Published By
The National Speleological Society

Editor-in-Chief
Malcolm S. Field
National Center of Environmental Assessment (8623P)
Office of Research and Development
U.S. Environmental Protection Agency
1200 Pennsylvania Avenue NW
Washington, DC 20460-0001
703-347-8601 Voice 703-347-8692 Fax
field.malcolm@epa.gov

Production Editor
Scott A. Engel
CH2M HILL
2095 Lakeside Centre Way, Suite 200
Knoxville, TN 37922
865-560-2954
scott.engel@ch2m.com

Journal Copy Editor
Bill Mixon

JOURNAL ADVISORY BOARD
Penelope Boston
Gareth Davies
Luis Espinasa
Derek Ford
Louise Hose
Leslie Melim
Wil Orndorf
Bill Shear
Dorothy Vesper

BOARD OF EDITORS
Anthropology
George Crothers
University of Kentucky
211 Lafferty Hall
gcrot@uky.edu

Conservation-Life Sciences
Julian J. Lewis & Salisa L. Lewis
Lewis & Associates, LLC,
lewisbioconsult@aol.com

Earth Sciences
Benjamin Schwartz
Department of Biology
Texas State University
bs37@tstxstate.edu

Robert Brinkman
Department of Geology, Environment, and Sustainability
Hofstra University
robert.brinkman@hofstra.edu

Maria Parise
National Research Council, Italy
m.parise@ba.ipp.cnr.it

Exploration
Paul Burger
Cave Resources Office
National Park Service - Carlsbad, NM
paul_burger@nps.gov

Microbiology
Kathleen H. Lavoie
Department of Biology
State University of New York, Plattsburgh,
lavoiekh@plattsburgh.edu

Paleontology
Greg McDonald
Park Museum Management Program
National Park Service, Fort Collins, CO
greg_mcdonald@nps.gov

Social Sciences
Joseph C. Douglas
History Department
Volunteer State Community College
joe.douglas@volstate.edu

Book Reviews
Arthur N. Palmer & Margaret V. Palmer
Department of Earth Sciences
State University of New York, Oneonta
palmeran@oneonta.edu

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; Phone (256) 852-1300; Fax (256) 851-9241, email: ns@caves.org. World Wide Web: http://www.caves.org/pub/journal/
Check the Journal website for subscription rates. 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 by the following ISI Thomson Services Science Citation Index Expanded, ISI Alerting Services, and Current Contents/Physical, Chemical, and Earth Sciences.

Copyright © 2014 by the National Speleological Society, Inc.

Front cover: Access to Bat Cave in Grand Canyon National Park from the Colorado River. Photo by Robert Pape.
BIOLOGY AND ECOLOGY OF BAT CAVE, GRAND CANYON NATIONAL PARK, ARIZONA

ROBERT B. PAPE
Department of Entomology, University of Arizona, Tucson, Arizona 85721, spinelessbiol@aol.com

Abstract: A study of the biology and ecology of Bat Cave, Grand Canyon National Park, was conducted during a series of four expeditions to the cave between 1994 and 2001. A total of 27 taxa, including 5 vertebrate and 22 macro-invertebrate species, were identified as elements of the ecology of the cave. Bat Cave is the type locality for Eschatomoxys pholeter Thomas and Pape (Coleoptera: Tenebrionidae) and an undescribed genus of tineid moth, both of which were discovered during this study. Bat Cave has the most species-rich macro-invertebrate ecology currently known in a cave in the park.

INTRODUCTION

This paper documents the results of a biological and ecological analysis of Bat Cave on the Colorado River within Grand Canyon National Park conducted during four expeditions to the cave between 1994 and 2001. The study focused on the macro-invertebrate elements present in the cave and did not include any microbiological sampling, identification, or analysis. Access to caves in the park is strictly regulated and requires either an approved research permit or a cave entry permit, granted through the Grand Canyon Division of Science and Resource Management.

CAVE INVERTEBRATES IN GRAND CANYON NATIONAL PARK

Grand Canyon National Park has extensive cave resources, but there has been little research on cave-inhabiting invertebrates in the park. The earliest effort was conducted by the Cave Research Foundation at Horseshoe Mesa in 1977 and 1978 (Welbourn, 1978). The scope of that project was not strictly biological, but did include a search for invertebrates in eight caves contained in the Mooney Falls member of the Redwall limestone. The effort produced a total of fourteen invertebrate species, including a new species of garypid pseudoscorpion (Archeolarca cavicola Muchmore). The lack of species diversity was attributed to the lack of available moisture in the caves. Peck (1980) looked at three caves in the Grand Canyon, all of which, like Bat Cave, were in the Muav limestone. All three of these caves contain active streams that have their outfall in major tributaries to the main gorge of the Colorado River below the North Rim of Grand Canyon. Peck’s effort identified a total of 15 invertebrate species from the 3 caves. One of those caves, Roaring Springs Cave, was revisited by Drost and Blinn in 1994 and 1995 (Drost and Blinn, 1997), and their effort increased the invertebrate species recorded from that cave from the original 10 found by Peck to 19, including the first records of aquatic invertebrates from a Grand Canyon Cave. A review of cave-invertebrate studies in the park, which included 9 reports addressing 16 caves, was prepared by Wynne et al. (2007). Their compilation resulted in a list of approximately 37 species of cave macro-invertebrates currently known from caves there. Wynne and others have recently performed invertebrate surveys in caves in the Grand Canyon–Parashant National Monument, and have already encountered several undescribed cave-inhabiting macro-invertebrate species, including three new genera. These include a camel cricket (Rhaphidophoridae), a barklouse (Psocoptera), and a macrosternodesmid millipede, Pratherodesmus (Shear et al., 2009; Wynne and Drost, 2009).

BAT CAVE

Bat Cave is located in the north wall of the main gorge of the Colorado River within Grand Canyon National Park (Fig. 1, insert). The entrance is at an elevation of 580 meters, approximately 260 meters above the river. The cave is formed along a major vertical fault in the Cambrian Muav limestone and consists of just over one thousand meters of surveyed passage, mostly along the single main trunk passage. From the entrance, the cave trends a few degrees east of north (Fig. 1). The cave is essentially horizontal, with a total vertical relief of only 75 meters.

Bat Cave is named for the large maternity colony of Mexican free-tailed bats (Tadarida brasiliensis I. Geoffroy) that resides there. The first record of the cave is from the 1930s. Shortly thereafter, there were efforts to mine the bat guano for fertilizer. These early efforts were mostly unproductive. In 1958, the property was purchased by the U.S. Guano Corporation. U.S. Guano invested 3.5 million dollars in the setup of the mining operation, only to find that the extent of the guano deposit was only 1 percent of their original estimate. Difficulties with the haul system across the canyon, and specifically the lack of a significant guano deposit, forced the abandonment of the mining operation in 1960 (Billingsley et al., 1997). The support towers for the haul system’s cables are still present on the top of the south rim of the canyon and below the cave on the...

Journal of Cave and Karst Studies, April 2014•1
north side of the river. A wooden plank-way, portions of which are elevated, traverses approximately the first 145 meters of the cave from an elevated entrance platform. Sections of large flanged pipe approximately 30 cm in diameter and other mining equipment remain in the cave. Most of the animal species associated with the cave are arthropods tied directly to the bat guano-supported food web.

MATERIALS AND METHODS

The four expeditions to Bat Cave were conducted on March 12, 1994; March 29 and 30, 1996; October 24, 1997; and October 26, 2001. Access to the cave was made up the river by boat from Lake Mead. Visits to the cave were scheduled seasonally and temporally to avoid disturbance.
to the resident bat population. Even so, at least some bats were present on all visits to the cave. During the March visits, no more than a few hundred bats were present at any time, mostly around the roost at survey station D1 (Fig. 1). All efforts were made to keep disturbance to the bats at a minimum. This roost is at the east edge of the main trunk passage, and the roost area can be skirted with minimal disturbance to the bats.

The 5 visits involved approximately 34 hours of observation and sampling of invertebrates in the cave. The methods were low impact, consisting of a visual search of passage floors, walls, crevices, ledges, and breakdown-boulder accumulations. Additional habitat niches have been created in Bat Cave by the presence of wooden walkways and machinery remaining from the abandoned mine operation. Particular attention was paid to floor debris, under which many invertebrates commonly shelter. Debris that was overturned was replaced after examination to minimize impact to microhabitats. The principal benefits of this type of documentation are minimal habitat disturbance, low animal population impact, and ease of sampling in the cave environment. Since obligate cave invertebrates may be small and may be adversely impacted by over-sampling, no pitfall or other trapping devices were used. Pitfall traps should only be utilized where the traps can be regularly monitored.

Initial sampling of specimens was kept to a minimum, and no more than six individuals of each type were taken. Both males and females were included in these samples for species where their sex was readily evident. Additional sampling for type-series specimens was later performed for the tenebrionid beetle and the undescribed tineid moth. Specimens were sampled with forceps or a camel hair brush dampened with ethanol. Specimens were placed in vials of ethanol for transport to the laboratory.

Two approximately 0.3 kg samples of fresh bat guano were taken on March 29, 1996, for Berlese extraction. Each of these samples was a composite of smaller sub-samples taken in closely spaced areas within an approximately 0.5 m² area near the apex of the guano deposits. One sample was taken at each of the two main roosts, near survey stations D1 and B7. Specimens were sampled under two park permits (9503-09-02 and GRCA-2001-SCI-0043).

Relative-humidity and ambient air-temperature measurements were recorded at several points along the main trunk passage of the cave on March 29, 1996 using a digital temperature/humidity meter (Hanna Instruments model HI 8565).

RESULTS AND DISCUSSION

A total of 27 taxa, including vertebrates, were identified from Bat Cave during this study. Table 1 is a taxonomic summary of the species documented and lists the assigned ecological group (troglolexene, troglobiole, troglobite, or accidental), guild (scavenger, fungivore, predator, parasite, or phytophage), and relative abundance (abundant, common, uncommon, or rare) for each species. Ecological groups used in this paper are defined as follows: A troglolexene is an animal that enters caves to fill some ecological need, such as obtaining food, water, shelter, etc., but that cannot survive without returning to the surface to meet some of its life-cycle requirements. A troglobiole is an animal that is capable of completing its life cycle within caves, but may also do so elsewhere. A troglobite is an obligate cave animal, which cannot live outside of the cave environment. The occurrence of parasites in the cave is in association with other species of animals, but because of their presence in the cave during part of their life cycle we consider them to be troglolexenes. Accidentals are just what the label implies, animals that wander or fall into the cave, would not normally occur in such habitats, and obtain no benefit from their presence in the cave.

**SPECIES ACCOUNTS**

**Phylum: Chordata**
- **Class: Aves**
  - **Order: Passeriformes**
    - **Family: Troglodytidae**
      - *Catherpes mexicanus* Swainson
        - A single canyon wren was observed in the entrance room of the cave on October 26, 2001. Canyon wrens are common in cave entrance areas, where they forage, shelter, and occasionally nest.

- **Class: Mammalia**
  - **Order: Chiroptera**
    - **Family: Vespertilionidae**
      - *Myotis californicus* Audubon & Bachman
        - The presence of this insectivorous bat at Bat Cave is known from acoustic monitoring that was conducted at the entrance in 1997 (Charles Drost, pers. comm). It is assumed that only small numbers of these bats use the cave. Their contribution to the nutrient input to the cave is greatly overshadowed by the population of the Mexican free-tailed bat.
    - *Myotis yumanensis* H. Allen
      - The presence of this insectivorous bat at Bat Cave is known from acoustic monitoring that was conducted at the entrance in 1997 (Charles Drost, pers. comm). It is assumed that only small numbers of these bats use the cave. Their contribution to the nutrient input to the cave is greatly overshadowed by the population of the Mexican free-tailed bat. This species almost always occurs near a permanent water source, and it is likely a relatively common animal in caves and crevices along the Colorado River.

- **Family: Molossidae**
  - *Tadarida brasiliensis* I. Geoffroy
  - The Mexican free-tailed bat maternity colony at Bat Cave is the major source of nutrient input into the cave, with its guano serving as the foundation of the cave’s invertebrate communities.
Table 1. Summary of fauna documented at Bat Cave, Grand Canyon National Park, Arizona.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Ecological Group</th>
<th>Guild</th>
<th>Abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Class Aves</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Order Passeriformes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Troglodytidae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Catherpes mexicanus</em></td>
<td>Trogloxene</td>
<td>Predator</td>
<td>Uncommon</td>
</tr>
<tr>
<td><strong>Class Mammalia</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Order Chiroptera</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vespertilionidae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Myotis californicus</em></td>
<td>Trogloxene</td>
<td>Predator</td>
<td>Uncommon?</td>
</tr>
<tr>
<td><em>Myotis yumanensis</em></td>
<td>Trogloxene</td>
<td>Predator</td>
<td>Uncommon?</td>
</tr>
<tr>
<td>Molossidae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Tadarida brasiliensis</em></td>
<td>Trogloxene</td>
<td>Predator</td>
<td>Abundant</td>
</tr>
<tr>
<td><strong>Order Carnivora</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Procyonidae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Bassariscus astutus</em></td>
<td>Trogloxene</td>
<td>Predator</td>
<td>Common</td>
</tr>
<tr>
<td><strong>Class Arachnida</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Order Sarcoptiformes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycyphagidae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Glycyphagus</em> sp.</td>
<td>Troglophile</td>
<td>Fungivore</td>
<td>Uncommon</td>
</tr>
<tr>
<td>Rosensteiniidae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Cubaglyphus</em> sp.</td>
<td>Guanophile</td>
<td>Scavenger</td>
<td>Common</td>
</tr>
<tr>
<td>Suidasidae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Sapracarus tuberculatus</em></td>
<td>Troglophile</td>
<td>Scavenger?</td>
<td>Rare</td>
</tr>
<tr>
<td><strong>Order: Mesostigmata</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macronyssidae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Chiroptonyssus robustipes</em></td>
<td>Troglophile</td>
<td>Parasite</td>
<td>Abundant</td>
</tr>
<tr>
<td><em>Mitonyssoides stercoralis</em></td>
<td>Troglophile</td>
<td>Predator</td>
<td>Common</td>
</tr>
<tr>
<td><strong>Order: Trombidiformes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pterygosomatidae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pimeliophilus</em> sp. (Undes.?)</td>
<td>Trogloxene</td>
<td>Parasite</td>
<td>Common</td>
</tr>
<tr>
<td><strong>Order Araneae</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Filistatidae?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>(Kukulcania</em> sp.?</td>
<td>Troglophile</td>
<td>Predator</td>
<td>Rare</td>
</tr>
<tr>
<td>Plectreuridae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Kibramoa suprenans</em></td>
<td>Troglophile</td>
<td>Predator</td>
<td>Common</td>
</tr>
<tr>
<td>Sicariidae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Loxosceles</em> sp.</td>
<td>Troglophile</td>
<td>Predator</td>
<td>Uncommon</td>
</tr>
<tr>
<td>Pholeidae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Psilochorus</em> sp.</td>
<td>Troglophile</td>
<td>Predator</td>
<td>Uncommon</td>
</tr>
<tr>
<td>Selenopidae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Selenops</em> sp.</td>
<td>Troglophene</td>
<td>Predator</td>
<td>Uncommon</td>
</tr>
<tr>
<td><strong>Order Pseudoscorpiones</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chernetidae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Neoallochernes stercoreus</em></td>
<td>Troglophile</td>
<td>Predator</td>
<td>Abundant</td>
</tr>
<tr>
<td><strong>Class Hexapoda</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Order Pscoptera</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Psyllipsocidae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Psyllipsocus ramburyi</em></td>
<td>Troglophile</td>
<td>Fungivore</td>
<td>Common</td>
</tr>
<tr>
<td><strong>Order Neuroptera</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myrmeleontidae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Eremoleon pallens</em></td>
<td>Troglophile</td>
<td>Predator</td>
<td>Common</td>
</tr>
</tbody>
</table>
food web. During the period the field work was conducted, the summer population of the bats was estimated at 285,000 (Charles Drost, pers. comm). The bats currently roost at two major sites within the cave. The primary site is at survey station B7, 265 meters from the entrance. The second, smaller roost is located at survey station D1, 229 meters into the cave. Overflow bats from these roosts probably congregate in wider sections in the main corridor closer to the entrance, between survey stations A11 and B2. Recent guano deposits in the front sections of the cave are not extensive. Currently, the bats seldom go deeper into the cave than the passage constriction at survey station B9, 308 meters from the entrance. No recent guano deposition was observed beyond survey station B9.

A large fossil guano deposit is present at the back of the cave beginning at survey station B14. Over a period of time the main cave passage was gradually cut off at a passage constriction at survey station B9. The bedrock ceiling of the cave passage at this point dips steeply downward. Evidently the annual accumulations of guano deposited at the roost centered at survey station B7 eventually increased in depth until the slope of the deposit met with the ceiling at survey station B9, isolating the back portion of the cave. Based on the presence of old mining pipes at this point, the back section of the cave was apparently reopened during the last mining efforts. A sample of the top of the fossil bat guano beyond station B9 was dated at 6.5 ka, and the bottom of the fossil guano deposit was dated at 14.5 ka (Wurster et al., 2008). The more recent date would represent the time of last regular use of the back portion of the cave by the large Tadarida colony.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Ecological Group</th>
<th>Guild</th>
<th>Abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Order Coleoptera</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dermestidae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Dermestes carnivorus</em></td>
<td>Troglophile</td>
<td>Scavenger</td>
<td>Rare</td>
</tr>
<tr>
<td>Tenebrionidae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Eschatomoxys pholet</em></td>
<td>Troglophile</td>
<td>Scavenger</td>
<td>Abundant</td>
</tr>
<tr>
<td>Curculionidae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Smicronyx imbricatus</em></td>
<td>Accidental</td>
<td>Phytophage</td>
<td>Uncommon</td>
</tr>
<tr>
<td>Order Siphonaptera</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ischnopsyllidae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Sternopsylla distincta</em></td>
<td>Trogloxene</td>
<td>Parasite</td>
<td>Common</td>
</tr>
<tr>
<td>Order Diptera</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muscidae</td>
<td></td>
<td>Trogloxene?</td>
<td>?</td>
</tr>
<tr>
<td><em>Fannia sp.</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Order Lepidoptera</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tineidae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>New genus and species</em></td>
<td>Troglophile</td>
<td>Scavenger</td>
<td>Common</td>
</tr>
<tr>
<td><em>Tinea (pallescentella?)</em></td>
<td>Troglophile?</td>
<td>Scavenger</td>
<td>Rare</td>
</tr>
<tr>
<td>Order Hymenoptera</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sphecidae</td>
<td></td>
<td>Trogloxene</td>
<td>Predator</td>
</tr>
<tr>
<td><em>Sceliphron caementarium</em></td>
<td>Trogloxene</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The bats currently roost at two major sites within the cave. The primary site is at survey station B7, 265 meters from the entrance. The second, smaller roost is located at survey station D1, 229 meters into the cave. Overflow bats from these roosts probably congregate in wider sections in the main corridor closer to the entrance, between survey stations A11 and B2. Recent guano deposits in the front sections of the cave are not extensive. Currently, the bats seldom go deeper into the cave than the passage constriction at survey station B9, 308 meters from the entrance. No recent guano deposition was observed beyond survey station B9.

