JOURNAL OF CAVE AND KARST STUDIES

August 2005
Volume 67 Number 2
ISSN 1090-6924
A Publication of the National Speleological Society

Journal of Cave and Karst Studies of the National Speleological Society

Volume 67 Number 2 August 2005

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The *Journal of Cave and Karst Studies* (ISSN 1090-6924, CPM Number #40065056) is a multi-disciplinary, refereed journal published three times a year by the National Speleological Society, 2813 Cave Avenue, Huntsville, Alabama 35810-4431 USA; (256) 852-1300; FAX (256) 851-9241, e-mail: nss@caves.org; World Wide Web: http://www.caves.org/pub/journal/. The annual subscription fee, worldwide, by surface mail, is $18 US. Airmail delivery outside the United States of both the *NSS News* and the *Journal of Cave and Karst Studies* is available for an additional fee of $40 (total $58); The *Journal of Cave and Karst Studies* is not available alone by airmail. Back issues and cumulative indices are available from the NSS office. POSTMASTER: send address changes to the *Journal of Cave and Karst Studies*, 2813 Cave Avenue, Huntsville, Alabama 35810-4431 USA.

The *Journal of Cave and Karst Studies* is covered the the following ISI Thomson Services: Science Citation Index Expanded, ISI Alerting Services, and Current Contents/Physical, Chemical, and Earth Sciences.

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Front cover: Elks Room in Wind Cave (photo by Peter and Ann Bosted). See John C. Moore, Paul Saunders, Greg Selby, Howard Horton, Marisa K. Chelius, Amanda Chapman, and Rodney D. Horrocks, p. 110.

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Malcolm S. Field - Why publish discussions and author responses to papers published in the *Journal of Cave and Karst Studies*? *Journal of Cave and Karst Studies*, v. 67, no. 2, p. 91.

WHY PUBLISH DISCUSSIONS AND AUTHOR RESPONSES TO PAPERS PUBLISHED IN THE *JOURNAL OF CAVE AND KARST STUDIES*?

Malcolm S. Field

In the last issue of the *Journal of Cave and Karst Studies* (April 2005; vol. 67, no. 1), two discussion articles (Barton and Pace, 2005; Davis, 2005) of a previously published paper (Hunter *et al*., 2004) were published. Responses by Hunter *et al*. [this issue] are also published. A question that some readers may be asking: Why is it necessary to include discussions and author responses in the *Journal of Cave and Karst Studies*?

Allowing for the development and publication of a discussion article in the *Journal* furnishes readers with different perspectives on the published research. By affording individuals the opportunity to publish discussion articles of previously published papers, it is then also necessary that the original authors be given an opportunity to respond.

Many top-rated peer-reviewed journals include provisions for comments or discussions of previously published articles to also be published for the benefit of their readers. Publishing these discussions allows for the correction of errors possibly included in the original paper, publication of additional insights gleaned from the original paper, and possible suggestions for future research based on the original research.

The publication of author responses to discussions is equally important. Ideally, responses would be published in the same issue as the discussion article so that readers can get both perspectives immediately, but this is not always feasible. An author-produced response to a discussion article allows for the correction of potential errors contained in the discussion, additional overlooked insights by both the original paper's author and the discussion author, or just acknowledgment of the discussion article's value.

The notion that the purpose of a discussion article might be to correct errors in a published paper might suggest a problem with the peer-review process. Peer-review does not necessarily mean that a paper has been thoroughly examined to ensure scientific "perfection," which is unrealistic. Rather, peer-review does ensure that the basic research concepts, methods, and conclusions are sound and reasonable.

In general, readers of scientific literature generally assume that when an article is published in a peer-reviewed journal it means that someone has checked the data and perhaps even replicated how the data was collected and analyzed, checked the equations used and calculations made, and checked that the stated conclusions are fully supported by the evidence presented (McIntyre, 2005). But peer-review does not guarantee any of this, especially because many, if not most, journal editors and reviewers work as volunteers.

The net effect is that influential papers can continue to be quoted for years without the data or methods ever being fully evaluated, let alone independently checked, even as future research projects or policies are developed based on the previous work. Publication of discussions of papers will not ensure that any errors contained in the original work will subsequently be caught and corrected. However, it is perhaps more probable that the errors will be caught by someone who has some related expertise in the subject matter and who has conducted a more in-depth analysis and prepared a discussion article of the original work.

Overall, it is believed that publication of discussions and responses will be good for karst science in general and the karst community at large. By regularly publishing discussions and responses, the science will necessarily improve as prospective authors become more careful in their research and presentation. In addition, controversial concepts brought out by the discussions and responses may lead to new directions for research and study.

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GEOMORPHIC HISTORY OF CRYSTAL CAVE, SOUTHERN SIERRA NEVADA, CALIFORNIA

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Cave development in mountainous regions is influenced by a number of factors, including steep catchments, highly variable allogenic recharge, large sediment fluxes, and rapid rates of canyon downcutting. Caves can help to quantify this latter process, provided their ages are determined. Here we investigate the history of 4.8 km long Crystal Cave, a complex, multiple level cave in the Sierra Nevada, through detailed geomorphic and geochronologic investigations. Crystal Cave is composed of six major levels spanning 64 m in elevation. The levels are comprised of large, low gradient conduit tubes, and are connected by numerous narrow, steeply descending canyon passages. Passages in the upstream end of the cave are significantly modified by collapse, while in the downstream section they are intact with an anastomotic maze overprinting. Dye tracing confirms that the cave stream originates from partial sinking of Yucca Creek to the north. Passage gradients, wall scallops, and sediment imbrication indicate that groundwater flowed consistently southeast through time, forming cave levels as bedrock incision of Cascade Creek lowered local base level. Although modern cave stream discharges are restricted to ~0.03 $m^3 s^{-1}$*, likely due to passage collapse near the sink point ca. 0.5 million years ago (Ma), bedrock scallops and coarse clastic sediment in upper levels indicate paleodischarges as much as three orders of magnitude greater prior to that time. Infrequent high discharge flood events played an important role in passage development and sediment transport. Cosmogenic* $^{26}Al/^{10}Be$ *burial dating of sediment suggests that the majority of Crystal Cave formed rapidly between ca. 1.2 and 0.5 Ma; rates of cave development approach theoretical maximums, presumably due to a combination of allogenic recharge highly undersaturated with respect to calcite, and physical erosion by transported sediment.*

INTRODUCTION

Mountainous regions impart a distinctive set of conditions that affect cave development. Among these are steep, often sparsely vegetated catchments, highly variable stream discharges, allogenic recharge undersaturated with respect to calcite, high sediment flux, and rapid rates of landscape erosion. Caves can play an important role in quantifying the topographic evolution of mountainous regions because their development is often closely tied to local base level position, which is set by the most deeply incised local stream. Provided their ages can be determined, caves in canyon walls can record rates of river incision and topographic development (e.g., Ford *et al*., 1981; Atkinson and Rowe, 1992; Sasowsky *et al.*, 1995; Farrant *et al.*, 1995; Springer *et al.*, 1997; Granger *et al.*, 1997, 2001; Stock *et al.*, 2004; Anthony and Granger, 2004). One fundamental challenge facing cave-related landscape evolution studies is reliably determining cave ages (Sasowsky, 1998; Stock *et al.*, 2005). Another challenge is discerning the history of cave development through observations, measurements, and interpretations of cave morphology. If these two challenges can be sufficiently resolved, and the resulting information integrated into a comprehensive geomorphic history, then caves can provide important constraints on the evolution of mountainous topography.

Stock *et al.* (2005) dated sediment and speleothem deposits from caves in the Sierra Nevada of California and concluded that cosmogenic $^{26}Al/^{10}Be$ burial dating of coarse clastic sediment provided the most reliable cave ages. Using the chronology provided by burial ages, we have investigated the geomorphology of Crystal Cave, a complex, multi-level system, to understand better how caves develop in mountain settings and how cave morphology relates to overall landscape evolution.

SETTING

Crystal Cave is located within Sequoia National Park, on the western slope of the southern Sierra Nevada (Fig. 1 inset). The Sierra Nevada is an asymmetric, west-tilted fault block with a mean altitude of ~2800 m and altitudes at the range crest as great as 4419 m (Bateman and Wahrhaftig, 1966; Wakabayashi and Sawyer, 2001). Below the glacially sculpted range crest, the broad western slope of the range descends in a series of undulating low relief upland surfaces punctuated by deeply incised river canyons. Most of the caves in the range have formed proximal to these rivers.

The Sierra Nevada presents a unique setting for cave forming processes. The climate of the study area is distinctly Mediterranean, with cool, wet winters and hot, dry summers. Groundwater recharge for Sierra Nevada caves is typically allogenic, derived from sinking streams that originate in granitic catchments. These drainages are often steep and may drop several thousand meters over very short (10–30 km) distances. Much of the precipitation in the southern Sierra Nevada falls in the winter as snow. Increasing spring temperatures lead

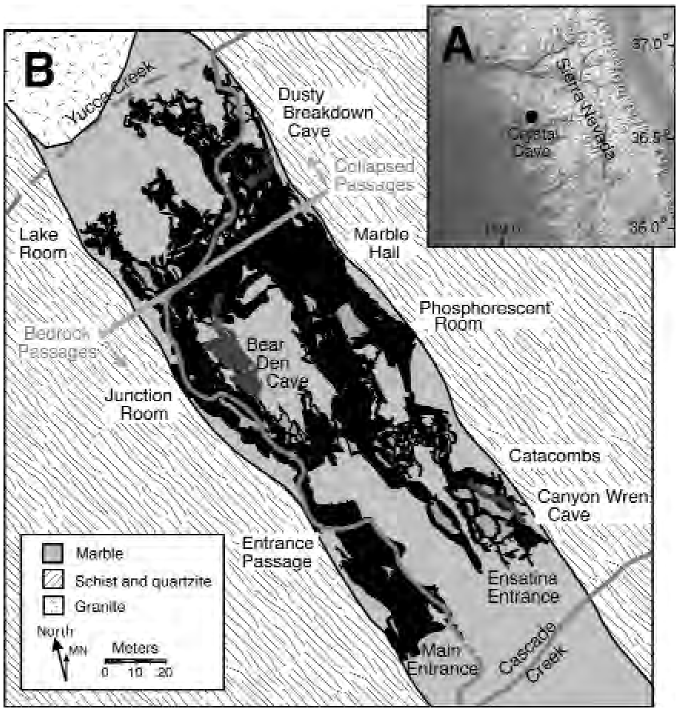

Figure 1. A: Location of Crystal Cave in the southern Sierra Nevada. B: Plan view of the cave showing passage layout, hydrology, and geology. Satellite caves shown in dark gray; streams shown by light gray lines, dashed where intermittent or inferred. Geology modified from Sisson and Moore (1994).

to dramatic seasonal run-off with stream discharges rising two or even three orders of magnitude over the course of days to weeks. Less frequent but much larger floods, resulting from unseasonably warm rainfall onto a dense snow pack, augment these annual floods. Recent events of this nature have occurred within the study area in 1957, 1969, 1997 and 2002. Because of the steep granitic catchments that supply water to Sierra Nevada caves, and the dominance of sinking stream recharge and flooding, sediment flux through cave passages is high, complicating cave conduit development.

Crystal Cave and adjacent small satellite caves sit at elevations ranging from 1386 to 1446 m, and occupy the lower 64 m of a marble ridge approximately 160 m long and 40 m wide (Fig. 1). Within this narrow ridge there are 4.87 km of surveyed cave passages. The extent of cave passages and the small size of the marble lens have produced a high percentage of voids within the bedrock, ~11%. The cave and ridge lie between Yucca and Cascade creeks, two tributaries of the North Fork of the Kaweah River. In the vicinity of Crystal Cave, these streams have incised as much as 250 m into the surrounding bedrock, forming deep canyons with steep (up to 55°) hillslopes. Although located near the western limit of Pleistocene glaciation, these drainages remained ice-free during glacial periods (Matthes, 1965), so their rugged relief is solely a product of stream incision.

GEOLOGIC AND STRUCTURAL CONTROLS ON CAVE DEVELOPMENT

Crystal Cave has formed within marble of the Sequoia pendant, one of many metamorphic pendants trending northwest across the Sierra Nevada. The Sequoia pendant is composed primarily of Jurassic and/or Triassic quartz-biotite schist interlayered with quartzite, but includes tabular masses of coarse-grained marble (Sisson and Moore, 1994). A general northwest trend of these rocks is evident despite strong deformation; beds typically strike northwest (~320°) and are nearly vertically oriented (~87° SW). The Sequoia Pendant is approximately 27 km long and 2 km wide, and is surrounded by Jurassic and Cretaceous plutonic rocks of the Sierra Nevada Batholith (Sisson and Moore, 1994). Crystal Cave has formed in a narrow lens of coarsely crystalline, vertically bedded marble. This lens is never more than 60 m wide, though it extends in a northwesterly orientation for nearly 2 km. The marble lens is bounded on both the northeast and southwest by biotite-feldspar-quartz schist (Fig. 1). Numerous thin (0.1–2 m) interbeds of schist and quartzite are present within the marble.

Most passages in Crystal Cave have developed on strike, parallel to the axis of the marble lens and the metamorphic pendent (Fig 1). Many cave walls are composed of schist, particularly along the margins of the cave, and these contacts provided the primary porosity for passage development.

DESCRIPTION OF CAVE PASSAGES

During a three-year-long effort, and with the help of many volunteers, we surveyed Crystal Cave using compasses, clinometers and fiberglass tapes. Fore- and back-sight readings along major routes and between entrances produced surveys with less than 1% error. Resurveys of poor loop closures (>2 standard deviations) in smaller passages and minor routes produced a total survey error of less than 2%. Crystal Cave currently stands at 4.77 km long, while adjacent Bear Den, Dusty Breakdown, and Canyon Wren caves are 46, 25, and 27 m long, respectively (Fig. 1).

The Crystal Cave system is composed of six distinct levels (the term “level” here refers to a specific phase of cave development and does not imply a lack of passage gradient). The levels have a combined vertical relief of 64 m (Fig. 2). Four of the levels contain the largest passages in the cave, primarily large, low gradient tubes and wide canyons (Oberhansley, 1946). Two levels have smaller passages and are shorter in length with more prominent vadose incision in passage floors. Steep, narrow, meandering vadose canyons connect all but one of the levels (Fig. 2).

The highest levels of the cave lie preferentially near the eastern, upstream edge of the marble, and subsequent lower levels show a progressive shift of cave development toward the western, downstream edge of the marble (Fig. 3). The lowest level of the cave contains a perennial stream. In the northern upstream portion of the cave extensive breakdown collapse is superimposed upon previously intact passages. The col-

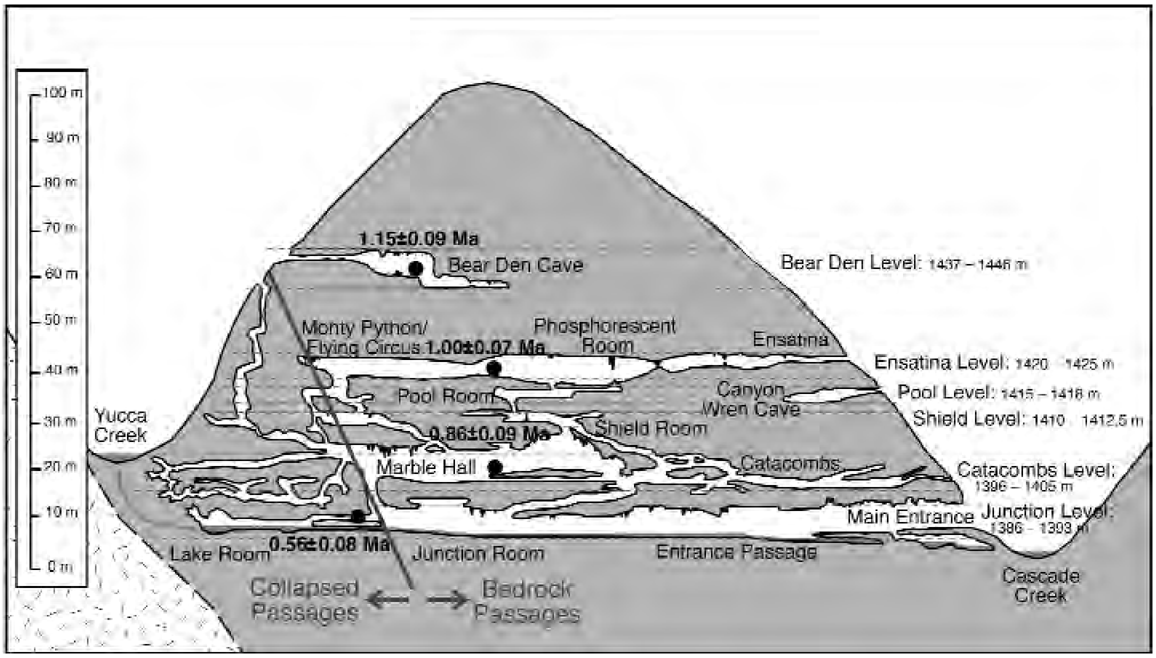

Figure 2. Profile view of Crystal Cave and surrounding caves showing pronounced cave levels. Highest level cave (Bear Den) formed first, while lowest level (Entrance Passage) contains an active vadose stream and several shallow phreatic pools. Vertical development of cave levels is controlled by incision rate of Cascade Creek; this rate is constrained by cosmogenic ^{26}Al/^{10}Be burial dates for coarse clastic sediments shown by black circles (Stock *et al.*, 2005).

lapsed area extends from base-level pools in the lowest sections of Crystal Cave to the overlying hillslope (Fig 2). Collapsed areas include Dusty Breakdown Cave and the northernmost 65 m of Crystal Cave, north of the Fault Room (Fig. 1).

The uppermost level of the cave system, the Bear Den level (Fig. 2), consists of a small upper canyon 0.3–0.5 m wide and 2–3 m tall and a lower section of broad rooms 3–10 m wide. Below the Bear Den level is a 12 m vertical gap in passage development. The next highest level, the Ensantina level, is a low-gradient passage containing the large Phosphorescent Room, 8 m wide and 4 m tall. Much of the Ensatina Passage lies along the eastern margin of the marble (Fig. 3). The upstream end of the Phosphorescent Room terminates in a large choke of sediment covered with flowstone. Two parallel and interconnected canyons (the Monty Python and Flying Circus passages) bifurcate from the northwest end of the Phosphorescent Room at a non-graded intersection. Four steep, narrow canyons approximately 1 m tall and 0.5 m wide connect the Ensatina level to the smaller Pool Room level below (Fig. 2). The relatively small Pool Room and Shield Room are distinguished from their connecting slot canyons by their very low gradients and broad cross sections. Although the downstream end of the Pool Room level terminates in flowstone, Canyon Wren Cave, 40 m to the southeast, is the apparent continuation of this level. Two steep slot canyons connect the Pool level to the Shield level, and five such canyons connect the Shield Room level to the lower-lying Catacombs level. The Catacombs level contains the most extensive development within the cave system, including Marble Hall (the largest room in the cave), the Dome Room, the Organ Room, the Cathedral and Junction balconies, the Catacombs, and numerous adjacent passages. The complex anastomotic mazes of the Catacombs make up the downstream terminus of this level of the cave adjacent to the Cascade Creek canyon. The Organ Room/Curtain Room area constitutes one of only two sections of the caves that developed perpendicular to strike, across the marble lens. The other passage, the Fault Room, is an enlarged fracture parallel to and at the margin of northern collapsed sections of the caves. Canyons connecting the Catacombs level to the lowest Junction level vary from gently dipping to vertical slots. The Junction level consists primarily of the large Entrance Passage, which is up to 6 m wide and 6 m tall and contains the active cave stream.

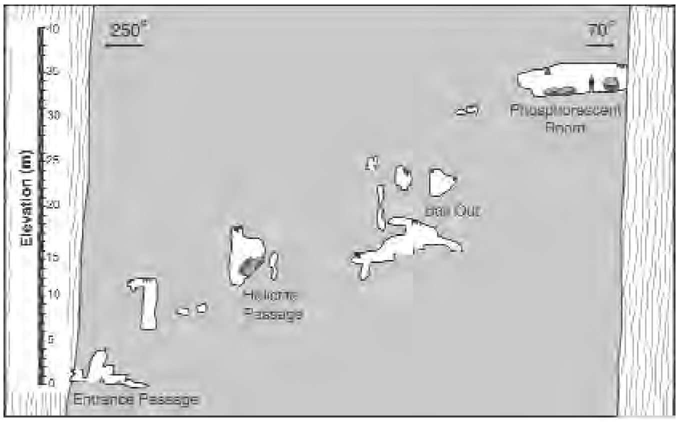

Figure 3. Cross-section view through Crystal Cave passages, normal to north-northwest trend of cave, showing progressive shift of cave development toward western, downstream edge of marble.

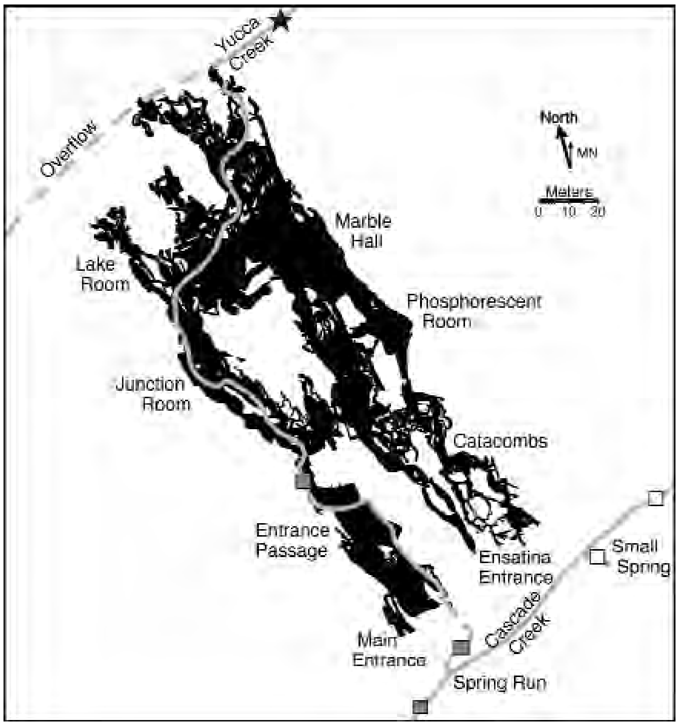

Figure 4. Plan view of Crystal Cave showing streams, locations of dye receptors, and results of dye trace experiment. Cave and surface streams shown by gray lines, dashed where intermittent. White squares show the locations of receptors that did not contain dye, and gray squares mark locations of receptors showing dye within 24 hours after the dye injection. Star indicates location of dye injection point.

PRESENT HYDROLOGIC SETTING

The active cave stream first appears in the northern portion of the cave through breakdown and subsequently flows through the large Entrance Passage to emerge in front of the Spider Web Gate Entrance as a small spring (Fig. 4). To test our assumption that the cave stream derives from sinking of Yucca Creek, we conducted a qualitative dye trace. We used dye receptors of ~10 g of activated coconut husk charcoal contained within mesh netting, and positioned them at various sites within the Crystal Cave hydrologic system (Fig. 4). We then injected 2 L of Rhodamine WT dye into Yucca Creek approximately 40 m upstream from the sink point. The following day we collected the receptors and, after rinsing with water and drying, eluted the dye with ~50 ml of 10% ammonium hydroxide in 50% aqueous 1-propanol solution (Smart and Brown, 1973). The dye trace produced positive results in three locations: (1) Yucca Creek downstream from the cave, (2) the cave stream, and (3) below the cave stream resurgence (Fig. 4). This test confirms that the cave stream originates from Yucca Creek. Transit time through the cave was less than 24 hours, suggesting that water storage within the cave is minimal.

Surface streams in the Sierra Nevada experience considerable seasonal and annual fluctuations in discharge, largely dependent on the amount of snow pack and rapidity of melt. However, the Crystal Cave stream presently appears to be immune to such discharge fluctuations. For example, we measured the discharge of the cave stream during 1994, a relatively dry year with only 48% of mean annual precipitation, and again in 1995, a wet year with 270% of mean annual precipitation. Discharge in early May of 1994 was 0.028 $m^3 s^{-1}$, and by September had dropped to 0.012 $m^3 s^{-1}$. Despite markedly greater precipitation, stream discharges in 1995 were similar to those in 1994. Discharge in mid-May of 1995 was 0.031 $m^3 s^{-1}$, and dropped to 0.014 $m^3 s^{-1}$ in September. The surprisingly small variance reveals that factors other than precipitation amount and runoff control discharge of the cave stream. Collapse and passage blockage in the northern, upstream sections of Crystal likely allowed a mantle of granitic sediments and landslide debris to constrict the sink area, limiting recharge into the cave. During times of low discharge, generally July through October, all of Yucca Creek diverts underground, while during times of high discharge, November to June, much of the flow remains on the surface (Fig. 4).

PALEOHYDROLOGY

Asymmetric bedrock scallops on cave surfaces can be used to infer both paleoflow direction and velocity (Curl, 1974). We examined 222 scallops at 17 locations within the major levels of the cave to understand better the paleohydrologic regimes that formed these passages. Measured scallops were selected based on abundance (i.e., the number of scallops present at a site) and the presence of distinct scallop margins needed for precise measurements. Scallop lengths were measured across their greatest length, and width measured normal to the length. Scallop orientations consistently show that the present pattern of water flow through the cave (from Yucca Creek to Cascade Creek) persisted throughout the duration of cave development. This observation is corroborated by both ceiling and floor gradients of major passages that dip from Yucca Creek toward Cascade Creek, and by occasional imbrication of coarse sediments within these passages.

Curl (1974) demonstrated a relation between mean scallop length, *L*, and the Reynolds number, Re_L, for scallops in both parallel wall and circular conduits. We determined mean scallop lengths for each set of scallop populations and used Curl's (1974) predicted relation between Reynolds number and the ratio of conduit width, D, to mean scallop length, *L*, in parallel wall conduits to determine Re_L values for each site. We then used the relation between *L* and Re_L to calculate mean flow velocity, v, through these conduits using the relation:

$$v = \upsilon \frac{\overline{\mathrm{Re}_L}}{\overline{L}} \qquad (1)$$

where υ is the kinematic viscosity (~0.013 $cm^2 s^{-1}$ for fresh water at 10°C; Curl, 1974). Scallop lengths indicate reasonable paleoflow velocities ranging from 0.05 to 0.96 $\mathrm{ms^{-1}}$ (Table 1). Multiplying flow velocities by passage cross-sectional areas yield paleodischarges at these sites that span two orders of magnitude, ranging from 0.12 to 15.19 $\mathrm{m^3s^{-1}}$ (Table 1). As a first order approximation, scallop measurements imply extremely variable discharges, presumably due to flood events, a common occurrence in steep mountainous catchments. This finding is in general accord with those of Lauritzen *et al.* (1983, 1985) who found that modern scallops in Norwegian caves preserved flood discharges three times larger than mean annual discharges. However, a potentially serious complication inherent to scallop-based discharge calculations is that passage cross-sectional areas may have enlarged considerably after scallops formed; thus, although the flow velocities determined from scallops may be accurate, calculated discharge values may be too large. In fact, we see abundant evidence for vadose enlargement of many passages after their initial development (see below).

