Journal of the Arkansas Academy of Science

Volume 65 Article 18

2011

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Abstract

During September 2008 through June 2011, we compiled a biological inventory of Meacham Cave in Independence County, AR. Compared to other caves in the region, Meacham Cave houses few vertebrates, but non-aquatic invertebrates were relatively common. A transiently-increased bacterial load in the cave's only pool of water indicated recent fecal contamination. The combination of vandalism, low vertebrate populations, and high coliform bacterial load reveals that human abuse of the cave has significantly disrupted its ecosystem. Gating the cave in such a way as to allow the movement of bats, salamanders and other animals, while excluding humans, may allow the cave ecosystem to recover. The close proximity of the cave to Lyon College makes it ideal for long-term investigation.

Introduction

Meacham Cave is privately-owned and located approximately 5 miles north of Lyon College in Independence County, AR (Lat 35.81°, Lon -91.61°). The cave is situated beneath the Batesville Sandstone formation (United States Geological Survey 2000). Most of the cave features were eroded from sandstone with secondary depositional calcite speleothems. The Little Rock Grotto of the National Speleological Society mapped Meacham Cave in 1996 (Little Rock Grotto 1997). Members of the COBRA (Cavers of the Batesville Region of Arkansas) Grotto added an additional passage to the map in 2009. Meacham is a relatively small cave that consists of one large chamber to which several smaller passages converge. The entrance faces east, and the 2 longest passages extend to the north and northwest. During wet weather, an ephemeral pool forms in the lowest part of the main chamber. Local knowledge of the cave's location, unfortunately, has resulted in extensive graffiti, breakage of speleothems and other vandalism. Vandals have damaged or removed all except the very highest formations. A fire ring inside the cave at the bottom of the entrance slope had been used recently.

Methods

The cave was explored in accordance with National Speleological Society guidelines (Jones and Dale 2009). We visited the cave 25 times between September 2008 and June 2011. Three OM-EL-USB data loggers (Omega Engineering, Inc., Stamford, CT) monitored temperature and humidity in the main chamber, and at the ends of the north and northwest passages. Soil texture was determined by volumetric assortment of soil particles (Sammis 2009). Replicate samples for soil texture analysis (n=3) were collected at approximately the same locations at which the data loggers were placed.

Macroscopic organisms – inside and outside of the cave – were counted and photographed for identification. Organisms were identified using commonly-available field guides and online resources (Behler and King 1979, Harvey 1986, Harvey et al. 1999, Milne and Milne 1980, Sealander and Heidt 1990, Trauth et al. 2004, Van Dyk 2011). In order to preserve the fragile cave biota, very few organisms, other than microbes, were collected or otherwise intentionally removed from the cave.

Soil and water samples were analyzed for microbial biomass via fluorescein diacetate (FDA) hydrolysis assays (Adam and Duncan 2001) modified for low-volume samples (Thomas et al. 2008). Replicate soil samples (n = 6) for the FDA assays were collected along a linear path from the bottom of the entrance to the back of the northeast passage of the cave. Replicate water samples (n=2-6) from the ephemeral pool were tested for coliform bacteria using Petrifilm (3M) media. Other cave bacteria were grown on low-nutrient agar plates (Spilde et al. 2005). Duplicate Petrifilm subsamples were incubated at 37°C and 44°C, to determine total coliforms and fecal coliforms, respectively, as recommended by the manufacturer. All other microbiological media were

incubated at 12°C - 13°C (near cave temperature). Cultured and *in situ* microbes were identified by 16S ribosomal RNA sequences amplified using "universal" primers (Boutte et al. 2006, Nubel et al. 1997): 352F 5'- CTCCTACGGGAGGCAGCAG-3' (forward) and 1492R 5'-TACGGYTACCTTGTTACGACTT-3' (reverse).