A large fossil guano deposit is present at the back of the cave beginning at survey station B14. Over a period of time the main cave passage was gradually cut off at a passage constriction at survey station B9. The bedrock ceiling of the cave passage at this point dips steeply downward. Evidently the annual accumulations of guano deposited at the roost centered at survey station B7 eventually increased in depth until the slope of the deposit met with the ceiling at survey station B9, isolating the back portion of the cave. Based on the presence of old mining pipes at this point, the back section of the cave was apparently reopened during the last mining efforts. A sample of the top of the fossil bat guano beyond station B9 was dated at 6.5 ka, and the bottom of the fossil guano deposit was dated at 14.5 ka (Wurster et al., 2008). The more recent date would represent the time of last regular use of the back portion of the cave by the large Tadarida colony.

Order: Carnivora
Family: Procyonidae
*Bassariscus astutus* Lichtenstein

Ringtail scats and tracks were observed throughout the front half of the cave, back to the active bat roosts at survey stations D1 and B7. A ringtail was observed on two of the five visits into the cave. Both were nocturnal sightings, which is the normal activity period for the animals. One sighting was at 1929 hrs on March 29, 1996, along the east wall of the cave adjacent to survey station B2, where a single animal was observed headed deeper into the cave. At the time there were a few hundred freetailed bats active at the roost at survey station D1. A second sighting was at 1930 hrs on October 24, 1997, between survey stations A5 and A6, where the main passage narrows. Along the west wall of the cave at this point there is a wooden walkway support structure that extends toward the middle of the passage. We had earlier noted that there were several dead Tadarida lying on and around this small structure (Fig. 2). There were abundant ringtail scats present along the west wall here. The ringtail was observed perched on the east edge of the support structure. This put it near the center of the walkway area and well within the flight path of any low-flying bats. There were still a few bats leaving the cave at this time. The ringtail was not observed attempting to take bats out of the air, but it is probable that is how it was capturing live bats. It is unknown why the several dead bats on the support structure noted earlier in the day had not been consumed by the ringtail. With the abundant bats as a dependable food source from at
least April through October and the natural shelter provided by the cave, it is possible that ringtails may also den in the cave. Ringtails would probably use the front entrance room for denning, since there is a jumble of rocks which could provide a secluded area for that purpose. Other rock-pile accumulations occur near survey station B2.

Based on sightings and an abundance of their scats, ringtails apparently regularly use caves in the southwestern U.S. They use caves for shelter, as a source of water and some foods, and probably den in caves, at least occasionally. Ringtails are opportunistic in their feeding, taking equally from animal and plant materials seasonally. Birds, wood rats, bats, mice, and other small rodents are commonly taken (Taylor, 1954; Murie, 1974; Poglayen-Neuwall and Toweill, 1988). Ringtails probably regularly take dying adult bats and young that fall onto cave floors below roosts. Records for lizards and snakes from two-thousand-year-old ringtail scats were documented in caves in the western part of Grand Canyon by Mead and Van Devender (1981).

**Figure 2.** Dead *Tadarida* killed by ringtail near survey stations A5 and A6; scale is 15 cm.

Phylum: Arthropoda
Class: Arachnida
Order: Sarcoptiformes
Family: Glycyphagidae
*Glycyphagus* sp.
This dust mite is likely fungivorous and likely feeds on molds associated with the bat guano.

Family: Rosensteiniidae
*Cubaglyphus* sp.
This animal is an undescribed species of *Cubaglyphus* (Barry O’Connor, pers. comm.).

Family: Suidasiidae
*Sapracarus tuberculatus* Fain & Philips
This is a species of very small mites that is known from a variety of habitats, including caves (Barry O’Connor, pers. comm.). The feeding habits of the species are not known (Barry O’Connor, pers. comm.). Other mite records from caves in Grand Canyon National Park are an anystid mite from several of the Horseshoe Mesa caves (Welbourn, 1978) and a rhagidiid mite (*Rhagidia cf. hilli*) from Roaring Springs Cave (Peck, 1980).

Order: Mesostigmata
Family: Macronyssidae
*Chiroptonyssus robustipes* Ewing
This mite is an obligate parasite of the Mexican freetailed bat. The bats groom them off their bodies and the mites drop to the guano pile below. It is not known whether any of the mites manage to return to the bats. Many of them are likely consumed by the various invertebrate predators in the guano pile. The mite population in the guano piles was not at its peak during our visits to the cave since the number of bats present was generally low. The fresh guano accumulation was quite limited when the Berlese samples were taken.

*Mitonyssoides stercoralis* Yunker, Lukoschus, and Giesen
This mite is the only species in its family (Macronyssidae) that is not a blood-feeding parasite on a vertebrate host, but is predatory on the rosensteiniid mites that occur in bat guano. *M. stercoralis* is found in the guano of both molossid and vespertilionid bats. The previously known range of the species was from Indiana in the U.S. to southern Brazil (Radovsky and Krantz, 2003). Presence of *M. stercoralis* in Grand Canyon National Park is a significant range extension for the species.

Order: Trombidiformes
Family: Pterygosomatidae
*Pimeliaphilus* sp.
Species of *Pimeliaphilus* are parasitic on lizards and a variety of arthropods (Field et al., 1966; Anderson, 1968; Ibrahim and Abdel-Rahman, 2011; Delfino et al., 2011). A single deutonymph and larvae of an undetermined species of *Pimeliaphilus* were sampled from *Eschatomoxys pholeter*
in the cave (Fig. 3). These mites are relatively common on the beetles, and are typically parasitic at these stages. This animal may be a new species of *Pimeliaphilus* (Barry OConnor, pers. comm).

**Order: Araneae**  
**Family: Filistatidae?**

*Kukulcania* sp.?  
A single large, blackish spider was found in the apron of its web on the north side of a large boulder in the middle of the main trunk passage of the cave at survey station A5. The spider eluded capture, but is believed to be *Kukulcania* sp.

**Family: Plectreuridae**

*Kibramoa suprenans* Chamberlin  
The population of this spider at Bat Cave is pale in color and may represent a troglophilic population (Darrell Ubick, pers. comm). No troglophilic plectreurid spider population has been previously recorded (Ribera and Juberthie, 1994). This spider is the only large invertebrate predator in the cave-invertebrate food web here. The primary food of the older, larger spiders is the tenebrionid beetle *Eschatomoxys pholeter*. Below their webs an accumulation of the beetle bodies is often evident (Fig. 4). The spiders are abundant wherever there is support for their webs. They build their webs along the base of the cave walls, among rock piles, and in and under the walkway and machinery left from the guano-mining operation. Some recesses in the walls of the cave are festooned with old webs containing their ecdysed skins, old egg cases, and carcasses of *E. pholeter* (Fig. 5). A close-up view of an individual abandoned web is shown in Fig. 6.

The juvenile spiders spin their webs across small depressions on the cave walls or in recesses or depressions in the guano deposits on the floor of the cave. The latter generally occur at the edge of the active guano deposition areas where they are proximal to smaller invertebrates present on the surface of the guano. Interestingly, this species has not been recorded in other caves in the park.

**Family: Sicariidae**

*Loxosceles* sp.  
There are several troglophilic and a few troglobitic species in this genus that occur in the tropics (Ribera and Juberthie, 1994). This species exhibits no obvious morphological modifications that would indicate that it is adapted for living in a cave environment, and it is probably an epigean form. This may be the same species recorded from Thunder Cave by Peck (1980) and from Roaring Springs Cave by Drost and Blinn (1997).

This spider is not a prominent member of the biota within the cave, but is probably relatively common outside.
the cave in rocky areas along the cliff faces. The single female found was located on the cave floor at the base of the west wall of the passage, approximately 64 meters from the cave entrance. A reduction of cave passage dimensions in this area has an ameliorating affect on the cave microclimate. Closer to the entrance, where passage dimensions are considerably larger, hot, dry desert air from outside the cave easily mixes with the cave atmosphere, resulting in significant drying of the front part of the cave. The area of this initial passage constriction is where invertebrates of the guano food-web are first encountered. The brown spider has encroached on the periphery of the active food web, where it is not in competition with the dominant plectreurid spider. When first observed, this spider had a muscid fly (*Fannia* sp.) in its chelicerae.

**Family: Pholcidae**  
*Psilochorus* sp.

This troglobile is a small relative of the common cellar spider. Members of this family are often a common element of invertebrate biota of caves in the western U.S. *Psilochorus* has not previously been recorded from caves in the park, but is likely to be present in habitats similar to that present in Bat Cave. There is some depigmentation in this species, but no observable reduction of the eyes. Only a small population of these delicate spiders is found in the cave, and they typically occur away from areas of intense invertebrate activity at the fresh guano deposits. Their more delicate webs and the situations where they build may indicate that mostly small airborne prey are taken. No prey debris was observed in their webs. Their diet may include

---

**Figure 5.** Several old webs of *Kibramoa suprenans* hanging from a wall in the cave; scale is 15 cm.

**Figure 6.** Single old web structure of *Kibramoa suprenans* showing white egg-case enclosures at top and aggregation of shed spider skins trapped in lower portion.
the guano moth and small dipterous species associated with the fungus-covered guano and dead bats in the cave.

Family: Selenopidae
Selenops sp.

The presence of Selenops sp. in the cave was limited to a couple of shed skins found on the walls of the first room of the cave within the first six meters from the entrance. These spiders are foragers that typically occur in rocky habitats, spending most of their time in rock piles, crevices, and caves. Their presence here is not a surprise. They apparently do not go deeply enough into the cave to capitalize on prey supported by the bat guano deposit, where Kibramoa suprenans is the dominant predator.

Order: Pseudoscorpiones
Family: Chernetidae
Neoallochernes stercoreus Turk

This is the first record of this pseudoscorpion associated with a bat colony in Arizona (William Muchmore, pers. comm). This species is apparently the common pseudoscorpion associated with active Mexican free-tailed bat guano deposits, and is found in many caves in Texas where free-tailed colonies reside (Muchmore, 1992). A mean density of 135 individuals/dm² was recorded for this species at Fern Cave, Val Verde Co., Texas (Mitchell and Reddell, 1971). The estimated density of this species in Bat Cave at the time of sampling is approximately one fourth that value. However, the sampling was not done during the summer peak of the bat population and active guano deposition.

Interestingly, the species has not been found at Carlsbad Caverns National Park, which has a free-tailed colony of approximately 700,000 bats. The pseudoscorpion species present there is Dinocheirus astutus Hoff (Muchmore, 1992). Likely prey of N. stercoreus in Bat Cave includes mites, the psocid, and collembola, if they are present. No collembola were observed on or sampled from the guano.

The only other pseudoscorpion known from a cave in Grand Canyon National Park is Archeolatca cavicola from Cave of the Domes on Horseshoe Mesa (Welbourn, 1978). A. cavicola shows slight morphological modifications for its subterranean habit (Muchmore, 1981). Other species in that genus have been reported from caves at Wupatki National Monument, Arizona, and Guadalupe Mountains National Park, Texas (Muchmore, 1981).

Pseudoscorpions are a common element of cave and guano deposit ecosystems. I have observed them in many caves in the southwestern U.S., often in caves that are quite dry and where food resources seem to be minimal. Overall, the group appears to be more tolerant of arid conditions than other arthropod predators. Additional studies in caves in the park should reveal additional pseudoscorpion species.

Class: Hexapoda
Order: Pscoptera
Family: Psyllipsocidae

Psyllipsocus ramburii Selys-Longchamps

This barklouse seems to be the common psocid found in caves in the southwestern U.S., and indeed, has a worldwide distribution (Badonnel and Lienhard 1994). Psocids occurring in cave environments are probably detritivores, feeding on microflora or other organic sources (Mockford, 1993). The guano deposits at Bat Cave likely provide a whole suite of potential nutrient sources for this species. P. ramburii is associated with cave cricket (Ceuthophilus nr. pinalensis) guano at Arkenstone Cave in southern Arizona. There it is the prey of the cave-adapted pseudoscorion Albiorix anopthalmus Muchmore (Mockford, 1993; Muchmore and Pape, 1999). It is likely to be a prey species for Neoallochernes stercoreus in Bat Cave. Psocids (Psyllipsocidae) were recorded from several of the Horseshoe Mesa caves (Welbourn, 1978), and they are likely the same species. P. ramburii was also recorded from a single specimen at Tapeats Cave (Peck, 1980).

Order: Neuroptera
Family: Myrmeleontidae
Eremoleon pallens Banks

The larval pits of this antlion species are common in the dry, silty soils of the entrance room of the cave. Adults were seen flying in the evenings in the main passage in the vicinity of survey station A7, and small groupings of their wings were found clustered on the floor of the passage where Tadarida had captured the animals in flight and hung up on the ceiling to feed on the insects. This is a new county record for E. pallens in Arizona (Mohave County), and the only record of the species from a cave. The type species for E. pallens was from a mine shaft on Picacho Peak in southern Arizona. At least one species of Eremoleon (E. longior Banks) has previously been recorded from caves (Adams, 1956).

Order: Coleoptera
Family: Dermestidae
Dermestes carnivorus Fabricius

Dermestid beetles are common faunal elements on bat-guano deposits in the New World and Asia (Mitchell and Reddell, 1971; Decu et al., 1998; Gnaspini and Trajano, 2000). Where they occur in association with bat guano, they are typically the dominant decomposer, and they may be present in enormous numbers. No dermestid species is established in the cave, and colonization attempts by dermestids, which likely occur on occasion, are apparently repelled by predatory or scavenging elements in the guano community, possibly including Eschatomoxys pholerter, which could feed on eggs and/or young larvae of dermestids. Only a single D. carnivorus was found in the cave, on October 26, 2001. The animal was probably attracted to the odor of the guano deposit, which is detectable even by humans at great distance. The beetle was found near the front part of the cave (near survey station A5).
Family: Tenebrionidae

*Eschatomoxys pholeter* Thomas and Pape

This beetle species was described using specimens taken at Bat Cave during this study. The species also occurs in other caves in western Grand Canyon National Park and the Grand Canyon–Parashant National Monument (Pape et al., 2007). The species is probably not strictly a cave species, and likely also occurs in dry situations among cliffs, where it is probably associated with fecal materials in rodent nests. This is similar to the habit of *E. tanneri* Sorensen and Stones, which has been recorded in southern Utah (Sorensen and Stones, 1959) and in Marble Canyon, Arizona (Pape et al., 2007). Peck (1980) recorded a single specimen of *Eschatomoxys* sp. from Thunder Cave, also in the Grand Canyon. The location of this record is about midway between the known distributions of *E. pholeter* and *E. tanneri*. The Thunder Cave record probably represents one of these two species, but since the disposition of the specimen is unknown it could not be examined. The specimen was taken off a wall of a dry upper passage above some (bat?) guano (Peck 1980).

Peck (1980) recognized that *Eschatomoxys* spp. show morphological adaptations to a subterranean habitat. The head and pronotum are narrowed, and there is a definite attenuation of the legs and antennae. He suggested that this morphology represents adaptation for scavenging in animal nests and burrows rather than being associated with caves. This seems reasonable, considering most records of the genus have not been from caves, except for *E. pholeter*, which has so far been recorded only from caves. The species probably colonizes caves opportunistically from adjacent shelter and crevice habitats where they are probably associated with rodents.

The greatest density of the beetles in the cave is on the active bat-guano deposits. Otherwise they are thinly distributed throughout the front portion of the cave, except in the entrance room, which may be too dry. They scavenge over the surface of the guano and probably feed on a variety of foods, including dead bats. They were observed feeding on the dead bats presumed to have been caught by a ringtail near the front part of the main passage of the cave (Pape et al., 2007: figure 9). There were no larvae of *E. pholeter* found in the guano deposits, and they were not present in the two guano samples removed from the cave. The larvae may be present only during times of the year when bat activity is at its peak. The beetle is the primary prey of the spider *Kibramoa suprenans* at Bat Cave.

*E. pholeter* is the only invertebrate species that was observed beyond the passage constriction at survey station B9. Only a couple of individuals were present there, and it is probable these were vagrants from the nearby active guano deposit. A search of the fossil guano deposit for invertebrate activity gave negative results, and the deposit is likely depleted of useable nutrients.

Family: Curculionidae

*Smicronyx imbricatus* Casey

This weevil has been recorded from the Gulf coastal plain and “central lowlands” (north-central plains) in Texas to the Sierra Nevada and coast-range sections of the Pacific mountain system, and it is widely distributed in the basin and range province (Anderson, 1962). I assume the weevils emerge in the cave from seeds contained in scats deposited by ringtails. *S. imbricatus* was found in moderate abundance at the peak of the guano deposit at survey station D1. The natural proclivity of many weevils is to climb up vegetation and other objects. The only thing available for them to climb in the cave is a mountain of guano, so they congregate at the top along with other invertebrates. The presence of this species is coincidentally associated with ringtail use of the cave.

