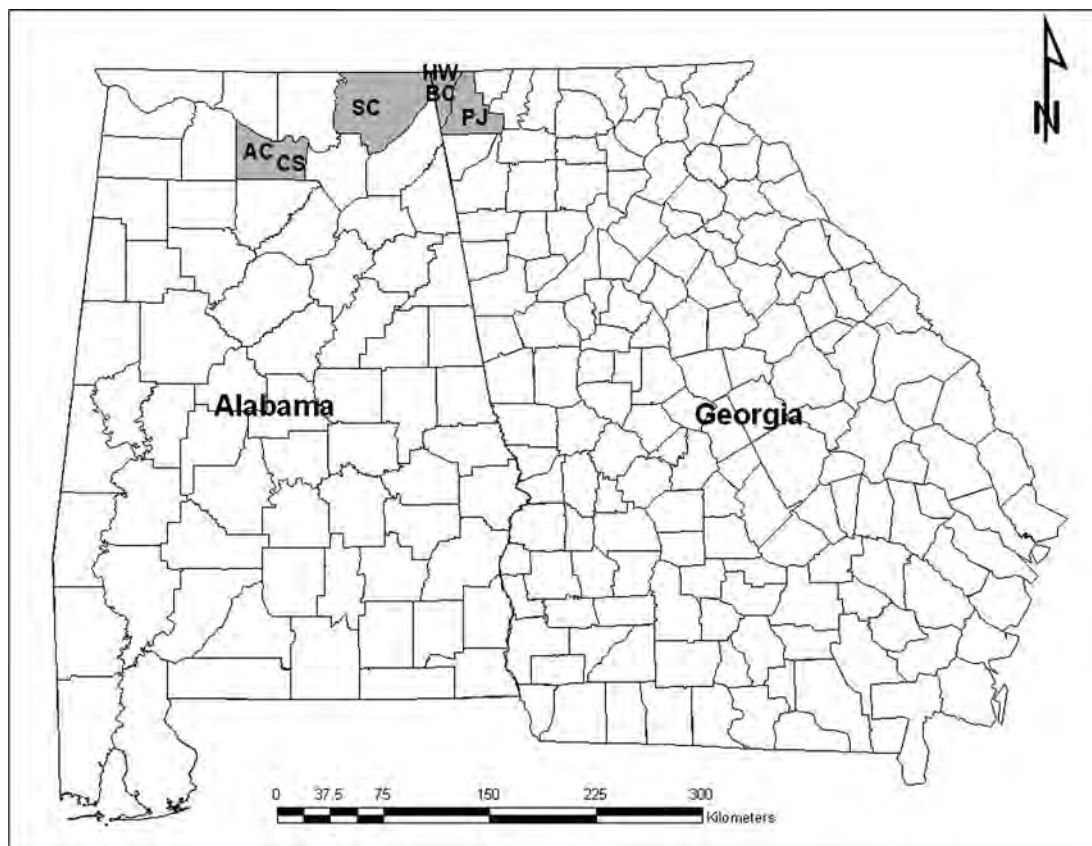
Figure 1. Approximate locations of the six caves in our study. AC=Anvil Cave, BC=Byers Cave, CS=Cave Springs Cave, HW=Howard's Waterfall Cave, PJ=Pettyjohns Cave, SC=Sauta Cave. Counties in which these caves are located have been shaded.

watershed or animals that excrete waste products within the cave. Coliform and environmental bacteria can enter a cave by water that drains into a cave from the watershed (Lerch et al., 2001; Kelly et al., 2009), primarily from a stream flowing directly into a cave or by seepage through soil or rock. Bats and other animals can also be major contributors of coliforms by excreting waste products within a cave ecosystem. Human visitors are another possible source of coliform bacteria within a cave ecosystem. Humans can bring bacteria into caves on the soles of their shoes and by dumping feces (Hunter et al., 2004). Some English caves showed contamination of certain bacterial species after cavers passed through (Moore and Sullivan, 1997, p. 80). Northup et al. (1997) found that cave areas frequently traversed by humans contained more bacterial species than less-frequented areas. Therefore, the recreational use of caves and human-induced land-use changes could lead to increases in coliform bacteria to levels considered a public health threat, while also affecting the natural bacterial assemblages in ways that are not yet understood.

In this study we tested water pools in six caves from northern Alabama and northwestern Georgia for abundance and species richness of coliform and other environ-

mental bacteria. We hypothesized that caves with more human or animal visitors or in a watershed with agricultural or sewage runoff would have higher bacterial abundance and species richness. We also hypothesized that abundance of coliforms and other bacteria would be greater closer to the entrance.

METHODS

STUDY SITES

Between January and July 2008, water samples were gathered and tested from six caves in northern Alabama (Anvil and Cave Springs Caves, Morgan Country; Sauta Cave, Jackson County) and northwestern Georgia (Byers and Howard's Waterfall Cave, Dade County; Pettyjohns Cave, Walker County) (Fig. 1). These six caves were chosen due to their length and accessibility, the presence of pools, and the different numbers of human and animal visitors at the caves. All of our collection sites are in horizontal caves in fossiliferous limestone.

SAMPLING PROCEDURE

Within each cave we aseptically took water samples from locations three or four different distances from the

entrance. At each location we took two 100 ml samples, generally from each of five cave pools. The pools had surface areas of 100 cm^2 to 1000 cm^2 and were separated by at least 1 meter. To avoid spikes of re-suspended bacteria from soil during storms, selected cave pools were not connected to any stream or runoff area and appeared to be supplied primarily by dripping stalactites. The selected pools rarely, if ever, become dry. Sauta and Cave Springs Caves contain streams that flow from their entrances. If isolated cave pools could not be found, stagnant areas of the creeks were sampled.

One water sample from each cave pool was stored at 2 to 4 °C for future cultures, and a second undiluted sample was poured and sealed into a Colisure quanti-tray (IDEXX Laboratories, Inc.) and placed in an incubator at 35 °C for up to 48 hours. Enzyme-based methods are widely accepted as a standard for detecting E. coli and other coliforms in water (Olstadt et al., 2007). Colisure products are approved by the Environmental Protection Agency for testing of drinking water and groundwater for total coliform bacteria and E. coli and are able to detect E. coli and other coliforms at one organism (up to 2419.6) per 100 ml (U.S. EPA, 1994; U.S. EPA, 1997; U.S. EPA, 1999, p. V-12–V-13). Coliforms produce the enzyme β-galactosidase, which is detected by cleaving Colisure's nutrient-indicator CPRG (chlorophenol red β-D-galactopranoside). The cleaving causes the water sample to change color over a 24 to 48 hour period from yellow to red or magenta, and E. coli causes the sample to fluoresce green when exposed to UV light because E. coli metabolizes the nutrient indicator MUG (4-methyllumbel-liferyl b-D-glucuronide). Colisure products detect E. coli and other coliforms with high confidence and suppress the growth of galactosidase-producing non-coliform organisms, thus resulting in a low rate of false positives (Olstadt et al., 2007). Our abundance tests are presumptive, but we did culture E. coli from all water samples, thus confirming the presence of E. coli.

BACTERIAL IDENTIFICATION PROCEDURE

A subsample (10 µl) of the water that had been stored at 2 to 4 °C was plated on MacConkey agar, a selective and differential plating medium used for isolating and differentiating lactose-fermenting from non-lactose fermenting gram-negative bacteria (BBL MacConkey II Agar). The cultures were incubated at 37 °C for ~48 hours. If no growth was present after 48 hours, pool water (10 µl) was inoculated into tryptic soy broth, incubated overnight and then plated back onto MacConkey agar. Any bacterial colonies that appeared morphologically (color, shape, or size) different were transferred to tryptic soy agar with 5% sheep blood. Qualitative oxidase and indole tests were completed using BBL DrySlide products. Bacteria that were oxidase negative and indole positive were again streaked on MacConkey and Simmon's Citrate media. MacConkey plates that were lactose positive with a strong precipitate around the colony and Simmon's Citrate

negative were determined to be E. coli. If MacConkey plates were lactose + with no precipitate or lactose − and the Simmon's Citrate was + or −, the bacteria were placed on a Trek Sensititre System GN ID Panel. A 0.5 McFarland suspension of the isolate was prepared in sterile distilled water and inoculated into the GN ID Panel. If a bacteria was not identified by the Trek Sensititre System, the bacteria was placed on a Bio Merieux API 20E or Remel Rap ID NF+ System for identification. All procedures followed standard methods (Clesceri et al., 1998).

Abundance and species richness data were analyzed with GLM (Statistix 9 program, Analytical Software, Tallahassee, Florida) to conduct one-way ANOVAs with distance intervals as the independent variable and coliform bacteria and E. coli abundances and total number of bacterial species cultured as dependent variables. The Tukey's multiple comparison procedure in the same program was used to determine differences in relative abundances of E. coli and other coliform bacteria and bacterial species among caves. Due to differences in location and number of water pools among caves, locations were assigned to three zones, the entrance zone 0–75 m, the intermediate zone 75–125 m, and the deep zone >125 m, for 166 bacterial abundances and species richness analysis.

RESULTS

We collected ten coliform and forty environmental bacterial species from the six caves, with at least twenty-seven of these species considered to be potentially pathogenic. Most bacterial species were isolated from Pettyjohns Cave, followed by Sauta and Cave Spring Caves (Table 1). There was little consistency in species occurrence between caves, with thirty-three species isolated from only one cave (Table 1). E. coli was the only species isolated from all caves. Cave Springs Cave had significantly higher E. coli and other coliform abundance compared to all other caves except Sauta Cave (Fig. 2). Neither species richness ($P = 0.8102$) or abundance ($P = 0.2572$) of E. coli and other coliform were significantly different among distance intervals when 163 data from all caves were pooled into different zones. However, many differences were detected between various distance zones within individual caves (Table 2). Because we did not dilute our water samples, some of our bacteria abundance numbers are conservative. Due to the heterogeneity and availability of water pools within caves, results will be discussed separately for each cave. The forty environmental bacteria species were not targeted and considered incidental in our cultures. Therefore, our species richness numbers do not reflect the true species richness of each cave.

ANVIL CAVE

E. coli and other coliform bacteria were significantly higher within the entrance zone compared to all other

Table 1. Bacteria species isolated from six caves located in North Alabama and Northwest Georgia. *Escherichia coli* is the only species cultivated from all caves.

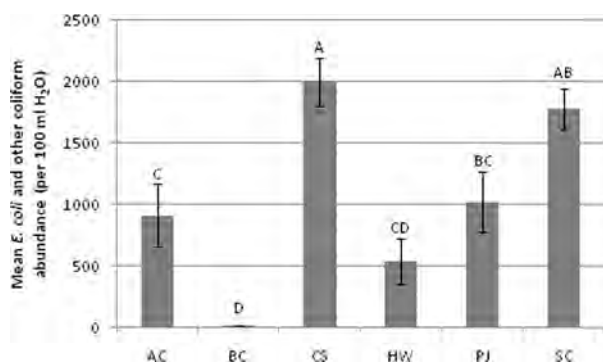| Bacteria Species	Anvil Cave	Byers Cave	Cave Springs	Howard's Waterfall	Pettyjohns Cave	Sauta Cave
Coliform Bacteria						
Citrobacter freundii	X				X	X
Enterobacter amnigenus	X					
Enterobacter cancerogenus				X	X	
Enterobacter intermedius	X					
Enterobacter sp.	X				X	
Escherichia coli	X	X	X	X	X	X
Klebsiella ozaenae					X	
Klebsiella oxytoca					X	
Klebsiella pneumo ss pneumoniae					X	
Pantoea agglomerans				X	X	X
Environmental Bacteria						
Acinetobacter baumannii					X	
Acinetobacter lwoffi			X		X	
Aeromonas caviae				X	X	X
Aeromonas hydrophilia		X			X	X
Aeromonas sp.						X
Aeromonas sobria						X
Bacillus sp. 1						X
Bacillus sp. 2		X	X			X
Brevundimonas diminuta					X	
Chromobacterium sp.						X
Chryseobacterium meningosepticum			X			
Comamonas testosterone					X	
Empedobacter brevis			X			
Hafnia alvei	X				X	
Kluyvera cryocrescens				X		
Listonella (Vibrio) anguillarum				X	X	
Micrococcus sp.			X			
Moraxella osloensis	X					
Myroides odoratum			X			
Ochrobactrum anthropi	X					
Proteus mirabilis			X			
Pseudomonas aeruginosa				X		
Pseudomonas aeruginosa/putida				X		
Pseudomonas alcaligenes	X	X	X	X	X	
Pseudomonas flourescens		X	X		X	X
Pseudomonas flourescens/putida		X	X			X
Pseudomonas mendocina			X			X
Pseudomonas pseudoalcaligenes						X
Pseudomonas stutzeri		X			X	X
Pseudomonas sp.			X	X	X	X
Psychrobacter phenylpyruvica		X				
Serratia fonticola						X
Serratia marcescens					X	
Serratia plymuthica					X	
Serratia proteamaculans					X	
Shewanella putrefaciens						X
Sphingomonas paucimobilis						X
Stentrophomonas maltophilia					X	
Vibrio alginolyticus	X					
Vibrio fluvialis	X					

Figure 2. Mean total abundance (+/−SE) (per 100 ml of H$_2$O) of *E. coli* and other coliform from six caves located in north Alabama and northwest Georgia. Columns with the same letter(s) are not significantly different at $P \leq 0.05$ according to Tukey's multiple comparison procedure. AC=Anvil Cave, BC=Byers Cave, CS=Cave Springs Cave, HW=Howard's Waterfall Cave, PJ=Pettyjohns Cave, SC=Sauta Cave.

distance zones, and the intermediate zone was significantly higher in coliforms than the deep zone. We collected eleven species of bacteria from Anvil Cave (Table 1), seven of which were isolated from the entrance zone. Two *Vibrio* species (*V. alginolyticus* and *V. fluvialis*) were isolated from Anvil Cave. They are common in estuarine and marine environments (Roberts et al., 1982), but can be causative agents of diseases in humans (West, 1989).

Byers Cave

Although not significantly different, *E. coli* and other coliforms were higher in abundance in the deep zone compared to the entrance zone (Table 2). Only eight bacteria species were isolated from Byers (Table 1) and, overall, *E. coli* and other coliforms were found in very low abundance compared to all other caves.

Cave Springs Cave

E. coli and other coliform abundance were significantly higher in the deep zone compared to the entrance zone (Table 2). Thirteen species of bacteria were isolated from Cave Springs Cave (Table 1). Within the intermediate zone, a *Bipolaris* spp. (dematiaceous fungus) was isolated, several species of which are considered to be a potential human pathogens.

Howard's Waterfall Cave

E. coli and other coliforms were higher in abundance in the entrance zone compared to the deep zone, but the difference was not significant (Table 2). We collected ten species of bacteria from Howard's Waterfall Cave (Table 1). Despite low abundance of coliforms within the deep zone, seven species of bacteria were isolated there, including *Listonella* (*Vibrio*) *anguillarum*. *L. anguillarum*

is primarily isolated from marine environments, but has on occasion been isolated from freshwater environments (Chen et al., 2006). It causes fish disease worldwide (Powell and Loutit, 1994).

Pettyjohns Cave

E. coli and other bacteria were found throughout our sampling transect. No significant differences in abundance were noted between the entrance and deep zone (Table 2). We collected twenty-four species of bacteria from Pettyjohns Cave (Table 1). *Listonella* (*Vibrio*) *anguillarum* was also isolated from the entrance zone of this cave.

Sauta Cave

E. coli and other coliform bacteria were not significantly different among the distance zones (Table 2). However, *E. coli* abundance did increase at deeper distance intervals. Nineteen species were collected within Sauta Cave (Table 1). One species collected, *Shewanella putrefaciens*, is a common environmental isolate that composes biofilms (McLeod et al., 2002) and can use multiple electron acceptors (including manganese) for aerobic respiration (Myers and Myers, 2001), which may allow for its survival in cave ecosystems.

Discussion

A great deal of heterogeneity existed among caves, such as location and separation of water pools. Although distance from an entrance is a variable that can influence bacterial abundance (as observed in Anvil Cave), other variables appear to be more important in controlling abundance and species richness of coliform bacteria in cave ecosystems. Outside influences, such as number of human and bat visitors and watershed differences, differed among caves, which probably accounted for the lack of correlation between *E. coli* and other bacteria abundances and species richness and distance from an entrance. Abundance levels of *E.coli* and other coliform bacteria fluctuated widely among water pools in the same distance zone of some caves, suggesting that isolated pools are not necessarily influenced by other nearby pools, and bacterial presence may depend more heavily on a direct source such as human or animal visitors rather than an indirect source such as watershed contamination.

The isolation of many potentially pathogenic bacteria from these cave waters suggests that these species are able to remain viable in a low metabolic state. *E. coli* has been shown to survive longer in soil kept at low temperatures (Teague et al., 1995), and Flint (1987) found *E. coli* surviving for extended periods of time in cold river water. Some of these coliforms and environmentals may be adhering to biofilms on the substrate, which could help sustain the species in cave environments. Epilithic biofilms have been shown to be an important energy source for aquatic cave communities (Simon et al., 2003). Biofilms

Table 2. Mean *E. coli* and other coliform abundance (+/−SE) (per 100 ml H$_2$O) from each cave and the various distance zones within each cave.

Cave	Entrance Zone	Intermediate Zone	Deep Zone
Anvil Cave	2419.6 (0)[a]	1035.8 (564.5)[b]	100.68 (52.3)[c]
Byers Cave	6.76 (3.1)[a]	NP	23.87 (7.8)[a]
Cave Springs Cave	1065.5 (553.1)[a]	2099 (320.7)[ab]	2419.6 (0)[b]
Howard's Waterfall Cave	823.86 (352.5)[a]	NP	253.3 (68.8)[a]
Pettyjohns Cave	1108.6 (336.3)[a]	NP	939.25 (361.8)[a]
Sauta Cave	1758.5 (298.9)[a]	1813 (173.3)[a]	1780.5 (288.4)[a]

Note: Within each cave means of various distance zones followed by the same letter(s) are not significantly different ($P \leqslant 0.05$) according the Tukey's multiple comparison procedure.

NP = No pools present.

Lower case letters indicate which caves have differences in bacterial abundance among the three zones.

can also trap nutrients (Costerton and Lappin-Scott, 1989), which can increase the survival of non-endemic bacteria (Boyle et al., 1991).

Although our cultivation techniques favored cultivation of coliform bacteria, we also isolated forty environmental chemoheterotrophic bacterial species. Our study highly underestimated environmental bacteria, because our cultivation techniques targeted coliform bacteria, and our result is not representative of the true bacterial species diversity. Many cave bacteria species may have been inhibited by our media or inoculation temperature, which do not mimic a cave environment. According to McNamara and Mitchell (2005), less than 10% of bacteria ($< 1\%$ in many instances) from an environmental sample can be cultivated by standard laboratory techniques.

Each cave illustrated unique physical differences that may help explain differences in abundance and species of bacteria. Anvil Cave, a maze cave, has over 20 km (13 miles) of passages and several entrances and receives few human visitors. Most human visitors and other animals will necessarily pass through an entrance zone, but they may become dispersed in the mazes beyond these zones, which could account for the higher *E. coli* and other coliform abundances and species richness within the first distance zone. Anvil Cave is, however, located primarily underneath pasture land that could be a source of bacteria.

Byers Cave, which is one of Georgia's largest caves, contains over 8 km (5 miles) of passages and contains a waterfall, and many pools are present within the deep zone, here > 400 m from the entrance. Byers Cave is not heavily trafficked by humans, and water seepage from the general watershed is a probable source for the bacteria in these distance zones. Byers Cave is located within a pristine wooded area that is far from any human development, which could account for the overall low abundance and species richness of bacteria.

Cave Springs and Sauta Caves both have large openings and contain streams roughly 3 to 5 meters wide that slowly drain towards the entrance. Large colonies of endangered gray bats (*Myotis grisescens*) inhabit Cave Springs and Sauta Caves, with maternity colonies formed during spring and summer. These caves are closed to the public and get only an occasional human visitor. Total organic matter in soil has been shown to increase in areas with larger numbers of bats in Cave Springs and Sauta Caves (Joshua Campbell et al., unpubl. data), which probably accounted for the high abundance of *E. coli* and other coliforms.

Howard's Waterfall Cave is a horizontal cave that is over 3 km long (2 miles long) and receives approximately 1000 human visitors per year, with most only exploring the first part of the cave, which could account for the increased abundance of *E. coli* and other coliforms within the entrance zone. However, watershed sources and other animals are also possible sources for the increased abundance of *E. coli* and other coliforms from the entrance zone. The higher number of species isolated from the deep zone (including *Listonella (Vibrio) anguillarum*) is an interesting result and may suggest a watershed source of bacteria, even though overall coliform and other bacteria abundance was low in this zone.

Pettyjohns Cave has over 10.5 km (6.5 miles) of passages and is a popular cave that draws approximately 10,000 visitors per year (Padgett, 1999). The majority of human visitors only explore near the entrance, which could explain the high numbers of bacteria within the entrance zone. In one area 300 to 325 meters in, a spike in *E. coli* and other coliforms was observed. Here the cave narrows, which forces any cavers to pass through any pools present, suggesting that the high number of human visitors could be a culprit in these spikes. Although the high number of species is likely due to the high number of people that visit this cave, watershed sources and other animals could also be contributing factors. Neither Howard's Waterfall nor Pettyjohns Caves contain fish, which suggests *Listonella (Vibrio) anguillarum* entered from the watershed through the aquifer, rather than by way of possibly diseased fish.

CONCLUSIONS

Due to the heterogeneity of these caves, general patterns can be difficult to discern. However, bacterial abundance and species richness in caves can be influenced by humans,

other animals such as bats, and watershed uses. Cave bacteria can be used as bioindicators of water quality, and high abundance could be an ecosystem warning signal. High levels of bacteria abundance, especially coliforms, could also be a human health concern. Humans could come into direct contact with contaminated pools by caving and indirectly through water wells. Overall, we found widespread fecal contamination in all caves, and people with scrapes or cuts should use caution while caving. Drinking directly from pools without filtration should be avoided at all times. Many organisms that live in caves are very sensitive to ecosystem disturbances and may have limited populations and slow reproduction times. How increases in non-native bacterial species affect cave macroinvertebrates and other animals should be investigated.

Acknowledgments

References

Boyle, M., Ford, T., Maki, J.S., and Mitchell, R., 1991, Biofilms and the survival of opportunistic pathogens in recycled water: Waste Management and Research, v. 9, p. 465–470, doi: 10.1177/0734242X9100900165.

Caumartin, V., 1963, Review of the microbiology of underground environments: Bulletin of the National Speleological Society, v. 25, p. 1–14.

Chen, C.Y., Chao, C.B., and Bowser, P.R., 2006, Infection of tilapia Oreochromis sp. by Vibrio vulnificus in fresh water and low-salinity environments: Journal of the World Aquaculture Society, v. 37, p. 82–88, doi: 10.1111/j.1749-7345.2006.00010.x.

Clesceri, L.S., Greenberg, A.E., and Eaton, A.D., eds., 1998, Standard Methods for the Examination of Water and Wastewater, 20th ed., APHA, Washington, D.C., 1325 p.

Costerton, J.W., and Lappin-Scott, H.M., 1989, Behavior of bacteria in biofilms: American Society for Microbiology News, v. 55, p. 650–654.

Culver, D.C., 1970, Analysis of simple cave communities I. Cave as islands: Evolution, v. 24, p. 463–474.

Culver, D.C., Deharveng, L., Bedos, A., Lewis, J.J., Madden, M., Reddell, J.R., Sket, B., Trontelj, P., and White, D., 2006, The mid-latitude biodiversity ridge in terrestrial cave fauna: Ecography, v. 29, p. 120–128, doi: 10.1111/j.2005.0906-7590.04435.x.

Davis, R.K., Hamilton, S., and Van Brahana, J., 2005, Escherichia coli survival in mantled karst springs and streams, northwest Arkansas Ozarks, USA: Journal of the American Water Resources Association, v. 41, p. 1279–1287, doi: 10.1111/j.752-1688.2005.tb03800.x.

Flint, K.P., 1987, The long-term survival of Escherichia coli in river water: Journal of Applied Bacteriology, v. 63, p. 261–270, doi: 10.1111/j.1365-2672.1987.tb04945.x.

Graening, G.O., and Brown, A.V., 2003, Ecosystem dynamics and pollution effects in an Ozark cave stream: Journal of the American Water Resources Association, v. 39, p. 1497–1507, doi: 10.1111/j.1752-1688.2003.tb04434.x.

Green, W., Ellicott, L., and Crawford, N., 1990, Investigation of nonpoint source pollution associated with karst aquifer systems: Transactions of the Kentucky Academy of Science, v. 51, p. 177–181.

Groth, I., and Saiz-Jimenez, C., 1999, Actinomycetes in hypogean environments: Geomicrobiology Journal, v. 16, p. 1–8.

Hunter, A.J., Northup, D.E., Dahm, C.N., and Boston, P.J., 2004, Persistent coliform contamination in Lechuguilla cave pools: Journal of Cave and Karst Studies, v. 66, p. 102–110.

Kelly, W.R., Panno, S.V., Hackley, K.C., Martinsek, A.T., Krapac, I.G., Weibel, C.P., and Storment, E.C., 2009, Bacteria contamination of groundwater in a mixed land-use karst region: Water Quality Exposure and Health, v. 1, p. 69–78, doi: 10.1007/s12403-009-0006-7.

Laiz, L., Groth, I., Schumann, P., Zezza, F., Felske, A., Hermosin, B., and Saiz-Jimenez, C., 2000, Microbiology of the stalactites from Grotta dei Cervi, Porto Badisco, Italy: International Microbiology, v. 3, p. 25–30.

Lerch, R.N., Erickson, J.M., and Wicks, C.M., 2001, Intensive water quality monitoring in two karst watersheds of Boone County, Missouri, in Rea, G.T., ed., Proceedings of the 2001 National Cave and Karst Management Symposium, Tucson, Arizona, Oct. 16–19, 2001, Tucson, Coronado National Forest, p. 151–168.

McLeod, E.S., MacDonald, R., and Brözel, V.S., 2002, Distribution of Shewanella putrefaciens and Desulfovibrio vulgaris in sulphidogenic biofilms of industial cooling water system determined by fluorescent in situ hybridization: Water SA, v. 28, p. 123–128.

McNamara, C.J., and Mitchell, R., 2005, Microbial deterioration of historic stone: Frontiers in Ecology and the Environment, v. 3, p. 445–451, doi: 10.1890/1540-9295(2005)003[0445:MDOHS]2.0.CO;2.

Moore, G.W., and Sullivan, G.N., 1997, Speleology: Caves and the Cave Environment, third edition: St. Louis, Cave Books, 176 p.

Myers, J.M., and Myers, C.R., 2001, Role for outer membrane cytochromes OmcA and OmcB of Shewanella putrefaciens MR-1 in reduction of manganese dioxide: Applied and Environmental Microbiology, v. 67, p. 260–269, doi: 10.1128/AEM.67.1.260-269.2001.

Northup, D.E., Beck, K.M., and Mallory, L.M., 1997, Human impact on the microbial communities of Lechuguilla cave: Is protection possible during active exploration?, abs., National Speleology Society Convention Program, Sullivan, Missouri, 55 p.

Northup, D.E., and Lavoie, K.H., 2001, Geomicrobiology of caves: A review: Geomicrobiology Journal, v. 18, p. 199–222, doi:10.1080/01490450152467750.

Olstadt, J., Schauer, J.J., Standridge, J., and Kluender, S., 2007, A comparison of ten USEPA approved total coliform/E. coli tests: Journal of Water and Health, v. 5, p. 267–282, doi: 10.2166/wh.2007.008.

Padgett, A., 1999, Pigeon Mountain: Thirty years of noninterference, in Rea, G.T., ed., Proceedings of the 1999 National Cave and Karst Management Symposium, Chatanooga, Tennessee, Southeastern Cave Conservancy, p. 143–147.

Peck, S.B., 1976, The effect of cave entrances on the distribution of cave-inhabiting terrestrial arthropods: International Journal of Speleology, v. 8, p. 309–321.

Poulson, T.L., and White, W.B., 1969, The cave environment: Science, v. 165, p. 971–981.

Powell, J.L., and Loutit, M.W., 1994, The detection of fish pathogen Vibrio anguillarum in water and fish using a species-specific DNA probe combined with membrane filtration: Microbial Ecology, v. 28, p. 375–383, doi:10.1007/BF00662030.

Roberts, N.C., Siebeling, R.J., Kaper, J.B., and Bradford, Jr., H.B., 1982, Vibrios in the Louisiana coast environment: Microbial Ecology, v. 8, p. 299–312, doi: 10.1007/BF02010670.

Simon, K.S., Benfield, E.F., and Macko, S.A., 2003, Food web structure and the role of epilithic biofilms in cave streams: Ecology, v. 84, p. 2395–2406.

Taylor, S.J., Krejca, J.K., and Denight, M.L., 2005, Foraging range and habitat use of Ceuthophilus secretus (Orthoptera: Rhaphidophoridae),

a key trogloxene in central Texas cave communities: American Midland Naturalist, v. 154, p. 97–114.

Teague, K.A., Wolf, D.C., and Vendrell, P.F., 1995, Survival of fecal contamination indicator organisms in soil: Arkansas Water Resources Center, Publication No. 173, 28 p.

U.S. EPA, 1994, National Primary and Secondary Drinking Water Regulations: Analytical Methods for Regulated Drinking Water Contaminants: *Federal Register*, 5 December 1994, 40 *Code of Federal Regulations* parts 141, 143 p.

U.S. EPA, 1997, Manual for the Certification of Laboratories Analyzing Drinking Water: Criteria and Procedures, Quality Assurance, fourth edition: Environmental Protection Agency document 815-B-97-001, 186 p.

U.S. EPA, 1999, National Primary and Secondary Drinking Water Regulations: Analytical Methods for Chemical and Microbiological Contaminants and Revisions to Laboratory Certification Requirements: Final Rule: *Federal Register*, 1 December 1999, p. 67449–67467, 40 *Code of Federal Regulations* parts 141, 143 p.

West, P.A., 1989, The human pathogenic vibrios—A public health update with environmental perspectives: Epidemiology and Infection, v. 103, p. 1–34, doi: 10.1017/S0950268800030326.

ESCHERICHIA COLI, OTHER COLIFORM, AND ENVIRONMENTAL CHEMOHETEROTROPHIC BACTERIA IN ISOLATED WATER POOLS FROM SIX CAVES IN NORTHERN ALABAMA AND NORTHWESTERN GEORGIA

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Abstract: *Escherichia coli* and other bacteria can be used as indicators of water quality within a cave ecosystem. However, bacterial species within caves have not been thoroughly documented, especially in the southeastern United States. Water from isolated pools was gathered along transects from six caves in northern Alabama and northwestern Georgia. We used cultivation techniques to isolate and characterize bacteria. Diversity of coliforms and some environmental genera were determined for each cave, and abundance was determined for *E. coli* and other coliforms. Distance from the entrance in most caves did not statistically correlate with abundance or species richness of bacteria. A total of fifty bacterial species and one fungal species were isolated from the six caves, with over half of these species considered potentially pathogenic in humans. Some species isolated, such as *Vibrio alginolyticus* and *V. fluvialis*, are considered primarily marine and are not expected isolates of cave waters. Most of the species we isolated have never been reported from limestone cave ecosystems. Overall, coliforms were found in all tested caves, indicating fecal contamination of all six caves.

INTRODUCTION

Microbial communities in caves are poorly understood (Groth and Saiz-Jimenez, 1999), but caves do support a complex microbial life (Northup and Lavoie, 2001; Caumartin, 1963). Caves contain unique populations that rely on both chemoautotrophic and heterotrophic interactions for survival. Many bacteria in caves are considered non-native species that have been transported into a cave via water, air, or animals (Northup and Lavoie, 2001), and how these bacteria interact and compete with endemic microflora is unknown. Alabama and Georgia are cave-rich states (Culver et al., 2006) that offer opportunities for microbial studies. Most caves in these two states are found in the Appalachian Plateaus and Interior Low Plateaus physiographic provinces and are formed in fossiliferous Paleozoic limestone. Despite its having a high density of caves, there is a scarcity of literature for this region concerning cave bacteria.

Most caves are considered to be nutrient (carbon) limited (Poulson and White, 1969; Culver, 1970), and cave-inhabiting organisms may be constrained by these limitations (Peck, 1976; Taylor et al., 2005). By breaking down organic matter and recycling nutrients, heterotrophic bacteria are important components of a cave ecosystem. Most bacterial communities in caves depend, like other heterotrophs, on allochthonous sources of organic matter (Laiz et al., 2000). However, large increases of bacteria in cave waters, associated with increased organic matter, can rob the waters of dissolved oxygen, which could cause massive changes in community structure of invertebrates and other organisms (Graening and Brown, 2003).

Coliform bacteria (Enterobacteriacae) are gram-negative, aerobic and facultatively anaerobic, rod-shaped bacteria that ferment lactose to produce acid. They produce β–galactosidase within 48 hours at 35 °C (Clesceri et al., 1998). Some coliforms are found in soil or vegetation, whereas fecal coliform are bacteria that usually live in the intestines of warm-blooded animals, making them a useful indicator of fecal contamination. Outside the intestines of warm-blooded animals, fecal coliform bacteria can survive for extended time periods. In karst aquifers, *E. coli* and other fecal coliform bacteria can remain viable for several months in water and stream sediments (Davis et al., 2005) and can be transported several kilometers in karst aquifers (Green et al., 1990). Lower temperatures found in cave environments reduce bacterial metabolism and increase their life span, allowing them to survive in these less-than-optimum conditions (Davis et al., 2005).

Coliform bacteria can be indicators for potential pathogens responsible for various waterborne diseases (Hunter et al., 2004) or be pathogens themselves, such as certain strains of *E. coli*. Coliform contamination can occur in caves (e.g. Lerch et al., 2001; Hunter et al., 2004; Kelly et al., 2009), with probable sources being the

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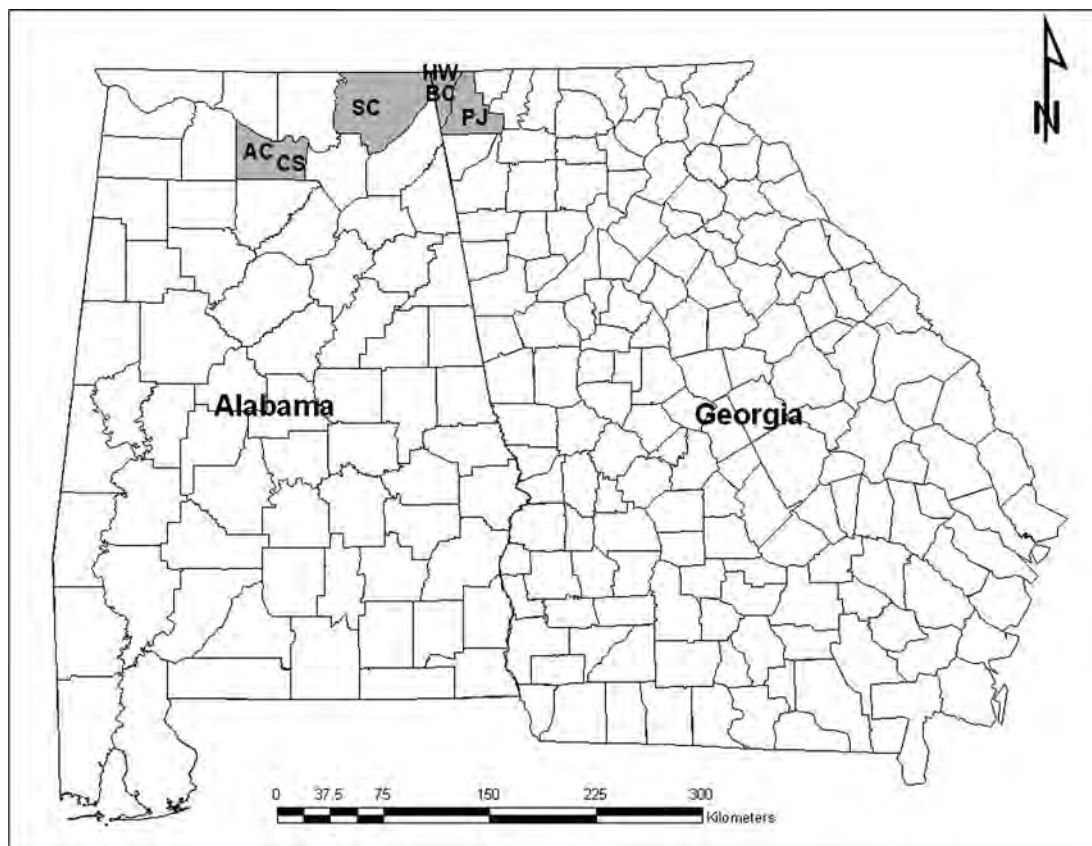
Figure 1. Approximate locations of the six caves in our study. AC=Anvil Cave, BC=Byers Cave, CS=Cave Springs Cave, HW=Howard's Waterfall Cave, PJ=Pettyjohns Cave, SC=Sauta Cave. Counties in which these caves are located have been shaded.

watershed or animals that excrete waste products within the cave. Coliform and environmental bacteria can enter a cave by water that drains into a cave from the watershed (Lerch et al., 2001; Kelly et al., 2009), primarily from a stream flowing directly into a cave or by seepage through soil or rock. Bats and other animals can also be major contributors of coliforms by excreting waste products within a cave ecosystem. Human visitors are another possible source of coliform bacteria within a cave ecosystem. Humans can bring bacteria into caves on the soles of their shoes and by dumping feces (Hunter et al., 2004). Some English caves showed contamination of certain bacterial species after cavers passed through (Moore and Sullivan, 1997, p. 80). Northup et al. (1997) found that cave areas frequently traversed by humans contained more bacterial species than less-frequented areas. Therefore, the recreational use of caves and human-induced land-use changes could lead to increases in coliform bacteria to levels considered a public health threat, while also affecting the natural bacterial assemblages in ways that are not yet understood.

In this study we tested water pools in six caves from northern Alabama and northwestern Georgia for abundance and species richness of coliform and other environ-

mental bacteria. We hypothesized that caves with more human or animal visitors or in a watershed with agricultural or sewage runoff would have higher bacterial abundance and species richness. We also hypothesized that abundance of coliforms and other bacteria would be greater closer to the entrance.

METHODS

STUDY SITES

Between January and July 2008, water samples were gathered and tested from six caves in northern Alabama (Anvil and Cave Springs Caves, Morgan Country; Sauta Cave, Jackson County) and northwestern Georgia (Byers and Howard's Waterfall Cave, Dade County; Pettyjohns Cave, Walker County) (Fig. 1). These six caves were chosen due to their length and accessibility, the presence of pools, and the different numbers of human and animal visitors at the caves. All of our collection sites are in horizontal caves in fossiliferous limestone.

SAMPLING PROCEDURE

Within each cave we aseptically took water samples from locations three or four different distances from the

entrance. At each location we took two 100 ml samples, generally from each of five cave pools. The pools had surface areas of 100 cm^2 to 1000 cm^2 and were separated by at least 1 meter. To avoid spikes of re-suspended bacteria from soil during storms, selected cave pools were not connected to any stream or runoff area and appeared to be supplied primarily by dripping stalactites. The selected pools rarely, if ever, become dry. Sauta and Cave Springs Caves contain streams that flow from their entrances. If isolated cave pools could not be found, stagnant areas of the creeks were sampled.

One water sample from each cave pool was stored at 2 to 4 °C for future cultures, and a second undiluted sample was poured and sealed into a Colisure quanti-tray (IDEXX Laboratories, Inc.) and placed in an incubator at 35 °C for up to 48 hours. Enzyme-based methods are widely accepted as a standard for detecting E. coli and other coliforms in water (Olstadt et al., 2007). Colisure products are approved by the Environmental Protection Agency for testing of drinking water and groundwater for total coliform bacteria and E. coli and are able to detect E. coli and other coliforms at one organism (up to 2419.6) per 100 ml (U.S. EPA, 1994; U.S. EPA, 1997; U.S. EPA, 1999, p. V-12–V-13). Coliforms produce the enzyme β-galactosidase, which is detected by cleaving Colisure's nutrient-indicator CPRG (chlorophenol red β-D-galactopranoside). The cleaving causes the water sample to change color over a 24 to 48 hour period from yellow to red or magenta, and E. coli causes the sample to fluoresce green when exposed to UV light because E. coli metabolizes the nutrient indicator MUG (4-methyllumbelliferyl b-D-glucuronide). Colisure products detect E. coli and other coliforms with high confidence and suppress the growth of galactosidase-producing non-coliform organisms, thus resulting in a low rate of false positives (Olstadt et al., 2007). Our abundance tests are presumptive, but we did culture E. coli from all water samples, thus confirming the presence of E. coli.

BACTERIAL IDENTIFICATION PROCEDURE

A subsample (10 μl) of the water that had been stored at 2 to 4 °C was plated on MacConkey agar, a selective and differential plating medium used for isolating and differentiating lactose-fermenting from non-lactose fermenting gram-negative bacteria (BBL MacConkey II Agar). The cultures were incubated at 37 °C for ~48 hours. If no growth was present after 48 hours, pool water (10 μl) was inoculated into tryptic soy broth, incubated overnight and then plated back onto MacConkey agar. Any bacterial colonies that appeared morphologically (color, shape, or size) different were transferred to tryptic soy agar with 5% sheep blood. Qualitative oxidase and indole tests were completed using BBL DrySlide products. Bacteria that were oxidase negative and indole positive were again streaked on MacConkey and Simmon's Citrate media. MacConkey plates that were lactose positive with a strong precipitate around the colony and Simmon's Citrate

negative were determined to be E. coli. If MacConkey plates were lactose + with no precipitate or lactose − and the Simmon's Citrate was + or −, the bacteria were placed on a Trek Sensititre System GN ID Panel. A 0.5 McFarland suspension of the isolate was prepared in sterile distilled water and inoculated into the GN ID Panel. If a bacteria was not identified by the Trek Sensititre System, the bacteria was placed on a Bio Merieux API 20E or Remel Rap ID NF+ System for identification. All procedures followed standard methods (Clesceri et al., 1998).

Abundance and species richness data were analyzed with GLM (Statistix 9 program, Analytical Software, Tallahassee, Florida) to conduct one-way ANOVAs with distance intervals as the independent variable and coliform bacteria and E. coli abundances and total number of bacterial species cultured as dependent variables. The Tukey's multiple comparison procedure in the same program was used to determine differences in relative abundances of E. coli and other coliform bacteria and bacterial species among caves. Due to differences in location and number of water pools among caves, locations were assigned to three zones, the entrance zone 0–75 m, the intermediate zone 75–125 m, and the deep zone >125 m, for 166 bacterial abundances and species richness analysis.

RESULTS

We collected ten coliform and forty environmental bacterial species from the six caves, with at least twenty-seven of these species considered to be potentially pathogenic. Most bacterial species were isolated from Pettyjohns Cave, followed by Sauta and Cave Spring Caves (Table 1). There was little consistency in species occurrence between caves, with thirty-three species isolated from only one cave (Table 1). E. coli was the only species isolated from all caves. Cave Springs Cave had significantly higher E. coli and other coliform abundance compared to all other caves except Sauta Cave (Fig. 2). Neither species richness ($P = 0.8102$) or abundance ($P = 0.2572$) of E. coli and other coliform were significantly different among distance intervals when 163 data from all caves were pooled into different zones. However, many differences were detected between various distance zones within individual caves (Table 2). Because we did not dilute our water samples, some of our bacteria abundance numbers are conservative. Due to the heterogeneity and availability of water pools within caves, results will be discussed separately for each cave. The forty environmental bacteria species were not targeted and considered incidental in our cultures. Therefore, our species richness numbers do not reflect the true species richness of each cave.

ANVIL CAVE

E. coli and other coliform bacteria were significantly higher within the entrance zone compared to all other

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Table 1. Bacteria species isolated from six caves located in North Alabama and Northwest Georgia. *Escherichia coli* is the only species cultivated from all caves.

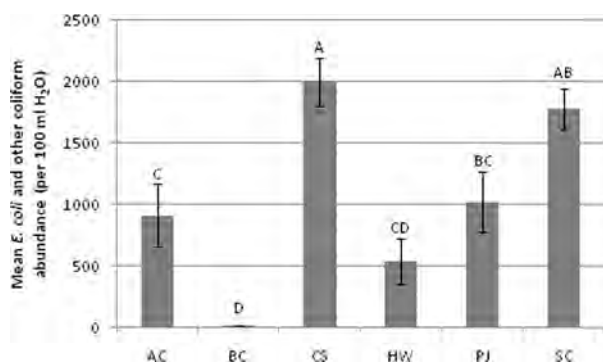| Bacteria Species	Anvil Cave	Byers Cave	Cave Springs	Howard's Waterfall	Pettyjohns Cave	Sauta Cave
Coliform Bacteria						
Citrobacter freundii	X				X	X
Enterobacter amnigenus	X					
Enterobacter cancerogenus				X	X	
Enterobacter intermedius	X					
Enterobacter sp.	X				X	
Escherichia coli	X	X	X	X	X	X
Klebsiella ozaenae					X	
Klebsiella oxytoca					X	
Klebsiella pneumo ss pneumoniae					X	
Pantoea agglomerans				X	X	X
Environmental Bacteria						
Acinetobacter baumannii					X	
Acinetobacter lwoffi			X		X	
Aeromonas caviae				X	X	X
Aeromonas hydrophilia		X			X	X
Aeromonas sp.						X
Aeromonas sobria						X
Bacillus sp. 1						X
Bacillus sp. 2		X	X			X
Brevundimonas diminuta					X	
Chromobacterium sp.						X
Chryseobacterium meningosepticum			X			
Comamonas testosterone					X	
Empedobacter brevis			X			
Hafnia alvei	X				X	
Kluyvera cryocrescens				X		
Listonella (Vibrio) anguillarum				X	X	
Micrococcus sp.			X			
Moraxella osloensis	X					
Myroides odoratum			X			
Ochrobactrum anthropi	X					
Proteus mirabilis			X			
Pseudomonas aeruginosa				X		
Pseudomonas aeruginosa/putida				X		
Pseudomonas alcaligenes	X	X	X	X	X	
Pseudomonas flourescens		X	X		X	X
Pseudomonas flourescens/putida		X	X			X
Pseudomonas mendocina			X			X
Pseudomonas pseudoalcaligenes						X
Pseudomonas stutzeri		X			X	X
Pseudomonas sp.			X	X	X	X
Psychrobacter phenylpyruvica		X				
Serratia fonticola						X
Serratia marcescens					X	
Serratia plymuthica					X	
Serratia proteamaculans					X	
Shewanella putrefaciens						X
Sphingomonas paucimobilis						X
Stentrophomonas maltophilia					X	
Vibrio alginolyticus	X					
Vibrio fluvialis	X					

Figure 2. Mean total abundance (+/−SE) (per 100 ml of H₂O) of *E. coli* and other coliform from six caves located in north Alabama and northwest Georgia. Columns with the same letter(s) are not significantly different at $P \le 0.05$ according to Tukey's multiple comparison procedure. AC=Anvil Cave, BC=Byers Cave, CS=Cave Springs Cave, HW=Howard's Waterfall Cave, PJ=Pettyjohns Cave, SC=Sauta Cave.

distance zones, and the intermediate zone was significantly higher in coliforms than the deep zone. We collected eleven species of bacteria from Anvil Cave (Table 1), seven of which were isolated from the entrance zone. Two *Vibrio* species (*V. alginolyticus* and *V. fluvialis*) were isolated from Anvil Cave. They are common in estuarine and marine environments (Roberts et al., 1982), but can be causative agents of diseases in humans (West, 1989).

Byers Cave

Although not significantly different, *E. coli* and other coliforms were higher in abundance in the deep zone compared to the entrance zone (Table 2). Only eight bacteria species were isolated from Byers (Table 1) and, overall, *E. coli* and other coliforms were found in very low abundance compared to all other caves.

Cave Springs Cave

E. coli and other coliform abundance were significantly higher in the deep zone compared to the entrance zone (Table 2). Thirteen species of bacteria were isolated from Cave Springs Cave (Table 1). Within the intermediate zone, a *Bipolaris* spp. (dematiaceous fungus) was isolated, several species of which are considered to be a potential human pathogens.

Howard's Waterfall Cave

E. coli and other coliforms were higher in abundance in the entrance zone compared to the deep zone, but the difference was not significant (Table 2). We collected ten species of bacteria from Howard's Waterfall Cave (Table 1). Despite low abundance of coliforms within the deep zone, seven species of bacteria were isolated there, including *Listonella* (*Vibrio*) *anguillarum. L. anguillarum*

is primarily isolated from marine environments, but has on occasion been isolated from freshwater environments (Chen et al., 2006). It causes fish disease worldwide (Powell and Loutit, 1994).

Pettyjohns Cave

E. coli and other bacteria were found throughout our sampling transect. No significant differences in abundance were noted between the entrance and deep zone (Table 2). We collected twenty-four species of bacteria from Pettyjohns Cave (Table 1). *Listonella* (*Vibrio*) *anguillarum* was also isolated from the entrance zone of this cave.

Sauta Cave

E. coli and other coliform bacteria were not significantly different among the distance zones (Table 2). However, *E. coli* abundance did increase at deeper distance intervals. Nineteen species were collected within Sauta Cave (Table 1). One species collected, *Shewanella putrefaciens,* is a common environmental isolate that composes biofilms (McLeod et al., 2002) and can use multiple electron acceptors (including manganese) for aerobic respiration (Myers and Myers, 2001), which may allow for its survival in cave ecosystems.