To check the accuracy of the scallop-based paleodischarges, we used an independent method for determining paleodischarges in these passages based on the size of clastic sediment particles. Sediment particles of a certain size require a critical shear stress generated by the flow to entrain them. For spherical particles, the relationship between critical shear stress, τ_c, and particle size, d, is described by the Shields equation (Shields, 1936):

$$\tau_c = \beta\left(\rho_p - \rho_f\right)gd \tag{2}$$

where β is the Shields function (0.056 for typical gravel beds), ρ_p is the particle density (2700 kg $\mathrm{m^{-3}}$), ρ_f is the fluid density (1000 kg $\mathrm{m^{-3}}$), g is gravitational acceleration (9.81 m $\mathrm{s^{-2}}$), and d is the sediment particle diameter in meters. We examined 63 sediment particles at 19 sites. At each site, we measured the population of largest spherical particle diameters, which best represent the maximum discharge conditions before the basal shear stress of the flow fell below the critical shear stress necessary to transport the particles. We then used τ_c values to calculate the critical flow depths, h_c, required to entrain the particles, using an expression for basal shear stress (Bagnold, 1966):

$$\tau_b = \rho_f g h_c S \tag{3}$$

where S is the local passage slope. We determined passage slopes by averaging over a length of ~30 m up and down passage from the sample sites; when possible, we used the slope of passage ceilings rather than floors, which are uneven due to accumulations of sediment and breakdown. We determined critical flow velocities, U_c, by combining the critical flow depths with two different methods for estimating the flow resistance. The first method uses a friction factor, f, which is a function of the Reynolds number and the relative conduit roughness:

$$U_c = \frac{\sqrt{8gh_cS}}{f} \tag{4}$$

where f is the friction factor, assumed to be 0.05, a value typical for turbulent flow in most cave conduits (Palmer, 1987). The second method of calculating the critical flow velocity utilizes a flow resistance based on hydraulic radius:

$$U_c = \frac{R_H{}^{0.66}S^{0.5}}{n} \tag{5}$$

where R_H is the hydraulic radius, determined using passage width and the critical flow depth calculated from Eq. 3. The variable n is known as Manning's roughness coefficient, and is defined as:

$$n = 0.32S^{0.38}h_c{}^{-0.16} \tag{6}$$

Again, we multiplied the critical flow velocities calculated by these two methods (friction factor f and Manning's n) by passage cross-sectional areas to derive maximum paleodischarges (Table 2). As with those based on scallops, the paleodischarges we calculate from sediment clast sizes are quite variable, ranging from 0.01 to 39.1 $\mathrm{m^3\ s^{-1}}$. All but one of these paleodischarges are greater than the present maximum stream discharge of ~0.03 $\mathrm{m^3\ s^{-1}}$. This is expected because most of the sediment clasts we used to calculate paleodischarges are larger than those in the active cave stream channel, and our observations indicate that even these smaller clasts are not being transported by the present low stream flow. Even when the considerable uncertainty in the paleodischarge measurements is taken into account, the large (up to 20 cm) sediment clasts in rooms such as the Phosphorescent Room demand much higher discharges than the present stream flow to transport them. Thus, there is considerable evidence implicating infrequent but very large discharge events in the dissolution and sedimentation of the cave. Greater discharges in the past likely reflect changing climatic conditions; the present warm and dry Holocene climate of the Sierra Nevada differs markedly from the cooler, wetter climates of glacial times that dominated most of the past ~2 Myr (e.g., Benson and Thompson, 1987; Hostetler and Clark, 1997; Bartlein *et al.*, 1998; Clark *et al.*, 2003). Paleodischarges recorded by scallops and sediment clasts likely occurred during large floods that caused cave passages to enlarge rapidly.

PHREATIC AND VADOSE DEVELOPMENT

In the absence of horizontal structural control, as is generally the case in vertically bedded rock, large, low-gradient passages are thought to form at or near the piezometric surface. The piezometric surface marks the position of the water table and therefore the transition between phreatic and vadose cave

Table 1. Flow velocities and discharges for Crystal Cave passages deduced from bedrock scallops.

Location	L[a] (m)	Conduit width (m)	D/L ratio[b]	Reynolds number[c]	Flow velocity (m s^{-1})	Cross-sectional area (m^2)	Discharge (m^3 s^{-1})
Ensatina Entrance	0.108	1.25	11.6	0.0250	0.303	2.08	0.63
Ensatina constriction 1	0.483	1.4	2.9	0.0190	0.051	2.29	0.12
Ensatina constriction 2	0.200	1.3	6.5	0.0210	0.137	2.01	0.28
Ensatina Passage ceiling	0.380	2.1	5.5	0.0205	0.071	5.08	0.36
Pool Room	0.084	0.7	8.3	0.0230	0.358	3.05	1.09
Pool Room	0.065	0.8	12.3	0.0250	0.503	3.76	1.39
Monty Python Passage	0.051	0.6	11.8	0.0250	0.641	1.01	0.64
Marble Hall Overlook	0.061	1.4	23.0	0.0285	0.611	2.63	1.60
Fat Man's Misery	0.055	0.4	7.3	0.0220	0.523	13.50	7.06
Catacombs Corridor	0.310	1.5	4.8	0.0200	0.084	15.87	1.34
Catacombs Corridor	0.043	1.7	39.5	0.0315	0.957	15.87	15.19
Catacombs Red Belly	0.136	0.7	5.1	0.0205	0.197	8.37	1.65
Catacombs Hedlund Complex	0.072	0.6	8.3	0.0230	0.418	2.87	1.20
Entrance Passage	0.124	4.7	37.9	0.0300	0.316	30.25	9.57
Entrance Passage	0.093	3.8	40.9	0.0305	0.429	16.35	7.01
Entrance Passage	0.086	7.0	61.4	0.0375	0.570	16.35	9.32
Entrance Passage	0.065	7.1	109.2	0.0380	0.764	16.35	12.49

[a] Mean scallop length for the population of scallops.

[b] Conduit width to mean scallop length

[c] Determined from relations of Curl (1974), assuming parallel wall conduits.

development. It is determined by the position of the most deeply incised local surface stream (Palmer, 1987). In classic models, large, low gradient passages are often portrayed as having formed along this surface, i.e., having a shallow phreatic origin (e.g., Ford and Ewers, 1978; White, 1988; Ford and Williams, 1989; Palmer, 1991). While undoubtedly many of the low-gradient passages in Crystal Cave did form in such conditions, we suspect that several of the large, low gradient passages reflect vadose, or at least paraphreatic (alternating vadose and phreatic) development, produced during the flood-induced re-establishment of conduit-filling phreatic conditions. Vadose features such as wall notches are found along passage walls from floor to ceiling (Figs. 5, 6) implying sediment retention for extended periods of time and either a low gradient vadose origin or strong vadose overprinting onto tubes formed in shallow phreatic conditions.

Keyhole-shaped passages are often taken to imply a developmental history of phreatic initiation followed by vadose incision (White, 1988; Ford and Williams, 1989). However, the morphology of keyhole passages varies greatly, and in Crystal Cave may point to other processes. In Crystal Cave, most canyons, such as the Flying Circus and the Catacombs Corridor, exhibit an even width from top to bottom. However, keyhole morphologies are found in the Catacombs, in steep canyons above and below the Shield Room Level, and possibly in the Monty Python Passage. The upper sections of these keyholes are less than 50% wider than the canyons below, and the passages remain steep, even at their ceilings. The sinuous top of the Monty Python passage widens to the outside of passage curves, implying development through vadose incision. Parallel passages with the same elevations generally form simultaneously, and the Python and Flying Circus passages are free of significant breakdown and secondary formations. The development of these passages and the unusual rising ceiling downstream in Monty Python may be explained by sediment aggradation and flooding forcing ground water into parallel routes, creating a paraphreatic environment. In other passages, such as the Ensantina Passage, widening at the ceiling is in the form of obvious vadose cuts

Figure 5. Wall notches in the Entrance Passage near the Main Entrance. Although low gradient and rounded cross-section of this passage suggest shallow phreatic development, extensive wall notches suggest significant, perhaps even dominant, vadose origin. Photograph by Shane Fryer.

Table 2. Flow velocities and discharges for Crystal Cave passages based on sediment clast size.

Location	Passage slope	Clast size (m)	Critical shear stress (N m^{-2})	Critical flow depth (m)	Velocity based on f [a] (m s^{-1})	Velocity based on n [b] (m s^{-1})	Discharge based on f ($\text{m}^3\,\text{s}^{-1}$)	Discharge based on n ($\text{m}^3\,\text{s}^{-1}$)
Ensatina Entrance	0.001	0.005	9.1	0.46	0.85	1.89	0.49	1.09
Ensatina constriction 1	0.001	0.042	45.3	3.88	2.47	2.87	13.41	15.57
Ensatina constriction 2	0.001	0.090	81.6	8.32	3.61	3.08	39.06	33.31
Ensatina Passage	0.020	0.090	81.6	0.42	3.61	3.76	3.15	3.29
Phosphorescent Room	0.020	0.195	181.3	0.90	5.32	7.08	21.56	28.70
Pool Room	0.010	0.020	18.1	0.18	1.70	1.46	0.22	0.19
Pool Room	0.010	0.020	18.1	0.18	1.70	1.60	0.25	0.24
Monty Python Passage	0.070	0.010	9.1	0.01	1.20	1.09	0.01	0.01
Marble Hall Overlook	0.011	0.045	45.3	0.38	2.55	2.63	1.35	1.39
Blue Passage	0.002	0.080	72.5	3.70	3.41	1.34	5.04	1.98
Catacombs Corridor	0.060	0.095	86.1	0.15	3.71	2.90	0.81	0.64
Catacombs Corridor	0.060	0.095	86.1	0.15	3.71	3.16	0.92	0.78
Catacombs Red Belly	0.005	0.109	98.8	2.01	3.98	1.97	5.61	2.78
Catacombs Hedlund Complex	0.040	0.009	8.2	0.02	1.14	1.10	0.01	0.01
Junction Balconies turnoff	0.100	0.050	45.3	0.05	2.69	4.34	0.41	0.66
Entrance Passage	0.025	0.040	36.3	0.15	2.41	5.61	1.74	4.06
Entrance Passage	0.010	0.030	27.2	0.28	2.09	4.82	2.20	5.08
Entrance Passage	0.010	0.030	27.2	0.28	2.09	7.24	4.05	14.05
Entrance Passage	0.010	0.030	27.2	0.28	2.09	7.31	4.11	14.39

[a] The friction factor, f, is assumed to be 0.05 (Palmer, 1987).

[b] Manning's roughness coefficient, n, determined using Equation 7.

and horizontal notches. We suspect that the development of keyhole passages may have been encouraged by the actions of vadose and paraphreatic floodwaters in concert with heavy sediment loads or other mechanisms for constricting open conduits; in this case their presence does not necessarily indicate phreatic initiation of passage development followed by vadose incision.

CAVE SEDIMENTS AND SEDIMENTATION

Clastic sediments are abundant in all levels of Crystal Cave (Oberhansley, 1946). Sediment deposits are typically clast-supported, poorly sorted gravels and cobbles in a sandy matrix; clast imbrication is generally uncommon. Clasts are predominantly composed of schistose, quartzite, and granitic lithologies. Silt and clay-sized particles are often found as capping layers and as matrix deposits between larger clasts. Exposed sediment sections range from a few centimeters to up to 5 m thick; some passages terminate in sediment fill. Remnant pockets of sediment are preserved in alcoves high on cave walls (Fig. 7). The various coarse-grained, poorly sorted, and voluminous sediment deposits suggest rapid sedimentation that periodically aggraded cave passages.

Secondary bedrock features, such as notches, ceiling channels, and pendants, suggest that certain passages experienced sediment aggradation for long time periods. Notches in passage walls form as low discharge cave streams meander across sediment-mantled channels (White, 1988). At low discharge, sediment transport is limited and the sediment protects bedrock from physical erosion. Yet the stream retains its ability to chemically erode bedrock, and incises horizontally into the wall where it runs against it. As meanders grow, their outer bends incise farther back into the walls, resulting in low, wide arcuate notches (Fig. 6). Wall notches are often mantled with fluvial sediment left after subsequent incision (Fig. 7). In other caves, wall notches whose ages correlate with interglacial peaks in global δ^{18}O curves suggest aggradation events in

Figure 6. Clastic sediments stranded within a wall notch near Marble Hall. Following notch creation in response to sediment aggradation, renewed incision formed underlying passage, leaving sediments stranded in notch. Photograph by Steven M. Bumgardner.

Figure 7. Coarse clastic sediments in an alcove in Dome Room of Crystal Cave. Photograph by Steven M. Bumgardner.

phase with global climate events (Farrant *et al.*, 1995). However, notches in Crystal Cave are clearly too numerous to relate to such large-scale climatic fluctuations. Instead, they likely formed during shorter-term aggradation events restricted to Crystal Cave.

Why did passages aggrade? Sediment aggradation in fluvial systems is generally assumed to have climatic and/or tectonic implications (e.g. Bull, 1991). Although cave sediments can plausibly record such events, interpretation of aggradation events in caves is considerably more complicated. Independent of both climate change and tectonics, variation in rates of cave sediment deposition and erosion may be affected simply by breakdown, entrance collapse, or other barriers that can sieve sediments to varying degrees or dam them entirely for long periods. Likewise, mass wasting and sinkhole creation events on the surface further influence cave sedimentation rates. However, we consider episodic large flood events, which mobilize and rapidly transport sediment, to be the most likely source of sediment aggradation in Crystal Cave passages.

Evidence from the Catacombs section of Crystal Cave (Fig. 1) supports the prevalence of previous high discharges accompanied by sediment aggradation. This area is a braided, curvilinear, anastomotic maze of now abandoned fossil passages formed on several sub-levels with a vertical extent of 11 m. Anastomotic mazes, common in Sierra Nevada caves, form when groundwater discharge overwhelms existing conduits, creating alternative flow routes (Palmer, 1975; Palmer, 1991). Such flooding is frequent in caves situated in steep mountainous catchments. Anastomotic maze development is compounded in passages blocked or constricted by breakdown, secondary mineral deposits, or sediment fill. Unlike typical anastomotic mazes that follow horizontal porosity, such as horizontal bedding, the Catacombs are largely oriented on strike along vertical foliation. Catacombs passages are generally narrow canyons less than 2 m tall, or elliptical tubes less than 1 m in diameter. The largest and lowest passage in the labyrinth is the Catacombs Corridor, a canyon passage up to 3 m tall and 1 m wide, that lies along the western margin of the maze (Figs. 1–3). That the Corridor was the dominant master conduit for the Catacombs is supported by its comparatively larger size, its lower position, and the coarser sediment clasts relative to adjacent, interconnected conduits. Although the Catacombs passages are free of collapse and secondary speleothems, sediments completely fill some adjacent passages, and multiple wall notches attest to prior sediment deposits in now open passages. These features link sediment aggradation with impoundment of the maze-forming waters. This may be particularly true for the Hedlund complex, where two interconnected loops leave and rejoin the Corridor in a morphology that would have allowed groundwater to bypass sediment constrictions.

Recent transport of cave sediments by floodwaters has been documented in Sierra Nevada caves at or near local base level. Late on January 2, 1997, the Kaweah River flooded when a warm tropical storm melted the local snow pack, raising the discharge of the Main Fork of the Kaweah River from 142 $m^3 s^{-1}$ to 1602 $m^3 s^{-1}$ in six hours. A 150 m long passage in Wild Child Cave, situated along the Marble Fork of the Kaweah, accumulated more than 175 m^3 of sediment during the event, in deposits as thick as 3 m. Floodwaters deposited gravels and cobbles in the main passage while silts and sands accumulated in alcoves. Ten m wide rooms were completely aggraded, and passages that were once easily negotiated upright were transformed into crawlways. Conversely, during the same flood event passages in nearby Lilburn Cave were enlarged by removal of sediment. Sediment excavated from these passages exited the cave at the Big Spring resurgence and formed paired terraces along the valley walls as the flood waned. These terraces contain ~1060 m^3 of mostly fine sand (John Tinsley, pers. comm.), a minimum estimate of the sediment volume removed from the cave because additional sediment was transported farther downstream during peak discharge.

Although such high discharge events have not been recorded historically for the Crystal Cave hydrologic system, this is very likely due to a combination of the episodic nature of such events (e.g., Kirchner *et al.*, 2001), a generally warmer and drier Holocene climate (e.g., Benson and Thompson, 1987; Hostetler and Clark, 1997; Bartlein *et al.*, 1998; Clark *et al.*, 2003), and the fact that, at present, groundwater recharge is limited by passage collapse in the upstream section of the cave. Higher discharges at a time when the Yucca Creek sink point was larger likely led to the significant aggradation events evident in most cave passages.

GEOCHRONOLOGY AND RATES OF PASSAGE DEVELOPMENT

The speleothem and clastic sediment deposits in Crystal Cave were the subjects of a geochronological study in which speleothem U-Th dating, sediment paleomagnetism, and cosmogenic $^{26}Al/^{10}Be$ sediment burial dating were employed to constrain the timing of cave development (Stock *et al.*, 2005). Comparison of these dating methods revealed that $^{26}Al/^{10}Be$

burial ages were markedly older than both speleothem ages and those implied by paleomagnetism of fine sediment. This is probably because coarse sediment is deposited at base level during the waning stages of cave development, while fine sediment is often deposited in cave passages above base level during floods; drip-type calcite speleothems may begin forming any time after groundwater abandonment, and often lag cave development significantly. Thus, we consider sediment burial ages to best reflect the age of Crystal Cave passages. These ages range from 1.15 ± 0.09 Ma for the uppermost Bear Den level to 0.56 ± 0.08 Ma for the lowermost Junction level (Fig. 2). As expected, burial ages are in correct stratigraphic order, i.e., the higher cave levels are sequentially older than the lower levels.

Cosmogenic burial ages suggest that Crystal Cave development began ~1.2 Ma and proceeded rapidly until ~0.5 Ma, after which time passage development slowed markedly. That 4.72 km of cave passages spanning 64 vertical m developed in just 0.7 Myr suggests rapid rates of cave development; although direct measurements of passage widening rates is complicated by the numerous aggradational events in the cave, simple calculations suggest that widening rates approached, and in some cases even exceeded, theoretical maximums of ~1 mm yr^{-1} based on dissolution kinetics (Palmer, 1991). We consider these rapid rates to be due primarily to large volumes of chemically aggressive allogenic recharge entering the cave during floods, which can dissolve cave passages rapidly (Palmer, 2001). Passage widening was likely enhanced by physical abrasion of cave surfaces by both coarse and fine sediments entrained by these floodwaters.

Sixty-four m of vertical cave development in 0.7 Myr yields a mean Cascade Creek incision rate of 0.09 mm yr^{-1}. The intermediate dated levels provide further detail, and show a marked decrease in the rate of incision from 0.18 to 0.015 mm yr^{-1} over 0.7 Myr (Fig. 2). This pattern of decreasing incision is found in many other drainages in the southern Sierra Nevada, including Yucca Creek downstream from Crystal Cave, and is thought to reflect the waning effects of Pliocene uplift of the range (Stock *et al.*, 2004).

CONCEPTUAL MODEL OF CAVE DEVELOPMENT

A series of long-lasting conditions influenced the general development of Crystal Cave, the most influential of which has been the vertical position of Cascade Creek. Over time, bedrock incision of Cascade Creek lowered local base level, exposing cave passages to free-surface streams carrying coarse clastic sediment from Yucca Creek, particularly during floods. Due to the chemically aggressive nature of floodwaters, their ability to carry coarse sediments that promote mechanical erosion, and the prevalence of seasonal flooding and episodic extreme floods in this region, we hypothesize that pulses of rapid cave development accompanied these floods. As bedrock incision progressively lowered the Cascade Creek channel, the cave stream was pirated to lower cave levels and upper passages received sediment only during floods. Eventually, old conduits were abandoned, protecting fluvial sediments from further erosion. Subsequent deposition was limited to breakdown from the cave walls and ceilings, and accumulation of calcite speleothems from meteoric seepage.

After ~0.5 Ma, the lowering of cave passages and the development of lower levels appears to have slowed considerably. At present, there is little active dissolution of cave passages, and the only enlargement of the cave is in the vadose channel the cave stream is incising into the floor of the Entrance Passage, and potentially in phreatic pools, such as Solstice Lake. We consider two reasons for the reduction in the rate of base level lowering, and thus cave development. The first possible reason relates to restrictions on the size of the cave stream resulting from passage collapse. Extensive collapse of previously large conduits in the northern, upstream portion of the cave likely restricted cave stream discharge, greatly reducing cave development. However, the level of Cascade Creek ultimately drives cave development, so the second possible reason is that the rate of bedrock incision of Cascade Creek has slowed down since ~0.5 Ma. In a study of many other caves in the region, Stock *et al.* (2004) demonstrated a marked reduction in incision rates for many southern Sierra Nevada rivers over the last ~1 million years. Although the reasons for this slowdown are not fully understood, that this pattern is shown throughout the southern part of the range suggests that it is also a plausible mechanism for reducing the rate of cave development. Of course, these two reasons are not mutually exclusive, and both may have served to slow development of Crystal Cave over the past 0.5 million years.

In addition, we hypothesize that the incision of Cascade Creek in front of the Crystal Cave system is punctuated by the upstream migration of knickpoints. A longitudinal profile of Cascade and Yucca Creek across the vertically bedded lithologies of the Sequoia metamorphic pendant shows zones of steep channel gradients (i.e., knickpoints) within the schist and quartzite lithologies, but relatively low gradients within the marble (Fig. 8). We suspect that once knickpoints work through the resistant lithologies downstream from the cave they translate rapidly through the marble portion of the streambed, presumably due to the additional erosional influence of chemical dissolution and the greater susceptibility of carbonates with low hardness to physical erosion. This leads to a rapid drop in local base level to which the cave stream must adjust. The shallow phreatic and/or low gradient vadose passages described above are abandoned for sections of steeper, lower canyons, leading to a new, lower base level. Thus, one plausible mechanism to explain the frequent changes from large, low gradient tubular passages to narrow, steep canyons is the periodic abrupt drop in local base level associated with rapid knickpoint migration in front of the cave.

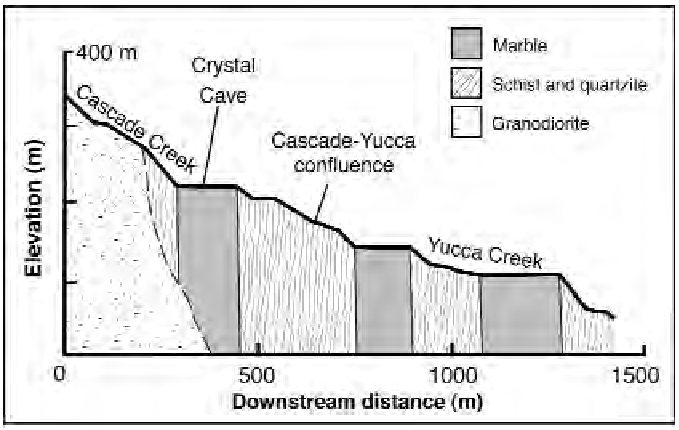

Figure 8. Longitudinal profile of Cascade Creek, and Yucca Creek below its confluence with Cascade Creek. Stepped profile, with prominent knickpoints, results primarily from contrasting vertically bedded lithologies. Channel gradients are low in relatively soft, chemically soluble marble and very steep in relatively hard, insoluble schist and quartzite. Knickpoints presumably migrate quickly upstream through marble lenses and stall on schist and quartzite, influencing local base level that controls development of Crystal Cave levels.

CONCLUSIONS

Our investigations of Crystal Cave suggest the following history of cave development:

Uppermost Bear Den level formed at ~1.2 Ma as water from Yucca Creek flowed southeast through the ridge toward Cascade Creek.

After an apparent ~200 kyr pause in passage development, but during which time Cascade Creek incised some 12 m, the Ensatina level formed at ~1.0 Ma. The Ensantina Passage and Phosphorescent Room formed first, while the parallel Monty Python and Flying Circus passages formed thereafter as a flood-deposited sediment choke in the north end of the Phosphorescent Room forced water into parallel passages.

After ~1.0 Ma, at least four small canyons developed rapidly, sequentially and headward between the Ensatina level and Pool level. The smaller Pool and Shield levels formed during apparently brief pauses in the midst of a period of otherwise rapid downward development.

Another series of small steep canyons formed below the Shield level with apparent headward migration of the active conduit.

Crystal Cave's primary level containing the Catacombs and many of the cave's largest rooms, including Marble Hall, formed at or slightly before ~0.8 Ma. The larger rooms and passages at this level may have formed due to a significant pause in the downcutting of Cascade Creek, or may reflect increased discharge as seen in scallops and sediment clasts from this level.

Collapse of the northern, upstream end of the cave ~0.5 Ma restricted input of Yucca Creek into the cave. This flow restriction, combined with decelerating Cascade Creek incision and Holocene climate change, limit present-day cave development to small phreatic pools in the upstream end of the cave and slight vadose modification of the floor of the Entrance Passage.

This detailed geomorphic history of cave development helps illustrate the unique conditions affecting cave development in mountainous regions, and provides an important context for interpreting rates of landscape evolution derived from dated cave deposits.

ACKNOWLEDGMENTS

Shane Fryer and Steve Bumgardner provided field assistance and discussions. We thank the many cavers that aided in the survey of Crystal Cave. Insightful field discussions with Darryl Granger, Arthur Palmer, Margaret Palmer, and Ira Sasowsky helped to refine many of our ideas about the geomorphic history of the cave. We acknowledge the work of Park Naturalist Frank R. Oberhansley, whose astute observations of Crystal Cave, published in 1946, were the stimulus for many of the subjects addressed in this paper.

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Tanja Pipan and David C. Culver - Estimating biodiversity in the epikarstic zone of a West Virginia cave. *Journal of Cave and Karst Studies*, v. 67, no. 2, p. 103–109.

ESTIMATING BIODIVERSITY IN THE EPIKARSTIC ZONE OF A WEST VIRGINIA CAVE

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A total of 13 ceiling drips in Organ Cave, West Virginia, USA, were sampled for fauna for three consecutive 10 day intervals. A total of 444 individuals from 10 copepod genera were found. Incidence functions revealed that 90 percent of the genera were found in eight samples, and that estimates of total diversity indicated only one or two genera had yet to be found. The overall rate of false negatives for different drips was 0.39 and the overall rate for different time intervals was 0.31, also suggesting that the sampling scheme was sufficient. Compared to nearby pools which serve as collection points for epikarst water, the drip samples were significantly different and more diverse. In addition to copepods, a wide variety of other invertebrates were found in drips, including many terrestrial insects that serve as part of the food base for the cave community. Direct sampling of drips is the preferred method at present for sampling the epikarst fauna.

INTRODUCTION

The uppermost zone of karst, the skin of karst, is "located within the vadose zone and is defined as the heterogeneous interface between unconsolidated material including soil, regolith, sediment, and vegetative debris, and solutionally altered carbonate rock that is partially saturated with water and capable of delaying or storing and locally rerouting vertical infiltration to the deeper regional phreatic zone of the underlying karst aquifer" (Jones *et al.*, 2004).

Known for decades by speleobiologists (Sket *et al.*, 2004), water in the epikarst zone was often termed percolating water and included as part of the vadose zone, the zone of karst above the permanently saturated (phreatic) zone. An extraordinarily complex and heterogeneous structure, it is home to a wide array of animals, mostly but not exclusively aquatic, that often rivals in diversity the rest of the karst aquifer (Pipan and Brancelj, 2004).

The position of the epikarst at the top of the karstic bedrock and the typical absence of enterable passages or voids have meant that sampling has had to be indirect. Water exiting from the epikarst appears in caves as drip and seeps from ceilings and walls. The exiting water often creates pools (often surrounded by rimstone) well above any streams that might be in the cave and well above the water table. The first collections of epikarst fauna were hand collections from these sites, and typically included macroscopic invertebrates, especially amphipods. More recently, drips have been collected directly, and special sampling devices have been designed to collect copepods and other microscopic invertebrates (Pipan, 2003; Brancelj, 2004). The use of these techniques has greatly increased the number of known epikarst species, especially from drips (Pipan and Brancelj, 2004).