Order: Siphonaptera
Family: Ischnopsyllidae

*Sternopsylla distincta* Rothschild

This flea is known from throughout the southern portion of the United States, where it is associated primarily with the Mexican free-tailed bat and the western bonneted bat (*Eumops perotis* Schinz) (Hubbard, 1968). It is found in large numbers on the active guano piles at Bat Cave when the bats are present. The bats groom the fleas off their bodies, and the fleas drop to the guano pile. There is no way for the fleas to return to the host once they are removed, and some may become prey of predators on the guano piles. There are currently no other records of fleas from caves in the park, but associations with birds, rodents, bats, and other cave-frequenting mammals makes their presence likely.

Order: Diptera
Family: Muscidae

*Fannia* sp.

A single individual of this muscid fly was found in the cave. It had been captured by the single female *Loxosceles* spider observed in the cave. Its association with the ecology of the cave, if any, is not known.

Order: Lepidoptera
Family: Tineidae

Tineid moth (new genus and species)

This animal is a new genus and species (Don Davis, pers. comm.). This small, weakly flying moth is common in the cave. They are readily noticed because their wing scales reflect a silvery color in the light from headlamps. The larvae of the moths probably derive nourishment directly from the fresh guano (Vandel, 1965) and would then be considered guanobionts. Alternatively, they may be fungivores. They may be preyed upon by spiders present in the cave (*Psilochorus* sp., *Kibramoa suprenans*, and *Loxosceles* sp.). The moths were apparently attracted to the dead *Tadarida* found near the front of the cave. This association is not understood. The only other record of a tineid moth
from a cave in the park is from one of the caves on Horseshoe Mesa (Welboun, 1978).

**Tinea (pallescensella?)**

A single individual of this animal was found in the cave. The moth is larger and distinctively different from the undescribed species listed above. The specimen was damaged and was not positively identifiable to species, but may be *T. pallescensella*. Its association with the ecology of the cave is not known.

Order: Hymenoptera  
Family: Sphecidae  
*Sceliphron caementarium* Drury  
The presence of this sphecid wasp was evident from the abundant multi-celled mud nests observed throughout the entrance area. Nests were present on the walls of the cave and on the wooden walkway supports, pulleys, and cables left from the guano mining operation. One of the nests contained sixteen cells. None of the nests were in use during our visits to the cave. *S. caementarium* provisions its nests with spiders. Due to the dry, sheltered environment inside the cave entrance, the old nest cells may have accumulated over a very long time.

**Cave Microclimate**

A series of temperature and relative humidity measurements were taken in the cave on March 29, 1996, beginning at the cave entrance (at 1617 hrs), and progressing deeper into the cave to the bat-occupied roost areas. Temperature and humidity at the cave entrance (drip line) were 17 °C and 15.4 percent, respectively. Both of these climatic parameters increased toward the rear of the cave at the time of our visit. The lower values at the cave entrance were the result of the large size of the entrance and its proximity to, and air exchange with, the arid desert environment outside of the cave. The microclimate of the cave at the bat roosts is greatly influenced by the presence of the bats, particularly when they are in residence in large numbers. Their presence results in a localized increase in both relative humidity and ambient air temperature. At the first bat roost (229 meters from the entrance; Fig. 1, survey station D1), where a few hundred bats were present, the values were 26 °C and 89.0 percent at fifteen centimeters above the guano deposit. Deeper in the cave, along the main trunk passage, the air temperature drops slightly at the high point in the cave at the main bat roost (265 meters from the entrance; Fig. 1, between survey stations B7 and B9). Here the air temperature was 25 °C and the relative humidity 100.0 percent. The slightly lower air temperature at this location may have been due to a larger volume of air that was less affected by the small number of bats present at the time the measurements were taken. Additionally, air movement along the main trunk passage may have caused some minor mixing with cooler air from the front part of the cave.

**Bat Cave Food Web**

The food web in the cave is supported primarily by the annual deposition of guano introduced by the Mexican free-tailed bat colony. While some bats are present throughout much of the year, the greatest numbers are in residence from April through September. The bats leave the cave after sunset each evening to feed on insects and return to roost in the cave and digest their food. Their urine and fecal pellets drop to the floor and build into large deposits over many years. The annual guano layer supports most of the arthropod activity, with layers from previous years, depleted of nutrients, showing little or no arthropod activity.

Adult bats that die of natural causes fall to the guano pile and provide additional nutrients. During the summer, juvenile bats that lose their grasp on the ceiling and are unable to fly also drop to the guano deposit and are consumed by arthropods and, probably, ringtails. Parasites that are groomed from the bats drop to the guano pile, where they become food sources for other microfauna. Ringtail scats provide a small amount of additional allochthonous nutrient input to the cave. The lack of a rhaphidophorine cave cricket at Bat Cave may be due to inadequate vegetation in proximity to the cave.

The diagram of the cave food web, as it is currently understood, is presented in Fig. 7. Most associations shown are assumed and are based on typical life habits of the taxa present. However, several of the associations were documented during the study, including the Mexican free-tailed bats feeding on the antlion (*E. pallens*), the ringtail feeding on both bats (*T. brasiliensis*) and the tenebroid beetle *E. pholeter*, the plectreurid spider (*K. suprenans*) also feeding on *E. pholeter*, and the brown spider *Loxosceles* sp. feeding on the muscid fly (*Fannia* sp.).

**Summary**

The annual deposit of *Tadarida* guano is the only nutrient input of any consequence entering Bat Cave. The entrance to the cave is horizontal, and the ceiling at the entrance overhangs the debris slope from the cave, precluding allochthonous organic debris washing into the cave and contributing nutrients. Only very small quantities of water penetrate the thick bedrock overburden at the main fault along which the cave is developed. The only water observed in the cave was several small drip points concentrated in an area along the west wall 230 meters from the entrance and at the top of the large guano deposit 265 meters from the entrance and very small quantities of condensate water found in the fossil guano deposit approximately 360 meters from the entrance. No arthropods were found at any of these water sources.

Most of the invertebrates present in Bat Cave are associated directly with the active guano deposit. Previous annual guano layers beneath the freshly accumulating layer are drier and appear mostly devoid of arthropod activity.
However, the drier condition of the lower layers may provide suitable habitat for eggs, larvae, or pupae of some species inhabiting the guano. The drier guano may inhibit potentially harmful fungi associated with the higher moisture content of the fresh guano. No arthropod activity was present in the nutrient-depleted fossil guano deposit at the rear of the cave.

Besides the presence of the large bat colony and the associated guano deposit, Bat Cave would appear to follow the pattern described by Welbourn (1978) for the caves at Horseshoe Mesa and many other caves in Grand Canyon National Park. Because of the generally arid climate in the region during the last ten to twelve thousand years (Van Devender, 1990), many caves, especially those with multiple entrances, have dried to a considerable degree. Cave invertebrates that may have been present in more mesic, wetter times were likely extirpated during this extended drying period. While Bat Cave is nearly devoid of free water sources, the humidity in the cave increases from the front to the rear of the cave. This is due to the cave having a single entrance, which minimizes air exchange with the arid exterior environment, and moisture contributed by the bats through their respiration and urination.

A total of 27 taxa, including five vertebrates and 22 macro-invertebrate species, were identified as elements of the ecology of Bat Cave. Two of the macro-invertebrates, *Eschatomoxys pholeter* (Coleoptera: Tenebrionidae) and an undescribed genus of tineid moth are species first discovered during this study. Additional study would likely reveal additional macro-invertebrate species in the cave. Bat Cave has the most species rich macro-invertebrate ecology currently known in a cave in the park.

**ACKNOWLEDGEMENTS**

I thank Robert A. Winfree, Senior Scientist at Grand Canyon National Park at the time this study was conducted, for his encouragement and cooperation with this project. Special thanks are due to Ray Keeler for handling all the logistics that made the expeditions to the cave possible. Ray also reviewed the manuscript and provided the plan map of the cave for Figure 1.

Sincere appreciation is extended to the following specialists who performed identifications: Barry M. O’Connor, University of Michigan (Acari); Darrell Ubick, California Academy of Sciences (Araneae); William Muchmore, University of Rochester (Pseudoscorpiones); Don Davis, National Museum of Natural History (Amphipoda, Oligochaeta); and Carl Funktion, Natural History Museum, London (Crustacea).
of Natural History, Smithsonian Institution (Lepidoptera); and Charles W. O’Brien, Florida A & M Univ. (Coleoptera: Curculionidae). I thank Carl Olson, University of Arizona Department of Entomology, for reviewing the manuscript. I also thank the three anonymous reviewers whose incorporat-ed suggestions improved this manuscript. I thank Charles Drost, USGS-CPRS, for providing the records of the two species of Myotis from Bat Cave. I thank my wife Esty Pape for assistance with graphics and proofreading of the manuscript. This study was partially funded with the aid of a grant from the National Speleological Society.

REFERENCES


MICROHABITAT INFLUENCES THE OCCURRENCE OF AIRBORNE FUNGI IN COPPER MINE IN POLAND

Wojciech Pusz1, Włodzimierz Kita1, and Ryszard Weber2

Abstract: From January to April 2012 we studied the occurrence of air-borne fungi in the Lubin mining site, property of KGHM Polska Miedź SA. The research was conducted in three copper-mining shafts: Bolesław, Lubin Zachodni (Lubin West shaft), and Lubin Główny (Lubin Main shaft) at about 610 to 850 meters below ground level. Air samples were collected between 6 and 9 a.m. using the impact method (Air Ideal 3P Sampler) onto Potato Dextrose Agar. The volume of air sampled for each agar plate was 50 liters. We found twenty-seven fungal species, the most numerous being Penicillium notatum, P. urticae, and Aspergillus flavus. As the application of log-linear and correspondence analyses have shown, the population of fungi varied considerably among the copper mine shafts or shaft parts. P. notatum and P. urticae were found to be the best adapted to grow in copper-mine conditions. The significant interaction among the shafts and the sample collection sites suggests a substantial microclimate influence on the population-size variations of studied fungal species in each shaft. The fungal-spore concentration in the majority of the shafts of this copper mine does not present a health risk to the mine workers. But it is enhanced in some portions of the mine, so it may constitute a health hazard locally.

INTRODUCTION

The copper-ore deposits near the town of Lubin were discovered during a geological survey in 1957. The construction of the Lubin copper-mining site began in 1960, laying a foundation for what later became the Copper Mining and Smelting Industrial Complex (KGHM). In the Lubin facility copper is mined at about 610 to 850 meters below ground level, and the operations cover approximately 160 km² of surface.

The microclimate in some Lubin mine shafts and galleries displays features similar to the tropical rainforest climate. At some points the temperatures rise to 33 °C and the relative humidity exceeds 90%. These conditions, with the presence of organic substances, creates a fairly favorable growth environment for microscopic fungi, which can be found on any material used in the mining process, especially on timber and all types of organic waste, as well as on insulators, rubber surfaces, or machinery tires (Piontek and Bednar, 2010). The miners going down to work bring fungi into the mine. The research conducted by Grzyb and Frączek (2010) in spa rooms of the underground salt mine in Bochnia has shown that the number of colony forming units obtained before the staff and clients entered the spa was considerably smaller than the CFU value in high tourist season.

The growth of fungi in the KGHM mine galleries and excavations is considerable and produces a great number of spores that spread through the ventilation system. Frequent air changes, which are characteristic to the microclimate of the KGHM Lubin mining site, result in significant variations of the number of CFU present in the studied air samples. Some airborne fungal species (*Aspergillus* spp., *Penicillium* spp., *Cladosporium* spp.) that occur in the mine excavations and galleries could cause allergies and fungal infections in some exposed workers (Heppleston et al., 1949; Gamboa et al., 1996; Obtułowicz, 2006; Cabral, 2010). The official limits of airborne fungal-spore concentration indoors used in Poland as safety standards (Polish Norm, 1989a, b, and 2007) do not apply to mine excavations or galleries. Most of the fungal spores found in the copper-mine air samples or on organic material such as timber safety structures built in the galleries produce mycotoxins. These can cause various diseases when introduced into the human body (Rusca et al., 2008; Ogórek et al., 2013). The aim of our research was to determine which species of airborne fungi occur in the copper mine and to identify their CFU concentrations.

MATERIALS AND METHODS

This study was performed from January to April 2012 in the Lubin mining facilities, owned by KGHM Polska Miedź SA. The measurements were taken in three shafts: Bolesław shaft, Lubin Zachodni (Lubin West shaft), and Lubin Główny (Lubin Main shaft), at about 610 to 850 meters below ground level. The samples were collected between 6 and 9 a.m. At this time of day four hundred miners work in the Lubin West shaft and the Lubin Main shaft, while approximately nine hundred miners work in the Bolesław shaft. We used the impact method and the Air

---

1 Wroclaw University of Environmental and Life Sciences, Department of Plant Protection, Division of Phytopathology and Mycology; pl. Grunwaldzki 24a, 50-363 Wroclaw, Poland, wojciech.pusz@up.wroc.pl
2 Institute of Soil Science and Plant Cultivation, State Research Institute in Pulawy; Department of Weed Science and Tillage Systems in Wroclaw
colonies grown on one plate per 1 m$^3$ was determined from air measurements at each site were performed three times, and the impactor was held at 1.5 m above the ground. The PDA plates were incubated for seven days at room temperature (22 °C). After incubation, the number of visible colonies was determined, and the fungi were identified into species according to their morphology. The number of colony forming units (CFU) was determined from PDA plates manufactured by Biocorp. Ideal 3P sampler to analyze the fungal load using PDA plates was incubated for seven days at room temperature (22 °C). After incubation, the number of visible colonies was determined, and the fungi were identified into species according to their morphology. The number of colony forming units (CFU) was determined from PDA plates manufactured by Biocorp.

The log-linear and correspondence analyses were performed in order to investigate the relationship among the shafts and the variations in the number of CFU for selected fungal species.

Log-linear analysis is used to examine quantity variations in cross-tabulation. It is also helpful in performing an analysis of variance if no individual observations can be made at any level of the objects experimented on (null values). The log-linear analysis applied to this study is based on Goodman (1978). All significant deviations of the observed quantities from the expected quantities indicate an interaction between the analyzed variables. After a logarithmic transformation of the expected values, the model is expressed as a linear model that, in its most simple form, can be expressed as follows:

$$\ln(E_{ij}) = M + \lambda_i^X + \lambda_j^Y + \lambda_{ij}^{XY}$$

where $E_{ij}$ are the expected values, $M$ is the overall mean of the expected values based on equal counts in each cell, $\lambda_i^X$ is the effect of the $i$th value of variable $X$, $\lambda_j^Y$ is the effect of the $j$th value of variable $Y$, and $\lambda_{ij}^{XY}$ is the interaction effect of the $i$th value of variable $X$ and the $j$th value of variable $Y$.

The log-linear model allows us to test the null hypothesis, in which there is no relationship between two or more of the analyzed variables. After eliminating non-significant interactions, the model can also be used to investigate the influence of specific factors on the variation of the studied populations. In order to assess a testing model that would be optimal for the specific factors of this experiment, the chi-squared test was calculated for main effects, then the extended log-linear models were tested, taking into account the second- and third-order interactions between the studied factors. An iterative procedure was applied to fit the model to the observed frequencies. The iterative algorithm was stopped when the fit and the observed marginal distributions converged with a ratio of 0.01.

In practice, these procedures usually eliminate insignificant interactions between observed variables from the model. In the next section, we discuss the structure of relationships between the shafts and the observed population size of each analyzed fungal species. The discussion is based on correspondence analysis, popularized by Hill (1974) and often also referred to as reciprocal averaging, homogeneity analysis, dual scaling, or canonical analysis of contingency tables. The analysis visualizes the associations among a set of points in a two-, or (maximum) three-dimensional space. Mapping the variations among the identified fungal species also allows for presenting the variability of the analyzed factors.

### RESULTS

Analyzing the air in the copper-mine galleries, we identified twenty-seven airborne fungal species with *Penicillium notatum*, *P. urticae*, and *Aspergillus flavus* being the most numerous. The significance assessment of main effects and interactions among the factors of this experiment is presented in Table 1.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Degrees of Freedom</th>
<th>Chi-squared (Partial Association)$^a$</th>
<th>Chi-squared (Marginal Association)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of shaft (1)</td>
<td>2</td>
<td>31477.4</td>
<td>31477.4</td>
</tr>
<tr>
<td>Part of shaft (2)</td>
<td>5</td>
<td>77662.7</td>
<td>77662.7</td>
</tr>
<tr>
<td>Fungal species (3)</td>
<td>10</td>
<td>114938.1</td>
<td>114938.1</td>
</tr>
<tr>
<td>1 x 2</td>
<td>10</td>
<td>32381.5</td>
<td>38421.0</td>
</tr>
<tr>
<td>1 x 3</td>
<td>20</td>
<td>24606.8</td>
<td>30646.2</td>
</tr>
<tr>
<td>2 x 3</td>
<td>50</td>
<td>24700.4</td>
<td>30739.8</td>
</tr>
</tbody>
</table>

$^a$ In all cases, the $p$ values is highly significant ($p \leq 0.01$).

Ideal 3P sampler to analyze the fungal load using PDA plates manufactured by Biocorp. The volume of air sampled for each plate was 50 liters. The measurements at each site were performed three times, and the impactor was held at 1.5 m above the ground.
population sizes and the type of shaft, as well as between the sample collection point and the number of identified fungal species, point to a considerable variation within the fungal population that depends on the factors examined during the experiment. As shown in Tables 2, 3, and 4, many more fungal species were identified in Bolesław shaft than in Lubin Main shaft. The *P. notatum* species occurred mostly in the Bolesław and Lubin West shafts. The Lubin Main shaft was colonized mostly by *P. urticae*, *P. waksmani*, and *P. commune*. Probably the climate conditions of the Lubin West shaft were not favorable to the growth of the *P. commune* (particularly high temperatures do not favor the growth of this species). Meanwhile, we observed that the population of the *A. flavus* species is relatively large in the Bolesław shaft, when compared to its size in other shafts. Finding a significant interaction between the fungal species and the parts of each shaft let us confirm that more fungi occurred in the timber storage areas of the shafts Bolesław and Lubin West than beside the conveyor-belt system of the Lubin Main shaft. Also, many more fungal colonies were collected in the pump room of the Main shaft than in the so-called air split of the West shaft.