DNA sequences for identification/phylogenetic analysis were obtained using two methods: culture-independent and culture-dependent. For culture-independent analysis, large environmental water samples (50+ mL) were filtered through 0.45 µm membranes using a filtration manifold (Millipore, Billerica, MA). Microbes were rinsed from the filters with Tris-EDTA (TE) buffer (pH 8). Microorganisms in the TE buffer were subjected to a CTAB, freeze-thaw DNA extraction method (Doyle and Doyle 1987) and the DNAs were precipitated using isopropanol. Microorganisms for culture-dependent analysis were obtained by picking colonies growing on culture media with a sterile loop and suspending the cells in 50 µL of TE buffer. DNA extraction employed the same CTAB method. All 16S DNAs were amplified over 35 cycles of the polymerase chain reaction (PCR) under the following conditions: 94°C for 50 seconds; 54°C for 50 seconds, 72°C for 50 seconds, and a final "polishing" step of 72°C for 10 minutes. Amplified DNAs were visualized using 1% agarose electrophoresis, then correct-sized bands were excised and the DNA purified from the gel matrix using the Cyclo-prep kit (Ameresco, Solon, OH). DNAs amplified for the culture-dependent method were sent directly for DNA sequencing (DNA Resource Center, Division of Agriculture, University of Arkansas, Fayetteville, AR). However, the heterogeneous DNAs from culture-independent PCR amplification were separated by cloning into the TOPO vector (Invitrogen, Carlsbad, CA) and positive clones selected for DNA sequencing.

DNA alignment and analysis used a combination Sequencher (Gene Codes, Ann Arbor, MI) and Se-Al (Oxford, UK). Related sequences were searched using the BLAST application on the National Center for Biotechnology Information (NCBI) nucleotide database. Phylogenetic analyses, tree building and bootstrapping used the maximum-likelihood method in PAUP* v. 4.0b10 (Swofford 2002). Trees were estimated using the HKY85 substitution model, set transition to transversion ratio, and approximated gamma distribution. All trees were swapped by random stepwise addition with tree-bisection-reconnection (TBR) branch swapping. Bootstrap values were obtained using the same HKY85 substitution model, conducted for 100 replicates.

Results and Discussion

Throughout the study period, the temperature in the back of the cave remained stable at 14°C. Near the entrance, however, the temperature varied between 12°C and 15°C. Relative humidity remained at 100% in the rear of the cave, and varied between 95-100% near the entrance. Clays dominated the soils in the back of the cave, whereas soil closer to the entrance contained approximately equal amounts of clay and silt. Sand comprised 10% or less of the cave soils (Figure 1).

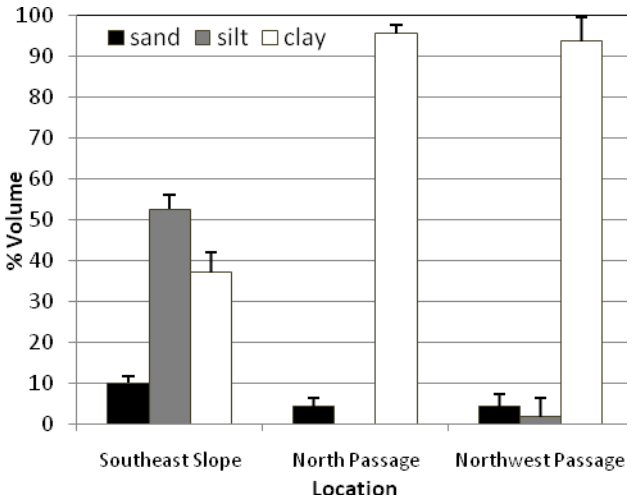

Figure 1. Soil texture in Meacham Cave. With the exception of the soil on the southeast slope (near the entrance), clays dominated the cave soils (n = 3, bars = s.d.). No silt was detected in samples from the North passage.

Total soil microbial biomass, as measured by FDA hydrolysis activity, was highest near the entrance (Figure 2). Presumably, this was due to the input of nutrients by leaf litter and other detritus that falls into the cave. Coliform bacteria were detected in every cave water sample (however, water was not present in the cave at every visit). The population of coliforms loosely correlated (r^2 = 0.56) with total microbial biomass in the water samples (Figures 3 & 4). With the exception of the first two samples, microbial populations in the water were relatively small. The source of the bacteria is unclear, but may have been due to animal waste deposited in or near the water just prior to the sampling period.

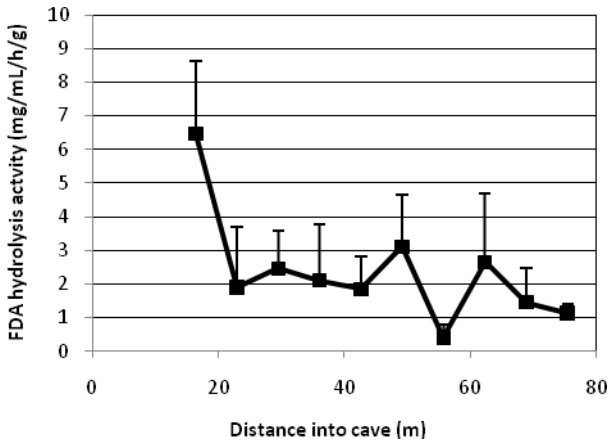

Figure 2. Soil microbial biomass as a function of distance from the entrance. The distance shown is actual distance along the cave floor surface, not horizontal distance (n = 6, bars = s.d.). Extensive leaf litter at the cave entrance made samples before 15 m impractical.