Discussion

A great deal of heterogeneity existed among caves, such as location and separation of water pools. Although distance from an entrance is a variable that can influence bacterial abundance (as observed in Anvil Cave), other variables appear to be more important in controlling abundance and species richness of coliform bacteria in cave ecosystems. Outside influences, such as number of human and bat visitors and watershed differences, differed among caves, which probably accounted for the lack of correlation between *E. coli* and other bacteria abundances and species richness and distance from an entrance. Abundance levels of *E.coli* and other coliform bacteria fluctuated widely among water pools in the same distance zone of some caves, suggesting that isolated pools are not necessarily influenced by other nearby pools, and bacterial presence may depend more heavily on a direct source such as human or animal visitors rather than an indirect source such as watershed contamination.

The isolation of many potentially pathogenic bacteria from these cave waters suggests that these species are able to remain viable in a low metabolic state. *E. coli* has been shown to survive longer in soil kept at low temperatures (Teague et al., 1995), and Flint (1987) found *E. coli* surviving for extended periods of time in cold river water. Some of these coliforms and environmentals may be adhering to biofilms on the substrate, which could help sustain the species in cave environments. Epilithic biofilms have been shown to be an important energy source for aquatic cave communities (Simon et al., 2003). Biofilms

Table 2. Mean *E. coli* and other coliform abundance (+/−SE) (per 100 ml H$_2$O) from each cave and the various distance zones within each cave.

Cave	Entrance Zone	Intermediate Zone	Deep Zone
Anvil Cave	2419.6 (0)[a]	1035.8 (564.5)[b]	100.68 (52.3)[c]
Byers Cave	6.76 (3.1)[a]	NP	23.87 (7.8)[a]
Cave Springs Cave	1065.5 (553.1)[a]	2099 (320.7)[ab]	2419.6 (0)[b]
Howard's Waterfall Cave	823.86 (352.5)[a]	NP	253.3 (68.8)[a]
Pettyjohns Cave	1108.6 (336.3)[a]	NP	939.25 (361.8)[a]
Sauta Cave	1758.5 (298.9)[a]	1813 (173.3)[a]	1780.5 (288.4)[a]

Note: Within each cave means of various distance zones followed by the same letter(s) are not significantly different ($P \leqslant 0.05$) according the Tukey's multiple comparison procedure.
NP = No pools present.
Lower case letters indicate which caves have differences in bacterial abundance among the three zones.

can also trap nutrients (Costerton and Lappin-Scott, 1989), which can increase the survival of non-endemic bacteria (Boyle et al., 1991).

Although our cultivation techniques favored cultivation of coliform bacteria, we also isolated forty environmental chemoheterotrophic bacterial species. Our study highly underestimated environmental bacteria, because our cultivation techniques targeted coliform bacteria, and our result is not representative of the true bacterial species diversity. Many cave bacteria species may have been inhibited by our media or inoculation temperature, which do not mimic a cave environment. According to McNamara and Mitchell (2005), less than 10% of bacteria ($< 1\%$ in many instances) from an environmental sample can be cultivated by standard laboratory techniques.

Each cave illustrated unique physical differences that may help explain differences in abundance and species of bacteria. Anvil Cave, a maze cave, has over 20 km (13 miles) of passages and several entrances and receives few human visitors. Most human visitors and other animals will necessarily pass through an entrance zone, but they may become dispersed in the mazes beyond these zones, which could account for the higher *E. coli* and other coliform abundances and species richness within the first distance zone. Anvil Cave is, however, located primarily underneath pasture land that could be a source of bacteria.

Byers Cave, which is one of Georgia's largest caves, contains over 8 km (5 miles) of passages and contains a waterfall, and many pools are present within the deep zone, here > 400 m from the entrance. Byers Cave is not heavily trafficked by humans, and water seepage from the general watershed is a probable source for the bacteria in these distance zones. Byers Cave is located within a pristine wooded area that is far from any human development, which could account for the overall low abundance and species richness of bacteria.

Cave Springs and Sauta Caves both have large openings and contain streams roughly 3 to 5 meters wide that slowly drain towards the entrance. Large colonies of endangered gray bats (*Myotis grisescens*) inhabit Cave Springs and Sauta Caves, with maternity colonies formed during spring and summer. These caves are closed to the public and get only an occasional human visitor. Total organic matter in soil has been shown to increase in areas with larger numbers of bats in Cave Springs and Sauta Caves (Joshua Campbell et al., unpubl. data), which probably accounted for the high abundance of *E. coli* and other coliforms.

Howard's Waterfall Cave is a horizontal cave that is over 3 km long (2 miles long) and receives approximately 1000 human visitors per year, with most only exploring the first part of the cave, which could account for the increased abundance of *E. coli* and other coliforms within the entrance zone. However, watershed sources and other animals are also possible sources for the increased abundance of *E. coli* and other coliforms from the entrance zone. The higher number of species isolated from the deep zone (including *Listonella (Vibrio) anguillarum*) is an interesting result and may suggest a watershed source of bacteria, even though overall coliform and other bacteria abundance was low in this zone.

Pettyjohns Cave has over 10.5 km (6.5 miles) of passages and is a popular cave that draws approximately 10,000 visitors per year (Padgett, 1999). The majority of human visitors only explore near the entrance, which could explain the high numbers of bacteria within the entrance zone. In one area 300 to 325 meters in, a spike in *E. coli* and other coliforms was observed. Here the cave narrows, which forces any cavers to pass through any pools present, suggesting that the high number of human visitors could be a culprit in these spikes. Although the high number of species is likely due to the high number of people that visit this cave, watershed sources and other animals could also be contributing factors. Neither Howard's Waterfall nor Pettyjohns Caves contain fish, which suggests *Listonella (Vibrio) anguillarum* entered from the watershed through the aquifer, rather than by way of possibly diseased fish.

CONCLUSIONS

Due to the heterogeneity of these caves, general patterns can be difficult to discern. However, bacterial abundance and species richness in caves can be influenced by humans,

other animals such as bats, and watershed uses. Cave bacteria can be used as bioindicators of water quality, and high abundance could be an ecosystem warning signal. High levels of bacteria abundance, especially coliforms, could also be a human health concern. Humans could come into direct contact with contaminated pools by caving and indirectly through water wells. Overall, we found widespread fecal contamination in all caves, and people with scrapes or cuts should use caution while caving. Drinking directly from pools without filtration should be avoided at all times. Many organisms that live in caves are very sensitive to ecosystem disturbances and may have limited populations and slow reproduction times. How increases in non-native bacterial species affect cave macroinvertebrates and other animals should be investigated.

References

Boyle, M., Ford, T., Maki, J.S., and Mitchell, R., 1991, Biofilms and the survival of opportunistic pathogens in recycled water: Waste Management and Research, v. 9, p. 465–470, doi: 10.1177/0734242X9100900165.

Caumartin, V., 1963, Review of the microbiology of underground environments: Bulletin of the National Speleological Society, v. 25, p. 1–14.

Chen, C.Y., Chao, C.B., and Bowser, P.R., 2006, Infection of tilapia Oreochromis sp. by Vibrio vulnificus in fresh water and low-salinity environments: Journal of the World Aquaculture Society, v. 37, p. 82–88, doi: 10.1111/j.1749-7345.2006.00010.x.

Clesceri, L.S., Greenberg, A.E., and Eaton, A.D., eds., 1998, Standard Methods for the Examination of Water and Wastewater, 20th ed., APHA, Washington, D.C., 1325 p.

Costerton, J.W., and Lappin-Scott, H.M., 1989, Behavior of bacteria in biofilms: American Society for Microbiology News, v. 55, p. 650–654.

Culver, D.C., 1970, Analysis of simple cave communities I. Cave as islands: Evolution, v. 24, p. 463–474.

Culver, D.C., Deharveng, L., Bedos, A., Lewis, J.J., Madden, M., Reddell, J.R., Sket, B., Trontelj, P., and White, D., 2006, The mid-latitude biodiversity ridge in terrestrial cave fauna: Ecography, v. 29, p. 120–128, doi: 10.1111/j.2005.0906-7590.04435.x.

Davis, R.K., Hamilton, S., and Van Brahana, J., 2005, Escherichia coli survival in mantled karst springs and streams, northwest Arkansas Ozarks, USA: Journal of the American Water Resources Association, v. 41, p. 1279–1287, doi: 10.1111/j.752-1688.2005.tb03800.x.

Flint, K.P., 1987, The long-term survival of Escherichia coli in river water: Journal of Applied Bacteriology, v. 63, p. 261–270, doi: 10.1111/j.1365-2672.1987.tb04945.x.

Graening, G.O., and Brown, A.V., 2003, Ecosystem dynamics and pollution effects in an Ozark cave stream: Journal of the American Water Resources Association, v. 39, p. 1497–1507, doi: 10.1111/j.1752-1688.2003.tb04434.x.

Green, W., Ellicott, L., and Crawford, N., 1990, Investigation of nonpoint source pollution associated with karst aquifer systems: Transactions of the Kentucky Academy of Science, v. 51, p. 177–181.

Groth, I., and Saiz-Jimenez, C., 1999, Actinomycetes in hypogean environments: Geomicrobiology Journal, v. 16, p. 1–8.

Hunter, A.J., Northup, D.E., Dahm, C.N., and Boston, P.J., 2004, Persistent coliform contamination in Lechuguilla cave pools: Journal of Cave and Karst Studies, v. 66, p. 102–110.

Kelly, W.R., Panno, S.V., Hackley, K.C., Martinsek, A.T., Krapac, I.G., Weibel, C.P., and Storment, E.C., 2009, Bacteria contamination of groundwater in a mixed land-use karst region: Water Quality Exposure and Health, v. 1, p. 69–78, doi: 10.1007/s12403-009-0006-7.

Laiz, L., Groth, I., Schumann, P., Zezza, F., Felske, A., Hermosin, B., and Saiz-Jimenez, C., 2000, Microbiology of the stalactites from Grotta dei Cervi, Porto Badisco, Italy: International Microbiology, v. 3, p. 25–30.

Lerch, R.N., Erickson, J.M., and Wicks, C.M., 2001, Intensive water quality monitoring in two karst watersheds of Boone County, Missouri, in Rea, G.T., ed., Proceedings of the 2001 National Cave and Karst Management Symposium, Tucson, Arizona, Oct. 16–19, 2001, Tucson, Coronado National Forest, p. 151–168.

McLeod, E.S., MacDonald, R., and Brözel, V.S., 2002, Distribution of Shewanella putrefaciens and Desulfovibrio vulgaris in sulphidogenic biofilms of industial cooling water system determined by fluorescent in situ hybridization: Water SA, v. 28, p. 123–128.

McNamara, C.J., and Mitchell, R., 2005, Microbial deterioration of historic stone: Frontiers in Ecology and the Environment, v. 3, p. 445–451, doi: 10.1890/1540-9295(2005)003[0445:MDOHS]2.0.CO;2.

Moore, G.W., and Sullivan, G.N., 1997, Speleology: Caves and the Cave Environment, third edition: St. Louis, Cave Books, 176 p.

Myers, J.M., and Myers, C.R., 2001, Role for outer membrane cytochromes OmcA and OmcB of Shewanella putrefaciens MR-1 in reduction of manganese dioxide: Applied and Environmental Microbiology, v. 67, p. 260–269, doi: 10.1128/AEM.67.1.260-269.2001.

Northup, D.E., Beck, K.M., and Mallory, L.M., 1997, Human impact on the microbial communities of Lechuguilla cave: Is protection possible during active exploration?, abs., National Speleology Society Convention Program, Sullivan, Missouri, 55 p.

Northup, D.E., and Lavoie, K.H., 2001, Geomicrobiology of caves: A review: Geomicrobiology Journal, v. 18, p. 199–222, doi:10.1080/01490450152467750.

Olstadt, J., Schauer, J.J., Standridge, J., and Kluender, S., 2007, A comparison of ten USEPA approved total coliform/E. coli tests: Journal of Water and Health, v. 5, p. 267–282, doi: 10.2166/wh.2007.008.

Padgett, A., 1999, Pigeon Mountain: Thirty years of noninterference, in Rea, G.T., ed., Proceedings of the 1999 National Cave and Karst Management Symposium, Chatanooga, Tennessee, Southeastern Cave Conservancy, p. 143–147.

Peck, S.B., 1976, The effect of cave entrances on the distribution of cave-inhabiting terrestrial arthropods: International Journal of Speleology, v. 8, p. 309–321.

Poulson, T.L., and White, W.B., 1969, The cave environment: Science, v. 165, p. 971–981.

Powell, J.L., and Loutit, M.W., 1994, The detection of fish pathogen Vibrio anguillarum in water and fish using a species-specific DNA probe combined with membrane filtration: Microbial Ecology, v. 28, p. 375–383, doi:10.1007/BF00662030.

Roberts, N.C., Siebeling, R.J., Kaper, J.B., and Bradford, Jr., H.B., 1982, Vibrios in the Louisiana coast environment: Microbial Ecology, v. 8, p. 299–312, doi: 10.1007/BF02010670.

Simon, K.S., Benfield, E.F., and Macko, S.A., 2003, Food web structure and the role of epilithic biofilms in cave streams: Ecology, v. 84, p. 2395–2406.

Taylor, S.J., Krejca, J.K., and Denight, M.L., 2005, Foraging range and habitat use of Ceuthophilus secretus (Orthoptera: Rhaphidophoridae),

a key trogloxene in central Texas cave communities: American Midland Naturalist, v. 154, p. 97–114.

Teague, K.A., Wolf, D.C., and Vendrell, P.F., 1995, Survival of fecal contamination indicator organisms in soil: Arkansas Water Resources Center, Publication No. 173, 28 p.

U.S. EPA, 1994, National Primary and Secondary Drinking Water Regulations: Analytical Methods for Regulated Drinking Water Contaminants: *Federal Register*, 5 December 1994, 40 *Code of Federal Regulations* parts 141, 143 p.

U.S. EPA, 1997, Manual for the Certification of Laboratories Analyzing Drinking Water: Criteria and Procedures, Quality Assurance, fourth edition: Environmental Protection Agency document 815-B-97-001, 186 p.

U.S. EPA, 1999, National Primary and Secondary Drinking Water Regulations: Analytical Methods for Chemical and Microbiological Contaminants and Revisions to Laboratory Certification Requirements: Final Rule: *Federal Register*, 1 December 1999, p. 67449–67467, 40 *Code of Federal Regulations* parts 141, 143 p.

West, P.A., 1989, The human pathogenic vibrios—A public health update with environmental perspectives: Epidemiology and Infection, v. 103, p. 1–34, doi: 10.1017/S0950268800030326.

SPATIAL AND TEMPORAL DISTRIBUTION OF PROTOZOA AT CUEVA DE LOS RISCOS, QUERÉTARO, MÉXICO

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Abstract: Protozoa are important members of ecosystems, but protozoa that inhabit caves are poorly known worldwide. In this work, we present data on the record and distribution of thirteen protozoa species in four underground biotopes (water, soil, bat guano, and moss), at Cueva de Los Riscos. The samples were taken in six different months over more than a year. Protozoa species were ciliates (eight species), flagellates (three species), amoeboid (one species), and heliozoan (one species). Five of these species are reported for the first time inside cave systems anywhere, and an additional three species are new records for Mexican caves. *Colpoda* was the ciliate genera found in all cave zones sampled, and it inhabited the four biotopes together with *Vorticella*. The biotopes with the highest specific richness were the moss, sampled near the main cave entrance, and the temporary or permanent water bodies, with ten species each. The greatest number of species was observed in April 2006 (dry season). With the exception of water, all biotopes are studied for the first time.

Introduction

A great extent of Mexican territory is formed by sedimentary rocks that permit the formation of caves, but the number, location, and biodiversity of Mexican caves is only partially known. Records of subterranean protozoan taxa in Mexico are scarce, and refer only to caves in San Luis Potosí and Guerrero states (Osorio-Tafall, 1943; Hoffmann et al., 1986, p. 206–207), in which thirty species of sarcodines and ciliates have been reported.

There are records of nine protozoa species from caves in North America. In Bermuda, Hill et al. (1986) recorded *Euplotes iliffei* in subterranean anchialine habitats and Small et al. (1986) recorded *Glauconema bermudense* in marine caves. Holsinger (1966) found *Paramecium multimicronucleatum* and *Spirostomum ambiguum* in Virginia, and Barr (1968) reported *Phacus* sp., *Paramecium* sp., *Halteria* sp., *Difflugia* sp., and *Peranema* sp. as free-living inhabitants of the Mammoth Cave system in Kentucky.

Protozoa are cosmopolitan and tolerate a wide range of physicochemical factors, including pH, temperature, oxygen concentration, and salinity. They are not randomly distributed, but live in microhabitats, small regions that may be as tiny as a few cubic centimeters, within a body of water or a moist environment such as soil, vegetation, or the bodies of plants and animals (Bamforth, 1985). They occupy many different biotopes, in the sense of Olenin and Ducrotoy (2006).

According to Hoffmann et al. (1986, chap. III), roots, leaf and animal debris, and the guano of bats provide the primary energy sources in open system caves. Indeed, guano is considered the most important biotope, but water, soil, and moss also play important roles as sustainable habitats for diverse communities of microorganisms and metazoan taxa.

The protozoan trophozoite or cyst phase enters caves in water flow or infiltration through soil, in air currents, and by troglophile fauna present in the cave (Golemansky and Bonnet, 1994) and accidental or trogloxene organisms. The aim of this work is to record and analyze protozoan spatial and temporal distribution associated with different biotopes over more than a year at Cueva de Los Riscos.

Materials and Methods

Cueva de Los Riscos is located in Jalpan de Serra, Querétaro, Mexico, at 1122 m asl, 21°11′38″N, 99°30′50″W. It is a mixed underground system (horizontal and vertical) with a length of 440 m (Lazcano-Sahagún, 1986a, p. 32; 1986b, p. 77–79), with four zones (A–D) and four entrances (Fig. 1); a detailed cave description is in Espino del Castillo et al. (2009).

Six visits were made to Cueva de Los Riscos from November 2005 to June 2007 (Table 1). Samples, including water, soil, bat guano, and moss, were collected where available in four large zones in the cave, A, B, C, and D (Fig. 1); other areas in narrow tunnels were not sampled. Access to the cave was done without special equipment, but for biosafety we wore face masks with filters (Milter disposable 3M 8210 N95).

Water samples were collected by using sterile pipettes; guano and soil samples were obtained with sterilized metallic

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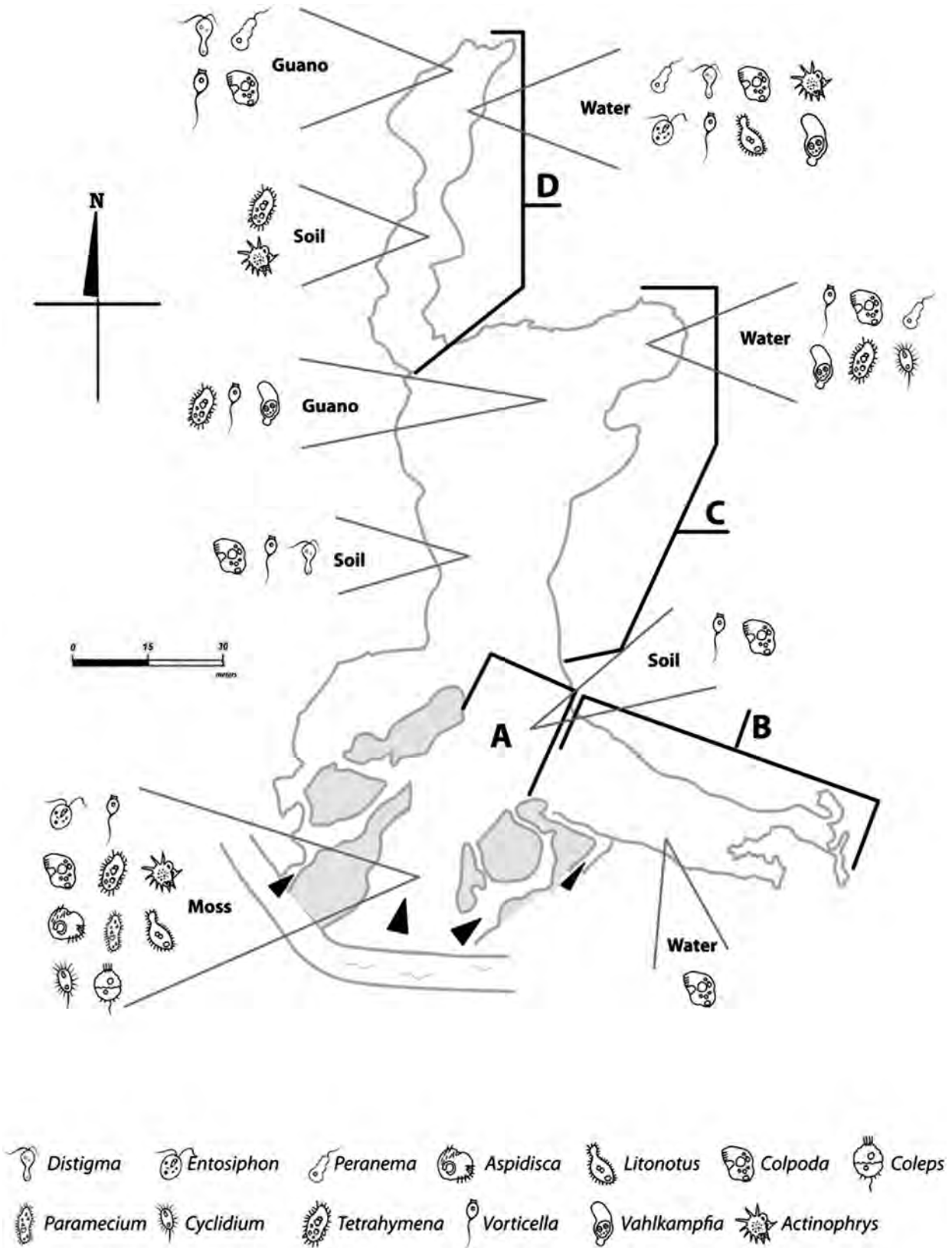
Figure 1. Cueva de Los Riscos map drawn by D. McKenzie of the Association for Mexican Cave Studies in 1966 (Lazcano Sahagún, 1986b, p. 79), modified by A. Espino del Castillo, M. Hernández, J.B. Morales-Malacara, and L. González of the Universidad Nacional Autónoma de México in March 2007, showing collecting places and species biodiversity at each biotope. Black arrow heads represent cave entrances.

Table 1. Distribution of protozoa genera recorded on each biotope during the study at Los Riscos Cave.

Zone	2005 Rainy Season November Genus	2005 Rainy Season November Biotopes	2006 Dry Season April Genus	2006 Dry Season April Biotopes	2006 Rainy Season June Genus	2006 Rainy Season June Biotopes	2006 Rainy Season October Genus	2006 Rainy Season October Biotopes	2007 Dry Season March Genus	2007 Dry Season March Biotopes	2007 Rainy Season June Genus	2007 Rainy Season June Biotopes
A	NC	NC	*Entosiphon*	Moss	…	…	…	…	…	…	…	…
A	NC	NC	*Vorticella*	Moss	…	…	…	…	*Entosiphon*	Moss	…	…
A	NC	NC	*Colpoda*	Moss	*Entosiphon*	Moss	…	…	*Vorticella*	Moss	*Entosiphon*	Moss
A	NC	NC	*Tetrahymena*	Moss	*Vorticella*	Moss	…	…	*Colpoda*	Moss	*Vorticella*	Moss
A	NC	NC	*Aspidisca*	Moss	*Colpoda*	Moss	*Vorticella*	Soil	*Litonotus*	Moss	*Colpoda*	Soil
A	NC	NC	*Paramecium*	Moss	*Cyclidium*	Moss	…	…	*Aspidisca*	Moss	*Tetrahymena*	Moss
A	NC	NC	*Cyclidium*	Moss	…	…	…	…	*Paramecium*	Moss	…	…
A	NC	NC	*Coleps*	Moss	…	…	…	…	*Cyclidium*	Moss	…	…
A	NC	NC	*Actinophrys*	Moss	…	…	…	…	…	…	…	…
B	NC	NC	NC	NC	*Colpoda*	Water	NC	NC	NC	NC	NC	NC
C	NC	NC	*Distigma*	Soil	…	…	*Vahlkampfia*	Water	*Vahlkampfia*	Guano	*Peranema*	Water
C	NC	NC	*Vorticella*	Soil, Water	*Vorticella*	Water	*Vorticella*	Water	*Vorticella*	Guano	*Tetrahymena*	Water
C	NC	NC	*Colpoda*	Soil	…	…	*Colpoda*	Water	*Tetrahymena*	Guano	*Cyclidium*	Water
C	NC	NC	…	…	…	…	*Tetrahymena*	Water	…	…	…	…
D	*Peranema*	Water, Guano	*Vorticella*	Water, Guano	*Peranema*	Guano	NA	NA	NA	NA	*Vahlkampfia*	Guano
D	*Distigma*	Water	*Colpoda*	Guano	*Distigma*	Guano	NA	NA	NA	NA	…	…
D	*Entosiphon*	Water	*Tetrahymena*	Soil	*Vorticella*	Water	NA	NA	NA	NA	…	…
D	*Vorticella*	Water, Guano	*Actinophrys*	Soil	…	…	NA	NA	NA	NA	…	…
D	*Colpoda*	Water	…	…	…	…	NA	NA	NA	NA	…	…
D	*Litonotus*	Water	…	…	…	…	NA	NA	NA	NA	…	…
D	*Actinophrys*	Water	…	…	…	…	NA	NA	NA	NA	…	…

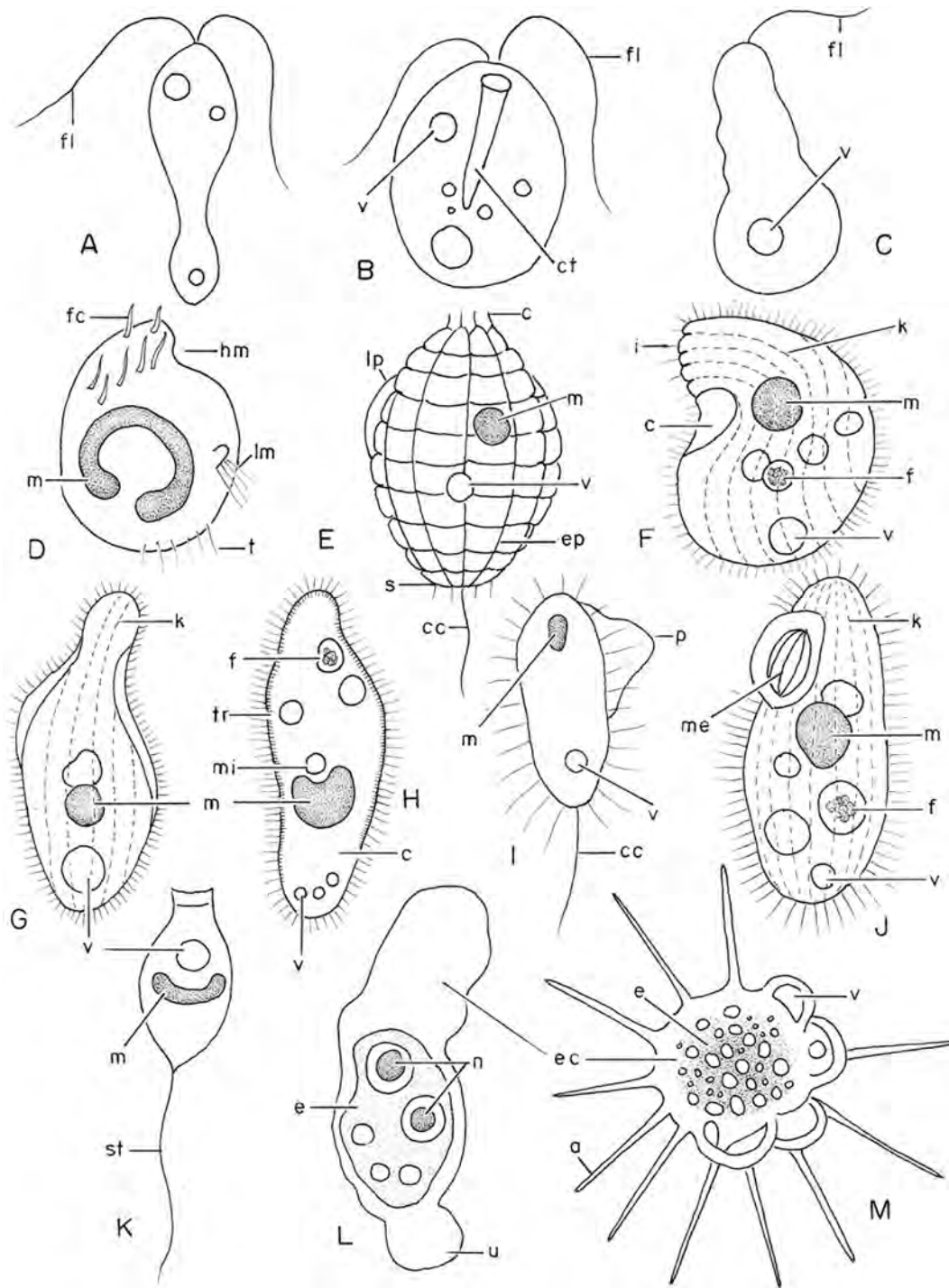Note: NC means no samples collected.
NA means no protozoa recorded.

Figure 2. Protozoa species biodiversity found at Cueva de Los Riscos. Data in parentheses correspond to length and width measurements in micrometers, except for *Actinophrys sol* which corresponds to diameter. A. *Distigma* sp. (8.0 × 2.5); B.*Entosiphon* sp. (20.0 × 12.0); C. *Peranema* sp. (17.0 × 4.0); D. *Aspidisca* sp. (40.0); E. *Coleps hirtus* (40.0 × 30); F. *Colpoda* sp. (36.0 × 30.0); G. *Litonotus* sp. (80.0 × 34.0); H. *Paramecium caudatum* (200.0 × 60.0); I. *Cyclidium* sp. (20.0 × 10.0); J. *Tetrahymena pyriformis* (35.0 × 24.0); K. *Vorticella* sp. (25.0 × 20.0); L. *Vahlkampfia* sp. (30.0 × 10.0); *Actinophrys sol* (25). Abbreviations: a = a×opodium; c = cytostome; cc = caudal cilium; ct = conical tubule; e = endoplasm; ec = ectoplasm; ep = ectoplasmatic plates; f = food vacuole; fc = frontoventral cirri; fl = flagellum; hm = higher adoral zone of membranelles; i = indentation; k = kinety; lm = lower adoral zone of membranelles; lp = lateral projection; m = macronucleus; me = membranelles; mi = micronucleus; n = nucleus; p = paroral membrane; s = spines; st = stalk; t = transversal cirri; tr = trichocysts; u = uroid; v = contractile vacuole.

spoons, and for moss we utilized forceps. Approximately 2 ml or 2 cc of each sample was placed in one sterilized Falcon tube containing 5 ml of nutritive sterile pea infusion (Patterson and Hedley, 1992, p.17–18). Additionally, 100 ml or 100 cc of each sample was collected in sterilized 100 ml glass jars. Moss samples were collected in plastic bags. All samples were transported in a cooler without ice, in order to maintain all samples in good condition.

All laboratory procedures started within 24 hours of collection. In all cases, to obtain polyxenic cultures (multiple species), 2 ml of each sample was added to natural infusions of straw, rice, wheat, pea, and corn (Lee et al. 1985; Jahn et al. 1979, p. 10–12) and chemical media (Chalkley, peptone, and RPMI-1640 Sigma) (Manwell, 1968, p. 559–572; Kudo, 1971, p. 848–852). The samples and cultures were maintained in the dark at 25 °C in an incubator, except for the moss samples, which were exposed to sunlight. To identify the protozoa and for a photomicrographic record, all the cultures were periodically examined by using phase-contrast, differential-inter-ference-contrast and bright-field microscopes (Nikon Labophot-2 with Nikon Digital Sight DS-2Mv and Nikon FX-35DX incorporated camera; Zeiss Axioskop 2 plus with Zeiss AxioCam MRC system). Diagnostic characters were studied with staining and impregnation techniques such as Harris hematoxylin, Klein, protargol, and butanol-nigrosine (Borror, 1969; Kudo, 1971, p. 863; Lee et al., 1985; Silva-Neto, 2000).

We utilized the Jaccard index to estimate the degree of similarity between biotopes with regard to genera. The similarity values obtained were summarized by clustering, using the UPGMA method (program NTSYS pc. v. 2.2, Exeter Software, Setauket, New York).

Results

We identified thirteen protozoa species, with three flagellates (*Entosiphon* sp., *Distigma* sp., *Peranema* sp.), one naked amoebae (*Vahlkampfia* sp.), one heliozoan (*Actinophrys sol* Ehrenberg), and eight ciliates [*Aspidisca* sp., *Litonotus* sp., *Colpoda* sp., *Coleps hirtus* (O. F. Müller), *Paramecium caudatum* Ehrenberg, *Cyclidium* sp., *Tetrahymena pyriformis* (Ehrenberg), and *Vorticella* sp.] (Table 1, Fig. 2). The protozoan distribution in relation to the cave zones and biotopes from all seasons is shown in Figure 1.

The species *Entosiphon* sp., *Distigma* sp., *Aspidisca* sp., *Litonotus* sp., and *Tetrahymena pyriformis* have not been recorded in caves anywhere in the world. The following species are new records for Mexican caves: *Actinophrys sol, Colpoda* sp., and *Paramecium caudatum* (Table 2).

The relative species richness among the cave biotopes, seasons, and zones, is shown in Figures 1 and 3. The most diverse biotopes were moss and water, and the soil was the least diverse (Table 1).

The greatest number of protozoan taxa was present in April 2006 (dry season), and the lowest was in October
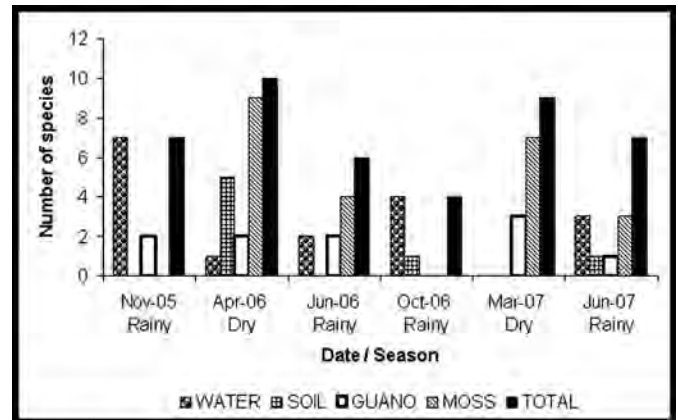

Figure 3. Number of protozoa species for each biotope and season at Cueva de Los Riscos. Note that some species appear in the totals for more than one biotope for each date.

(rainy season) of the same year, when flagellates were absent, with Zone A having the highest diversity

The genus *Colpoda* was found on all biotopes, zones, and periods. *Vorticella* sp. and *Tetrahymena pyriformis* were recorded in all biotopes and during all periods. The genus *Coleps* was recorded only in Zone A moss in April 2006.

Entosiphon was recorded in samples from five months (rainy and dry seasons), except for October 2006. The heliozoan *Actinophrys sol* was observed only in November 2005 (rainy season) and April 2006 (dry season). The amoeboid genus *Vahlkampfia* was recorded three times: October 2006 (rainy season), March 2007 (dry season), and June 2007 (rainy season).

In Zone B, we found only the genus *Colpoda*, which was observed in a draining water sample.

From the Jaccard similarity index two clusters were obtained; the first grouped the biotopes water, guano, and soil, and the second cluster included only the moss biotope. We obtained a similarity index of 0.6 between the water and guano biotopes, a similarity of 0.54 when both biotopes were compared with the soil, and a similarity of 0.38 when these three biotopes were compared with the moss.

Discussion

According to Corliss (2002), the protists are cosmopolitan in overall distribution, and, in particular, most protozoa play roles mainly as phagotrophs (particulate consumers). Free-living species have a very broad distribution as planktonic or benthic forms. Free-swimming flagellates and ciliates are the most important consumers of bacteria in aquatic and terrestrial ecosystems.

Considering the functional groups of protozoa in ecosystems proposed by Pratt and Cairns (1985), the species found in the biotopes at Cueva de Los Riscos

Table 2. Genera recorded in this study, with previous cave records, if any.

Previous Records				Los Riscos Cave[a]	Record
Europe	USA	México	References		
···	*Peranema* sp.	···	Barr, 1986	*Peranema* sp.	P
···	···	*Peranema* sp.	Hoffmann et al., 1986		
···	···	*Vahlkampfia* sp.	Hoffmann et al., 1986	*Vahlkampfia* sp.	P
V. limax	···	···	Golemansky and Bonnet, 1994		
V. magna	···	···	Golemansky and Bonnet, 1994		
V. tachipodia	···	···	Golemansky and Bonnet, 1994		
Cyclidium oligotrichum	···	···	Carey et al., 2001	*Cyclidium* sp.	P
C. fuscum	···	···	Carey et al., 2001		
C. glaucoma	···	···	Golemansky and Bonnet, 1994		
···	···	*Cyclidium* sp.	Hoffmann et al., 1986		
···	···	···			
···	···	*Tetrahymena* sp.	Hoffmann et al., 1986	*Tetrahymena pyriformis*	P
···	···	*Vorticella* sp.	Hoffmann et al., 1986	*Vorticella* sp.	P
···	···	*Vorticella* sp. cf. *microstoma*	Osorio-Tafall, 1943		
Colpoda sp.	···	···	Barr, 1986	*Colpoda* sp.	M
C. steinii	···	···	Golemansky and Bonnet, 1994		
Paramecium caudatum	···	···	Golemansky and Bonnet, 1994	*Paramecium caudatum*	M
···	*Paramecium* sp.	···	Barr, 1986		
···	*P. micromultinucleatum*	···	Holsinger, 1966		
···	···	*P. aurelia*	Hoffmann et al., 1986		
Actinophrys sol	···	···	Golemansky and Bonnet, 1994	*Actinophrys sol*	M
A. vesiculata	···	···	Golemansky and Bonnet, 1994		
···	···	*Actinophrys* sp.	Osorio-Tafall, 1943		
···	···	*Coleps* sp.	Hoffmann et al., 1986	*Coleps hirtus*	W
···	···	*Coleps* sp. cf. *hirtus*	Osorio-Tafall, 1943		
···	···	···	···	*Entosiphon* sp.	W
···	···	···	···	*Distigma* sp.	W
···	···	···	···	*Aspidisca* sp.	W
···	···	···	···	*Litonotus* sp.	W

[a] This study.
W—New world and Mexican record for caves.
M—New Mexican record for caves.
P—Previously recorded in Mexican caves.

correspond to bactivores-detritivores (*Aspidisca* sp., *Colpoda* sp., *Coleps hirtus*, *Cyclidium* sp., *Litonotus* sp., *Paramecium caudatum*, *Tetrahymena pyriformis*, *Vorticella* sp., *Entosiphon* sp. and *Peranema* sp.), saprotophs (*Distigma* sp.), and non-selective omnivores (*Actinophrys sol*). Foissner and Berger (1996) reported some species of *Litonotus* as predators. The main functional role of substrate-associated protozoa is the processing of dead organic matter and its associated bacterial flora (Pratt and Cairns, 1985).

Members of genera *Coleps, Vorticella, Tetrahymena* and *Paramecium* are very common in many ecosystems and have been previously reported in some cave biotopes (Table 2). Previous records of Mexican cave protozoa include species that were observed only in water samples, in contrast to our findings from multiple biotopes. As shown in Figure 1, *Vorticella* sp., a detritivorous genus, was recorded in all biotopes of the cave; therefore we consider this species as having a broad distribution.

The flagellates are typically smaller, but much more abundant than ciliates and often mixotrophic in their nutrition; they occupy both planktonic and benthic levels (Corliss, 2002). We found the genera *Entosiphon, Distigma*, and *Peranema* as benthic organisms.

The samples collected during October 2006 (rainy season) had the lowest diversity, with only four genera, probably because of the excessive water flow in the cave, as compared with April and May, that probably washed out protozoan populations. However, some puddles remained as propitious microhabitats for the development of protozoan communities and other organisms like small metazoans.

For the water biotope, we observed the highest species richness during November 2005 and October 2006 (rainy season). For the soil biotope we found the highest number of species during the spring months; however samples were not obtained for all collections during this period. The bat guano biotope presented only slight differences in species richness among seasons (Figure 3). April 2006 and March 2007 (dry season) had the highest number of species inhabiting the moss substrate.

We conclude for this cave system that the spring months, corresponding to the dry season, have the highest protozoan diversity.

According to Finlay et al. (1998), the same ciliate species are found wherever their preferred habitat is found. Free-living ciliates may be ubiquitous, as they are continually being distributed by effective passive dispersal; these statements can be taken into account when we analyze the protozoa recorded previously in world caves.

According to Hausman and Hülsmann (1996), some species of *Colpoda* can resist lower temperatures, which favors its establishment in several habitats. However, caves are systems where microclimate conditions are almost constant through the year in dark zones, and this environmental stability could be a dominant factor for some protozoa species found throughout the cave, such as *Colpoda* sp. and *Vorticella* sp.

Cyst production by protozoa is sometimes just part of the life-cycle, but often is a response to unfavorable environmental factors, such as desiccation, temperature, or starvation, and is triggered in response to these conditions. Cyst formation has been documented in species of the genera *Colpoda, Vahlkampfia, Actinophrys*, and *Paramecium* (Hausmann and Hülsmann, 1996, p. 154–157), which were found at Cueva de Los Riscos, and this capability facilitates the presence of these species in different seasons inside the cave.

The differences in specific richness in the cave zones can be explained as a function of the type of biotopes present. The greatest values were obtained in Zone A (the light zone), followed by Zone D (the darkest and deepest zone), and Zone C (the twilight and dark zone). The elevated specific richness of Zone A could be explained because of the presence of moss, a biotope that favors protozoan population growth. In Zone C, we found small bodies of water formed by temporary water sources, and also permanent gour pools, which provide a more suitable habitat for protozoan communities. Water facilitates oxygen uptake, contains food resources, has surface tension for movement and dispersion, and is a medium that also facilitates reproduction; this explains the record of seven protozoa genera. In Zone D, permanently dark and where vampire bats (*Desmodus rotundus* (Geoffroy) and *Diphylla ecaudata* Spix) were present in all seasons, the predominant biotope was the guano of the hematophagus bat, which had fewer species than the moss and water substrates, but provides nutrients such as nitrogen compounds for protozoa and is, according to Hoffmann et al. (1986, chap. III), a major energy source in caves, supporting five genera of heterotrophic protozoa. Zone B (twilight zone), had the lowest specific richness, but this can be due the small number of samples collected there because of its inaccessibility.

The Jaccard index demonstrated a similarity degree of 0.6 between the water and the bat guano biotopes, which shared mainly bactivore-detritivore species. Water and guano biotopes shared four species with the soil biotope, for a 0.54 similarity index. The biotopes guano, water, and soil were distributed into the darkest areas of the cave and proved to be suitable substrates for the protozoan colonization and establishment.

Soil is a microhabitat that could be frequently exposed to variable degrees of desiccation affecting the ciliates, flagellates and amoebaes. That could explain why we only found six species (three ciliates, one flagellate, and the heliozoan), as compared with other biotopes. However, a cave system usually exhibits high humidity, preventing desiccation. Nevertheless, protozoan diversity in soil was lowest, probably due to other factors, such as granulometric and physical characteristics that could prevent free movement and dispersion, among other functions.

The community assemblages of several taxa of protozoans in the same time and place could be explained by their trophic roles in relation to the type and availability of food resources. The protozoa have a wide spectrum of food requirements, and these were available in the different cave biotopes documented. Broad tolerances of the most common taxa suggest that some species should be found in nearly every natural system (Pratt and Cairns, 1985) and explain why most of the species recorded in the present work also have been reported in habitats other than caves worldwide. In respect to this particular cave ecosystem, previous data refer only to protozoa from water samples of caves. We found that the all the biotopes considered in this study were suitable for many protozoa, favoring reproduction and providing food sources, among others requirements.

Conclusions

We sampled four biotopes in Cueva de Los Riscos that were inhabited by thirteen protozoa species. Each biotope provided favorable conditions, but they harbored different species compositions and richness throughout the dry and rainy seasons. We recorded for the first time five protozoa species in caves worldwide; an additional three species are new records for Mexican caves. With these data we conclude that protozoa have a wide distribution in cave systems, but more studies are needed to complete the records in these ecosystems.

Acknowledgments

References

Bamforth, S.S., 1985, Ecology of protozoa, in Lee, J.J., Bovee, E.C., and Hutner, S.H., eds., An Illustrated Guide to the Protozoa: Lawrence, Kansas, Society of Protozoologists, p. 8–15.

Barr, T.C., 1968, Ecological studies in the Mammoth Cave System of Kentucky. I. The Biota. International Journal of Speleology, v. 3, no. 1–2, p. 147–204.

Borror, A.C., 1969, Application of the stain-fixative Nigrosin-HgCl2-Formalin to fragile or contractile ciliates: Transactions of the American Microscopical Society, v. 88, no. 3, p. 454–458.

Carey, P.G., Sargent, A.J., Martínez Taberner, A., Ramón, G., and Moyà, G., 2001, Ecology of cavernicolous ciliates from the anchialine lagoons of Mallorca. Hydrobiologia, v. 448, p. 193–201.

Corliss, J.O., 2002, Biodiversity and biocomplexity of the protists and an overview of their significant roles in maintenance of our biosphere: Acta Protozoologica, v. 41, p. 199–219.

Espino del Castillo, A., Castaño-Meneses, G., Dávila-Montes, M.J., Miranda-Anaya, M., Morales-Malacara, J.B., and Paredes-León, R., 2009, Seasonal distribution and circadian activity in the troglophile long-footed robber frog, Eleutherodactylus longipes (Anura: Brachycephalidae) at Los Riscos Cave, Querétaro, Mexico: Field and laboratory studies: Journal of Cave and Karst Studies, v. 71, no. 1, p. 24–31.

Finlay, B.J., Esteban, G.F., and Fenchel, T., 1998, Protozoan Diversity: Converging estimates of the global number of free-living ciliate species: Protist, v. 149, p. 29–37.

Foissner, W., and Berger, H., 1996, A user-friendly guide to the ciliates (Protozoa, Ciliophora) commonly used by hydrobiologists as bioindicators in rivers, lakes, and waste waters, with notes on their ecology: Freshwater Biology, v. 35, p. 375–482.

Golemansky, V., and Bonnet, L., 1994, Protozoa, In: Juberthie, C., and V. Decu, V., eds., Encyclopaedia Bioespeologica: Société de Biospéologie, p. 23–33.

Hausmann, K., and Hülsmann, N., 1996, Protozoology: Stuttgart, Georg Thieme Verlag, 338 p.

Hill, B.F., Small, E.B., and Iliffe, T.M., 1986, Euplotes iliffei n. sp.; a new species of Euplotes (Ciliophora, Hypotrichida) from the marine caves of Bermuda. Journal of the Washington Academy of Sciences, v. 76, no. 4, p. 244–249.

Hoffmann, A., Palacios-Vargas, J.G., and Morales-Malacara, J.B., 1986, Manual de Bioespeleología (con nuevas aportaciones de Morelos y Guerrero, Méx.): Universidad Nacional Autónoma de México, 274 p.

Holsinger, J.R., 1966, A preliminary study on the effects of organic pollution of Banners Corner Cave, Virginia. International Journal of Speleology, v. 2, no. 1–2, p. 75–89.

Jahn, T.L., Bovee, E.C., and Jahn, F.F., 1979, How to Know the Protozoa: Dubuque, Iowa, Wm. C. Brown, 279 p.

Kudo, R.R., 1971, Protozoology: Springfield, Illinois, Charles C. Thomas, 1174 p.

Lazcano-Sahagún, C., 1986a, Cavernas de la Sierra Gorda. v. I: Universidad Nacional Autónoma de Querétaro, Mexico, 181 p.

Lazcano-Sahagún, C., 1986b, Cavernas de la Sierra Gorda. v. II: Universidad Nacional Autónoma de Querétaro, Mexico, 206 p.

Lee, J.J., Small, E.B., Lynn, D.H., and Bovee, E.C., 1985, Some techniques for collecting, cultivating and observing Protozoa, in Lee, J.J., Bovee, E.C., and Hutner, S.H., eds., An Illustrated Guide to the Protozoa: Lawrence, Kansas, Society of Protozoologists, p. 1–7.

Manwell, R.D., 1968. Introduction to Protozoology, second edition. New York, Dover, 642 p.

Olenin, S., and Ducrotoy, J.P., 2006, The concept of biotope in marine ecology and coastal management. Marine Pollution Bulletin, v. 53, p. 20–29.

Osorio-Tafall, B.F., 1943, Observaciones sobre la fauna acuática de las cuevas de la región de Valles, San Luis Potosí (México): Revista de la Sociedad Mexicana de Historia Natural, v. 4, no. 1–2, p. 43–71.

Patterson, D.J., and Hedley, S., 1992, Free-Living Freshwater Protozoa: A Colour Guide: London, Wolfe Publishing, 223 p.

Pratt, J.R., and Cairns, J., 1985, Functional groups in the Protozoa: roles in differing ecosysrems: Journal of Protozoology, v. 32, no. 3, p. 415–423, doi: 10.1111/j.1550-7408.1985.tb04037.x.

Silva-Neto, I.D., 2000, Improvement of silver impregnation technique (Protargol) to obtain morphological features of protists ciliates, flagellates and opalinates: Revista Brasileira de Biologia, v. 60, no. 3, p. 451–459, doi: 10.1590/S0034-71082000000300010.