The relationship between the type of shaft and the sampling point on the one hand, and the population size of the fungal species on the other, are discussed below, where the correspondence plots of the fungal response to the analyzed factors represent the data in two dimensions, displaying the full information on the interactions among the shafts (Fig. 1).

If we look at the two-dimensional position of each species in the plot, we can identify three groups of fungi with different population sizes, depending on shaft and sample collection point. The first group involves *P.*

### Table 2. Fungal population sizes in various parts of Bolesław shaft (CFU/m³).

<table>
<thead>
<tr>
<th>Fungi</th>
<th>Timber Storage</th>
<th>Maintenance Chamber</th>
<th>Dump Hopper</th>
<th>Gob Fence</th>
<th>Machine Chamber</th>
<th>Mouth</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aspergillus flavus</em></td>
<td>7560</td>
<td>220</td>
<td>760</td>
<td>240</td>
<td>680</td>
<td>1</td>
<td>9461</td>
</tr>
<tr>
<td><em>Aspergillus niger</em></td>
<td>0</td>
<td>40</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>41</td>
</tr>
<tr>
<td><em>Botrytis cinerea</em></td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>40</td>
<td>43</td>
</tr>
<tr>
<td><em>Penicillium citrinum</em></td>
<td>0</td>
<td>0</td>
<td>240</td>
<td>1</td>
<td>12</td>
<td>0</td>
<td>253</td>
</tr>
<tr>
<td><em>Penicillium commune</em></td>
<td>0</td>
<td>0</td>
<td>92</td>
<td>1840</td>
<td>0</td>
<td>0</td>
<td>1932</td>
</tr>
<tr>
<td><em>Penicillium expansum</em></td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td><em>Penicillium luteum</em></td>
<td>0</td>
<td>340</td>
<td>1</td>
<td>12</td>
<td>1</td>
<td>1</td>
<td>355</td>
</tr>
<tr>
<td><em>Penicillium notatum</em></td>
<td>8528</td>
<td>240</td>
<td>6520</td>
<td>9560</td>
<td>326</td>
<td>60</td>
<td>25234</td>
</tr>
<tr>
<td><em>Penicillium urticae</em></td>
<td>4420</td>
<td>380</td>
<td>440</td>
<td>1360</td>
<td>240</td>
<td>1</td>
<td>6841</td>
</tr>
<tr>
<td><em>Penicillium vermiculatum</em></td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td><em>Penicillium waksmani</em></td>
<td>960</td>
<td>1</td>
<td>420</td>
<td>680</td>
<td>80</td>
<td>0</td>
<td>2141</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>21470</strong></td>
<td><strong>1223</strong></td>
<td><strong>8474</strong></td>
<td><strong>13694</strong></td>
<td><strong>1341</strong></td>
<td><strong>105</strong></td>
<td><strong>46307</strong></td>
</tr>
</tbody>
</table>

### Table 3. Fungal populations in various parts of Lubin West shaft (CFU/m³).

<table>
<thead>
<tr>
<th>Fungi</th>
<th>Timber Storage</th>
<th>Transfer Point</th>
<th>Dump Hopper</th>
<th>Air Split</th>
<th>Machine Chamber</th>
<th>Mouth</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aspergillus flavus</em></td>
<td>740</td>
<td>1580</td>
<td>60</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2382</td>
</tr>
<tr>
<td><em>Aspergillus niger</em></td>
<td>60</td>
<td>200</td>
<td>1</td>
<td>40</td>
<td>1</td>
<td>1</td>
<td>303</td>
</tr>
<tr>
<td><em>Botrytis cinerea</em></td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td><em>Penicillium citrinum</em></td>
<td>0</td>
<td>560</td>
<td>1</td>
<td>0</td>
<td>40</td>
<td>140</td>
<td>741</td>
</tr>
<tr>
<td><em>Penicillium commune</em></td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><em>Penicillium expansum</em></td>
<td>1220</td>
<td>840</td>
<td>40</td>
<td>1</td>
<td>0</td>
<td>60</td>
<td>2161</td>
</tr>
<tr>
<td><em>Penicillium luteum</em></td>
<td>560</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>562</td>
</tr>
<tr>
<td><em>Penicillium notatum</em></td>
<td>7780</td>
<td>780</td>
<td>40</td>
<td>60</td>
<td>80</td>
<td>220</td>
<td>8960</td>
</tr>
<tr>
<td><em>Penicillium urticae</em></td>
<td>5340</td>
<td>2520</td>
<td>60</td>
<td>100</td>
<td>0</td>
<td>20</td>
<td>8040</td>
</tr>
<tr>
<td><em>Penicillium vermiculatum</em></td>
<td>720</td>
<td>1180</td>
<td>60</td>
<td>260</td>
<td>1</td>
<td>1</td>
<td>2222</td>
</tr>
<tr>
<td><em>Penicillium waksmani</em></td>
<td>3300</td>
<td>0</td>
<td>40</td>
<td>80</td>
<td>60</td>
<td>100</td>
<td>3580</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>19721</strong></td>
<td><strong>7661</strong></td>
<td><strong>303</strong></td>
<td><strong>545</strong></td>
<td><strong>182</strong></td>
<td><strong>542</strong></td>
<td><strong>28954</strong></td>
</tr>
</tbody>
</table>
notatum and *A. flavus*, the second group is formed by *A. niger*, *P. vermiculatum*, *P. expansum*, *P. luteum*, *P. urticae*, and *P. waksmani* and the third group with greater variation in fungal population sizes of *Botrytis cinerea*, *P. citrinum*, and *P. commune*. As pictured by the length and direction of the vectors starting at the origin of the coordinate system, each shaft influences the population size of each fungal species in many different ways. The considerable length of the vector associated with the Main shaft indicates that this variable has a considerable discriminating power, which contributes to differentiating the population size of each fungal species. The orthographic projections of the points, which represent the fungal species, onto vectors associated with the variables, confirm the increase in *A. flavus* and *P. notatum* populations in Bolesław shaft. On the other hand, the position of the point that represents the size variations of the *P. expansum* population suggests that this fungus is well adapted to grow in the climate conditions of the Lubin West shaft. Also, the projection of the point representing the variability of *P. citrinum*, on the extended vector associated with Lubin Main shaft, confirms the increased growth of its population in the Lubin Main shaft conditions.

The second column of Table 5 displays the row masses, which are the total relative frequencies of each row. These can be interpreted as information on the significance of each row (shaft) for the population size variations within the analyzed fungal species. Thus, the climate conditions in Bolesław shaft stimulated the growth of fungal populations to the largest extent, while the conditions in the Main shaft decreased the population sizes of the analyzed species. The

Table 4. Fungal populations in various parts of Lubin Main shaft (CFU/m³).

<table>
<thead>
<tr>
<th>Fungi</th>
<th>Belt Conveyor</th>
<th>Foreman’s Office</th>
<th>Dump Hopper</th>
<th>Main pump Room</th>
<th>Machine Chamber</th>
<th>Mouth</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aspergillus flavus</em></td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>102</td>
</tr>
<tr>
<td><em>Aspergillus niger</em></td>
<td>0</td>
<td>60</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>62</td>
</tr>
<tr>
<td><em>Botrytis cinerea</em></td>
<td>80</td>
<td>4</td>
<td>80</td>
<td>0</td>
<td>20</td>
<td>1</td>
<td>185</td>
</tr>
<tr>
<td><em>Penicillium citrinum</em></td>
<td>120</td>
<td>40</td>
<td>1</td>
<td>560</td>
<td>0</td>
<td>200</td>
<td>921</td>
</tr>
<tr>
<td><em>Penicillium commune</em></td>
<td>180</td>
<td>0</td>
<td>300</td>
<td>560</td>
<td>0</td>
<td>0</td>
<td>1040</td>
</tr>
<tr>
<td><em>Penicillium expansum</em></td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>40</td>
<td>42</td>
</tr>
<tr>
<td><em>Penicillium luteum</em></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td><em>Penicillium notatum</em></td>
<td>80</td>
<td>1</td>
<td>4</td>
<td>80</td>
<td>0</td>
<td>0</td>
<td>425</td>
</tr>
<tr>
<td><em>Penicillium urticae</em></td>
<td>40</td>
<td>100</td>
<td>140</td>
<td>2240</td>
<td>7</td>
<td>40</td>
<td>2567</td>
</tr>
<tr>
<td><em>Penicillium vermiculatum</em></td>
<td>0</td>
<td>80</td>
<td>100</td>
<td>540</td>
<td>1</td>
<td>0</td>
<td>721</td>
</tr>
<tr>
<td><em>Penicillium waksmani</em></td>
<td>0</td>
<td>40</td>
<td>0</td>
<td>1080</td>
<td>720</td>
<td>80</td>
<td>1320</td>
</tr>
<tr>
<td>Total</td>
<td>502</td>
<td>426</td>
<td>626</td>
<td>5062</td>
<td>149</td>
<td>623</td>
<td>7388</td>
</tr>
</tbody>
</table>

Figure 1. Biplot of population-size variations within the fungal species occurring in different KGHM mine shafts (V1 – dimension 1, V2 – dimension 2). Dimensions 1 and 2 show the size of the variation of the number of species of fungi (variance).
representation quality of the biplot points determines how precisely each shaft can be evaluated in a space reduced to two dimensions. Values close to 1 indicate that the species have been represented well in the two-dimensional space. Reducing the eleven-dimensional space to a two-dimensional space for the purpose of this experiment still allows for a full presentation of the original variations. The relative inertia of each point shows each variable’s absolute contribution to the principal inertia of the original space of eleven dimensions that described the three shafts. The term inertia in correspondence analysis is used by analogy with the definition in physics of moment of inertia, which stands for the integral of mass times the square of the distance to the centroid (Greenacre, 1984). Inertia is defined as the total Pearson Chi-square for the two-way interaction divided by the total sum of the spore numbers of the sampled fungi species.

As suggested by relative inertia values, an increased population size variation within the identified fungal species occurred in Lubin Main shaft. The next columns present the contribution of partial inertia values of the shafts Boleslaw, Lubin West, and Lubin Main, to each dimension. The correspondence analysis also allows us to evaluate the similarities among the fungal species and to present them in a space defined by these three shafts. As shown in Table 6, both the mass value and the relative inertia values in the two dimensions are highest for the species occurred in Lubin Main shaft. The next columns show the size of the variation of the number of species of fungi (variance).

<table>
<thead>
<tr>
<th>Fungal Species</th>
<th>Mass</th>
<th>Relative Inertia</th>
<th>Inertia in Dimension 1</th>
<th>Inertia in Dimension 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus flavus</td>
<td>0.144527</td>
<td>0.096281</td>
<td>0.132044</td>
<td>0.002185</td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>0.004912</td>
<td>0.012152</td>
<td>0.013403</td>
<td>0.008860</td>
</tr>
<tr>
<td>Botrytis cinerea</td>
<td>0.005324</td>
<td>0.023762</td>
<td>0.003242</td>
<td>0.07750</td>
</tr>
<tr>
<td>Penicillium citrinum</td>
<td>0.020629</td>
<td>0.164708</td>
<td>0.169629</td>
<td>0.151762</td>
</tr>
<tr>
<td>Penicillium commune</td>
<td>0.035971</td>
<td>0.114969</td>
<td>0.004670</td>
<td>0.405176</td>
</tr>
<tr>
<td>Penicillium expansum</td>
<td>0.026691</td>
<td>0.132466</td>
<td>0.071906</td>
<td>0.291805</td>
</tr>
<tr>
<td>Penicillium luteum</td>
<td>0.011131</td>
<td>0.010491</td>
<td>0.001376</td>
<td>0.034473</td>
</tr>
<tr>
<td>Penicillium notatum</td>
<td>0.418868</td>
<td>0.168344</td>
<td>0.230110</td>
<td>0.005833</td>
</tr>
<tr>
<td>Penicillium urticae</td>
<td>0.211110</td>
<td>0.073696</td>
<td>0.100904</td>
<td>0.002110</td>
</tr>
<tr>
<td>Penicillium verruculosum</td>
<td>0.035645</td>
<td>0.131304</td>
<td>0.173635</td>
<td>0.019927</td>
</tr>
<tr>
<td>Penicillium waksmani</td>
<td>0.085192</td>
<td>0.071826</td>
<td>0.099081</td>
<td>0.000118</td>
</tr>
</tbody>
</table>

Note: The inertia term is used in correspondence analysis and is analogous to that in terms of variance statistics. Inertia dimensions 1 and 2 show the size of the variation of the number of species of fungi (variance).

Table 6. Inertia contribution by fungal species (Columns).

Table 5. Inertia contribution by shaft (Rows).

<table>
<thead>
<tr>
<th>Intertia Contributions by Shaft</th>
<th>Relative Inertia</th>
<th>Inertia in Dimension 1</th>
<th>Inertia in Dimension 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boleslaw</td>
<td>0.560285</td>
<td>0.296208</td>
<td>0.389829</td>
</tr>
<tr>
<td>Lubin West</td>
<td>0.350325</td>
<td>0.281218</td>
<td>0.227731</td>
</tr>
<tr>
<td>Lubin Main</td>
<td>0.089390</td>
<td>0.422574</td>
<td>0.382440</td>
</tr>
</tbody>
</table>

Note: The inertia term is used in correspondence analysis and is analogous to that in terms of variance statistics. Inertia dimension 1 and 2 show the size of the variation of the number of species of fungi (variance).
be an important part of indoor bioaerosols since some of them can produce mycotoxins, which are hazardous to human health (Cabral, 2010). Some of these fungal species can also cause mycoses or other respiratory diseases (Gamboa et al., 1996). Nevertheless, to confirm these results, we would need to perform a series of in vitro or molecular tests, because not all fungal isolates sampled from the atmosphere are able to produce mycotoxins (Ren et al., 1999; Nielsen, 2003).

**Conclusions**

The log-linear and correspondence analyses show significant size variations within the fungal populations in each of the copper mine’s shafts, as well as in parts of these shafts.

Based on their numbers and abundance, *Penicillium notatum* and *Penicillium urticae* turned out to be the fungal species best adapted to grow in a copper-mine environment.

There are significant differences among the shafts and the sample collection points, suggesting a considerable influence of each shaft’s microclimate on the population size variations of the identified fungal species. The fungal spore concentrations in the analyzed shafts do not pose a health hazard to miners.

**Acknowledgements**

We wish to thank to the management staff of KGHM for their acceptance concerning our conducting the research in the mine of the Lubin Mining Company. In particular, our thanks go to the Director of the LMC, Mr. Krzysztof Tkaczuk. We also want to thank Mr Wojciech Sobieszek, the director for safety issues, for his kindness and assistance during our work in the mine. Many thanks go as well to safety inspectors Artur Poniedziałek, Dariusz Mróz, Krzysztof Sokolowski, and Wojciech Was. Additionally, we acknowledge the kindness and invaluable hints given to us by all the foremen of the LMC during our underground work. Finally, special thanks go to Ms. Agata Kaczmarek from the Division of Phytopathology and Mycology of the Plant Protection Department at the Wrocław University of Environmental and Life Sciences for her help in the preparation of the mycological analyses.

**References**


Polish Norm, 1989a, PN-89/Z-04111/02: Air Purification Testing. Determining the Concentration of Bacteria in the Atmosphere (ambient concentration) by Aspiration and Sedimentation Sampling [In Polish]: Polski Komitet Normalizacji Mar i Jakości (Polish Committee for Measurements and Quality Standards).

Polish Norm, 1989b, PN-89/Z-04111/03: Air Purification Testing. Microbiological testing. Determining the Concentration of Microscopic Fungi in the Atmosphere (ambient concentration) by Aspiration and Sedimentation sampling [In Polish]: Polski Komitet Normalizacji Mar i Jakości (Polish Committee for Measurements and Quality Standards).


MILLIPEDES (DIPLOPODA) FROM CAVES OF PORTUGAL

ANA SOFIA P.S. REBOLEIRA1 AND HENRIK ENGHOFF2

Abstract: Millipedes play an important role in the decomposition of organic matter in the subterranean environment. Despite the existence of several cave-adapted species of millipedes in adjacent geographic areas, their study has been largely ignored in Portugal. Over the last decade, intense fieldwork in caves of the mainland and the island of Madeira has provided new data about the distribution and diversity of millipedes. A review of millipedes from caves of Portugal is presented, listing fourteen species belonging to eight families, among which six species are considered troglobions. The distribution of millipedes in caves of Portugal is discussed and compared with the troglobiont biodiversity in the overall Iberian Peninsula and the Macaronesian archipelagos.

INTRODUCTION

Millipedes play an important role in the decomposition of organic matter, and several species around the world have adapted to subterranean life, being found from cave entrances to almost 2000 meters depth (Culver and Shear, 2012; Golovatch and Kime, 2009; Sendra and Reboleira, 2012). Although the millipede faunas of many European countries are relatively well studied, this is not true of Portugal. Thus, only 44 millipede species are listed from the Portuguese mainland in Fauna Europaea, compared to 178 species from the Spanish mainland, 264 species from the French mainland, and 440 species from the Italian mainland (Enghoff and Kime, 2009). The primitive status of knowledge of Portuguese mainland millipedes stands in contrast to the well-documented diversity of millipede fauna in the Atlantic archipelagos of Madeira and Azores (Enghoff, 1982, 1983a,b, 1992; 2010; Enghoff and Borges, 2005).

What is true of millipedes in general, is true of Portuguese cave millipedes. Knowledge about terrestrial invertebrates from caves of Portugal has increased significantly in recent years, but troglobiont millipedes from Portugal were unknown until last year (Reboleira et al., 2013a). In mainland Portugal, the presence of millipedes in caves was first documented by Barros Machado, but despite several species having been recorded from caves, no troglobiont species had been discovered (Machado, 1946; Reboleira et al., 2011a; Reboleira et al., 2013a).