In this paper we report on the differences in faunal composition between drips and pools. Collections from drips represent animals exiting from the epikarst, in the same sense that harpacticoid copepods collected by drift nets at springs by Rouch (1970) represented both losses from the system and a way to assess the dynamics and diversity of the system, data which Rouch used with great success in his multi-year study of the Baget karst basin in France. He called this phenomenon "auto-hémorragie." While the animals collected in drips may not be the entire epikarst fauna, it contains no other elements except for the possibility of surface-dwelling species being flushed through the system. By way of contrast, pools not only collect the animals from drips, there is also the possibility of interaction among these animals as well as the possibility of different levels of reproduction. In addition, there is the possibility that invertebrates living in other subterranean waters, especially streams, may colonize pools especially during times of flooding.

In particular, we have intensively studied the drip and pool fauna of a cave in southern West Virginia, USA, and compare both the copepod and non-copepod fauna in these habitats. We also look at measures of sampling completeness to estimate whether we have found all of the species present.

MATERIAL AND METHODS

A short-term field study was conducted in the Organ cave system (West Virginia) from May 17 to June 20, 2004. Rains are frequent in late spring and early summer and drips are usually more active than at other times of the year. The area receives an average of 95 cm of precipitation annually and during the sampling period precipitation was 24 cm. Jones (1997) provides more details on climate in the area. We chose three sampling sites of the Organ cave system: the Lipps, Sively No. 2, and Sively No. 3 sections of the cave (see Stevens 1988 for a detailed map and description). In the Lipps section we collected from five drips within 4 m of each other. In the Sively 2 and Sively 3 sections we found and sampled four drips in each, separated by distances of up to 100 m. In the first case of Sively 2 the distance between the second and the fourth drip was around 7 m, and the distance between the second and the fourth drip of Sively 3 was 1 m. Thus we had four clusters of distances – sets of drips less then 1 m apart, sets less then 10 m apart, sets less then 100 m apart, and sets of drips less then 1500 m apart.

The water from a drip was directed through a funnel into a plastic container. A 2 cm by 3 cm area on each of two sides of the square container was cut out and covered with a net (mesh size 60 µm) to retain animals in the container. Samples were collected three times at approximately 10-day intervals. Pools within 5 m of the drips were sampled by aspiration of the water filtered through the collecting container described above. In Lipps and Sively #2, there was one pool within the area and in Sively #3 there were two pools. Each pool was in an area of rimstone with many subdivisions and intercalations. Each of these four pools was sampled at the beginning and at the end of the study. The volume of water aspirated ranged from 0.2 L to 10 L, depending on the volume of water in pools.

Non-copepod specimens were usually identified to order but occasionally to species. Copepods, which are largely undescribed from North American caves (but see Reid [2004] for cave *Diacyclops* from Indiana), were identified to genus using Williamson and Reid (2001).

Patterns of similarity between faunas were assessed using log-likelihood contingency tables, using JMP™ (SAS Institute, Cary, NC, USA). Genera incidence curves were determined using the Mao-Tau function of Colwell *et al.* (2004), using EstimateS (Colwell, 2004). In addition, we calculated the total expected number of genera based on Chao's function S_{Chao1} and Smith and van Belle's jack-knife procedure, S_{boot}, where

$$S_{Chao1} = S_{obs} + \frac{F_1^{\,2}}{2(F_2+1)} - \frac{F_1 F_2}{2(F_2+1)^2} \qquad (1)$$

$$S_{boot} = S_{obs} + \sum_{k=1}^{S_{obs}} (1-p_k)^m \qquad (2)$$

(Colwell, 2004) where F_i is the number of genera that have exactly i individuals when all samples are pooled, S_{obs} is the observed number of genera, p_k is the proportion of samples that contain genus k, and m is the total number of samples.

RESULTS

A total of 11 genera of copepods were collected, and a total of 14 morphological species were identified. Identification to species and description of new species requires further taxonomic work. We have taken the conservative approach and analyzed only generic diversity. It appears that for most genera, only one species is present (Table 1). Their morphology would indicate that at least seven species in five genera are adapted to and likely limited to subterranean habitats (see Reid and Strayer, 1994). A total of 10 genera were collected from drips and seven were collected from pools.

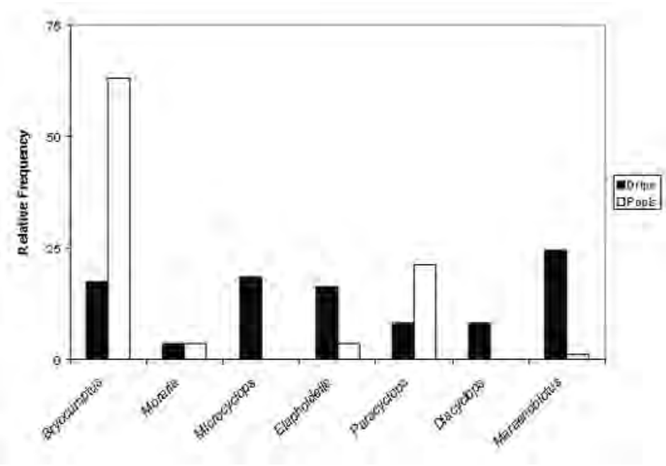

Figure 1. Relative abundance of different copepod genera in drips and in pools. Only the seven most common genera are shown, ones common enough for statistical analysis (see Table 1). Frequencies in each habitat sum to 100%.

A total of 444 copepods were collected in drips and 84 in pools. The difference in abundance is a reflection of the relatively few opportunities for collection from pools. Pools were thoroughly aspirated on the first sampling trip and subsequent samples were much reduced in abundance. The relative abundance of the seven most common genera is shown in Figure 1. In pools, the harpacticoid *Bryocamptus* was the most abundant genus, representing nearly two-thirds of the individuals collected. The only other common genus was the cyclopoid *Paracyclops*. In drips, the harpacticoids *Maraenobiotus*, *Elaphoidella*, and *Bryocamptus* and the cyclopoid *Microcyclops* were all common, each accounting for between 16 and 25 percent of individuals collected. The cyclopoid *Ectocyclops*, of which only three total specimens were found, was the only genus not represented in drips. By contrast, several common genera were missing from pools, including *Microcyclops* and *Diacyclops*; the abundant *Maraenobiotus* was only represented by a single specimen from pools. *Parastenocaris* (20 individuals), *Eucyclops* (2 individuals), and *Epactophanes* (two individuals) were found only in drips. The differences in relative abundance of the more common genera are quite evident in Figure 1 and Table 2. Among the seven most common genera, there was a large, statistically significant difference in relative proportions between the two habitats (log-likelihood G = 136.7, df = 6, $p < 0.001$).

In addition to copepods, other invertebrates were found in drips and pools. A total of 454 other invertebrates (and one salamander) was found in drips and 100 other invertebrates

Table 1. List of genera found in drips and drip pools in Organ Cave. The number of morphologically distinct species and the number of troglomorphic species are also shown.

Genera found in Organ Cave	Number of morphospecies	Number of troglomorphs
Cyclopoida		
Diacyclops	1	1
Ectocyclops	1	0
Eucyclops	1	0
Microcyclops	1	0
Paracyclops	2	0
Harpacticoida		
Bryocamptus	1	1
Elaphoidella	2	2
Epactophanes	1	0
Maraenobiotus	1	0
Moraria	1	1
Parastenocaris	2	2

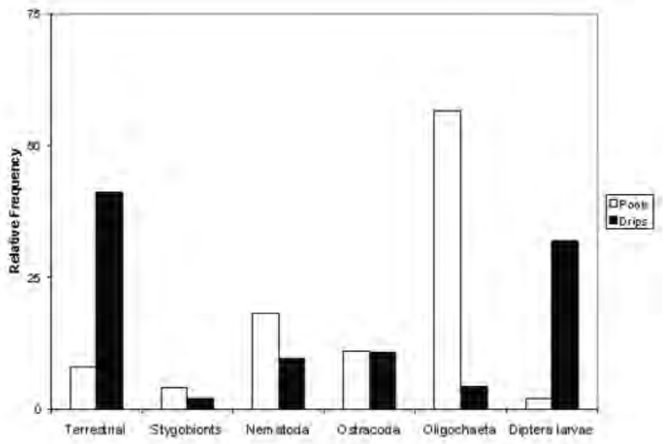

Figure 2. Relative abundance of non-copepods in drips and in pools.

were found in pools. They belonged to a wide variety of groups and many of them were not identifiable below class or order (Figure 2). Taken together, they rival the copepods in abundance. Included among the terrestrial species (presumably ones washed in through the epikarst (see Gibert, 1986) were Acarina, Collembola, Coleoptera and Diplopoda. The small but interesting category of stygobionts included the amphipod *Stygobromus*, the isopod *Caecidotea holsingeri*, a bathynellid and the archiannelid *Troglochaetus*. In pools, oligochaetes and nematodes predominated, probably because these benthic dwellers can find suitable microhabitat in the sediment of drip pools. Given the previous work of the French biologists Delay (1968) and Gibert (1986), it is not surprising that terrestrial accidentals are well represented. More unexpected was the large number of Diptera larvae. However, 95 of the 137 specimens collected were taken from a single drip and a single sampling interval. All groups were found in drips but some terrestrial accidentals (millipedes) and some rare stygobionts (archiannelids and bathynellids) were missing from pools.

In general, drips provided a more complete sample than pools, in the case of both copepods and other invertebrates. This is indicated primarily by the greater generic richness in drip samples, but also by the significant differences in composition between drips and pools (Tables 2 and 3). Drips provide a more or less unbiased sample except for any mortality that occurs in the sampling bottle during the 10 days of the sample. By contrast, pools are clearly biased samples. Groups are missing, and some groups are overrepresented. We do not have sufficient understanding of these creatures to know why particular copepods are proportionately more common in pools, but it is certainly true that pools are subject to environmental fluctuations (especially drying), that there are some microhabitats that may be quite abundant in pools, and that the relatively simple physical structure of pools may make predation more important.

False negatives are cases where a species was initially found, but was not recollected in subsequent samples (Tyre *et al.* 2003). When individual drips and individual 10-day sampling intervals were considered, overall rates of false negatives were quite high, averaging 0.71 (Table 4). However, rates of false negatives were much lower for the entire time course of a single drip, averaging 0.39. If all four (or five in the case of Lipps) drips were combined for one 10-day interval, rates were also lower, averaging 0.31. As a rule of thumb (Tyre *et al.,* 2003), rates higher than 0.50 indicated repeat sampling was advisable. By way of contrast, Schneider (pers. comm.) found false negative rates higher than 0.50 for collections of macroscopic terrestrial and aquatic cave fauna. Rates in Sively #2 were highest, presumably because of the lower number of animals found at this site. Overall, the results indicate that sufficient sampling intensity was employed in Organ Cave, and in fact false negatives reached an acceptable level after 10 days of sampling.

A better sense of the thoroughness of sampling can be gained from the genera accumulation curve shown in Figure 3. Based on Colwell *et al.* (2004) sample-based rarefaction, the accumulation curve together with 95% confidence intervals is shown. It rapidly begins to reach an asymptote, reaching 75 percent of the maximum after four samples, 90 percent of the maximum after eight samples, and 95 percent of the maximum after 10 of 13 samples. This asymptotic relationship is nearly unprecedented: examples of failure of sampling in caves to reach an asymptote abound (Culver *et al.*, 2004; Schneider and Culver, 2004). The number of genera unique to a single drip within the study sites also declined, indicating that sampling was thorough.

An alternative approach to sampling completeness is to use estimates of the total number of genera, based not on accumulation curves, but rather on the internal structure of species abundance, especially the number of genera in one (singleton)

Table 2. Comparison of observed and expected numbers of individuals of different copepod genera in drips and in pools. Overall there were highly significant differences (log–likelihood test, G = 136.7, df = 6, p < 0.001).

Genera found in Organ Cave	Pools		Drips	
	Observed	Expected	Observed	Expected
Bryocamptus	53	20.0	78	110.99
Diacyclops	0	5.7	37	31.40
Elaphoidella	3	11.6	73	64.40
Maraenobiotus	1	16.8	109	93.20
Microcyclops	0	12.5	82	69.50
Moraria	3	2.9	16	16.10
Paracyclops	18	8.4	37	46.60

Table 3. Comparison of observed and expected numbers of individuals of different non–copepod groups in drips and in pools. Overall there were highly significant differences (log–likelihood test, G = 192.99, df = 5, p <0.001).

Non–copepod groups found in Organ Cave	Pools		Drips	
	Observed	Expected	Observed	Expected
Diptera larvae	2	26.11	137	112.89
Nematoda	18	11.08	41	47.92
Oligochaeta	56	14.09	19	60.91
Ostracoda	46	46.29	11	10.71
Stygobionts[a]	9	10.56	4	2.44
Terrrestrials[b]	8	34.57	176	149.44

[a]Includes *Stygobromus pollostus* (Amphipoda), *Caecidotea holsingeri* (Isopoda), *Troglochaetus* (Archiannelida), and Bathynellacea.

[b]Includes mostly non–troglobitic Acarina, Coleoptera, Collembola, and Diptera.

or two (doubleton) drips (Colwell and Coddington, 1994). We looked at this at the scale of individual drips and sites as well as overall combined sites since there are differences in generic composition among sites and drips (Culver *et al.* 2005). At all scales there was good agreement between the number of observed and the total number of predicted genera (Table 5). Because of the particularities of the bootstrap formula, S_4, predicted number of genera can actually be less than the observed number of genera if there are doubletons, but no singletons, and this happened in several cases. For the three sites, only Sively #2 appeared to be under-sampled, with seven observed genera and 11 predicted genera. This is in accord with the overall smaller sample size in Sively #2 (Table 5). For Organ Cave taken as a whole, there was good agreement between observed and expected numbers of genera (10 vs. 12).

Discussion

The easiest way to sample the epikarstic fauna of a cave is to sample pools. The sampling can be easily done in a single visit and it is relatively easy and quick to collect the samples. Unfortunately, pool sampling is far from an unbiased sample of the epikarst, and indeed common species may be completely missing from pools. For example, in our study *Diacyclops* and *Microcyclops* were completely missing from pools and *Maraenobiotus* was nearly so (Fig. 1, Table 1). Of course, many genera of copepods were found in drip pools, but unless drips are sampled directly, there are likely to be gaps in faunal lists. The same is true for non-copepod species although we had less detailed information on these. One non-copepod component of epikarst communities that is under-represented in pools is the terrestrial arthropods entering the cave through drips. They included Collembola, Diplopoda, Acarina, and Coleoptera, with Collembola being numerically dominant. The assemblage is interesting both because the animals are food input into the cave and because it is a sample of terrestrial epikarst fauna. We know virtually nothing about this fauna, although it is likely similar to the non-karstic MSS, "milieu souterrain superficial" (Juberthie *et al.*, 1980).

We only saw one genus unique to pools in Organ Cave—*Ectocyclops*, only three of which were found. In her study of the Postojna-Planina Cave System (PPCS), Pipan (2003) sampled both drips and pools in a similar way. She found a total of 23 species of copepods; of these 10 were found in both drips and pools, five were found only in drips, and eight were found only in pools. The major difference between the two studies was the presence of several species in pools that were not found in drips. The reasons for the difference are not clear, but in general the drips in Pipan's study yielded fewer individuals than in the Organ Cave study. In PPCS she found only 505

Table 4. Rates of false negatives.

Section	Type	Frequency	N
Lipps	by drip by time	0.71	24
Sively #2	by drip by time	0.92	12
Sively #3	by drip by time	0.65	23
Total	by drip by time	0.71	55
Lipps	by drip	0.16	19
Sively #2	by drip	0.60	10
Sively #3	by drip	0.53	15
Total	by drip	0.39	44
Lipps	by time	0.10	11
Sively #2	by time	0.67	9
Sively #3	by time	0.25	16
Total	by time	0.31	36

individuals in 20 drips sampled for five months and 1574 individuals taken in two collections in pools. This contrasts with the current study where 444 individuals were taken in 13 drips and 84 individuals were taken in pools in 30 days. We have no information about drip rates in the Slovenian sites, so total volume of dripping water cannot be compared. The difference in abundance in pools is probably mostly a reflection of the amount of volume or surface area of pools collected, but the differences between drips may reflect real differences between the "bleeding" from the epikarst in the two caves or it may reflect more short-term temporal differences. The total generic richness of copepods in the two cave systems was not that different. PPCS had a total of 14 copepod genera compared to 11 in Organ Cave; there were eight genera from PPCS in drips compared to 10 in Organ Cave; there were 12 genera in pools in PPCS and only seven in Organ Cave.

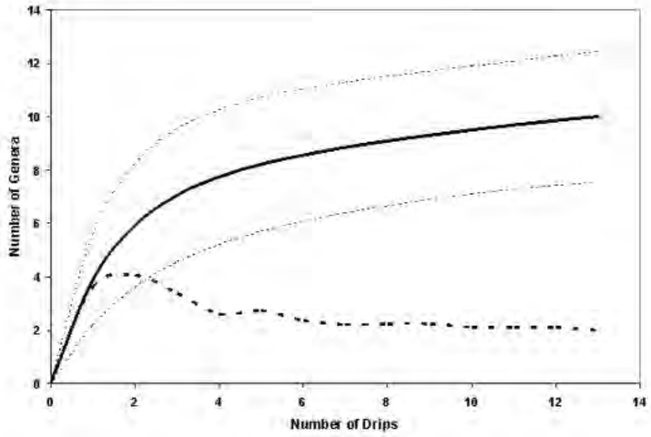

Figure 3. Copepod genera accumulation curve based on Mao-Tau procedure of Colwell *et al.* (2004). The solid line is the accumulation curve and the two dotted lines are the 95% confidence intervals. The heavy dashed line is the number of genera unique to a single drip.

When we examined the results of drip collections more thoroughly, it was clear from a variety of perspectives that the cave was well sampled for epikarstic copepods. This is especially the case for the generic accumulation curve (Fig. 3). Not only did it reach 90% of the total after eight of 13 samples, but the number of single drip genera declined to two after seven samples. It is likely that few if any genera are limited to a single drip within the set of study sites, but we simply do not know enough about the distribution of animals in the epikarst to state this with any certainty. The reaching of an asymptote is not a trivial point. It is rare to find a thoroughly sampled cave or cave region for macro-invertebrates. Culver *et al.* (2004) showed that even in the relatively well-studied Classic

Table 5. Estimates of genera richness based on Chao's S_1 and Burnham and Overton's S_4. See text for details.

Cave	Drip No.	Total S (S_{obs})	Total N (n_3)	Singletons (n_1)	Doubletons (n_2)	S_{Chao1} (Chao's)	S_{boot} (bootstrap)
Lipps	1	1	1	1	0	–	0.0
Lipps	2	6	97	0	2	6.0	4.0
Lipps	3	5	21	0	0		4.9
Lipps	4	4	38	1	0	–	5.9
Lipps	5	7	65	0	2	7.0	5.1
Lipps	Combined	7	223	0	1	7.0	6.0
Sively #2	1	1	1	1	0		0.0
Sively #2	2	2	5	1	0		3.4
Sively #2	3	2	2	2	0		4.5
Sively #2	4	5	11	2	1	7.0	8.0
Sively #2	Combined	7	19	3	2	9.2	11.0
Sively #3	1	1	1	1	0		0.0
Sively #3	2	6	52	1	1	6.5	7.0
Sively #3	3	8	138	0	1	8.0	7.0
Sively #3	4	3	11	1	0		4.7
Sively #3	Combined	9	202	1	0		10.0
Organ Cave	Combined	10	444	2	0		12.0

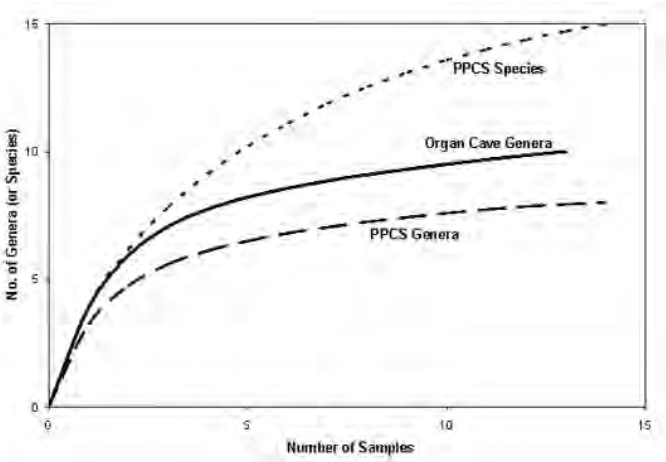

Figure 4. Comparison of accumulation curves of genera and species in Postojna Planina Cave System and genera in Organ Cave, based on the Mao-Tau procedure of Colwell *et al.* (2004).

Karst of Slovenia that the species accumulation curves for 100 km² hexagons were not reaching an asymptote. Stoch (pers. comm.) reported that it took more than ten samples of a single cave (Antro di Bagnoli near Trieste, Italy) for the species accumulation curve to reach an asymptote. In Schneider and Culver's (2004) study of more than 60 caves in a 15 km² area in West Virginia, USA, the species accumulation curve never reached an asymptote. In the present study, the number of copepod genera begins to reach an asymptote (90 percent saturation) after eight samples.

We suspect that the ability to more or less completely sample the species in the epikarst by collecting from 10 or more drips is quite general. Pipan's data on PPCS can also be examined in this way, both for genera and species. The shapes of the accumulation curves for Organ Cave and PPCS are compared in Figure 4. The shapes of the accumulation curves for genera are quite similar, with 90% of the genera found after an average of eight samples. It took 10 of 14 samples to find 90% of the species.

Likewise, analysis of patterns of false negatives (Table 3) and predicted numbers of genera (Table 4) indicated sufficient sampling. Values of false negatives were less than 0.40 for individual drips or individual dates, indicating that a strategy of either a single time period with multiple drips or a single drip over a longer time period would keep false negatives below the critical value of 0.50 (Tyre *et al.*, 2003). Predicted and observed numbers of genera (Table 4) were remarkably close with only Sively #2 indicating that any additional sampling would likely yield more genera.

What generalities about the amount of drip sampling required can be gleaned from this study? The first is that given the heterogeneity of the fauna in individual seeps, sampling in multiple seeps would be advisable. For example, seeps in the Sively #2 site were generally less productive for reasons that cannot be adequately explained by differences in flow rate (Culver *et al.* 2005). Second, at least in Organ Cave, a ten day sample of 10–13 drips would probably have been sufficient (see Tables 3 and 4). Of course we do not know if the scale of heterogeneity we observed is typical of caves.

Finally, we note the extraordinary diversity of the epikarstic copepod assemblage in Organ Cave. Although many species await description, it appears that there are at least 14 species of copepods among the 11 genera, seven of which are troglomorphic and likely stygobitic. By contrast, the streams of Organ Cave, a well-studied system (Culver *et al.* 1994), harbor only a total of 6 stygobionts. The only copepods found in the stream (sampled with a modified Hess sampler) were two stygophilic species: *Brycamptus nivali* and *Bryocamptus* nr. *morrisoni* (Culver *et al.*, 1994). When the epikarst fauna in Organ Cave and elsewhere in North America is fully described (see Reid 2004), it may well dominate the lists of stygobitic fauna.

ACKNOWLEDGMENTS

We thank the owners of Organ Cave, Mr. and Mrs. Sam Morgan, for permission to enter Organ Cave. Daniel Fong, Horton Hobbs III, Jeff Hajenga, and David Cowan helped with field work. DCC and TP were supported by a grant from the Cave Conservancy Foundation.

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THE DISTRIBUTION AND LIFE HISTORY OF *ARRHOPALITES CAECUS* (TULLBERG): ORDER: COLLEMBOLA, IN WIND CAVE, SOUTH DAKOTA, USA

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Individuals of the collembolan species Arrhopalites caecus *(Tullberg) were collected from drip pools within Wind Cave, South Dakota, at Methodist Church adjacent to the Natural Entrance Tour Route and Room Draculum near survey marker NP-39. Specimens were identified as* A. caecus *using direct interference and scanning electron microscopy. Molecular analysis of the D2 region of 28S rDNA was performed and the sequences were deposited in Genbank (accession number AY239037). We determined that our population of* A. caecus *reproduced parthenogenetically by successively isolating and rearing eggs through the F4 generation on 9:5 plaster:charcoal media maintained at 21°C, and by the absence of males. Molecular analysis of 16S rDNA for bacteria within our specimens failed to detect the α-proteobacterium (Rickettseales)* Wolbachia. *Generation times, fecundity, and molt frequency were consistent with other reports for Collembola.*

INTRODUCTION

Arrhopalites caecus (Tullberg) was initially described and studied by Tullberg (1871) and Böerner (1906) and has been found in caves and surface localities in Europe and North America (Christiansen, 1966). The genus *Arrhopalites* is one of the more widespread cave forms of Collembola within the Holarctic region (Vandel, 1965). Although a recent catalog of the genus *Arrhopalites* in North American caves (Zeppelini and Christiansen, 2003) did not include caves of the Black Hills of South Dakota, the genus has been collected in both Wind Cave and Jewel Cave. Christiansen and Bellinger (1980, 1998) reported finding *Arrhopalites caecus* in Custer County, South Dakota (the county where Wind Cave is located), while Moore *et al.* (1996) found the species in both Wind Cave and nearby Jewel Cave.

Reports on the geographic distribution of and morphological description of *A. caecus* are available, but little information on its behavior, and to our knowledge, no information on the D2 region of its 28s rDNA or its life history have been reported. Given the ease that we were able to initiate viable cultures from single individuals, we suspected that *A. caecus* might reproduce parthenogenetically. The objectives of our study were as follows: 1) determine the nucleotide sequence of the D2 region of the 28s rDNA of *A. caecus*, 2) determine the extent of the distribution of *A. caecus* within Wind Cave, and 3) observe and document the life history of *A. caecus*.

SITE DESCRIPTION

Wind Cave is located in Wind Cave National Park on the southeastern flank of the Black Hills of South Dakota, near the town of Hot Springs (Fig. 1). Wind Cave is a phreatic dissolution cave in the Madison Limestone and overlying Minnelusa Formation, that formed during the uplift of the Black Hills between 65 and 40 million years ago and continued to be modified during the erosion that has occurred over the past 40 million years. The Madison Limestone was deposited over 330 million years ago during the life and evaporation of a warm shallow sea, while the Minnelusa Formation, consisting of sandstone, shales, and carbonates, was deposited 300 million years ago during a subsequent advance and retreat of a later sea (Palmer and Palmer, 2000). Wind Cave is a network maze cave with five recognized levels and up to 76 meters of vertical relief at any given point. The cave only has two natural entrances and is fairly well sealed by the overlying Minnelusa Formation. The cave is dry, except where it lies underneath modern drainages or where it hits the water table at its deepest point (Horrocks and Szukalski, 2002). The upper levels of the cave started draining 350,000 years ago and were completely air-filled by 155,000 years ago (Ford, 1993). Surface vegetation of the landscape overlying Wind Cave has been described as northern mixed grass-prairie (80%) with woodlands (20%) dominated by *Pinus ponderosa* Dougl. (Coppock *et al.,* 1983; Whicker and Detling, 1988).