Small, E.B., Heisler, J., Sniezek, J., and Iliffe, T.M., 1986, Glauconema bermudense n. sp. (Scuticociliatida, Oligohymenophorea), a troglobitic ciliophoran from Bermudian marine caves. Stygologia, v. 2, no. 1–2, p. 167–179.

METHODOLOGY TO ASSESS WATER PRESENCE ON SPELEOTHEMS DURING PERIODS OF LOW PRECIPITATION, WITH IMPLICATIONS FOR RECHARGE SOURCES – KARTCHNER CAVERNS, ARIZONA

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Abstract: Beginning in January 2005, recharge processes and the presence of water on speleothems were monitored in Kartchner Caverns during a 44-month period when annual rainfall rates were 6 to 18 percent below the long-term mean. Electrical-resistance sensors designed to detect the presence of water were used to identify ephemeral streamflow in the channels overlying the cave as well as the movement of water within the cave system. Direct infiltration of precipitation through overhead rocks provided consistent inflow to the cave, but precipitation rates and subsequent infiltration rates were reduced during the comparatively dry years. Ephemeral stream-channel recharge through autogenic and allogenic processes, the predominant recharge mechanism during wetter periods, was limited to two low-volume events. From visual observations, it appeared that recharge from channel infiltration was equal to or less than recharge from overhead infiltration. Electrical-resistance sensors were able to detect thin films of water on speleothems, including stalactites, ribbons, and stalagmites. These films of water were directly attributed to overhead infiltration of precipitation. Periods of low precipitation resulted in decreased speleothem wetness.

INTRODUCTION

Kartchner Caverns, located in southeastern Arizona, USA, was opened to the public in November 1999 (Fig. 1). Prior to its opening, a baseline investigation of the cave's internal and external climate, geology, and hydrogeology was conducted to aid in identifying future impacts from development. The baseline investigation was conducted in the 1980s and early 1990s, a period that coincided with higher than average precipitation conditions. Results from the baseline investigation are documented in a special issue of the *Journal of Cave and Karst Studies* (vol. 61, no. 2, 1999). After the initial baseline investigation, observations of precipitation and groundwater levels continued. Concurrent to the opening of the cave to visitation was the onset of drier than average conditions (circa mid-1990s). During the late 1990s, moisture on the cave's formations noticeably declined. The decline was hypothesized to be a consequence of decreased precipitation causing decreased inflow to the cave, the introduction of drier air into the cave with visitors, or both (Rick Toomey, pers. comm.).

During the baseline investigation two primary mechanisms of inflow to the cave were identified as contributors of water to the cave: direct infiltration of overhead precipitation and infiltration of surface runoff in the stream channels that surround the cave (Graf, 1999). The direct infiltration of overhead precipitation has been defined by White (2003) as diffuse infiltration and categorized as autogenic recharge because the recharge water originates over the karst landscape and percolates in place. Infiltration of surface runoff through stream channels is a combination of discrete autogenic recharge (Lerch et al., 2005) and concentrated allogenic recharge (White, 1988, p. 281). In the former, precipitation over the karst landscape is concentrated into losing stream channels and infiltrates into the cave system. Allogenic recharge originates as precipitation over nonkarst areas upstream of the cave system and is concentrated into losing stream channels that are situated adjacent to the cave system. Groundwater inflow from adjacent aquifers was considered a possible source of cave water, but the initial investigators did not observe direct inflow from up-gradient aquifers, except in conjunction with streamflow in adjacent surface channels (Graf, 1999).

The objectives of this study were to determine if the observed declines in water flow on speleothems could be attributed to climate fluctuation and to identify the relative contributions of sources of inflow to the cave system during drier-than-average conditions.

To meet these objectives, it was necessary to use a novel method for detecting the presence of water on speleothems. Electrical-resistance (ER) sensors have been used to determine the presence of flow in ephemeral stream channels based on the ability of water to conduct electricity (Blasch et al., 2002). ER sensors were constructed using modified TidbiT sensors (Onset Corporation, Bourne, Massachusetts) as described by Blasch et al. (2002). ER sensors have been successfully used to monitor streamflow timing in coarse-grained alluvial channels (Blasch et al., 2002) and bedrock channels (Adams et al., 2006) and on
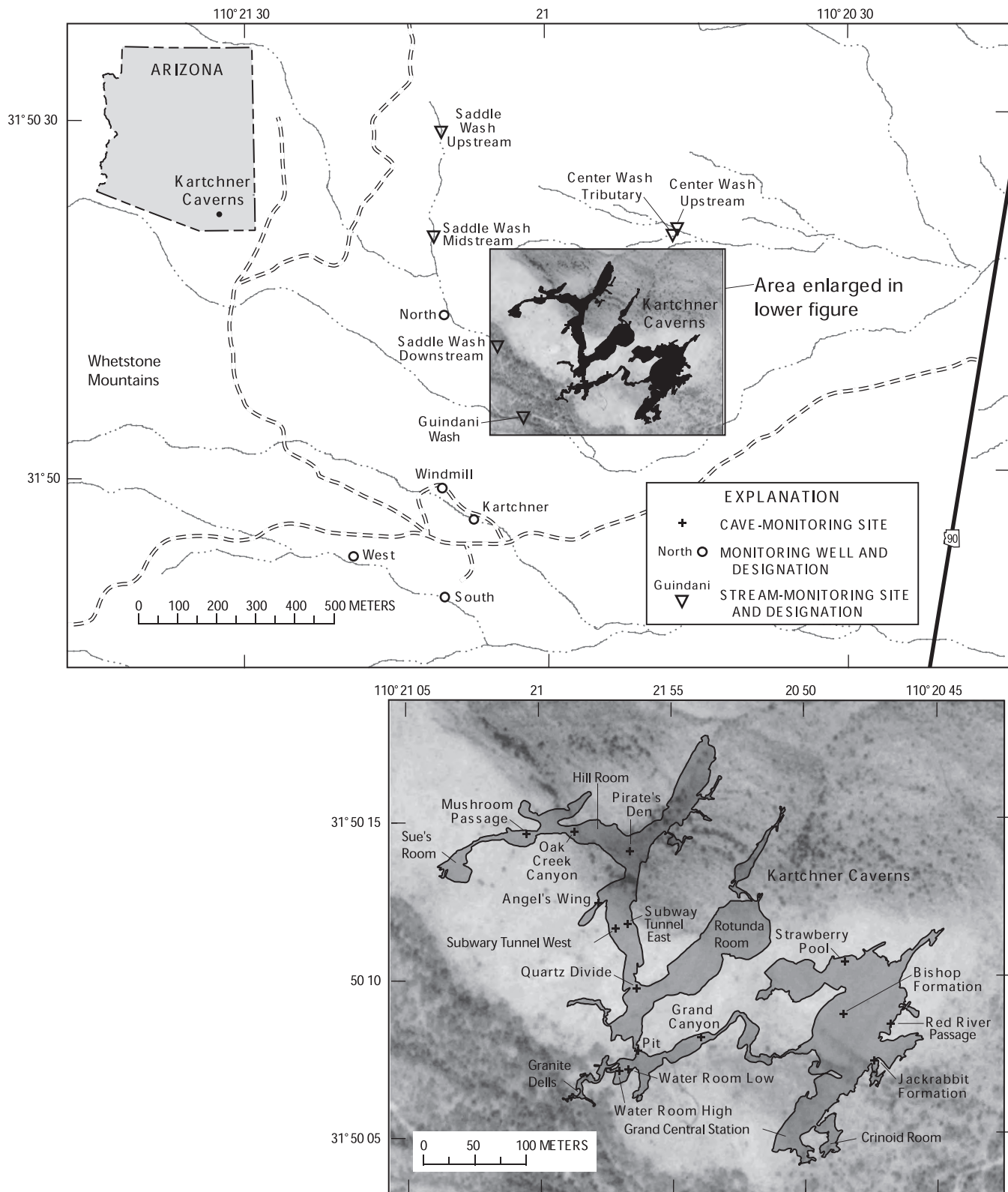
Figure 1. Map of Kartchner Caverns study area and monitoring sites.

bedrock walls. ER sensors should be useful for monitoring the presence of water within the cave environment, including cave walls, formations, pools, and drainage channels. An additional objective of the study was to evaluate the use of electrical resistance sensors for monitoring formation wetness and water flow within a cave.

STUDY AREA

Kartchner Caverns is located approximately 75 km east of Tucson and 30 km west of Tombstone at the base of the Whetstone Mountains in southern Arizona. A full description of the hydrogeology of Kartchner Caverns State Park was presented by Graf (1999) as part of the Kartchner Caverns State Park Symposium special issue of the *Journal of Cave and Karst Studies* (vol. 61, no. 2, 1999). Regional geologic history and dating of speleothems and sediments indicates that the cave formed roughly 200 ka ago, with the main speleothem development occurring between then and 70 ka (Hill, 1999). Kartchner Caverns formed at the base of the Whetstone Mountains within a ridge of Mississippian-age Escabrosa Limestone. The ridge itself is at the surface of the Kartchner block that has been displaced downward from the Whetstone Mountains to the west, while the San Pedro alluvial basin to the east of the Kartchner block has subsided even farther.

A major fault on its western boundary separates the Kartchner block from the Precambrian-age Pinal Schist and the Whetstone Mountains. The upthrown side of the fault is Pinal Schist, which extends to a greater depth than the Escabrosa Limestone of the Kartchner block on the downthrown side of the fault. The fault is within 0.25 km of the known extent of Kartchner Caverns. The Pinal Schist is overlain by alluvial sediments, called the granite wash, of Illinoian age (Melton, 1965) that in turn are overlain by the pediment surface called the Whetstone surface. Gray (1967) describes the granite wash as a combination of alluvial sediments, decomposed granite, mud flow, and alluvial fan sediments. The depth of the granite wash, as approximated by Graf (1999), is on the order of tens of meters. Using non-pumping water-level measurements, pump tests, and specific electrical conductivity measurements, Graf (1999) concluded that the groundwater yield in the granite wash is small and that connectivity in the formation is poor. Similarly, groundwater yields in the Pinal Schist are low.

The main basin-and-range fault separating the Kartchner block from the San Pedro alluvial basin to the east was mapped by Graf (1999) using gravity data from Lange et al. (1990). The basin-and-range fault is located less than 0.5 km from the known eastern boundary of Kartchner Caverns. The San Pedro alluvial basin immediately east of the fault is composed of a course alluvium overlain by granite wash. To the east, the coarse alluvium deposition is replaced by the finer-grained Pliocene and Pleistocene sediments of the St. David Formation. Groundwater levels measured within the St. David Formation indicate flow from the edge of the Whetstones eastward toward the San Pedro River.

Kartchner Caverns is in highly faulted limestone and formed under shallow-phreatic conditions (Graf, 1999). The regional water-table conditions that existed during its formation are no longer present. Depth to water ranges today from tens to hundreds of meters in the vicinity of the cave.

The channels of three ephemeral streams bound the cave (Fig. 1). Guindani Wash originates in the Whetstone Mountains and traverses the southern boundary of the cave from west to east. Center Wash originates on the northern slopes of the cave ridge and flows west to east. Center Wash empties into Guindani Wash northeast of the cave boundary. Saddle Wash originates northwest of the cave and runs along the western and southwestern edge of the cave, eventually merging into Guindani Wash. Both Saddle Wash and Guindani Wash overlie fault boundaries in proximity of the cave.

The explored regions of Kartchner Caverns range in elevation from about 1400 m near the Red River Passage at the eastern end of the cave to about 1425 m in Sue's Room at the western end of the cave (Fig. 1). Sediment depths within the rooms vary, so the actual elevations of the bedrock floors are unknown. The stream channels adjacent to the cave system are all above 1432 m (4700 ft) in elevation, allowing infiltrating water to travel vertically towards the cave system. Saddle Wash passes about 15 meters from the mapped boundaries of the Granite Dells and Guindani Wash passes about 90 meters from the mapped boundaries of Grand Central Station (Fig. 1).

The conceptual flow model for the cave system was originally developed during the baseline investigation of the cave. Components of the flow system were documented by Graf (1999), but important unpublished data still reside with the Kartchner Caverns Cave Resources Unit. As indicated, the primary sources of inflow to the cave originate as overhead precipitation and channel recharge. Graf (1999) noted that the water table is about 200 m below the known depth of the cave, and thus unsaturated conditions and processes are present.

Graf (1999) estimated that inflow from channel infiltration of ephemeral streamflow events accounted for the majority of the water entering the cave and that the remaining inflow was from direct overhead infiltration of precipitation. Buecher (1992, 1999) estimated about 230,000 L of water (7.6 mm over the surface area of the cave) enters the cave through overhead infiltration from precipitation by way of faults, fractures, Darcy flow through permeable beds, and flow down the surface of impermeable, dipping beds (Jagnow, 1999). Using drip studies, Buecher estimated that it took 4 to 12 days for water to percolate from the surface to the ceiling of the cave. This translated into an average groundwater flow

rate of 15 m/day through the overhead limestone and pediment.

Three ephemeral stream reaches were identified as contributing areas to the cave through observations, geophysics (Lange et al., 1990, and Lange, 1999), and dye tracers (Buecher, 1992). Fluorescein dye was used in September 1990 to confirm the connection between the ephemeral flow in Saddle Wash downstream from the North well and Sue's Room. Rhodamine WT dye was used in January 1991 to confirm the connection between the junction of Saddle and Guindani Washes and the Granite Dells. Flow from Guindani Wash upstream from the trail was also observed in the Crinoid Room.

Graf (1999) suggested the conditions generally necessary before recharge waters are observable in the cave. These conditions include surface flows in Guindani or Saddle Washes for more than one week and excess soil moisture exceeding 30.5 mm in one month or 38.1 mm over two consecutive months as computed by the Thornthwaite potential-evapotranspiration method.

Once water enters the cave, it tends to pond in place and slowly drain through the cave floor. If the flow rate into the cave is higher than the draining rate, ponding will increase until water spills over to neighboring rooms (Graf, 1999). For large flood events this process continues until flow is dispersed throughout most of the cave. Some rooms remain disconnected from the overall flow system due to impermeable units. In general, flow is from west to east, because the surface stream channels are on the western and southern borders of the cave and room elevations within the cave decrease from west to east. Eventually, water flowing through the cave drains through the Red River Passage, at 1400 m the lowest recorded point in the cave. Graf (1999) estimated an overall drainage rate based on the three largest flood events to be about 22 L/min, though drainage rates in the rooms vary. It is important to note that the structure of the cave below the mapped rooms (Fig. 1) is unknown. Thus constraints on draining and flow between rooms within the cave are not entirely understood.

METHOD

Hydrologic parameters were monitored inside and outside of the cave in order to describe the interaction between the surface and subsurface flow system and monitor speleothem wetness.

OUTSIDE THE CAVE
Monitoring of precipitation, streamflow, and groundwater levels outside the cave started well before this study. Precipitation has been measured about 1 kilometer southeast of the cave and recorded on an event basis using a 20.32-cm bucket rain gauge with an accuracy of 0.25 cm. A second bucket rain gauge with an accuracy of 0.50 cm was used to collect weekly measurements. The rain-gauge record started in January 1992. In order to quantify flow

in an ephemeral stream, continuous monitoring (15-minute interval) in Guindani Wash (Fig. 1) began about January 2000.

For this study, ephemeral streamflow presence was monitored using TidbiT electrical-resistance (ER) sensors and TidbiT temperature sensors installed in stream channels surrounding the cave (Fig. 1). Monitoring began in December 2004, and recording intervals were set to 1 hour. Sensors were installed in perforated PVC housings and tethered to trees adjacent to the channels. Boulders were placed over the sensors to maintain their positions in the lowest portion of the cross-section and to shield the sensors from direct rainfall.

Three sets of ER and temperature sensors were installed in Saddle Wash. The upstream sensors were placed north of Kartchner Hill. The midstream sensors were installed about 275 meters downstream of the upstream sensors, and the downstream sensors were installed about 400 m downstream of the midstream sensors. The middle set of sensors was placed near a dye injection location used by Graf (1999) during an earlier surface channel recharge investigation.

One set of sensors was installed in Center Wash and an ER sensor was installed in a tributary wash to Center Wash. The unnamed tributary wash originates on the north side of Kartchner Caverns Hill and trends to the northeast into Center Wash.

Groundwater-level monitoring started during the baseline investigation. Monthly groundwater levels were measured in five wells (Fig. 1) during this investigation using a Solinst water level meter. Water temperatures were also measured in the North and West wells on a monthly basis.

INSIDE THE CAVE
ER sensors and temperature sensors were installed within the cave drainage channels (Fig. 1). To preserve the cave environment, only selected regions of the cave were accessible, and within these regions only prescribed footpaths could be used for sensor installation. Thus some lower-elevation drainage channels were not accessible for monitoring. The lowest-elevation locations in the rooms that could be reached from the foot paths were instrumented. Other placement considerations included consistency with previous dye-trace sites and records of water presence from the baseline investigation. Previous investigations observed entrainment of fines during periods of inflow to the cave. Consequently, sensors were installed about 1 cm from the sediment surface to avoid deposition of fines on the electrodes.

ER sensors were installed at the bottom of an area that collects flow referred to as the Strawberry Pool. The sensors were placed in a low section of the pool. Water enters the pool from low-flow features and dripping features. ER sensors were installed in the thalweg of a drainage channel called the Red River Passage. The Red

River Passage is a drain at the eastern edge of the cave. Flow rates in the cave are small enough that the sensors were not put into protective housings. Sensors were installed in the upper and lower regions of the Water Room and in Mushroom Passage exactly where the dye-trace receptors had been placed. Sensors were also installed in the Grand Canyon, the Pit, Quartz Divide, Angel's Wing, Subway Tunnel East, Subway Tunnel West, entrance to the Pirate's Den, and the Hill Room (the sensor location is called Oak Creek Canyon).

A second type of ER sensor that was installed on cave formations was the four-channel external HOBO sensors (Onset Corporation, Bourne, Massachusetts) with open leads. These data-loggers have four channels and sensor wires about 20 cm in length. The leads of the wires were exposed at the end by 1 cm and were mounted on the formations using flagging tape (Fig. 2). Each data-logger was housed in a sealed plastic bag containing desiccant.

An initial group of sensors was installed on December 4, 2004, on the Bishop formation and on the Jackrabbit formation. The Bishop formation is a large column that is one of the monumental formations within the cave. The leads on the Bishop formation were installed on a stalactite and in wet and dry alcoves. The Jackrabbit formation is another significant formation mass with a considerable number of soda straws, stalactites, and ribbon features. Leads were installed on a soda straw and a ribbon tip and on the ground below the formation.

Finally, an In-Situ Level Troll 500 pressure transducer was installed in the Subway Tunnel East (Fig. 1), an area with significant ponding, to determine the rate at which water drained. The Subway Tunnel is at a lower elevation and receives flow from upstream rooms.

<center>RESULTS AND DISCUSSION</center>

ELECTRICAL RESISTANCE SENSOR PERFORMANCE
Electrical Resistance Sensor (TidbiT)

The Strawberry Room was one of the wettest areas monitored within the cave on the basis of sensor data and visual observations. The Strawberry Room is easily accessible within the cave, and therefore, more visual observations were obtained during the investigation. These visual observations were used to confirm the presence or absence of water as measured by the ER sensors.

Water was detected by the ER sensor in the summer of 2005 and summer of 2008 (Fig. 3). Four visual observations during the summer of 2005 and 2008 were in agreement with the ER sensor. Seven additional visual observations during dry periods were also confirmed. These data are consistent with successful detections in ephemeral channels previously documented (Blasch et al., 2002). The success of the ER sensors within the cave environment is attributed to the lack of physical hazards such as high streamflow velocities, debris, and scour or deposition processes.
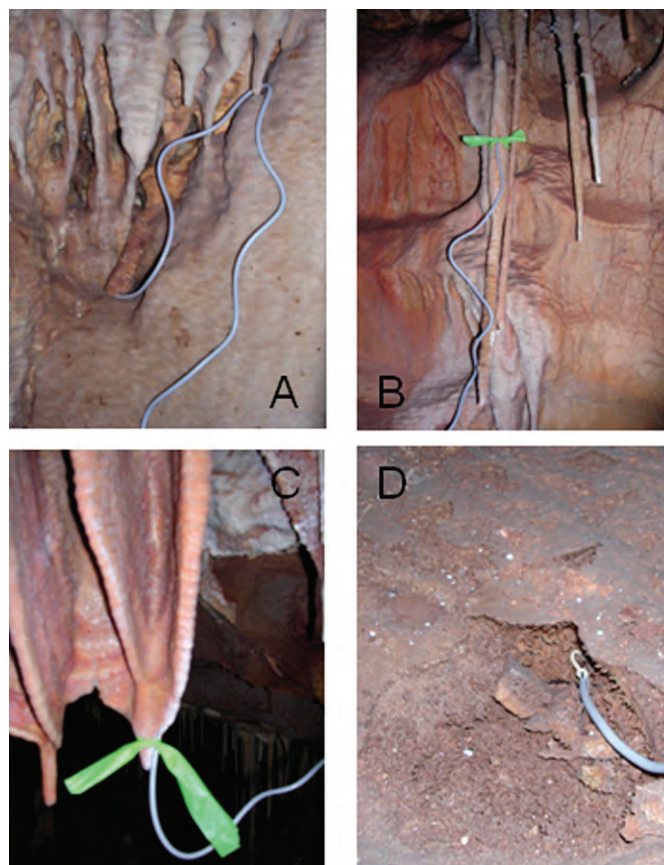

Figure 2. Placement of HOBO electrical resistance sensors on the Bishop Formation (A) and Jackrabbit Formation stalactite (B), ribbon (C), and (D) ground below.

Electrical Resistance Sensor (HOBO)

The HOBO sensors on the Jackrabbit formation recorded data for almost the entire period of study. The data reveals a more complex signature than the TidbiT ER sensors (Fig. 4). This may be attributed to the measurement of thin films of water on the formations or exposure of the circuitry to the humid environment. Voltages recorded before and after disconnecting the sensors from the data-logger, required for downloading, were noticeably different. During data analysis the recorded voltages were adjusted based on the voltage readings prior to the disconnection. With these corrections, the presence of water on the formations is indicated with lower voltage output, implying a higher conductivity.

During the winter of 2005, the spring and summer of 2007, and the summer of 2008 water drops were seen on the tip of the ribbon formation. The HOBO ER sensor recorded a lower voltage (higher conductivity) during these periods as indicated by the light gray points in Figure 4. The sensor on the floor recorded wet and dry conditions consistent with visual observations. The response recorded on the stalactite is not as conclusive (Fig. 4). The decrease in voltage from October 2006 to June 2007 cannot be
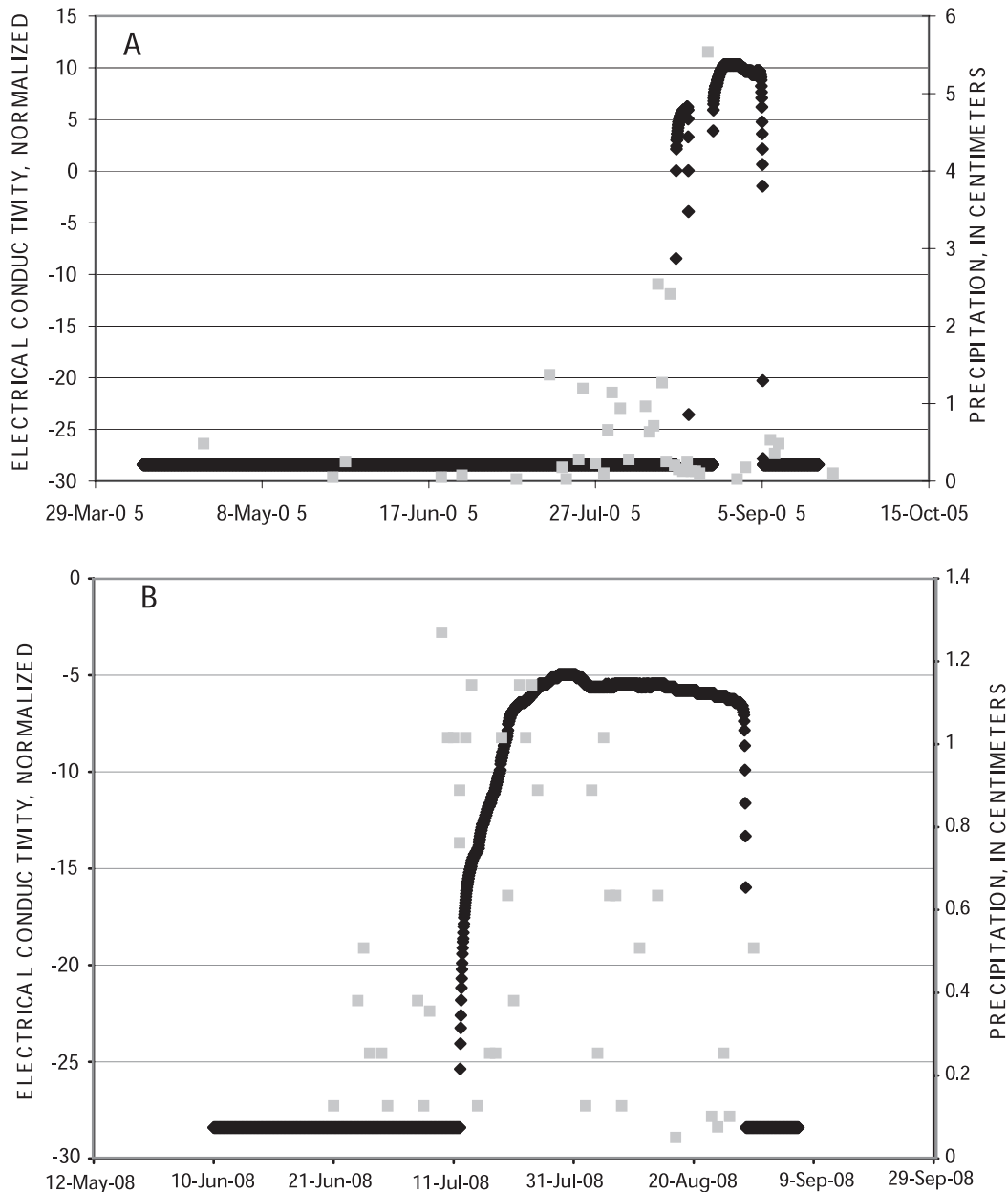
Figure 3. Measurements of normalized electrical conductivity in the pool of the Strawberry Room for (A) 2005 and (B) 2008. Daily precipitation represented by gray squares.

explained. During this time period visual observations indicated the formation was dry.

The sensors on the Bishop formation recorded wetness on the formation consistent with observations until the instruments failed in the humid environment. The last recorded day was April 9, 2005.

Within the cave, the success of the two types of ER sensors differed. The modified TidbiT sensors were well suited for cave channels, ponding areas, and formations with ponding surfaces. The short leads and the weight of the instruments prohibited them from being installed on delicate formations or small fractures. The flexibility of the long leads from the 4-Channel HOBOs was well suited for installation on the delicate formations, but the high humidity of the cave environment was harmful to the HOBO's circuitry and made necessary frequent visits to change the desiccant packaged with the HOBO. One HOBO failed within the first year, likely owing to the high-humidity environment. Output from the HOBOs was also not as clear as output from the TidbiTs. The proprietary nature of HOBO and TidbiT circuitry prohibited a clear explanation for the difference in readings. For cave channels and ponding situations the TidbiTs performed well, but a combination of the TidbiT's ruggedness and
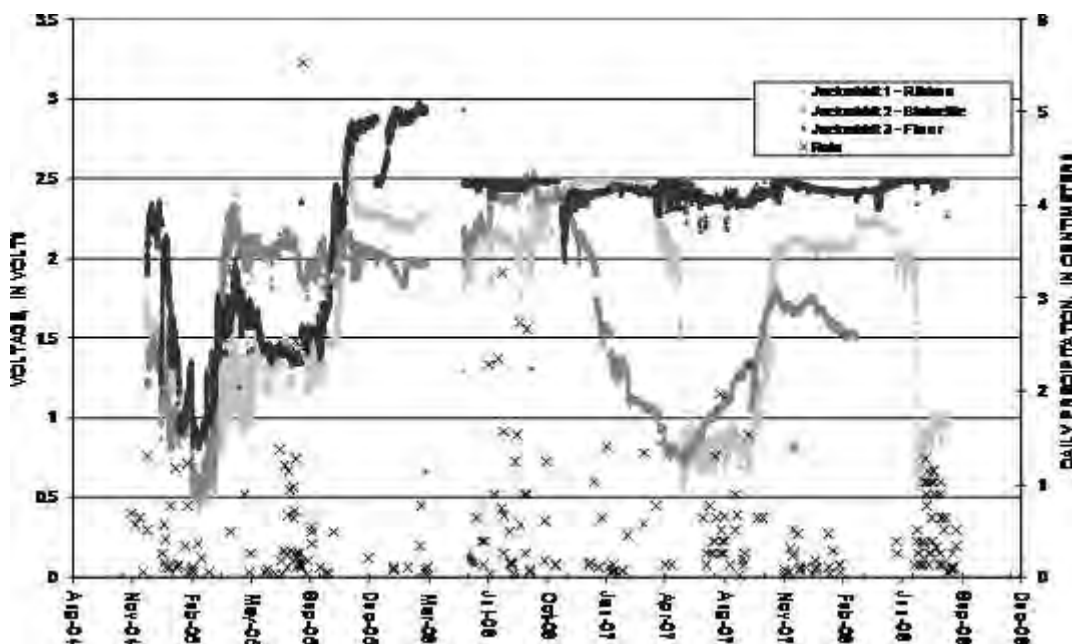
Figure 4. HOBO data for the presence of water on the Jackrabbit Formation. Daily precipitation represented by crosses.

output with the four-channel and long-lead capability of the HOBOs would be an optimal sensor for cave-formation monitoring.

WATER INFLOW AND MOVEMENT IN THE CAVE SYSTEM
External Cave Environment

Climate conditions at Kartchner Caverns were drier than the long-term mean. Mean annual precipitation monitored at Tombstone, Arizona, (National Climatic Data Center, 2008a) was 36 cm from 1900 to 2005 (Fig. 5).

Rainfall measured at Kartchner Caverns from 1992 to 2007 averaged 34 cm. During this study, annual rainfall values at Kartchner Caverns were 32, 31, and 28 cm per year, respectively. This translates into a reduction of 6 to 18 percent from mean annual rainfall. The Palmer Drought Severity Index (PDSI) for the southeast region of Arizona was used as an indicator of dryness and moisture storage (Fig. 6). PDSI values were obtained from the National Oceanic and Atmospheric Administration's National Climate Data Center (National Climatic Data Center,

Figure 5. Annual precipitation for Tombstone, Arizona, (gray diamonds) and annual precipitation for Kartchner Caverns (black squares).
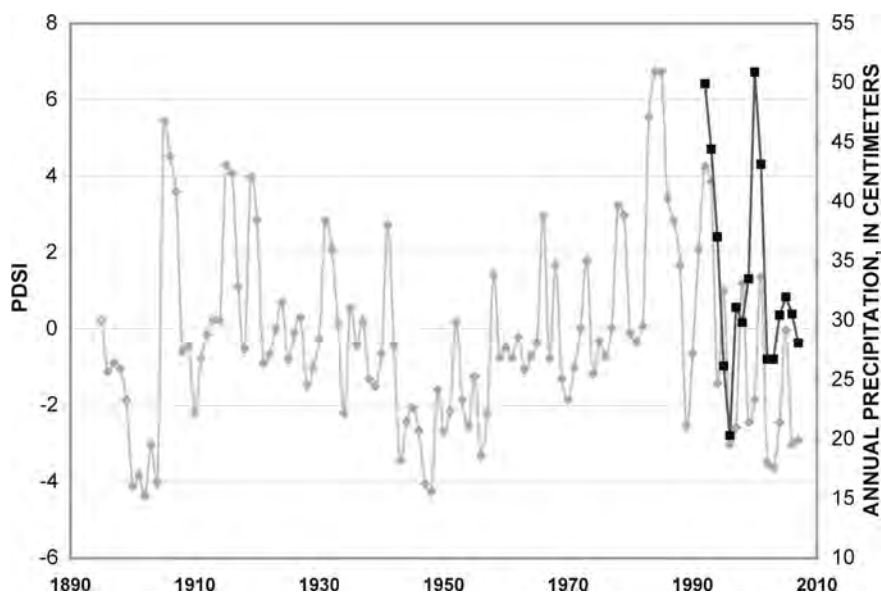
Figure 6. The Palmer Drought Severity Index (PDSI) for the southeast region of Arizona (National Oceanic and Atmospheric Administration's National Climate Data Center, 2008b) and annual precipitation for Kartchner Caverns (black squares).

2008b). Since 1994 the average annual PDSI has risen above normal only three years (1995, 1998, and 2001). The initial baseline investigations were conducted after six years of above normal values (1982–1987). During the baseline investigation from 1988 to 1992, the years 1989 and 1990 were below normal.

Ephemeral streamflow events, previously identified as the predominant source of recharge to the cave, are produced by high precipitation rates. There were no data on streamflow events collected during the baseline study in the early 1990s to compare. Streamflow events were detected in the ephemeral streams about nine times per year in Saddle Wash and about five times per year in Central Wash (Fig. 7). With the exception of the event on December 1, 2007, in upstream Saddle Wash, the remaining flow events occurred during the wet season (July–September) or during the fall (October–November). The wet season is a period of above-average precipitation (24 cm for the three-month period) due to high-intensity, convective rain storms. Average streamflow event duration for Saddle Wash (midstream) was 79 hours and the median event duration was 8 hours. Average event duration for Center Wash (main stem) was 27 hours and the median event duration was 5 hours.

Ephemeral streamflow events in Guindani Wash were rare. Streamflow events did not occur during 2003, 2004, 2006, or 2007. There were seven streamflow events in 2000 and two events in 2001. There was insufficient data for 2002. One flow event was detected in 2005 from 14–31 August, 2005 (Fig. 7). This event was associated with a measured rainfall of 2.4 cm on August 14, 2005, and 5.5 cm on August 23, 2005. Flow was observed during the July 22,

2008 event, but the gauge sustained damage during this high-flow event and the data were lost.

On the basis of rainfall and streamflow data, a rainfall intensity of 1.2 cm d^{-1} at Kartchner Caverns is generally required to generate runoff in the surface channels. Lower intensity rainfall events of 0.6 cm d^{-1} generated runoff if the event succeeded several days of rainfall. Winter storm intensities generally were not high enough to produce runoff during this period of study, though Graf (1999) observed earlier winter streamflow events.

Based on Graf's (1999) personal communication with Robert Buecher, one of the requirements for observed flow in the cave was at least one continuous week of ephemeral streamflow within Guindani or Saddle Wash. From December 2004 through September 2008 there was only one event in Saddle and Center Washes that was more than a week in duration. The 2008 wet-season event that started on July 22 caused flow for 36 days in Saddle Wash. Guindani Wash had flow that exceeded one week during this same time period, as well as a 14-day event during September 2005.

The full extent of the cave both laterally and vertically is unknown. Recent geophysical exploration supports the existence of large voids the size of the Rotunda Room to the west of the explored cave perimeter (Dale Rucker, personal communication, October 2007). Existence of these voids and their connection to Kartchner Caverns has not been verified. Voids between the known cave system and the adjacent washes could be important for the transmission of water from the washes into the known cave system. The size and location of voids affects the storage capacity and can reduce the volume and rate of flow into the
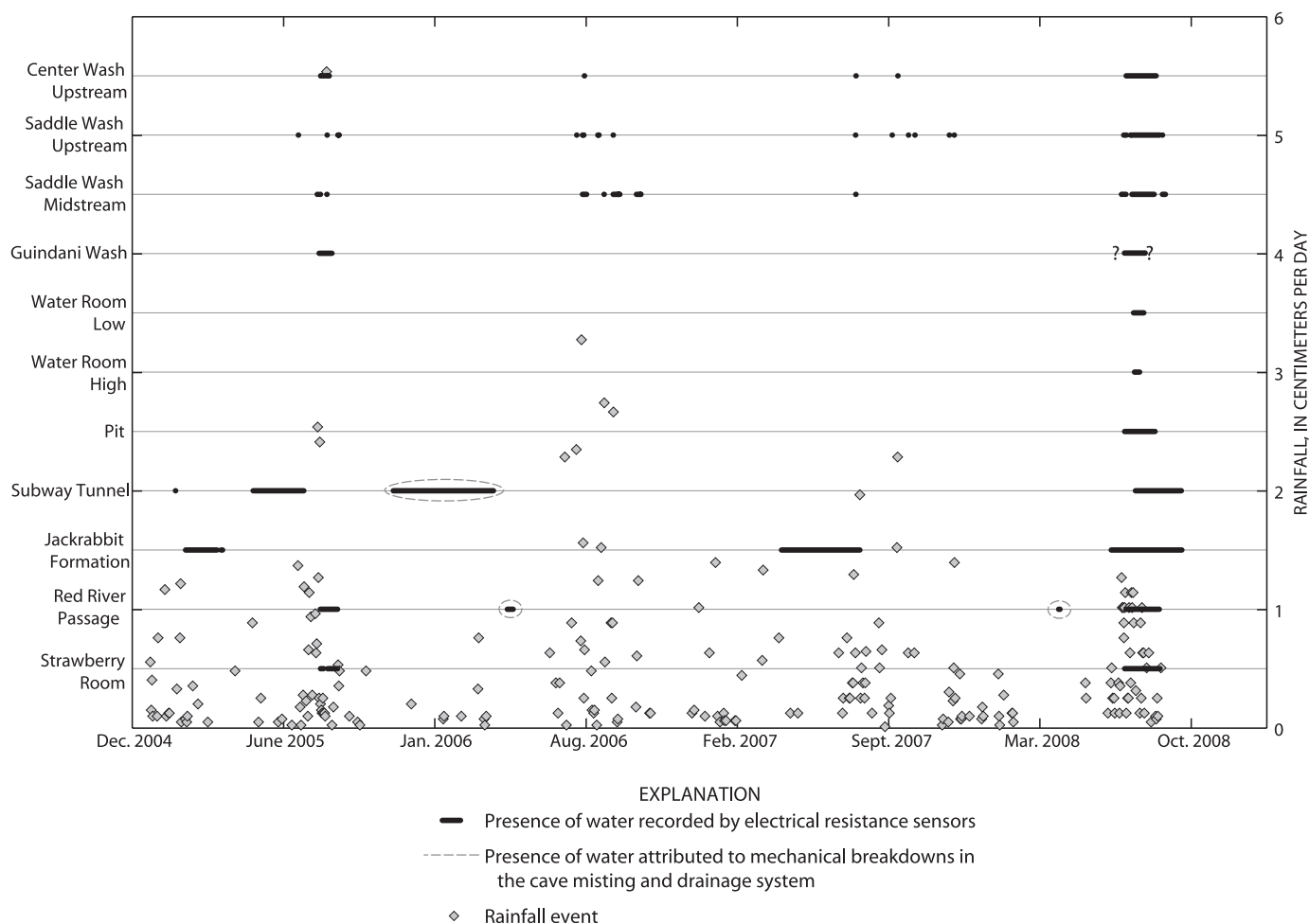
Figure 7. Water occurrence within the ephemeral stream channels outside the cave and formations and rooms within the cave, Kartchner Caverns, Arizona. Only monitoring sites with a nearly full period of record are shown. Daily precipitation totals represented by gray diamonds.

currently mapped and monitored portions of the cave system.

Groundwater levels in the adjacent granite wash and Pinal schist aquifers declined during the period of this investigation, but water levels remained higher than the lowest cave elevations monitored during the study (Fig. 8). Water levels were about 1 to 10 meters lower than historic water levels measured in 1995 and 2000. Graf (1999) analyzed historic water levels and concluded that groundwater inflow to the cave from the adjacent aquifer was negligible with the exception of infiltration over the fault boundaries underlying Saddle and Guindani Washes. Groundwater inflow to the cave was not observed during this investigation.

Internal Cave Environment

Measurements of humidity and temperature obtained from the Kartchner Cave Resources Unit shows a nearly undetectable increase in temperature and humidity for the Strawberry Room from September 2000 to September 2008

(Fig. 9). A general analysis of trend shows temperature increased from 21.44 °C to 21.67 °C and humidity increased from 97.9 percent to 98.2 percent. Over the same time period, measurements at Red River Passage showed an increase in temperature from 21.28 °C to 21.44 °C and almost no change in humidity when outliers are removed.

Water was not detected in the channel observation sites within the Mushroom Passage, Oak Creek Canyon, intersection with the Pirate's Den, Angel's Wing, or the Grand Canyon for the duration of the investigation. Water often observed in footprints along the paths within some of these rooms probably came from infiltration of rainfall and dripping from the ceiling. The ponding was not significant enough in these rooms to cause runoff to the drainage channels being monitored.

The Strawberry Room is on the northeastern edge of the cave and is the farthest monitoring site from the surface stream channels. Water was noticeable in the summer of 2005 and the winter and summer of 2008. Pooling in this room due to rainfall occurred within one day of a rainfall
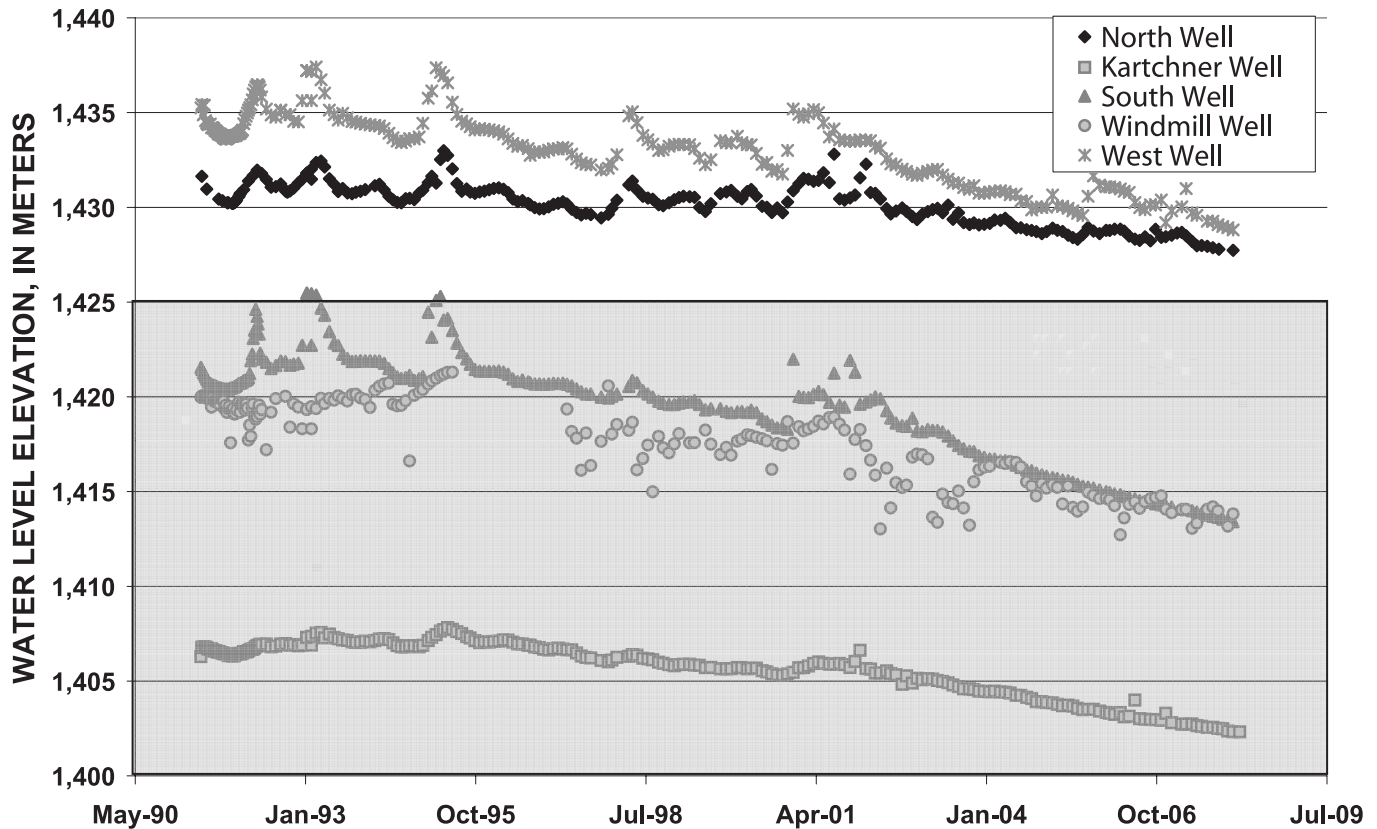
Figure 8. Groundwater levels (elevations) for wells adjacent to Kartchner Caverns. The patterned box indicates known depths of the cave system.

event. On August 15, 2005, ponded water was detected. This followed seven days with rain and a wet July. In particular, August 11 and August 14 both had rainfalls of about 2.5 cm. On August 18 the ponded water was no longer present. However, a rainfall event on August 23 produced about 5.5 cm over the cave system. Within one day (August 24), another pulse of water pooled in the Strawberry Room (Fig. 3A). The longest recorded ponding occurred from July 12, 2008, to August 28, 2008 (Fig. 3B).

Ponded water was recorded in the Red River Passage in September 2005 and April 2006. During the winter of 2007/2008 there was evidence of water, but the ER sensor had failed. Flow also was recorded in July and August of 2008 for a period of 44 days. Water detected at the Red River Passage during September 2005 and the September 2008 heavy-rainfall events was probably a combination of rainfall and infiltration from Guindani Wash (Fig. 7). The Red River Passage showed periods of wetting during April 2006 and April 2008 when there was no rain or streamflow activity. Personnel at Kartchner Caverns suspect that a floor sump near the Red Passage overflowed, discharging water into the Red River Passage. The floor sump drains water that is introduced through a plumbing system to clean foreign contamination from the paved trails.

The summer wet season of 2008 was the only time water was recorded by the Water Room High and Water Room

Low sensors and in the Pit. Given these rooms' proximities to Saddle Wash, this water is likely to have originated as infiltration from this steam during the long period of flow.

The record of water detected in the Subway Tunnel was not consistent with the precipitation or streamflow records (Figs. 7 and 10). The source of the water was traced to leaking misters in the Rotunda Room. Several misters had been installed at the entrance of the cave to reduce foreign contamination such as lint from being deposited into the cave by visitors. Leaking mister sprayers were replaced December 14, 2005, January 21, 2005, and March 30, 2005. In each case the water level immediately declined in the Subway Tunnel after the replacements. These events provided the data to conclude that a flow of water into the Subway Tunnel from the Rotunda Room occurred through sediments, thus establishing a hydraulic connection between these rooms. Additionally, in order for water to travel from the Rotunda Room to the Subway Tunnel, the horizontal rate of travel had to be higher than the vertical drainage rate of the sediments within the Rotunda Room. The water level in the Subway Tunnel drops approximately 0.8 cm/d, according to the pressure transducer, when no water is flowing into it.

Climate Impacts and Future Considerations.

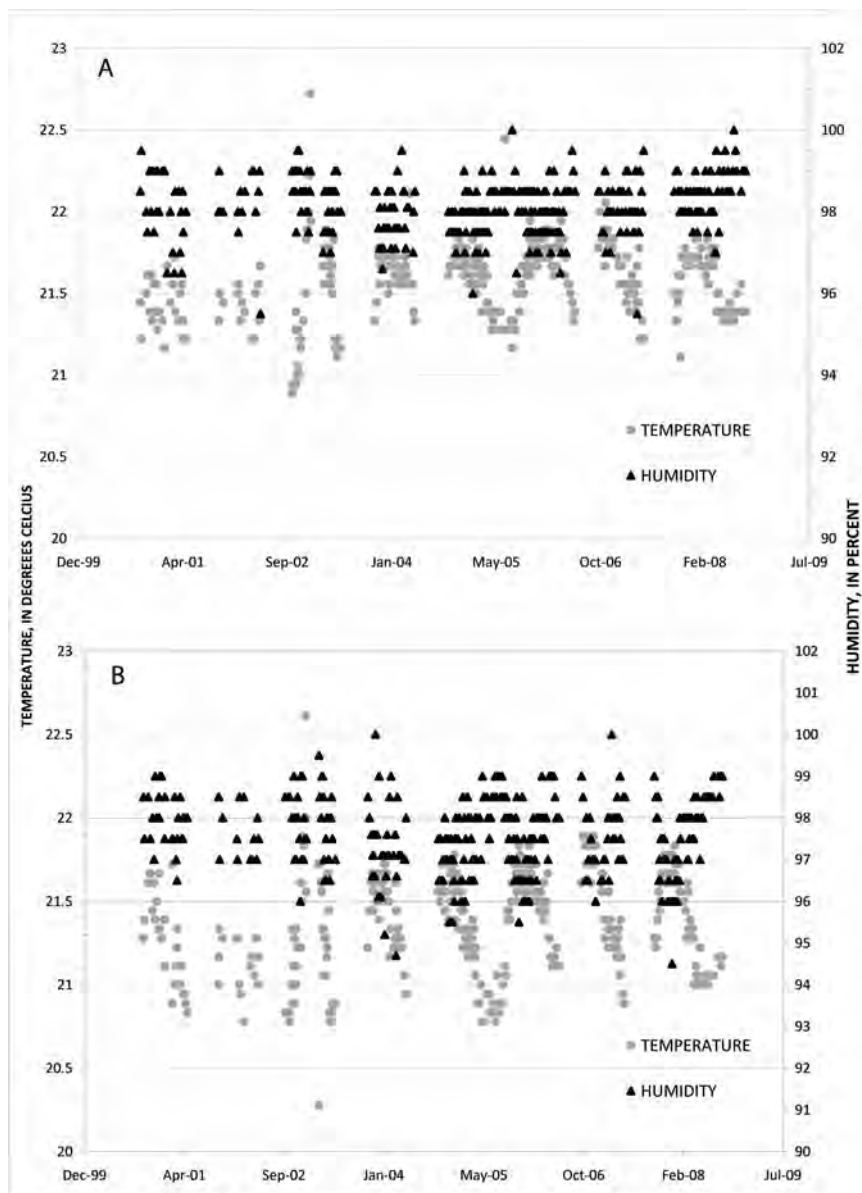Compared to the period of the baseline investigation, the magnitude of recharge observed within Kartchner

Figure 9. Humidity and temperature for the Strawberry Room (A) and the Red River Passage (B).