Intense fieldwork in recent years in caves of mainland Portugal and on Madeira Island is synthetized here, listing new species for Portugal and new distribution data for millipedes in caves.

MATERIAL AND METHODS

Specimens were collected in caves of Portugal from 1989 to 2013, mainly in karst caves of mainland Portugal and in the lava tubes of Madeira (Fig. 1). Identification of species was made using the collection of the Zoological Museum of University of Copenhagen and the pertinent literature.

RESULTS

A total of fourteen species, belonging to eight families, were identified. Troglobions are represented by six species, five from karst caves in mainland and one from lava tubes in Madeira (Table 1).

Most of the studied caves lack troglobiont millipedes; only three massifs have yielded troglobions so far: Sicó and Estremoz-Cano, with one new species of Chordeumata-tidae each in families Chamaesomatidae and Opisthocheiridae, respectively, and the Algarve, with two species of Julida in family Blaniulidae and one of Polydesmida in family Paradoxosomatidae (Table 1). In the Portuguese Atlantic archipelagos, only one troglobiont species of Julida, family Julidae, has been recorded so far, in caves of Madeira.

1 Departamento de Biología Animal, Universidad de La Laguna, 38206 La Laguna, Spain, sreboleira@ua.pt
2 Natural History Museum of Denmark (Zoological Museum), University of Copenhagen, Universitetsparken 15, DK-2100 København Ø–Denmark, henghoff@snm.ku.dk
Figure 1. Locations of sampled caves in Portugal.

Table 1. Millipedes from caves of Portugal.

<table>
<thead>
<tr>
<th>Family/Species</th>
<th>Life Style</th>
<th>Distribution in Caves</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haplobainosomatidae</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Haplobainosoma lusitanum</em></td>
<td>Troglophile</td>
<td>Sicó, Estremenho, and Montejunto massifs</td>
</tr>
<tr>
<td>Chamaesomatidae</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Scutogona</em> n. sp.</td>
<td>Troglobiont</td>
<td>Sicó massif</td>
</tr>
<tr>
<td>Opisthocheiridae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gen. indet. sp. indet.</td>
<td>Troglobiont</td>
<td>Estremoz-Cano massif</td>
</tr>
<tr>
<td>Dorypetalidae</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Lusitanipus alternans</em></td>
<td>Epigean</td>
<td>Outil-Cantanhede and Sicó massifs</td>
</tr>
<tr>
<td>Blaniulidae</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Acipes bifilum</em></td>
<td>Troglobiont</td>
<td>Eastern Algarve massif</td>
</tr>
<tr>
<td><em>Acipes machadoi</em></td>
<td>Troglobiont</td>
<td>Central Algarve massif</td>
</tr>
<tr>
<td><em>Blaniulus guttulatus</em></td>
<td>Epigean</td>
<td>Madeira</td>
</tr>
<tr>
<td>Julidae</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Cylindroiulus</em> sp. 1</td>
<td>Epigean</td>
<td>Estremenho massif</td>
</tr>
<tr>
<td><em>Cylindroiulus</em> sp. 2</td>
<td>Troglobiont</td>
<td>Madeira</td>
</tr>
<tr>
<td><em>Choneiulus palmatus</em></td>
<td>Epigean</td>
<td>Madeira</td>
</tr>
<tr>
<td><em>Nopoiulus kochii</em></td>
<td>Epigean</td>
<td>Madeira</td>
</tr>
<tr>
<td>Polydesmidae</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Polydesmus coriaceus coriaceus</em></td>
<td>Troglophile</td>
<td>Outil-Cantanhede and Sicó massifs and Madeira</td>
</tr>
<tr>
<td>Paradoxosomatidae</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Oxidus gracilis</em></td>
<td>Epigean</td>
<td>Outil-Cantanhede massif and Madeira</td>
</tr>
<tr>
<td><em>Boreviulisoma barrocalense</em></td>
<td>Troglobiont</td>
<td>Algarve</td>
</tr>
</tbody>
</table>
**LIST OF PORTUGUESE CAVE MILLIPEDES**

Class Diplopoda
Order Chordeumatida
Family Haplobainosomatidae

**Haplobainosoma lusitanum** Verhoeff, 1900

*Studied material:* Portugal, Estremenho massif; Gruta da Contenda, 27 January 2007, 1 ♂; Algar do Ladoeiro, 6 January 2007, 1 ♂, 2 ♀; 6 February 2007, 1 ♂, 3 ♀; Sicó massif; Gruta da Cerâmica, 29 August 2009, 1 ♂; Montejunto massif; Algar do Javali, 1 ♂, 2 ♀, 4 juveniles; 24 December 2009, 3 ♂, 4 ♀.

Remarks: Epigean species, often found near cave entrances of central Portugal. Also present in the Azores (Terceira, Pico, Faial, São Miguel, and Santa Maria) (BDBA, 2013).

Family Chamaesomatidae

**Scutogona sp.**

*Localities:* Portugal, Sicó massif; Gruta da Arrifana, 13 January 2013, 3 ♂, 7 ♀, 1 juvenile; Gruta da Senhora da Estrela, 11 June 2009, 1 ♂, 1 ♀; same data but 11 June 2009, 1 ♀.

Remarks: Troglobiont, blind and depigmented, found in two caves of Sicó massif.

Family Opisthocheiridae

*gen. indet. sp. indet.*

*Locality:* Portugal, Estremoz-Cano massif; Gruta de Santo António, 10 October 2008 1 ♂; 22 May 2009, 3 ♀; 4 September 2009, 2 ♂, 2 ♀.

Remarks: Troglobiont, blind and depigmented, only found in one cave of the Alentejo. The allocation of this species to the family Opisthocheiridae is tentative (J.-P. Mauriès, 2013).

Order Callipodida
Family Dorypetalidae

**Lusitanipus (Silvestria) alternans** (Verhoeff, 1893)

*Lysiopetalum alternans* Verhoeff, 1893

*Silvestria alternans:* Mauriès, 1978: 575

*Studied material:* Portugal, Sico massif; Penela, Gruta do Soprador do Carvalho, 30 August 2009, 2 ♂, 15 ♀; Condeixa, Gruta da Arrifana, 12 January 2013, 1 juvenile; Algar das Corujeiras, 10 July 2011, 1 ♂; Outil-Cantanhede massif; Portunhos, Gruta d’el Rey, 12 December 2008, 1 ♂ and 1 ♀; 8 October 2008, 2 ♀ and 4 ♂; 19 May 2009, 3 ♂ and 3 ♀.

Previous records: Portugal, Coimbra, Serra da Estrela and Bucão, no date available (Machado, 1946); Arcozelo das Maias, Viseu district, 28 March 1993, in oak wood (D.T. Bilton unpublished, D. Kime pers. com.).

Remarks: Troglophile species, found in caves of Sicó and Cantanhede-Outil massifs.

Order Julida
Family Blaniulidae

**Acipes bifilum** Enghoff and Reboleira, 2013

*Studied material:* Algarve massif, Moncarapacho, Gruta da Senhora, 29 December 2012, 1 ♂.

Remarks: Troglobiont, only known from type locality in the Algarve massif.

**Acipes machadoi** Enghoff and Reboleira, 2013

*Studied material:* Portugal, Algarve massif, Gruta do Vale Telheiro, 30 June 2011, 1 ♂ and 1 juvenile.

Remarks: Troglobiont, only known from type locality in the Algarve massif.

Figure 2. Millipede species of caves in Portugal, the surface species *Lusitanipus alternans* at left and the troglobiont species *Boreviulisoma barrocalense* at right.
**Blaniulus guttulatus** (Fabricius, 1798)


Remarks: Epigean species.

**Cylindroiulus** sp. 1


Remarks: Troglobiont species.

**Choneiulus palma** (Nemec, 1895)


Remarks: Epigean species.

**Nopoiulus kochii** (Gervais, 1847)


Remarks: Epigean species.

Order Polydesmida

Family Polydesmidae

**Polydesmus coriaceus coriaceus** Porat, 1870


Family Paradoxosomatidae

**Oxidus gracilis** (C.L. Koch, 1847)


Remarks: Introduced species, colonizes superficial caves and cave entrances. Found also in Azores, all islands except in São Jorge (BDBA, 2013).

**Boreviulisoma barrocalense** Reboleira and Enghoff, 2013


Remarks: Troglobiont, only known from type locality in the Algarve massif.

**Discussion and Final Remarks**

The majority of the collected millipede species in caves of mainland Portugal occur in the most superficial parts of the caves, where they play an important role in the decomposition of organic matter that comes from the surface. This is the case of the troglophilic *Haplobainosoma lusitanum*, widely distributed in caves of central Portugal, including Estremenho, Sicó, and Montejunto massifs. Also, *Polydesmus coriaceus coriaceus* has cave populations, especially where bat colonies are present and consequently large amounts of guano are available.

The known distribution of troglobiont species of millipedes in mainland Portugal is, for now, confined to three massifs (Fig. 3): Sicó, Estremoz-Cano, and Algarve, the latter being the richest, with three troglobiont species of millipedes.

The two troglobiont species *Acipes machadoi* and *Boreviulisoma barrocalense* inhabit the same cave, Vale Telheiro, coincidentally the richest cave in troglobiont species of Portugal (Enghoff and Reboleira, 2013; Rebo- leira and Enghoff, 2013).

Among all the central karst areas, which include the largest and higher extension of karst in Portugal, only one troglobiont species, *Scutogona* sp., was found, in several caves of the Sicó massif.

Mainland Portugal has been separated into two main biospeleological areas, the Lusitanian district in central Portugal and the Baetic district in the south; the latter includes the Algarve and extends through the Spanish Andalusia (Bellés, 1987; Reboleira et al., 2011a). The pattern of troglobiont richness for millipedes in the south of Portugal matches the patterns for troglomorphic species in other arthropod groups that inhabit caves and is influenced by biogeographical factors of the Iberian Peninsula (Reboleira et al., 2010a, 2010b, 2010c, 2011b, 2012a, 2012b, 2013a, 2013b).

Within the Iberian Peninsula overall, the richest areas in cave millipedes are located in the north and eastern areas, where several species, mainly in the order Julida, are known, namely the genera *Mesoiulus* in the Cantabrian and Vasque mountains, *Blaniulus* (= *Typhloblaniulus*) in the Pyrenees, and *Synius* (= *Paratyphloblaniulus*) in Catalonia. Another rich area for troglobiont millipedes is located in the south of the Iberian Peninsula, including the Spanish Andalusia and the Portuguese Algarve, represented by troglobiont species of julids of the genera *Acipes*, *Dolichoitus*, and *Euzkadiulus* (= *Iberoius*) (Bellés, 1987; Enghoff and Reboleira, 2013). Chordeumatidans are also a
diverse group in the caves in the Iberian Peninsula, represented by thirteen genera, mainly distributed in northeastern Spain (Mauriés, 1975; Mauriés and Vicente, 1977a, 1977b; Vicente, 1980, 1981; Vicente and Mauriés, 1980; Bellés, 1987; Mauriés, 2012, 2013) and now with two troglobiont species in the western part of the Iberian Peninsula. Another interesting millipede order, so far with no troglobiont representatives in Portugal, is the Glomerida, which is represented by seven troglobiont species in continental Spain, mostly distributed in the north, with the exception of Glomeris albida Mauriés and Vicente, 1977, known from one cave in Andalusia (Mauriés and Vicente, 1977a; Bellés, 1987), and one species on Tenerife (Golovatch and Enghoff, 2003). True troglobiont Polydesmida are only described from Cantabria, the relict Cantabrodesmus lorioli Mauriés, 1971, and recently from southern Portugal, Borevitilisoma barrocalense (Mauriés, 1971; Reboleira and Enghoff, 2013).

A troglobiont new species of Cylindroiulus is recorded in Madeira, where several species of epigean millipedes are frequently found in caves. The Azores lack troglobiont millipedes (Reboleira et al. 2011a), whereas in the Canary Islands, eight species of troglobiont millipedes are known, four on Tenerife, three on Gran Canaria, and one on El Hierro (Enghoff, 2002, 2012; Golovatch and Enghoff, 2003).

This suggests that, although the knowledge of the Portuguese cave millipede fauna increased with this work, sampling in caves has been mostly neglected. Complete sampling of the richness of millipedes in the subterranean ecosystems of Portugal requires a consistent sampling effort.

Acknowledgements

This study was supported by the SYNTHESYS Project (DK-TAF-2416), financed by European Community Research Infrastructure Action under the FP7 Capacities Program, and by Fauna Ibérica X (Ref.CGL2010-22267-C07-01), which allowed A.S.R. to visit the Natural History Museum of Denmark. We are grateful to Marian Ramos for providing the support from Fauna Ibérica. We express our gratitude to the caving clubs NEUA, CEAE-LPN, GPS, Geonauta, and CEEAA for kind support in the fieldwork in mainland Portugal. We thank Jean-Paul Mauriés, Paris, for his preliminary evaluation of the new chordeumatidan genus, and Nesrine Akkari and David Koon-Bong Cheung for all kinds of help during A.S.P.S.R.’s stay in Copenhagen.

References


ASSESSMENT OF FORWARD OSMOSIS AS A POSSIBLE MITIGATION STRATEGY FOR URINE MANAGEMENT DURING EXTENDED CAVE EXPLORATION

CATHERINE H. BORER*, WARREN J. STILES, JOSHUA C. STEVENSON, AND KATHERINE E. CABANILLAS
Biology Department and Environmental Science Program, Berry College, 2277 Martha Berry Hwy., Mount Berry, GA 30149, U.S.A.

Abstract: Extended expeditions into caves for the purpose of survey, exploration, and scientific studies pose unique challenges to cavers, but also create the potential for environmental degradation as a result of human activities. Human waste disposal can present particular challenges during extended trips underground. Urine may cause rapid microbe proliferation and substantial odor when deposited in areas that do not receive frequent flooding, but weight and bulk make it impractical to carry many days worth of urine to the surface. In this study, we evaluated the feasibility of a forward osmosis system to concentrate nitrogen-containing and carbon-containing compounds, which would allow for cleaner treated liquid to be deposited in the cave. The concentrated waste solution, with lower weight and volume than the raw urine, could then be removed from the cave. In our analysis of volume and chemical changes of a urine solution treated over the course of a week-long trial, we determined that the system, as tested, does reduce the weight and concentrate the chemical constituents in urine, allowing some chemical separation from the treated liquid. Unfortunately, the drawbacks of this system (chemical breakthrough, added weight of the system, and unknown ecological effects associated with substantial sodium chloride additions to the cave) outweigh the benefits. We do not recommend this forward osmosis system, as tested, as an effective mitigation strategy, although other treatment strategies may hold promise.

INTRODUCTION

Caves are unusual and delicate ecosystems that must be carefully preserved. In many caves, such as Lechuguilla Cave in Carlsbad Caverns National Park, U.S.A., regulations mandate that all solid waste must be removed from the cave (National Park Service, 2006), and where regulation is absent, conscientious cavers follow this same practice. In contrast, liquid waste is occasionally deposited in caves, posing an added environmental threat during exploration. In many caves, continuous stream flow or periodic flooding dilutes and flushes out contaminants, which may mitigate ecological damage from urine deposition. However, this removal by groundwater does not occur in Lechuguilla Cave, which has no known surface streams entering the cave and only minimal internal water flow (Cunningham et al., 1995; Davis, 2000).

The normal range of urine output in a healthy, well-hydrated adult is 800 to 2500 mL per 24 hour period (Fischbach and Dunning, 2008), with a typical daily output of roughly 1200 to 1500 mL of urine. Thus, during a seven day expedition, which is common on exploration and survey trips in Lechuguilla (National Park Service, 2006), a single caver may produce 11 liters (11 kg) of urine. It is not feasible to carry this volume or weight out of the cave in addition to the substantial gear needed for expedition activities. A mandate of urine removal during a week-long expedition could encourage cavers to decrease their liquid intake to reduce their urine output, leading to dehydration. However, even mild dehydration can lead to impaired cognitive performance, motor skills, and judgment (Lieberman, 2007), decreasing expedition productivity, reducing the quality of the survey or scientific data collected, and increasing the potential for accidents. Therefore, the Cave Resources Office (CRO) at Carlsbad Caverns National Park has designated sacrificial areas within Lechuguilla for urine deposition during longer expeditions (National Park Service, 2006).

Many unique microbial species have been described from Lechuguilla Cave, (e.g., Cunningham et al., 1995; Johnston et al., 2012), and alterations to microbial communities have been attributed to human activities (e.g., Northup et al., 1997; Hunter et al., 2004; Johnston et al., 2012). Thus, depositing urine in the cave may result in substantial ecological impacts in urine dump sites, and in areas that are hydrologically connected to them. When urine is added to the cave ecosystem, it can cause rapid growth (blooms) of opportunistic microbe populations (Lavoie, 1995), which have been reported to be primarily endemic cave species that utilize both nitrogen and organic carbon from the urine (Johnston et al., 2012).

Waste treatment possibilities are limited by substantial challenges. Weight, bulk, and energy requirements of supplies and materials must be minimized. All equipment...
must be rugged, easy to operate, and safe, and the intentional addition of foreign microbes must be avoided. In this project, we evaluated one possible method to mitigate the ecological and aesthetic effects of human waste deposition. Our goal was to reduce the urine’s volume and weight by forward osmosis, so a concentrated urine solution containing most of the urine’s nitrogen and carbon could be removed from the cave, while a more inert saltwater solution with most of the urine’s volume and weight could be deposited in the cave. In this study, we evaluated the feasibility of treating human urine via a commercially available, portable water filtration osmosis system (X-Pack, Hydration Technology Innovations, Scottsdale, AZ).

MATERIALS AND METHODS

Each X-Pack unit is a rugged, portable (130 g), two-chambered plastic enclosure for treating drinking water. Its two chambers have re-sealable ports for adding and removing liquids, and are separated by a semi-permeable membrane with a pore size of 0.3–0.5 nm (Hydration Technology Innovations, 2010). In this study, we used urine as the input liquid rather than dirty water, and we used table salt for the osmotic draw solution because it is inexpensive, reasonably inert, readily available, and inorganic. For health and safety reasons, each researcher used his/her own urine to test previously unused X-Packs.