METHODS

SURVEYS OF SOILS AND SEDIMENTS

We determined the distribution of *A. caecus* within Wind Cave using a combination of strategies including the systematic and opportunistic sampling of soils, sediments and water surfaces, and sampling with pitfall traps conducted from June 1992 through October 2004. For the systematic sampling of the cave a 152.4 x 152.4 m (actual dimension was 500 x 500 ft) grid was positioned over a map of the cave, and sediments

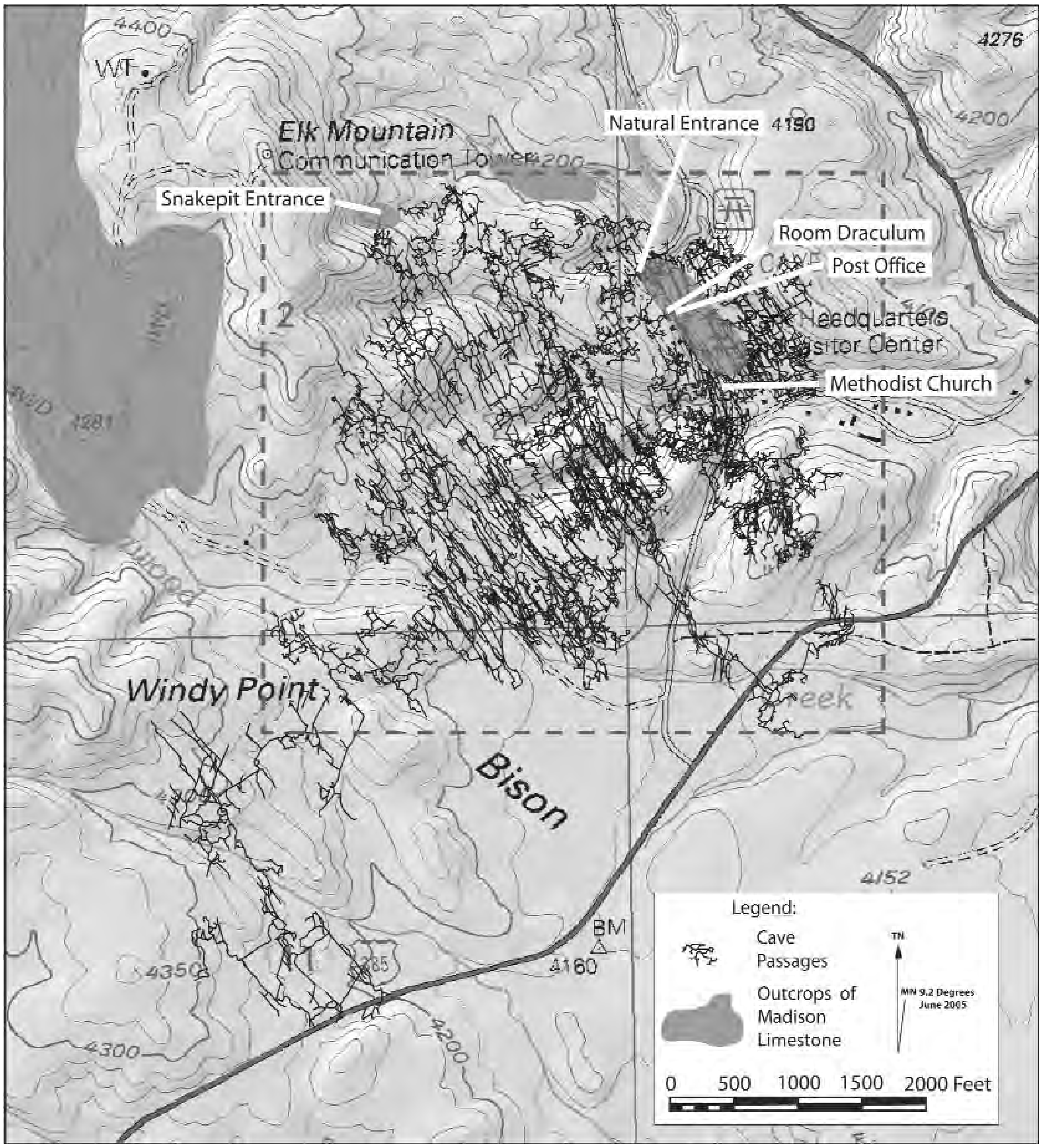

Figure 1. Topographic map with an overlay of the current known extent of Wind Cave (adapted from Horrocks and Szukalski, 2002). The box framed with dashed lines approximates the extent of the 1992–1996 survey, where sampling occurred at 152.4 x 152.4 m grid intersections. The Natural Entrance and Snake Pit are known entry points into the cave.

were sampled at locations closest to the grid intersections. For the systematic sampling of surface soils, three 5 cm diameter x 20 cm deep cores were taken at 10 m intervals along each of three 100 m transects spaced 200 m from one another, perpendicular in orientation to Wind Cave Canyon. Collembola were extracted from the soils using modified Tullgren funnels (Moore *et al.*, 2000) and identified using the keys of Christiansen and Bellinger (1998) and Janssens (2003). Opportunistic sampling from different locations occurred on survey trips or in conjunction with other studies. Sampling included pitfall traps lined with a 9:5 plaster charcoal, open Petri dishes lined with 2% water agar, collection of specimens from the surface of drip pools using brushes and aspirators, and extraction from sediments, as described above.

COLLECTION AND REARING

We selected four individuals from laboratory colonies of *A. caecus* established using specimens collected from pools at Methodist Church adjacent to the tour path on the Natural Entrance Tour Route and Room Draculum near marker NP-39. Each individual was placed in a fresh vial containing a medium of plaster and charcoal in a 9:5 ratio (Snider *et al.,* 1969) and fed baker's yeast. All vials were kept moist and incubated at 21°C and observed daily. There were a few 1-day and 2-day interruptions throughout the study, and one 4-day interruption for the F2 generation. Beginning with the parental generation (P), as eggs were laid, each egg was separated into its own fresh vial containing the 9:5 ratio plaster-charcoal medium.

The date that the egg was laid and identification of the parent were recorded. When an egg hatched, the date was recorded and a grain of baker's yeast added. Individuals were observed daily (with a few interruptions as noted above), with the date of each of its molts and its death recorded. When subsequent generations began to lay eggs, the same process of separating eggs and recording molts and death was followed until laboratory supplies were exhausted. Excluding the four parents, 90 individuals across four generations were studied.

IDENTIFICATION

Specimens from the field and laboratory cultures were identified using light microscopy, scanning electron microscopy, and molecular phylogenetic methods using 28S rDNA. For identification with light microscopy, adults were selected from the cultures, killed in 70% ethanol, and cleared in dilute Hoyer's solution. Cleared specimens were mounted in Hoyer's solution and observed under an Olympus BH-2 outfitted with Direct Interference Contrast. For scanning electron microscopy, specimens were killed and dehydrated using hexamethyldisilazane (Nation, 1983). Specimens were mounted and sputter-coated with 15 µm of gold and examined using a Joel JSM-5200 scanning electron microscope.

For molecular analysis and identification, DNA was extracted and purified using a commercial DNA extraction kit (Mo Bio Laboratories, CA). The D2 region of 28S rDNA was PCR-amplified using primers C2 (5'-GAAAAGAACTTTGRARAGAGAGT-3') (Friedrich and Tautz, 1997) and D2Coll (5'- ACCACGCATGCWTTAGATTG- 3') (D'Haese 2002). Purified DNA was used as a template in 50 µl PCR reaction containing 1X Taq buffer (Eppendorf), 2 mM $Mg(Oac)_2$, 0.5X TaqMaster PCR Enhancer (Eppendorf), 200 µM dNTPs, 20 pmol of each primer, and 0.5 U Taq polymerase. Touchdown PCR was used with a 3 minute denaturation at 95°C followed by 30 cycles at 94°C for 15 seconds, annealing for 15 seconds, and extension at 72°C for 20 seconds, with a final extension at 72°C for 7 minutes. Annealing began at 62°C and decreased 0.5°C each successive cycle until 58°C was achieved for the remaining 20 cycles. The PCR product was purified using the Micron PCR purification kit (Millipore, MA). The ABI cycle sequencing kit was used by the University of Colorado Cancer Center Sequencing and Analysis Core to sequence the entire PCR product using primers C2 and D2 Coll.

AMPLIFICATION OF ENDOSYMBIONT 16S rDNA

We attempted PCR amplification of the α-proteobacterium *Wolbachia* from individual *Arrhopalites caecus* (Tullberg) and *Folsomia candida* (Willem) using the methods described above. We targeted our PCR-amplification to 16S rDNA using the primers ftsZunif, ftsZunir (Lo *et al.* 2002), ftsZf1, ftsZr1, ftsZAdf, ftsZAdr, ftsZ1, ftsZ2 (Werren *et al.*, 1995), and ftsZcolr (Czarnetzki and Tebbe, 2004) specific to *ftsZ*, a cell division gene; primers wspf and wspr (Braig *et al.*, 1998), specific to *wsp* which encodes a cell surface protein, and primers 16SAf, 16SAr (Werren *et al.*, 1995) 27f, 1492r (Lane, 1991), specific to 16S rDNA. Because these amplifications failed, double PCR was employed using primer pairs ftsZunif and ftsZunir, ftsZf1 and ftszr1. In this case, one µL of PCR product from the initial round of amplification was used as template in the subsequent PCR containing the same primer pairs used in the first round of amplification. Additionally, double PCR using internal primers (ftsZunif and ftsZunir, ftsZ1 and ftsZunir, ftsZ1and ftsZcolr) was used after an initial amplification with ftsZunif and ftsZcolr and the internal primers ftsZunif and ftsZunir were used after amplification with ftsZf1 and ftsZr1.

RESULTS

IDENTIFICATION

We identified adult specimens collected from the field and from our laboratory cultures as *Arrhopalites caecus* using the keys developed by Christiansen and Bellinger (1998) and Janssens (2003). Adult *A. caecus* possessed a reddish-brown pigmentation across their dorsal surface and a bleached variation of this coloration along their ventral surface. Specimens possessed a pair of antennae that were longer than the width of the head (Fig. 2A). The fourth antennal segment was longer than the third antennal segment and possesses faint evidence of subdivision (Fig. 2A). The reduced eyes were 1+1 (Fig. 2B). Spine-like medial cephalic setae were present (Fig. 2C). The ventral surface of the dentes possessed 3 proximal setae (Ve_3, Ve_4, and Ve_5), with the medial Ve_1 setae being spine-like (Fig. 2D). The dorsal surface of the dentes possessed proximal e_6 setae (Fig. 2D). The unguis of the second leg appeared to possess a weak tunica (Fig. 2E). The anal valve possessed spines (Fig. 2F).

At the time of identification, no *A. caecus* molecular sequences had been deposited in Genbank. The D2 region of 28S rDNA (397 bases) was sequenced and deposited in Genbank with the accession number AY239037. The closest relative in the database was *A. sericus*, sharing 84% sequence identity with *A. caecus*.

DISTRIBUTION

A. caecus was collected from only two of the over 50 sites that were sampled in Wind Cave from 1992-2004 (see Fig. 1). Specimens were sighted and collected at several locations within the Room Draculum and along the Natural Entrance Tour Route in a drip pool at the Methodist Church (Fig. 3). Room Draculum is located between 18–22 m below the floor of Wind Cave Canyon and is found in the Upper Middle Level of Wind Cave. The presence of stream-rounded cobbles in this room indicates that the area may have possessed an entrance at some point in the distant past. Cobbles found at the potential entrance appear to match Tertiary-aged cobbles found on abandoned terraces within Wind Cave National Park. Methodist Church is located 44–49 m below that same drainage and is located in the Middle Level of Wind Cave. These sites are sep-

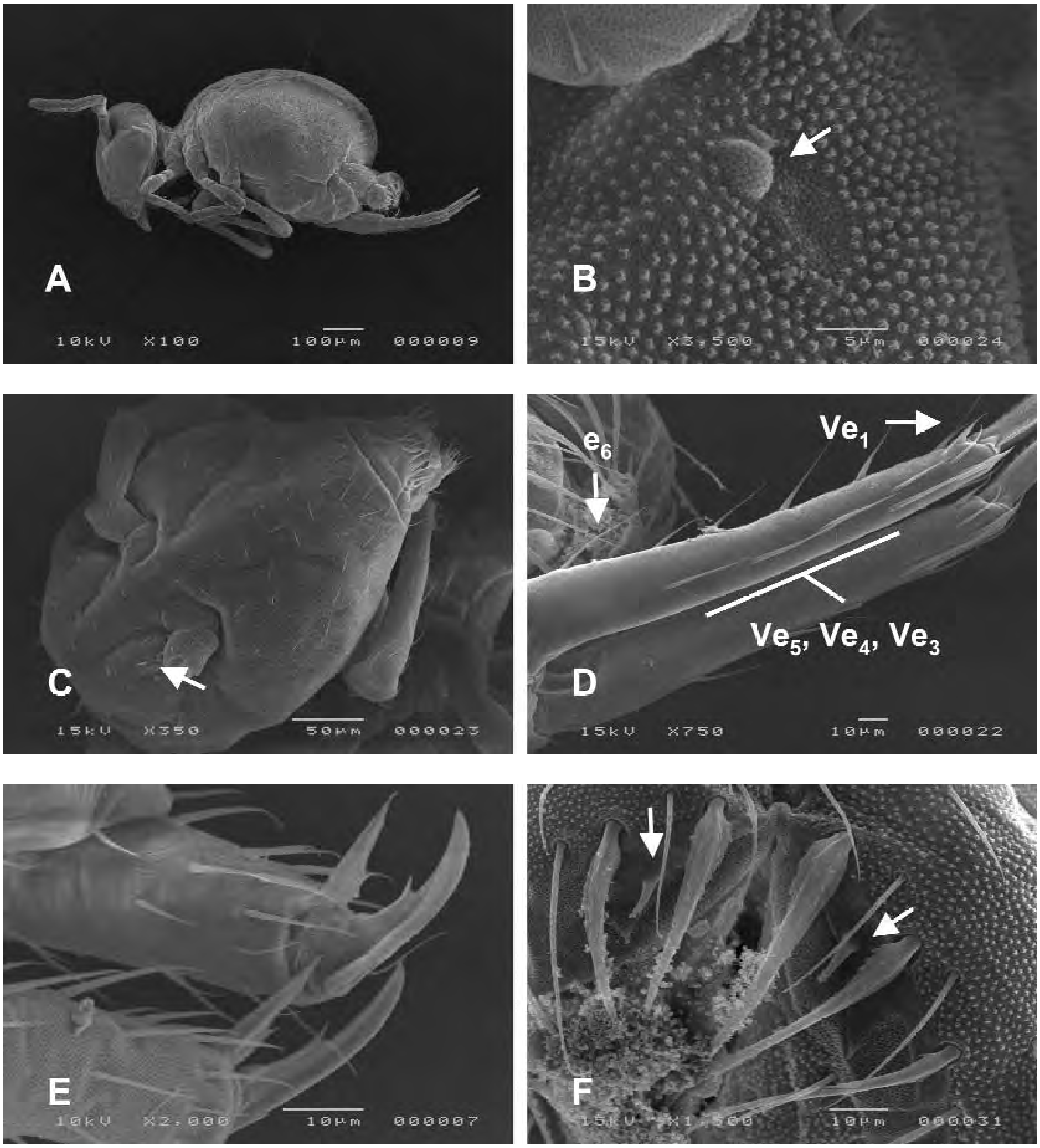

Figure 2. Scanning electron micrographs of morphological features used to identify *A. caecus*. A) Lateral view of an adult *A. caecus*, fourth antennal segment more than half as long as third, antennal segment longer than head; B) Single ocellus (arrow) and adjacent post antennal organ (depression); C) Spinelike dorsal cephalic setae; D) Ve setae complex the dens, ventral surface of dens possesses 3 proximal setae; E) Tarsal setae, absence of clavate tenate hairs; F) pair of anal spines (arrows).

arated horizontally by 107 m and vertically by 27 m. No specimens were found in soil samples taken outside the cave during surface soil surveys.

LIFE HISTORY

Eggs were round to slightly oblong and measured 0.154 ± 0.013 mm (n = 8) at the long ends and were white with a rough surface. As is typical for Collembola the eggs darkened with age. Gestation averaged 22.2 ± 5.2 days, ranging from 16.2 ± 6.3 days for the F1 generation and to 28.8 ± 5.6 days for the F4 generation (Table 1). Juveniles were white for the first two instars, and darkened after subsequent molts to a tan to rust color. Instar duration ranged from 5.2 ± 2.7 to 20 ± 2.8 days, with the first and second instars being longer than subsequent instars (Table 1).

The majority of individuals matured to adults during the second instar. Of the individuals that hatched from eggs laid during the study, eight individuals produced their first eggs within the first instar, and 50 individuals produced their first eggs within the second instar (Fig. 4). Our assessment of 381 egg laying events found that the average clutch size was 3.5 ± 0.4 eggs, with individuals producing between 1 and 18 eggs with an average of 1.6 ± 0.3 events per instar (Table 2). Fecundity of successive generations declined with reduced numbers of events per instar, and maximum and mean clutch sizes (Table 2).

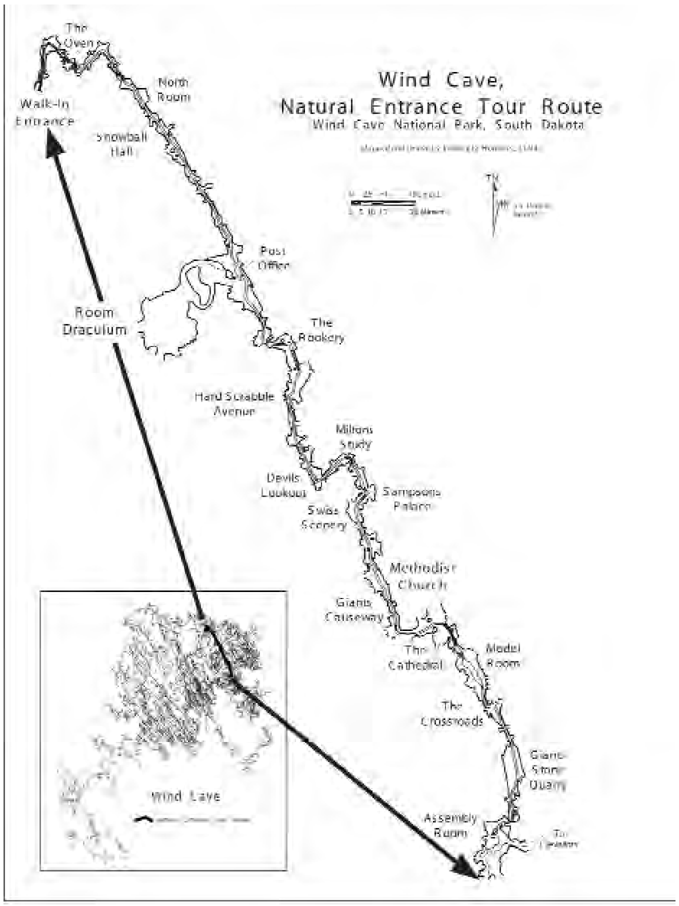

Figure 3. Populations of *Arrhopalites caecus* were discovered in Room Draculum and Methodist Church, and reported in the Post Office (K. Christensen *pers. comm.* 2003).

Figure 5 presents a survivorship curve and timeline constructed from averages across the F1 through F4 generations. For a study cohort of 128 eggs for which we had complete information, 78% hatched and 51% matured to adulthood (2nd Instar). The survivorship curve drops precipitously after the 2nd instar with fewer than 5% surviving to the 5th instar. A single individual from the F2 generation survived 10 instars (died during the 11th instar).

Our population of *A. caecus* reproduced parthenogenetically. Isolated eggs collected and reared to adults from the parental through the F3 generation produced viable offspring, i.e., F4 offspring and eggs (F5 generation) were produced. No males were found in the samples collected from the field or within our laboratory cultures.

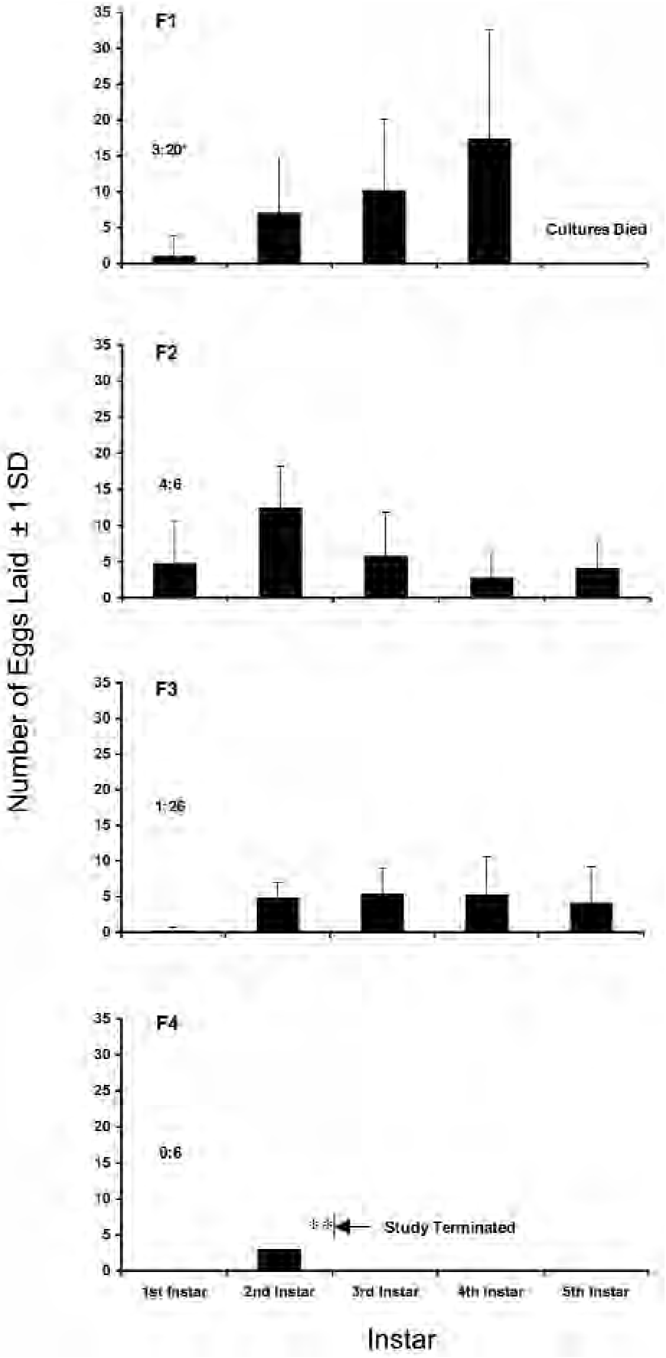

Figure 4. Fecundity of the F1 through F4 generations of *A. caecus*, as indexed by egg production (number of eggs ± S.E.) by instar. The ratio appearing above the bars for the 1st instar represents the number of individuals that matured in the 1st instar: total number of individuals that matured. The instar in which an individual matured was marked by the appearance of eggs.

THE ENDOSYMBIONT *WOLBACHIA*

We were unable to amplify α-proteobacterium *Wolbachia* from *A. caecus* but were able to amplify *Wolbachia* from *F. candida* that we had also collected from Wind Cave (Fig. 6). *FtsZ* and 16S rDNA primers that have PCR amplified *Wolbachia* within *F. candida* (Czarnetzki and Tebbe, 2004; Lo *et al.,* 2002) and the sister group A members (Hong *et al.*, 2002; Werren *et al.*, 1995) failed to generate a PCR product with *A. caecus.* Given that initial attempts to PCR amplify *Wolbachia* associating with *Folsomia* (Lo *et al.,* 2002) and

Table 1. Egg development times (days ± S.D.) and instar durations (days ± S.D.) for the F1 through F4 generations. The number of eggs or individuals is in parentheses.

Generation	Egg Development Time	Instar Duration								
		1st Instar	2nd Instar	3rd Instar	4th Instar	5th Instar	6th Instar	7th Instar	8th Instar	9th Instar
F1	16.2 ± 6.3 (25)	10.2 ± 8.1 (20)	11.4 ± 9.1 (14)	5.4 ± 5.4 (7)	8.5 ± 6.4 (2)	N.A.[a]	N.A.	N.A.	N.A.	N.A.
F2	22.6 ± 8.1 (15)	20.0 ± 2.8 (6)	16.9 ± 1.9 (7)	8.2 ± 0.8 (5)	12.2 ± 2.5 (5)	5.2 ± 2.7 (5)	12.0 ± 3.5 (4)	7.7 ± 0.7 (2)	9.5 ± 3.5 (2)	9.0 ± N.A (1)
F3	21.1 ± 9.4 (25)	14.0 ± 1.6 (26)	15.5 ± 1.5 (24)	7.9 ± 2.0 (19)	10.3 ± 2.4 (18)	9.1 ± 1.8 (11)	N.A.	N.A.	N.A.	N.A.
F4	28.8 ± 5.6 (25)	13.0 ± 1.6 (6)	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Aggregated Generations	22.2 ± 5.2 (4)	14.3 ± 4.1 (4)	14.6 ± 2.8 (3)	7.2 ± 1.5 (3)	10.3 ± 1.9 (3)	7.1 ± 2.8 (2)	12.0 ± 3.5[b] (1)	7.6 ± 0.7[b] (1)	9.5 ± 3.5[b] (1)	9.0 ± N.A. (1)

[a] N.A.= no data available, all the organisms within the generation died, or a single organism remained.

[b] Mean and standard deviation of the F2 generation are reported, as no data are available for instars 6 – 9 for the F1, F3, and F4 generations.

Table 2. The mean number of egg laying events (events ± S.D.) and clutch sizes (number eggs ± S.D.) for instars > 2 for the F1 through F4 generations. The number of events or eggs is in parentheses.

Generation	Events >2nd Instar	Clutch Size		
		Individual	Pooled	Maximum
F1	2.0 ± 0.8 (4)	4.3 ± 1.4 (21)	4.1 ± 3.4 (143)	18 (143)
F2	1.5 ± 0.7 (10)	3.7 ± 0.5 (16)	3.6 ± 2.3 (126)	13 (126)
F3	1.4 ± 0.4 (5)	3.4 ± 1.0 (26)	3.5 ± 2.1 (121)	12 (121)
F4	N.A.	3.0 ± N.A. (1)	3.0 ± N.A. (1)	1 (1)
Grand Mean ± S.D.	1.6 ± 0.3 (3)	3.6 ± 0.6 (4)	3.5 ± 0.4 (4)	18 (381)

unrelated hosts (Jeyaprahash and Hoy, 2000) have produced false-negative results, and the parthenogenetic life history of *A. caecus*, it is possible that *Wolbachia* is present within *A. caecus* but is intractable using the methods described here. Therefore, we are pursuing the use of universal 16S rDNA PCR primers in conjunction with cloning and sequencing to reveal all bacteria associating with *A. caecus.*

Discussion

Our specimens keyed directly to *Arrhopalites caecus* using the keys of Christiansen and Bellinger (1998) for Nearctic *Arrhopalites*. Spine-like medial cephalic setae are clearly visible, as is the full complement of the proximal ventral Ve_1-Ve_3 setae. The key provided by Janssens (2003), which focuses on *Arrhopalites* worldwide, failed at the final couplet to definitely distinguish our specimens as either *A. caecus* or *A. minutus,* given our conservative interpretation of the more subjective characteristics. Consistent with *A. caecus* our specimens possessed dorsal dental seta e_6. However, consistent with *A. minutus*, the fourth antennal segment is arguably not subdivided and the second unguis does not possess a pronounced tunica, i.e., arguably absent. Moreover, consistent with *A. minutus*, the anal valves possess a single spine rather than a pair of spines. Christiansen and Bellinger (1998) note that for Nearctic species, the absence of a subdivided fourth antennae segment may occur for *A. caecus*, and the presence of anal spines was a useful but not a definitive diagnostic character for the species.