Caverns from both ephemeral streamflow infiltration (autogenic and allogenic recharge) and direct infiltration of precipitation (autogenic recharge) was less. There are not sufficient data to compare the total volume of water from each of these recharge mechanisms, but through visual observations the amount of water introduced through streamflow infiltration (two possible events over 44 months) was not noticeably higher than the amount introduced by direct infiltration. This would have to be verified with a more detailed study, but is in contrast to the results from the baseline period of above average rainfall.

During this investigation, autogenic recharge from direct infiltration occurred throughout the entire period of the investigation. One consideration for cave manage-ment is to identify the higher and lower permeability fracture zones overlying the cave through the continued use of ER sensors and drip monitoring. To aid in this endeavor, precipitation measurements should be initiated above the cave. These data would be useful for producing a detailed assessment of vulnerability of formations to low-rainfall years.

Both climate fluctuations and the opening of the cave to visitors are likely contributing to changes in water and water vapor transport within the cave, but the data above show that decreased precipitation and overhead infiltration of water into the cave contributes directly to the reduction of water on the formations throughout the cave. Even if opening of the cave is increasing the inflow of dry air near the entrance, humidity measurements near the formations
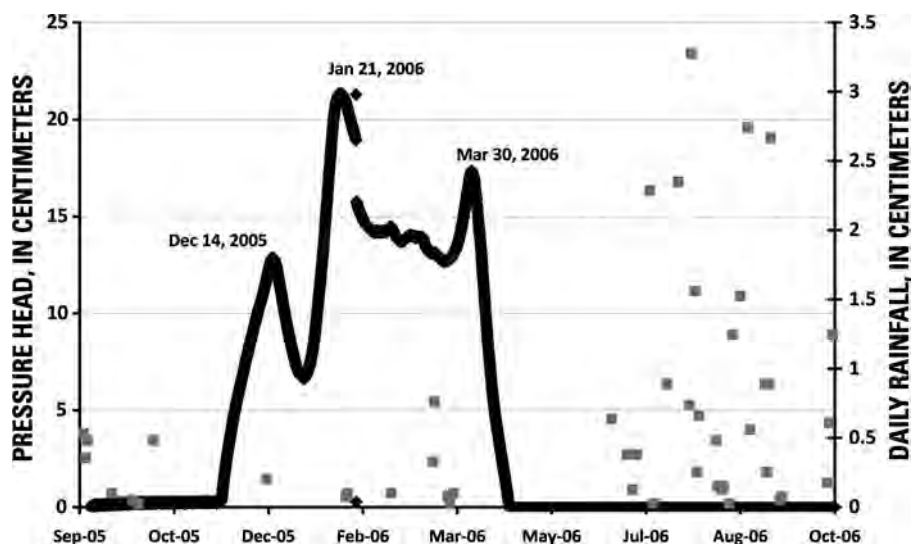
Figure 10. Pressure-head (depth) measurements of ponded water in the Subway Tunnel. Daily precipitation shown as gray squares.

show from no change to a slight increase. While more detailed air-inflow studies are in progress at the cave entrance, humidity measurements, speleothem drip-rate measurements, and ER measurements should continue to be conducted throughout the cave for assistance with future visitor management.

CONCLUSIONS

During this investigation, recharge of water to Kartchner Caverns from surface channel infiltration, overland infiltration from precipitation, and groundwater inflow was monitored using electrical-resistance sensors, temperature sensors, pressure transducers, and groundwater levels. The investigation coincided with a period of less-than-average rainfall, permitting the comparison of results from this study to the baseline investigations at Kartchner Caverns conducted during a period of higher-than-average rainfall. Monitoring of ephemeral streamflow in the surface channels overlying the cave and the presence of water in the cave itself indicated that the primary difference between wet and dry climate periods is the almost complete absence of any surface channel infiltration recharging the cave system during dry periods. Additionally, overhead autogenic recharge from infiltrating precipitation decreased during drier periods. Humidity measured in the cave at the monitoring points away from the cave entrance was consistent with wetter periods. This would indicate that, although the amount of water flowing on speleothems decreased during the drier period, existing drops on cave speleothems do not more readily evaporate. Finally, electrical-resistance sensors proved valuable for wetness detection on speleothems and within cave drainage channels. Encapsulated circuitry was necessary for instrument integrity within the humid cave environment.

REFERENCES

Adams, E., Monroe, S., Springer, A., Blasch, K.W., and Bills, D.J., 2006, Electrical resistance sensors record spring flow timing, Grand Canyon, Arizona: Ground Water, v. 44, no. 5, p. 630–641. doi: 10.1111/j.1745-6584.2006.00223.x.

Blasch, K., Ferré, P., Christensen, A., and Hoffmann, J.P., 2002, A new field method to determine streamflow timing using electrical resistance sensors: Vadose Zone Journal, v. 1, p. 289–299.

Buecher, R.H., 1992, Final report, environmental and geologic studies for Kartchner Caverns State Park, Arizona Conservation Projects, Inc., 328 p.

Buecher, R.H., 1999, Microclimate study of Kartchner Caverns, Arizona: Journal of Cave and Karst Studies, v. 61, no. 2, p. 108–120.

Graf, C.G., 1999, Hydrogeology of Kartchner Caverns State Park, Arizona: Journal of Cave and Karst Studies, v. 61, no. 2, p. 59–67.

Gray, R.S., 1967, Petrography of the Upper Cenozoic non-marine sediments in the San Pedro Valley, Arizona: Journal of Sedimentary Petrology, v. 37, p. 774–789.

Hill, C.A., 1999, Overview of Kartchner Caverns, Arizona: Journal of Cave and Karst Studies, v. 61, p. 41–43.

Jagnow, D.H., 1999, Geology of Kartchner Caverns State Park, Arizona: Journal of Cave and Karst Studies, v. 61, no. 2, p. 49–58.

Lange, A.L., 1999, Geophysical Studies at Kartchner Caverns State Park, Arizona: Journal of Cave and Karst Studies, v. 61, no. 2, p. 68–72.

Lange, A.L., Gustafson, E.P., and Walen, P.A., 1990, Geophysical field studies at Kartchner Caverns State Park, Arizona, Arizona Conservation Projects, Inc, 49 p.

Lerch, R.N., Wicks, C.M., and Moss, P.L., 2005, Hydrologic characterization of two karst recharge areas in Boone County, Missouri: Journal of Cave and Karst Studies, v. 67, no. 3, p. 158–173.

Melton, M.A., 1965, The geomorphic and paleoclimatic significance of alluvial deposits in southern Arizona: Journal of Geology, v. 73, p. 1–38. doi: 10.1086/627044.

National Climatic Data Center, 2008a, Climate Summaries, http://www.wrcc.dri.edu/summary/climsmaz.html, [accessed September 14, 2008].

National Climatic Data Center, 2008b, U.S. Temperature-Precipitation-Drought Index (DSI-9640), http://www7.ncdc.noaa.gov/CDO/CDODivisionalSelect.jsp#, [accessed September 14, 2008].

White, W.B., 1988, Geomorphology and Hydrology of Karst Terrains, New York, Oxford University Press, 464 p.

White, W.B., 2003, Conceptual models for karstic aquifers: Speleogenesis and Evolution of Karst Aquifers , v. 1, no. 1, 6 p., re-published from:, Palmer, A.N., Palmer, M.V., and Sasowsky, I.D., eds., 1999, Karst Modeling, Karst Waters Institute Special Publication 5, p. 11–16.

ESCHERICHIA COLI, OTHER COLIFORM, AND ENVIRONMENTAL CHEMOHETEROTROPHIC BACTERIA IN ISOLATED WATER POOLS FROM SIX CAVES IN NORTHERN ALABAMA AND NORTHWESTERN GEORGIA

Joshua W. Campbell[1], Anna Watson[1], Cindy Watson[2], Hannah Ball[1], and Richard Pirkle[1]

Abstract: *Escherichia coli* and other bacteria can be used as indicators of water quality within a cave ecosystem. However, bacterial species within caves have not been thoroughly documented, especially in the southeastern United States. Water from isolated pools was gathered along transects from six caves in northern Alabama and northwestern Georgia. We used cultivation techniques to isolate and characterize bacteria. Diversity of coliforms and some environmental genera were determined for each cave, and abundance was determined for *E. coli* and other coliforms. Distance from the entrance in most caves did not statistically correlate with abundance or species richness of bacteria. A total of fifty bacterial species and one fungal species were isolated from the six caves, with over half of these species considered potentially pathogenic in humans. Some species isolated, such as *Vibrio alginolyticus* and *V. fluvialis*, are considered primarily marine and are not expected isolates of cave waters. Most of the species we isolated have never been reported from limestone cave ecosystems. Overall, coliforms were found in all tested caves, indicating fecal contamination of all six caves.

INTRODUCTION

Microbial communities in caves are poorly understood (Groth and Saiz-Jimenez, 1999), but caves do support a complex microbial life (Northup and Lavoie, 2001; Caumartin, 1963). Caves contain unique populations that rely on both chemoautotrophic and heterotrophic interactions for survival. Many bacteria in caves are considered non-native species that have been transported into a cave via water, air, or animals (Northup and Lavoie, 2001), and how these bacteria interact and compete with endemic microflora is unknown. Alabama and Georgia are cave-rich states (Culver et al., 2006) that offer opportunities for microbial studies. Most caves in these two states are found in the Appalachian Plateaus and Interior Low Plateaus physiographic provinces and are formed in fossiliferous Paleozoic limestone. Despite its having a high density of caves, there is a scarcity of literature for this region concerning cave bacteria.

Most caves are considered to be nutrient (carbon) limited (Poulson and White, 1969; Culver, 1970), and cave-inhabiting organisms may be constrained by these limitations (Peck, 1976; Taylor et al., 2005). By breaking down organic matter and recycling nutrients, heterotrophic bacteria are important components of a cave ecosystem. Most bacterial communities in caves depend, like other heterotrophs, on allochthonous sources of organic matter (Laiz et al., 2000). However, large increases of bacteria in cave waters, associated with increased organic matter, can rob the waters of dissolved oxygen, which could cause massive changes in community structure of invertebrates and other organisms (Graening and Brown, 2003).

Coliform bacteria (Enterobacteriacae) are gram-negative, aerobic and facultatively anaerobic, rod-shaped bacteria that ferment lactose to produce acid. They produce β–galactosidase within 48 hours at 35 °C (Clesceri et al., 1998). Some coliforms are found in soil or vegetation, whereas fecal coliform are bacteria that usually live in the intestines of warm-blooded animals, making them a useful indicator of fecal contamination. Outside the intestines of warm-blooded animals, fecal coliform bacteria can survive for extended time periods. In karst aquifers, *E. coli* and other fecal coliform bacteria can remain viable for several months in water and stream sediments (Davis et al., 2005) and can be transported several kilometers in karst aquifers (Green et al., 1990). Lower temperatures found in cave environments reduce bacterial metabolism and increase their life span, allowing them to survive in these less-than-optimum conditions (Davis et al., 2005).

Coliform bacteria can be indicators for potential pathogens responsible for various waterborne diseases (Hunter et al., 2004) or be pathogens themselves, such as certain strains of *E. coli*. Coliform contamination can occur in caves (e.g. Lerch et al., 2001; Hunter et al., 2004; Kelly et al., 2009), with probable sources being the

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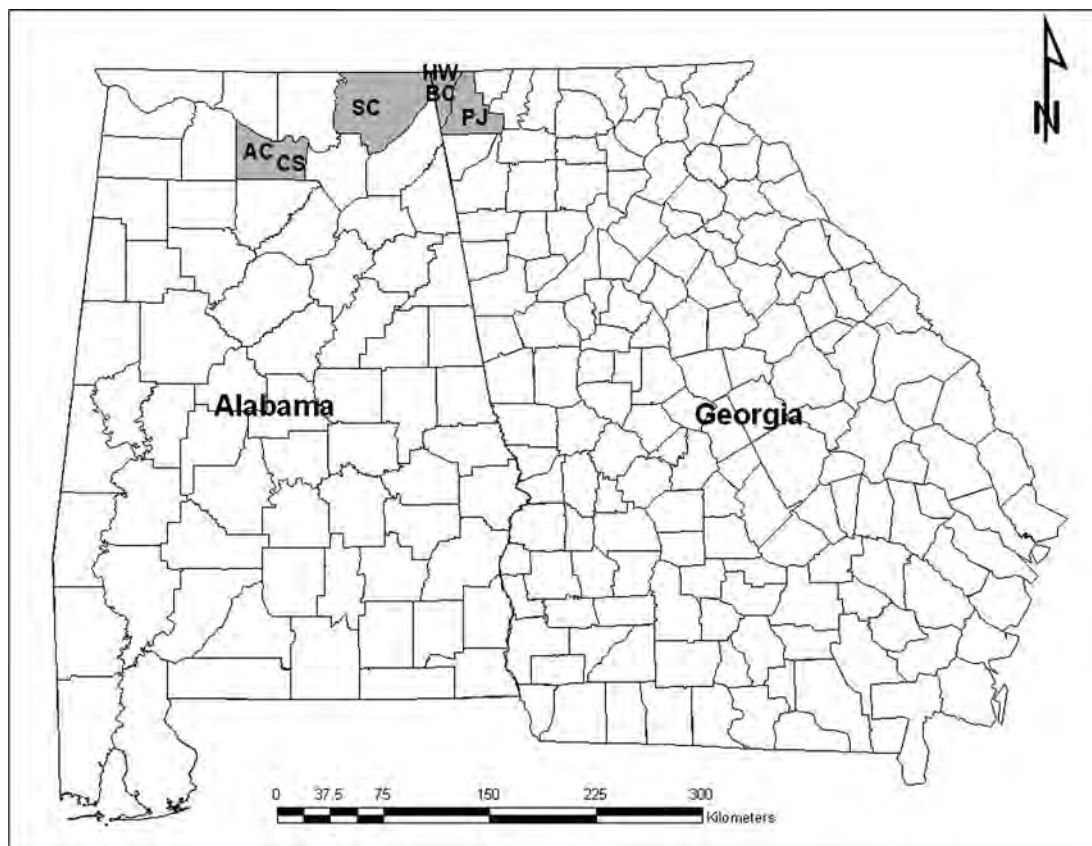
Figure 1. Approximate locations of the six caves in our study. AC=Anvil Cave, BC=Byers Cave, CS=Cave Springs Cave, HW=Howard's Waterfall Cave, PJ=Pettyjohns Cave, SC=Sauta Cave. Counties in which these caves are located have been shaded.

watershed or animals that excrete waste products within the cave. Coliform and environmental bacteria can enter a cave by water that drains into a cave from the watershed (Lerch et al., 2001; Kelly et al., 2009), primarily from a stream flowing directly into a cave or by seepage through soil or rock. Bats and other animals can also be major contributors of coliforms by excreting waste products within a cave ecosystem. Human visitors are another possible source of coliform bacteria within a cave ecosystem. Humans can bring bacteria into caves on the soles of their shoes and by dumping feces (Hunter et al., 2004). Some English caves showed contamination of certain bacterial species after cavers passed through (Moore and Sullivan, 1997, p. 80). Northup et al. (1997) found that cave areas frequently traversed by humans contained more bacterial species than less-frequented areas. Therefore, the recreational use of caves and human-induced land-use changes could lead to increases in coliform bacteria to levels considered a public health threat, while also affecting the natural bacterial assemblages in ways that are not yet understood.

In this study we tested water pools in six caves from northern Alabama and northwestern Georgia for abundance and species richness of coliform and other environ-

mental bacteria. We hypothesized that caves with more human or animal visitors or in a watershed with agricultural or sewage runoff would have higher bacterial abundance and species richness. We also hypothesized that abundance of coliforms and other bacteria would be greater closer to the entrance.

METHODS

STUDY SITES

Between January and July 2008, water samples were gathered and tested from six caves in northern Alabama (Anvil and Cave Springs Caves, Morgan Country; Sauta Cave, Jackson County) and northwestern Georgia (Byers and Howard's Waterfall Cave, Dade County; Pettyjohns Cave, Walker County) (Fig. 1). These six caves were chosen due to their length and accessibility, the presence of pools, and the different numbers of human and animal visitors at the caves. All of our collection sites are in horizontal caves in fossiliferous limestone.

SAMPLING PROCEDURE

Within each cave we aseptically took water samples from locations three or four different distances from the

entrance. At each location we took two 100 ml samples, generally from each of five cave pools. The pools had surface areas of 100 cm^2 to 1000 cm^2 and were separated by at least 1 meter. To avoid spikes of re-suspended bacteria from soil during storms, selected cave pools were not connected to any stream or runoff area and appeared to be supplied primarily by dripping stalactites. The selected pools rarely, if ever, become dry. Sauta and Cave Springs Caves contain streams that flow from their entrances. If isolated cave pools could not be found, stagnant areas of the creeks were sampled.

One water sample from each cave pool was stored at 2 to 4 °C for future cultures, and a second undiluted sample was poured and sealed into a Colisure quanti-tray (IDEXX Laboratories, Inc.) and placed in an incubator at 35 °C for up to 48 hours. Enzyme-based methods are widely accepted as a standard for detecting E. coli and other coliforms in water (Olstadt et al., 2007). Colisure products are approved by the Environmental Protection Agency for testing of drinking water and groundwater for total coliform bacteria and E. coli and are able to detect E. coli and other coliforms at one organism (up to 2419.6) per 100 ml (U.S. EPA, 1994; U.S. EPA, 1997; U.S. EPA, 1999, p. V-12–V-13). Coliforms produce the enzyme β-galactosidase, which is detected by cleaving Colisure's nutrient-indicator CPRG (chlorophenol red β-D-galactopranoside). The cleaving causes the water sample to change color over a 24 to 48 hour period from yellow to red or magenta, and E. coli causes the sample to fluoresce green when exposed to UV light because E. coli metabolizes the nutrient indicator MUG (4-methyllumbelliferyl b-D-glucuronide). Colisure products detect E. coli and other coliforms with high confidence and suppress the growth of galactosidase-producing non-coliform organisms, thus resulting in a low rate of false positives (Olstadt et al., 2007). Our abundance tests are presumptive, but we did culture E. coli from all water samples, thus confirming the presence of E. coli.

Bacterial Identification Procedure

A subsample (10 μl) of the water that had been stored at 2 to 4 °C was plated on MacConkey agar, a selective and differential plating medium used for isolating and differentiating lactose-fermenting from non-lactose fermenting gram-negative bacteria (BBL MacConkey II Agar). The cultures were incubated at 37 °C for ~48 hours. If no growth was present after 48 hours, pool water (10 μl) was inoculated into tryptic soy broth, incubated overnight and then plated back onto MacConkey agar. Any bacterial colonies that appeared morphologically (color, shape, or size) different were transferred to tryptic soy agar with 5% sheep blood. Qualitative oxidase and indole tests were completed using BBL DrySlide products. Bacteria that were oxidase negative and indole positive were again streaked on MacConkey and Simmon's Citrate media. MacConkey plates that were lactose positive with a strong precipitate around the colony and Simmon's Citrate

negative were determined to be E. coli. If MacConkey plates were lactose + with no precipitate or lactose − and the Simmon's Citrate was + or −, the bacteria were placed on a Trek Sensititre System GN ID Panel. A 0.5 McFarland suspension of the isolate was prepared in sterile distilled water and inoculated into the GN ID Panel. If a bacteria was not identified by the Trek Sensititre System, the bacteria was placed on a Bio Merieux API 20E or Remel Rap ID NF+ System for identification. All procedures followed standard methods (Clesceri et al., 1998).

Abundance and species richness data were analyzed with GLM (Statistix 9 program, Analytical Software, Tallahassee, Florida) to conduct one-way ANOVAs with distance intervals as the independent variable and coliform bacteria and E. coli abundances and total number of bacterial species cultured as dependent variables. The Tukey's multiple comparison procedure in the same program was used to determine differences in relative abundances of E. coli and other coliform bacteria and bacterial species among caves. Due to differences in location and number of water pools among caves, locations were assigned to three zones, the entrance zone 0–75 m, the intermediate zone 75–125 m, and the deep zone >125 m, for 166 bacterial abundances and species richness analysis.

Results

We collected ten coliform and forty environmental bacterial species from the six caves, with at least twenty-seven of these species considered to be potentially pathogenic. Most bacterial species were isolated from Pettyjohns Cave, followed by Sauta and Cave Spring Caves (Table 1). There was little consistency in species occurrence between caves, with thirty-three species isolated from only one cave (Table 1). E. coli was the only species isolated from all caves. Cave Springs Cave had significantly higher E. coli and other coliform abundance compared to all other caves except Sauta Cave (Fig. 2). Neither species richness ($P = 0.8102$) or abundance ($P = 0.2572$) of E. coli and other coliform were significantly different among distance intervals when 163 data from all caves were pooled into different zones. However, many differences were detected between various distance zones within individual caves (Table 2). Because we did not dilute our water samples, some of our bacteria abundance numbers are conservative. Due to the heterogeneity and availability of water pools within caves, results will be discussed separately for each cave. The forty environmental bacteria species were not targeted and considered incidental in our cultures. Therefore, our species richness numbers do not reflect the true species richness of each cave.

Anvil Cave

E. coli and other coliform bacteria were significantly higher within the entrance zone compared to all other

ESCHERICHIA COLI, OTHER COLIFORM, AND ENVIRONMENTAL CHEMOHETEROTROPHIC BACTERIA IN ISOLATED WATER POOLS FROM SIX CAVES IN NORTHERN ALABAMA AND NORTHWESTERN GEORGIA

Table 1. Bacteria species isolated from six caves located in North Alabama and Northwest Georgia. *Escherichia coli* is the only species cultivated from all caves.

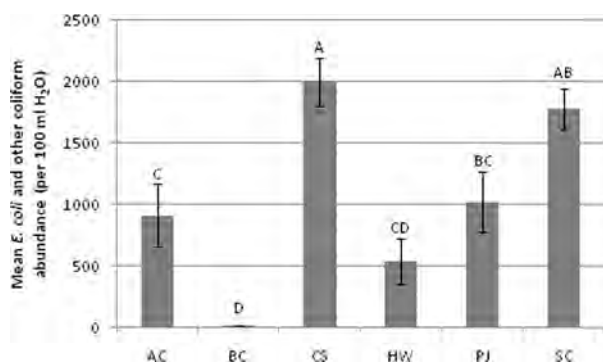| Bacteria Species	Anvil Cave	Byers Cave	Cave Springs	Howard's Waterfall	Pettyjohns Cave	Sauta Cave
Coliform Bacteria						
Citrobacter freundii	X				X	X
Enterobacter amnigenus	X					
Enterobacter cancerogenus				X	X	
Enterobacter intermedius	X					
Enterobacter sp.	X				X	
Escherichia coli	X	X	X	X	X	X
Klebsiella ozaenae					X	
Klebsiella oxytoca					X	
Klebsiella pneumo ss pneumoniae					X	
Pantoea agglomerans				X	X	X
Environmental Bacteria						
Acinetobacter baumannii					X	
Acinetobacter lwoffi			X		X	
Aeromonas caviae				X	X	X
Aeromonas hydrophilia		X			X	X
Aeromonas sp.						X
Aeromonas sobria						X
Bacillus sp. 1						X
Bacillus sp. 2		X	X			X
Brevundimonas diminuta					X	
Chromobacterium sp.						X
Chryseobacterium meningosepticum			X			
Comamonas testosterone					X	
Empedobacter brevis			X			
Hafnia alvei	X				X	
Kluyvera cryocrescens				X		
Listonella (Vibrio) anguillarum				X	X	
Micrococcus sp.			X			
Moraxella osloensis	X					
Myroides odoratum			X			
Ochrobactrum anthropi	X					
Proteus mirabilis			X			
Pseudomonas aeruginosa				X		
Pseudomonas aeruginosa/putida				X		
Pseudomonas alcaligenes	X	X	X	X	X	
Pseudomonas flourescens		X	X		X	X
Pseudomonas flourescens/putida		X	X			X
Pseudomonas mendocina			X			X
Pseudomonas pseudoalcaligenes						X
Pseudomonas stutzeri		X			X	X
Pseudomonas sp.			X	X	X	X
Psychrobacter phenylpyruvica		X				
Serratia fonticola						X
Serratia marcescens					X	
Serratia plymuthica					X	
Serratia proteamaculans					X	
Shewanella putrefaciens						X
Sphingomonas paucimobilis						X
Stentrophomonas maltophilia					X	
Vibrio alginolyticus	X					
Vibrio fluvialis	X					

Figure 2. Mean total abundance (+/−SE) (per 100 ml of H$_2$O) of *E. coli* and other coliform from six caves located in north Alabama and northwest Georgia. Columns with the same letter(s) are not significantly different at $P \leq 0.05$ according to Tukey's multiple comparison procedure. AC=Anvil Cave, BC=Byers Cave, CS=Cave Springs Cave, HW=Howard's Waterfall Cave, PJ=Pettyjohns Cave, SC=Sauta Cave.

distance zones, and the intermediate zone was significantly higher in coliforms than the deep zone. We collected eleven species of bacteria from Anvil Cave (Table 1), seven of which were isolated from the entrance zone. Two *Vibrio* species (*V. alginolyticus* and *V. fluvialis*) were isolated from Anvil Cave. They are common in estuarine and marine environments (Roberts et al., 1982), but can be causative agents of diseases in humans (West, 1989).

Byers Cave

Although not significantly different, *E. coli* and other coliforms were higher in abundance in the deep zone compared to the entrance zone (Table 2). Only eight bacteria species were isolated from Byers (Table 1) and, overall, *E. coli* and other coliforms were found in very low abundance compared to all other caves.

Cave Springs Cave

E. coli and other coliform abundance were significantly higher in the deep zone compared to the entrance zone (Table 2). Thirteen species of bacteria were isolated from Cave Springs Cave (Table 1). Within the intermediate zone, a *Bipolaris* spp. (dematiaceous fungus) was isolated, several species of which are considered to be a potential human pathogens.

Howard's Waterfall Cave

E. coli and other coliforms were higher in abundance in the entrance zone compared to the deep zone, but the difference was not significant (Table 2). We collected ten species of bacteria from Howard's Waterfall Cave (Table 1). Despite low abundance of coliforms within the deep zone, seven species of bacteria were isolated there, including *Listonella* (*Vibrio*) *anguillarum. L. anguillarum*

is primarily isolated from marine environments, but has on occasion been isolated from freshwater environments (Chen et al., 2006). It causes fish disease worldwide (Powell and Loutit, 1994).

Pettyjohns Cave

E. coli and other bacteria were found throughout our sampling transect. No significant differences in abundance were noted between the entrance and deep zone (Table 2). We collected twenty-four species of bacteria from Pettyjohns Cave (Table 1). *Listonella* (*Vibrio*) *anguillarum* was also isolated from the entrance zone of this cave.

Sauta Cave

E. coli and other coliform bacteria were not significantly different among the distance zones (Table 2). However, *E. coli* abundance did increase at deeper distance intervals. Nineteen species were collected within Sauta Cave (Table 1). One species collected, *Shewanella putrefaciens,* is a common environmental isolate that composes biofilms (McLeod et al., 2002) and can use multiple electron acceptors (including manganese) for aerobic respiration (Myers and Myers, 2001), which may allow for its survival in cave ecosystems.

Discussion

A great deal of heterogeneity existed among caves, such as location and separation of water pools. Although distance from an entrance is a variable that can influence bacterial abundance (as observed in Anvil Cave), other variables appear to be more important in controlling abundance and species richness of coliform bacteria in cave ecosystems. Outside influences, such as number of human and bat visitors and watershed differences, differed among caves, which probably accounted for the lack of correlation between *E. coli* and other bacteria abundances and species richness and distance from an entrance. Abundance levels of *E.coli* and other coliform bacteria fluctuated widely among water pools in the same distance zone of some caves, suggesting that isolated pools are not necessarily influenced by other nearby pools, and bacterial presence may depend more heavily on a direct source such as human or animal visitors rather than an indirect source such as watershed contamination.

The isolation of many potentially pathogenic bacteria from these cave waters suggests that these species are able to remain viable in a low metabolic state. *E. coli* has been shown to survive longer in soil kept at low temperatures (Teague et al., 1995), and Flint (1987) found *E. coli* surviving for extended periods of time in cold river water. Some of these coliforms and environmentals may be adhering to biofilms on the substrate, which could help sustain the species in cave environments. Epilithic biofilms have been shown to be an important energy source for aquatic cave communities (Simon et al., 2003). Biofilms

Table 2. Mean *E. coli* and other coliform abundance (+/−SE) (per 100 ml H$_2$O) from each cave and the various distance zones within each cave.

Cave	Entrance Zone	Intermediate Zone	Deep Zone
Anvil Cave	2419.6 (0)[a]	1035.8 (564.5)[b]	100.68 (52.3)[c]
Byers Cave	6.76 (3.1)[a]	NP	23.87 (7.8)[a]
Cave Springs Cave	1065.5 (553.1)[a]	2099 (320.7)[ab]	2419.6 (0)[b]
Howard's Waterfall Cave	823.86 (352.5)[a]	NP	253.3 (68.8)[a]
Pettyjohns Cave	1108.6 (336.3)[a]	NP	939.25 (361.8)[a]
Sauta Cave	1758.5 (298.9)[a]	1813 (173.3)[a]	1780.5 (288.4)[a]

Note: Within each cave means of various distance zones followed by the same letter(s) are not significantly different ($P \leqslant 0.05$) according the Tukey's multiple comparison procedure.

NP = No pools present.

Lower case letters indicate which caves have differences in bacterial abundance among the three zones.

can also trap nutrients (Costerton and Lappin-Scott, 1989), which can increase the survival of non-endemic bacteria (Boyle et al., 1991).

Although our cultivation techniques favored cultivation of coliform bacteria, we also isolated forty environmental chemoheterotrophic bacterial species. Our study highly underestimated environmental bacteria, because our cultivation techniques targeted coliform bacteria, and our result is not representative of the true bacterial species diversity. Many cave bacteria species may have been inhibited by our media or inoculation temperature, which do not mimic a cave environment. According to McNamara and Mitchell (2005), less than 10% of bacteria ($< 1\%$ in many instances) from an environmental sample can be cultivated by standard laboratory techniques.

Each cave illustrated unique physical differences that may help explain differences in abundance and species of bacteria. Anvil Cave, a maze cave, has over 20 km (13 miles) of passages and several entrances and receives few human visitors. Most human visitors and other animals will necessarily pass through an entrance zone, but they may become dispersed in the mazes beyond these zones, which could account for the higher *E. coli* and other coliform abundances and species richness within the first distance zone. Anvil Cave is, however, located primarily underneath pasture land that could be a source of bacteria.

Byers Cave, which is one of Georgia's largest caves, contains over 8 km (5 miles) of passages and contains a waterfall, and many pools are present within the deep zone, here > 400 m from the entrance. Byers Cave is not heavily trafficked by humans, and water seepage from the general watershed is a probable source for the bacteria in these distance zones. Byers Cave is located within a pristine wooded area that is far from any human development, which could account for the overall low abundance and species richness of bacteria.

Cave Springs and Sauta Caves both have large openings and contain streams roughly 3 to 5 meters wide that slowly drain towards the entrance. Large colonies of endangered gray bats (*Myotis grisescens*) inhabit Cave Springs and Sauta Caves, with maternity colonies formed during spring

and summer. These caves are closed to the public and get only an occasional human visitor. Total organic matter in soil has been shown to increase in areas with larger numbers of bats in Cave Springs and Sauta Caves (Joshua Campbell et al., unpubl. data), which probably accounted for the high abundance of *E. coli* and other coliforms.

Howard's Waterfall Cave is a horizontal cave that is over 3 km long (2 miles long) and receives approximately 1000 human visitors per year, with most only exploring the first part of the cave, which could account for the increased abundance of *E. coli* and other coliforms within the entrance zone. However, watershed sources and other animals are also possible sources for the increased abundance of *E. coli* and other coliforms from the entrance zone. The higher number of species isolated from the deep zone (including *Listonella (Vibrio) anguillarum*) is an interesting result and may suggest a watershed source of bacteria, even though overall coliform and other bacteria abundance was low in this zone.

Pettyjohns Cave has over 10.5 km (6.5 miles) of passages and is a popular cave that draws approximately 10,000 visitors per year (Padgett, 1999). The majority of human visitors only explore near the entrance, which could explain the high numbers of bacteria within the entrance zone. In one area 300 to 325 meters in, a spike in *E. coli* and other coliforms was observed. Here the cave narrows, which forces any cavers to pass through any pools present, suggesting that the high number of human visitors could be a culprit in these spikes. Although the high number of species is likely due to the high number of people that visit this cave, watershed sources and other animals could also be contributing factors. Neither Howard's Waterfall nor Pettyjohns Caves contain fish, which suggests *Listonella (Vibrio) anguillarum* entered from the watershed through the aquifer, rather than by way of possibly diseased fish.

CONCLUSIONS

Due to the heterogeneity of these caves, general patterns can be difficult to discern. However, bacterial abundance and species richness in caves can be influenced by humans,

other animals such as bats, and watershed uses. Cave bacteria can be used as bioindicators of water quality, and high abundance could be an ecosystem warning signal. High levels of bacteria abundance, especially coliforms, could also be a human health concern. Humans could come into direct contact with contaminated pools by caving and indirectly through water wells. Overall, we found widespread fecal contamination in all caves, and people with scrapes or cuts should use caution while caving. Drinking directly from pools without filtration should be avoided at all times. Many organisms that live in caves are very sensitive to ecosystem disturbances and may have limited populations and slow reproduction times. How increases in non-native bacterial species affect cave macroinvertebrates and other animals should be investigated.

References

Boyle, M., Ford, T., Maki, J.S., and Mitchell, R., 1991, Biofilms and the survival of opportunistic pathogens in recycled water: Waste Management and Research, v. 9, p. 465–470, doi: 10.1177/0734242X9100900165.

Caumartin, V., 1963, Review of the microbiology of underground environments: Bulletin of the National Speleological Society, v. 25, p. 1–14.

Chen, C.Y., Chao, C.B., and Bowser, P.R., 2006, Infection of tilapia Oreochromis sp. by Vibrio vulnificus in fresh water and low-salinity environments: Journal of the World Aquaculture Society, v. 37, p. 82–88, doi: 10.1111/j.1749-7345.2006.00010.x.

Clesceri, L.S., Greenberg, A.E., and Eaton, A.D., eds., 1998, Standard Methods for the Examination of Water and Wastewater, 20th ed., APHA, Washington, D.C., 1325 p.

Costerton, J.W., and Lappin-Scott, H.M., 1989, Behavior of bacteria in biofilms: American Society for Microbiology News, v. 55, p. 650–654.

Culver, D.C., 1970, Analysis of simple cave communities I. Cave as islands: Evolution, v. 24, p. 463–474.

Culver, D.C., Deharveng, L., Bedos, A., Lewis, J.J., Madden, M., Reddell, J.R., Sket, B., Trontelj, P., and White, D., 2006, The mid-latitude biodiversity ridge in terrestrial cave fauna: Ecography, v. 29, p. 120–128, doi: 10.1111/j.2005.0906-7590.04435.x.

Davis, R.K., Hamilton, S., and Van Brahana, J., 2005, Escherichia coli survival in mantled karst springs and streams, northwest Arkansas Ozarks, USA: Journal of the American Water Resources Association, v. 41, p. 1279–1287, doi: 10.1111/j.752-1688.2005.tb03800.x.

Flint, K.P., 1987, The long-term survival of Escherichia coli in river water: Journal of Applied Bacteriology, v. 63, p. 261–270, doi: 10.1111/j.1365-2672.1987.tb04945.x.

Graening, G.O., and Brown, A.V., 2003, Ecosystem dynamics and pollution effects in an Ozark cave stream: Journal of the American Water Resources Association, v. 39, p. 1497–1507, doi: 10.1111/j.1752-1688.2003.tb04434.x.

Green, W., Ellicott, L., and Crawford, N., 1990, Investigation of nonpoint source pollution associated with karst aquifer systems: Transactions of the Kentucky Academy of Science, v. 51, p. 177–181.

Groth, I., and Saiz-Jimenez, C., 1999, Actinomycetes in hypogean environments: Geomicrobiology Journal, v. 16, p. 1–8.

Hunter, A.J., Northup, D.E., Dahm, C.N., and Boston, P.J., 2004, Persistent coliform contamination in Lechuguilla cave pools: Journal of Cave and Karst Studies, v. 66, p. 102–110.

Kelly, W.R., Panno, S.V., Hackley, K.C., Martinsek, A.T., Krapac, I.G., Weibel, C.P., and Storment, E.C., 2009, Bacteria contamination of groundwater in a mixed land-use karst region: Water Quality Exposure and Health, v. 1, p. 69–78, doi: 10.1007/s12403-009-0006-7.

Laiz, L., Groth, I., Schumann, P., Zezza, F., Felske, A., Hermosin, B., and Saiz-Jimenez, C., 2000, Microbiology of the stalactites from Grotta dei Cervi, Porto Badisco, Italy: International Microbiology, v. 3, p. 25–30.

Lerch, R.N., Erickson, J.M., and Wicks, C.M., 2001, Intensive water quality monitoring in two karst watersheds of Boone County, Missouri, in Rea, G.T., ed., Proceedings of the 2001 National Cave and Karst Management Symposium, Tucson, Arizona, Oct. 16–19, 2001, Tucson, Coronado National Forest, p. 151–168.

McLeod, E.S., MacDonald, R., and Brözel, V.S., 2002, Distribution of Shewanella putrefaciens and Desulfovibrio vulgaris in sulphidogenic biofilms of industial cooling water system determined by fluorescent in situ hybridization: Water SA, v. 28, p. 123–128.

McNamara, C.J., and Mitchell, R., 2005, Microbial deterioration of historic stone: Frontiers in Ecology and the Environment, v. 3, p. 445–451, doi: 10.1890/1540-9295(2005)003[0445:MDOHS]2.0.CO;2.

Moore, G.W., and Sullivan, G.N., 1997, Speleology: Caves and the Cave Environment, third edition: St. Louis, Cave Books, 176 p.

Myers, J.M., and Myers, C.R., 2001, Role for outer membrane cytochromes OmcA and OmcB of Shewanella putrefaciens MR-1 in reduction of manganese dioxide: Applied and Environmental Microbiology, v. 67, p. 260–269, doi: 10.1128/AEM.67.1.260-269.2001.

Northup, D.E., Beck, K.M., and Mallory, L.M., 1997, Human impact on the microbial communities of Lechuguilla cave: Is protection possible during active exploration?, abs., National Speleology Society Convention Program, Sullivan, Missouri, 55 p.

Northup, D.E., and Lavoie, K.H., 2001, Geomicrobiology of caves: A review: Geomicrobiology Journal, v. 18, p. 199–222, doi:10.1080/01490450152467750.

Olstadt, J., Schauer, J.J., Standridge, J., and Kluender, S., 2007, A comparison of ten USEPA approved total coliform/E. coli tests: Journal of Water and Health, v. 5, p. 267–282, doi: 10.2166/wh.2007.008.

Padgett, A., 1999, Pigeon Mountain: Thirty years of noninterference, in Rea, G.T., ed., Proceedings of the 1999 National Cave and Karst Management Symposium, Chatanooga, Tennessee, Southeastern Cave Conservancy, p. 143–147.

Peck, S.B., 1976, The effect of cave entrances on the distribution of cave-inhabiting terrestrial arthropods: International Journal of Speleology, v. 8, p. 309–321.

Poulson, T.L., and White, W.B., 1969, The cave environment: Science, v. 165, p. 971–981.

Powell, J.L., and Loutit, M.W., 1994, The detection of fish pathogen Vibrio anguillarum in water and fish using a species-specific DNA probe combined with membrane filtration: Microbial Ecology, v. 28, p. 375–383, doi:10.1007/BF00662030.

Roberts, N.C., Siebeling, R.J., Kaper, J.B., and Bradford, Jr., H.B., 1982, Vibrios in the Louisiana coast environment: Microbial Ecology, v. 8, p. 299–312, doi: 10.1007/BF02010670.

Simon, K.S., Benfield, E.F., and Macko, S.A., 2003, Food web structure and the role of epilithic biofilms in cave streams: Ecology, v. 84, p. 2395–2406.

Taylor, S.J., Krejca, J.K., and Denight, M.L., 2005, Foraging range and habitat use of Ceuthophilus secretus (Orthoptera: Rhaphidophoridae),

a key trogloxene in central Texas cave communities: American Midland Naturalist, v. 154, p. 97–114.

Teague, K.A., Wolf, D.C., and Vendrell, P.F., 1995, Survival of fecal contamination indicator organisms in soil: Arkansas Water Resources Center, Publication No. 173, 28 p.

U.S. EPA, 1994, National Primary and Secondary Drinking Water Regulations: Analytical Methods for Regulated Drinking Water Contaminants: Federal Register, 5 December 1994, 40 Code of Federal Regulations parts 141, 143 p.

U.S. EPA, 1997, Manual for the Certification of Laboratories Analyzing Drinking Water: Criteria and Procedures, Quality Assurance, fourth edition: Environmental Protection Agency document 815-B-97-001, 186 p.

U.S. EPA, 1999, National Primary and Secondary Drinking Water Regulations: Analytical Methods for Chemical and Microbiological Contaminants and Revisions to Laboratory Certification Requirements: Final Rule: Federal Register, 1 December 1999, p. 67449–67467, 40 Code of Federal Regulations parts 141, 143 p.

West, P.A., 1989, The human pathogenic vibrios—A public health update with environmental perspectives: Epidemiology and Infection, v. 103, p. 1–34, doi: 10.1017/S0950268800030326.

THE MAMMALIAN FAUNA OF ABISMO IGUATEMI, SOUTHEASTERN BRAZIL

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Abstract: The Quaternary vertebrate fauna record of South America is characterized by the predominance of mammals, and the study of cave deposits can provide important information on their diversity and distribution. In Brazil, cave deposits have preserved remarkable fossil remains, including both large and small vertebrates, although the former have been the focus of most paleontological works. The fossils studied here came from Abismo Iguatemi, a karstic fissure located in the municipality of Apiaí, upper Ribeira River valley, São Paulo, Brazil. Blocks of sediment collected from its floor yielded a large sample of micro-remains, mainly composed of fragmentary small vertebrate specimens. Taphonomic parameters suggest that the fossil elements entered the cave either by entrapment or transported by rain runoff, as partially decayed carcasses or isolated elements. A total of 35 taxa were recorded in Abismo Iguatemi, four of which are extinct. The number of identified specimens per taxon (NISP index) is the best estimator of number of individuals at the burial site. The comparison of this fauna to that of other Quaternary deposits and to the present biodiversity of different areas reveals low similarity. The identification of fossil organisms with different ecological requirements (extinct savannah organisms and extant dense-forest organisms) suggests the existence of time averaging and may reflect environmental changes in the vicinity of the cave during the late Pleistocene and Holocene.

INTRODUCTION

The abundance and diversity of mammals, including the megafauna, is remarkable in the Quaternary fossil record of South America. Important information on the mammalian diversity can be obtained through the study of cave deposits. Caves sometimes act as natural traps for living animals and burial sites for organic remains. These are often protected from the normal processes of disintegration, such as scavenging, erosion, and fluvial transportation (Behrensmeyer and Hook, 1992). In the cave deposits, reworking and redeposition of skeletal remains by floods (Salles et al., 1999) and other fluvial activity (Sutcliffe, 1970) are common. The reliability of fossil deposits can be estimated by their completeness and by temporal or spatial averaging (Behrensmeyer and Kidwell, 1985; Behrensmeyer and Hook, 1992; Behrensmeyer et al., 2000). Karst deposits usually represent local habitats (i.e., the remains are not transported beyond the life habitat of the fauna) and may be averaged over time intervals of 100 to 10,000 years (Behrensmeyer, 1988; Andrews, 1990, p. 93–95; Behrensmeyer and Hook, 1992). These deposits typically include both large and small fossil vertebrates and are especially important for the latter, which are less commonly preserved in other depositional contexts (Andrews and Evans, 1983; Andrews, 1990, p. 1; Fernández-Jalvo and Andrews, 1992).

In Brazil, following the pioneer work of Peter Wilhelm Lund (Lund, 1840), cave deposits provided a great deal of well-preserved fossil remains (Paula-Couto, 1953, p. 26–33), and they have been continuously explored (Cartelle and Hartwig, 1996; Lessa et al., 1998; Salles et al., 1999; Santos et al., 2002). Although the Quaternary fossil record shows high abundance and diversity of small mammals, these have been mostly disregarded in paleontological works in Brazil (Salles et al., 1999). Stratigraphic control has often been poor, and taphonomic and geochronologic studies are scarce (Auler et al., 2006). Concerning their age, Brazilian cave deposits are loosely dated as late Quaternary. Some authors (e.g., Cartelle, 1995) consider their faunas as contemporaneous and representative of the Pleistocene-Holocene transition. Doubts about the synchronicity and age of these deposits have long been recognized (Paula-Couto, 1975) and are supported by the broad range of U-series dates reported for mammalian faunas of northeastern Brazilian caves (Auler et al., 2006). Moreover, recent work has produced Pleistocene records for living genera, as well as early Holocene ages for extinct taxa (Baffa et al., 2000; Neves and Piló, 2003; Auler et al., 2006, Hubbe et al., 2007).

The fossil remains studied here were collected during September 2001 from a karstic fissure known as Abismo Iguatemi. Along with the blocks of sediment containing the micro-remains, macrofossils were recovered from the bottom of the cave, but no stratigraphic or taphonomic information was recorded. The site was first explored in November 1999 by Ferreira and Karmann, (2002), who retrieved paleontological material, including remains of a

ground sloth (*Catonyx cuvieri*), and the skull of a saber-tooth cat (*Smilodon populator*). Postcranial elements possibly associated with that skull were described later (Castro and Langer, 2008).

STUDY AREA

Abismo Iguatemi is located about 5 km southwest of the town of Apiaí, upper Ribeira River valley, São Paulo, Brazil (Fig. 1), in an area presently covered by ombrophilous dense (Mata Atlântica) and secondary forests (Kronka, 2005, p. 53). Many caves in the region were previously studied (Ameghino, 1907; Krone, 1950; Paula-Couto, 1954, 1973; Lino et al., 1979; Barros Barreto et al., 1982) and yielded mainly megafauna remains. The 15 m deep and 44 m long fissure is formed in the intensely folded rocks of the Açungui Group (Middle Proterozoic), which includes metamorphized dolomitic limestones intercalated with insoluble lithologies (Auler and Farrant, 1996). The deposits in the cave are mainly composed of black to reddish clay associated with larger clasts of limestone, filite, and calcite crusts. The poor sorting of the grains is probably related to water percolating through the fissure, as it is located in the middle of a gentle slope (Ferreira and Karmann, 2002). The present access to the cave is limited to a small vertical slot.

ANALYSES AND RESULTS

The blocks of sediment were disintegrated in water and screen-washed using a 1 mm mesh. The recovered micro-remains are mainly fragmentary small vertebrate specimens ranging from a few millimeters to about 10 cm and are housed at Museu de Zoologia da Universidade de São Paulo. Several studies have demonstrated that a wide assortment of paleontological aspects, such as hydrodynamic sorting and preservational properties, are affected by the bone size (see Kowalewski and Hoffmeister, 2003). To avoid any bias related to the choice of discrete size ranges, the total fraction of bioclasts was analyzed. This presumably provides a more accurate interpretation of the fossil assemblage by sampling a greater spectrum of organisms (Rodrigues and Simões, 2004). The fragmentation and weathering (polishing and abrasion) of the fossils restricted their taxonomic assignments. Out of 4,534 recovered remains, 3% were taxonomically identified at generic or specific level, 13% at suprageneric levels (minimally class), and 41% were only anatomically identified. Besides mammalian specimens, which represented 74% of the taxonomically identified sample, amphibian (15%), reptilian (6%), and avian (5%) material was also collected.

TAPHONOMIC ASPECTS

The taxonomically or anatomically identified mammal elements are mostly fragmented and not articulated, except for series of a maximum of three vertebrae of small, indeterminate mammals. Large forms include only *Smilodon populator*, *Catonyx cuvieri*, and *Glyptodon clavipes*, the remains of which exhibit similar weathering to the remains of the small taxa. The polished and abraded surfaces, mostly concentrated on the extremities of the fossils, may be related to either pluvial transport into or reworking inside the cave. Spatial distribution of the bones is scattered, except for the remains of *Smilodon populator* and *Cebus* cf. *C. nigritus*, which were clustered, probably representing the disarticulation of single skeletons. Bone fractures are straight and uniform, a pattern frequently related to completely or partially mineralized material (Holz and Simões, 2002, p. 73), which also suggests reworking.