In our first test, 1.5 liters of urine was added to the input side of an unused X-Pack and 150 g non-iodized table salt (NaCl) was placed in the output, or draw side, after which the unit was gently agitated, and then allowed to equilibrate. We analyzed the raw urine prior to setup, and the concentrated urine on the input side and the draw (treated) liquid after 8, 16, and 24 hours. A second test was performed for 48-hours. For all analyses, we measured nitrate, nitrite, and ammonia using an aquarium water test kit (Master Test Kit, Aquarium Pharmaceuticals, Inc., Chalfont, PA), and pH via 1 to 14 scale pH paper. We evaluated urea, the primary nitrogen-containing constituent of urine (Fischbach and Dunning, 2008), with a Blood Urea Nitrogen kit (Stanbio, Boerne, TX) using a spectrophotometer in comparison with a standard curve of known urea concentrations.

In a second test, the input side of an unused X-Pack was initially filled with a 24 hour sample of urine (minus an aliquot removed for chemical testing), and the draw side was primed with 150 g of non-iodized table salt. The urine was allowed to process in the X-Pack for 24 hours, after which the treated (draw) solution and the concentrated urine on the input side were removed from the unit. The input side was then refilled with a new 24-hour urine sample and 150 g of salt was added to the draw side. This same procedure was repeated each day for a week, using a single X-Pack and urine from the same healthy volunteer throughout the week. The mean urine volume that was added each day during the six-day testing period was 873 mL (range, 760 to 1030 mL), somewhat lower than a typical 24 hour sample, because liquid was removed for chemical testing prior to filling the X-Pack each day. For each 24 hour processing period, the initial raw urine added to the X-Pack, the concentrated urine on the input side, and the treated (draw) liquid were chemically evaluated as described.

RESULTS AND DISCUSSION

Water movement via osmosis is driven by a concentration gradient of solutes across a selectively permeable membrane. In our study, we created this gradient by putting a concentrated salt solution on one side of the X-Pack membrane and urine on the other side. We expected the water to spontaneously move out of the urine toward the more concentrated salt side of the membrane. The results from our first test (Table 1) suggest that 24 hours is an appropriate time for osmosis in the X-Pack to reduce the urine volume. This 24-hour processing period is also logistically feasible for cave expeditions, during which X-Packs could be left in urine dump areas and solutions could be exchanged as part of a daily routine. In all tests, the pH remained stable at just above pH 6, and the nitrite was below 0.25 mg L\(^{-1}\). After the 24-hour incubation period,

<table>
<thead>
<tr>
<th>Hours</th>
<th>Percent Volume, Concentrated Urine</th>
<th>Percent Volume, Treated (draw) Liquid</th>
<th>Percent N, Concentrated Urine</th>
<th>Percent N, Treated (draw) Liquid</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>52.8</td>
<td>47.2</td>
<td>69.7</td>
<td>30.3</td>
</tr>
<tr>
<td>12</td>
<td>43.6</td>
<td>56.4</td>
<td>53.0</td>
<td>47.0</td>
</tr>
<tr>
<td>16</td>
<td>40.4</td>
<td>59.6</td>
<td>75.2</td>
<td>24.8</td>
</tr>
<tr>
<td>24</td>
<td>22.7</td>
<td>77.3</td>
<td>30.6</td>
<td>69.4</td>
</tr>
<tr>
<td>48</td>
<td>15.0</td>
<td>85.0</td>
<td>24.5</td>
<td>75.5</td>
</tr>
</tbody>
</table>
ammonia and urea concentrations showed the greatest difference between the concentrated urine on the input side and the treated (draw) liquid. In our 48-hour test, 80% of the original urine volume moved by osmosis to the treated (draw) side of the membrane during that period. We also evaluated the possibility of reusing a single X-pack daily during a seven-day expedition. In this test, the concentrated urine was reduced to roughly 47% of its original volume (Fig. 1). Based on a typical volume of 1.5 L day\(^{-1}\), this would result in a carry-out volume of 4.9 L, or about 4.9 kg per person after six days. Urea in the concentrated urine remained greater than in the treated (draw) solution (Fig. 2). With six days of treatment, roughly 67% of the urea from the raw urine remained in the X-Pack, which would be removed from the cave. This separation is a great improvement over depositing raw urine in the cave, but the urea that would be deposited after X-Pack treatment (33% of the urea from the raw urine) is substantially greater than we had desired. As with urea, some ammonia accumulated on the treated (draw) side of the membrane, suggesting that some ammonia passed through the membrane. Over the course of the week, a small amount of nitrogen was lost from the system, likely as a result of microbial activity releasing gaseous ammonia, or incorporating nitrogen into proteins and other biological molecules that we did not evaluate. Overall, approximately 10 g of urea would be deposited in the cave with the treated (draw) solution over a six-day period, which is just

Figure 1. Cumulative volumes during the 7-day X-Pack test, during which all liquid was removed from both sides of the membrane daily. The input side was refilled with fresh urine, and 150 g NaCl was added to the draw side daily.

Figure 2. Cumulative urea amount during the 7-day X-Pack test, during which liquid was removed from both sides of the membrane daily, the input side was refilled with fresh urine, and 150 g NaCl was added to the draw side daily.

28 • Journal of Cave and Karst Studies, April 2014
under a quarter of the roughly 44 g in the raw urine (Fig. 2). The X-Pack membrane appears to impede but not completely prevent passage of the nitrogen-containing molecules present in urine. The membrane’s 0.3 to 0.5 nm pore size allows the passage of water (molecular weight [MW] 18.02), and thus may be too large to prevent the passage of other small molecules such as ammonia (MW 17.03), but it may impede the passage of urea (MW 60.06).

There are a number of drawbacks to the use of the X-Pack for urine treatment. Importantly, a substantial amount of nitrogen-containing compounds would be left in the cave with the treated (draw) solution. Ecological impacts such as altered biological and mineralogical processes, which we did not evaluate, could occur as a result of the deposition of the table salt (NaCl) used to produce the concentration gradient across the membrane. This technique would also increase caver expenses, which are already substantial. Each X-Pack costs over $50 U.S. when purchased commercially. Despite greatly reducing the total weight and volume of urine, each caver using this system for a week would have to carry into the cave an additional 1.23 kg (130 g X-pack plus 1.1 kg salt). At the end of a week-long expedition, each caver would then carry out the used X-Pack, plus about 4.9 kg (4.9 L) of concentrated urine (assuming 1.5 L of urine per day). Cavers move more efficiently with smaller and lighter packs, increasing safety and reducing the likelihood of inadvertent damage to the cave. This filtration method would add substantially to caver pack weight and a considerable portion of the total nitrogen compounds would still be deposited in the cave. Because of these drawbacks, we do not recommend this system, as tested.

**CONCLUSIONS**

In this study, we evaluated a possible strategy to mitigate human impacts during multi-day cave expeditions. We determined that the X-Pack system, as tested, does reduce the weight and concentrate the chemical constituents in urine, however, the drawbacks (chemical breakthrough, added weight and volume of the system, and unknown ecological effects associated with substantial sodium chloride additions to the cave) outweigh this system’s benefits. We do not recommend this forward osmosis system, as tested, as an effective mitigation strategy. Other possible urine treatment methods are being evaluated.

**ACKNOWLEDGMENTS**

We are grateful to the Dogwood City Grotto’s competitive grants program for financial support of this project. We also thank Hydration Technology Innovations for donating the X-Packs we tested. Many thanks to Ron Miller and Art Fortini for their work in the early stages of this project and for their many helpful conversations and suggestions. We thank Kathy Lavoie, Ron Miller, Art Fortini, Mary Castro, and two anonymous reviewers for their helpful comments on earlier drafts of this manuscript.

**REFERENCES**


ECOTOURISM IN THE STATE FOREST KARST OF PUERTO RICO

ANDREA HALL AND MICK DAY
Department of Geography, University of Wisconsin-Milwaukee, PO Box 413, Milwaukee, WI 53201, andrea.brooke.hall@gmail.com, mickday@uwm.edu

Abstract: Ecotourism and nature-based tourism in karst landscapes are often focused on protected areas and are significant both economically and because of potential impacts. Karst covers nearly a third of the highly urbanized island of Puerto Rico, especially adjacent to the north coast and in the southwest. Much of the island’s nature-oriented tourism is focused on the karst, because it is the least-fragmented remaining habitat. The authors conducted a literature review and collected data during field research in 2009. The results indicate that the five state (or commonwealth) forests located within the karst of Puerto Rico are a primary focus of ecotourism because they are readily accessible and represent an important resource for low-impact recreation and education. The forests are used by residents and visitors, and they provide opportunities for appreciation and enjoyment of the karst landscape. Ecotourism activities focused on the state forests include hiking, bird-watching, and learning about nature. Without the state forests, levels of ecotourism within the karst would be considerably constrained. So, although they are limited in numbers and extent, they provide a critical recreational and economic resource in the karst landscape.

INTRODUCTION

Protected areas play a critical role in human use of karst areas, particularly in providing a buffer against exploitation of the natural landscape and in the burgeoning realms of recreation and tourism. Karst landscapes provide significant venues for ecotourism (e.g., Bundschuh et al., 2007), and the protected areas within karst can be important for promoting recreational and tourism opportunities (Scott et al., 2004). Few studies have examined this topic in detail, with most past studies specifically related to tourist-cave development and conservation (Lobo and Moretti, 2009; Cigna and Burri, 2000) and not ecotourism within the karst environment as a whole; a particularly striking example of the latter case is presented here.

Caribbean karst landscapes have been subject to long-term and intense human pressure, and tourism is a human activity that is of increasing significance (Day, 1993). Tourism impacts karst, particularly because of the relative fragility of the landscape (Day, 2010a,b, 2011), and even eco-related activities can damage the environment, particularly in karst landscapes (Coghlan, 2008). Overall, tourism, which necessarily involves human-environment interactions, can have severe effects on caves and other components of karst ecosystems. Caves in particular are vulnerable because of the focused intensive nature of visitor use (Ford and Williams, 2007; Pulido-Bosch et al., 1997).

KARST IN PUERTO RICO

The island of Puerto Rico is located in the Caribbean, between the Caribbean Sea and the North Atlantic Ocean, east of the Dominican Republic, and about 1,600 km (1,000 mi) southeast of Miami, Florida (Fig. 1). Puerto Rico consists of three physiographic regions: a volcanic central mountainous area (the Cordillera Central) of Late Jurassic to Eocene age, a marginal Tertiary karst belt, and a discontinuous coastal plain (Monroe, 1976; Gardner et al., 1987; Troester, 1992). Carbonate deposition began in the early Cretaceous period but peaked in the early Tertiary period, with deposition of the limestones beginning and ending earlier in the south than the north. After active volcanism and tectonism ceased, extensive middle Oligocene to Pliocene limestones and terrigenous sediments were deposited over the older rocks, particularly along the northern flanks of the mountains, forming the northern karst belt (Monroe, 1976). In the southern karst region, much of the limestone is buried under deep alluvial deposits. The southern limestone is intensely faulted, while the rocks in the north are cut by very few faults (Lugo et al., 2001).

There are marked climatic differences between the northern and southern portions of Puerto Rico, with the north being characterized by a moist, humid climate, while the south is much drier. The southern karst is in a dry, rain-shadow location and, superimposed upon the lithological and structural differences noted above, karstification is limited because reduced rainfall inhibits the rate of dissolution (Lugo et al., 2001). Differing lithologies, structures, and climates lead to different rates of karstification and more- or less-developed features, with the north having better-developed karst with more distinctive karst landforms such as cockpits and towers.

Karst covers between 28% (Lugo et al., 2001) and 34% of Puerto Rico (Monroe, 1976) and is most extensive in the
northern karst belt, which is the best documented (Monroe, 1976; Troester, 1992; Lugo et al., 2001). The northern karst belt extends about 70 km east-west parallel to the northern coast west of San Juan, with a maximum width of about 22 km south of Arecibo. This karst belt encompasses approximately 1,600 km$^2$ or about 20% of the land area of Puerto Rico (Giusti, 1978) and accounts for about 90% of the karst in Puerto Rico, with the residual 10% in the south and in scattered outcrops in the Cordillera Central (Fig. 2).

Six distinct limestone formations are recognized in northern Puerto Rico. In ascending order, they are the San Sebastián Formation, the Lares Limestone, the Cibao Formation, the Aguada Limestone, the Aymamón Lime- stone, and the Camuy Formation (Monroe, 1976; Giusti, 1978). The northern karst belt includes extensive areas of dry valleys and sinkholes, together with more dramatic landforms such as cockpits and mogotes (Monroe, 1976; Day, 1978, 2004; Day and Tang, 2004). Mogotes occur particularly along the northern edge of the northern karst belt, and they are perhaps the most distinctive and obvious landforms of the northern karst (Lugo et al., 2001). There are also numerous cave systems (Miller, 2009) and through-flowing allogenic rivers (Monroe, 1976; Lugo et al., 2001).

In the southern karst, four limestone formations are recognized, the Juana Díaz Formation (Oligocene and Miocene age), the Ponce Limestone (Miocene age), the Guánajibo Formation (late Miocene and possibly Pliocene age) and the Parguera Limestone (Early Cretaceous age) (Lugo et al., 2001). The southern limestone is largely of coral-reef origin and marine limestones outcrop in chalk cliffs of the Juana Díaz Formation near Ponce. This area contains several large caves and closed depressions. Natural windows and rock-shelter caves also occur in the south (Lugo et al., 2001). The landforms in the southern karst region are not as dramatic as those in the north, but the southern karst has been largely ignored and merits further study.

Throughout the Caribbean, human activities have had widespread adverse impacts on karst landscapes (Day, 1993, 2010a,b, 2011), and these are predicted to increase (Day and Chenoweth, 2009). In this context, the northern karst belt of Puerto Rico has an interesting history of colonial agricultural expansion and contraction, followed by depopulation and then urban and industrial encroachment (Pico, 1974; Lugo et al., 2001). It has been regarded as one of the world’s most endangered karst areas (Tronvig and Belson, 1999), and it has been a focus of karst-conservation efforts on the island (Kueny and Day, 1998; Mujica-Ortiz and Day, 2001). Paradoxically, although the karst is under extreme human pressure, it still represents the least fragmented natural habitat in Puerto Rico.
making its use, conservation, and management all the more critical (Lugo et al., 2001). One approach to reconciling conservation and human activities is through the designation of protected natural areas within which human activities, including tourism, are controlled and closely monitored (Kueny and Day, 1998).

ECOTOURISM IN PUERTO RICO

The definition of ecotourism is complex, multiple definitions exist, and the term has varied meanings to different people and organizations. Although there are a plethora of definitions available to choose from, most definitions include certain criteria. Fennell (2001), for example, suggests that ecotourism is a type of specialty travel that is nature-oriented, promotes conservation, protects local culture, benefits the local population, and promotes education. One of the first (and the most widely-accepted) formal definitions of ecotourism was presented by Ceballos-Lascurain in 1987 as “traveling to relatively undisturbed or uncontaminated natural areas with the specific objective of studying, admiring, and enjoying the scenery and its wild plants and animals, as well as any existing cultural manifestations (both past and present) found in these areas” (Ceballos-Lascurain, 1987: p. 14).

Activities conducted under the title of ecotourism are varied and can take place in many different environments (Ceballos-Lascurain, 1996). Examples include such low-impact activities as hiking, cycling, rafting, bird-watching, and astronomical observation. Others are limited to specific landscapes and thus are more specialized; caving and scuba diving are examples. Both general and specialized ecotourism occur in karst landscapes, and tourism is becoming an important aspect of human use of karst areas, with attendant impacts (Huppert et al., 1993; Day, 2010a). The karst also contains diverse and endemic plant and animal populations and provides a wide variety of outdoor activities, while supporting biological, ecological, and geomorphological diversity (Lugo et al., 2001).

Increasingly aware of sustainability issues, Puerto Rico has turned to ecotourism and sustainable development in an effort to conserve and protect its natural resources (Frederique, 2004). With a land area of 13,790 km² and a population of nearly 4 million people (Central Intelligence Agency, 2010), Puerto Rico has a population density of more than 430.5 people per km², second in the Caribbean only to Barbados (Scarpaci and Portela, 2009). Sustainable development and ecotourism have become particularly important national issues precisely because of the island’s small size and high population density. Protected areas are among the most important venues for ecotourism activity in the Caribbean region (Weaver, 1998; Farrell and Marion, 2001), and well-planned sustainable tourism development should lead not to the deterioration of natural areas but to their conservation (Page and Dowling, 2002).

Specialized tourism in karst landscapes and in protected areas is in some ways analogous to other tourism with particular themes or restricted to specific landscape types, such as religious tourism (Vukonić, 1996), mountain tourism (Nepal and Chipeniuk, 2005), tourism on coral reefs (Hawkins and Roberts, 1994), and archeological tourism (Wallace, 2005). The potential of these distinctive or niche versions of ecotourism is increasing (Weaver,
At the same time, as protected areas provide a mechanism via which protection of karst areas may be accomplished, they also represent important potential locations for karst-focused tourism and ecotourism (Day and Hall, 2012).

The Caribbean karst, including Puerto Rico, appeals to tourists because of its natural environment and its unique topography, with impressive sinkholes, mogotes, dry valleys, springs, caves, and underground rivers that create unique visitor experiences. The karst also contains diverse and endemic plant and animal populations and provides a wide variety of outdoor activities, while supporting considerable biological, ecological, and geomorphological diversity (Lugo et al., 2001). Tropical karst is often visually striking and creates an immediate and lasting impression on visitors (Day and Hall, 2012). Natural landscapes are valued highly by many tourists and are central to their experience (Mowforth et al., 2008). More significantly, ecotourism is, according to most definitions, dependent on an enjoyable and low-impact experience within the natural environment (Mowforth et al., 2008). It is estimated, internationally, that between 40 and 60% of tourism is directed toward the appreciation of natural areas (Frederique, 2004), and this suggests that a significant portion of Puerto Rico’s tourism must be directed towards the karst, since it represents a significant proportion of the remaining wilderness on the island (Lugo et al., 2001). This is cause both for concern, because of the karst’s fragility and the potential impacts of tourism, and for optimism, because ecotourism may provide a vehicle for conservation.