Previous surveys within Wind Cave have provided conflicting reports on the distribution of *Arrhopalites*. Peck (1959) surveyed Wind Cave and did not report *Arrhopalites* but did report, albeit tentatively, a closely related genus *Pararrhopalites*. We suspect that this earlier survey may have based the identification on a juvenile of *A. caecus*. Our finding *A. caecus* in Room Draculum and at Methodist Church in Wind Cave confirms the earlier report by Christiansen (1966). Though the location within Wind Cave was not disclosed in the 1966 paper, Christiansen communicated finding *A. caecus* near the Post Office room (Christiansen, personal communication, 2003). If we combine the collection at the Post Office

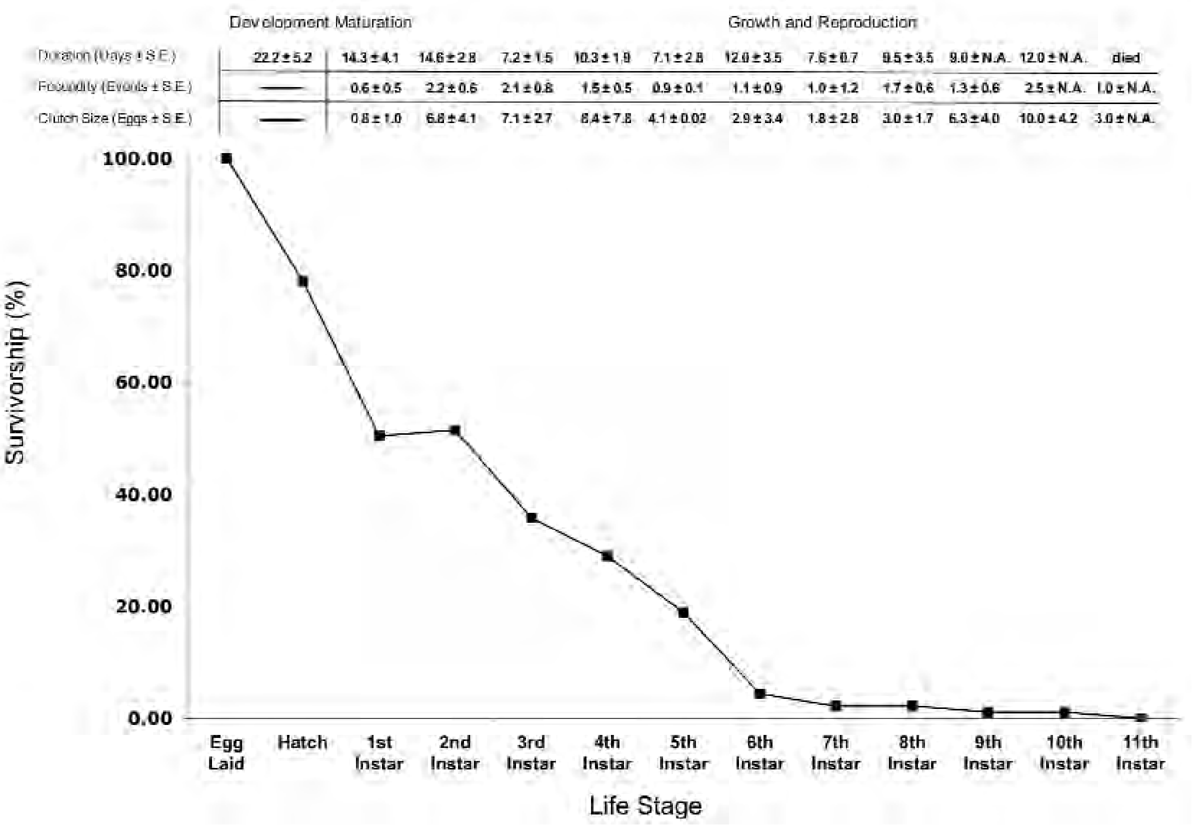

Figure 5. Survivorship curve generated from the compilation of the unweighted averages of data across instars. The time line presented above the survivorship curve includes the generation means presented in Table 1.

with our collections at Methodist Church and Room Draculum, the distribution of *A. caecus* within Wind Cave is localized since the Post Office is the jump point to Room Draculum and is along the same tour route as Methodist Church (Fig. 1, Fig. 3). Moreover, all sites possess or are near drip pools that originate from the above ground drainage of Wind Cave Canyon. These sites represent a fraction of the soils and drip pools beneath other drainages throughout Wind Cave (Horrocks and Szukalski, 2002).

Several hypotheses could explain why *A. caecus* was collected in a restricted region of the cave and appears not to have expanded further within Wind Cave. Three possibilities include limitations in the amount of available energy, a low colonization rate, and the compatibility of sediments. The latter two explanations are unlikely for several reasons. *A. caecus* co-occurs with the collembolans *Folsomia candida* (Willem) and three species in the family Entomobryidae and with a campodied dipluran (Moore *et al.*, 1996). Given the age of the cave and the vagile nature of the animal, *A. ceacus* has had ample opportunity to colonize even the more remote sections of the cave, as did the other species. The dearth of energy inputs into the cave is the more likely explanation. Previous studies in Wind Cave demonstrated a precipitous collapse of the trophic structure correlating with energy inputs (Moore *et al.,* 1996; Jesser, 1998; Moore and de Ruiter, 2000) in a manner consistent with current theory (Oksanen *et al.,* 1981; Moore *et al.,* 2003). The Methodist Church site received higher rates of energy input in the form of human skin cells, hair and clothing lint than Room Draculum owing its proximity to the tour route. Neither site supported predators of Collembola. Other sites receiving lower rates of energy inputs supported only bacteria, fungi and Protozoa. Similar relationships between the occurrence and abundance of collembola and energy inputs have been reported (Christiansen, 1961; Christiansen *et al.*, 1961). In their study of Hunters Cave in the Galena Limestone of the Kansan Drift in northeastern Iowa, Christiansen *et al.* (1961) reported that densities of *A. caecus* and species of *Megalothorax*, *Isotoma*, *Tullbergia*, *Tomocerus*, *Oncopodura*, and *Onychiurus* were strongly correlated with the organic matter content of the substrate, and weakly correlated with the particle size of the substrate.

Samples taken outside the Natural Entrance and from soils along the Wind Cave Canyon drainage did not yield any *A. caecus*. The only other reports of *Arrhopalites* in the Black Hills of South Dakota that we could find were from Jewel Cave, where the senior author reported collecting *A. caecus* at a single site – a pool formed atop a boulder adjacent to the tour route in the Tape Room (Moore *et al*., 1996). An earlier survey by Olson (1977) reported finding *F. candida* and *Entomobrya troglobita* within Jewel Cave, but no record of *A. caecus.*

There have been many publications on the taxonomic classifications and geographic distribution of *Arrhopalites*, but few detailed accounts of life history. This study represents the first comprehensive study of the life history of *Arrhopalites caecus*. The specifics of the life history are not new for Collembola as a whole, but do in some instances represent extremes for the group or new findings for the genus *Arrhopalites* (Hopkin, 1997). Average clutch size and frequency of egg laying were similar to other Collembola (Snider, 1973; Hopkin, 1997). Embryonic development for *A. caecus* was considerably longer at 22.2 ± 5.2 days than that of other species, which ranged from 6–11 days, when reared at similar temperatures (Snider, 1973; Von Allmen and Zettel, 1983; van Straalen and Joose, 1985). The relatively high mortality rate for eggs (~22%) was due largely to fungal contamination, as many of the eggs that did not hatch were covered with a dense mat of fungal hyphae. The frequency of molts and instar durations are comparable to several species (Hopkin, 1997; Snider, 1973; Krool and Bauer, 1987). The decline in fecundity that we observed with successive generations was likely due to an inadequacy in the yeast as the sole source of the diet (Fig. 4).

Our results suggest that *A. caecus* reaches sexual maturity during the 1st Instar, as eight of the 58 hatchlings produced eggs prior to molting. However, these results are misleading, as our records suggest that six of the eight early maturing individuals may have indeed molted to the 2nd instar but were not recorded. Four of the eight early maturing individuals occurred in the F2 generation, where we had a 4-day gap in data collection coincident with when the first molt might have occurred based on our observations from the F1, F3 and F4 generations (see discrepancy in Table 2). Our laboratory notes indicated that two of the three early maturing individuals from the F1 generation had grown significantly and developed pigmentation before a molt was recorded, indicating that molts had occurred but went unnoticed. Nonetheless, maturation during the second instar is early. Hopkin (1997) reported only 1 species, *Mesaphorura krausberi* (*sensu* Hale 1965) reaching sexual maturity at this early instar, with most species reaching maturity between instar five and eight.

We concluded that *A. caecus* exhibited parthenogenetic reproduction using the conservative criterion of Hopkin (1997), which stipulated the appearance of F4 offspring from the isolated eggs of successive generations. We suspect *A. caecus* is also capable of sexual reproduction, but could not draw this conclusion from our study as we did not encounter any males or observe any spermatophore-like structures in our laboratory cultures. Parthenogenesis may help explain the wide global distribution of *Arrhopalites*, the rarity of encountering males (Christiansen and Bellinger, 1996), as well as historic difficulties in collection (Zeppelini and Christiansen, 2003).

Parthenogenicity offers several selective advantages to populations in cave environments, particularly those with extremely low energy inputs that are patchily distributed throughout the cave. Parthenogenetic reproduction is common among euedaphic Collembola living in stable environments (Hopkin, 1997). Caves offer stable environments, but the dearth of energy poses additional pressures as successful colonization of a site requires either the establishment of gravid females that successfully hatch both males and females, or the successful establishment of both males and females. Parthenogenetic reproduction allows a colony to be established by the immigration of a single female. Once established, this colony could retain reproductive viability at very low densities, especially in low temperature and low energy-input caves, making the likelihood of encountering an individual low.

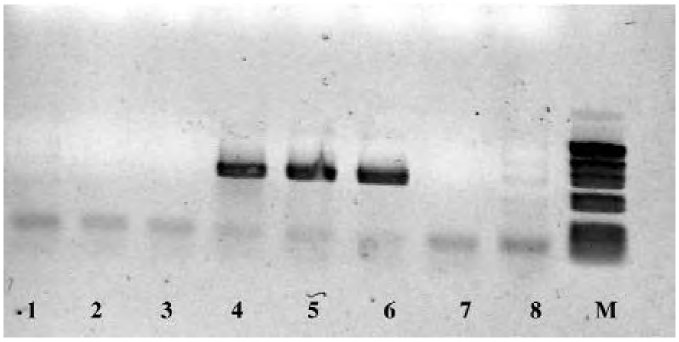

Figure 6. PCR amplification of *Arrhopalites caecus* and *Folsomia candida* using a gradient of annealing temperatures with primers ftsZunif and ftsZunir (Lo *et al.* 2002). Lanes: 1-3, 8, *Arrhopalites caecus*; 4-6, *Folsomia candida*; 7, negative control; M, marker Phi X174 *Hae* III digest. Lanes: 1,4,7, 50º C anneal; 2, 5, 53º C anneal; 3, 6, 56º C anneal; 8, 48º C anneal.

While we were able to confirm that the endosymbiotic *Wolbachia*, an α-proteobacterium (Rickettseales) is present within *F. candida* (Fig. 6) isolated from Wind Cave, we could find no evidence of *Wolbachia* in *A. caecus*. Our search included using primers to detect the protein-coding *FtsZ* gene and 16S rRNA gene primers that have PCR amplified *Wolbachia* within *F. candida. Wolbachia* is maternally transferred, infecting the gonadal tissues. Vandekerckhove *et al.* (1999) noted that the bacterium has been shown to enhance host fertility (Hoerauf *et al.,* 1999) and lead to reproductive isolation of the host population by causing cytoplasmic incompatibility (Bourtzis *et al.,* 1996; Breeuwer, 1997) or inducing parthenogenesis by the feminization of males (Juchault and Legrand, 1989; Juchault *et al.*, 1994) or by initiating thelytokous (= mother-to-daughter) parthenogenesis (Stouthamer *et al.,* 1990; Zchori-Fein *et al.*, 1995). To date six subgroups of *Wolbachia* (designated A-F) have been isolated, with subgroups A and B infecting various arthropods, subgroups C and D infecting filarial nematodes, subgroup E infecting arthropleonan Collembola (*F. candida*, *Mesaphorura italica, M. macrocheata*, *Paratulbergia calliphygos*) and subgroup F infecting termites. We could find no reports of *Wolbachia* infections within symphyleonan Collembola like *A. caecus*.

CONCLUSIONS

The distribution and life history of *A. caecus* in Wind Cave makes sense when viewed from current evolutionary and ecological perspectives. From an evolutionary context, *A. caecus* possesses all the life history traits favored by natural selection; rapid maturation, early and high fecundity, and low mortality.

Moreover, parthenogenesis is common among collembolans that reside in stable environs (Stam *et al.,* 1996). From an ecological standpoint, *A. caecus* occupies sediments within sections of the cave that possess a stable climate with sufficient energy to meet its growth and reproductive needs, but not enough energy to support a stable population of predators (Roberts, 1974; Moore *et al.,* 1993). Issues that are yet to be resolved for *A. caecus* in Wind Cave are its longevity and details of the physiological basis of its parthenogenesis, as we could find no evidence of infection by the α-proteobacterium *Wolbachia*.

ACKNOWLEDGMENTS

We thank the NPS personnel at both Wind Cave National Park and Jewel Cave National Monument for their assistance with this effort, with special gratitude to Kate Cannon, Thomas Casey, James Nepstad, Ross Rice and Michael Wiles. A special thanks to Darwin Bonnet, Lynn Jones, Renee Jesser, Gina Lipari, Ann Ratcliffe, and countless others from the UNC Soil Ecology Laboratory for their assistance in collecting and rearing *A. caecus*, maintaining the cultures, and their observations. We also wish to thank Angela Morrow, Ken Cochran and the staff of the UNC Imaging Suite for providing the SEM micrographs, Lori Reinsvold for her technical assistance with the figures, and three anonymous reviewers for their helpful comments and constructive criticisms. Funds to conduct the study were provided by the National Science Foundation (NSF grants DEB-9257710 and DEB-9815925), the U.S. National Park Service, and the UNC Sponsored Programs and Academic Research Center.

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USING STATE-WIDE GIS DATA TO IDENTIFY THE COINCIDENCE BETWEEN SINKHOLES AND GEOLOGIC STRUCTURE

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The Kentucky GIS coverage of sinkholes, completed in 2003, consists of 101,176 polygons representing the uppermost closed contour of every karst sinkhole identified using USGS 1:24,000 scale topographic maps. This resource is a useful tool for delineating karst landscapes in Kentucky because karstified limestones underlie 55% of the areal surface of the state. For hydrologic studies, alignments of sinkholes commonly indicate preferential flowpaths for groundwater; and this information aids in large-scale planning and zoning. In this paper, I demonstrate the effectiveness of using this sinkhole coverage as a tool for delimiting structural features of Kentucky.

INTRODUCTION

The relationship between karst features and geologic structures is well documented. At the largest scale, geologic structures determine the exposure of limestones to karst processes (White *et al.*, 1970). At a medium scale geologic structures determine the flow paths in karst aquifers (Parizek, 1976; Nelson, 1988). At a small scale, geologic structures have a significant impact upon the ultimate morphology of conduit systems (Ford and Ewers, 1978; White, 1988; Palmer, 1991).

Sinkholes are a common feature used to identify subsurface lineaments or "photo-linears" (Littlefield *et al.*, 1984; Howard, 1968; Kastning, 1983; Orndorff and Lagueux, 2000). Thus, sinkhole alignments are considered an indication of preferential flowpaths for groundwater (Elvrum, 1994; Taylor, 1992). The low matrix permeability characteristics of Kentucky limestones enhance the correlation between sinkholes, secondary permeability, and groundwater flowpaths (Worthington *et al.*, 2000). Secondary permeability features, such as fractures, faults, and bedding planes, are frequent in telogenetic limestones, such as those in Kentucky, due to brittle deformation during uplift and exhumation (Vacher and Mylroie, 2002).

THE KENTUCKY SINKHOLE GIS COVERAGE

From 2000 through 2003, the Kentucky Geological Survey (KGS), in association with the Kentucky Speleological Survey (KSS) digitized all karst sinkholes in Kentucky using the upper-most closed contour present on 1:24,000 scale topographic maps (Paylor *et al.*, 2003). The complete GIS shapefile coverage is available to the public on the KGS website (www.uky.edu/KGS). The numbers are staggering. In Kentucky, karstified limestones underlie 55% of the land area. In these karst regions, 101,176 topographic sinkholes occupy 4% of the total land area of the state (Figure 1a).

The sinkhole delineation project began in response to individual project needs (Florea *et al.*, 2002); however, by completion it became clear that the GIS coverage would become a

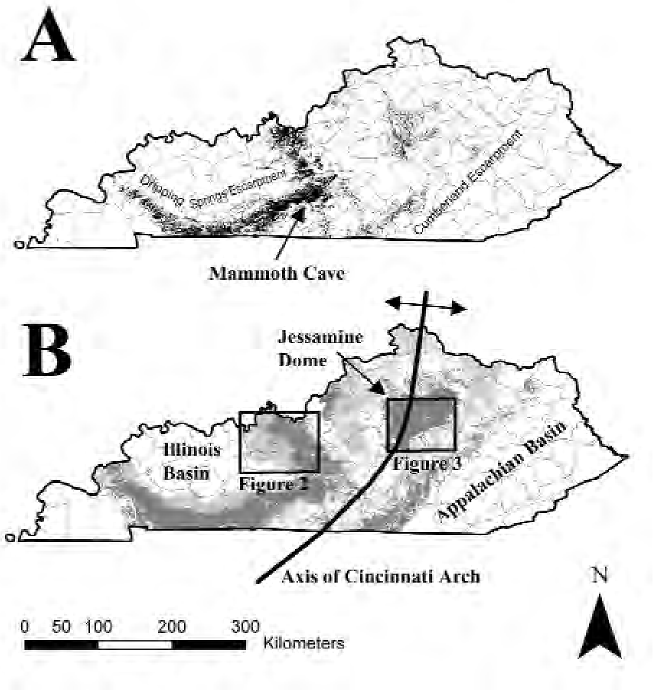

Figure 1. State-wide GIS coverages of sinkhole data (A) and karst occurrence (B) for Kentucky (Paylor and Currens, 2002). Black areas in A denote digitized sinkholes. Dark-gray areas in B denote areas underlain by limestones with a high potential for karstification. Light-gray areas denote areas underlain by limestones with low to moderate potential for karstification. Sinkholes in A correlate well with highly karstified limestones from B. The exposure of the highly karstified limestones and thus sinkholes correlate to large-scale structural features noted in B. The bounding boxes for Figures 2 and 3 are shown in B. High-density sinkhole plains are visible near Mammoth Cave in A.

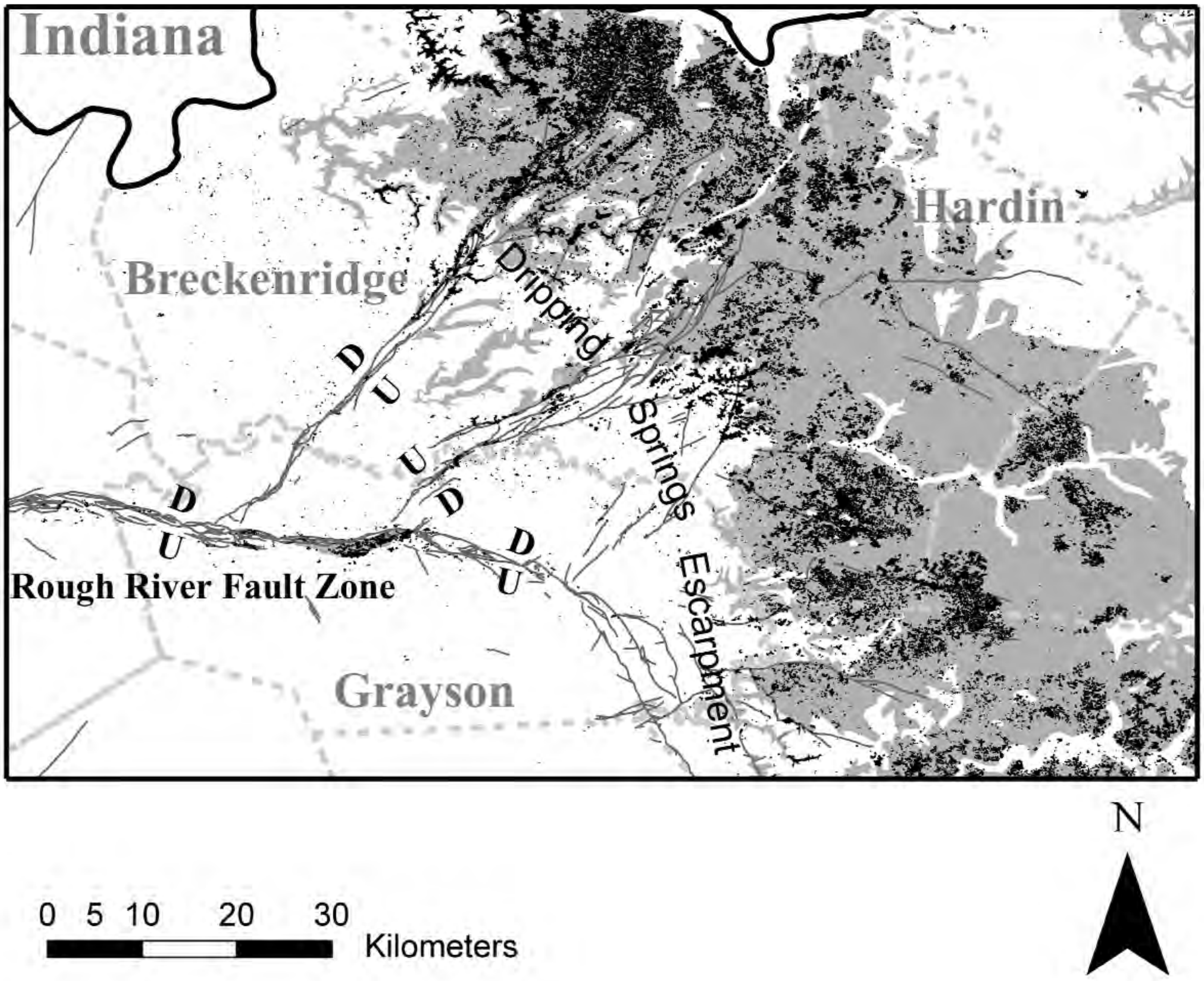

Figure 2. Sinkholes (black polygons), highly karstified limestones (dark-gray area), and faults (solid, dark-gray lines) in Breckenridge, Grayson, and Hardin Counties, Kentucky. Sense of motion for major fault zones is denoted by U for the up-thrown side and D for the down-thrown side. The Dripping Springs Escarpment separates the highly karstified Mississippian limestones from the Pennsylvanian sandstones and shales to the west. A good correlation exists between sinkhole alignments and portions of the Rough River Fault Zone, especially in areas of Grayson County where the up-thrown side of normal faulting has exposed limestones in a region otherwise dominated by sandstone and shale.

valuable resource to state agencies, environmental and transportation industries, and the public. Patterns emerged in the sinkhole distribution during digitization which correlate to geological boundaries and features and reflect the underlying geologic structure of Kentucky.

EXAMPLES OF CORRELATING SINKHOLES WITH STRUCTURAL FEATURES

LARGE-SCALE

The state-wide sinkhole map conveys large-scale geological information (Figure 1a and b). Regions of sinkhole development correlate to the exposure of karstified limestones (Paylor and Currens, 2002). In the Inner Bluegrass region along the axis of the Cincinnati Arch the Ordovician-age Lexington Limestone is exposed at the center of the Jessamine Dome. On the east flank of the arch, Mississippian-age limestones plunge into the Appalachian Basin along the Cumberland Escarpment. These same Mississippian limestones border the Dripping Springs Escarpment and outline the edge of the Illinois Basin on the west side of the arch. The high-density sinkhole plains in the Mammoth Cave region are clearly visible.

Additionally, the occurrence of some sinkhole groupings correlates to systems of faults. In Breckenridge, Grayson, and Hardin Counties it is possible to trace portions of the Rough

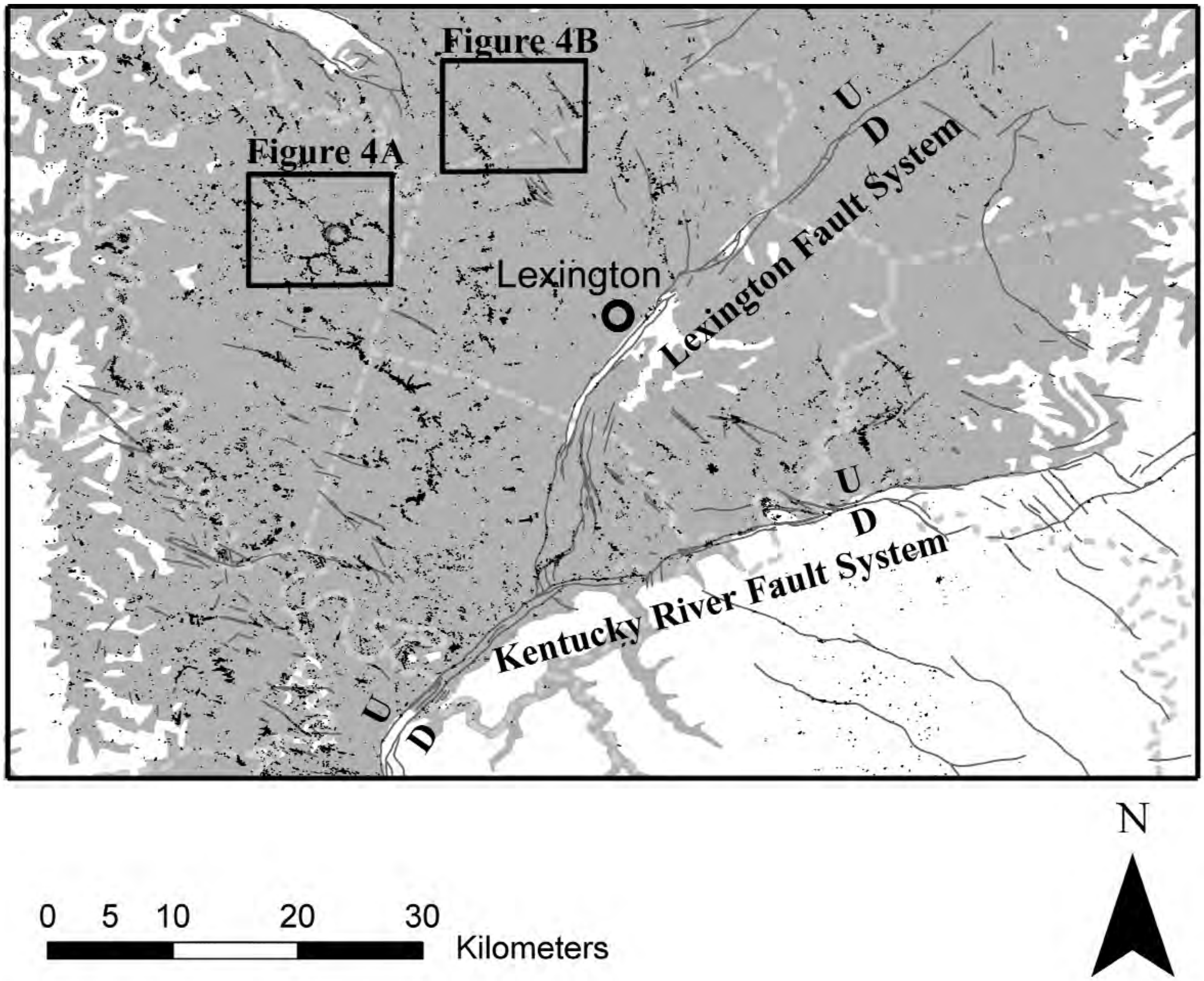

Figure 3. Sinkholes (black polygons), highly karstified limestones (dark-gray area), and faults (solid, dark-gray lines) in a portion of the Inner Bluegrass Region near Lexington. Sense of motion for major fault zones are denoted by U for the up-thrown side and D for the down-thrown side. The Kentucky River and Lexington Fault Systems act as a geologic boundary between the highly karstified Lexington Limestone to the north and west (the up-thrown side) and the argillaceous Clay's Ferry Formation to the south and east (the down-thrown side). The dominant NW alignment of sinkholes in the Inner Bluegrass is clear in the north western portion of the figure. The bounding boxes for Figures 4A and 4B are shown.