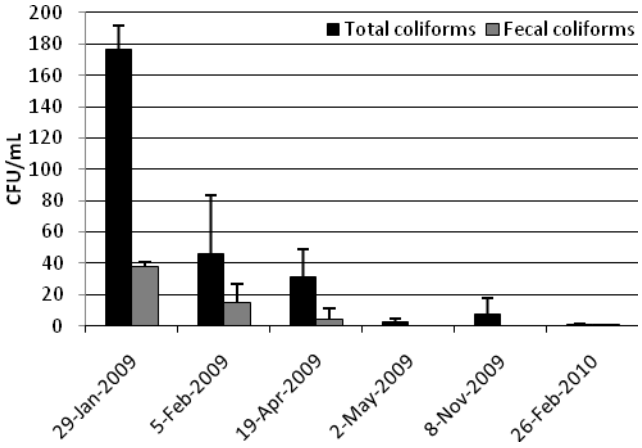

Figure 3. Coliforms and fecal coliforms in cave water (in order from left, n = 2, 8, 4, 4, 2, 8; bars = s.d.; CFU = colony forming units). Fecal coliforms were not detected in the last three samples.

Coliform and fecal (thermotolerant) coliform bacteria are standard indicator organisms of fecal contamination. In particular, the thermotolerant bacterium, *Escherichia coli*, is a specific indicator of fecal contamination. Other coliforms can be present without necessarily being of fecal origin (reviewed in (Moe 2002, Toranzos et al. 2002). A small number of vertebrates live in the cave (Table 1), and we saw feces and nests of small animals in many areas of the cave. In addition, we observed small amounts of bat guano pellets in the water during most visits. Finally, we cannot rule out the possibility of human contributions to the fecal coliform population. Even the most careful and conscientious explorers can contaminate cave water by their presence (Hunter et al. 2004).

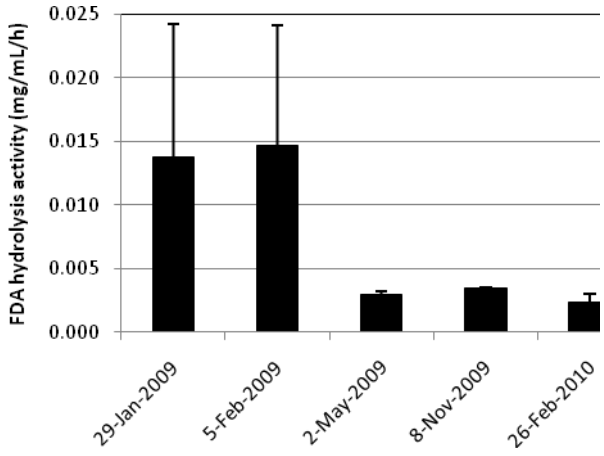

Figure 4. Water microbial biomass. Samples from around the pool were averaged for each date (in order from left, n = 12, 32, 4, 2, 7; bars = s.d.).