The analyzed remains include 559 isolated teeth and 226 disarticulated vertebrae, which make a 2.47 ratio, consistent with fluvial transportation (Behrensmeyer and Dechant-Boaz, 1980). In this case, however, the small proportion of vertebrae may be related to depletion during their transport by rain runoff or reworking inside the cave. Hydraulic sorting was estimated by Voorhies Groups (Voorhies, 1969; Behrensmeyer, 1975), which classify bioclasts based on their dispersal trends. The allochthonous nature of the Abismo Iguatemi fossil assemblage is corroborated by the minor proportion of skeletal elements that tend to resist transportation, i.e., Group III, and the prevalence of Groups I and II (Table 1), composed of, respectively, remains that tend to be promptly removed from carcasses and elements removed gradually. Accordingly, it is assumed that most of the fossilized mammalian fauna lived in habitats in the vicinity of the cave, not inside it.

Given the nearly vertical entrance of Abismo Iguatemi, likely sources of bioclasts are (1) the fall of animals into the natural trap, (2) floating carcasses or (3) isolated specimens of animals that died upslope and were washed into the cave, and (4) the animals that lived and died inside the cave. Based on the discussion above, we believe that most of the recovered material entered the cave from the third source, although the clustered remains with non-overlapping elements were probably accumulated by the first or second sources. The relatively scarce chiropteran record suggests that bats did not inhabit the cave; otherwise this group would be represented by more complete fossils and would be more abundant.

COUNTING INDIVIDUALS: MNI VERSUS NISP INDICES

Relative abundance of taxa in fossil assemblages is frequently estimated by the determination of the number of individuals per taxon. The numerical results may vary markedly with the adoption of different counting criteria, but the context of bone accumulation provides the required basis to decide which is more appropriate (Badgley, 1986). If the material is widely dispersed due to extensive transportation and accumulated as isolated specimens, it
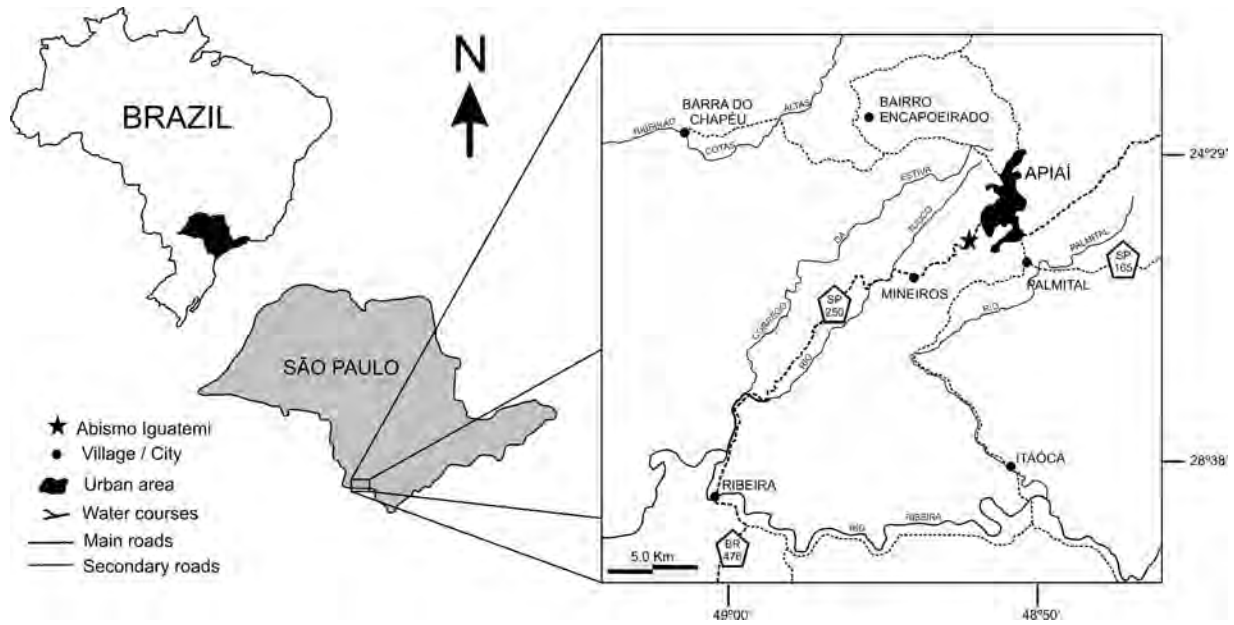
Figure 1. Location of Abismo Iguatemi, São Paulo, Brazil.

Table 1. Number and relative abundance of skeletal elements per Voorhies Groups.

Skeletal Elements	Number of Elements			Relative Abundance (%)
	Taxonomically Identified	Anatomically Identified Only	Total	
Group I				
Astragalus	1	16	17	0.67
Calcaneus	0	23	23	0.91
Phalanges	0	130	130	5.14
Ribs	11	73	84	3.32
Vertebrae	2	224	226	8.94
Isolated teeth	527	32	559	22.10
Scapula	0	7	7	0.28
Osteoderm	3	0	3	0.12
Subtotal	544	505	1049	41.48
Group II				
Femora	1	107	108	4.27
Humeri	0	36	36	1.42
Ulnae	1	24	25	0.99
Pelvis	2	16	18	0.71
Radius	1	0	1	0.04
Other long bones	27	1161	1188	46.98
Subtotal	32	1344	1376	54.41
Group III				
Mandible	64	0	64	2.53
Maxillae	34	0	34	1.34
Skull	1	5	6	0.24
Subtotal	99	5	104	4.11
Total	675	1854	2529	100

is suitable to infer the number of individuals based on the number of specimens of each taxon (NISP index). This method assumes that the probability of association (i.e., that more than one skeletal element of a fossil assemblage belongs to the same individual) is zero. In contrast, determining the minimum number of individuals represented by multiple skeletal elements (MNI index) assumes a high probability of association and is appropriate for material that accumulated in articulated form and was later disarticulated by reworking, as occurs in natural traps (Badgley, 1986). Both approaches were tested here in an attempt to infer which better fits the studied case. Tooth locus determination was ambiguous for the molars of some rodents, especially for teeth considerably weathered on the occlusal surface that probably belonged to senile individuals. Accordingly, tooth locus was not used to determine MNI for Rodentia, so this index may be an underestimate for the group.

A total of 35 taxa were recorded in Abismo Iguatemi (Figs. 2 and 3), four of which are extinct, and the number of individuals per taxon estimated by both NISP and MNI indices is provided in Table 2. Badgley (1986) suggested adopting the MNI index for natural-trap deposits because their fossil assemblages include articulated material, clustered skeletal elements of the same taxon, and relatively little bone damage. However, fossils from Abismo Iguatemi do not fit this combination of features: articulated sequences are rare, most material has a scattered spatial distribution and is polished or abraded. Indeed, the choice of the counting method must be based upon underlying assumptions on the probability of skeletal association, given the accumulation processes inferred from the taphonomic characteristics of the fossil assemblage. In Abismo Iguatemi, the MNI index is preferable if bioclastic sources 1, 2, or 4 (see above) occurred, while the NISP index is appropriate if mode 3 was the case. Relative abundance, using either NISP or MNI indices (Fig. 4), reveals that rodents are the most abundant mammalian group recovered from Abismo Iguatemi, as also seen in the living fauna of the area (Silva, 2001).

COMPARING SITES

The mammalian fauna of Abismo Iguatemi was compared with that of several other Brazilian Quaternary deposits and with the present diversity of different modern habitats (Table 3). Because species diversity consists of richness (the number of species) and evenness (or equitability) of species abundances, quantitative coefficients of similarity, i.e., Bray-Curtis and Morisita-Horn indexes, are considered more accurate than qualitative indexes (Magurran, 1988, p. 95–96). However, because not all of the faunas with which Abismo Iguatemi was compared include abundance, Jaccard's qualitative index was adopted. This is based on the number of species in each sample, which were counted and weighted equally. Ten faunal lists were used for comparison, including five

Quaternary fossils sites and five extant ecosystems. The latter include three areas of ombrophilous dense forest (Vaz, 2005), two of which are near Abismo Iguatemi (Pianca, 2001; Instituto Ambiental do Paraná, 2002, p. VI 22–27), as well as two savannah areas, one in the state of São Paulo (Lyra-Jorge and Pivello, 2005; Talamoni, 1996) and another in Central Brazil (Passamani, 2004). Out of the fossil assemblages used for comparison, three are located in the municipality of Iporanga, near Abismo Iguatemi (Ameghino, 1907; Paula-Couto, 1973; Lino et al., 1979; Barros Barreto et al., 1982), and two in areas presently covered by savannah in Central Brazil (Souza-Cunha and Guimarães, 1982; Salles et al., 1999). For comparison to the extant faunistic inventories, extinct taxa of Abismo Iguatemi (*Smilodon populator*, *Catonyx cuvieri*, and *Glyptodon clavipes*) were not considered. In order to match the restricted collection efforts of some inventories (e.g., non-flying mammals or exclusively small mammals), some taxa recorded in Abismo Iguatemi were excluded from the comparisons. In addition, because various faunal lists were uncertain about identification to species, the comparisons were conducted at the generic level.

Although generally low, Jaccard indices are higher for comparisons with living faunas (30% similarity on average) than with other fossil assemblages (17% on average). They are never above to 36% and 25%, respectively. Comparisons to faunas of either dense forest or savannah are similar. This may be a consequence either of time-averaging in Abismo Iguatemi or many taxa with wide geographic distributions, living in different phytophysiognomies (see below).

DISCUSSION AND CONCLUSIONS

Regardless of the difficulties associated with the study of fossil assemblages composed of fragmentary remains, the attempt to extract additional information from Abismo Iguatemi revealed that skeletal remains probably entered this cave partially articulated (trapped or transported by rain runoff as partial or complete decaying carcasses) as well as in the form of isolated elements. The former was probably the case for the clustered elements of *Smilodon populator*, *Catonyx cuvieri*, and *Cebus* cf. *C. nigritus*. For the other taxa, the deposition of isolated specimens by transport was presumably more frequent. In this case, NISP values seem to better fit the faunistic composition of the studied deposit.

Although Brazilian cave deposits usually have been assigned to the Pleistocene-Holocene boundary, new radiometric dates challenge this assumption, suggesting a time span encompassing much of the upper Pleistocene and early Holocene (Auler et al., 2006; Hubbe et al., 2007). Specimens of *Catonyx cuvieri* and *Smilodon populator* from Abismo Iguatemi provided ^{14}C AMS ages of 10,800 (BETA 230974) and 14,580 (BETA 183566) years BP respectively (A. Hubbe, pers. comm., 2008), confirming a
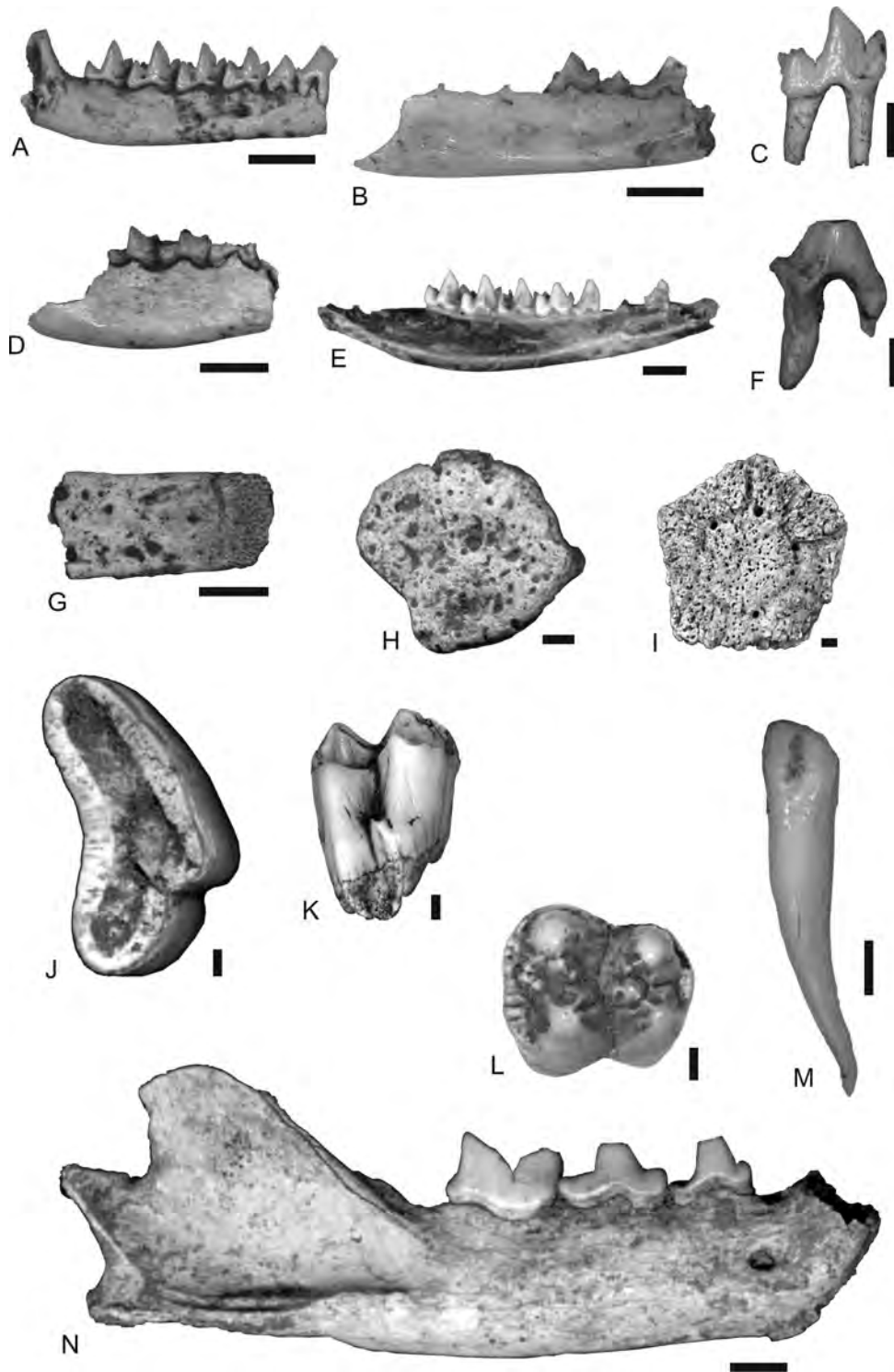
Figure 2. Marsupials, xenarthrans, artiodactylans, and carnivorans from Abismo Iguatemi. A. *Cryptonanus* **sp., left mandibular fragment in lingual view; B.** *Gracilinanus* **sp., right mandibular fragment in labial view; C.** *Philander* **sp., probable M_2 in labial view; D.** *Monodelphis* **sp., right mandibular fragment in labial view; E.** *Metachirus nudicaudatus*, **right mandibular fragment in labial view; F.** *Didelphis aurita*, **probable right P^2 in lingual view; G. Dasypodidae indet., osteoderm fragment in external view; H. Glyptodontidae indet., osteoderm fragment in external view; I.** *Glyptodon clavipes*, **osteoderm in external view; J.** *Catonyx cuvieri*, **right M^2 in occlusal view; K.** *Mazama gouazoubira*, **molar in labial view; L.** *Pecari tajacu*; **probable right M^2 in occlusal view; M.** *Cerdocyon thous*, **lower left incisor in labial view; N.** *Leopardus wiedii*, **left mandibular ramus in labial view. Scale bars = 2mm (A–D, F, G, K–M), = 4mm (E, H, I, N).**
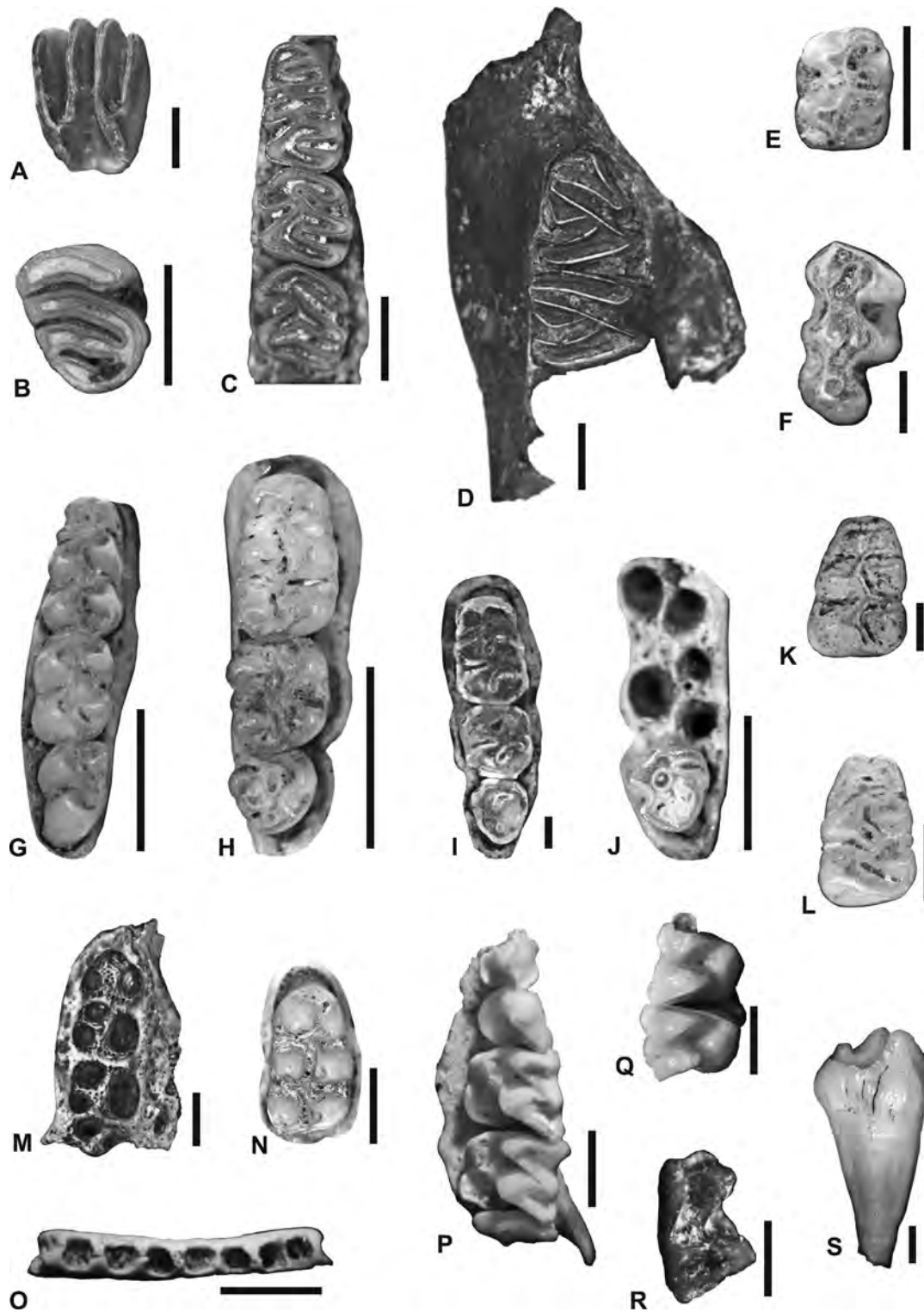
Figure 3. Rodents, bats, and primate from Abismo Iguatemi. A–R, occlusal views: A. *Kannabateomys* **sp., molar fragment; B.** *Trinomys* **sp., molar; C.** *Phyllomys* **sp., left mandibular fragment; D.** *Cavia* **sp., left mandibular fragment; E.** *Oryzomys* **cf.** *O. capito*, **molar; F.** *Blarinomys breviceps*, **M¹; G.** *Akodon* **sp., left mandibular fragment; H.** *Oligoryzomys* **sp., right maxillar fragment; I.** *Oryzomys* **sp., left maxillar fragment; J.** *Calomys* **sp., left maxillar fragment; K.** *Oryzomys* **cf.** *O. angouya*, **left M₁; L.** *Wilfredomys* **sp., M¹; M.** *Sciurus aestuans*, **right maxillar fragment; N.** *Wilfredomys oenax*, **right M¹; O.** *Carollia* **sp., right mandibular fragment; P.** *Mimon benettii*, **left maxillar fragment; Q.** *Molossus* **sp., right M¹⁻²; R.** *Myotis* **sp., M³. S.** *Alouatta* **cf.** *A. fusca*, **probable left M₁ in lingual view. Scale bars = 1mm (E–G, K, L, Q, R); = 2mm (A–D, H–J, M–P, S).**

Table 2. Identified taxa and numbers of individual according to NISP and MNI indexes.

Taxon	NISP index	MNI index
Marsupialia		
Cryptonanus sp.	1	1
Didelphis aurita	1	1
Gracilinanus sp.	2	2
Metachirus nudicaudatus	2	2
Monodelphis sp.	1	1
Philander sp.	6	2
Subtotal	13	9
Xenarthra		
Dasypodidae indet.	1	1
Glyptodon clavipes[a]	1	1
Glyptodontidae indet.[a]	1	1
Catonyx cuvieri[a]	17	1
Subtotal	20	4
Carnivora		
Cerdocyon thous	1	1
Leopardus wiedii	1	1
Smilodon populator[a]	21	1
Subtotal	23	3
Chiroptera		
Molossus sp.	2	1
Carollia sp.	2	1
Mimon benettii	6	2
Myotis sp.	1	1
Subtotal	11	5
Primates		
Cebus cf. *C. nigritus*	154	1
Alouatta cf. *A. fusca*	1	1
Subtotal	155	2
Artiodactyla		
Mazama gouazoubira	1	1
Pecari tajacu	1	1
Subtotal	2	2
Rodentia		
Kannabateomys sp.	1	1
Phyllomys sp.	57	7
Trinomys sp.	16	2
Cavia sp.	9	1
Akodon sp.	4	1
Blarinomys breviceps	1	1
Calomys sp.	1	1
Oligoryzomys sp.	3	2
Oryzomys sp.	13	2
Oryzomys cf. *O. angouya*	2	1
Oryzomys cf. *O. capito*	1	1
Wilfredomys sp.	1	1
Wilfredomys oenax	1	1
Sciurus aestuans	1	1
Subtotal	111	23
Total	335	48

[a] Extinct Taxa.
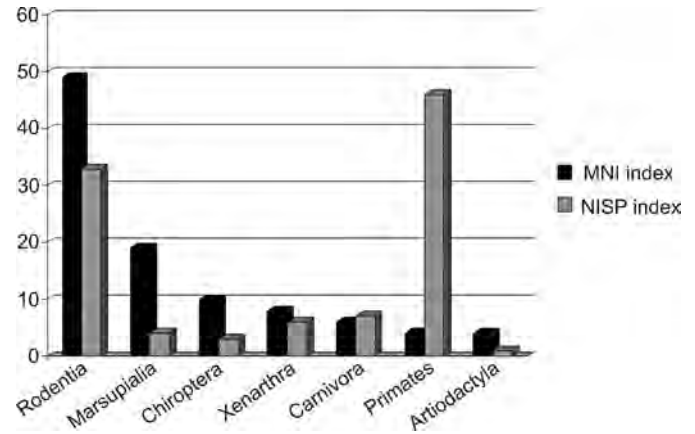
Figure 4. Relative abundance (%) of individuals identified to generic or specific level using MNI and NISP indices.

late Pleistocene age for these extinct species. Because of the likelihood of time-averaging in the deposit (see below), it is possible that its extant taxa are Holocene in age. This could be confirmed by radiometric dates. In the absence of these, and considering the poor stratigraphic control during the collection of the Abismo Iguatemi fauna, a more accurate chronologic assignation is unfeasible.

Along with absolute dates and taphonomic signatures, paleoecological data can also be used to identify time-averaging in Quaternary assemblages. Paleoenvironmental inferences are best based on the known habitat requirements of extant species, or on fossils with adaptations to particular environments (Auler et al., 2006). Almost half of the extant taxa recorded in Abismo Iguatemi are not informative for this sort of inference, because they occur over a wide geographic range, in both savannah and dense forested areas (Weksler et al., 1999; Wilson and Reeder, 2005, p. 6–7, 12–17, 412, 440–441, 501–517, 540, 578, 644, 656, 1106–1109, 1140–1143, 1552–1553; Voss et al., 2005). On the other hand, among the extinct taxa, *Glyptodon clavipes* and *Catonyx cuvieri* are usually considered open-country herbivores (Vizcaíno and Bargo, 1998; Ubilla and Perea, 1999; Perez et al., 2000), possibly preyed upon by *Smilodon populator*, which is less confidently assigned to savannah habitats (Paula-Couto, 1979, p. 338–340; Kurtén and Werdelin, 1990). Small mammals are usually considered good proxies for climatic conditions (Avery, 1982), and some living taxa identified in the cave are endemic to ombrophilous dense forest (Mata Atlântica), e.g., *Didelphis aurita* (Silva, 2001), *Blarinomys breviceps* (Silva et al., 2003), *Kannabateomys* sp., *Wilfredomys* sp., *Trinomys* sp., and *Phyllomys* sp. (Leite, 2003, p. 101).

It is possible that all of the recovered taxa coexisted in a mosaic of savannah and moist habitats or, contrary to the currently accepted notion, that the extinct *Glyptodon clavipes*, *Catonyx cuvieri*, and *Smilodon populator* may have also inhabited forested areas, as has been inferred for other megafaunal elements in the Brazilian Amazon

Table 3. Similarity indexes (C$_{Jaccard}$) between mammalian fauna of Abismo Iguatemi and other faunal inventories. N(A) Number of taxa considered in Abismo Iguatemi; N(B) Number of taxa considered in the other studies; N(AB) Number of taxa represented in both sites; AI is Abismo Iguatemi faunistic assemblage.

Fauna Type	N(A)	N(B)	N(AB)	C$_{Jaccard}$
Quaternary Fossils Assemblages				
AI/Karstic caves, Iporanga, SP[a]	29	15	4	0.10
AI/Abismo do Fóssil, Iporanga, SP[b]	29	21	10	0.25
AI/Abismo Ponta de Flecha, Iporanga, SP[c]	29	17	5	0.12
AI/Caves in Serra da Mesa, GO[d]	29	41	11	0.19
AI/Lapa Vermelha, MG[e]	29	21	8	0.19
Living Faunas				
AI/Parque do Zizo, São Miguel Arcanjo, SP[f]	6	11	4	0.31
AI/Pedra Branca, Paraty, RJ[g]	22	31	14	0.36
AI/Parque das Lauráceas, Adrianópolis, PR[h]	26	31	10	0.21
AI/Vicinity of Palmas, TO[i]	16	23	9	0.30
AI/Pé-de-Gigante and Jataí, Luís Antônio, SP[j]	22	36	14	0.32

[a] Ameghino (1907) and further alterations by Paula-Couto (1973).
[b] Lino et al. (1979).
[c] Barros Barreto et al. (1982).
[d] Salles et al. (1999).
[e] Souza-Cunha and Guimarães (1982).
[f] Pianca (2000).
[g] Vaz (2005).
[h] IAP (2002).
[i] Passamani (2004).
[j] Talamoni (1996), Lyra-Jorge and Pivello (2005).

(Rossetti et al., 2004; MacFadden, 2005). Otherwise, we suggest that the occurrence in Abismo Iguatemi of organisms interpreted as having different ecological requirements is related to time-averaging, instead of spatial averaging, because karstic deposits have minimum transport beyond the immediate paleocommunity habitat (Behrensmeyer and Hook, 1992). Indeed, this may be an example of an Environmentally Condensed Assemblage, where extended time-averaging allows finding specimens associated with different environments, of ecologically unrelated species, and with varied taphonomic signatures (Kowalewski, 1996). The existence of two typical faunistic groups (extinct open field or savannah and extant ombrophilous dense forest organisms) suggests that environmental changes occurred in the Holocene of South America, when the establishment of a more humid phase would have caused the expansion of landscapes with denser vegetation and the extinction of the megafauna that inhabited open physiognomies (see de Vivo and Carmingnotto, 2004). Some of the discussed uncertainties could be resolved with further studies; the Holocene age of the extant taxa could be confirmed by radiometric dating, while the habitat requirements of the herbivores could be determined by stable isotope methods.

References

Ameghino, F., 1907, Notas sobre una pequeña colección de huesos de mamíferos procedentes de las grutas calcarías de Iporanga, en el estado de São Paulo, Brazil: Revista do Museu Paulista, v. 3, p. 59–124.

Andrews, P.J., and Evans, E.M.N., 1983, Small mammal bone accumulations produced by mammalian carnivores: Paleobiology, v. 9, no. 3, p. 289–307.

Andrews, P.J., 1990, Owls, caves and fossils: predation, preservation and accumulation of small mammal bones in caves, with an analysis of the Pleistocene cave faunas from Westbury-sub-Mendip, Somerset, UK: London, British Museum (Natural History), viii, 231 p.

Auler, A.S., and Farrant, A.R., 1996, A brief introduction to karst and caves in Brazil: Proceedings of the University of Bristol Spelaeological Society, v. 20, p. 187–200.

Auler, A.S., Pilo, L.B., Smart, P.L., Wang, X.F., Hoffmann, D., Richards, D.A., Edwards, R.L., Neves, W.A., and Cheng, H., 2006, U-series dating and taphonomy of Quaternary vertebrates from Brazilian caves: Palaeogeography Palaeoclimatology Palaeoecology, v. 240, no. 3–4, p. 508–522, doi: 10.1016/j.palaeo.2006.03.002.

Avery, D.M., 1982, Micromammals as palaeoenvironmental indicators and an interpretation of the Late Quaternary in the southern Cape Province, South Africa: Annals of the South African Museum, v. 85, no. 2, p. 183–374.

Badgley, C., 1986, Counting individuals in mammalian fossil assemblages from fluvial environments: Palaios, v. 1, no. 3, p. 328–338.

Baffa, O., Brunetti, A., Karmann, I., and Neto, C.M.D., 2000, ESR dating of a toxodon tooth from a Brazilian karstic cave: Applied Radiation and Isotopes, v. 52, no. 5, p. 1345–1349.

Barros Barreto, C.N.G., De Blasis, P.A.D., Dias-Neto, C.M., Karmann, I., Lino, C.F., and Robrahn, E.M., 1982, Abismo Ponta de Flecha: um projeto arqueológico, paleontológico e geológico no médio Ribeira de Iguape, São Paulo: Revista de Pré-História, v. 3, p. 195–215.

Behrensmeyer, A.K., 1988, Vertebrate preservation in fluvial channels: Palaeogeography Palaeoclimatology Palaeoecology, v. 63, no. 1–3, p. 183–199.

Behrensmeyer, A.K., and Dechant-Boaz, D.E., 1980, The recent bones of Amboseli Park, Kenya, in relation to East African paleoecology, in Behrensmeyer, A.K., and Hill, A.P., eds., Fossils in the Making, Chicago, University of Chicago Press, Prehistoric Archeology and Ecology series, p. 77–92.

Behrensmeyer, A.K., and Hook, R.W., 1992, Paleoenvironmental contexts and taphonomic modes, in Behrensmeyer, A.K., Damuth, J.D., DiMichelle, W.A., Potts, R., Sues, H.-D., and Wing, S.L., eds., Terrestrial Ecosystems Through Time: Evolutionary Paleoecology of Terrestrial Plants and Animals, Chicago, University of Chicago Press, p. 15–136.

Behrensmeyer, A.K., and Kidwell, S.M., 1985, Taphonomy's contributions to paleobiology: Paleobiology, v. 11, no. 1, p. 105–119.

Behrensmeyer, A.K., Kidwell, S.M., and Gastaldo, R.A., 2000, Taphonomy and paleobiology: Paleobiology, v. 26, no. 4, p. 103–147.

Cartelle, C., 1995, A fauna local de mamíferos pleistocênicos da Toca da Boa Vista (Laje dos Negros, BA) [Thesis]: Belo Horizonte, Universidade Federal de Minas Gerais, 131 p.

Cartelle, C., and Hartwig, W.C., 1996, A new extinct primate among the Pleistocene megafauna of Bahia, Brazil: Proceedings of the National Academy of Sciences of the United States of America, v. 93, no. 13, p. 6405–6409.

Castro, M.C., and Langer, M.C., 2008, New postcranial remains of Smilodon populator Lund, 1842 from south-central Brazil: Revista Brasileira de Paleontologia, v. 11, no. 3, p. 199–206, doi: 10.4072/rbp.2008.3.06.

de Vivo, M., and Carmignotto, A.P., 2004, Holocene vegetation change and the mammal faunas of South America and Africa: Journal of Biogeography, v. 31, no. 6, p. 943–957, doi: 10.1111/j.1365-2699.2004.01068.x.

Fernández-Jalvo, Y., and Andrews, P., 1992, Small mammal taphonomy of Gran Dolina, Atapuerca (Burgos) Spain: Journal of Archaeological Science, v. 19, no. 4, p. 407–428.

Ferreira, N.B., and Karmann, I., 2002, Descobertas paleontológicas na região de Apiaí-SP: Boletim Informativo Geovisão, v. 10, p. 7–8.

Holz, M., and Simões, M.G., 2002, Elementos fundamentais de tafonomia: Porto Alegre, Editora da Universidade-UFRGS, 231 p.

Hubbe, A., Hubbe, M., and Neves, W., 2007, Early holocene survival of megafauna in South America: Journal of Biogeography, v. 34, no. 9, p. 1642–1646, doi: 10.1111/j.1365-2699.2007.01744.x.

Instituto Ambiental do Paraná, 2002, Plano de manejo do Parque Estadual das Lauráceas: Curitiba, Secretaria de Estado do Meio Ambiente e Recursos Hídricos, 348 p.

Kowalewski, M., 1996, Time-averaging, overcompleteness, and the geological record: Journal of Geology, v. 104, no. 3, p. 317–326.

Kowalewski, M., and Hoffmeister, A.P., 2003, Sieves and fossils: Effects of mesh size on paleontological patterns: Palaios, v. 18, no. 4–5, p. 460–469, doi: 10.1086/629827.

Krone, R., 1950, As grutas calcárias do Vale do rio Ribeira de Iguape: O Instituto Geográfico e Geológico, v. 8, p. 248–297. (Reprint from Arquivos do Museu Nacional, 1909, Rio de Janeiro.)

Kronka, F.J.N., ed., 2005, Inventário florestal da vegetação natural do estado de São Paulo: São Paulo, Imprensa Oficial, 200 p.

Kurtén, B., and Werdelin, L., 1990, Relationships between North and South American Smilodon: Journal of Vertebrate Paleontology, v. 10, no. 2, p. 158–169, doi: 10.1080/02724634.1990.10011804.

Leite, Y.L.R., 2003, Evolution and systematics of the Atlantic tree rats, genus Phyllomys (Rodentia, Echimyidae), with description of two new species: University of California Publications in Zoology 132, 118 p.

Lessa, G., Cartelle, C., Faria, H.D., and Gonçalves, P.R., 1998, Novos achados de mamíferos carnívoros do Pleistoceno final-Holoceno em grutas calcárias do estado da Bahia: Acta Geologica Leopoldensia, v. 21, p. 157–169.

Lino, C.F., Dias-Neto, C.M., Trajano, E., Gusso, G.L.N., Karmann, I., and Rodrigues, R., 1979, Paleontologia do Vale do Ribeira-exploração I, Abismo do Fóssil (SP-145): Resultados parciais, in Proceedings, II Simpósio Regional de Geologia: Rio Claro, p. 257–268.

Lund, P.W., 1840, View of the fauna of Brazil previous to the last geological revolution [translated from Danish original (1938) by W. Bilton]: Magazine of Natural History, v. 4, p. 1–8, 49–57, 105–112, 153–161, 207–213, 251–259, 307–317, 373–389.

Lyra-Jorge, M.C., and Pivello, V.R., 2005, Mamíferos, in Pivello, V.R., and Varanda, E.M., eds., O Cerrado Pé-de-Gigante (Parque Estadual de Vassununga, São Paulo): Ecologia e Conservação, São Paulo, Secretaria do Meio Ambiente, p. 133–148.

MacFadden, B.J., 2005, Diet and habitat of toxodont megaherbivores (Mammalia, Notoungulata) from the late Quaternary of South and Central America: Quaternary Research, v. 64, no. 2, p. 113–124.

Magurran, A.E., 1988, Ecology diversity and its measurement: Princeton, Princeton University Press, 179 p.

Neves, W.A., and Piló, L.B., 2003, Solving Lund's dilemma: new AMS dates confirm that humans and megafauna coexisted at Lagoa Santa: Current Research in the Pleistocene, v. 20, p. 57–60.

Passamani, M., 2004, Levantamento de pequenos mamíferos não-voadores da região central do Tocantins: Humanitas, Revista do Centro Universitário Luterano de Palmas, v. 4–6, p. 55–60.

Paula-Couto, C., 1953, Paleontologia brasileira: mamíferos: Rio de Janeiro, Instituto Nacional do Livro, 516 p.

Paula-Couto, C., 1954, Megatérios intertropicais do Pleistoceno: Anais da Academia Brasileira de Ciências, v. 26, p. 447–463.

Paula-Couto, C., 1973, Edentados fósseis de São Paulo: Anais da Academia Brasileira de Ciências, v. 45, p. 261–275.

Paula-Couto, C., 1975, Mamíferos fósseis do Quaternário do sudeste brasileiro: Boletim Paranaense de Geociências, v. 33, p. 89–132.

Paula-Couto, C., 1979, Tratado de Paleomastozoologia: Rio de Janeiro, Academia Brasileira de Ciências, 590 p.

Perez, L.M., Scillato-Yané, G.J., and Vizcaíno, S.F., 2000, Morphofunctional analysis of the hyoid apparatus of Glyptodon cf. clavipes Owen (Cingulata : Glyptodontidae): Ameghiniana, v. 37, no. 3, p. 293–299.

Pianca, C.C., 2001, Levantamento de mamíferos e de sua caça em uma área preservada de mata atlântica no sudeste de São Paulo: Pontifícia Universidade de São Paulo, 45 p.

Rodrigues, S.C., and Simões, M.G., 2004, Taphonomy of brachiopod shells from the Ubatuba Bay, Northern of São Paulo coast: can methodological decisions mask the (paleo)environmental inferences?, in Proceedings, Congresso Brasileiro de Geologia, XLII. Araxá, Sociedade Brasileira de Geologia, p. 904.

Rossetti, D.F., de Toledo, P.M., Moraes-Santos, H.M., and Santos, A.E.D., 2004, Reconstructing habitats in central Amazonia using megafauna, sedimentology, radiocarbon, and isotope analyses: Quaternary Research, v. 61, no. 3, p. 289–300.

Salles, L.O., Carvalho, G.S., Weksler, M., Sicuro, F.L., Abreu, F., Camardella, A.R., Guedes, P.G., Avilla, L.S., Abrantes, E.A.P., Sahate, V., and Costa, I.S.A., 1999, Mammals from the Quaternary of Serra da Mesa (Goiás, Brazil): Publicações Avulsas do Museu Nacional, v. 78, p. 1–15.

Santos, M.F.C.F., Lima-Filho, F.P., and Bergqvist, L.P., 2002, Fósseis pleistocênicos da Ravina do Leon, Lajedo de Soledade, Apodi/RN: Revista de Geologia, v. 15, p. 23–29.

Silva, C.R., 2001, Riqueza e diversidade de mamíferos não-voadores em um mosaico formado por plantios de Eucalyptus saligna e remanescentes de Floresta Atlântica no município de Pilar do Sul-SP [M.Sc. Thesis], Piracicaba, Universidade de São Paulo, 81 p.

Silva, F.M., Barreto, A.M.F., Sá, F.B., and Silva, A.C.L., 2003, Levantamento das ocorrências de fósseis de megafauna pleistocênica do estado de Pernambuco: Paleontologia em Destaque, v. 4, p. 53–54.

Souza-Cunha, F.L., and Guimarães, M.L., 1982, A fauna sub-recente de vertebrados do "Grande Abrigo da Lapa Vermelha Emperaire (P.L.)" de Pedro Leopoldo, estado de Minas Gerais: Revista do Museu Paulista, v. 28, p. 235–272.

Sutcliffe, A.J., 1970, A section of an imaginary bone cave: Studies in Speleology, v. 2, no. 2, p. 79–80.

Talamoni, S.A., 1996, Ecologia de uma comunidade de pequenos mamíferos da Estação Ecológica de Jataí, Município de Luís Antônio-SP [Ph.D. Thesis], São Carlos, Universidade Federal de São Carlos, 173 p.

Ubilla, M., and Perea, D., 1999, Quaternary vertebrates of Uruguay: a biostratigraphic, biogeographic and climatic overview, *in* Tonni, E.P., and Cione, A.L., eds., Quaternary Vertebrate Paleontology in Southern South America, Leiden, the Netherlands, Balkema, Quaternary of South America and Antartic Peninsula 12, p. 75–90.

Vaz, S.M., 2005, Mamíferos coletados em Pedra Branca, município de Paraty, Rio de Janeiro, Brasil: Revista Brasileira de Zoologia, v. 22, no. 4, p. 1164–1169, doi: 10.1590/S0101-81752005000400051.

Vizcaíno, S.F., and Bargo, M.S., 1998, The masticatory apparatus of the armadillo *Eutatus* (Mammalia, Cingulata) and some allied genera: paleobiology and evolution: Paleobiology, v. 24, no. 3, p. 371–383.

Voorhies, M.R., 1969, Taphonomy and Population Dynamics of an Early Pliocene Vertebrate Fauna, Knox, County, Nebraska: Laramie, University of Wyoming, Contributions to Geology special paper 1, 69 p.

Voss, R.S., Lunde, D.P., and Jansa, S.A., 2005, On the Contents of *Gracilinanus* Gardner and Creighton, 1989, with the Description of a Previously Unrecognized Clade of Small Didelphid Marsupials, New York, American Museum of Natural History, Novitates no. 3482, 34 p.

Weksler, M., Geise, L., and Cerqueira, R., 1999, A new species of *Oryzomys* (Rodentia, Sigmodontinae) from southeast Brazil, with comments on the classifications of *O. capito* species group: Zoological Journal of the Linnean Society, v. 125, p. 445–462, doi: 10.1111/j.1096-3642.1999.tb00600.x.

Wilson, D.E., and Reeder, D.M., 2005, Mammal Species of the World: A Taxonomic and Geographic Reference, 3rd edition: Baltimore, Johns Hopkins University Press, 2,142 p.

CAVE MILLIPEDS OF THE UNITED STATES. IX. A NEW SPECIES OF THE GENUS *TAIYUTYLA* (DIPLOPODA, CHORDEUMATIDA, CONOTYLIDAE) FROM CAVES IN SEQUOIA AND YOSEMITE NATIONAL PARKS, CALIFORNIA, USA

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Abstract: During surveys of cave life in Sequoia and Kings Canyon National Parks and Yosemite National Park, *Taiyutyla loftinae,* a new species of conotylid milliped, was collected and is described below. The new species occurs in eleven marble caves distributed throughout Sequoia National Park (Tulare County, California), two granite talus caves, and a single surface locality in Yosemite National Park (Mariposa County, California) and is best considered troglophilic, not troglobitic.

INTRODUCTION

Species of the milliped genus *Taiyutyla* Chamberlin 1952 are a dominant feature of the diplopod fauna of the Pacific Coast states of the United States and the Canadian province of British Columbia, from the Sierra Nevada of central California to Vancouver Island (Shear, 1971, 1976, 2004); a possible undescribed species, so far known only from female specimens, may extend the range of the genus north to Alaska and the Yukon (Shelley et al, 2009). Farther inland, species are also known from Montana and Idaho (Shear, 2004). While some species of the genus have been collected from caves and are known only from those habitats, none show troglomorphy, and they are best characterized as troglophiles.

Sixteen species have been named, and at least that number of additional species are awaiting description. Given the relatively limited distributions of most of them, even more may be expected in the future. Shear (2004) briefly discussed the difficulties in delimiting the genera *Conotyla, Plumatyla*, and *Taiyutyla* in the context of describing *Taiyutyla lupus* Shear 2004 from Vancouver Island. That species has gonopod characters that combine those of both *Taiyutyla* and *Conotyla,* the former genus limited to North America west of the Continental Divide and the latter to North America east of the Mississippi River. *Taiyutyla loftinae* n. sp., described below, is similar to *T. lupus* in this respect, and even shares the unusual somatic feature (for *Taiyutyla*) of prominent, recurved paranota on the metazonites. It may be that a new generic name will be required for these species in the future, when the additional undescribed species of *Taiyutyla* have been thoroughly studied.

Meanwhile, this species is described below because it is important for conservation purposes to provide a name for it. All specimens are deposited in the California Academy of Sciences collections, San Francisco, California.

TAXONOMY

Family Conotylidae Cook 1896
Genus *Taiyutyla* Chamberlin 1952
Taiyutyla loftinae, n. sp.
Figs. 1–13, Map 1
Suggested vernacular name: Loftin's Cave Milliped.

TYPES

Male holotype and male and female paratypes from Paradise Cave, Sequoia and Kings Canyon National Parks, Tulare Co., California, collected 30 April 2004 by J. Krejca et al. Additional material from other localities is listed below.

ETYMOLOGY

The new species is named for Vivian Loftin, who dedicates her time to cave science, conservation, and exploration and whose effervescent attitude has kept spirits high throughout numerous biological research expeditions.

DIAGNOSIS

The two-branched anterior gonopods and their position relative to the posterior gonopod colpocoxites are unique among California *Taiyutyla* species.