Protected Areas in the Puerto Rican Karst

Karst landscapes of the Caribbean are in particular need of conservation and sound environmental management because they are inherently fragile and vulnerable to environmental change and human impacts (Day, 1993; Kueny and Day, 1998). The IUCN World Commission on Protected Areas (WCPA) has recognized karst landscapes, including those in the Caribbean, as being at particular risk of degradation and warranting protection (Watson et al., 1997). Pressures on the Caribbean’s karst resources are of great concern for environmental, economic, and social reasons (Kueny and Day, 1998; Day, 2010a,b, 2011), and the establishment of protected areas is one way these resources can be conserved. In Puerto Rico, the karst merits conservation because it “not only a significant portion of the total land area of the island, but it is a particularly important area in terms of its environmental assets” (Lugo et al., 2001: p. 82). As elsewhere in the Caribbean, the designation of protected areas within the Puerto Rican karst is not highly sophisticated, and few areas are protected specifically because they are karst (Mujica-Ortiz and Day, 2001).

In particular, the karst of Puerto Rico merits conservation to preserve its biodiversity, to promote the recovery of endangered species, to maintain its wilderness nature and spectacular scenery, to ensure scientific and educational opportunities, to maintain open space and recreational potential, and to protect its many environmental functions (Lugo et al., 2001). Recreational potential is inextricably linked to the karst’s tourism and ecotourism activities. In addition, the karst of Puerto Rico is extremely important because it possesses spectacular scenery reflecting its diverse landforms, rugged topography, unusual landscapes and contrasting vistas (Lugo et al., 2001). The karst is diverse geomorphologically, hydrologically, and ecologically; it harbors valuable natural resources such as flora and fauna and many endemic and endangered species; and it is economically important due to its water supply (its aquifers and rivers), mining potential (limestone and dolostone), agricultural potential (both subsistence and commercial agriculture), and construction and manufacturing activities (Lugo et al., 2001). Many Taíno archeological sites, including pictographs and petroglyphs, are located in the karst, especially in caves (Frank, 1993). These sites are valuable and should be preserved to protect the culture and history of the island.

Most of the legislation pertaining to protected areas within the Puerto Rican karst has been enacted since 1980, although some legislation is older (Mujića-Ortiz and Day, 2001). In many instances, protection extends only to specific karst features, such as caves, rather than to the broader scale of drainage basins or ecoregions. Prior to 1999, only 71 km² of the karst in Puerto Rico was protected, representing less than 3% of the total karst area, well below average for the Caribbean (Kueny and Day, 1998; Mujica-Ortiz and Day, 2001). Lugo et al. (2001) recommended that an additional 39,000 ha of the northern karst belt, representing 27% of that belt and 16% of the total karst, should be designated as public lands. In 1999, Law 292 (known as Ley para la Proteccion y Conservacion de la Fisiografia Karsica de Puerto Rico, or more simply, Ley del Karso) was passed to protect 35% of the karst. The Departamento de Recursos Naturales y Ambientales de Puerto Rico was given the responsibility of completing a study within two years of the law’s passage to determine the areas of the karst most needing protection and conservation. This study was not completed until 2008, and implementation has been slow. Figure 3 shows the karst areas protected under the law, as well as state forests and other protected areas. The karst is vulnerable because amendments to Ley del Karso (for example, to authorize construction projects in the karst) are a constant threat and could impact the protected karst areas, including the protected areas in the northern karst belt, the southern karst, and the outcrops in the interior of the island, including natural reserves and state forests (Ciudadanos Del Karso, 2010).

One particularly important mechanism promoting karst protection in Puerto Rico is the creation of state or commonwealth forests, which are the most numerous...
category of protected areas and occupy the greatest area within the karst (Mujica-Ortiz and Day, 2001). Under the conditions of Puerto Rico Law Number 133, Ley de Bosques de Puerto Rico, enacted in 1975, state forests are multiple-use areas managed for sustainable use of water resources, timber, wildlife and ecotourism (Frederique, 2004).

There are five state forests in the Puerto Rican karst, Río Abajo, Guajataca, Cambalache, and Vega Alta in the northern karst belt and Guánica in the south (Fig. 4). Of these, Guánica State Forest is the largest, with an area of 4,006 hectares. Río Abajo State Forest is the second largest, at 2,339 hectares, Guajataca State Forest covers 954 hectares, Cambalache 648 hectares, and Vega Alta 504 hectares.

Although the Puerto Rican state forests are not explicitly categorized by IUCN’s (International Union for Conservation of Nature) management categories, broadly they fall within protected area category VI, protected areas with sustainable use of natural resources, “with most of the area in a natural condition, where a proportion is under sustainable natural resource management, and where low-level non-industrial use of natural resources compatible with nature conservation is seen as one of the main aims of the area.” (IUCN, 2012). Such protected areas incorporate multiple goals and, to be considered successful, must be managed to reconcile conservation objectives with human activities such as ecotourism.

**METHODS**

Because the state forests are the most numerous and largest protected areas in the Puerto Rican karst, they were selected for further investigation. Ecotourism activities...
located in the coastal areas. Ninety percent of the state forest and reserve is also the site of several mangrove forests. This is because of the semi-arid climate the karst is not as pronounced as in the northern karst belt (DRNA, 2010).

The state forests were then targeted for intensive field investigation. Each state forest was visited, its ecotourism resources and activities were documented, and semi-structured interviews were conducted with the forest rangers. Approximate annual tourist numbers collected from the state forests provide a preliminary, if conservative, measure of tourism activity within the karst-based state forests. They also identify the relative significance of the different state forests. Pressures on the karst environment may or may not be directly proportional to the number of visitors participating in karst-based activities. Visitor numbers were reported by rangers as monthly totals and were used to calculate annual totals, resulting in a relatively wide range in annual visitor numbers for each forest. These numbers were then refined on the basis of further information, acquired primarily through ranger interviews. Further details of the research methods are provided by Hall (2010).

**RESULTS**

Located in southwestern Puerto Rico (Fig. 4) in the municipality of the same name, Guánica State Forest is unique among the state forests, both in its location and because it is a UNESCO Biosphere Reserve. Guánica was designated a commonwealth forest in 1917 and subsequently became a forest reserve in 1919 with an area of 3,035 hectares (7,500 acres). Additional land was incorporated in 1931, and the forest now covers 4,006 hectares (9,900 acres), making it the largest of the karstic state forests. Guánica was designated a United Nations International Biosphere Reserve in 1981, and it is now managed cooperatively by the Departamento de Recursos Naturales y Ambientales de Puerto Rico and UNESCO (DRNA, 2010). As a UNESCO biosphere reserve, Guánica is a location where “monitoring, research, education, and training activities are encouraged to support sustainable development” and “the challenge of the biosphere reserve is to educate the growing population to appreciate and maintain Guánica’s biological diversity” (UNESCO MAB, 2011).

The Guánica State Forest and Biosphere Reserve is one of the best examples of subtropical dry forest in the United States, and it incorporates two predominant forest types, semi-deciduous forest and cactus forest (Nellis, 1999). The state forest and reserve is also the site of several mangrove cays located in the coastal areas. Ninety percent of the forest is developed on limestone substrate, although because of the semi-arid climate the karst is not as pronounced as in the northern karst belt (DRNA, 2010). Guánica has the greatest bird and plant diversity of any area in Puerto Rico (DRNA, 2010), and it is considered the premier bird-watching site on the island (Nellis, 1999). Eleven of Puerto Rico’s fourteen endemic bird species have been recorded in the forest, and over 700 species of plants, including 248 tree species, have been recorded (Nellis, 1999; Mora, 2009). Of the plants, forty-eight are endangered and sixteen are endemic to Guánica (Nellis, 1999).

The biosphere reserve is managed as a multi-use area to foster “harmonious relationships between human activities and the maintenance of the natural ecological integrity” (UNESCO MAB, 2011). Attractions in Guánica include hiking trails, bird-watching, and picnic facilities. There are 57 km (36 miles) of trails and old roads, including twelve major officially recommended trails. Most visits are day-trips, and camping is not permitted. Visitor records indicate that between 800 and 2,000 people visit Guánica each month, or 9,600 to 24,000 annually, making it the most heavily used of the karst state forests. Because of its international ornithological renown, actual totals are probably at the high end of this range. Usage is highly seasonal, and visitor numbers in some years have reached 20,000 during the summer months alone. Access to the forest is relatively tightly controlled, and an annual total of 20,000 to 24,000 visitors is probably realistic. The ranger confirmed that many people visit the forest to observe and document birds, noting that many educational groups, from primary school to university level, also visit. On request, the ranger presents charlas, or informational talks to these groups, focusing on the unique ecology of the forest. Families and social and religious groups also frequent the picnic sites.

Interviews indicate that visitors to Guánica include Puerto Ricans, travelers from the contiguous US, and international visitors, primarily Europeans, in approximately equal numbers. Visitors from all three categories were encountered during the field visit, and represent a greater diversity than was indicated or observed at any other state forest. Perhaps reflecting the blend of visitors, the forest office provides trail guides and literature about Guánica’s natural resources and ecology, including a description of the dry subtropical forest. General literature on the natural resources of Puerto Rico is also readily available.

The other four karst state forests, Cambalache, Río Abajo, Guajataca, and Vega Alta, are located within the northern karst belt, and they share some characteristics in terms of terrain and visitor usage, although they differ somewhat in area, contiguity, history, specificity of purpose, and visitor numbers. All four are readily accessible from San Juan and the north coast and, like Guánica, each has a role in the island’s karst ecotourism. Cambalache State Forest is located in the municipalities of...
Barceloneta and Arecibo, Río Abajo is located in the municipalities of Utuado and Arecibo between the Tamán River and Lago Dos Bocas, Guajataca is in the municipality of Isabela, and Vega Alta is in the municipalities of Vega Alta and Vega Baja (Fig. 4).

The northern state forests have broadly similar histories in terms of their establishment dates and their management purposes. Cambalache was established in 1943 through an agreement between the United States Forest Service and the Autoridad de Tierras de Puerto Rico to implement a forestry program within the karst terrain. In 1975, with the passing of Law 133 (Ley de Bosques de Puerto Rico) the DRNA took over forest management responsibility, the principal function of which is now preservation and conservation of resources for public enjoyment (DRNA, 2010). Río Abajo was also created in 1943 to protect significant drainage basins and aquifers and to implement forest management (DRNA, 2010). Guajataca was donated by the Puerto Rico Reconstruction Administration to the government of Puerto Rico in 1943. Vega Alta was created in 1951, and like the others, is managed by the DRNA, although it is owned by the Autoridad de Tierras de Puerto Rico.

The Cambalache State Forest area of 648 hectares (1,600 acres) is distributed between seven separate areas, five in the municipality of Arecibo and two in the municipality of Barceloneta (DRNA, 2010). Cambalache is one of the few preserved areas of lowland karst forest in Puerto Rico and includes more than 150 species of trees, 10 of which are rare or in danger of extinction and at least 15 of which are endemic to Puerto Rico (Nellis, 1999). Seventy percent of the forest covers residual hills or mogotes, providing a dramatic landscape. Cambalache supports the majority of the lowland-forest bird species in Puerto Rico, with the avifauna totaling about 45 species, including 34 permanent resident species and 10 of Puerto Rico’s 16 endemic species (DRNA, 2010).

Major recreational attractions in Cambalache include camping areas, gazebos, and barbecue and picnic facilities. There is an 8 km (about 5 mi) network of trails for walking, hiking, and mountain biking, and the park encourages astronomical observation, landscape appreciation, bird-watching, hiking, cycling, photography, meditation, and camping.

Visitor records indicate that between 400 and 1,000 people visit Cambalache State Forest each month, or between 4,800 and 12,000 visitors annually. Visitor records probably underestimate total numbers because access is not tightly controlled and not all visitors are recorded. On these grounds, it is estimated that probable visitor numbers are in the upper reach of the range reported. Visitors frequently use the trails for walking, biking, and bird watching, and many school groups visit the forest as part of educational projects. The forest ranger gives presentations about the ecology of the forest, the karst landscape and their importance. Upon reservation, caminatas or nature walks can also be arranged, and literature about the forest is available to visitors who inquire. According to the ranger, the majority of recorded visitors to Cambalache are from the US mainland or international locations. The ranger was of the opinion that relatively few local people utilize the forest, although he described a good relationship with the surrounding community.

Río Abajo covers a contiguous area of 2,339 hectares (5,780 acres) and includes a variety of karst landforms, including cockpits, dry valleys, mogotes, and caves (Nellis, 1999; DRNA, 2010). The majority of the forest is classified as subtropical moist forest, and it includes 175 tree species, 47 of which are threatened or in danger of extinction, and 8 of which are endemic. Following deforestation during the 1940s, the Puerto Rican government started an ongoing reforestation program, with Río Abajo as a focal area (DRNA, 2010).

Recreational facilities in Río Abajo include campsites, hiking trails, and access to a cave system. Visitor records indicate that between 500 and 1,000 people visit Río Abajo each month, or between 6,000 and 12,000 annually. Like Cambalache, visitor records probably underestimate total numbers because access is not tightly controlled and not all visitors are recorded. Camping and hiking are particularly popular activities at Río Abajo, and the cave is a focal point for visitors. School and university classes visit to study and complete educational projects. The ranger gives charlas upon reservation and discusses the ecology of the forest and its relationship to the karst landscape. Trails in Río Abajo are not well marked, and perhaps this is one reason why, according to the ranger, Puerto Ricans are more numerous than international visitors to the forest.

Guajataca covers a smaller contiguous area than Río Abajo (954 hectares or 2,357 acres) (DRNA, 2010) and is classified as subtropical moist forest (Nellis, 1999). The forest is considered a unique natural area because of its distinctive tropical karst topography, including caves, sinkholes, and residual hills (Nellis, 1999). In Guajataca, 186 species of trees have been recorded, of which 23 are endemic to Puerto Rico (Nellis, 1999). The avifauna includes 45 bird species, 8 of which are endemic. Other notable fauna include the Puerto Rican boa, one of the 13 endangered species found in the forest (DRNA, 2010).

Guajataca is designated for multiple uses, including wildlife protection, soil and water conservation, timber production, and outdoor recreation (DRNA, 2010). Attractions include the unique flora and fauna, scenic vistas, Cueva del Viento, a lookout tower that provides an excellent view of the karst landscape, and forty-six hiking trails, totaling 43.5 km (27 mi.), plus picnic sites and camping facilities. According to the rangers, the camping, picnic, and hiking facilities are what attract most people, although the karst topography, and particularly Cueva del Viento, also attracts visitors. The flora and fauna, especially the endemic species, also attract specialized visitors. Charlas and literature about the forest are also available.
Visitor records indicate that between 500 and 1,000 people visit Guajataca every month, or 6,000 to 12,000 annually. Like elsewhere, visitor records probably underestimate total numbers because access is not tightly controlled and not all visitors are recorded. A majority of the visitors are from the United States and Europe, although many others are from within Puerto Rico, including families and educational, religious, social, and environmental groups, particularly those involved in tree planting.

Vega Alta, the smallest of the northern karst forests, consists of six non-contiguous areas, and its principal purpose is conservation of the natural area, including the karst landscape (DRNA, 2010). The forest covers a total of 504 hectares (1,245 acres) of moist subtropical forest based primarily on mogote and ridgetop karst topography (Nellis, 1999; DRNA, 2010). There are at least 72 tree species, 6 native plant species, and 15 bird species, including 3 endemics (DRNA, 2010).

Attractions in Vega Alta include three poorly marked hiking trails and a small picnic area near the rather inconspicuous ranger station. The trails and picnic area are available daily and excursions are available upon reservation, although the ranger is only available at this location on Tuesday and Thursday mornings. According to the ranger, 500 to 1,000 people visit Vega Alta annually, although visitors are not documented here as accurately as in the other state forests. Because the forest is fragmented and because its principal objective is conservation of the flora, fauna and karst landscape, tourism is largely not encouraged. There are few international visitors and most visitors are local residents and/or school groups.

Analysis

The state forests are an important ecotourism focus in the karst landscape of Puerto Rico. Numerous ecotourism activities take place in the karst landscape, and many of these are focused primarily or exclusively on the five state forests. Cumulatively, annual visitor numbers total between 27,000 and 61,000, probably at the upper end of this range. Overall, it is estimated that between 25% and 40% of ecotourism in Puerto Rico takes place within the karst (Hall, 2010), and on the basis of the visitor numbers above, approximately 5 to 15% of the ecotourism activity within the karst takes place within the state forests. This range is an estimate and is probably conservative.

One reason why visitors are drawn to the state forests in the karst is that tourism guidebooks draw particular attention to them, both as attractive and interesting destinations and as sites for nature-based activities. Some of this attention is simply because the forests represent accessible natural areas, but it at least partly reflects the realization and acknowledgement that the karst itself is a worthwhile destination, not only for its relatively undisturbed nature, but also because of the inherent character-istics of the karst landscape and its ecology. Thus, the state forests are significant both as sites for general ecotourism activities and as sites for ecotourism focusing on the specific nature of the karst itself.

The protected areas, as represented by the state forests, play several pivotal and intersecting roles with respect to tourism within the karst. First, they are representative of the least fragmented natural habitat in Puerto Rico, and are thus automatic candidates for ecotourism, which, by definition, focuses on an enjoyable experience within natural areas with minimal (low impact) human disturbance (Mowforth et al., 2008; Ceballos-Lascurain, 1996). Although one of the goals of ecotourism is minimal human disturbance, “protected area visitation may degrade natural resources, particularly in areas of concentrated visitor activities like trails and recreation sites.” (Farrell and Marion, 2001: p. 215). As visitor numbers increase, this may become a matter of increasing concern because of the inherent vulnerability of the karst landscape.