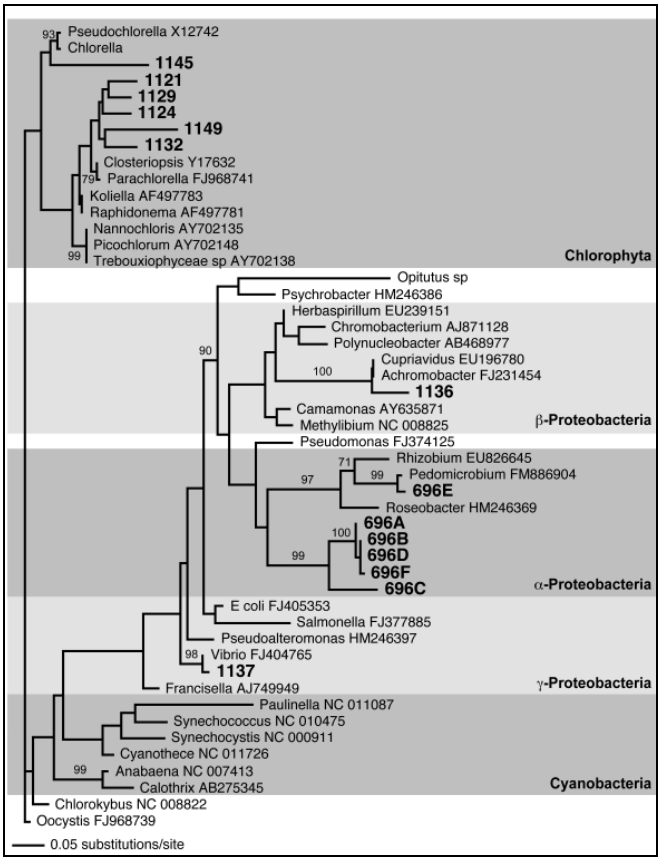

Figure 5. Phylogenetic tree of isolated microorganisms. Taxa identified with numbers (only) are sequences generated from this study. All of the isolates from Meacham Cave grouped into the Chlorophyta and Proteobacteria divisions. Only bootstrap values greater than 70% are shown.

Table 1. Animals in and around Meacham Cave.

Outside of the cave (within 5 m of entrance):
Vertebrates
Eumeces fasciatus (Five-lined skink)
Plethodon serratus (Southern red-backed salamander)
Sciurus carolinensis (Gray squirrel)
Invertebrates
Acrosternum hilare (Green stink bug)
Bombus sp. (Bumblebee)
Camponotus pennsylvaticus (Black carpenter ant)
Ceuthophilus maculatus (Spotted camel cricket)
Leiobunum sp. (Eastern daddy longlegs)
Leucage venusta (Venusta orchard spider)
Musca domestica (House fly)
Pardosa sp. (Thin-legged wolf spider)
Phiddipus audax (Bold jumping spider)
Pisauridae family (Nursery web spider)
Thysanura sp. (Jumping bristletail)
Inside of the cave:
Vertebrates
Eptesicus fuscus (Big brown bat)
Perimyotis (formerly *Pipistrellis*) *subflavus* (Tri-colored bat, formerly Eastern pipistrelle)
Eurycea lucifuga (Cave salamander)
Lithobates (formerly *Rana*) *palustris* (Pickerel frog)
Invertebrates
Abacion sp. (Millipede)
Arrhopalitidae family (Globular springtails, two different)*
Cambala minor (Lesser cave-loving millipede)*
Cantheridae family (Soldier beetle larva)
Causeyella sp. (Cave millipede)*
Ceuthophilus maculatus (Spotted camel cricket)*
Culicidae family (Mosquito)
Dolomedes tenebrosus (Dark fishing spider)
Heleomyzidae family (Heleomyzid fly)
Leiodidae family (Round fungus beetle larva)
Linyphiidae family (Sheet-weaving spider)
Litocampa sp. (Cave two-pronged bristletail)*
Lumbricus terrestris (Earthworm)
Macrocera nobilis larva (Ozark webworm)*
Oxidus gracilus (Greenhouse millipede)
Parajulidae family (Millipede)
Patera perigrapta (Engraved bladetooth snail)
Phoridae family (Humpbacked fly)
Pseudopolydesmus sp. (Millipede)
Psychodidae family (Moth fly)
Rhagidiidae family (Mite)
Sinella sp. (Springtail)*
Sphaeroceridae family (Dung fly)*
Tineola bisselliela (Clothes moth)
Tomoceridae family (Springtail)

*Indicates likely troglophilic or troglobitic organisms.

Aside from the coliforms, two major microbial groups were identified by 16s ribosomal gene sequences (Figure 5). The number of identified isolates was disappointingly low, and probably was the result of difficulties in extracting DNA from the microorganisms and environmental samples. All of the bacteria that we identified were members of the Proteobacteria division. The Proteobacteria division is the single largest group in both epigean and hypogean environments (Romero 2009) and includes the coliforms. Isolate 696E, a culture-independent sample, was identified as being homologous with *Pedomicrobium*, a Mn-oxidizing bacterium (Larsen et al. 1999) that has been implicated with MnO_2 deposits in caves (Northup et al. 2003). In some caves, metal-oxidizing bacteria, like *Pedomicrobium*, are the chemoautotrophic bases of food webs (Barton et al. 2007, Northup et al. 2003, Spilde et al. 2005).