DESCRIPTION

Male: length, 8.5 mm, greatest width (measured across paranota), 1.75 mm. Twenty-two to 24 ocelli in five rows in triangular eyepatch. Antennae long, slender. Pigmentation light brown to pale tan, faintly mottled darker purplish brown on head and anterior segments. Segments posterior to fifth with prominent, narrow paranota extending

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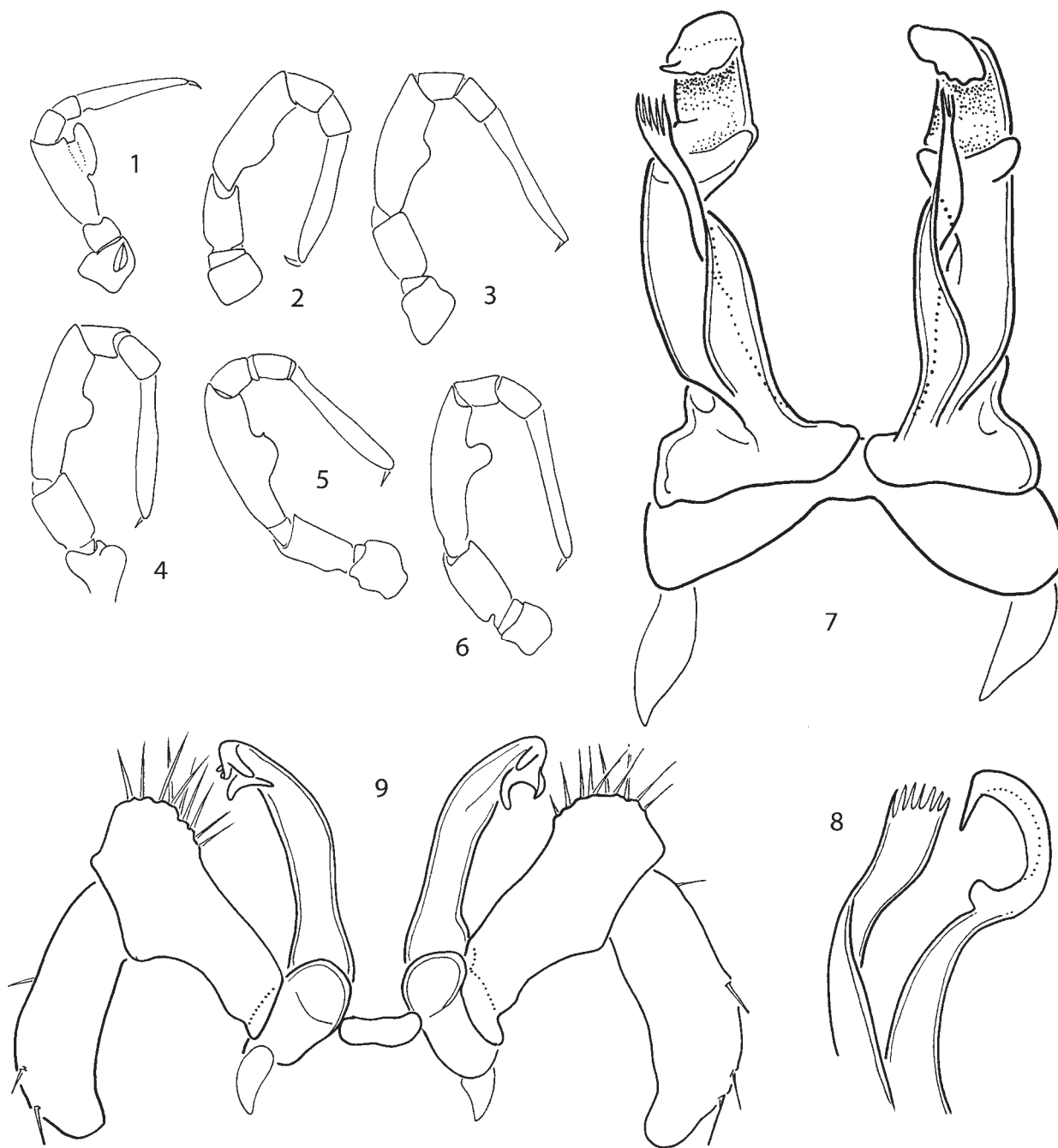
Figures. 1–9. Male *Taiyutyla loftinae,* n. sp. : Figs. 1–6: right pregonopodal legs, posterior views, drawn at 40×. (1) leg 2; (2) leg 3; (3) leg 4; (4) leg 5; (5) leg 6; (6) leg 7. Figs. 7, 8: anterior gonopods, drawn at 100×. (7) anterior view; (8) lateral view of distal part of right gonopod. Fig. 9: posterior gonopods, posterior view, drawn at 100×. Drawings not to scale.

approximately one-fourth metazonite width from lateral declevity of segment, slightly curved posteriorly, bearing two lateralmost segmental setae of each side (Fig. 12). Legpairs 2–7 with modified femora, pairs 4–7 larger than postgonopodal legs (Figs. 1–6). Femur two (Fig. 1) with large, distal, capitate knob bearing minute teeth; femora 3–7 with midlength median swelling, progressively larger on more posterior legs, forming distinct knob on seventh femur (Figs. 1–6). Anterior gonopods (Figs. 7, 8) with two branches, anterior flattened, lamellate, pectinate distally; posterior curved posteriorly, then sharply hooked anteriorly; in situ posterior branch passes laterally around posterior gonopod colpcoxites on each side. Posterior gonopods (Fig. 9) with distal and proximal telopodite articles subequal; colpocoxites simple, unbranched, with complex apices (Figs. 10, 11). Coxae of legpair 10 with prominent glands. Coxae of legpair 11 with long, mesobasal hooks projecting posteriorly.
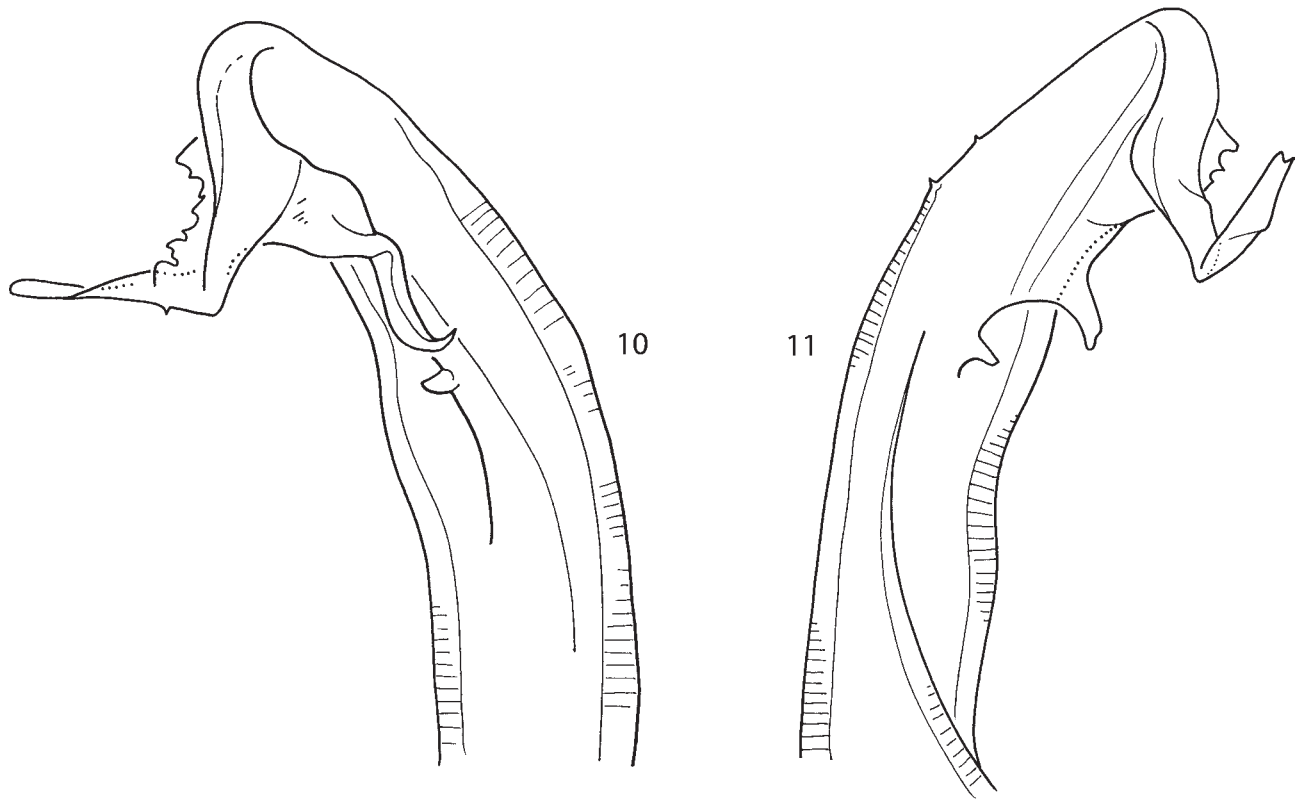
Figures. 10, 11. Male *Taiytyla loftinae*, n. sp., distal parts of posterior gonopod colpocoxites, posterior views, drawn at 400×. (10) right; (11) left.

Female: length, 9.1 mm, greatest width 1.8 mm. Nonsexual characters as in male (Fig.12).

DISTRIBUTION

Caves in Yosemite National Park and Sequoia and Kings Canyon National Parks, Mariposa and Tulare counties, California; see records listed below and Map 1. We do not provide geographical coordinates for the localities here, in the interests of conservation. The habitats in which *T. loftinae* occurs are delicate and sensitive to disturbance; the caves involved might be located only with some difficulty by those not familiar with them, and we believe we should not make that task any easier. The caves of Sequoia and Kings Canyon National Parks (Tulare County) occur in isolated marble bands separated by non-karstic granite, and as such they appear on maps in discrete
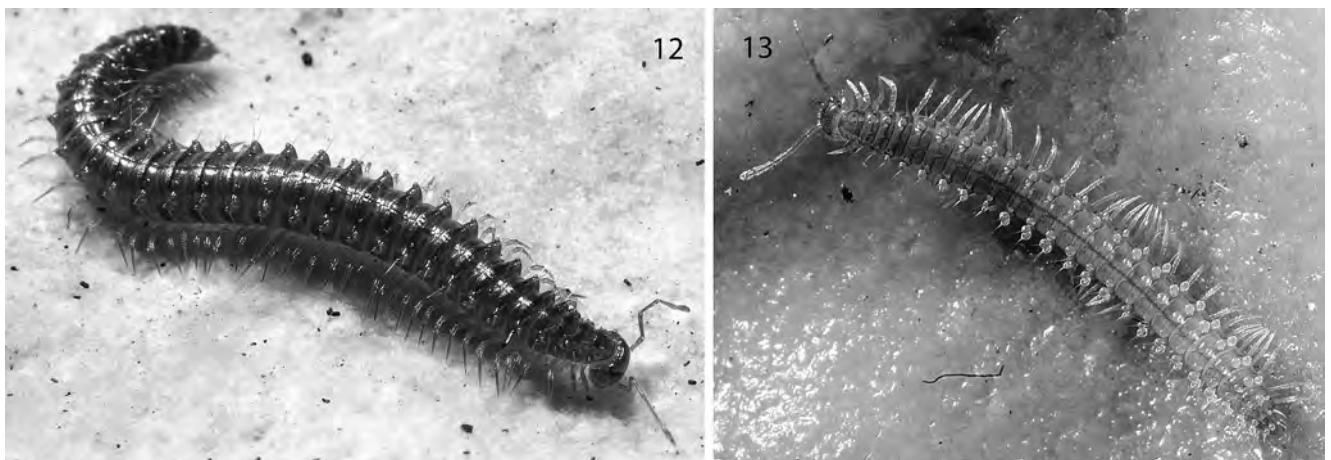
Figures. 12, 13. Live specimens of *Taiyutyla loftinae* in habitat. (12) female in 18th Hole Cave; (13) juvenile in Paradise Cave, showing presumed defensive droplets on the segmental setae.
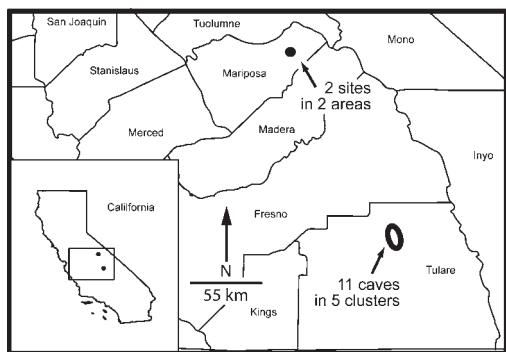
Map 1. Central California, showing distribution of *Taiyutyla loftinae* in two widely seperated areas.

clusters (see map in Shear and Krejca 2007). Five of these clusters were sampled during a multi-year inventory of cave fauna in the park, and we record *T. loftinae* from eleven caves, distributed through all five clusters. However, only juvenile specimens are known from Jordan Cave, the single Cluster 4 cave in which *Taiyutyla* specimens were taken (but note that they were observed but not collected in Bulah Cave, also in Cluster 4, though not mapped). Because of the strong similarities of these specimens to adult *T. loftinae,* we believe them to be the same species and so record them. Similarly, male specimens have not been collected in Lange Cave or Pet Cemetery Cave, both in Cluster 3, but *T. loftinae* was found in the other five caves in this cluster in which *Taiyutyla* was collected, so we have no reason to think these caves do not also support *T. loftinae,* especially given the near-identity of females from Lange Cave with females associated with *T. loftinae* males.

Nearly 200 km separate the two presently known areas of distribution for *T. loftinae,* a rather remarkable range for a *Taiyutyla* species, suggesting that it may be widespread in suitable habitats in the central Sierra Nevada.

Records: CALIFORNIA: *MARIPOSA CO.,* Yosemite National Park, Spider Cave, 3 and 6 March 2009, Krejca, McDermid, Stock, Sas, male, female, juveniles; Indian Cave, 3 and 4 March 2009, Krejca, McDermid, Stock, Sas, male, juveniles; talus slope above Spider Cave, 2 March 2009, Sas, McDermid, female. *TULARE CO.,* Sequoia and Kings Canyon National Parks, 8th Hole Cave, 12 May 2004, Krejca, Sprouse, Snow, Fryer, 2 males, 2 females; 12 August 2003, Krejca, Despain, Olberg, 2 females, 15 juveniles; Bear Den Cave, 18 July 2003, Krejca, Loftin, Fryer, Walck, juvenile, 1 May 2004, Krejca, Snow, Sprouse, 2 males, 2 females, 5 juveniles; Bulah Cave, August 2002, Krejca, Fryer, Olmbeck (sight record); Crystal Cave, 17 July 2003, Krejca, Loftin, juvenile, 10 August 2003, Krejca, Taylor, male; Hurricane Crawl Cave, 16 July 2003, Krejca, Loftin, Snow, Fryer, male, 9 juveniles; Jordan Cave, 12 July 2004, Krejca, Gluesenkamp, Fryer, female; Lange Cave, 6 May 2004, Krejca,

Sprouse, Fryer, Walck, 5 females, 2 juveniles; Wilburn Cave, 15 May 2004, Krejca, Snow, Fryer, Boiano, male; Palmer's Cave, 27 July 2003, Krejca, Loftin, male; Pet Cemetery Cave, 11 May 2004, Krejca, Loftin, Fryer, Walck, female, 3 juveniles; Weiss Raum Cave, 10 May 2004, Olmbeck, 2 males, 2 females, juvenile.

HABITAT

All of the Tulare County localities are caves formed by gradual dissolution of marble by water. The caves with this species in Mariposa County are granite talus caves formed instantaneously as a result of a rock avalanche (Wieczorek et al. 1999). The habitat in these granite talus caves is very similar to that in caves formed in sedimentary rock by dissolution, with true darkness, humidity at or near saturation, and limited nutrient input. These granite talus caves have a variety of passageways, narrow squeezes and large rooms, and are not extensive (< 100 m long, < 20 m deep). A handful of marble caves also exist in Yosemite National Park in the Forsyth Mountain area (Tuolumne County), and inventories of four of these caves did not yield *T. loftinae.* Possibly the elevation is too high (2900 meters) or the detectability is low enough that the brief visits there did not turn up any specimens. For maps and descriptions of Sequoia and Kings Canyon National Parks caves, see Despain (2003).

In addition to cave localities, we collected one individual from underneath rocks on a granite talus slope near Spider Cave. This surface locality is somewhat contiguous with the caves there, considering that the talus slopes are laterally extensive and deep. This finding supports our ecological designation of this species as a troglophile.

Elevations where this species were recorded range from 1100 meters to 2900 meters. Extensive sampling effort (25% of the significant caves visited, and greater than 25% of the cave visits) occurred at lower-elevation sites, between 500 and 1000 meters, but the species was not found there. Some sampling effort also occurred above 3000 meters, with no records of *T. loftinae.* Based on the results of this sampling effort, we propose that the range of the species may be limited to higher elevations, but not above 3000 m. However, it is not limited to a certain type of karst geology.

MICROHABITAT
Temperature data are available for the microhabitat of 78 of the 85 collected individuals. The temperature average for those 78 individuals is 9.4 °C (range = 2.5° to 12°, standard deviation = 1.6°). Humidity for 14 collections averaged 95% (range = 91.8 to 100%, standard deviation 3.3%). In-cave location data are available for 53 individuals, with an average distance into the cave from the entrance of 56 meters (range = 3 to 180 meters, standard deviation = 68). Time spent searching by surveyors gives an idea of abundance, and is available for 82 individuals

collected. The average time spent to find an individual was 69 person-minutes (range = 25 to 261 person-minutes, standard deviation = 50). Substrate data for 40 individuals showed the majority (83%) were found approximately equally in the following environments: under rocks (on silt or other types of floors), on woody debris, or on rocks that were on a floor of woody debris. The remainder were found in other environments, such as calcite ceilings, bedrock walls, and silt floors.

REMARKS

The specimens of *T. loftinae* were collected in connection with a survey of the fauna of the caves of Sequoia and Kings Canyon National Parks and Yosemite National Park carried out under the direction of JKK. Other collections from this work have already been reported on; Shear and Krejca (2007) described two new species of the striariid milliped genus *Amplaria* (*A. muiri* Shear & Krejca 2007 and *A. adamsi* Shear & Krejca 2007), and Shear and Shelley (2008) described a new genus and species of macrosternodesmid, *Sequoiadesmus krejcae* Shear & Shelley 2008, all from Sequoia and Kings Canyon National Parks. Shear et al. (2009), in a paper on new cave-dwelling polydesmidans from the southwestern states, named *Pratherodesmus despaini* Shear 2009, another macrosternodesmid from Sequoia, and yet another Sequoia *Pratherodesmus* species awaits the collection of further specimens for description. Of these species, it is likely that only *Amplaria muiri,* which shows reduced pigmentation and ocellus number when compared to other, surface-dwelling *Amplaria,* may be cave-limited. The other species so far described show no obvious adaptations to life underground, as exemplified by *Taiyutyla loftinae,* which has pigmentation and the standard number of ocelli for surface *Taiyutyla* in the same size range. The legs and antennae of *T. loftinae* are not inordinately elongate. We expect that collecting on the surface in the parks will reveal the presence of this species, but work has so far focused on the caves, making the exact ecological status of the millipedes found there difficult to clarify.

Taiyutyla loftinae is not a typical *Taiyutyla* species in the conformation of its gonopods. The two-branched anterior gonopods are found otherwise only in *T. variata* Shear 1976 (Josephine Co., Oregon) and *T. lupus* Shear 2004 (Vancouver Island, British Columbia), with the *loftinae* form closest to *T. lupus.* Having the posterior branch of the anterior gonopod pass lateral to the posterior gonopod colpocoxite is characteristic of the genus *Conotyla,* and among *Taiyutyla* species is found only in *T. lupus* and *T. loftinae.* The posterior gonopod colpocoxites, however, are much simpler than are generally found in *Taiyutyla*, lacking additional basal branches and an otherwise characteristic mid-distal, posterior fimbriate region or process. Like *T. lupus, T. loftinae* also has very prominent paranota (see Fig. 5, Shear, 2004). Further-

more, *T. loftinae* is evidently unique among described *Taiyutyla* species, and indeed all known conotylids, in the modifications of male legpair 2, a strong, oblong prefemoral process (Fig. 1). All the pregonopodal legpairs except the first have modified prefemora.

Whether some division of the genus *Taiyutyla* will be justifiable in the future depends on the study of a substantial number of undescribed species currently in collections; it is also quite likely that additional species will turn up in numbers, if systematic collecting for them in California is ever undertaken.

As shown in Fig. 13, living animals often show droplets attached to the segmental setae. One explanation for this might be condensation in the saturated cave environment, but they do not appear on all specimens (Fig. 12, for example). Trichopetalid millipeds often show very similar droplets (Shear, 2003; 2010) that emerge from the sockets at the bases of the setae and work their way out to the tips. One of us (WS) has hypothesized that this is part of a defensive strategy, since in the trichopetalids the droplets are very sticky. If *Taiyutyla loftinae* is exhibiting the same behavior, this is the first report of it from a conotylid milliped.

REFERENCES

Chamberlin, R.V., 1952, Two Oregon millipeds of the order Chordeumid, Natural History Miscellanea (Chicago Academy of Sciences), no. 113, p. 1–3.

Despain, J., 2003, Hidden Beneath the Mountains: Caves of Sequoia and Kings Canyon National Parks, Dayton, Ohio, Cave Books, 128 p.

Shear, W.A., 1971, The milliped family Conotylidae in North America, with a description of the new family Adritylidae (Diplopoda: Chordeumida): Bulletin of the Museum of Comparative Zoology, v. 141, p. 55–98.

Shear, W.A., 1976, The milliped family Conotylidae (Diplopoda, Chordeumida): Revision of the genus *Taiyutyla*, with notes on recently proposed taxa: American Museum Novitates, no. 2600, 22 p.

Shear, W.A., 2003, The milliped family Trichopetalidae, Part 1: Introduction and genera *Trigenotyla* Causey, *Nannopetalum*, n. gen., and *Causeyella* n. gen. (Diplopoda: Choredumatida, Cleidogonoidea.: Zootaxa 321, p. 1–36.

Shear, W.A., 2004, Two new species in the milliped genus *Taiyutyla* from British Columbia, Canada, and Washington, USA (Diplopoda, Chordeumatida, Contylidae): Myriapodologica, v. 8, p. 13–20.

Shear, W.A., 2010, The milliped family Trichopetalidae, Part 2: The genera *Trichopetalum, Zygonopus* and *Scoterpes* (Diplopoda: Chordeumatida, Cleidogonoidea: Zootaxa 2385, p. 1–62.

Shear, W.A., and Krejca, J.K., 2007, Revalidation of the milliped genus *Amplaria* Chamberlin 1941 (Diplopod, Chordeumatida, Striariidae) and description of two new species from caves in Sequoia and Kings Canyon National Parks, California: Zootaxa, v. 1532, p. 23–39.

Shear, W.A., and Shelley, R.M., 2008, Cave millipeds of the United States. VI. *Sequoiadesmus krejcae*, n. gen., n. sp., from Sequoia and Kings Canyon National Parks, California, USA (Diplopoda, Polydesmida, Polydesmidea, Macrosternodesmidae): Zootaxa, v. 1693, p. 41–48.

Shear, W.A., Taylor, S.J., Wynne, J.J., and Krejca, J.K., 2009, Cave millipeds of the United States. VIII. New genera and species of polydesmidan millipeds from caves in the southwestern United States (Diplopoda, Polydesmida, Macrosternodesmidae): Zootaxa, v. 2151, p. 47–65.

Shelley, R.M., Medrano, M.F., Shear, W.A., Ovaska, K., White, K.J., and Havard, E.I., 2009, Distribution extensions of the milliped families Conotylidae and Rhiscosomididae (Diplopoda: Chordeumatida) into northern coastal British Columbia and southern Alaska: Insecta Mundi, article no. 71, p. 1–6.

Wieczorek, G.F., Morrissey, M.M., Iovine, G., and Godt, J., 1999, Rock-fall potential in the Yosemite Valley, California: U.S. Geological Survey Open-file Report 99-578, URL http://pubs.usgs.gov/of/1999/ofr-99-0578/ [accessed 9 August 2009].

CONTAMINANT TRANSPORT IN TWO CENTRAL MISSOURI KARST RECHARGE AREAS

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Abstract: Karst watersheds with significant losing streams represent a particularly vulnerable setting for groundwater contamination because of the direct connection to surface water. Because of the existing agricultural land-use and future likelihood of urbanization, two losing-stream karst basins were chosen for intensive monitoring in Boone County, Missouri: Hunters Cave and Devils Icebox. Both caves were formed in Burlington Limestone and have similar recharge areas (33 to 34 km^2) and land uses. Year-round monitoring was conducted from April 1999 through March 2002 to characterize the water quality of the main cave streams relative to herbicide, nutrient, and sediment contamination. Water sampling entailed grab samples at regular intervals and runoff-event samples collected using automated sampling equipment. Total nitrogen, phosphorus, and sediment concentrations and loads were consistently higher in the Devils Icebox stream compared to Hunters Cave. Median total N fluxes were 96 g km^{-2} d^{-1} at Devils Icebox and 30 g km^{-2} d^{-1} at Hunters Cave, while median total P fluxes were 8.5 g km^{-2} d^{-1} at Devils Icebox and 3.3 g km^{-2} d^{-1} at Hunters Cave. Herbicides or their metabolites were detected in more than 80% of the samples from both cave streams, and herbicide concentrations and areal loss rates were generally similar between the sites. Overall, the greater loads and mass flux of contaminants in the Devils Icebox recharge area compared to Hunters Cave was a result of both greater stream discharge and the occurrence of more cropped fields (94%) on claypan soils with high runoff potential. These claypan soils are known to be especially problematic with respect to surface transport of contaminants. Prevailing land use has significantly degraded the water quality in both recharge areas, but a watershed plan has been developed for the Bonne Femme watershed, which encompasses these two recharge areas. With the baseline data collected in this study, the impact of changing land uses and the implementation of management practices or new ordinances designed to improve water quality can be documented.

INTRODUCTION

The vulnerability of groundwater to contamination in karst recharge areas has been well established over the last 25 years (Hallberg et al., 1985; Boyer and Pasquarell, 1996; Younos et al., 2001; Vesper et al., 2001; Boyer, 2005), prompting the development of new methods to assess the vulnerability of karst aquifers (Gogu and Dassargues, 2000; Andreo et al., 2006). Contaminants such as nutrients (Hallberg et al., 1985; Boyer and Pasquarell, 1995; Currens, 2002; Panno and Kelly, 2004; Crain, 2006), pesticides (Pasquarell and Boyer, 1996; Currens, 2002; Panno and Kelly, 2004; Crain, 2006), sediment (Hallberg et al., 1985; Mahler et al., 1999; Crain, 2006), heavy metals (Vesper and White, 2003; Vesper et al., 2001), organic solvents (Loop and White, 2001; Vesper et al., 2001), petroleum products (Ruhe et al., 1980), fecal bacteria (Pasquarell and Boyer, 1995; Lerch et al., 2002; Pronk et al., 2006), and endocrine disruptors (Wicks et al., 2004) have been documented in karst aquifers. These contaminants may be harmful to humans exposed through drinking water obtained from

karst aquifers and through recreational activities in caves. The establishment of maximum contaminant levels for drinking water (USEPA, 1996) and whole-body contact standards for fecal coliforms (e.g., USEPA, 2002) reflect the health concerns associated with exposure to these contaminants. In addition, cave-adapted organisms (i.e., stygobites and troglobites) may also be harmed by the presence of these contaminants in cave streams and drip waters (Elliott, 2000; Taylor et al., 2000; Spanjer and Cipollini, 2006), leading to disruption of karst ecosystems.

The wide array of contaminants impacting karst aquifers indicates that private on-site sewer systems and land uses such as urban development, agricultural production, industrial production, and military activities are all potential threats to groundwater quality. It is now well accepted that surface land uses directly impact the water quality of karst aquifers, especially those with discrete, or allogenic, recharge mechanisms (Betson, 1977; Ruhe et al.,

1980; Boyer and Pasquarell, 1994; Currens, 2002; Panno and Kelly, 2004; Lerch et al., 2005). In southeastern West Virginia, Boyer and Pasquarell (1994) found strong linear relationships between the percentage of a karst recharge area in agricultural use with the concentrations of nitrate-N, fecal coliforms, and triazine herbicides. In urban areas, the impact of increased impervious surfaces on allogenically recharged karst aquifers has been shown to be very similar to that on surface streams (Booth et al., 2002), resulting in greater discharge velocity, peak discharge, and volume of storm-water runoff (Betson, 1977; Ruhe et al., 1980). These hydrologic impacts have led to the development of strategies for managing urban growth to protect karst-water resources (Butler, 1987; Frueh et al., 2008).

Within central Missouri, the recharge area of the Devils Icebox has been extensively studied (St. Ivany, 1988; Halihan et al., 1998; Wicks, 1997; Wicks and Engeln, 1997; Lerch et al., 2005; Dogwiler et al., 2007), but the recharge area of nearby Hunters Cave was only recently documented (Lerch et al., 2005). The two recharge areas are located within the Bonne Femme Creek watershed located due south of Columbia (Fig. 1). The Devils Icebox recharge area is approximately 34.0 km^2, and it is composed of two distinct parts, an allogenic recharge area, corresponding to upper Bonne Femme Creek, and a discrete recharge area, encompassing the Pierpont sinkhole plain (Fig. 1) (Lerch et al., 2005). The majority of the streamflow in Devils Icebox derives from the allogenic portion of the recharge area. The Hunters Cave recharge area encompasses approximately 33.3 km^2, and its recharge is predominantly allogenic, occurring through a fault conduit connecting two tributaries of Turkey Creek to the uppermost part of the cave stream and a conduit connecting Bass Creek to the lower portion of the cave (Fig. 2). Both cave streams show rapid response to precipitation, and the resurgences have large variations in discharge (Wicks, 1997; Halihan et al., 1998; Lerch et al., 2005).

Although a number of studies have measured water quality or quantity in karst aquifers (Hallberg et al., 1985; Boyer and Pasquarell, 1995; Currens, 2002; Wicks et al., 2004; Panno and Kelly, 2004; Dogwiler et al., 2007), few studies to date have intensively monitored both water quantity and quality for a broad range of agricultural contaminants over multiple years. Thus, data on contaminant loads and their seasonality are lacking in the literature. Documenting current loads is critical for establishing baseline conditions so that the effects of future changes in land use on water quality and quantity can be assessed. Furthermore, load data are crucial for evaluating the effectiveness of management practices or growth-management strategies that may be implemented in karst aquifers for the purpose of improving or protecting water quality (Frueh et al., 2008). Frequent monitoring over multiple years also provides a more accurate assessment of the range of contaminant concentrations present in the aquifer, especially for contaminants with seasonal inputs and limited environmental persistence such as herbicides. This information is essential for accurately assessing the potential toxicity of contaminants on karst ecosystems.

The study presented here was conducted to assess the transport of agricultural contaminants within the Devils Icebox and Hunters Cave recharge areas. The Bonne Femme watershed at present is predominantly agricultural, but it is rapidly urbanizing due to growth of the cities of Ashland and Columbia. This study was initiated before significant changes in urban development and impervious surface occurred so that the effect of changing land use on the quality and quantity of water in these two karst recharge areas could be evaluated in the future. The objectives of this study were to characterize the concentrations and determine the loads of sediment, nutrients, and commonly used soil-applied herbicides in the Devils Icebox and Hunters Cave recharge areas by intensive monitoring of the resurgences of both caves.

Materials and Methods

Site Descriptions and Land Use

The caves were formed in the Burlington Limestone (Osagean Series, Mississippian System) (Wicks, 1997). The upper (eastern) portions of both recharge areas are covered by clay-rich Pleistocene glacial and loess deposits (St. Ivany, 1988). These low-permeability, fertile soils are generally in the Mexico-Putnam or Mexico-Leonard soil associations (USDA-NRCS, 2001). The lower (western) portions of each recharge area are characterized by residual soils of the Weller-Bardley-Clinkenbeard association and are the areas with karst features, including the two cave entrances. Both caves exhibit rudimentary branching patterns, with smaller side passages that are tributaries to the primary cave streams (Fig. 3). Devils Icebox is currently listed as Missouri's seventh longest cave, at 10.76 km (Gulden, 2010). The main trunk passage is the primary stream conduit, and it extends for approximately 6.4 km before reaching a sump. The cave system's downstream end is a spring located in Rock Bridge Memorial State Park. The length of Hunters Cave is 2.54 km, which currently makes it the 36th longest cave in Missouri. The main passage is also the primary stream conduit, and it extends for approximately 1.25 km before reaching a sump. The spring resurgence discharges directly into Bass Creek within the Three Creeks Conservation Area. Additional details about these sites can be found in Lerch et al. (2005).

Land-use information for the major classes of forest, urban, impervious, cropland, grasslands, wetlands, and open water was obtained from 30 m resolution Landsat imagery data collected from 2000 to 2004. The data were classified by the Missouri Resource Assessment Partnership, and files for Boone and Audrain Counties were downloaded from the Missouri Spatial Data Information
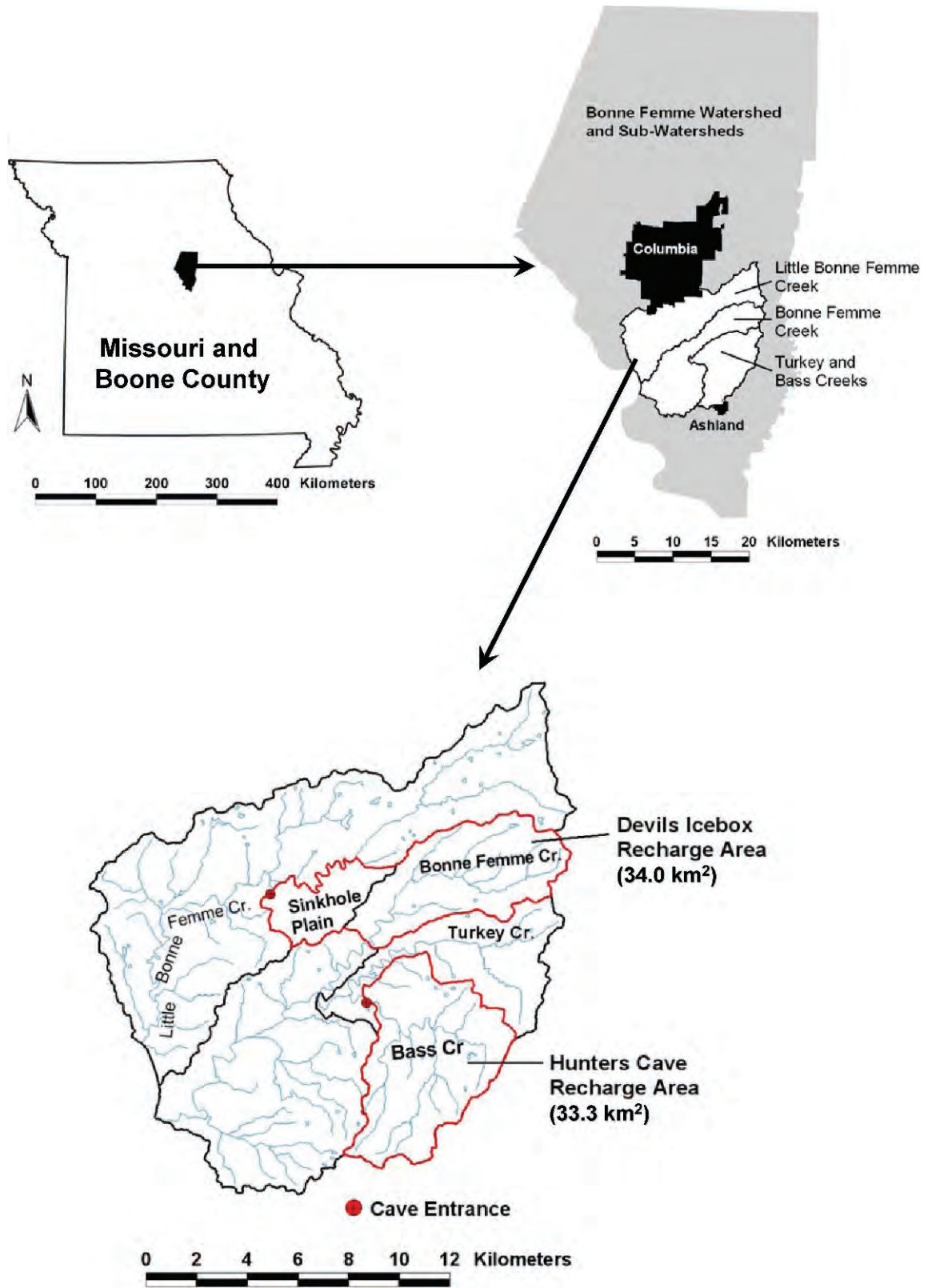
Figure 1. Location and hydrologic setting of the two karst recharge areas.
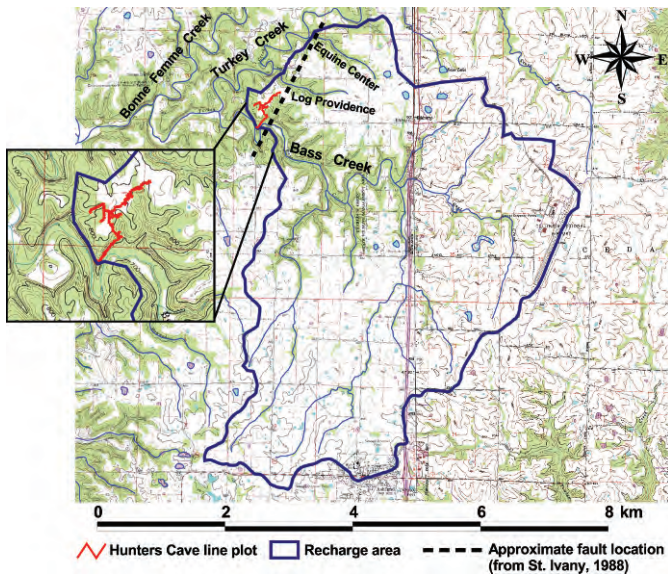
Figure 2. Hunters Cave recharge area delineation. Inset shows the meander cut-off created by the flow path from Bass Creek to the cave stream via Angel Spring. The fault shown indicates the likely conduit from the Turkey Creek tributaries to the upper cave stream.

Figure 3. Line plot diagrams of the two cave systems. (Note the difference in scale for the two caves.).

Service (2005). Because of their close proximity and similarity in geology and soils, both recharge areas have similar land use and cover, with about 80% of the recharge areas composed of grasslands or row crops (Fig. 4). However, the Hunters Cave recharge area had a higher proportion of grasslands and a lower proportion of row crops than Devils Icebox. Row crops within the Devils Icebox recharge area were mainly concentrated within the upper Bonne Femme watershed, while row crops within the Hunters Cave recharge area were more evenly distributed (Fig. 4). In both recharge areas, row crops were predominantly corn and soybeans. Grasslands were utilized for hay production and as range land, with cattle and horses the predominant livestock. The percentage of forest was nearly identical within both recharge areas. The Hunters Cave recharge area has a small amount of urban impervious cover at the Columbia Regional Airport in its eastern part and in commercial and residential developments in Ashland. The Devils Icebox recharge area currently has no significant amounts of either urban impervious or urban vegetation cover, and residential areas were limited to low-density developments not detected using the 30 m resolution data.

Crop-specific data for the herbicides monitored (see Analytical Procedures) were estimated for 1999 to 2001 growing seasons using the approach of Lerch and Blanchard (2003). The fraction of row crops in corn, soybeans, and sorghum for Boone County were calculated from data available at USDA's National Agricultural Statistics Service (USDA-NASS, no date). Multiplying this
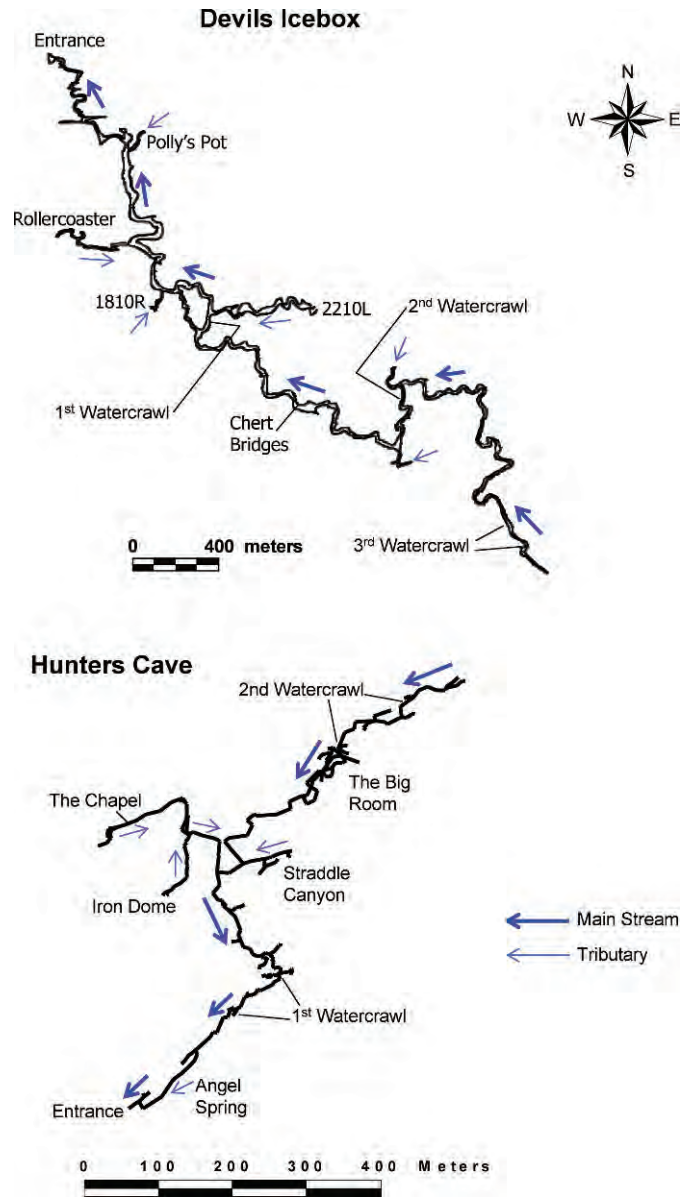
fraction by the row-crop areas from the Landsat data provided an estimate of the corn, soybeans, and sorghum areas within each recharge area. This approach assumes that the proportions of specific crops in all of Boone County was applicable to the two recharge areas. Data from statewide farmer surveys were obtained from the USDA-NASS (2000–2002), including the fraction of each crop treated with a given herbicide and the average application rate. Estimates of the treated areas and total input mass for each herbicide were calculated using this information. This allowed for reporting of the herbicide losses on a treated-area basis, rather than on the basis of the entire recharge area, since herbicides were only used on row crops.
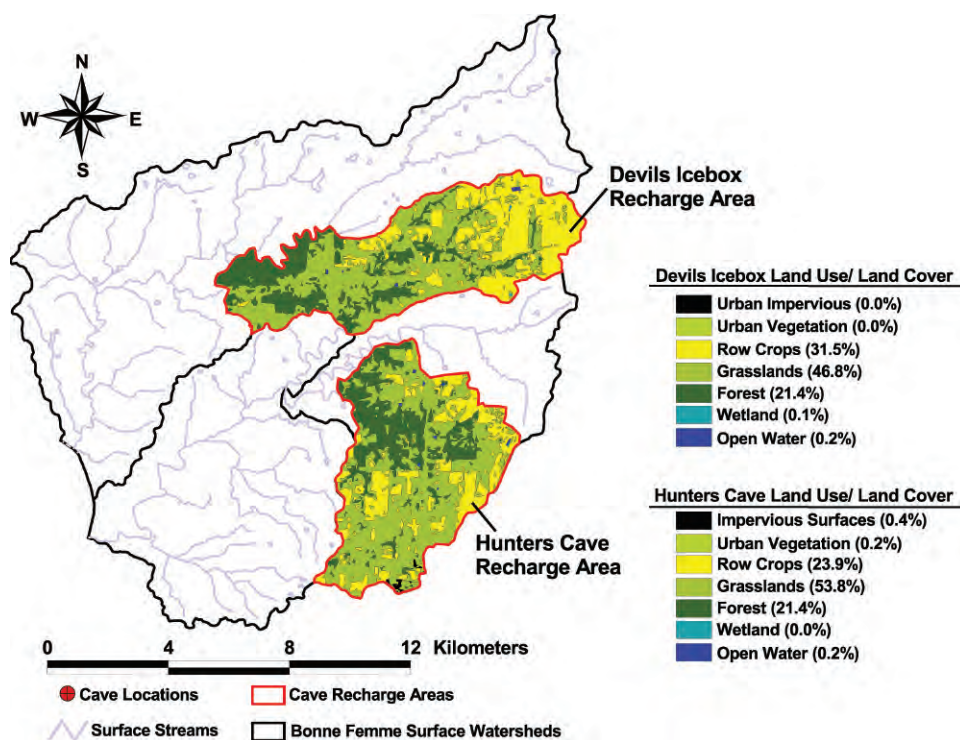
Figure 4. Land use–land cover for Devils Icebox and Hunters Cave recharge areas.

MONITORING PROCEDURES

Hydrological, chemical, and physical monitoring of the water was conducted near the resurgence of each cave from April 1999 to March 2002. The Devils Icebox monitoring station was located within a large karst window approximately 30 m downstream of the resurgence (Halihan et al., 1998). The Hunters Cave monitoring station was located approximately 15 m into the cave (i.e., upstream of the resurgence). All instrumentation was placed in stilling wells for protection against turbulent flow and to reduce data variability. Hydrological monitoring consisted of measuring stage height at 5-minute intervals with submerged pressure-transducer probes (Global Waters Instrumentation, Inc., Gold River, California, and Hach Co., Loveland, Colorado). Stage height was then used to compute stream discharge, as detailed in Lerch et al. (2005). Turbidity was measured at 15-minute intervals using YSI 6920 Sondes (YSI, Inc., Yellow Springs, Ohio) and expressed as nephelometric turbidity units (NTU). The turbidity probes were cleaned and calibrated every 4 to 6 weeks.

Water samples were collected under baseflow and runoff conditions for determination of nutrient, herbicide, and sediment (runoff samples only) concentrations. Grab samples were collected at regular intervals under baseflow conditions, weekly from April through June and twice monthly from July through March. Storm runoff samples at Devils Icebox were collected with an Isco 2900 automatic sampler (Teledyne Isco, Inc. Lincoln, Nebraska) from April 1999 to July 2000 and a Sigma 900 automatic sampler (Hach Co., Loveland, Colorado) from August 2000 to March 2002. At Hunters Cave, a Sigma 900 automatic sampler was used throughout the study. All automatic samplers were equipped with 24, 500 mL high-density-polyethylene sample bottles. For the purpose of sample collection, a runoff event was defined as a 1.5-fold increase in stage height. The stage height for triggering runoff sample collection was adjusted periodically based on ambient baseflow conditions. From April 1999 to July 2000, the automatic samplers were programmed to collect samples at 30-minute intervals throughout the course of an event, so sample collection occurred over a 12-hour period at both sites. By July 2000, a sufficient number of runoff events had been observed to assess the efficacy of the existing sampler programming. This evaluation showed that the existing programming was not sampling the later part of some runoff events at both sites. From August 2000 to March 2002, the samplers were re-programmed using variable time intervals, with decreasing sample frequency through the course of an event. Sampling intervals ranged from 5 minutes to 4 hours, with the programs designed to collect samples for an event length of 24 h at Hunters Cave and 36 h at Devils Icebox. The total number of samples collected at each site during the study for herbicide and nutrient analyses were 1031 at Hunters Cave and 765 at Devils Icebox. The higher number of samples at Hunters Cave resulted from the greater number of runoff events at this site over the course of the study.

Additional monitoring was also performed to compare contaminant concentrations between the caves and their primary surface streams, Bonne Femme Creek for Devils Icebox and Bass Creek for Hunters Cave. Bonne Femme Creek was sampled at U.S. highway 63, upstream of the losing reach to Devils Icebox, and Bass Creek was sampled above the Hunters Cave spring resurgence. Grab samples were collected quarterly from the fourth quarter of 2003 through the third quarter of 2007, for a total of 16 samples collected at each site.

ANALYTICAL PROCEDURES

All samples were analyzed for nutrients and herbicides, and suspended-sediment analyses were also conducted for selected runoff events at both sites from 1999 to 2001. All samples were transported to the laboratory on ice and then refrigerated at 2 to 4 °C until filtered. Herbicide and dissolved-nutrient samples were filtered through 0.45 μm nylon filters within 72 hours of collection.

Nutrient analyses included total and dissolved inorganic nitrogen and phosphorus species determined by standard colorimetric methods (Greenberg et al., 1992) using a Lachat flow injection system (Lachat Instruments, Loveland, Colorado). Total N and P were determined on thoroughly mixed, unfiltered 60 mL samples by autoclave digestion with potassium persulfate (Nydahl, 1978), which quantitatively converts all N forms to nitrate (NO_3^-) and all P forms to orthophosphate (PO_4^{3-}) that were then determined colorimetrically. Nitrate+nitrite-N were determined by the cadmium reduction method (Greenberg et al., 1992). Since nitrite would not be expected to be significant in these samples, the nitrate+nitrite-N will be subsequently referred to as nitrate-N (NO$_3$-N). Ammonium-N (NH$_4$-N) was determined by the phenate method, and orthophosphate-P (PO$_4$-P) was determined by the ascorbic acid method (Greenberg et al., 1992). Method detection limits were 0.10 mg L^{-1} for total N and NO$_3$-N, 0.020 mg L^{-1} for NH$_4$-N, and 0.005 mg L^{-1} for total P and PO$_4$-P. Organic N and P were computed as the difference between their respective total and inorganic concentrations.

Herbicide analyses were conducted for several of the commonly used soil-applied corn and soybean herbicides: atrazine [6-chloro-N^2-ethyl-N^4-(1-methylethyl)-1,3,5-triazine-2,4-diamine], alachlor [2-chloro-N-(2,6-diethylphenyl)-N-(methoxy-methyl)acetamide], acetochlor [2-chloro-N-(ethoxymethyl)-N-(2-ethyl-6-methylphenyl)acetamide], metolachlor [2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)acetamide], and metribuzin [4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4H)-one]. The stable atrazine metabolites deethylatrazine (DEA) [6-chloro-N^4-(1-methylethyl)-1,3,5-triazine-2,4-diamine] and deisopropylatrazine (DIA) [6-chloro-N^2-ethyl-1,3,5-triazine-2,4-diamine] were also analyzed. For all herbicides and metabolites, analyses were conducted by passing 200 mL samples, spiked with 100 ng of

terbutylazine, through C$_{18}$ solid-phase extraction cartridges. The analytes were eluted in ethyl acetate and evaporated to about 0.3 mL under a stream of ultrapure N$_2$ in a 30 °C water bath. Phenanthrene-d10 was then added as an internal standard. The herbicides and metabolites were quantified by gas chromatography/mass spectrometry using a Varian 3400 gas chromatograph with a Saturn 2000 ion-trap mass-selective detector (Varian, Inc., Harbor City, California). Detection limits in μg L^{-1} were atrazine, 0.003; alachlor, 0.003; acetochlor, 0.006; metolachlor, 0.002; metribuzin, 0.008; DEA, 0.004; and DIA, 0.008. Additional details of the herbicide analyses are in Lerch and Blanchard (2003).

Suspended-sediment analyses were performed by the evaporation method (Brakensiek et al., 1979) to develop a relationship between its concentration and turbidity. The method calls for adding a flocculant, but instead, samples were allowed to settle over a period of days to weeks, depending upon the clay content of the samples, before gravimetric analysis was performed. At Devils Icebox, 83 samples from eight runoff events in 1999 and 2000 were analyzed. At Hunters Cave, 300 samples from 19 runoff events in 1999 to 2001 were analyzed. Regression analysis was then used to correlate the suspended-sediment data to the turbidity data collected by the YSI Sonde. Given the absence of light in the cave streams, it was assumed that the algal contribution to the YSI turbidity data was negligible, so that the turbidity of the water was solely a function of suspended sediments. Turbidity data were selected for regression analysis using two methods, either choosing the data point closest in time to that of the runoff-sample collection or computing the average of the turbidity data that bracketed the runoff-sample collection time. At both sites, the turbidity data closest to the runoff-sample collection resulted in higher coefficients of determination (r^2); and therefore, these regression equations were chosen to compute suspended sediment concentrations from the 15-minute turbidity data. The initial regression analyses for both sites resulted in non-significant and negative y-intercepts, resulting in negative concentration estimates when turbidity values were low (e.g., ≤70 NTU). To avoid this, the regression line was forced through zero, resulting in the following equations, where SS is suspended sediments: for Devils Icebox, $SS = 1.37 \times Turbidity$ ($r^2 = 0.71$; $p < 0.001$), and for Hunters Cave, $SS = 1.30 \times Turbidity$ ($r^2 = 0.70$; $p < .001$).