River Fault zone using sinkhole alignments (Figure 2). Geologic offsets due to normal faulting in Grayson County expose Mississippian limestones in a region otherwise dominated by Pennsylvanian sandstones and shales (Figure 2).

Fault offsets often produce geologic boundaries for karst development. The division between the Inner and Outer Bluegrass physiographic provinces occurs at the boundary between the karst-rich Lexington Limestone and the argillaceous Clay's Ferry Formation — both of Ordovician age. South and east of Lexington this boundary occurs due to normal faulting along the Kentucky River and Lexington Fault Systems (Figure 3).

MEDIUM-SCALE

The dominant control on sinkhole alignments in the Inner Bluegrass region is a series of NW-trending fractures (Thrailkill, 1992; Elvrum, 1994, and references therein). Taylor (1992) found it difficult to ascribe these fractures to a specific tectonic event even though Stafford (1962) claimed that the fractures resulted from tensional forces related to the uplift of the Cincinnati Arch and Jessamine Dome. Irrespective of their origin, these fracture traces play an important role in the direction of groundwater flow (Taylor, 1992). These NW alignments are clearly visible in the sinkhole GIS data (Figures 3 and 4). Also visible is a circular and radiating alignment of

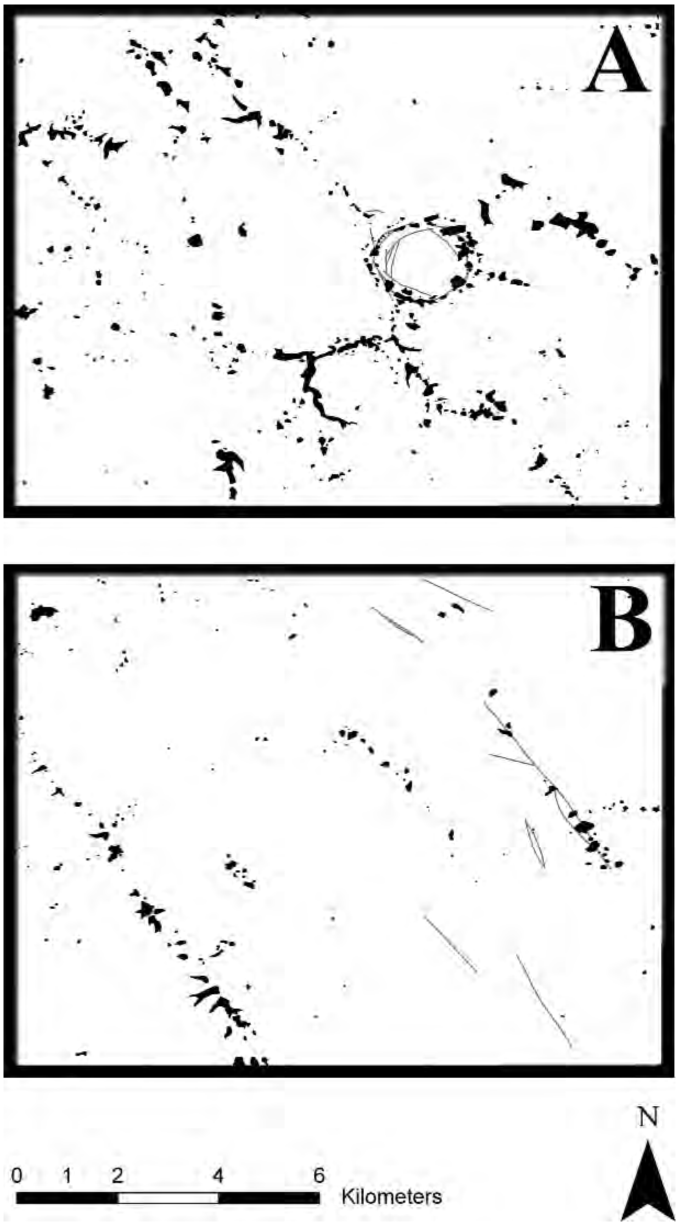

Figure 4. Sinkholes (black polygons) and faults (solid, dark-gray lines) for select portions of the Inner Bluegrass Region expanded from portions of Figure 3. In A the Versailles impact structure is clearly visible as a ring of sinkholes. These correlate closely with a ring of mapped faults. The down-thrown side of the faults is toward the center of the impact structure. While some sinkhole alignments correlate with known faults or fractures in B, other sinkhole alignments indicate the presence of otherwise unknown structural features.

sinkholes that correlates to the Versailles impact structure (Figure 4a). Discovered in 1962, this mile-wide circular structure within the Lexington Limestone is of late Ordovician age and consists of a brecciated central dome, and a marginal structural depression partly bounded by normal faults (Black, 1964).

Interestingly, while some sinkholes align with known faults, other faults have no overlying sinkholes, and many sinkhole alignments suggest currently unknown faults or fracture traces. Taylor (1992) found that only 12% of sinkhole alignments in the Inner Bluegrass overlie fault zones and hypothesized that mylonitization (fault gouge) could prevent groundwater flow. Fractures, with little or no offset and fault gouge, would enhance rather than reduce groundwater flow. These fracture traces are not included in the available GIS coverage of faults.

Considerable supporting data suggests that fractures are the primary control of sinkhole alignments in the Inner Bluegrass. For instance, in the areas surrounding Figure 4, Taylor (1992) found peaks on a rose diagram for sinkhole alignments at 310°–330° and 340°–360°. Elvrum (1994) found similar peaks on rose diagrams for topographic map fracture traces (310°–330° and 340°–360°), sinkhole long-axis orientations (320°–330° and 350°–360°), and color infra-red photolinears (330°–350°).

Sinkhole GIS data, combined with field reconnaissance, is a proven tool for tracing significant medium-scale structural features in Kentucky (Florea, 2002). Additionally, the ability of the sinkhole GIS data to assist in identifying yet unknown structural features is a valuable resource unanticipated at the beginning of digitization.

CONCLUSIONS

The vast number of digitized sinkholes now available in the Kentucky sinkhole GIS coverage provides a useful tool for delineating karst regions, promoting accurate hydrologic studies, and assisting planning and zoning. The correlation between structural features and sinkhole occurrence is well documented in the literature and clearly demonstrated using the Kentucky GIS data. Using the data, it is easy to identify regions of karstified limestone exposure, geologic boundaries, and faults and fracture traces. Of particular interest is the possibility of identifying previously unknown structural features using state-wide sinkhole data.

ACKNOWLEDGEMENTS

I wish to thank the Kentucky Geological Survey and the Kentucky Speleological Survey for the interest they have displayed over the years in the sinkhole GIS project and for the dedication they have shown to see the project to completion. Specifically, I wish to acknowledge Jim Currens and Randy Paylor at the KGS for their involvement. Beth Fratesi conducted an internal review of this paper. Significant improvements to the manuscript are the result of an anonymous reviewer and Christopher Connors.

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COMET CONES: A VARIETY OF CAVE CONE FROM FORT STANTON CAVE, NEW MEXICO

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The name "comet cones" is suggested for a variety of cave cone, after their physical likeness with the images of comets. The comet cones of Fort Stanton Cave are constructed of millimeter-sized calcite cave rafts. A pre-existing stream environment is responsible for the small size of the rafts as well as the development of a "comet" tail on the cones. Drips from condensate water sank the rafts and formed the cones.

INTRODUCTION

An unusual type of cave cone occurs in Fort Stanton Cave. We refer to these as "comet cones." Their shallow height, millimeter-sized rafts, and tail, result in a resemblance to images of comets (Fig. 1a). These comet cones differ from common cave cones because they have formed in a more dynamic environment (gentle stream) and are constructed of very small rafts. We present a brief description of the comet cones, discuss their unique origin, and suggest the comet cone as a new variety of cave cone.

Cave cones are a speleothem subtype of cave rafts, and two common varieties of cave cones are "volcano cones" and "tower cones" (Hill and Forti 1997). Cave cones are constructed of rafts. Rafts form when calcite crystal assemblages precipitate on the surface of still water. Individual rafts can grow up to 10s of centimeters in diameter. Cave cones form when dripping water impacts floating cave rafts, sinking them, and subsequently piling the rafts under the drip point. The piles of rafts form the stalagmite-like cones. The heights of cave cones are limited by the depth of the pools in which they develop. We show an example of a nicely formed volcano cone from Cavenee Caverns, central New Mexico, in Figure 2. In contrast to the common varieties, comet cones form in gently flowing, shallow water. When a water drip sinks the tiny rafts, the pile is thin and a small tail forms in the direction of flow.

DESCRIPTION AND COMPOSITION OF COMET CONES

The comet cones of Fort Stanton Cave are thin cave cones with tails (Fig. 1a). They are only 1–3 centimeters high, 15–30 centimeters wide, and 30–50 centimeters long. They are constructed of millimeter-sized cave rafts. X-ray diffraction shows that the rafts consist of low magnesian calcite. The mineral assemblage of the comet cones was calcite, quartz, and dolomite. The minor to trace amounts of quartz and dolomite are probably detrital components. Quartz and dolomite are not common cave-authigenic minerals, and small amounts of these would be expected in this stream passage environment.

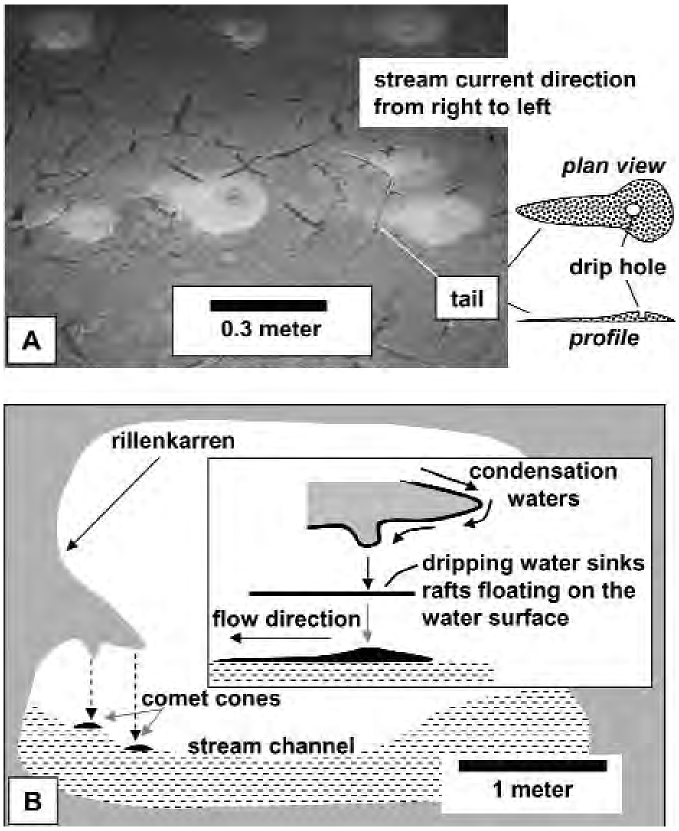

Figure 1. Depositional environment and morphology of the Fort Stanton Cave comet cones. (A) Image and sketch of cave cone morphology. (B) Cross-section of the Fort Stanton passage shows the importance of the shelf and condensate water to the origin of the cave cones.

ORIGIN OF COMET CONES

These cones have formed on stream silt and mud under a shelf along the margin of the cave passage (Fig. 1b). While this area is dry today, the stream water level must have been at about 0.1 to 0.5 m deep when the comet cones formed. Broad

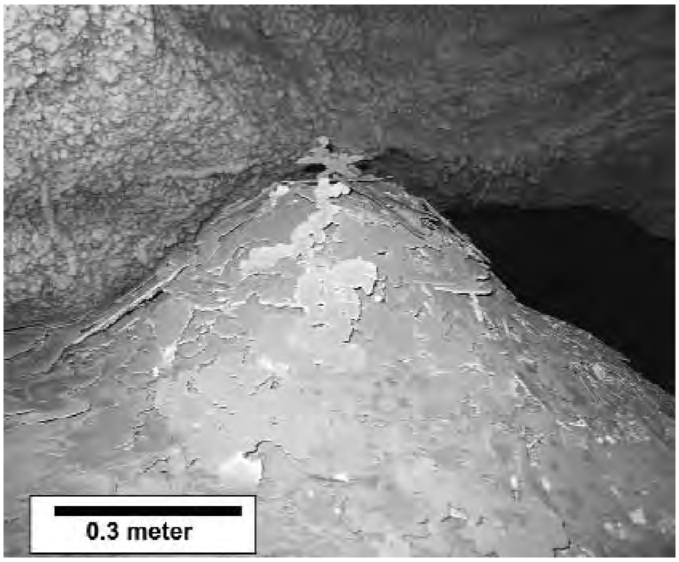

Figure 2. Example of well-developed cave cone. This cave cone that has formed in Cavenee Caverns, central New Mexico, is the volcano cone variety.

rillenkarren along the cave walls indicates that water vapor condensed along the upper walls and ceiling of the passage and then this condensate water descended to the shelf. The condensate water dripped from the lip of the shelf, and at projections under the shelf, and sank small cave rafts floating on the surface of the stream. The pre-existing stream flow, while very gentle, transported some of the rafts in the downstream direction past the cones. Deposition of the rafts by the gentle current tapered off away from the cones in the down stream direction to give the cones a comet's tail. Sometime after the water level descended below the cones and before the passage became dry as it is today, dripping continued and formed shallow drip holes in the center of the cones.

The comet cones of Fort Stanton Cave are delicate and probably short-lived features. It is likely that this area of the cave could become hydrologically active again and destroy the cones, or continue the cone-forming process in the not-too-distant future perhaps when climatic conditions are wetter. Even with their delicate, short-lived existence, we suggest the comet cone as a new variety of cave cone.

ACKNOWLEDGMENTS

We thank Kathy Eli for bringing the cones to our attention. Sam Bono noticed the unusual shapes of the cones and coined the term "comet cones." We are very grateful to John Corcoran for the trip to this part of Fort Stanton Cave. In Fort Stanton Cave the cones are referred to as Kat's Cometary Cones. Art and Peggy Palmer also provided helpful discussions. We are grateful to Wes Cavenee for allowing access to Cavenee Caverns, where the image was taken for Figure 1. We appreciate Mike Bilbo, Bill Murray, and the Bureau of Land Management for issuance of permits to study the comet cones in Fort Stanton Cave.

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MEASUREMENT OF pH FOR FIELD STUDIES IN KARST AREAS

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The determination of pH in karst waters is important for evaluating such chemical processes as cave growth, speleothem deposition, and overall water chemistry. Relatively small errors in pH readings can result in significant misinterpretations of the chemical processes taking place. For example, a pH error of 0.5 units would produce a correlative error in SIcalcite of 0.5. To ensure accuracy, pH must be measured in the field, but the conditions in karst settings make this hard to accomplish, and there is minimal published guidance available. Actions that help to improve data quality include: use of a good meter/electrode (accurate to 2 decimal places), careful preparation before field activities, cautious transport of instruments, frequent calibration, measurement in a beaker (not the water body), and allowance of time for equilibration. Instruments that allow measurement of very small samples, samples in wells, or continuous monitoring are available, but are more expensive and usually not as accurate.

INTRODUCTION

Water chemistry is an important factor controlling the growth of caves, the deposition of speleothems, and the suitability for healthy biota. Because of this, chemical studies are frequently conducted in karst areas. The measurement of hydrogen ion activity (commonly expressed as pH, the negative log of the activity) is an important component of such field investigations. pH is used to compare the acidity of different waters, to calculate CO_2 partial pressures, to determine the saturation state of the water with respect to calcite or other minerals, and for other geochemical modeling (see chapter 7, White, 1988 for review, examples, and discussion of these items). pH must be measured in the field, because changes during transport and storage of water samples will cause it to vary considerably. This sort of instability has also been recognized for other parameters (Baedecker and Cozzarelli, 1992). Laboratory-measured pH of natural waters is, therefore, not generally suitable for accurate geochemical calculations. The acquisition of field pH data in karst settings, however, is challenging due to the conditions encountered (remoteness, harsh transport, pervasive mud and water, etc.). Such difficulties are not addressed in the guidance literature or standards regarding the measurement of pH (American Society for Testing and Materials, 1990, Clesceri *et al*., 1999).

Over the course of various projects, and with suggestions from other karst geochemists, we have considered many of the challenges of accurate pH measurement. We have also developed strategies to accomplish this goal. In the present paper we examine the importance of pH measurement to cave and karst studies, explain some of the special concerns, and make suggestions (based on our experience) about how to collect accurate pH values in these settings. This is done in the hope that the information presented may be useful to other researchers. We do not address the theories of pH or its measurement, which are available elsewhere (e.g. Langmuir, 1997; Bates, 1973).

IMPORTANCE OF ACCURATE pH MEASUREMENT

There are many reasons (some mentioned above) why the accurate measurement of pH in karst terranes is important. As an illustration of this, we demonstrate the effect of possible errors in pH measurement on the calculation of calcite saturation index (SIcalcite), an important derived parameter, for several natural water samples. SI is given by the equation

$$SI_C = \log\frac{a_{Ca^{2+}}a_{CO_3^{2-}}}{K_C} = \log\frac{\gamma_{Ca^{2+}}\left[Ca^{2+}\right]\gamma_{HCO_3^-}\left[HCO_3^-\right]K_2}{10^{-pH}K_C}$$

where a is the activity of the given aqueous species, and K_{calcite} is the (temperature-dependent) equilibrium constant for the dissolution of that mineral (White, 1988). The expression on the far right is the one most often used, because it employs the commonly measured pH, Ca, and alkalinity parameters. In that expression the values in brackets are molar concentrations of the species, K_2 is the calculated activity coefficient for the species, and γ is the 2nd dissociation constant for carbonic acid.

A saturation index of less than zero indicates that the particular water is capable of dissolving calcite, hence can enlarge a cave. A saturation index greater than zero shows that calcite can be precipitated and that speleothems may grow. For purposes of illustration, we used analyses of three water samples taken from Scott Hollow Cave, West Virginia (Table 1). These waters are representative of those found in temperate karst regions throughout the world.

Laboratory data (major ions) along with field parameters (pH, conductance, temperature) for the samples were entered

Table 1. Geochmical parameters for three water samples from Scott Hollow Cave, West Virginia (Davis, 1999)

Location	Craigs Creek		John's Flowstone
Sample Date	2/21/1998	5/17/1998	2/21/1998
Temp (°C)	10.7	12.3	11.1
pH	7.78	7.41	7.8
Conductivity	303	361	294
TDS	196.9	191.1	191.1
$SI_{Calcite}$	0.099	-0.144	0.096
Cl	4.1	3.8	2.2
HCO_3	162.0	206.8	150.4
NO_3	11.0	11.8	9.7
PO_4	0.15	nd	0.09
SO_4	10.9	10.6	11.1
Ca	55.0	55.0	55.0
F	0.10	0.10	nd
K	0.8	1.5	0.4
Mg	4.5	5.0	4.2
Na	1.3	1.5	1.1
Si	35	35	35

Note: Values given in mg/L except pH and SI (std units) and conductivity (microsiemens). nd = not detected.

into the computer program WATEQ4F (Ball & Nordstrom, 1991) for calculation of SI. The results are shown in Figure 1. The symbols on the lines show the original values of SI as calculated for each sample, along with sample pH (actual pH of each of the three samples varied from 7.18 to 7.78, but was normalized to zero to aid visual comparison in this graph). The lines have a constant slope (a consequence of the algebraic relationship between pH and SI) but are offset. The position of the original data points shows that sample 1 was just oversaturated, and sample 2 and 3 were undersaturated to differing degrees. For any error in pH of 0.1 units, an error of 0.1 SI units is generated. It can be seen in the figure that, in natural waters, such errors can easily make the difference between considering the system to be under- or oversaturated with respect to calcite. This in turn could lead to misinterpretation of the processes going on within the cave.

Selection of a pH determination method

A review of any major scientific supply catalog shows that there are numerous instruments and supplies available for determining pH. Instruments may also be rented from environmental suppliers. Table 2 summarizes the features of several typical methods/instruments.

The least expensive methods employ titration and/or colorimetry. The materials required for these methods are also compact and robust, but unfortunately do not provide the precision needed for quantitative geochemical analysis. For this reason, electrometric methods (meters) are most commonly employed in karst geochemical studies. Some meters have a built-in electrode, but most use an external replaceable one which is purchased separately. Factors that come into consideration in selecting a meter/electrode include: cost, size, weight, sturdiness, accuracy, and precision.

Table 2. Comparison of pH determination methods.

Method	Make/ Model	Precison/ Resolution	Accuracy (+/-)	Analytical Range	Cost (US$)	Temp. Range (°C)	Supplier
Colorimetric pH Test Strips[a]	pHydrion Comparator[b]	1	1	0 to 13	14	n/a	Thomas Scientific
	Colorphast Store Set	0.5	0.5	0 to 14	128	n/a	Cole-Parmer
Field Titration/ Color Wheel[a]	Hach Color Disc/ Bromthymol Blue	0.1	0.1	5.5 to 8.5	98	n/a	Dynamic Aqua-Supply
Electrometric (pH meters)[c]	LaMotte PockeTestor 2[d]	0.1	0.1	0 to 14	89	0 to 50	Thomas Scientific
	Cardy Twin[d, e]	0.01	0.1	2 to 12	238	5 to 40	Cole-Parmer
	Hanna Checker[f]	0.01	0.2	0 to 14	35	0 to 50	Thomas Scientific
	Corning 307[f]	0.01	0.01	0 to 14	187	0 to 50	Thomas Scientific
	Hanna HI-9024[g]	0.01	0.01	0 to 14	349	0 to 100	Thomas Scientific
	Accumet AP62[g]	0.01	0.01	-1.99 to 19.99	620	-5 to 100	Fisher Scientific
	Orion 525A[h]	0.001	0.002	-2 to 19.999	1389	-5 to 105	Fisher Scientific
	Corning 455[h]	0.001	0.001	-2 to 19.999	2688	-30 to 130	Fisher Scientific

Note: The cost of electrodes for the pH meters can be an additional $65 to $450. The cost of pH buffers can add an additional $20 to $100.

[a] May be more variable depending on the quality of the operator's color match assessment.

[b] Includes comparator and test papers.

[c] Variability may be seen in accuracy and precision values depending on the type and make of the electrodes that are used

[d] Pocket model with non replaceable probe.

[e] Able to measure samples as small as 150 µL.

[f] Stick model with replaceable probes.

[g] Portable models.

[h] Bench model.

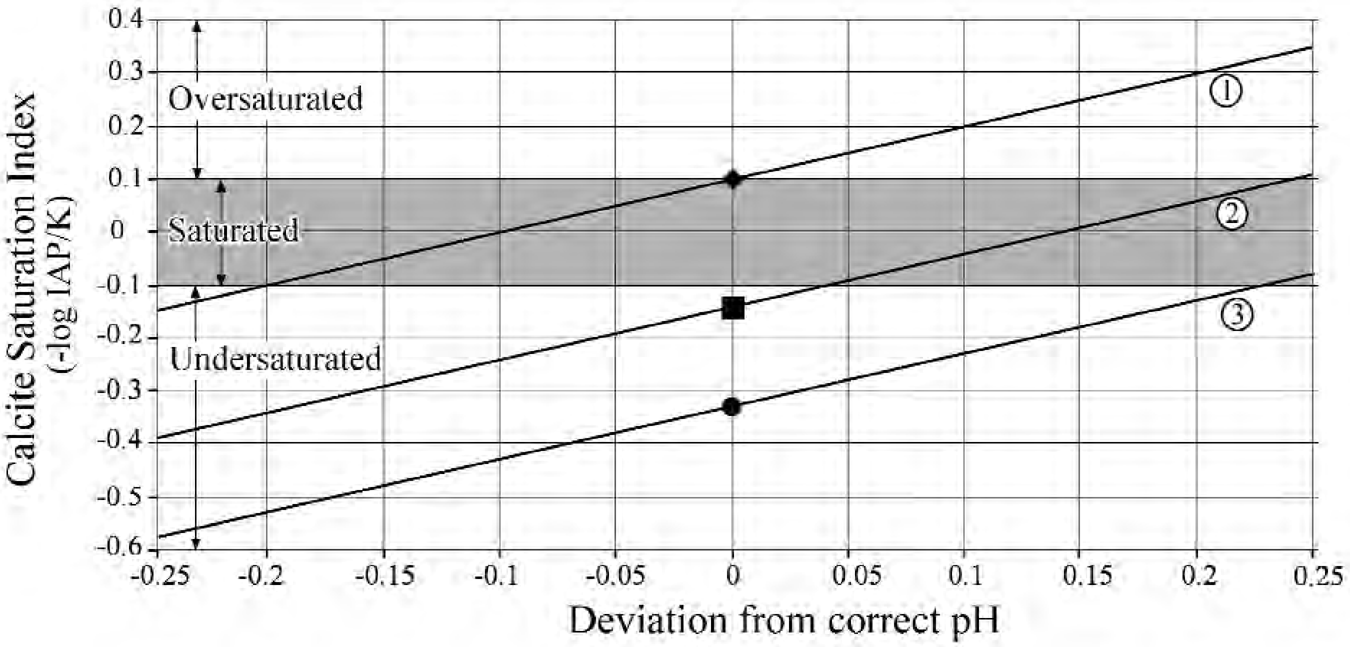

Figure 1. Fluctuations in the saturation index of calcite ($SI_{calcite}$) as a function of pH variation. The samples were collected from Scott Hollow Cave in West Virginia (Davis 1999) and showed a pH range from 7.18 to 7.78. For ease of visual comparison, measured pH was normalized to zero for this plot. A relative pH of zero indicates measured pH values as obtained in the field. Sample 1 was collected from Craig's Creek on February 21, 1998. Sample 2 was collected on May 17, 1998 from Craig's Creek, and Sample 3 was collected from John's Flowstone on February 21, 1998. This graph illustrates how small variations in measured field pH could result in misinterpretation regarding the degree of saturation of calcite in any particular sample.

There are many meters on the market, with varying sizes and features (Table 2). There is a rough correlation between accuracy and cost. For most geochemical studies, a meter with accuracy on the order of 0.01 pH units is desirable. More expensive models may offer additional features such as 3-point calibration, storage of measurements, download capabilities, etc. While these may be sought-after for specific applications, they are not crucial.

Features that are most essential are

1. Automatic temperature compensation. pH is highly temperature dependent, and it is essential that a correction be made either manually or automatically. This dependence is apparent on charts provided with the buffer solutions. As an example, pH "7" buffer has a value of 7.06 at 10 degrees C, and a pH of 6.99 at 30 degrees C.
2. Minimum 2 point auto calibration with slope and offset. Meters/electrodes require calibration to assure accuracy. Some of the less expensive models use only 1 point calibration.
3. Automatic buffer recognition, with temperature compensation. The values of pH buffer solutions vary with temperature, and this must be accounted for during calibration. A meter that includes the tables of the various buffer values is advantageous.
4. Compact, water-resistant, and robust meter design. The nature of karst environments dictates that the instrument should be compact and easy to carry, while at the same time able to withstand rough handling. Waterproofing is ideal, but such meters are less common. Water resistance is useful for brief, accidental water contact.
5. Combination electrodes with a plastic (resin) body. Glass body electrodes have some good characteristics, but are too easily broken in the field. For this reason plastic body electrodes are recommended. A combination electrode includes both the reference and measurement electrode in one piece, minimizing weight and the number of cables needed. The electrode should include some sort of tip protector (bulb shield) for use while measuring and for transport. Note that electrodes have a limited life, whether being used or stored on the shelf. While in service and carefully maintained, a 1- to 2-year life may be expected. Do not order a replacement until it is needed.