Members of the Chlorophyta division (green algae) comprised the other major group of microbes identified from the cave. The role and origin of algae in the cave is unclear. While light from the entrance is visible from the pool, the amount of incident light (<1 µmol photons m^{-2} s^{-1}) is far below the threshold of 12-28 µmol photons m^{-2} s^{-1} required for photosynthesis in chlorophytes (Richardson et al. 1983). Studies from Hungarian caves indicate that some algae utilize heterotrophic metabolism in place of photosynthesis (Claus 1962, Claus 1964, Hahdu 1966, Kol 1967). Another possibility is that epigean algae are carried by water as it percolates into the cave from the surface. In show caves, which are periodically illuminated by floodlights, algae may receive enough energy to grow at a slow rate, and may become "nuisance organisms" to cave owners (Aley 2004, Smith and Olson 2007). In wild caves, non-facultatively heterotrophic algae would eventually die in the cave, if not for the fortuitous collection by cave biologists.

In both microbial divisions, more specific identifications were not possible for two major reasons. First, BLAST comparisons often yielded close matches with "environmental samples" – uncharacterized organisms within the same taxon. Second, the "universal" primers used are not always long enough to allow species-level comparisons.

Table 1 lists the animals observed to date. Most of the epigean organisms were found within 5 meters of the entrance. Hypogean organisms were found throughout the cave, but were concentrated near the entrance and the main chamber. Cataloguing of the epigean organisms occurred during February through April 2009. Cataloguing of the hypogean organisms occurred throughout the 25 visits. We did not find any aquatic invertebrates during any of the visits – probably due to the transient nature of the cave pool. Invertebrates were more common and more diverse than vertebrates. The total number of vertebrates

found during any visit seemed low as compared to other caves in the area. We found no more than three bats, frogs or salamanders during each visit with one notable exception – 12 Western slimy salamanders (*Plethodon albagula*) were found during a single visit in June 2010.

For comparison, Cave Point Cave and Logan Point Cave (both in neighboring Stone County), are of similar or smaller volumes than Meacham Cave, but during a typical visit to Cave Point Cave, we have found 50 or more bats and 5 or more salamanders. During a single visit to Logan Point Cave, we found 3 bats and 20 salamanders (unpublished data). While the numbers of salamanders seem low in comparison to the two other caves mentioned, a survey of salamander use of 93 small caves in Crawford County (northwestern Arkansas), found average numbers of salamanders similar to what we reported here for Meacham Cave (Briggler and Prather 2006). A similar study on tri-colored bats (*Perimyotis subflavus*) in 54 northwestern Arkansas caves found >3 bats per visit for caves similar in length to Meacham Cave (Briggler and Prather 2003). However, that study also indicated that the bats preferred hibernacula with east-facing entrances; Meacham Cave's entrance faces west.

The seemingly low number of salamanders may actually be normal for a cave the size of Meacham, but the low bat count may be due to other causes. Specifically, the burning of wood in the fire pit, noted previously, may be responsible for the low bat population. A study that modeled smoke and fire effects on tree-dwelling bats showed that carbon monoxide poisoning and ear burns occurred during wildfires and prescribed fires (Dickinson et al. 2010). In the arboreal environment, CO poisoning was critical only directly above flames. However, in the enclosed environment of a cave, we would expect CO concentrations to be higher with smaller fire sources. Additionally, smoke irritants and CO may have more intense effects on bats in torpor or hibernation (Dickinson et al. 2009).

Conclusions

Leaf litter and other organic matter from the surface are the likely sources of energy for Meacham Cave's ecosystem. The algae found in the cave were not in suitable locations for photosynthesis, and bat guano was scarce. A single potentially chemosynthetic bacterial isolate was found, but the contribution of chemosynthesis to the cave's ecosystem probably is minimal at best. The number and diversity of vertebrates is low, especially for bats, and may be due, in part, to human abuse of the cave.

Meacham Cave's proximity to Lyon College makes it a useful long-term research site, and our results provide a baseline for continuing studies. As the threat of white nose syndrome – a fatal disease of bats associated with the fungus *Geomyces destructans* – continues to grow, the low bat population is of particular concern (Blehert et al. 2009, Chaturvedi et al. 2010, Gargas et al. 2009). Gating the cave entrance to prevent entry by vandals – while allowing ingress and egress of cave animals – may help to increase the populations of bats and other cave animals.

Acknowledgements

The Arkansas Space Grant Consortium this research. Members of the Little Rock and COBRA Grottos of the National Speleological Society assisted with some of the cave visits. The authors thank Mr. Doyle Rogers for allowing us to study his cave. We also thank Dr. Rowland Shelley of the North Carolina Museum of Natural Sciences and Mr. Mike Slay of The Nature Conservancy for assistance with invertebrate identification.

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