The constraint imposed when using regression through the origin requires an alternative to the ordinary least squares computation r^2. The computed values were determined using the approach described by Eisenhauer (2003). As can be seen from the resulting equations, there was a very significant correlation between turbidity and suspended sediments, and the slope of the regression lines was very similar between sites. This approach provided the equivalent of about 35,000 suspended sediment estimations per site per year.
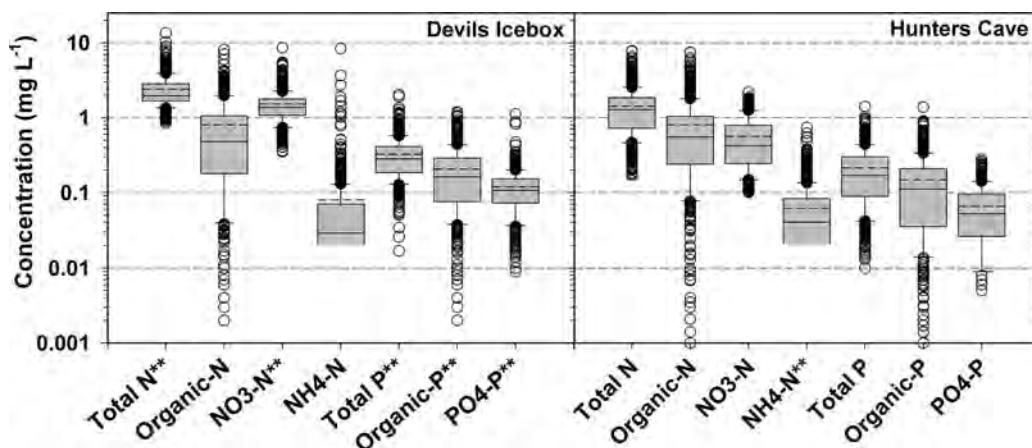
Figure 5. Box plot diagrams of nutrient concentrations in the Devils Icebox and Hunters Cave. The boxes show the 25th to 75th percentile concentrations with the median (solid line) and mean (dashed line) concentrations shown within the boxes. Whiskers represent the 10th and 90th percentiles and ○ represent outliers. ** = significantly different at p = 0.01 based on the Mann-Whitney U test.

Load Computations and Statistical Analyses

Contaminant loads were computed using concentration data from grab and automated samples and from the computed suspended-sediment concentrations combined with the 5-minute discharge data. Linear interpolation was used to estimate concentrations for any un-sampled period. The concentrations were then multiplied by the discharge to determine the load for each 5-minute interval. The 5-minute load data were then aggregated to a daily, quarterly, or annual basis as needed. The data were expressed as contaminant flux (e.g., $g \ km^{-2} \ d^{-1}$) for the daily data, percent of the annual load for the quarterly data, and areal loss rates (e.g., $kg \ ha^{-1} \ y^{-1}$) for the annual data. Nutrient and sediment areal-loss rates were based on the entire recharge area, and herbicide loss rates were expressed on a treated-area basis. Since the concentration and areal-loss data were not normally distributed based on the Kolmogorov-Smirnov test, statistical differences between sites were determined using the Mann-Whitney U-test. The U-test is a non-parametric test of the differences in mean rank between two data sets. For comparisons of concentration data between sites, the *a priori* level of significance was chosen to be $\alpha = 0.05$ because of the large number of observations for each contaminant ($n > 700$). This was also the significance level used for U-tests to determine differences in contaminant concentrations between the caves and their primary surface streams ($n = 16$). For the areal-loss data, the *a priori* level of significance was chosen to be $\alpha = 0.10$, because comparisons between sites were only performed on the annual data ($n = 3$).

Results and Discussion

Hydrology

The area-normalized discharge from the Devils Icebox resurgence (43,500 to 74,700 $m^3 \ km^{-2}$) was consistently greater than the Hunters Cave resurgence (19,600 to 43,300 $m^3 \ km^{-2}$) during three years of monitoring (Lerch et al., 2005). In addition, the Devils Icebox recharge area was seen to have much greater peak discharge during runoff events, greater water-storage capacity, and longer flow paths than the Hunters Cave recharge area. Hunters Cave had more frequent runoff events, greater median instantaneous discharge, and more pronounced seasonal changes in discharge, water temperature, and dissolved oxygen than Devils Icebox. The Hunters Cave discharge characteristics suggest that the areal extent and size of sub-surface conduits are very limited in this recharge area. In contrast, the Devils Icebox resurgence is characterized by a sub-surface conduit system that is both greater in volume and areal extent than Hunters Cave (Lerch et al., 2005).

Nutrient Concentrations

Nitrogen concentration data showed that Devils Icebox had significantly greater concentrations than Hunters Cave for total N and NO_3-N (Fig. 5). Total N concentrations showed a similar range between sites, but Devils Icebox had 68% greater mean and 54% greater median concentrations than Hunters Cave. At both sites, total N concentrations showed a very narrow range between the 10th and 90th percentiles (2.07 mg L^{-1} at Hunters Cave and 2.52 mg L^{-1} at Devils Icebox), indicating consistently high total N contamination over time. This was especially the case at Devils Icebox, where only eight samples out of 765 (i.e., 1.1%) were below 1 mg L^{-1}, but 37% of the samples collected at Hunters Cave were below 1 mg L^{-1}. Among the nutrient analytes measured or computed, NO_3-N showed the highest relative difference between sites, with mean concentrations that were 2.7 times greater at Devils Icebox (1.52 mg L^{-1}) than Hunters Cave (0.57 mg L^{-1}). Similar to total N, the distribution of NO_3-N concentrations also showed a narrow range between 10th and 90th

Table 1. Comparison of mean nitrogen and phosphorus concentrations between the caves and their primary surface streams based on quarterly samples collected from 2003 to 2007 ($n = 16$). Bold type indicates a significant difference between mean ranks for the Mann-Whitney U-test ($\alpha = 0.05$). The p-values represent the probability of observing a more extreme value for the U statistic.

Nutrient	Devils Icebox, mg L^{-1}	Bonne Femme Cr., mg L^{-1}	p-value	Hunters Cave, mg L^{-1}	Bass Cr., mg L^{-1}	p-value
Total N	**2.02**	**1.26**	**0.017**	**0.65**	**1.37**	**0.005**
NO_3-N	**1.62**	**0.97**	**0.004**	**0.26**	**0.87**	**0.009**
NH_4-N	0.03	0.07	0.080	0.02	0.03	0.087
Total P	0.19	0.30	0.152	0.10	0.10	0.494
PO_4-P	**0.13**	**0.18**	**0.007**	0.05	0.05	0.152

percentiles at both sites, but concentrations at Devils Icebox were greater throughout the distribution. Organic-N concentrations showed nearly equal distributions, and the mean concentration at both sites was 0.80 mg L^{-1}. Despite significantly lower total N and NO_3-N concentrations at Hunters Cave, the relative difference between the total N and inorganic N analytes was nearly identical between sites. Although NH_4-N at both sites had the lowest overall concentrations of any nutrient species monitored, Hunters Cave had significantly greater NH_4-N concentrations than Devils Icebox. Median NH_4-N concentrations were 0.04 mg L^{-1} at Hunters Cave and 0.03 mg L^{-1} at Devils Icebox. The consistently low NH_4-N concentrations were not expected, given the near certainty of ammonia-based fertilizer inputs from row-crop areas and the high fecal coliform levels observed at both sites (Lerch et al., 2002), indicating significant wastewater inputs. However, dissolved O_2 concentrations at both sites were generally at or slightly above saturation throughout this study (Lerch et al., 2005), and the NH_4^+ was apparently being oxidized to NO_3^- during transport.

Concentrations of the three P analytes were significantly greater at Devils Icebox than Hunters Cave (Fig. 5). The range in 10th to 90th percentile concentrations was similar between sites, indicating similar variation in P inputs, but Devils Icebox was consistently greater in both mean and median P concentrations. For example, average Devils Icebox concentrations were 1.5 times greater for total P, 1.4 times greater for organic-P, and 1.8 times greater for PO_4-P than Hunters Cave. Only NO_3-N had a greater relative difference between sites than PO_4-P. Since PO_4-P is quite insoluble at the pH of 7.5 to 7.8 observed under typical baseflow conditions for the cave streams (Lerch et al., 2005), it was expected that PO_4-P concentrations at Devils Icebox would be lower than Hunters Cave, given the greater possibility for PO_4^{3-} precipitation to occur along its much longer flow path (Lerch et al., 2005). Despite this, all P analytes were greater at Devils Icebox, demonstrating that the Devils Icebox recharge area was either more vulnerable to P transport or had much greater P inputs to its recharge area (see below).

Quarterly samples were collected from the caves and their primary surface streams to assess any differences in N and P concentrations that may occur along the subsurface flow paths. These data showed that for Devils Icebox total N and NO_3-N significantly increased, and NH_4-N slightly decreased compared to Bonne Femme Creek (Table 1). Apparently the Pierpont sinkhole plain, which lies between the losing reach of Bonne Femme Creek and the cave stream resurgence, is the source of the additional N to the Devils Icebox cave stream. The decrease in average NH_4-N concentrations from 0.07 mg L^{-1} in Bonne Femme Creek to 0.03 mg L^{-1} in Devils Icebox suggests that some minor losses occurred along the subsurface flow path, but the input concentrations were already very low before recharge to the Devils Icebox aquifer had occurred. At Hunters Cave, all N analytes were lower than Bass Creek, and total N and NO_3-N concentrations were significantly lower. For the P analytes, total P and PO_4-P were lower in Devils Icebox than Bonne Femme Creek, indicating that some PO_4^{3-} precipitation occurred in the subsurface, and the differences were statistically significant for PO_4-P. Comparison of Hunters Cave to Bass Creek showed that the two sites had equal average total P and PO_4-P concentrations. The finding that total N and NO_3-N significantly decreased while total P and PO_4-P were unchanged between Hunters Cave and Bass Creek suggests that the Turkey Creek tributaries had much lower N concentrations but similar P concentrations to that of Bass Creek.

The concentrations of N and P species observed in this study were generally within the lower range reported for karst aquifers (Boyer and Pasquarell, 1996; Johnson, 2002; Katz, 2004; Panno and Kelly, 2004). In The Hole cave in West Virginia, Boyer and Pasquarell (1996) reported median NO_3-N concentrations in the main cave stream and its tributaries ranging from 3.40 to 16.58 mg L^{-1}. Tributaries impacted by a dairy operation had much greater NO_3-N concentrations than those in tributaries draining pasture land (Boyer and Pasquarell, 1996), which were similar to the NO_3-N concentrations observed in Devils Icebox and Hunters Cave. Katz (2004) reported that NO_3-N concentrations in northern Florida springs ranged

Table 2. Herbicide detections and concentrations for Hunters Cave and Devils Icebox recharge areas.

Herbicide	Devils Icebox			Hunters Cave		
	Detection Frequency, %	Concentration Range, $\mu g\ L^{-1}$	Median, $\mu g\ L^{-1}$	Detection Frequency, %	Concentration Range, $\mu g\ L^{-1}$	Median, $\mu g\ L^{-1}$
Atrazine	94.9	<0.003–36.9	0.058	77.4	<0.003–75.6	0.036
DIA	36.3	<0.008–4.42	<0.008	41.0	<0.008–0.703	<0.008
DEA	88.2	<0.004–6.18	0.086	67.8	<0.004–1.02	0.028
Metribuzin	37.6	<0.008–0.280	<0.008	40.3	<0.008–0.356	<0.008
Acetochlor	18.7	<0.006–5.82	<0.006	9.0	<0.006–2.58	<0.006
Alachlor	45.2	<0.003–0.400	<0.003	48.4	<0.003–5.17	<0.003
Metolachlor	57.0	<0.002–4.06	0.005	24.1	<0.002–0.206	<0.002

from 0.50 to 4.2 mg L^{-1}, levels that were very similar to those observed in this study. Samples collected from 35 springs discharging from carbonate bedrock in the Upper Tennessee Valley and Ridge physiographic province gave median concentrations of N and P species considerably lower than those reported here (Johnson, 2002). Currens (2002) reported average annual concentrations of NO_3-N and PO_4-P in the intensively row-cropped Pleasant Grove Spring recharge area in Kentucky. The average NO_3-N concentrations ranged from 4.20 to 4.98 mg L^{-1} over a 7-year period, levels that were about 8 and 3 times greater than the average NO_3-N concentrations in Devils Icebox and Hunters Cave, respectively. In the same study, median PO_4-P concentrations in the first four years were similar to or lower than those reported here, but in the last two years increased to concentrations that were 2 to 5 times greater than those in Hunters Cave or Devils Icebox.
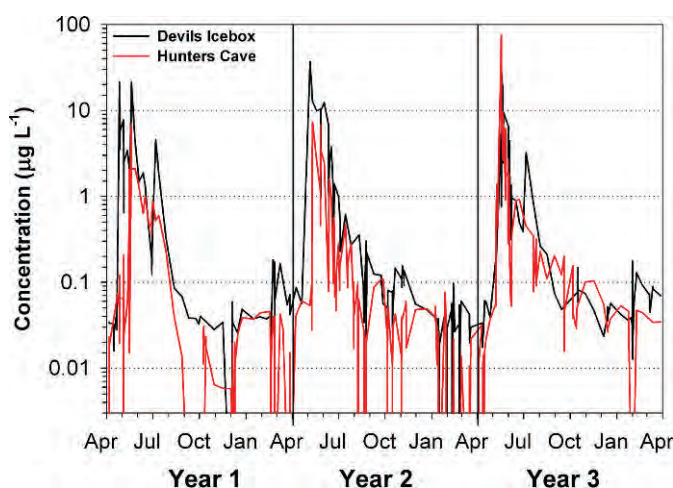
Figure 6. Atrazine concentrations in the Devils Icebox and Hunters Cave from April 1999 through March 2002. Graph represents all samples collected over the study (n = 765 for Devils Icebox; n = 1031 for Hunters Cave).

HERBICIDE CONCENTRATIONS

Herbicides were frequently detected at both sites (Table 2). Overall, 96% of Devils Icebox samples and 85% of Hunters Cave samples had a detection of at least one herbicide or metabolite compound. At both sites, atrazine and its DEA metabolite were most commonly detected, but the frequency was much greater for both compounds at Devils Icebox. The DIA metabolite was detected much less often than atrazine and DEA at both sites. The frequency of alachlor and metribuzin detections was similar between sites, but acetochlor and metolachlor were more often detected at Devils Icebox. Despite the frequent detections, herbicide concentrations were quite low overall, with median concentrations generally below the detection limits and none greater than 0.100 $\mu g\ L^{-1}$. As illustrated by the changes in atrazine at both sites (Fig. 6), herbicide concentrations showed a typical seasonal trend, with the greatest concentrations occurring in spring, followed by an exponential decrease to very low levels (e.g., <0.100 $\mu g\ L^{-1}$) by late summer. In general, herbicide detection frequency and median concentrations of these two karst aquifers were considerably less than those in surface streams of this region (Lerch and Blanchard, 2003). However, they were similar to the levels of herbicides reported in other karst aquifers (Pasquarell and Boyer, 1996; Currens, 2002; Panno and Kelly, 2004). Peak concentrations of atrazine and metolachlor in Devils Icebox and Hunters Cave were very similar to those reported for karst aquifers in Kentucky (Currens, 2002) and Illinois (Taylor et al., 2000; Panno and Kelly, 2004) in which the relative proportion of crop land within these recharge areas was similar to or greater than that of Devils Icebox and Hunters Cave.

CONTAMINANT LOADS

Based on the large annual mass transport of suspended sediment (>100,000 kg) and nutrients (>100 kg P and >1000 kg N) at each site, it is believed that the monitored contaminants were derived primarily from allochthonous sources distributed throughout the recharge areas. The

Table 3. Annual nutrient loads for Devils Icebox and Hunters Cave recharge areas.

Contaminant	Devils Icebox, kg				Hunters Cave, kg			
	Year 1	Year 2	Year 3	Mean	Year 1	Year 2	Year 3	Mean
Suspended Sediment	449,000	881,000	387,000	572,000	69,000	154,000	113,000	112,000
Total N	3,740	6,110	3,330	4,390	730	1,760	1,100	1,197
Organic-N	1,700	2,050	636	1,460	415	1,100	494	670
NO_3-N	1,960	3,940	2,520	2,810	288	600	524	471
NH_4-N	79.0	121	178	126	26.5	62.6	81.6	56.9
Total P	518	969	391	626	98	240	188	175
Organic-P	348	652	170	390	64	174	96	111
PO_4-P	170	317	221	236	34	67	92	64

herbicide transport data (Tables 3 and 4) further support this assumption, as the use of herbicides would have been restricted to farm fields within the recharge areas, and the seasonal nature of the herbicide concentrations indicated that new inputs occurred annually. Although autochthonous sediments could be potential sources of contaminants, the mass transported annually suggests that they were trivial compared to allochthonous sources. For example, the average annual mass of sediment transported was 112,000 kg at Hunters Cave and 572,000 kg at Devils Icebox (Tables 3 and 4). Sources of such large amounts of suspended sediment, if primarily derived from re-suspension of autochthonous bed and bank sediments, would be readily observed along the stream channels within the caves. However, personal observation of these caves over time has shown minor erosion of bed and bank sediments, but sediment deposition was frequently observed in the stream channels following large runoff events.

The quarterly distribution of contaminant loads and stream discharge for Devils Icebox and Hunters Cave, averaged over all three years, is shown in Figure 7. For Devils Icebox, total N and P loads by quarter of the year were nearly identical and strongly tied to seasonal discharge. In the second quarter, 45% of the year's discharge occurred along with 49% of the total N load

and 48% of the total P load. For suspended sediment, 50% of the annual load occurred in the second quarter, but it differed from the total N, total P, and discharge distributions in the other quarters. For example, 21% of the annual sediment load was transported in the third quarter while only 12% of the discharge, 10% of the total N, and 13% of the total P transport occurred during this quarter. For Hunters Cave, nutrient and sediment loads were more evenly distributed over the year, and loads were not as strongly associated with discharge in the first, third, and fourth quarters as was observed at Devils Icebox (Fig. 7). Similar to Devils Icebox, second quarter discharge and loads of sediment, total N, and total P accounted for the highest proportion of the year's load, with 34% of the annual discharge, 37% of the sediment load, 41% of the total N load, and 31% of the total P load. However, the third and fourth quarter loads of sediments and nutrients were proportionally much greater at Hunters Cave compared to Devils Icebox. Hunters Cave discharge was most disproportionate to sediment and nutrient loads in the first quarter, when 27% of the annual discharge occurred, compared to 16% of the sediment load, 22% of the total N load, and 20% of the total P load. The high relative sediment transport in the third quarter of the year at both sites was largely attributed to a single runoff event

Table 4. Annual herbicide loads for Devils Icebox and Hunters Cave recharge areas.

Contaminant	Devils Icebox, g				Hunters Cave, g			
	Year 1	Year 2	Year 3	Mean	Year 1	Year 2	Year 3	Mean
Atrazine	3,950	3,010	2,230	3,060	287	463	1,730	827
DEA[a]	807	1,180	663	883	47.2	174	180	134
DIA[b]	291	1,180	330	600	23.2	89.8	108	73.7
Metribuzin	105	37.5	15.3	52.4	34.9	22.5	2.68	20.0
Acetochlor	402	40.1	2.34	148	2.10	2.13	6.44	3.56
Alachlor	49.7	28.1	18.0	31.9	28.8	50.6	25.3	34.9
Metolachlor	165	113	352	210	1.88	1.43	6.59	3.30

[a] DEA = Deethylatrazine.
[b] DIA = Deisopropylatrazine.
Note: Both metabolite loads are expressed on an atrazine basis by correcting for the difference in molecular weight of the metabolites relative to that of atrazine.
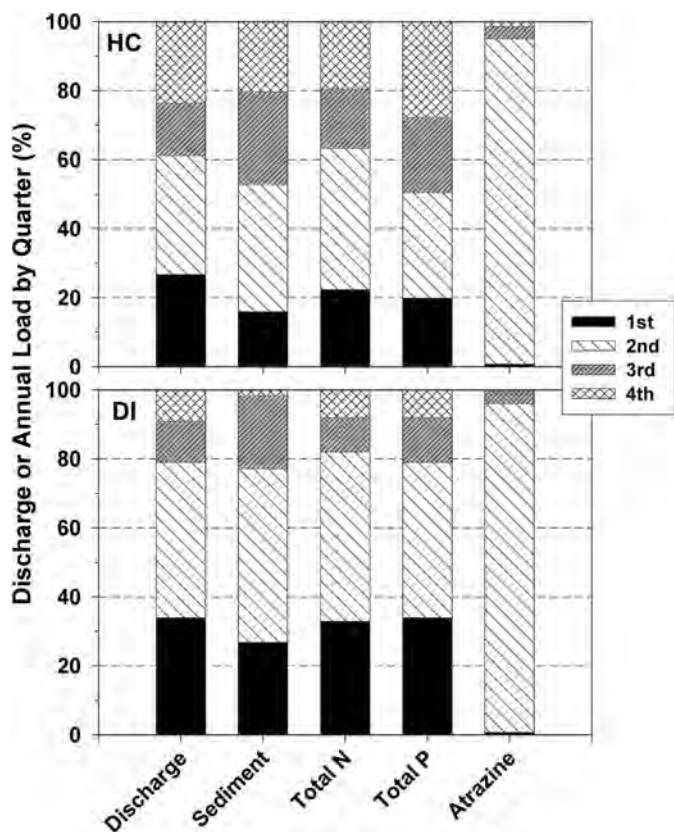
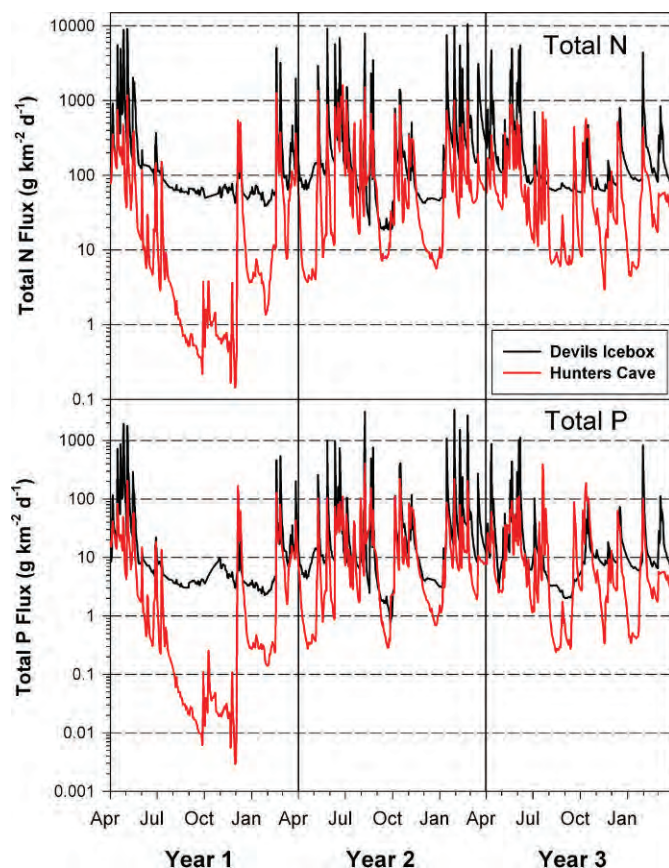Figure 7. Quarterly distribution of discharge, suspended sediment, total N, total P, and atrazine loads for Devils Icebox and Hunters Cave.

Figure 8. Estimated daily flux from each square kilometer of source area of total N and total P in Devils Icebox and Hunters Cave.

that occurred in August 2000. This event had the highest discharge and sediment transport of any event in the three-year monitoring period. The strong relationship between seasonal discharge and total N and P transport at Devils Icebox indicated that nutrient transport was significant even under baseflow conditions, while at Hunters Cave the majority of the nutrient transport occurred during runoff events. This is not to say that sediment-bound N and P transport at Devils Icebox was unimportant; the highest concentrations and daily loads of both nutrients always occurred during runoff events (see below).

The seasonal distribution of atrazine loads was very similar at both sites, but it showed a very different pattern than discharge and nutrients. In the second quarter, atrazine loads accounted for 95% of the annual load at Devils Icebox and 94% of the annual load at Hunters Cave, demonstrating the extreme seasonality typical of herbicide transport (Thurman et al., 1991; Lerch et al., 1995). The seasonal atrazine transport pattern was also representative of the other herbicides monitored. The seasonal dependence of atrazine transport occurs because farmers apply herbicides during a relatively narrow window in April and May and they have relatively short persistence in the environment.

Nutrient Flux

With the intensive monitoring approach used in this study, the flux of contaminants could be determined on a daily basis, providing highly time-resolved estimates of contaminant inputs to the two recharge areas. Of particular importance to the stygobites is the flux of nutrients occurring in the cave streams. High nutrient fluxes have been associated with the loss of stygobite diversity and changes in the distribution and abundance of species within caves (Elliott, 2000). Total fluxes of all types of N and P from each square kilometer of recharge area are shown as examples of the daily flux data (Fig. 8) that can be generated for any of the contaminants monitored in this study. Total N in Devils Icebox ranged from 19 to 11,000 g km^{-2} d^{-1}, with a median flux of 96 g km^{-2} d^{-1}. At Hunters Cave, total N ranged from 0.14 to 1600 g km^{-2} d^{-1}, with a median flux of 30 g km^{-2} d^{-1}. For total P, Devils Icebox ranged from 0.81 to 3400 g km^{-2} d^{-1}, and Hunters Cave ranged from 0.003 to 410 g km^{-2} d^{-1}. Median P flux was 8.5 g km^{-2} d^{-1} in Devils Icebox and 3.3 g km^{-2} d^{-1} in Hunters Cave. Thus, the median fluxes of total N and total P were about three times greater in Devils Icebox than Hunters Cave. The N

and P fluxes were dominated by runoff events, resulting in sharp increases of several orders of magnitude in the daily fluxes for days in which runoff events occurred, regardless of the time of year (Fig. 8). If the inputs of N and P were primarily derived from fertilizer sources, these would have been greatest in spring, following fertilizer application to crop and pasture fields. However, the peak spring fluxes were very similar to those observed in other seasons. For instance, in Year 2, similarly high N and P fluxes were observed in spring, summer, and winter at both sites (Fig. 8). Thus, runoff events with enough energy to cause significant erosion were transporting large quantities of sorbed N and P, in addition to soluble forms of the nutrients.

There have been only a few studies that have reported nutrient fluxes or loads for karst aquifers (Boyer and Pasquarell, 1996; Currens, 2002; Panno and Kelly, 2004; Katz et al., 2009). Of these studies, only two, Boyer and Pasquarell (1996) and Panno and Kelly (2004), were conducted in recharge areas with agricultural land use intensity comparable to the Devils Icebox and Hunters Cave recharge areas. Boyer and Pasquarell (1996) reported NO_3-N loads for the basin drained by The Hole in West Virginia on three separate days. These loads were divided by the known area of the basin (14.5 km^2) (Boyer, 2005) for the sake of comparability, resulting in NO_3-N fluxes that ranged from 250 to 3500 g km^{-2} d^{-1}. Panno and Kelly (2004) reported average NO_3-N fluxes of approximately 5600 g km^{-2} d^{-1} for a southwest Illinois recharge area. The study by Currens (2002) was conducted in an intensively (69 to 78%) row-cropped recharge area, resulting in average NO_3-N fluxes ranging from 3400 to 13,000 g km^{-2} d^{-1}. The data presented for Devils Icebox and Hunters Cave are total N fluxes, of which the NO_3-N was a subset, indicating that reported N loads and fluxes in other karst aquifers with significant agricultural land uses were much greater than those for Devils Icebox and Hunters Cave. Using data from Currens (2002), estimated average PO_4-P fluxes in the Pleasant Grove Spring recharge area ranged from 31 to 727 g km^{-2} d^{-1}, fluxes that were 4 to 220 times greater than the total P fluxes in Devils Icebox and Hunters Cave.

AREAL LOSS RATES

To facilitate comparisons between sites, annual contaminant loads were computed on an areal loss basis (Fig. 9). With the exception of NH_4-N loss rates, N, P, and sediment losses were significantly greater at Devils Icebox compared to Hunters Cave. Areal loss rates of NO_3-N and suspended sediment at Devils Icebox were more than 5 times greater than at Hunters Cave. Total N and P and PO_4-P loss rates were all more than 3 times greater at Devils Icebox than at Hunters Cave. The herbicide transport data showed that only metolachlor loss rates were significantly greater at Devils Icebox compared to Hunters Cave (Fig. 9), but atrazine and acetochlor losses
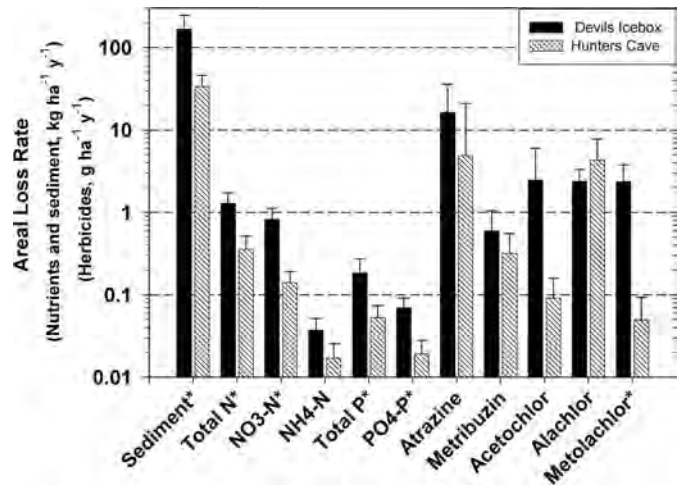

Figure 9. Average annual areal loss rates of suspended sediment, nutrients, and herbicides for Devils Icebox and Hunters Cave. Entire source area used for the first six columns; only treated row-crop area used for the last five. Note that areal loss rates for atrazine include the contribution of deethylatrazine and deisopropylatrazine. Error bars indicate one standard deviation. * = significantly different at p = 0.05 based on the Mann-Whitney U test.

were also much greater at Devils Icebox, while alachlor losses were greater at Hunters Cave. The large variation in atrazine and acetochlor losses precluded statistically significant differences from being discerned. Metribuzin loss rates were similar between sites.

Herbicide and suspended sediment loss rates from the Devils Icebox and Hunters Cave recharge areas were generally much lower than those measured for other surface watersheds of the Midwestern US (Saxton et al., 1971; Larson et al., 1983; Lerch and Blanchard, 2003). Suspended sediment transport was one to two orders of magnitude below that typically measured for agricultural watersheds (Saxton et al., 1971; Larson et al., 1983). In karst aquifers, suspended sediment loss rates reported by Currens (2002) ranged from 530 to 1500 kg ha^{-1}, rates that were 3 to 43 times greater than those reported here and were similar to surface watersheds. Panno and Kelly (2004) and Currens (2002) reported NO_3-N areal loss rates that were 20 to 190 times greater than those reported for Devils Icebox and Hunters Cave. However, areal loss rates of atrazine, metolachlor, and alachlor in these same two studies (Panno and Kelly, 2004; Currens, 2002) were very similar to those observed for Devils Icebox and Hunters Cave. It should be noted, though, that the loss rates reported here were on a treated-area basis, while those reported by Panno and Kelly (2004) were based on the total row-crop area, and those reported by Currens (2002) were reported for the entire recharge area. Thus, the herbicide loss rates for Devils Icebox and Hunters Cave
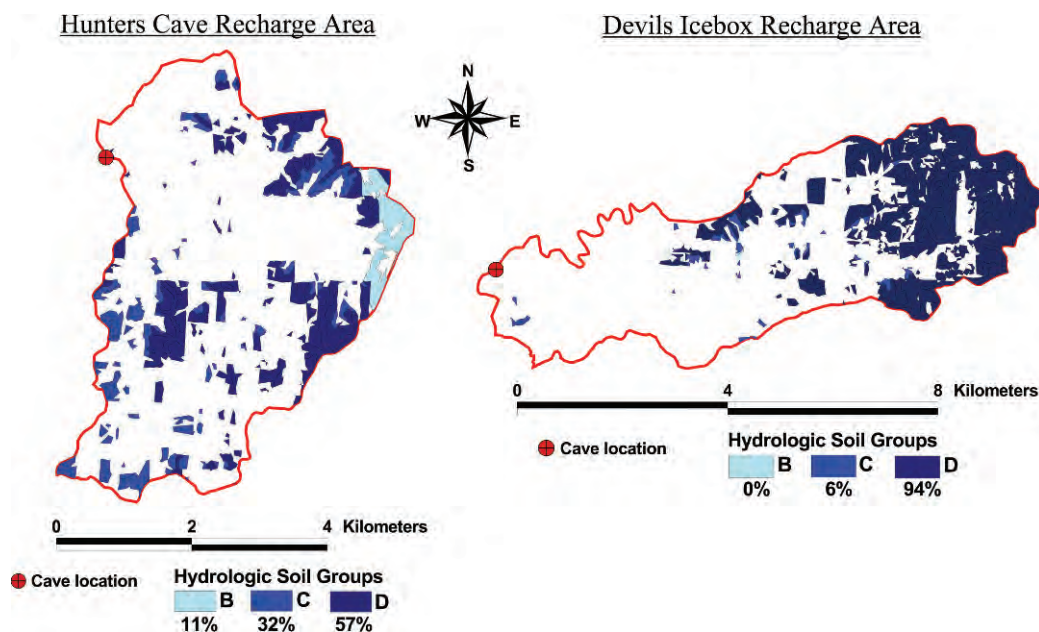
Figure 10. Hydrologic soil groups in the row-crop areas in each recharge area. Numbers below the color keys represents the proportion of row crops occurring on each soil type.

were most likely lower than those reported by Panno and Kelly (2004) and Currens (2002).

The higher areal loss rates of Devils Icebox compared to Hunters Cave were related to its consistently greater discharge and contaminant concentrations. While greater discharge is a function of the recharge area characteristics (Lerch et al., 2005), the greater observed concentrations at Devils Icebox were related to the occurrence of row crops on soils with high runoff potential. Hydrologic Soil Groups (HSG) represent one way to characterize the runoff potential of soils (USDA-NRCS, 2009). There are four categories of HSG, A through D, with HSGA having the lowest and HSGD the highest runoff potential, and they have been shown to be valid indicators of watershed vulnerability to herbicide transport (Lerch and Blanchard, 2003). Since runoff potential is critical to surface transport of contaminants, it follows that watersheds or recharge areas dominated by HSGC and D soils would be the most vulnerable to stream contamination. A GIS-based analysis of the intersection of row-crop areas with the HSG, by soil mapping unit, within each recharge area showed that 94% of the row-crop areas in the Devils Icebox recharge area occurred on HSGD soils compared to only 57% of the row-crop areas for Hunters Cave (Fig. 10). Nearly all the row crops on HSGD soils within the Devils Icebox area lie above the losing reach of Bonne Femme Creek. These claypan soils with high runoff potential are known to be especially problematic with respect to surface transport of sediment, nutrients, and herbicides (Lerch and Blanchard, 2003; Lerch et al., 2008).

Conclusions

The magnitude and seasonality of contaminant loads in both recharge areas showed that the contaminants were primarily derived from allochthonous sources distributed throughout the recharge areas. Devils Icebox generally had greater concentrations, loads, and fluxes of suspended sediment, nitrogen, phosphorus, and herbicides than Hunters Cave. The greater loads and fluxes at Devils Icebox were due to a combination of greater stream discharge, resulting from differences in recharge-area characteristics, and the extremely high proportion of row crops on claypan soils with high runoff potential. Compared to other agricultural karst recharge areas in the Midwestern US, Devils Icebox and Hunters Cave generally had lower nutrient concentrations and much lower nutrient and herbicide fluxes, but peak concentrations of atrazine, alachlor, and metolachlor were similar to more intensively cropped recharge areas. In both recharge areas, prevailing land management has significantly degraded water quality. Therefore, funding was obtained to develop a stakeholder-led watershed plan for the Bonne Femme watershed (Frueh et al., 2008), with the primary goal of improving water quality. The plan was completed in 2007 and is available on the Web (Bonne Femme Stakeholder Committee, 2007). It has a number of detailed recommendations for karst protection, including limiting stormwater runoff to pre-development levels in karst recharge areas, use of economic incentives to reduce urban development on karst (e.g., transfer of development rights

or conservation easements), new zoning regulations to provide special protections to karst recharge areas (e.g., require no-discharge on-site sewer systems), and adoption of a stream-buffer ordinance that limits construction within the 100-year flood plain of all stream channels in the watershed. With the data collected from this study, the impact of implementing these management practices and changes in land use can be documented.

REFERENCES

Andreo, B., Goldscheider, N., Iñaki, V., Vias, J.M., Neukum, C., Sinreich, M., Jiménez, P., Brechenmacher, J., Carrasco, F., Hötzl, H., Perles, M.J., and Zwahlen, F., 2006, Karst groundwater protection: First application of a pan-European approach to vulnerability, hazard, and risk mapping in the Sierra de Líbar (Southern Spain): Science of the Total Environment, v. 357, p. 54–73.

Betson, R.P., 1977, The hydrology of karst urban areas, in Dilamarter, R., and Csallany, S., eds., Hydrologic Problems in Karst Regions, Bowling Green, Western Kentucky University, p. 162–175.

Bonne Femme Stakeholder Committee, 2007, Bonne Femme Watershed Plan, http://www.cavewatershed.org/plan.asp.

Booth, D.B., Hartley, D., and Jackson, R., 2002, Forest cover, impervious-surface area, and the mitigation of stormwater impacts: Journal of the American Water Resources Association, v. 38, p. 835–845. DOI: 10.1111/j.1752-1688.2002.tb01000.x.

Boyer, D.G., 2005, Water quality improvement program effectiveness for carbonate aquifers in grazed land watersheds: Journal of the American Water Resources Association, v. 41, p. 291–300. DOI: 10.1111/j.1752-1688.2005.tb03735.x.

Boyer, D.G., and Pasquarell, G.C., 1994, Agricultural land use impacts on karst groundwater quality, in Marston, R.A., and Hasfurther, V.R., eds., Proceedings of the Effects of Human-Induced Changes on Hydrologic Systems Symposium, Jackson Hole, Wyoming, American Water Resources Association Technical Publication Series 94-3, p. 791–799.

Boyer, D.G., and Pasquarell, G.C., 1995, Nitrate concentrations in karst springs in an extensively grazed area: Journal of the American Water Resources Association, v. 31, p. 729–736. DOI: 10.1111/j.1752-1688.1995.tb03397.x.

Boyer, D.G., and Pasquarell, G.C., 1996, Agricultural land use effects on nitrate concentrations in a mature karst aquifer: Journal of the American Water Resources Association, v. 32, p. 565–573. DOI: 10.1111/j.1752-1688.1996.tb04054.x.

Brakensiek, D.L., Osborn, H.B., and Rawls, W.J., eds., 1979, Field Manual for Research in Agricultural Hydrology: United States Department of Agriculture, Science and Education Administration Agricultural Handbook 224, Revised, 547 p.

Butler, K.S., 1987, Urban growth management and groundwater protection: Austin, TX, in Page, G.W., ed., Planning for Groundwater Protection, New York, Academic Press, p. 261–287.

Crain, A.S., 2006, Concentrations of Nutrients, Pesticides, and Suspended Sediment in the Karst Terrane of the Sinking Creek Basin, Kentucky,

2004: U.S. Geological Survey, Open File Report 2006-1091, http://pubs.usgs.gov/of/2006/1091/ofr20061091.pdf.

Currens, J.C., 2002, Changes in groundwater quality in a conduit-flow-dominated karst aquifer, following BMP implementation: Environmental Geology, v. 42, p. 525–531. DOI: 10.1007/s00254-001-0515-6.

Dogwiler, T., Wicks, C.M., and Jenzen, E., 2007, An assessment of the applicability of the heat pulse method toward the determination of infiltration rates in karst losing-stream reaches: Journal of Cave and Karst Studies, v. 69, p. 237–242.

Eisenhauer, J.G., 2003, Regression through the origin: Teaching Statistics, v. 25, p. 76–80. DOI: 10.1111/1467-9639.00136.

Elliott, W.R., 2000, Conservation of the North American cave and karst biota in Wilkens, H., Culver, D.C., and Humphreys, W.F., eds., Subterranean Ecosystems, Amsterdam, Elsevier, Ecosystems of the World 30, p. 665–689.

Frueh, T., Campbell, R., and Lerch, R.N., 2008, Successful stakeholder-led watershed planning, in Elliott, W.R., ed., Proceedings of the 18th National Cave and Karst Management Symposium, NCKMS Steering Committee, p. 144–153.

Gogu, R.C., and Dassargues, A., 2000, Current trends and future challenges in groundwater vulnerability assessment using overlay and index methods: Environmental Geology, v. 39, p. 549–559. DOI: 10.1007/s002540050466.

Greenberg, A.E., Eaton, A.D., and Clesceri, L.S., eds., 1992, Standard Methods for the Examination of Water and Wastewater, 18th edition, Washington, DC, American Public Health Association, Part 4500, p. 4–80, 4–89, 4–155.

Gulden, B., 2010, US longest caves by state: NSS GEO^2 Committee on Long and Deep Caves, http://caverbob.com/state.htm [accessed May 7, 2010].

Halihan, T., Wicks, C.M., and Engeln, J.F., 1998, Physical response of a karst basin to flood pulses: Example of the Devil's Icebox cave system (Missouri, USA): Journal of Hydrology, v. 204, p. 24–36.

Hallberg, G.R., Libra, R.D., and Hoyer, B.E., 1985, Nonpoint source contamination of ground water in karst-carbonate aquifers in Iowa, in Perspectives on Nonpoint Source Pollution, Proceedings of a National Conference, Kansas City Mo., Washington, DC, United States Environmental Protection Agency, Criteria and Standards Division, report 440/5-85-001, p. 109–114.

Johnson, G.C., 2002, Water Quality of Springs in the Valley and Ridge Physiographic Province in the Upper Tennessee River Basin, 1997, U.S. Geological Survey, National Water Quality Assessment Program, Water-Resources Investigations Report 02-4180, 24 p.

Katz, B.G., 2004, Sources of nitrate contamination and age of water in large karstic springs of Florida: Environmental Geology, v. 46, p. 689–706. DOI: 10.1007/s00254-004-1061-9.

Katz, B.G., Sepulveda, A.A., and Verdi, R.J., 2009, Estimating nitrogen loading to ground water and assessing vulnerability to nitrate contamination in a large karstic springs basin, Florida: Journal of the American Water Resources Association, v. 45, p. 607–627. DOI: 10.1111/j.1752-1688.2009.00309.x.

Larson, W.E., Pierce, F.J., and Dowdy, R.H., 1983, The threat of soil erosion to long-term crop productivity: Science, v. 219, p. 458–465. DOI:10.1126/science.219.4584.458.

Lerch, R.N., Donald, W.W., Li, Y.X., and Alberts, E.E., 1995, Hydroxylated atrazine degradation products in a small Missouri stream: Environmental Science and Technology, v. 29, p. 2759–2768. DOI: 10.1021/es00011a010.

Lerch, R.N., Erickson, J.M., and Wicks, C.M., 2002, Intensive monitoring in two karst basins of Boone County, Missouri, in Rea, G.T., ed., Proceedings of the 15th National Cave and Karst Management Symposium, United States Department of Agriculture, Colonado National Forest, p. 157–168.

Lerch, R.N., and Blanchard, P.E., 2003, Watershed vulnerability to herbicide transport in northern Missouri and southern Iowa streams: Environmental Science and Technology, v. 37, p. 5518–5527. DOI: 10.1021/es030431s.

Lerch, R.N., Wicks, C.M., and Moss, P.L., 2005, Hydrologic characterization of two karst recharge areas in Boone County, Missouri: Journal of Cave and Karst Studies, v. 67, p. 158–173.

Lerch, R.N., Sadler, E.J., Kitchen, N.R., Sudduth, K.A., Kremer, R.J., Myers, D.B., Baffaut, C., Anderson, S.H., and Lin, C.H., 2008, Overview of the Mark Twain Lake/Salt River Basin conservation

effects assessment project: Journal of Soil and Water Conservation, v. 63, p. 345–359. DOI:10.2489/jswc.63.6.345.

Loop, C.M., and White, W.B., 2001, A conceptual model for DNAPL transport in karst ground water basins: Ground Water, v. 39, p. 119–127. DOI: 10.1111/j.1745-6584.2001.tb00357.x.

Mahler, B.J., Lynch, L., and Bennett, P.C., 1999, Mobile sediment in an urbanizing karst aquifer: Implications for contaminant transport: Environmental Geology, v. 39, p. 25–38. DOI: 10.1007/s002540050434.

Missouri Spatial Data Information Service, 2005, Missouri Land Use Land Cover, http://www.msdis.missouri.edu/data/lulc/lulc05.htm [accessed August 11, 2008].

Nydahl, F., 1978, On the peroxodisulphate oxidation of total nitrogen in waters to nitrate: Water Research, v. 12, p. 1123–1130. DOI:10.1016/0043-1354(78)90060-X.

Panno, S.V., and Kelly, W.R., 2004, Nitrate and herbicide loading in two groundwater basins of Illinois' sinkhole plain: Journal of Hydrology, v. 290, p. 229–242.

Pasquarell, G.C., and Boyer, D.G., 1995, Agricultural impacts on bacterial water quality in karst groundwater: Journal of Environmental Quality, v. 24, p. 959–969. DOI:10.2134/jeq1995.00472425002400050026x.

Pasquarell, G.C., and Boyer, D.G., 1996, Herbicides in karst groundwater in southeast West Virginia: Journal of Environmental Quality, v. 25, p. 755–765. DOI:10.2134/jeq1996.00472425002500040015x.

Pronk, M., Goldscheider, N., and Zopfi, J., 2006, Dynamics and interaction of organic carbon, turbidity and bacteria in a karst aquifer system: Hydrogeology Journal, v. 14, p. 473–484. DOI: 10.1007/s10040-005-0454-5.

Ruhe, R.V., Clark, D.W., and Epstein, M.L., 1980, Urban hydrology in karst and water quality—Inorganic and organic systems, Indiana University at Bloomington Water Resources Research Center, Report of Investigations No. 9, 139 p.

Saxton, K.E., Spomer, R.G., and Kramer, L.A., 1971, Hydrology and erosion of loessial watersheds: Journal of the Hydraulics Division [American Society of Civil Engineers], v. 97, no. 11, p. 1835–1851.

Spanjer, G.R., and Cipollini, M.L., 2006, Relationship between physiochemical factors and distribution of stygobitic crayfishes in southeastern caves: Southeastern Naturalist, v. 5, p. 17–26.

St. Ivany, G., 1988, Geologic and hydrologic characteristics of the Gans–Bonne Femme and Clear Creek watersheds and the Pierpont karst plain near Rock Bridge Memorial State Park, Boone County, Missouri, Missouri Department of Natural Resources, Division of Geology and Land Survey, Open File Report 88-70, 15 p.

Taylor, S.J., Webb, D.W., and Panno, S.V., 2000, Spatial and temporal analyses of the bacterial fauna and water, sediment, and amphipod tissue chemistry within the range of *Gammarus acherondytes*, Illinois Natural History Survey, Center for Biodiversity. Technical Report 2000(18), 115 p.

Thurman, E.M., Goolsby, D.A., Meyer, M.T., and Kolpin, D.W., 1991, Herbicides in surface waters of the Midwestern United States: The effect of the spring flush: Environmental Science and Technology, v. 25, p. 1794–1796. DOI: 10.1021/es00022a018.

USDA-NASS, 2000–2002, Boone County Planted Area Data for Corn, Sorghum, and Soybean: United States Department of Agriculture, National Agricultural Statistics Service, http://www.nass.usda.gov/Statistics_by_State/Missouri/index.asp.

USDA-NASS, no date, US Department of Agriculture, National Agricultural Statistics Service Quick Stats, http://www.nass.usda.gov/QuickStats/index.asp [accessed June 23, 2009].

USDA-NRCS, 2001, Soil Survey of Boone County, Missouri, United States Department of Agriculture-Natural Resources Conservation Service, 318 p. [makes the author part of this entry agree with the way it's cited in the text. And the publication is dated 2001, not 2002]

USDA-NRCS, 2009, Hydrologic soil groups, chap. 7: United States Department of Agriculture-Natural Resources Conservation Service, Hydrology National Engineering Handbook, Part 630 (H-210_NEH-630), 13 p.

USEPA, 1996, United State Environmental Protection Agency, Drinking Water Regulations and Health Advisories, 23 p.

USEPA, 2002, Implementation guidance for ambient water quality criteria for bacteria: United States Environmental Protection Agency, EPA-823-B-02-003, http://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=20003PSB.txt.

Vesper, D.J., Loop, C.M., and White, W.B., 2001, Contaminant transport in karst aquifers: Speleogenesis and Evolution of Karst Aquifers, Theoretical and Applied Karstology, v. 13–14, p. 101–111.

Vesper, D.J., and White, W.B., 2003, Metal transport to karst springs during storm flow: An example from Fort Campbell, Kentucky/Tennessee, USA: Journal of Hydrology, v. 276, p. 20–36. DOI:10.1016/S0022-1694(03)00023-4.

Wicks, C.M., 1997, Origins of groundwater in a fluviokarst basin: Bonne Femme basin in central Missouri, USA: Hydrogeology Journal, v. 5, p. 89–96. DOI: 10.1007/s100400050124.

Wicks, C.M., and Engeln, J.F., 1997, Geochemical evolution of a karst stream in Devils Icebox Cave, Missouri, USA: Journal of Hydrology, v. 198, p. 30–41. DOI:10.1016/S0022-1694(96)03328-8.

Wicks, C., Kelley, C., and Peterson, E., 2004, Estrogen in a karstic aquifer: Ground Water, v. 42, p. 384–389. DOI: 10.1111/j.1745-6584.2004.tb02686.x.

Younos, T., Kaurish, F.W., Brown, T., and de Leon, R., 2001, Determining the source of stream contamination in a karst-water system, southwest Virginia, USA: Journal of the American Water Resources Association, v. 37, p. 327–334. DOI: 10.1111/j.1752-1688.2001.tb00972.x.