FIELD MEASUREMENTS

Our discussion emphasizes the most common conditions for pH measurement in karst studies, manual collection of data at cave or surface streams, or springs. Determination of pH is usually accomplished at the same time as measurement of other field parameters (such as conductance and dissolved oxygen), and the collection of water samples. pH measurement

Table 3. List of suggested materials for field measurement of pH in karst investigations. Electrode filling solution may be needed for certain electrodes. For many electrodes, pH 7 buffer may be temporarily used in place of electrode storage solution. The manufacturer can provide information on this.

Meter and spare batteries
Electrode, with bulb protectors
Temperature probe
Two 100 mL HDPE beakers
100 mL LDPE wash bottle
Deionized water, volume as required
Fresh pH buffers, in 60 mL wide mouth HDPE bottles
Lint free wipes
Field notebook & pencils
Lightweight padded case to hold meter, etc.
Copy of instruction manual
Laminated instruction sheet
Electrode storage solution[a]
Electrode filling solution[a]

[a] Optional items.

can regularly be accomplished in 15 minutes or so, although electrode equilibration may take longer in waters with low total dissolved solids (TDS).

PREPARATION

To assure that the best quality data are collected, preparation should be made before arriving in the field. Table 3 lists the suggested instruments and supplies for undertaking pH measurements. It is critical that any person who will be making the measurements becomes familiar with the process by reading the owner's manual and conducting trial measurements in the lab just beforehand. This also serves as a check that the instrument is working. A photocopy of the manual (perhaps photoreduced) should be brought into the field in case questions arise. If a condensed instruction sheet can be laminated, it is also very useful.

The meter, electrodes, and buffers should be packed in a padded container for protection. Water-proof hardshell cases such as those made by Pelican™ may be desirable, but are a bit heavy. A lightly padded soft case such as that shown in Figure 2 may be sufficient. If waterproofing is required for transit, a kayaker's "dry bag" may be employed. Electrodes can be destroyed by subzero temperatures. If there is a chance of freezing, then precautions must be taken during transport. This can be done by carrying the electrode within a cooler, or in an inside coat pocket. The bulb of the electrode should always be protected and kept immersed in electrode storage solution. If the bulb dries out or is scratched, the electrode may be rendered unusable.

Figure 2. Example of a kit for field pH measurement in karst settings.

MEASUREMENT PROCEDURE

Care should be taken to keep the equipment clean. If there is a level place near the water source, a cloth may be placed down to work on, or work may be done in the carrying case. A 2-point calibration (according to manufacturer instructions) should be conducted. Buffers should be chosen to bracket the expected pH of the sample. Most karst waters are in the pH 7 to 8 range, although some karst streams contaminated with mine drainage may be as low as 3.3 (Sasowsky & White, 1993, Webb & Sasowsky, 1994). The automatic temperature compensation (ATC) probe must also be placed in the buffer. The electrode and ATC probe should be rinsed with de-ionized water and shaken dry between using different buffers, and the glass bulb at the end of the electrode should be protected to avoid any chance of scratching. Some workers immerse the closed bottles of buffer in the water body being sampled so that the buffers will be at the same temperature as the water being measured. This is not a practice that we have followed, because the ATC probe and meter software are designed to compensate for this, and several informal field comparisons that we have made have not generated any difference in readings. Calibration is time-consuming, so some researchers calibrate only once at the beginning of a day, and use this for the remainder. In our experience, this usually provides good

Table 4. Summary of salient procedures for accurate pH measurement in karst settings.

Do	Do Not
Measure pH in the field	Use old electrodes
Use a good quality meter & electrode that are accurate to 2 decimal places	Use old buffer solutions
Allow readings to stabilize	Allow electrode to freeze
Calibrate meter frequently	Measure in moving water
Measure to 2 decimal places and record temperatures	Let the electrode bulb dry out

results. However, the highest data reliability will be obtained by calibration at each measurement location.

When calibration is complete, a sample of the water should be collected in a plastic beaker, and the electrode and ATC probe (rinsed with DI water) placed in the beaker. Use of a beaker is vital for two reasons. First, an accurate reading is not possible in moving water, due to generation of a streaming potential. Second, it is usually difficult to safely hold an electrode in a natural water body. It is also useful to have a second beaker available for temporarily holding the electrode.

Once the electrode is placed in the sample, the meter will usually give a pH reading within one minute, but this should not be considered accurate (even if the display gives a "locked" value). The electrode and probe should remain in the sample water for 5 minutes or so, until the meter readings stabilize. This allows the electrode to come into thermal and ionic equilibrium with the water being measured. After the value has stabilized, a fresh sample should be collected, and measurement should be made again. If there is still drift during measurement, an additional fresh sample should be collected until the reading has stabilized. Drift during pH measurement of karst waters is common, and is caused by three factors: degassing of CO_2, thermal disequilibrium between electrodes and water, and the relatively low ionic strength of the solution (response time for many pH electrodes is inversely proportional to the ionic strength). It is important to be patient, because drift on the order of 0.50 pH units is not unusual. Other tasks, such as collection of samples for elemental analysis, may be done while waiting. Final measurement should always be made on a fresh sample, because of potential ambient effects (warming, degassing, etc.).

When the final measurement has been made, it is recorded in the field book along with the water temperature. The meter is then turned off, and the electrode is packaged to keep safe and moistened.

MEASUREMENT IN SPECIAL INSTANCES

The majority of karst water samples are collected from streams and springs, and pH may be effectively measured using the procedures and equipment listed above. There are, however, several special instances which arise in karst studies, where different approaches and instruments may be needed. These include dripwater sampling, measurement of pH in wells, and situations where continuous monitoring is required.

The primary difficulty with dripwater sampling is lack of adequate sample size for usual measurement methods. Whereas in a cave stream a 100 mL sample can be collected instantaneously, a dripping stalactite might require over 24 hours to provide such volume. This delay would make it impossible to accurately measure pH using standard instrumentation, because the sample would not be fresh when measurement occurred. Using a specially designed, small-volume, pH instrument can circumvent this. Once such unit is the "Cardy" model (Cole-Parmer) which requires only 0.1 mL of sample. An unfortunate limitation is that it is only accurate to 0.1 pH unit.

Measurement of pH in well waters can be conducted either by bringing a sample to the surface and measuring as described in the previous section, or by using an instrument that measures *in situ*. Samples can be brought out of the well using a bailer or a pump. Continual slow pumping will probably provide the most representative sample. If depth-specific samples are desired, a Kemmerer sample vessel (or similar) may be employed. In each case some disturbance of the water chemistry may occur. *In situ* measurement can be made by using a pH electrode with long cable (hard to find), or by deploying a continuous monitoring device with a data logger. The latter option is discussed below.

In situations where a detailed, long term record of pH is needed, it may be desirable to install a continuous monitoring device/remote probe. These automated instruments have mainly seen use for flooding and storm pulse studies, where such detail is required. If there is a malfunction, data may be lost. This can be caused by electrical problems, electrode drift, or biofouling. The systems offer the benefit of multiple, closely-spaced readings, but drawbacks such as expense, lower accuracy, and maintenance make their use limited. One such device is the Yellow Springs Instruments (YSI, Inc.) 6 Series, which measures many different parameters. It has a resolution of 0.01 pH units, but an accuracy of only 0.2.

CONCLUSIONS

pH is a critical value to measure accurately, because H+ participates in many geologically important reactions. Since pH is a logarithmic scale, small changes in measured values correspond to large changes in hydrogen ion activity. pH must be measured in the field. The approaches we present are based upon our experience, and can be used in addition to information available from manufacturers and other published sources. Table 4 summarizes the most critical points. Accurate determination requires forethought and careful field work, especially given the challenges of karst environments.

ACKNOWLEDGEMENTS

We thank William B. White for introducing Ira Sasowsky to the use of geochemistry for the solution of geologic problems. We acknowledge the help of the following individuals and organizations: Katie McKibben (Ohio EPA NWDO); Annabelle Foos and Rebecca Bixby (University of Akron). Heather Raymond, Carol Wicks, and an anonymous reviewer provided thoughtful comments. The research for this publication was financed in part through a grant from the Ohio Environmental Protection Agency and the United States Environmental Protection Agency, under the provisions of Section 319(h) of the Clean Water Act (01(h)EPA-11). The use of trade names is for example only and does not reflect the endorsement of the authors or any associated agencies.

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PERSISTENT COLIFORM CONTAMINATION IN LECHUGUILLA CAVE POOLS
Response: Barton and Pace Discussion

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Barton and Pace (2005) criticized the results of Hunter *et al.* (2004) in terms of (1) the accuracy of our results suggesting the presence of fecal contamination, and in particular, *E. coli*, in pools in Lechuguilla Cave and (2) the assertion that *E. coli* can persist in the environment. In this response we clarify certain methodologies, further address the issue of persistence of *E. coli* in the environment, and present additional tests that confirm the presence of *E. coli* in previous samples from 2004.

The persistence of *E. coli* and other coliforms in natural systems is a complex issue (e.g., Szewzyk *et al.*, 2000; Byrd & Colwell, 1993). Since the Hunter *et al.* (2004) manuscript was accepted for publication in the *Journal of Cave and Karst Studies*, we have delved further into the literature of coliform persistence in other environments and conducted additional experiments to try to confirm or rule out our previous results. We discuss those here and recap our previous efforts.

We too have been surprised by the apparent persistence of organisms normally requiring high organic nutrient levels in an oligotrophic cave environment. Thus, we have kept an eye on the Red Lake area for a number of years. While some of our tests have been presumptive, they have consistently shown the presence of coliforms and in some instances *E. coli* over the course of six years from 1999 through our most recent samples taken in December 2004. The persistence of high nutrient-requiring coliforms in a low nutrient cave environment over relatively long periods of time can be explained by three possibilities: 1) a surface source of coliform input is reaching the Red Lake area through fracture percolation, 2) reinfection through human visitation in spite of official closure of the area, or 3) the coliforms are actually persisting in the environment and not dying off as one might initially expect. We believe that to make the case for the first possibility would be difficult. If the cave underlies heavy human or mammal population areas then it might be plausible, but the low annual volume of surface precipitation input (especially severe over the past seven years of drought) and the low mammal and other vertebrate density common to the desert environment above Lechuguilla Cave conspire to make this an implausible scenario. The second possibility of illicit visits is beyond our ability to determine and is a matter for NPS personnel. We like to believe that this is not happening. The third possibility appears to us as the most plausible; that is, that our collective ignorance in the microbiological community about the real lives of organisms in nature means we have a drastically incomplete picture of them. They may well be capable of things that our imaginations have not yet grasped.

To summarize our prior observations, presumptive *E. coli* was detected in Red Lake during the January 1999 trip by Boston much to her surprise (Boston, 1999). Additionally, we assayed for the presumptive presence of *E. coli* and other coliforms in the soils of Huapache Camp near Red Lake (1994–1995), and on the trails leading to Red Lake during a 2000 trip (Northup *et al.*, 1997; Northup *et al.*, 2000). The confirmed *E. coli* contamination present in the soils and small pools adjacent to Red Lake is indicative of fecal contamination (DOH, 2004). These tests were originally conducted because of an outbreak of digestive tract illness in a caving party that had drunk water from a siphon hose and spigot at the Red Lake pool.

In the paper under discussion here, drinking water pools were first tested using the positive/negative TC-5 coliform indicator kits (Hunter *et al.*, 2004). Pools that tested positive were then re-tested for total coliforms using U.S. Geological Survey water sampling protocols in the National Field Manual for the Collection of Water-Quality Data (Webb *et al.*, 1998). Total coliforms were quantified using the membrane filtration technique, an accepted and approved technique by hydrologists and microbiologists for total coliform sampling (Webb *et al.*, 1998). *E. coli* was not tested in drinking water resources during the 2000 or 2001 trips (Hunter *et al.*, 2004, Table 2).

In December of 2004, samples were collected at Red Lake once again. We used clinical Chromagar™ of various types and a series of dilution plates to get a better handle on any presence of coliforms, especially *E. coli* (Alonso *et al.*, 1996). Chromagar™ media utilize the principle of species-indicative enzymes. Cleavage of various substrates and reaction with specific dyes yields diagnostically colored colonies. For example, the enzyme ßgalactosidase releases a dye that produces pink colonies in the presence of *E. coli*, whereas another chromogen is targeted towards ßglucosidase releasing a blue dye that indicates *Enterococcus* species. Such chromogenic media are revolutionizing the screening and detection in water quality and the public health field. The Becton Dickinson Company even claims that no further tests are required for a very high confidence detection rate of many organisms including *E. coli* and enterococci and minimal false positives. However, we did follow up on all putative *E. coli* tests with the spot indole test (Miller & Wright, 1982) and a gram stain. Chromagar™ *E. coli* yielded positives on three of seven samples. Chromagar™ EEC yielded positive *E. coli* tests on the same three samples, and positive for other coliforms on six of the seven samples. Chromagar™ Orientation, which differentiates between coliforms and other typical urinary tract and scar pathogens, yielded four positives for *E. coli*, five positives for *Enterococcus*, and one positive for *Pseudomonas.* Chromagar™ O157 fortunately yielded no positives for this enterohaemorrhagic strain of *E. coli* (Bettelheim, 1998). These were presence/absence tests, not intended to quantify the number of bacteria present in the water column and surfaces. All samples were also grown on EMB (Eosin-Methylene Blue) Agar. The same four samples that had yielded *E. coli* positives on various Chromagar media also developed the characteristic metallic green sheen byproduct of glucose metabolism that interacts with the medium dyes and indicates *E. coli* on many of the colonies. The repeated confirmation of *E. coli* and other coliform presence by a number of different presumptive tests has increased our confidence that we are really seeing them in the Red Lake samples. Based on these results, we have collected sample colonies from these tests for later DNA analysis that is not yet completed.

When testing drinking water, total coliforms are used to determine water treatment adequacy and distribution system integrity (EPA, 2005). The absence of total coliforms minimizes the likelihood that fecal pathogens are present (EPA, 2005). Thus, total coliforms are used to determine the vulnerability of a system to fecal contamination (EPA, 2005). Drinking-water resources sampled during the 2001 trip (noted in Table 2, Hunter *et al.*, 2004) are positive for total coliforms and are therefore vulnerable to fecal contamination (EPA, 2005).

The Total Coliform Rule (published 29 June 1989/effective 31 December 1990) set both EPA Maximum Contaminant Level Goals (MCLGs) for health and Maximum Contaminant Levels (MCLs) as legal limits for total coliform levels in drinking water (EPA, 2005). The EPA MCL for coliform bacteria in drinking water is zero (or no) total coliform per 100 ml of water (EPA, 2005). There have been waterborne-disease outbreaks in which researchers have found very low levels of coliforms, suggesting that any level indicates some health risk (EPA, 2005). Given the positive indication of total coliforms using two separate tests (TC-5 Total Coliform, Membrane Filtration) on three different occasions (Hunter *et al.*, 2004, Table 2), park service management recommendations were made to limit access to the water resources in question if the coliforms persisted. Further management recommendations included the following: 1) Quantify the number of coliforms in all pools routinely, 2) identify the major sources of coliforms (i.e. surface infiltration, dirty boots, hands, etc.), 3) measure total and dissolved organic carbon present in pools with/without biofilms, 4) identify dominant species present within siphoning hose biofilms using molecular methods (Hunter, 2001).

The MPN tests carried out by Boston only identified coliforms within Red Lake pool during 1999 (Hunter *et al.*, 2004, Tables 1 and 2). As noted in (Hunter *et al.*, 2004, Tables 1 and 2), "ND" stands for the convention of Not Determined (Hunter *et al.*, 2004).

Regarding the biofilm experiment: The same loop size was used for each "loop-full" of *E. coli* starter culture. Any variability in original numbers of *E. coli* organisms added would have also been reflected in the control (as described in the methods for the coliform growth preference experiment in Hunter *et al.*, 2004). Any variance in initial quantity of *E. coli* cells added was also taken into account with the triplicate vials and triplicate plating for each of those vials using 1:100,00 serial dilutions, the results of which were averaged to produce the line graph in (Hunter *et al.*, 2004, Fig. 5 and 6). Colony count data from the *E. coli/Hyphomicrobium*/biofilm experiment were analyzed using the analysis of variance (ANOVA) procedure in the SAS software release 6.12 (SAS Institute, Cary, NC, USA). Signficant differences among treatments were detected (P = 0.0001). This experiment emphasized *E. coli* growth preference in medium containing biofilm over growth in medium containing cultured *Hyphomicrobium*-like organisms.

Persistence, as noted in the article title, references total coliform contamination. For purposes of this study, *E. coli* was not specifically tested for during the 2000 and 2001 sampling trips. *E. coli* was used in the lab "coliform growth preference" experiment, however, to determine if *E. coli* had a preference towards biofilm that would potentially be of interest if the positive total coliform results of this study were followed up by more specific *E. coli* field tests in the future. Regardless, the soils and Red Lake water tested prior to the 2000 and 2001 sampling were positive for *E. coli.* Additionally, total coliforms were repeatedly tested and positively identified during that two-year period.

Total coliforms represent a health risk, and if they are still present, then additional testing identifying the source should be done. Precautions should still be taken by those using the above mentioned water resources.

The matter of persistence in the environment of human-associated bacteria including *E. coli* and other coliforms is of both academic and practical management interest. In a seminal paper, Byrd and Colwell (1993) reported *E. coli* persistence over long periods of time (more than three years) in a starved state in artificial seawater while retaining both culturability and the viability of their indigenous plasmids. In another study, Barcina *et al.* (1997) concluded that besides nutrient scarcity, the most negative factors on survival of allochthonous bacteria (i.e. those introduced from elsewhere) in aquatic surface systems were temperature, osmotic stress, visible light, and grazing by protozoa. We note that caves are thermally quite stable, moist, and dark places without either visible light or particularly ultraviolet radiation sources. We have seen no evidence of significant protist activity in any samples from pool waters (Hobbs, unpublished data), wall rock, or other materials in Lechuguilla Cave. A temperature study of *E. coli* cells starved for carbon and/or nitrogen showed that their temperature optima departed from the usual 37°C (~ human body temperature) and survivability of starved cells was greater at 20°C (Nelson *et al*., 1996). This metabolic downshifting indicates that such cells radically adjust their so-called "normal" behavior to meet environmental exigencies.

Interestingly, protection by biofilm has been reported for *E coli* and other pathogens. For example, Camper *et al*. (1985) have shown colonization of biofilms developed from tap water organisms by *E. coli*. Momba & colleagues (1999) have suggested that biofilms may provide significant protection for introduced pathogens in groundwater systems. We have suggested that the slimy biofilms found on introduced siphon hoses in Red Lake might be helping the survival and recoverability of viable *E. coli* and other coliforms. If this proves to be a factor, then it will have major management implications for how we should obtain water from cave pools without changing their inherent microbiology.

In conclusion, we believe that the accumulated evidence of human-associated coliforms in the Red Lake over the course of a number of years of observation warrants further monitoring. In addition, more refined attempts to determine whether we are seeing unusual persistence in these organisms or the result of subsequent reintroductions by human carriers requires additional investigations.

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PERSISTENT COLIFORM CONTAMINATION IN LECHUGUILLA CAVE POOLS

Response: Davis Forum

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Rationale for recommendations limiting certain drinking water resources and research study site access is tri-fold.

1) The level of total coliform is beyond the acceptable level (0 colony forming units/100 ml) recommended by the EPA (EPA, 2005). Iodine tablets have proven successful with killing bacteria and viruses in water over time; however, consuming more than 0.5 to 1 ppm per day (1 liter of water correctly diluted equals ~1.19 ppm iodine concentration) of residual iodine is not recommended, especially for those who are iodine deficient or have known thyroid problems (Singer, 1995). Boiling the water or using a water filter would not be the lightest solution (given the added gas and filter weight), but it would be the safest.
2) On a world scale, more than 90% of species diversity is found in the microbial world — hence, micro-organisms are tremendously important in the context of biodiversity (De Poorter, 1998). Introducing a non-native organism into a new environment can lead to disturbance of the native micro-flora and the extirpation of viable microbes.
3) Costly and time-consuming microbial longevity studies have been in place since the early 1990s by numerous researchers. An influx of coliform bacteria to these water-resource sites may well disturb the native communities currently being studied. Unless researchers and explorers can develop methods to continue exploration without disturbing the microflora, then these areas should remain off limits until at least preliminary microbiological studies have been completed.

The campsite at Red Lake was closed due to coliform contamination in the soils, on surfaces, and in small pools around Red Lake itself including the outer and inner surfaces of the siphon hosing and the spigot (Boston, 1999; Northup *et al.*, 1997; Northup *et al.*, 2000). Continuing to camp at this site, while coliforms are still apparently present, will only further spread the organisms onto the trails and further into the cave via cavers' boots. We fully expected this to be a very short duration closure, based on the common notion that coliforms will die out very quickly in low nutrient environments. We were very surprised that this did not occur. Hair, lint, sweat and dirt from clothing or boots can all add organics to water resources, and this organic source may have helped the organisms to remain longer. When it is necessary to obtain water, via a pitcher directly from the poolside (i.e. Big Lake and Lake Louise), clean (preferably sterile) rubber gloves and boot covers should be used to help prevent further contamination. When water is obtained from a siphoning hose via a spigot, clean gloves should be worn and precaution should be taken not to touch the nozzle directly. Contaminants and introduced organisms from dirty hands could migrate up the hoses creating 1) slime-coated hoses and 2) pool contamination.

Crossing unexplored water leads with minimal contamination would involve having explorers strip off their clothing, bathe in anti-bacterial wipes (including helmet/light), remove boots/socks and put on clean slippers (rubber soles that can also be wiped), then inflating a clean rubber raft (applying anti-bacterial wipes or solutions to the bottom) and getting into it without touching the water. Paddles would need to be cleaned and wiped with anti-bacterial wipes and all gear/clothes placed into a large plastic garbage bag and transported in the raft. Research slides hanging in the pools would need to be left undisturbed with minimal water turbulence.

As an explorer (Hunter, 2005), I understand the frustrations when an obstacle impedes forward movement into new leads. However, the cave will always be there, but once opened to exploration, if not approached very carefully, the organisms we wish to study may not be.

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ABSTRACT

Groundwater is a significant component of the world's water balance and accounts for >90 % of usable freshwater. Around the world groundwater is an important source of water for major cities, towns, industries, agriculture and forestry. Groundwater plays a role in the ecological processes and "health" of many surface ecosystems, and is the critical habitat for subterranean aquatic animals (stygofauna). Over-abstraction or contamination of groundwater resources may imperil the survival of stygofauna and other groundwater-dependent ecosystems (GDEs). In two karst areas in Western Australia (Yanchep and Leeuwin-Naturaliste Ridge), rich stygofauna communities occur in cave waters containing submerged tree roots. These aquatic root mat communities were listed as critically endangered because of declining groundwater levels, presumably caused by lower rainfall, groundwater abstraction, and/or forest plantations. Investigation of the hydrology and ecology of the cave systems was considered essential for the conservation and recovery of these threatened ecological communities (TECs). This thesis investigated the hydrology and ecology of one of the TECs, located in the Jewel Cave karst system in the Leeuwin-Naturaliste Ridge. A multi-disciplinary approach was used to explore aspects pertinent to the hydrology and ecology of the groundwater system.

Thermoluminescence dating of the limestone suggested that development of the karst system dates from the Early Pleistocene and that caves have been available for colonization by groundwater-fauna since that time. Speleogenesis of the watertable maze caves occurred in a flank margin setting during earlier periods of wetter climate and/or elevated base levels. Field mapping and leveling were used to determine hydrologic relationships between caves and the boundaries of the karst aquifer. Monitoring of groundwater levels was undertaken to characterize the conditions of recharge, storage, flow and discharge. A hydrogeologic model of the karst system was developed.

The groundwater hydrograph for the last 50 years was reconstructed from old photographs and records while radiometric dating and leveling of stratigraphic horizons enabled reconstruction of a history of watertable fluctuations spanning the Holocene to Late Pleistocene. The watertable fluctuations over the previous 50 years did not exceed the range of fluctuations experienced in the Quaternary history, including a period 11,000 to 13,000 years ago when the watertable was lower than the present level.

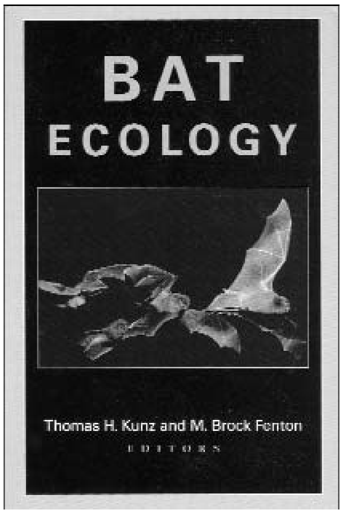
The recent groundwater decline in Jewel Cave was not reflected in the annual rainfall trend, which was above average during the period (1976 to 1988) when the major drop in water levels occurred. Groundwater abstraction and tree plantations in nearby catchments have not contributed to the groundwater decline as previously suggested. The period of major watertable decline coincided with a substantial reduction in fire frequency within the karst catchment. The resultant increase in understorey vegetation and ground litter may have contributed to a reduction in groundwater recharge, through increased evapotranspiration and interception of rainfall. To better understand the relationships between rainfall, vegetation and fire and their effects on groundwater recharge, an experiment is proposed that involves a prescribed burn of the cave catchment with before-after monitoring of rainfall, leaf-area, ground litter, soil moisture, vadose infiltration and groundwater levels.

Molecular genetic techniques (allozyme electrophoresis and mitochondrial DNA) were used to assess the species and population boundaries of two genera and species of cave dwelling Amphipoda. Populations of both species were largely panmictic which was consistent with the hydrogeologic model. The molecular data supported the conclusion that both species of amphipod have survived lower watertable levels experienced in the caves during the Late Pleistocene. A mechanism for the colonization and isolation of populations in caves is proposed.

Multi Dimensional Scaling was used to investigate patterns in groundwater biodiversity including species diversity, species assemblages, habitat associations and biogeography. Faunal patterns were related to abiotic environmental parameters. Investigation of hydrochemistry and water quality characterized the ecological water requirements (EWR) of the TEC and established a baseline against which to evaluate potential impacts such as groundwater pollution.

The conservation status of the listed TEC was significantly improved by increasing the number of known occurrences and distribution range of the community (from 10 m^2 to > 2 x 10^6 m^2), and by showing that earlier perceived threatening processes (rainfall decline, groundwater pumping, tree plantations) were either ameliorated or inoperative within this catchment. The GDE in the Jewel Cave karst system may not have been endangered by the major phase of watertable decline experienced 1975–1987, or by the relatively stable level experienced up until 2000. However, if the present trend of declining rainfall in southwest Western Australia continues, and the cave watertable declines > 0.5 m below the present level, then the GDE may become more vulnerable to extinction.

The occurrence and distribution of aquatic root mat communities and related groundwater fauna in other karst catchments in the Leeuwin-Naturaliste Ridge is substantially greater than previously thought; however, some of these are predicted to be threatened by groundwater pumping and pollution associated with increasing urban and rural developments. The taxonomy of most stygofauna taxa and the distribution of root mat communities is too poorly known to enable proper assessment of their conservation requirements. A regional-scale survey of stygofauna in southwest Western Australia is required to address this problem. In the interim, conservation actions for the listed TECs need to be focused at the most appropriate spatial scale, which is the karst drainage system and catchment area. Conservation of GDEs in Western Australia will benefit from understanding and integration with abiotic groundwater system processes, especially hydrogeologic and geomorphic processes.