THE USE OF A KARSTIC CAVE SYSTEM IN A STUDY OF ACTIVE TECTONICS: FAULT MOVEMENTS RECORDED AT DRINY CAVE, MALÉ KARPATY MTS (SLOVAKIA)

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Abstract: This paper reports on a study of active tectonics undertaken in the intracratonic setting of central Europe in the junction zone between Eastern Alps and Western Carpathians. The study site is focused on the karstic system of Driny Cave in the Malé Karpaty Mts, Slovakia. A range of geological, geomorphological, and in situ displacement data are presented. From previous geological mapping and our slickenside analyses, it is clear that the cave system has developed along significant fault structures. Further geomorphological investigations pointed towards ongoing faulting and block movements. For example, a number of slope failures can be seen on the hillsides above the cave and numerous fresh speleothem breaks can be observed within the cave. To test this hypothesis, three optical-mechanical crack gauges were installed in 2005. These gauges confirmed and quantified the ongoing movements. The NNE-SSW striking fault has recorded a strike-slip trend of 0.1 mm/year and a normal fault trend of 0.03 mm/year. The NW-SE striking fault has recorded a strike-slip trend of 0.04 mm/year. In addition, it has been possible to define their precise kinematics. Moreover, different strike-slip mechanisms along two transverse fault systems point to a horizontal stress field orientation. These results confirm the existence of active tectonic structures within central Europe. It is considered that the methodology described here can also be applied in other intracratonic settings where karstic cave systems are present. This would help define potentially seismogenic areas where unambiguous evidence for active faulting is lacking.

INTRODUCTION

The identification and characterization of active fault structures represents a crucial first step in any attempt to define potentially seismogenic areas. In the intracratonic setting of central Europe, such elementary work is often difficult to undertake because active fault movement is slow in comparison to more obviously seismic areas. Accordingly, fault scarps are unlikely to be generated because the overall denudation rate generally exceeds the rate of displacement. The problem is further compounded as the relevant faults are seldom exposed at the surface due to the extensive vegetation coverage (Štěpančíková et al., 2010). As a result, we have instead chosen to specifically examine karstic cave systems for evidence of active faulting. These systems are better able to preserve a record of displacement because the activity is documented within a three dimensional framework unaffected by subsequent erosion. In addition, underground systems are largely shielded from climatic effects such as seasonal massif dilations (Briestenský et al., 2010). It is, therefore, considered that karstic cave systems represent an ideal natural laboratory within which to study active tectonics.

This paper reports on a study of active tectonics undertaken at Driny Cave in the Malé Karpaty Mts, western Slovakia (Fig. 1). The cave is located close to the broadly NE-SW striking Smolenice Fault. This marginal fault is a significant morphostructural feature that separates the Malé Karpaty Mts from the adjacent Blatnianska priehlbina Depression. The cave itself is also of particular importance as it is the only show cave in this part of the country (Slovak Caves Administration, 2006). In addition to the geological and geomorphological mapping of the cave system, three optical-mechanical crack gauges have been installed to record fault displacements. These crack gauges have been regularly monitored since their installation in 2005.

DRINY CAVE

Driny Cave (48°30′01″N, 17°24′27″E) is located near the town of Smolenice in the district of Trnava, western Slovakia. The cave is situated in the Smolenice Karst, the most significant karst area in the Malé Karpaty Mts. The entrance to the cave is located on the western slope of Driny Hill (434 m asl) at an altitude of 399 m asl (Fig. 2a). It is a network cave consisting of intersecting fault-controlled fissure passages, sensu Palmer (1991). Driny Cave is a typical fissure cave conditioned by distinct

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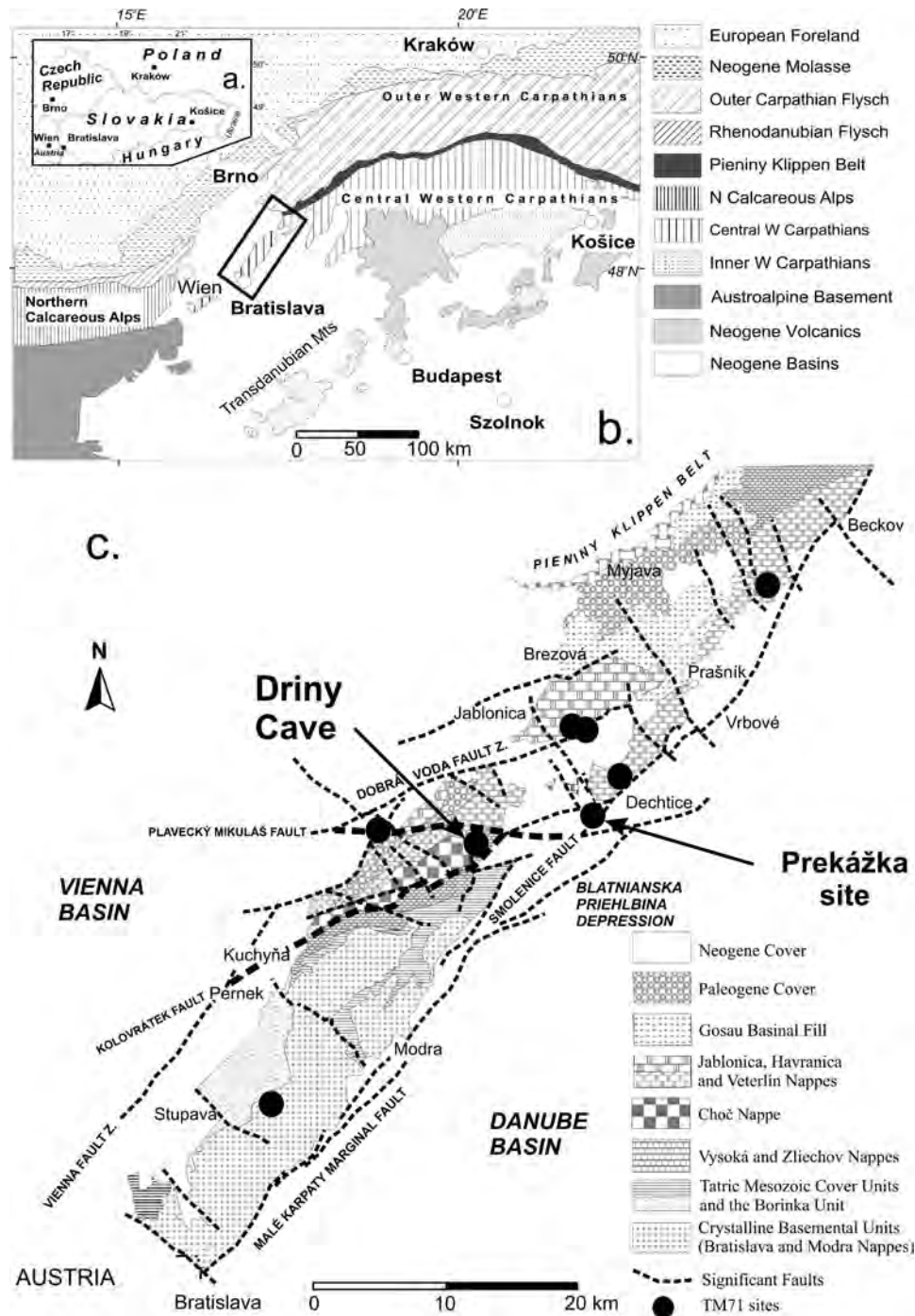
Figure 1. The geology of the Malé Karpaty Mts (modified after: Grabowski et al., 2010; Briestenský and Stemberk, 2008; Briestenský et al., 2010) with the regional network of optical-mechanical crack gauges also marked. A detailed geological map of the study area is presented in Figure 5. Inset: the location of the study area within the context of central Europe.

vertical faults (Fig. 2b). It consists of narrow fissure passages, from one to three meters wide (e.g. Collaborators' Passage, Beňovský's Passage, Passage of Hope), and small halls that are sometimes enlarged at the intersection of fault systems (e.g. Slovak Speleological Society Hall). The cave reaches a length of 680 m and has a vertical span of 40 m. Discovery Chimney, with a depth of 36 m,

descends from the smaller upper entrance (430 m asl) to the intersection between Entrance Passage and Collaborators' Passage (Droppa, 1951, Mitter, 1983, Bella, 2003). The cave has formed in the lower Cretaceous brown-grey chert limestones of the Vysoká Nappe (Michalík et al., 1992). It is thought that the cave originated as a result of corrosion by atmospheric waters seeping along steep failures
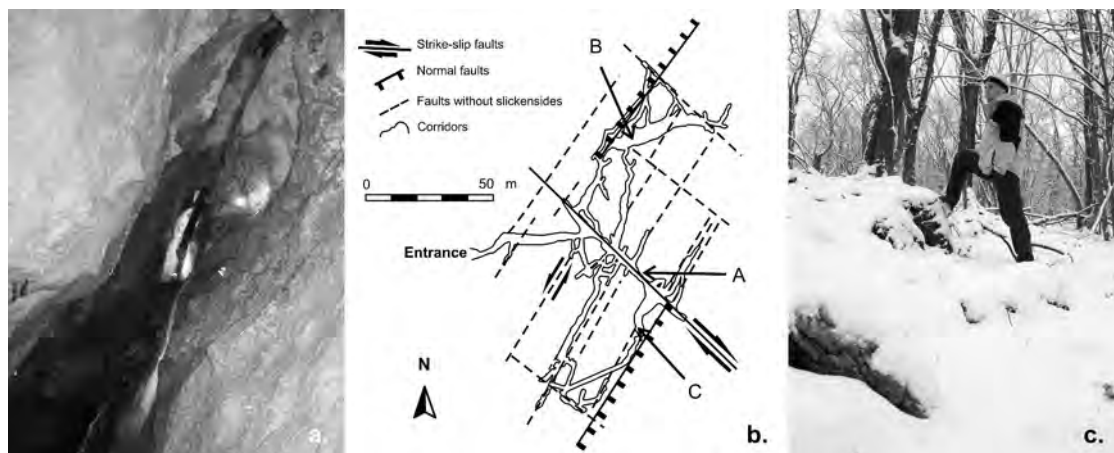
Figure 2. The expression of faults associated with the cave: (a) The entrance to Driny Cave, a fissure passage enlarged by corrosion (Photo: P. Bella); (b) A schematic map of the faults crossing Driny Cave (A: Collaborators' Passage; B: Slovak Speleological Society Hall; C: Chimney Passage). The faults are specified by their displacement mechanism; (c) A terrain step directly above 'Chimney Passage' in Driny Cave.

(Droppa, 1951). The upper, usually narrow, parts of many fissures and several vertical chimneys have been enlarged by corrosion in the vadose zone. In addition to the vadose morphological forms, ceiling pockets, blind holes, oval connecting passages, and water level notches are also noted. The ceiling pockets have been produced by mixing corrosion, the blind holes occur on both the ceilings and the walls, and the oval connecting passages join parallel fissure passages. These morphological and genetic features provide evidence for the phreatic and epiphreatic modeling of the cave as a result of the localized flooding of some passages. Such flooding was caused by the accumulation of clay sediments, which led to blockages within the lower parts of the fissure. Several lateral notches have been sculpted by corrosion directly beneath the contact of the clay sediments, with the rock walls acting as subsidiary features in the overall morphology of the cave (Bella, 2006). The cave is richly decorated with speleothems, mainly comprised of flowstone draperies, stalagmites, and stalactites. In addition, the cave contains small flowstone pools supplied with water from percolating rainfall and melting snow. In the proximal part of the cave, at the base of Discovery Chimney, air temperature fluctuates between 5.6 and 8.7°C. This variability is due to the influence of the terrestrial climate through the open upper entrance (Zelinka, 2000). At the distal end of the cave, air temperature is far more constant and ranges between 7.1 and 7.8°C.

TECTONIC STRUCTURES AND FAULTS

The cave system is located in the Malé Karpaty Mts, adjacent to the Blatnianska priehlbina Depression. These two distinct morphostructural units are separated by the Smolenice Fault (strike: NE-SW, NNE-SSW). This fault represents the southeastern marginal fault of the Malé

Karpaty Mts and has a vertical throw of over 2000 m (Maglay et al., 2006). In addition, significant structures are associated with the transverse faults of the Malé Karpaty Mts (strike: NW-SE). To the northwest of the cave, the Smolenice Fault intersects the seismically active Vienna Fault, a sinistral strike-slip transfer fault. Locally, this fault is called the Dobrá Voda Fault. The latter transverse fault system is thought to be responsible for dividing the main ridge of the Malé Karpaty Mts into a series of discrete morphostructural blocks during the Quaternary. It has recently been determined that movement on these NW-SE striking faults are dextral strike-slip (Kováč et al., 2002).

The seismically active Smolenice Fault is characterized by frequent earthquakes. The most notable recent event was recorded on 13th March 2006 close to the town of Vrbové, which recorded a magnitude of $M_L = 3.2$ (Briestenský et al., 2007). This earthquake registered significant normal faulting along the marginal Smolenice Fault and demonstrated active subsidence of the Blatnianska priehlbina Depression. The displacements were recorded using an optical-mechanical crack gauge located at Prekážka, to the northeast of Driny Cave (Fig. 3). In total, the period of seismic activity lasted for six months (March–August 2006). During this period, displacement oscillations (i.e. strike-slip movements and vertical shifts) were also registered in Driny Cave (Fig. 10, Site 3). Earthquake magnitudes are usually lower and energy is normally released through smaller, frequent, earthquakes. Therefore, long-term fault trends are dominant here.

METHODOLOGY

The study was instigated after it was noted that significant speleothem damage is associated with fault outcrops within the cave system (Šebela, 2008). A number
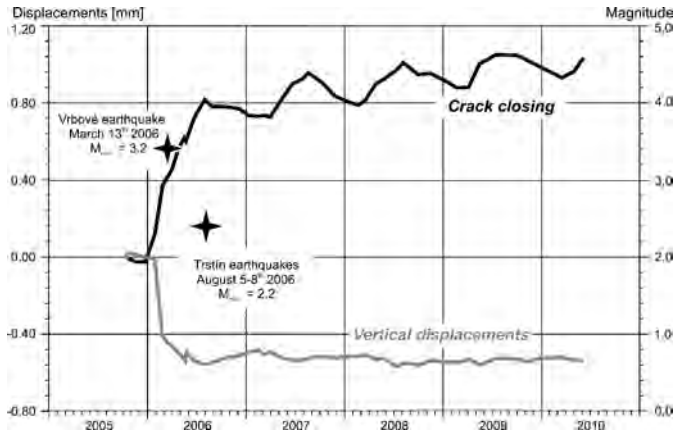
Figure 3. The displacements registered during the most recent phases of seismic activity at Prekážka. Measurements were recorded using an optical-mechanical crack gauge.
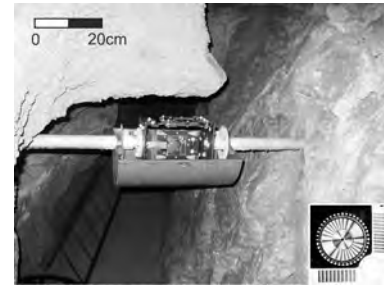

Figure 4. An optical-mechanical crack gauge installed across a significant fault in Bowl Passage. The cover of the instrument (top half removed) shields it against falling rocks. Inset: an example of the moiré (optical-mechanical) effect on the mirror gauge set during a reading.

of previous studies have shown that such damage is indicative of active tectonics (e.g., Kashima, 1993; Gilli, 2005). Consequently, a range of geological, geomorphological, and in situ displacement data have been collected. The geological data are used to suggest the origins of the cave system, the geomorphological data are used to provide evidence for fault movements, and the in situ displacement data are able to quantify activity at the present day.

GEOLOGICAL AND GEOMORPHOLOGICAL ANALYSES

The detailed geological mapping of the study area has previously been published by one of the authors (Michalík et al., 1992). In this study, fault outcrops at the surface were examined for evidence of movement. Within the cave, slickensides were used to determine the sense of fault movement was using the criteria of Petit (1987). The area around Driny Cave was extensively examined for slope failures. These were recorded on base maps using methods adopted from engineering geology (Rybář, 1973). Their precise position and geometry were documented. Within the cave, particular focus was placed on the type and distribution of sinter damage. Where any damage was observed, any clear evidence for the cause was sought.

IN SITU DISPLACEMENT MONITORING

Displacement monitoring has been undertaken using three optical-mechanical crack gauges, referred to as TM-71s. A detailed account of the device has recently been provided in Stemberk et al. (2010). In brief, the relative movement between adjacent fault blocks can be recorded by two identical grid systems when they are mechanically connected (Košt'ák, 1991, 2006) (Fig. 4). It is possible to calculate the three dimensional movement between adjacent blocks with an accuracy of greater of 0.01 mm/yr, while the resolution for angular deviations is $\pm 1.6 \times 10^{-4}$ grad. A number of studies have demonstrated the value of this type of instrument (e.g., Stemberk & Štěpančíková,

2003; Stemberk and Košt'ák, 2007; 2008; Briestenský and Stemberk, 2008). As the instruments are sited underground, the faults are largely shielded from climatic effects such as seasonal massif dilations (Briestenský et al., 2010). It is emphasized that the crack gauges do not require electricity or any other power source and, therefore, the device cannot be affected by changes in the electrical or magnetic field.

The crack gauges were installed across the three most significant faults. These were determined by the presence of tiny, fresh, cracks in the speleothems located close to the fault outcrops. The first gauge was placed across a NW-SE striking dextral strike-slip fault ($75° \rightarrow 040°$, i.e. dip→dip direction) that forms the main corridor of the cave (Fig. 2b, Site A). The second gauge was placed across a NNE-SSW striking normal fault ($70° \rightarrow 290°$) in Slovak Speleological Society Hall that dips to the WNW (Fig. 2b, Site B). The third gauge was placed across a NNE-SSW striking normal fault ($70° \rightarrow 110°$) close to the Chimney Passage that dips to the ESE (Fig. 2b, Site C). Displacement monitoring began in the fall of 2005, with data recorded at the sites every two weeks since that time.

RESULTS

GEOLOGICAL AND GEOMORPHOLOGICAL OBSERVATIONS
Geological Observations and Slickenside Analysis

The geological mapping and detailed examination of fault outcrops was undertaken in three cave systems: Driny Cave, Pod zavesenou Cave, and Pod orechom Cave. These analyses demonstrated that the most significant faults to cross the study area are the NW-SE striking dextral strike slip faults. An example of this type of fault is located in 'Collaborators' Passage' of Driny Cave, where there are slickensides preserved on the northeastern cave wall. The sense of previous fault displacements was determined using striations and slickencrysts. It was seen that the displacement mechanism was consistent across all cave corridors
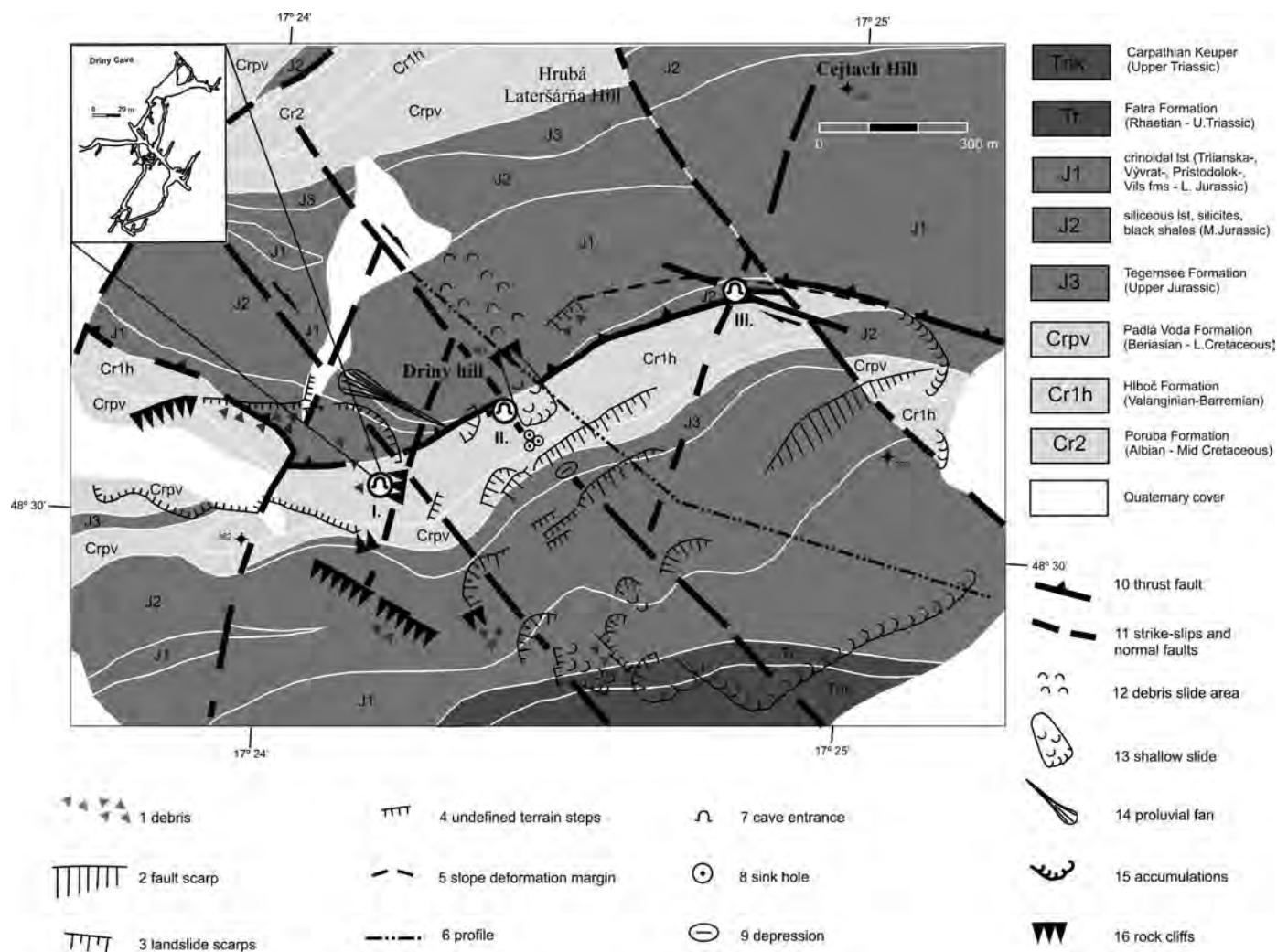
Figure 5. A map of faults and slope failures in the vicinity of Driny Cave (I: Driny Cave; II: Pod zavesenou Cave; III: Pod orechom Cave).

aligned NW-SE. These major fault structures provide the focus for both geomorphological processes at the surface and karstological processes within the cave system. In contrast, the transverse NNE-SSW striking faults show an association with vertical displacements initiated by grav-itectonic processes. The striations associated with normal faulting are preserved in both Slovak Speleological Society Hall and Chimney Passage. Due to the larger size of the cave system, these slickensides were only observed in Driny Cave. Unfortunately, many of the slickensides within cave system are concealed by calcite due to the significant sinter production irrespective of strike orientation. Nonetheless, this general scheme appears to be applicable for both the NW-SE dextral strike slip faults and the NNE-SSW transverse faults within Driny Cave.

Geomorphological Observations: Slope Failures

At the surface, a number of slope failures have been observed (Fig. 5). The most significant of these was an

extensive rockslide noted on the southeastern sector of Driny Hill. The deformation has a width of 1,075 m and extends downslope for more than 200 m. This deforma-tional area is characteristic of a partial scarp formed by a consecutive sequence of slope deformations (Fig. 6). The western part of the rockslide is constrained by a notably deep valley that reflects the presence of a NW-SE striking fault. The fault is not exposed at the surface but has been identified within the cave system. It is located in Collaborators' Passage and controls the development of the gallery in Driny Cave. The eastern part of the rockslide is constrained by a NW-SE dextral striking fault. This fault is also found in the small nearby cave of Pod orechom, which has a total length of 34 m. At the base of the rockslide is a significant accumulation of debris with a frontal wall approximately 20 m in height. In addition to the significant scarps with a characteristically curved shape, less conspicuous terrain steps also occur here. For example, one is located above Chimney Passage (Fig. 2c). The strike
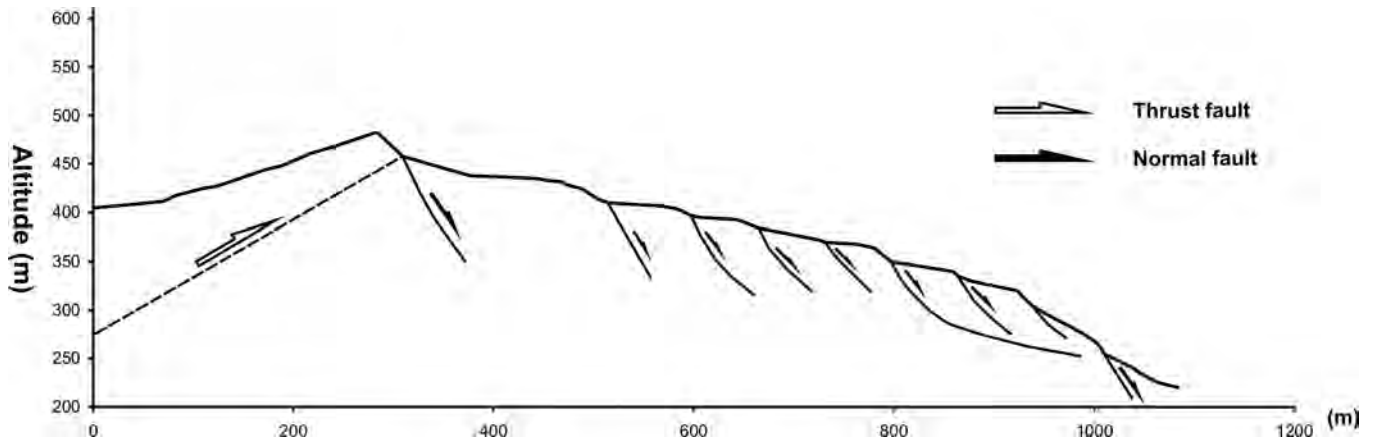
Figure 6. A profile through the slope failures affecting the southeastern side of Driny Hill. The location of the profile is depicted by the line crossing Figure 5.

and dip direction of the terrain step is identical to that of the normal fault that controls the development of the underlying gallery. The Pod orechom cave has developed along the same fault system as Driny Cave, and also displays evidence for the intersecting NW-SE and NNE-SSW striking faults.

In addition, the small cave of Pod zavesenou is located within the northern slope of the deformed area. It is a short cave of only 3 meters, which has developed at the intersection of a significant thrust fault and younger NW-SE dextral strike slip fault (Fig. 7b). The occurrence of a thrust fault here was previously described by Michalík et al. (1992). According to our studies, the NW-SE fault system forms the dominant system. This controls the overall elevation of Driny Hill, as well as the development of the cave galleries. Furthermore, the southern slope of the deformed area rests upon the active normal faults that form part of the Smolenice Marginal Fault System (SMFS). This system forms the northwestern boundary between the Malé

Karpaty Mts and the Blatnianska priehlbina Depression. Its recent activity was described earlier. Therefore, it can clearly be seen that the development of the slope deformation on the southeastern side of Driny Hill was influenced by occurrence of the fault intersection.

The surveyed valley in front of the entrance to Driny Cave, on the western side of Driny Hill, does not visibly reflect this slope deformation origin. It is possible to only find significant amphitheatre cliffs with debris and terrain steps. In contrast, normal fault slickensides were found in the Slovak Speleological Society Hall during the surveying of Driny Cave. The dip direction of this structure is orientated towards the valley. Recent activity has been recorded by a crack gauge. The fault is actually located in the scarp of the slope deformation. Dosedla (1974) and Bella (2006) both supposed that possible gravitational slope block movements occurred close to the entrance of the cave. The motion of the blocks is documented by open failures on surface (Fig. 8).
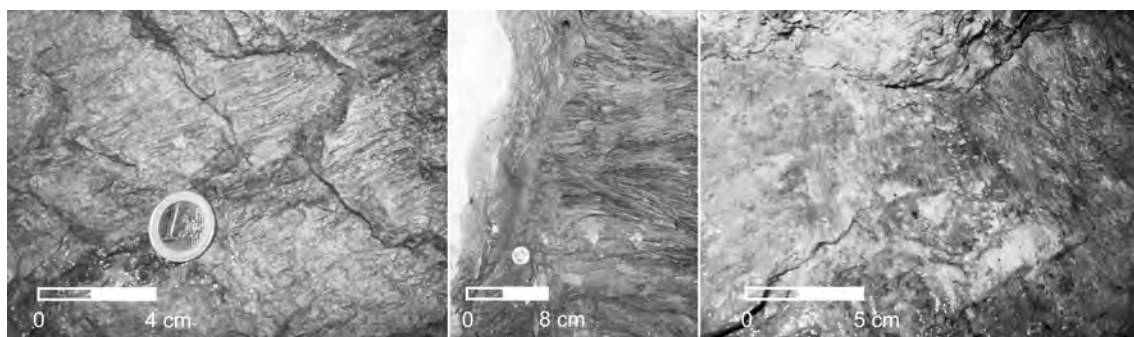

Figure 7. Faults with slickensides in nearby caves: (a) A slickenside on a NW-SE striking fault. This fault creates the passage of Pod orechom Cave; (b) A thrust fault, on the right hand side, cut by younger dextral strike-slip fault, on the left hand side, in Pod zavesenou Cave; (c) Slickenside on the fault plane below Site No. 1 in Driny Cave. The fracture steps, striations, and slickencrysts define the previous normal faulting along the fault plane.

Figure 8. An amphitheatre cliff with an open crack in vicinity of the entrance to Driny Cave. This crack is indicative of block movements in the scarp area.

Geomorphological Observations: Speleothem Damage

Within the cave, numerous broken or tilted stalagmites have been identified. These are usually assumed to reflect previous fault activity or sensitivity of the underground spaces to nearby earthquakes of significant power

(Fig. 9b). The freshly broken speleothems follow the fault strike in Driny Cave showing recent block displacements. Due to the fact that fresh cracks follow the blocks contacts and are found on the ceiling without any significant sinter crust or decoration (Fig. 9a), we could exclude all other principal causes recognized for speleothem breakage such as instability of the ground, collapse of the cave floor, water flow, ice, and anthropogenic impacts.

IN SITU DISPLACEMENT MONITORING

Over the past five years, the behavior of the faults has shown significant patterns. The NW-SE striking fault in 'Collaborators' Passage' (Driny 3) has displayed subsidence of the northeastern block by 0.03 mm/yr and dextral strike-slip displacement trend of 0.04 mm/yr (Fig. 10) The dextral strike-slip results are in full accordance with previous geological ideas regarding the mechanism of the transverse Malé Karpaty strike-slip fault displacements (Kováč et al., 2002). In addition, the observed sense of displacement reflects a continuation of the horizontal dextral shift previously noted on slickensides in the Collaborators' Passage. Furthermore, minor seasonal massif dilations have been noted at Driny 3. The crack opening component is associated with a peak-to-peak amplitude of 0.05 mm (Briestenský et al., 2010) and vertical seasonal variation is associated with a peak-to-peak amplitude of 0.07 mm. Such changes were not identified at the two other sites (Driny 1 and Driny 2).

The optical-mechanical crack gauges have also provided further understanding of the rock massif behavior. In contrast to the dextral strike-slips recorded along the NW-SE striking fault, sinistral strike-slip displacements have been recorded along the NNE-SSW directed faults (Fig. 10, Driny 1). The recognized sense of displacement reflects a continuation of the horizontal sinistral shift previously noted on a slickenside in the western part of the cave (Fig. 2b).This fault mechanism is due to the fact that the local stress field compression is thought to have recently been oriented in a NNW-SSE direction (or in
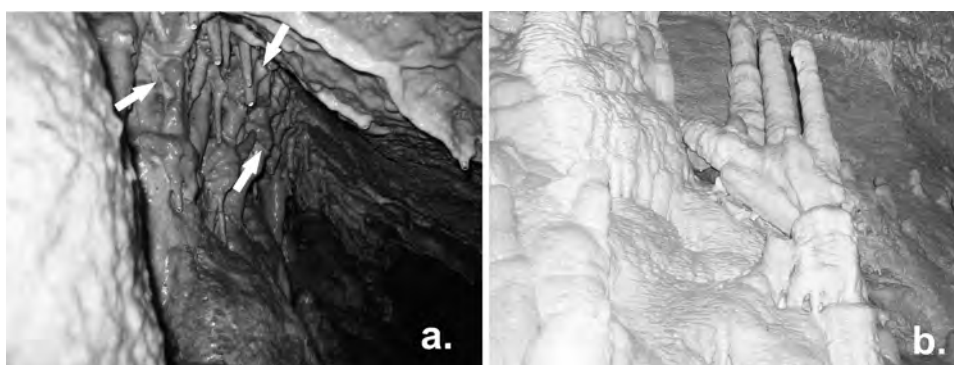

Figure 9. Damaged sinter decoration: (a) tiny cracks freshly healed by white sinter occur along the block contacts and affect ceiling sinter decoration across the entire cave system. The picture is taken close to Site 3; (b) a broken stalagmite in Driny Cave.
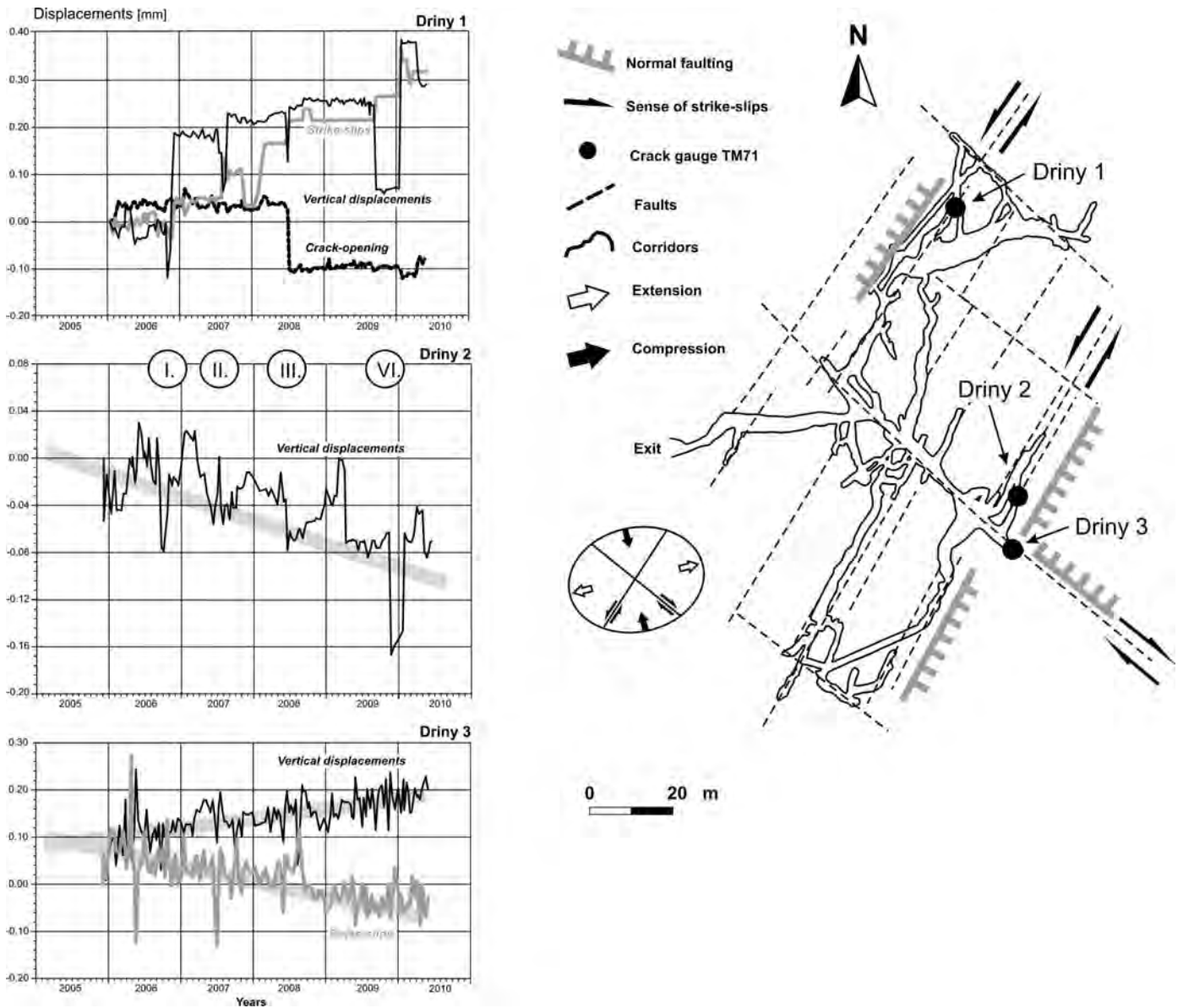
Figure 10. Significant displacements registered at the monitored sites (left) and the sense of recent horizontal strike-slip and normal fault displacements along the observed faults in Driny Cave (right). The black dots show the location of the crack gauges.

close declination) with extension in an ENE-WSW direction (Fig. 10). The sense of regional 2D deformation ellipse model is derived from McClay (1987). The sinistral strike-slip displacements are consistent with the interpreted displacement mechanism along the Smolenice Fault, in addition to the other NE-SW and N-S striking faults in the Malé Karpaty Mts (Kováč et al., 2002).

The vertical displacement recorded along the fault in Slovak Speleological Society Hall (Fig. 10, Driny 1) shows significant subsidence of the WWN block. This is in full accordance with the sense of fault displacement observed on the slickensides noted at the site. The active vertical block movements support our idea of a subsidence-

related origin for the valley in front of the entrance to Driny Cave. The subsidence of the WWN block is usually preceded by subsidence of the opposite EES block. This refers not only to one block shift, but also displacements in the block zone tend to subside in a westerly-dipping direction. The two block displacements are displayed as oscillations in the graph (Fig. 10, Driny 1). The most significant horizontal opening occurred at beginning of July 2008. The total shift of the WWN block reached 0.125 mm (Fig. 10, Driny 1).

These subsidence trends caused by block fault displacements are seen in the measurements recorded at Site 2 (Fig. 10, Driny 2). The total vertical trend, with a SE dip

direction, is 0.03 mm/yr. This vertical displacement is associated with the vast slope deformation above the village of Smolenice, which has affected the southeastern side of Driny Hill. These displacements are still active, as shown by the continuous formation of the slope deformation on the western side of Driny Hill. The most visible vertical displacements oscillations (two block movements) occurred at the same time at both Sites 1 and 2. Four periods of significant vertical displacement have been recorded (Fig. 10) along the NNE-SSW striking fault system, without any relationship to peak-to-peak seasonal amplitude. This demonstrates the sensitivity of the whole gravitectonic system to stress changes within the rock massif.

DISCUSSION

SUMMARY OF RESULTS

An important relationship between surface phenomena and faults cutting the cave has been identified. The geomorphological investigation of the adjacent area has shown significant slope deformation on the southeastern and western sides of Driny Hill. This deformation appears to reflect the underlying influence of two fault systems, the Smolenice Fault System and the transverse Malé Karpaty Mts System. These fault systems are now known to have also influenced the development of passages in Driny Cave as the cave system follows the strike of these two faults. It is clear that fresh sinter breakage in the cave is due to active block fault movement. In addition, slickensides preserved within the cave have allowed us to determine fault mechanisms. The sinter damage and slickensides have been used to help select appropriate sites for fault displacement monitoring. Three optical-mechanical crack gauges have been regularly monitored for the past five years. These data reveal continuing displacement activity characterized by dextral strike-slip movements along NW-SE striking faults and sinistral strike-slip movements along NNE-SSW striking faults. From this, it is deduced that the present stress field is subject to compression in a NNW-SSE direction. The vertical displacements observed along the faults demonstrate ongoing subsidence on the southeastern and western sides of Driny Hill. During the monitoring period, a significant period of earthquake tremors occurred from spring to fall 2006. At this time, all of the monitored structures in Driny Cave were affected by sudden reversals in their sense of movement (or displacement oscillations) with significant vertical and strike-slip displacements recorded. In total, four displacement oscillation events can be recognized. These appear to have been initiated along faults within the Blatnianska priehlbina Depression. It is proposed that these events reflect either higher levels of seismic activity or active basin development. Either way, it is clear that tectonic activity in the nearby Blatnianska priehlbina Depression has an influence on the fault displacements recorded within Driny Hill.

THE ADVANTAGES OF USING CLOSELY SPACED CRACK GAUGES

At the beginning of 2002, a monitoring network of optical-mechanical crack gauges (EU-TecNet) was established across central Europe (Stemberk et al., 2003). The network incorporates the most significant tectonic structures of the Bohemian Massif and Western Carpathians. A number of recent studies have shown that this monitoring net is able to record aseismic fault displacements with a high degree of precision (e.g., Šebela et al., 2005; Briestenský et al., 2007; 2010; Štěpančíková et al., 2008). Unfortunately due to the considerable distances between monitoring sites, understanding the precise kinematics can be complex. For example, one of the most significant problems encountered during such measurements relates to the interpretation of sudden reversals in the sense of movement. It is frequently unclear as to whether one fault block remains entirely stable while the adjacent block is active or whether there is motion on both blocks. Therefore, the three monitoring crack gauges installed at Driny Cave provide a unique opportunity to examine displacement along adjacent fault systems. From these, it has been possible to define the precise kinematics of the fault movements and thereby constraining the underlying mechanism. These results also help to define the total amount of displacement along the fault. In addition, the closely spaced crack gauges are able to reveal recent changes in the local stress field orientation (Briestenský and Stemberk, 2008).

THE ADVANTAGES OF USING KARSTIC CAVE SYSTEMS

To counter the problems associated with identifying active tectonic structures in intracratonic settings, we chose to specifically examine karstic cave systems for evidence of active faulting. These systems are better able to preserve a record of displacement because the activity is recorded within a three dimensional framework unaffected by subsequent erosion. In particular, it is suggested that karstic cave systems have four main advantages. First, fresh sinter breakage is frequently due to active block fault movements. In this study, the subsequent measurements recorded in the cave have corroborated this idea. Second, the slickensides preserved within the cave have allowed us to determine fault mechanisms. Third, the geometry of the individual passages within the cave system is ideal for the installation of optical-mechanical crack gauges. Fourth, there is no significant correlation between fault displacements and precipitation. It should be noted that a minor correlation between fault displacement and seasonal massif dilation can be indentified but this does not affect the overall trend. The influence of climate decreases with depth below the surface. This is discussed further in Briestenský et al. (2010).

CONCLUSIONS

This study has successfully integrated geological, geomorphological, and in situ displacement data recorded around and within a karstic cave system. These results

demonstrate the existence of active tectonic structures within the intracratonic setting of central Europe in the junction zone between Eastern Alps and Western Carpathians. In particular:

- The cave system has developed along significant fault structures, which can be seen from geological mapping and slickenside analyses.
- The possibility of ongoing faulting and block movements were suggested by the considerable number of slope failures on hillsides above the cave and the numerous fresh speleothem breaks observed within the cave.
- The notion of active faulting and block movements has been confirmed and quantified using three optical-mechanical crack gauges.
- It has been possible to define the precise fault kinematics. Different strike-slip mechanisms along two transverse fault systems point to a horizontal stress field orientation.

It is considered that the methodology described here can also be applied in other intracratonic settings where karstic cave systems are present. This would help define potentially seismogenic areas where unambiguous evidence for active faulting is lacking.

Acknowledgements

References

Bella, P., 2003, Slovensko sprístupnené jaskyne [Slovakia show caves]: Slovak Caves Administration, Liptovský Mikuláš, p. 38–41.

Bella, P., 2006, Príspevok k morfológii a genéze jaskyne Driny [A contribution to the morphology and genesis of Driny Cave]: Aragonit, v. 11, p. 4–9.

Briestenský, M., Stemberk, J., and Petro, L., 2007, Displacements registered around March 13th 2006 Vrbové earthquake, M = 3.2 (Western Carpathians): Geologica Carpathica, v. 58, p. 487–493.

Briestenský, M., and Stemberk, J., 2008, Monitoring mikropohybov v jaskyniach Západného Slovenska [Monitoring microdisplacements in the caves of western Slovakia]: Slovenský kras, v. 46, p. 77–83.

Briestenský, M., Košťák, B., Stemberk, J., Petro, L., Vozár, J., and Fojtíková, L., 2010, Active tectonic fault microdisplacement analyses: a comparison of results from surface and underground monitoring in western Slovakia: Acta Geodynamica et Geomaterialia, v. 7, p. 387–397.

Droppa, A., 1951, Smolenický kras v Malých Karpatoch [Smolenice Karst in the Small Carpathians]: Zemepisný sborník [Geographical Proceedings], v. 3, p. 7–52.

Dosedla, J., 1974, Příspěvek k poznání krasu v Malých Karpatech [Contribution to the knowledge of karst in the Small Carpathians]: Sborník Československé Společnosti Zeměpisné [Proceedings of Czechoslovak Geographical Society], v. 52, p. 88–91.

Gilli, E., 2005, Review on the use of natural cave speleothems as palaeoseismic or neotectonics indicators: Comptes Rendus Geosciences, v. 337, p. 1208–1215.

Grabowski, J., Michalík, J., Pszczólkowski, A., and Lintnerová, O., 2010, Magneto- and isotope stratigraphy around the Jurassic/Cretaceous boundary in the Vysoká Unit (Malé Karpaty Mountains, Slovakia): correlations and tectonic implications: Geologica Carpathica, v. 4, (in press).

Kashima, N., 1993, Fracture of speleothems in Hoshino-no-ana Cave, Minami-Daito Island, Okinawa Prefecture, Southwest Japan: Journal of the Speleological Society of Japan, v. 18, p. 33–41.

Košťák, B., 1991, Combined indicator using moiré technique in Sorum, G. Field Measurements in Geomechanics, Rotterdam, Balkema, p. 53–60.

Košťák, B., 2006, Deformation effects in rock massifs and their long-term monitoring: Quarterly Journal of Engineering Geology and Hydrogeology, v. 39, p. 249–258.

Kováč, M., Bielik, M., Hók, J., Kronome, B., Labák, P., Moczo, P., Plašienka, D., Šefara, J., and Šujan, M., 2002, Seismic activity and neotectonic evolution of the Western Carpathians (Slovakia): EGU Stephen Mueller Special Publication, Series 3, p. 167–184.

Maglay, J., editor, 2006, Geological Map of the Danube Lowland-Trnavská Pahorkatina Upland: State Geological Institute of Dionýz Štúr, scale 1:50 000, 1 sheet.

McClay, K.R., 1987, The Mapping of Geological Structures: Milton Keynes, Open University Press, 161 p.

Michalík, J., Reháková, D., and Marko, F., 1992, Stratigrafia a tektonika spodnokriedovej vápencovej sekvencie v profile jaskyne Driny (Vysoká jednotka, Malé Karpaty Mts) [Stratigraphy and tectonics of lower Cretaceous limestone sequence in a profile from Driny Cave (Vysocká Unit, Small Carpathians)]: Mineralia Slovaca, v. 24, p. 235–243.

Mitter, P., 1983, Geomorfologická rajonizácia krasu Malých Karpát [Geomorphological zoning of the karst Small Carpathians]: Slovenský kras, v. 21, p. 3–34.

Palmer, A.N., 1991, Origin and morphology of limestone caves: GSA Bulletin, v. 103, p. 1–21.

Petit, J.P., 1987, Criteria for the sense of movement on the fault surfaces in brittle rocks: Journal of Structural Geology, v. 9, p. 585–608.

Rybář, J., 1973, Representation of Landslides in Engineering Geological Maps: Landslide, v. 1, p. 15–21.

Slovak Caves Administration, 2006, Driny Cave: www.ssj.sk/jaskyne/spristupnene/driny [accessed: 06.26.2010].

Šebela, S., 2008, Broken speleothemes as indicator of tectonic movements: Acta Carsologica, v. 37, p. 52–62.

Šebela, S., Gosar, A., Košťák, B., and Stemberk, J., 2005, Monitoring of active faults in Slovenia: Acta Geodynamica et Geomaterialia, v. 1, p. 45–57.

Stemberk, J., and Košťák, B., 2007, 3-D trend of aseismic creep along active faults in western part of the Gulf of Corinth, Greece: Acta Geodynamica et Geomaterialia, v. 4, p. 53–65.

Stemberk, J., and Košťák, B., 2008, Recent tectonic microdisplacements registered in Bedřichov Tunnel "B" in the Jizerské Hory Mts (N Bohemia): Acta Geodynamica et Geomaterialia, v. 5, p. 377–388.

Stemberk, J., Košťák, B., and Cacoń, S., 2010, A tectonic pressure pulse and increased geodynamic activity recorded from the long-term monitoring of faults in Europe: Tectonophysics, v. 487, p. 1–12.

Stemberk, J., Košťák, B., and Vilímek, V., 2003, 3-D monitoring of active tectonic structures: Journal of Geodynamics, v. 36, p. 103–112.

Stemberk, J., and Štěpančíková, P., 2003, Tectonic setting and monitoring of recent tectonic deformation in the Rychlebské Hory Mts: Acta Montana, v. 24, p. 153–161.

Štěpančíková, P., Stemberk, J., Vilímek, V., and Košťák, B., 2008, Neotectonic development of drainage networks in the East Sudeten Mountains and monitoring of recent fault displacements (Czech Republic): Geomorphology, v. 102, p. 68–80.

Štěpančíková, P., Hók, J., Nývlt, D., Dohnal, J., Sýkorová, I., and Stemberk, J., 2010, Active tectonics research using trenching technique on the south-eastern section of the Sudetic Marginal Fault (NE Bohemian Massif, central Europe): Tectonophysics, v. 485, p. 269–282.

Zelinka, J., 2000, Speleoklimatický monitoring jaskyne Driny [Speleoclimatic monitoring in Driny Cave] in Proceedings, Výskum, využívanie a ochrana jaskýň [Research, Use, and Conservation of Caves], 2nd, Liptovský Mikuláš: Liptovský Mikuláš, Slovak Caves Administration, p. 168–179.

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