Bat Ecology

Thomas H. Kunz and M. Brock Fenton, eds. The University of Chicago Press, 798 p., 19 halftones, 112 line drawings, 28 tables, 6 x 9 inches. Available from http://www.press.uchicago.edu/cgi bin/hfs.cgi/00/15369.ctl. ISBN 0-226-46206-4 (hardbound), $55.00. ISBN 0 226 46207 2 (softbound) available fall 2005, $35.00.

Bats are the second-largest group of mammals, with approximately 1100 species currently known and new species described at the rate of several per year. Overall, the information about their life history is small in comparison to other orders. But the level of interest and rate of bat research has greatly accelerated in the last decade or so and continues to grow. Bat Ecology nicely summarizes much of the current state of knowledge and points out many of the areas in which we are still deficient.

This hefty (>1kg) book consists of 15 chapters by 30 contributing authors, divided into 3 sections.

Part 1, Life History and Social Biology, starts with "Ecology of Cavity and Foliage Roosting Bats" by Tom Kunz and Linda Lumsden, emphasizing natural roosts such as tree cavities, loose bark, bird nests, leaf structures, open foliage, termite and ant nests, and so on. Next, John Altringham and Brock Fenton discuss "Sensory Ecology and Communication in the Chiroptera". This is a rapidly growing field, as we learn more about the auditory, olfactory, visual, and contact cues used by bats. Gerry Wilkinson and Gary McCracken follow with one of the best titles in the book, "Bats and Balls: Sexual Selection and Sperm Competition in Chiroptera", which is just what it sounds like. In "Ecology of Bat Migration," Ted Fleming and Peggy Eby explain the types, advantages, and consequences of migration and concisely summarize the similarities and differences in migration between bats and birds. The last topic in this section, "Life Histories of Bats: Life in the Slow Lane," by Robert Barclay and Lawrence Harder, contains an analysis of a number of reproductive, developmental, and longevity factors of numerous bat families in comparison to other similarly sized insectivores (shrews), with speculation on their evolutionary ecology.

Part 2, Functional Ecology, begins with a chapter by Sharon Swartz, Patricia Freeman, and Elizabeth Stockwell entitled "Ecomorphology of Bats: Comparative and Experimental Approaches Relating Structural Design to Ecology." Analyses of body mass, skull shape, etc., show that form does indeed follow function. Gareth Jones and Jens Rydell follow with "Attack and Defense: Interactions between Echolocating Bats and their Prey," another look at echolocation, prey selection, foraging strategies, capture success, and the various insect strategies to avoid being eaten. In line with the feeding theme, Otto von Halverson and York Winter look at food resources and energy costs in "Glossophagine Bats and Their Flowers: Costs and Benefits for Plants and Pollinators." Betsy Dumont supplements that chapter nicely with "Bats and Fruit: an Ecomorphological Approach." She covers fruit distribution and abundance and how bats partition and process the fruit. "Physiological Ecology and Energetics of Bats" by John Speakman and Don Thomas follows. The thermodynamic equations may turn off casual readers, but, in summary, interactions between body temperature and ambient temperature and associated energy budgets control everything from torpor to reproduction to flight.

Part 3, Macroecology, opens with a detailed chapter by Nancy Simmons and Tenley Conway on "Evolution and Ecological Diversity in Bats," which traces the fossil history of bats, the evolution of flight and echolocation, feeding specializations, and diversification of body size. "Trophic Strategies, Niche Partitioning, and Patterns of Ecological Organization," by Bruce Patterson, Michael Willig, and Richard Stevens, delves deeply into resource partitioning, and taught me a new word: animalivory (what most of us would call carnivory). The same authors also contribute "Patterns of Range Size, Richness, and Body Size in Chiroptera." Sharon Messenger, Chuck Rupprecht, and Jean Smith offer "Bats, Emerging Virus Infections, and the Rabies Paradigm," which covers one of the most commonly overlooked causes of bat mortality, disease. They also address how the way we perceive and prevent known diseases (such as rabies) and emerging diseases may impact our conservation efforts. The final chapter, "Conservation Ecology of Bats" by Paul Racey and Abigail Entwistle, discusses the need for additional knowledge of current status, threats, ecological requirements, and development of conservation approaches for the world's bats.

I had mixed feelings about reviewing this book. While I know many of the authors personally, I still found several chapters difficult to understand because I have little background in those particular disciplines, even with more than 20 years of experience working with bats. However the last chapter, "Conservation Ecology," should be read by every caver who thinks he knows something about bats. One of the best features of the book is the inclusion of suggestions for future research. Other selling points are that the book is well edited, the photos and figures are clear, and the literature cited is extremely thorough, totaling some 168 pages. The normal Subject Index is supplemented by contributors' contact information, a Species Index, and an Author Index. On the down side, I had my copy less than a week before the binding tore at the back cover. For $55, one expects it to last a little longer.

Another weakness, at least from a caver's perspective, is the failure to include almost any material on cave roosts and cave roosting ecology. The largest section devoted to caves, 8 pages, is in the section on rabies. Another half page, in the Conservation Ecology chapter, points out the importance and fragility of caves. I realize that the majority of bat species are not cave dwellers, but not to include caves (or artificial roosts) in a chapter similar to the one for cavity and foliage roosting bats seems a disservice. Perhaps no prominent author was available to write it.

This book is definitely not for the average bat lover, but it is an invaluable reference for more involved researchers . It is not a picture book, not a field guide, and doesn't tell you how to capture or study bats. But there is a wealth of information for those who can wade through the jargon. If one wants to start a scholarly bat library, this book belongs right next to Kunz's earlier *Ecology of Bats* (1982), John Hill and James Smith's *Bats – A Natural History* (1984), Paul Racey and Susan Swift's *Ecology, Evolution, and Behaviour of Bats* (1995), John Altringham's *Bats – Biology and Behaviour* (1996), Kunz and Racey's *Bat Biology and Conservation* (1998), and the all-too-rare *Biology of Bats*, edited by William Wimsatt (1970).

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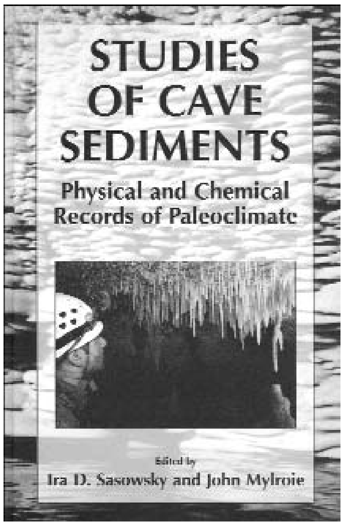

STUDIES OF CAVE SEDIMENTS
Physical and Chemical Records of Paleoclimate
Edited by
Ira D. Sasowsky and John Mylroie

Studies of Cave Sediments: Physical and Chemical Records of Paleoclimate

Ira D. Sasowsky and John Mylroie, eds., 2004. New York, Kluwer Academic/Plenum Publishers, 329 p. ISBN 0-306-47827-7, hardbound, 6.4 x 9.5 inches, $155. Available on-line at http://www.springeronline.com.

This book has the stated goal of demonstrating to the scientific community the utility of cave deposits and the sophistication of current studies in the field. It is based mainly on papers presented at a symposium at a national meeting of the Geological Society of America. Paleoclimate is the theme woven throughout this book. The two major kinds of cave deposits, detrital and chemical, apply to paleoclimates in different ways. Climatic evidence from detrital sediments is indirect, as it is based mainly on interpretations of past flow conditions and grain petrology. Carbonate speleothems give a more explicit record of temperature and vegetation cover with time.

The 17 chapters in this book are divided almost equally between the two topics, but the combined length of those on chemical precipitates is about 1.5 times longer. Some of the chapters on detrital sediments do not specifically address climate, but they are solid complements to those that do. There are three kinds of chapters: descriptions of new projects, updates of older work, and broad syntheses inspired by the symposium topic. This book contains a good balance of all three. Illustrations are clear and well reproduced. Photos and diagrams include a mix of color and black-and-white.

In the book's preface, the editors give a good introduction to the subject and the papers in the volume, and they also throw their gauntlet into the ring to defend karst studies against the agnostic hordes. The chapters on detrital sediments include the following: Rachel Bosch and William White describe through several case studies the variety of transport phenomena and sediment facies in karst aquifers. Barbara Mahler and coauthors use data from the Edwards Aquifer of Texas to demonstrate the mobility of sediment and that up to 90% of bacterial contamination is associated with surface-derived sediment. R.J. Musgrave and J.A. Webb use paleomagnetic analysis to show the great antiquity (late Tertiary) of certain Australian caves compared to those in other more tectonically active continents. Ira Sasowsky and coauthors apply paleomagnetics and stratigraphic data to sediments in Kooken Cave, Pennsylvania, to illustrate how rapidly thick sediments can accumulate in a flood-prone cave. Using clay mineralogy, Leo Lynch and coauthors trace the detrital sediment in Barton Spring, Texas, mainly to the surface catchment areas up to tens of kilometers away. Elizabeth Knapp and coauthors show that sediments in certain Virginia caves provide a record that of warm/wet and cold/dry climates. France Šušteršič gives examples from Slovenia of how sediments can be used to recognize ancient unroofed caves and interpret their original catchment areas.

The chapters on speleothems begin with a broad but detailed overview of the topic by William White. This chapter provides a convenient reference for the more specific contributions that follow. Jeffrey Dorale and coauthors describe the techniques and constraints involved in U-series speleothem dating. Russell Harmon and coauthors discuss the use of stable isotopes in speleothems to obtain paleoclimate data, as well as the uncertainties in the method. Peter Kolesar and Alan Riggs show how the various depositional facies in Devil's Hole, Nevada, relate to their depositional environment. Christopher Spötl and coauthors use data from a cave in the Austrian Alps to contrast the carbon-oxygen isotopic signatures in interglacial deposits with those of cold-climate deposits.

Stein-Erik Lauritzen and Joyce Lundberg use speleothem data from near the Arctic Circle in Norway to decipher the climate and vegetation during the "super-interglacial" of about 500 ka. Steven Turgeon and Joyce Lundberg establish a speleothem chronology in Oregon Caves and correlate it with ice cores, showing the global nature of the signal. Victor Polyak and Necip Güven describe silicate deposition in caves and show that amorphous silica is most common in association with rapidly deposited calcium carbonates, whereas quartz and trioctahedral smectite are products of slower deposition. Finally, Donald McFarlane and Joyce Lundberg describe catastrophic flood deposits in West Indies caves caused by abrupt climate changes during the last interglacial stage. The book's most serious omission is the topic of dating quartz-rich sediments by cosmogenic radionuclides, as the potential authors (limited to one or two) were unavailable at the time of writing.

The dual nature of the subject and the wide range of topics and analytical approaches might give the superficial impression of a heterogeneous group of papers cobbled together into a book. On the contrary, these topics are well integrated into a fairly coherent picture. Even the local studies provide wide-ranging concepts. Although, much of the information is available elsewhere, it is scattered throughout the literature. The only other book that covers comparable ground is the proceedings of a symposium held about 10 years ago in Norway (Lauritzen, 1996). Although it covers similar topics, it consists mainly of short, site-specific papers and has limited distribution.

Paleoclimatology has become such a popular and well-funded field that there are many researchers who know little about caves but are using speleothems to further their research. Let us hope that the Sasowsky-Mylroie book is a step toward broadening their perspective.

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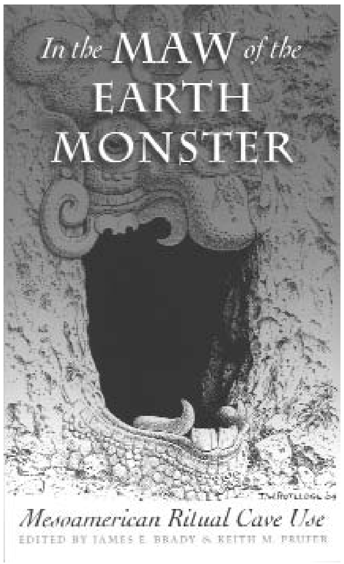

In the Maw of the Earth Monster: Mesoamerican Ritual Cave Use

James E. Brady and Keith M. Prufer, eds., 2005. Austin, TX, The Linda Schele Series in Maya and Pre-Columbian Studies, University of Texas Press, 445 p. First Edition, ISBN 0-292-70586-7, hardcover with dust jacket, 9.25 x 6.125 inches, $60.

This highly anticipated book is the first edited volume specifically about ritual cave use among Mesoamerican people, from Pre-Columbian times to the present. *In the Maw of the Earth Monster* is based on symposia presented at annual meetings of the American Anthropological Association and Society for American Archaeology in the mid-to-late 1990s. The 20 contributors come from various fields including archaeology, linguistics, cultural anthropology, art history and epigraphy. Its multi-disciplinary approach is the strength of this volume.

The book is organized into sections on Central Mexico, Oaxaca and the Maya area. The latter region dominates the book and accounts for more than half the chapters. Chapter 1 presents a brief history of Mesoamerican cave interpretation and summarizes the more significant contributions, including those of J. Eric Thompson, Doris Heyden, Barbara MacLeod and Dennis Puleston, Andrea Stone, and Evon Vogt. The editors point out that many scholars were not aware of works by their colleagues in other regions, and that this lack of collaboration may have hindered scholarly advancement in the field. With the rise of Mesoamerican cave specialists in the late 1980s, scholars became more multidisciplinary and methodical in their approaches to cave research, and as a result "interpretations have tended to be less speculative and more heavily grounded in data." The section on Central Mexico (Part 1) begins with an updated paper by Doris Heyden that was originally published almost 30 years ago but which never received proper recognition because it was written in Spanish. Heyden surveys colonial documents and modern ethnography in relation to creation myths and modern ceremonies and/or rites of passage related to caves. Her data demonstrate that rituals concerning all phases of life from birth to death can be linked to caves.

Alan Sandstrom offers a rare glimpse into a modern cave pilgrimage carried out by Nahuas (Nahuatl speakers) in the Huasteca region of Veracruz, Mexico. He discusses how Nahua beliefs stem from observations of natural phenomena and an understanding of how geographic features such as caves relate to the landscape. Because of the observed link between caves, water, and rain, the pilgrimage petitioned the cave spirits for rain during an unprecedented drought. The importance of caves in the Mesoamerican rain/water complex was underscored by the arduous 12-hour trek to the top of a sacred mountain in weather so hot that several pilgrims fainted.

The last chapter in Part 1 examines a complex of artificial caves at the archaeological site of Acatzingo Viejo, Puebla. The authors, Manuel Aguilar, Miguel Jaen, Tim Tucker, and James Brady, relate their field data to the sixteenth-century map of Cuauhtinchan No. 2 and argue that the caves represent a constructed Chicomoztoc, i.e., a mythical "Place of the Seven Caves." Many indigenous groups in Mexico consider Chicomoztoc to be a sacred place of their origin. Thus, the cave complex at Acatzingo Viejo is part of a long tradition in Central Mexico of constructing sacred spaces, as at Cholula, Teotihuacán, and Xochicalco.

Part 2, on Oaxaca, begins with Janet Fitzsimmons' site report on Blade Cave, which was investigated in the mid-1980s as part of the Huautla Cave Project. Her chapter offers a detailed description of the artifacts discovered in the cave along with numerous illustrations showing their context. Fitzsimmons argues that Blade Cave was a place where elite practitioners conducted rituals for rain and propagation.

In another chapter on Oaxaca, Carlos Rincón Mautner presents an interesting analysis of the Colossal Natural Bridge, a large cave in the northern Mixteca region. Armed with ethnohistorical data, Rincón makes comparisons between art in the cave and images from codices to argue that the cave is actually depicted in the codices from the Coixtlahuaca Basin, and that the cave relates to regional stories of Quetzalcoatl and origin myths.

Part 3, on the Maya region, begins with Evon Vogt and David Stuart's work on ancient glyphs and modern ethnography of Highland Chiapas. The article starts with the first publication of Stuart's deciphering of the glyph CH'EEN as "cave." The authors argue that caves are viewed by both the ancient and contemporary Maya as places of political or communal importance, and that caves provide religious and political identities for many communities. Finally, caves serve as sacred locations where elaborate rituals take place that involve communication with powers of rain, water, and lightning.

Two chapters concern investigations in Belize. Keith Prufer discusses the socio-political roles played by contemporary ritual specialists and relates these to his archaeological investigations in the Maya Mountains. A chapter by Jaime Awe, Cameron Griffith, and Sherry Gibbs documents the erection of stelae and other megalithic monuments in three caves in western Belize. They suggest a pattern of cave stelae/monument placement and argue that they were assembled to demarcate sacred space within the caves.

Two chapters focus on the relationship of artifact distribution in caves to cave morphology and use of sacred space. Andrea Stone believes that by examining the material remains in caves in a spatially based context, we can better understand the order conceptualized by the ancient Maya for use of sacred space. From ethnographic data she suggests that deliberate placement of materials by a spiritual leader was necessary to achieve successful ceremonies, and that therefore the use of space and the distribution of artifacts is non-random. With a similar goal, Holley Moyes used GIS in her spatial analysis of the Main Chamber of Actun Tunichil Muknal in western Belize. Moyes used cluster analysis to examine artifact distribution and suggests a cave-use model derived from a basic quincuncial (four directions with a center) arrangement encircled by boundary markers that delineate sacred space.

Several chapters offer interpretations of contemporary cave use based on direct observations of rituals performed in caves by indigenous practitioners. Abigail Adams and James Brady discuss the Q'eqchi' Maya religion and its relation to the sacred geography of Alta Verapaz, Guatemala. They focus on the archeological context of rituals related to a cave pilgrimage and examine the gender roles surrounding the rituals. Pierre Colas has translated from German perhaps the earliest ethnographic article describing a Maya cave ceremony. Jaroslaw Petryshyn's 1968 account of a pilgrimage to a cave in the Lacandon area is augmented by the editors' notes that provide additional data from other investigators. The article summarizes the little that is known about Lacandon cave ritual.

Rounding out the Maya area are two chapters covering the Yucatan Peninsula. Dominique Rissolo conducted a regional cave survey in the Yalahau region of Quintana Roo, which examined the spatial relationship of caves to surface sites and their sacred roles as places for acquiring resources such as water, speleothems, and white rock. In agreement with Prufer, Rissolo finds that rockshelters were treated as sacred space by the ancient Maya. In the second chapter on Yucatan, Clifford Brown examines karst geomorphology at Mayapan to demonstrate that settlement choices were influenced by the locations of caves and cenotes. Not only was civic and ceremonial architecture arranged with religious significance, but also residential groups with emphasis on family lineages and ancestor worship which were related to caves and cenotes.

The final chapter consists of concluding comments by Prufer and Brady. They note the significance of this volume to Mesoamerican cave studies and how interpretations have changed from speculation to those that are empirically grounded from data on archaeology, ethnography, ethnohistory, and art history.

This book is a valuable contribution, not only because it is the first book published on Mesoamerican cave use, but it also reveals how the field has matured in recent years. It also offers the final contributions of pioneers Evon Vogt, who passed away in 2004, and Doris Heyden, who was incapacitated by a stroke in 1999. The book presents compelling information on central Mexico and Oaxaca, but their under-representation relative to the Maya area demonstrates the need for more investigations in those areas. Finally, while the field has suffered from a lack of the ethnographic data, this volume offers a glimpse at the richness of information available in several ethnographic chapters that provide an unprecedented amount of published material.

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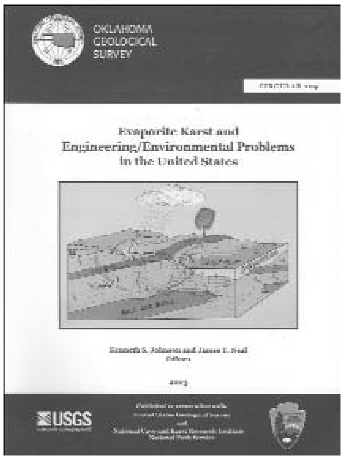

Evaporite Karst and Engineering/Environmental Problems in the United States

Kenneth S. Johnson and James T. Neal, eds., 2003. Oklahoma Geological Survey Circular 109. US Geological Survey and National Cave and Karst Research Institute, National Park Service, 353 p. ISBN 0078-4397, softbound, 8.5 x 11 inches, $20.00 plus $4.00 shipping. Order from Publication Sales, Oklahoma Geological Survey, University of Oklahoma, 100 East Boyd St., Suite N-131, Norman, OK 73019 or on-line at http://www.ogs.ou.edu/pubs.htm.

This is the first book that specifically addresses evaporite karst in the United States. It is based on papers presented at a theme session at the 2002 annual meeting of the Geological Society of America. Its 50 authors include the most authoritative American researchers in evaporite karst. The two editors are among them. It is interesting to note the very little overlap between this group and those who specialize in carbonate karst. This is not as odd as it may seem, because a book on engineering problems tends to draw mainly from the pool of regional engineers and environmental scientists – in this case, from those who work in the semi-arid West.

Stability problems in evaporites are caused by their rapid dissolution rate, which accentuates the problems typical of karst in general. These problems are accelerated where inflow of fresh water takes place from the surface (e.g., along well casings and into mines). Collapse at the surface is often caused by evaporite dissolution more than 1000 feet below. In some areas of the humid eastern states, salt mining has caused large-scale land subsidence.

The introductory section of the book includes a description of evaporite karst problems, the need for evaporite karst maps, and the effects of karst processes on gypsum mining. A variety of maps show the distribution of evaporite karst in the US. The remaining chapters are devoted to a mixture of broad overviews and detailed case studies of evaporite-related problems. Most of the coverage focuses on the western US, where evaporite strata are exposed, but there are also individual chapters on Michigan, New York, Virginia, and Louisiana, where evaporites are present below the surface. The overall emphasis is on site-specific problems, rather than on the geomorphic or geochemical aspects of karst.

Topics in the book fall into several categories: (1) overviews of evaporite karst regions, which include cave descriptions and discussion of geomorphic processes, (2) techniques for locating subsurface voids by geophysical and subsurface imaging techniques, as well as surveying and GPS applications, (3) effect of evaporites on groundwater flow, hydraulic conductivity, and water quality, and (4) engineering problems, such as subsidence and collapse, and how to avoid and remediate them. The role of evaporites in gas explosions, stability of petroleum reserves, waste-isolation practices, dam failures, breccia pipes, and mining are also described. The negative effects of pork-barrel economics are well demonstrated. There is inevitably some overlap in subject matter between chapters, but the unique aspects of each region give each chapter a fresh field for discussion. Photos are grayscale and span a wide range of definition from good to poor. Diagrams are clear and well presented.

This is a substantial and important contribution to a field that is poorly represented in the American literature. It is most useful for land managers and engineers who need to recognize and circumvent the problems of building on evaporites, but it can also serve general readers as a good introduction to evaporite karst. Such readers will find it clearly written and not overly technical. In comparison, the book *Gypsum karst of the world*, edited by Alexander Klimchouk and others, gives greater attention to geomorphic and geochemical aspects, and of course global coverage. José Calaforra's *Karstologia de yesos* (Gypsum karst studies) has a similar approach and is useful even to those with a limited knowledge of Spanish. Regrettably, all three books have limited distribution.

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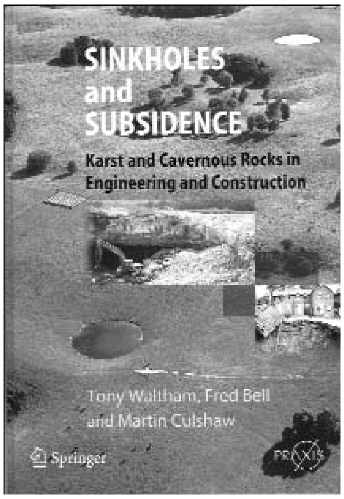

Sinkholes and Subsidence: Karst and Cavernous Rocks in Engineering and Construction

Tony Waltham, Fred Bell, and Martin Culshaw, 2005. New York, Springer-Verlag, 382 p. ISBN 3-540-20725-2, hardbound, 6.9 x 9.7 inches, $189. Order on-line at www.springeronline.com.

This book was written by geologists for engineers. It provides a thorough and practical overview of subsidence processes, effects, detection, and remediation. Those who design structures in karst terranes may be competent engineers, but they rarely have an adequate understanding of the subsurface processes that cause land instability. There is a great communication gap between the professional engineer, whose training is quantitative and standardized, and the karst specialist whose background is more qualitative and field-oriented. Engineers act on what they see at the surface and in boreholes. Karst specialists argue that surface collapse cannot be understood without detailed knowledge of caves and subsurface processes. Neither side is right or wrong, but because engineers are the ones who design and sign off on construction projects, the burden is on them to understand what they are doing. This book should go a long way toward bridging the gap. It is comprehensive, covering every type of collapse whether generated by karst processes or not, and its scope is international.

It opens with a description of rock types, their properties, and modes of dissolution. This is followed by a chapter on sinkhole classification that is well illustrated by diagrams that emphasize that a sinkhole is merely part of a large underground system, and that any impact on one will affect the other. There are chapters on rock failure and soil failure as important mechanisms in forming sinkholes. A discussion of buried sinkholes and karren demonstrates the uncertainty of building on unconsolidated material in karst, where foundations must be fixed to bedrock that varies greatly and unpredictably in depth. A chapter on rock failure due to imposed loads over caves includes a discussion of the effects of stress on caves with varied roof types. Sinkholes induced by water-table decline are described along with several case studies, such as the catastrophic collapse due to mine dewatering in the gold-mining district of South Africa. Coverage includes insoluble rock and pseudokarst. There is a good section on the instability and collapse of lava caves.

Later chapters examine how to avoid stability problems in karst through the use of geophysical methods, remote sensing, and direct observation such as well borings. Remedial measures are described. Non-engineers will be interested in the various ways in which attempts are made to stabilize sinkholes, for example with thick plastic reinforcement. The book also includes assessment strategies concerning hazards, risks, and insurance. The final section consists of 16 case studies by invited specialists who describe subsidence problems around the world. Five are from the eastern and south-central US. There is also a short but helpful glossary that clarifies ambiguous terms such as "rockhead."

The book is remarkably compact, with no superfluous parts and little redundancy. The presentation is seamless and well organized despite the triple authorship and international team of guest contributors. The grayscale photo reproduction is very sharp, with wide tonal gradation, and the diagrams are clear and informative. Classifications are meaningful and easily applied; they are not mere exercises in nomenclature. Although quantitative in places, the book focuses on fundamental concepts without becoming trapped in a morass of experimental data.

Other books on this topic have different perspectives and goals. The several books by Petar Milanovic (e.g., 2000) are based mainly on his experience as an engineer with a strong background in karst, but his viewpoint is strongest at the surface and gets fuzzy with depth. Barry Beck and his associates have provided a series of volumes on sinkholes and related topics that are based on professionally oriented karst conferences held biennially in the US (e.g., Beck, 1984). These are compilations of papers presented at the conferences, and they contain a great deal of technical information of a highly site-specific and individualistic nature. Johnson and Neal (2003) have assembled a similar volume on evaporite karst problems in the US, based on a similar conference (see review elsewhere in this issue). In contrast to these books, the Waltham-Bell-Culshaw volume presents a more unified synthesis and fewer descriptions of site-specific engineering strategies.

We recommend this book enthusiastically to anyone involved in the engineering aspects of karst, as well as to karst specialists. In presentation and utility it is in a class by itself. It is easy to imagine that engineers who read this book will look forward with eager anticipation to the next catastrophic collapse, so they can attack the problem with newly gained confidence.

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