Second, they provide access, facilitating entry into the karst and utilization of the landscape. Major roads across the karst often follow the major valleys, and many minor roads follow courses of dry valleys. These roads represent the primary routes via which the majority of visitors enter and traverse the karst, although others access the karst on foot, on horse, or via rivers by tube, canoe, kayak, or swimming (Hall and Day, 2011). Not entirely incidentally, the state forests are located away from major through-flowing rivers, and the primarily road access provides a mechanism for regulation and control of tourist activity within the karst, for example, through the opening and closing of access gates on a daily or longer-term basis. Forest rangers play a role in disseminating and enforcing environmental protection regulations.

Third, the forests within the karst provide a location for a wide variety of ecotourism activities, such as hiking, birdwatching, astronomy, and cave exploration, some of which might take place in any natural area, but others are specific to the karst. In this sense, the forests function as both general and specific ecotourism sites (Hall, in preparation). In addition, the forests play many important roles within the local community environment. According to a study of public knowledge and perception of the karst forests (López-Marrero et al., 2011), local residents believe that the forests provide multiple services, with the most significant roles being as a source of forest products, as a mechanism of temperature regulation, as a habitat for flora and fauna, as an aid in air purification, and by providing recreational facilities. Sixty percent of respondents specifically associated the Puerto Rican karst forests with recreational opportunities.

Fourth, the forests play a significant educational role within the karst. With the possible exception of Vega Alta, the forests place an emphasis on providing opportunities for educational activities within ecotourism, focusing on karst hydrology, geomorphology, and biogeography.
Through the forests, ecotourists learn about karst and cave development, karst ecology, and other aspects of karst science. The forest staffs also play an important role in communicating information (either via talks or by simply distributing literature) about all that the forest has to offer in the way of wildlife and other opportunities, as well as threats to the karst, such as the potential for water contamination and rapid transfer of pollutants. Additionally, the forests represent an important habitat for wildlife and thus serve as a major focus for wildlife viewing and tracking, particularly bird-watching, which is one of the primary ecotourism activities (Raffaele, 1989). Land protection and ecotourism use of protected areas often result in positive attitudes toward protection that are stimulated by environmental education (Eagles, 1999). The situation in Puerto Rico broadly mirrors other karst regions. For example, in Belize, education about the karst is provided by wardens at protected areas operated by the Belize Audubon Society (Bundschuh et al., 2007).

Many of the ecotourism activities within the karst use the protected areas in multiple and complementary ways, combining access, entertainment, and education. In themselves, the forests represent both controlled habitats and recreational sites within the karst, and they play multifaceted roles. For example, hiking is a basic component of much of the karst-based ecotourism, either as the primary activity or as a means to other ends, such as climbing, cave exploration, or bird-watching. Many hiking trails enter the karst via protected areas, and many organized hikes begin or end there, particularly providing post-hiking relaxation opportunities. This recreational focus within the protected areas is peculiar to Puerto Rico; other regional protected karst areas place a greater emphasis on conservation, with less concern for tourism (Bundschuh et al., 2007; Day, 2010a, 2011).

Karst itself is of variable importance to the ecotourism activities (Hall, 2010; Hall, in preparation). Some, such as caving, are karst-specific, while others are less intimately focused on the karst itself. Ecotourism activities within protected areas predominantly fall into the latter category, but nevertheless, the protected areas themselves represent an important locational focus. Although ecotourism activities take place throughout the karst, water resources such as rivers, sinks, and springs are often an important focus (Hall and Day, 2011). Because the karst forests are such accessible venues for local recreation, ecotourism here is perhaps less karst-specific than in other karst areas of Central America and the Caribbean, where visiting the karst requires greater planning and dedication (Bundschuh et al., 2007).

Although all of the protected areas provide potential ecotourism sites, numerically the Guánica State Forest is currently the most important protected area for ecotourism in the karst of Puerto Rico. This reflects its status as a UNESCO Biosphere Reserve and its importance as a bird-watching destination, which more than compensate for its relative inaccessibility from San Juan. The attraction of the Guánica avifauna owes much to the karst topography and vegetation, so the ecotourism here is moderately karst-specific, more so than in many other regional karst tourism sites (Day and Hall, 2012).

Within the northern karst belt, the Río Abajo, Guajataca, and Cambalache State Forests currently report similar visitor numbers, with Vega Alta reporting significantly fewer visitors. These numbers are probably conservative, but they appear reasonable considering factors such as scenic attractions, accessibility, size, recreational versus conservation emphasis, and proximity to other attractions. Guánica is the largest of the state forests and also attracts the most visitors, while Vega Alta is the smallest and has the least visitors. Guajataca, Cambalache, and Río Abajo State Forests attract approximately the same numbers of visitors, although Río Abajo is significantly larger than the other two. Given the state forest areas, numbers of visitors and visitor densities probably fall centrally within the ranges reported elsewhere (Bundschuh et al., 2007; Day and Hall, 2012).

There are several factors that may contribute to Río Abajo (2,339 ha), Guajataca (954 ha) and Cambalache (648 ha) state forests attracting approximately the same visitor traffic including their proximity to each other, as well as to other scenic attractions, access, and recreational and scenic opportunities. All three state forests are located in the northwest karst belt (Fig. 4) and all three are easily accessible via major roadways. They are within easy driving distance of each other, and they are also in close proximity to karst-based tourist attractions such as the Arecibo Observatory and the Río Camuy Cave Park.

All three state forests offer similar outdoor recreational activities, including designated recreational areas, camping, trails for hiking, mountain biking, and walking, and picnic facilities. All three offer scenic vistas of the karst landscape. Río Abajo and Guajataca State Forests have significantly more hiking trails than Cambalache State Forest. Río Abajo and Guajataca State Forests both contain publicly accessible caves; Cambalache does not. Most guidebooks list the three state forests together when suggesting outdoor recreational options in the northern karst landscape.

It is also important to consider the recreational carrying capacity of the state forests, in other words, “the character of use that can be supported over a specified time by an area developed at a certain level without causing excessive damage to either the physical environment or the experience of the visitor” (Lime and Stankey, 1971: p. 175). Management strategies to promote conservation through education can significantly change visitor attitudes toward the environment and, in turn, help to decrease recreational impact on physical resources. For example, brochures about low-impact camping distributed at the entrance station in a forest campground reduced tree damage and litter in campsites by between 50 and 80% (Oliver et al., 1985).
It is unknown to the authors whether or not specific recreational carrying capacities have been calculated for the karst state forests in Puerto Rico, but further research into this is indicated. Current levels of visitor activity at each of the forests suggest that such carrying capacities, if they have been established, are not be exceeded at present and are unlikely to be surpassed in the immediate future. Carrying capacities within karst landscapes may be even more important considerations than in other landscapes because of their inherent fragility, and they have already proved to be important tools elsewhere within karst landscapes, for example in assessing appropriate visitor numbers for tourist caves (Cigna and Burri, 2000). Assessing the broader recreational capacities of the landscapes within the state forests represents a greater challenge, but needs further consideration.

There is some inherent conflict in the promotion of ecotourism in karst landscapes in general, as exemplified in the contradictions involved in attempting to protect caves yet also introducing and exposing them to visiting tourists (Fleury, 2009). Overall, the karst state forests appear to be successful in providing ecotourism opportunities and reconciling these activities with conservation goals. Although self-promotion by the forests is minimal, information about them is readily available via tourist literature, particularly through the internet, and by means of the Departamento de Recursos Naturales y Ambientales website (DRNA, 2010). Both residents and visitors appear well aware of the forests, although the relative proportions of each visiting specific forests varies, for example with Vega Alta having mostly local usage and international birdwatchers focusing on Guánica. All of the forests provide recreational and educational opportunities, but these are well-focused and their scope does not appear to conflict with broader ecological and conservation goals.

Recreational pressures within the forests are heaviest near access points, in designated high-usage locations such as picnic areas, along designated trails, and at sites such as lookout points and cave entrances. These pressures, which are exacerbated during weekends and over public holidays, appear to be generally well-managed, with only minimal vegetative degradation in picnic areas and limited vandalism at cave entrances. Trails appear generally in good condition, and there is little apparent adverse impact on the overall karst landscape or on the vegetation or wildlife. Littering is a recurrent problem in heavily visited areas, but is manageable and decreases rapidly away from picnic areas and short trails. The forests are afflicted by barely adequate financial and personnel resources, but they nevertheless manage to fulfill important roles both in karst landscape conservation and ecotourism. Again, a paucity of parallel studies makes it difficult to compare this situation to that in other regional karst areas, but there are certainly parallels with protected and recreational karst areas in Belize (Bundschuh et al., 2007) and elsewhere in the Caribbean (Day, 2010b, 2011).

Although the state forests are broadly comparable to other IUCN Category VI protected areas regionally and internationally, they are restricted in size, reflecting the limited area and considerable population density of Puerto Rico, but this does not seem to compromise their effectiveness. They share some similarities with U.S. mainland state forests (Ceballos-Lascurain, 1996), although the latter generally have a greater emphasis on conservation and a lesser role in recreational ecotourism. They may be more similar to U.S. mainland state parks, where recreational activities share equal prominence with ecosystem conservation.

It is difficult to compare directly the importance of ecotourism in the Puerto Rican karst forests with that in other karst landscapes, even within Central America and the Caribbean, but there exist some broad similarities, even though visitor numbers themselves here are far lower than at other specific karst sites in the region (Day and Hall, 2012). As a percentage of a country’s overall tourism, the Puerto Rican karst is somewhat lower than in Belize (Bundschuh et al., 2007) and Jamaica (Day and Hall, 2012), but it is probably representative of broader patterns elsewhere in the karst of Central America and the Caribbean. What sets the Puerto Rican karst state forests apart, however, is their relatively small areas, their accessibility, their primacy as ecotourism foci, and their importance to local populations as opposed to international visitors (with more variation seen in the Guánica case).

CONCLUSIONS

Although not every visitor or resident wants to explore Puerto Rico’s natural areas, a significant number and percentage of both are increasingly interested in alternative or ecotourism, and it is clear that the karst represents an important focus for this sort of activity. Although there are alternative destinations, such as El Yunque (the Caribbean National Rainforest) and sites in the Cordillera Central, the karst constitutes much of the remaining natural landscape in Puerto Rico.

Even though the state forests are limited in area in the karst of Puerto Rico, they play a pivotal role in influencing ecotourism in the karst, which is itself a major tourism focus. In particular, the state forests provide access, user-friendly sites, information, and educational opportunities that are used by residents and visitors alike to maximize their use and enjoyment of the karst. The state forests provide access to and through the karst landscape. Many of the ecotourism activities within the state forests, such as hiking and bird-watching, are strongly linked to the karst landscape and its ecology. The state forests also play a significant educational role, particularly in making available information about the development and formation of karst landforms.

The state forests play a pivotal role in human use of karst areas, particularly in recreation and tourism. Karst
landscapes provide significant venues for ecotourism, and the forests play a major role in promoting recreational activities and tourism opportunities. Without them, levels of ecotourism within the karst would be considerably reduced, so, although limited in numbers and area, they provide critical recreational and economic resources in the Puerto Rican karst landscape.

Finally, although the Puerto Rican karst state forests are small in number and area, and although their visitor numbers are relatively low in comparison with other tropical karst venues (Day and Hall, 2012), they represent a critical conservation and recreational element within a densely populated Caribbean island with few remaining intact ecosystems (Lugo et al., 2001). Puerto Rico has a stronger economic base than many other countries in Central America and the Caribbean (Central Intelligence Agency, 2010), so costs of, and revenues from, protected-area tourism play only a minor economic role, one that is

REFERENCES


PERCEPTIONS AND PREVALENCE OF CAVING-SKILLS TRAINING IN THE UNITED STATES AND THE UNITED KINGDOM

AARON J. BIRD1,2*, MELISSA SAWA1, AND MIKE WILES3

Abstract: Results are presented of a study of perceptions of caving-skills training. Information in the current study was obtained from questionnaires submitted between May 2011 and February 2012 by recreational cavers, researchers, and others who visit caves for enjoyment, exploration, research, or work. Respondents overwhelmingly support a connection between training and safety during cave visits. In the United States, there is an even split in numbers of people who report having had formal and informal caving-skills training. In the United Kingdom, more respondents report having had informal training than formal. In both the US and UK, experience level is high among respondents, but is not a statistically significant predictor for training type, although large majorities agree training is valuable. Outcomes from this research are used as a basis for discussion of the efficacy of caving-skills training programs in the United States and for discussion of caving-skills training already present in other countries where caving is prevalent, represented here by the United Kingdom.

INTRODUCTION

Many people enter and explore caves for recreation or work activities. While it is currently unknown how many people visit caves in the US for recreation, the National Speleological Society reports its membership at 10,100 (Wm Shrewsbury, NSS President, personal communication, 24 March 2012), which is an indicator of the number of people who are aware of caves and value them. Caves are popular in other parts of the world, as well. In France, it is estimated that approximately 7,500 people are members of the French Federation of Speleology. The UK has approximately 5,750 members of its clubs and organizations (BCA, 2011). Many other countries have national level federations related to cave exploration and cave science, including Spain, Portugal, Italy, Belgium, Austria, Bulgaria, Croatia, Slovenia, Russia, China, and Mexico.

Recreation is a primary reason why people enter caves, but not the only one. There are a significant number of people in the US and elsewhere whose workplaces are in caves or directly related to caves. In government and private-sector workplaces there are cave guides, biologists, geologists, archeologists, historians, academics, and law enforcement officers, to name just a few occupations working in and around caves. In particular, there are employees in the US National Park Service, Forest Service, and Bureau of Land Management whose jobs are directly related to caves and who may require specialized caving training to safely do their jobs (Goodbar, 2007). Unlike the recreational users of caves, this group has many more specific policies, rules, and regulations governing their time in caves. All US workplaces are required to remain in compliance with federal and state regulations, and one of the most important ways to accomplish this is through training (OSHA, 1998). There are many known hazards in caves that can pose risks, especially for workers who spend more time over the course of a year in caves than recreational visitors. In the park service, caves are treated more as natural resources than as workplaces. Historically, there has been little formal training concerning workplace hazards, even for work done along developed tour routes. Risk assessments (Goodbar, 2007) and risk estimates (Field, 2007) are used to ascertain true risk to humans from specific cave hazards. The outcomes of such risk analyses are used to prepare strategies, which will usually include training to prevent injuries, illnesses, and fatalities.

Each year in US caves, an average of 50 injuries and 3.3 fatalities are reported (Keeler, 2011, p. 7–9; Stella-Watts et al., 2012). Since 1986, there has been only one year, 2005, in which no fatalities were reported in US caves. In total, over the 22-year period from 1986 to 2008, 1,159 injuries and 75 fatalities in US caves have been recorded (Keeler, 2011). In UK caves, the raw numbers are lower. There were 22 reported incidents in 2010 and no fatalities, with an average of 28 incidents per year since 2006 (BCRC, 2010).

Caving is believed by many to be a dangerous activity. It is known to be associated with fatalities and severe injuries, including fractures, soft tissue injuries, and lacerations (Stella-Watts et al., 2012). Most injuries in caves are due to falls, which are also the primary cause of injuries for the broader population. Stella-Watts et al. reported a slightly different range of years from Keeler (2011), 1980 to 2008, in which there were 81 documented

* Corresponding Author: bird2@oakland.edu
1 School of Health Sciences, Oakland University, Rochester, MI 48309
2 CD-adapco, Northville, MI
3 Jewel Cave National Monument, Custer, SD 57730
fatalities and 1,159 injuries in US caves. Most of these fatalities and injuries occurred in undeveloped caves, where rescue and recovery can be extremely difficult. The total number of yearly visits to undeveloped caves is unknown. An estimated 2 million people visit US national park and monument caves each year (Hooker and Shalit, 2000), and the vast majority of these visits are to developed caves on park properties. Therefore, it is not possible to calculate an accurate rate of injury and fatality incidence for undeveloped caves in the US. Such a calculation has been attempted for caving incidents in the United Kingdom that occurred from 1988 to 1992, with the resulting value described as a Fatal Accident Rate (FAR) (Mohr, 2000). The UK caving FAR value of 157 was second highest among a number of UK sports, less than hang gliding and parasailing (FAR = 200) but significantly higher than rock climbing (FAR = 30 to 60), sailing (FAR = 20), and swimming (FAR = 12). This number seems quite high, as there are many years in the UK when no caving fatalities were reported. The FAR value presented here is based on a speculative visitation rate in UK caves, which if correct, shows that UK cavers are very well prepared when they enter caves. However, if the speculative visitation number is off, then a wide range of actual FAR values could be possible.

It is widely believed that training and education are useful for increasing awareness of hazards, with an end goal of training being the reduction or elimination of injuries, illnesses, and fatalities. Bird et al. (2003) provides a number of motivational approaches for enhancing safer behaviors, with arguably the most important one being behavioral reinforcement. Burke et al. (2006) reported that training involving behavioral modeling, practicing, and dialogue was effective for improving knowledge about hazards. This is further supported by the work of Robson et al. (2012), who also found evidence that training was effective for bringing about behavioral changes. However, the link to reduction of injuries, illnesses, and fatalities is not always clear. One reason for this is that trainers cannot easily compare the success of their training, as measured by tests or certifications, with the eventual incidence of problems (Goldenhar et al., 2001), because few injuries occur soon after training. The time lag between demonstration of learning outcomes, i.e., showing knowledge about hazards, and the occurrence of an injury make it difficult to track all of the factors that caused the undesired incident to occur.

The United States is one of just a few first world countries without a formal nation-wide caving-skills training program. One possible explanation for this may be that caving is perceived to be reasonably safe, and thus training is not needed. Another explanation could be that cavers are relatively few in numbers when compared to other sports, so there are simply too few cavers to support the infrastructure needed for a national caving training entity in the US. Still another explanation is that informal training—where skilled cavers personally train those with less skill to become safer cavers—currently meets the US need for development of safe-caving skills. This process is ongoing at many US national parks and monuments, such as Jewel Cave in South Dakota, where off-trail rules and situational awareness are taught to trip leaders so that knowledge and skills can be informally passed on to trip participants. A fourth explanation is that regional differences in US caves require different caving techniques and equipment. It is also possible that cavers develop beliefs about caving that are based on their individual attitudes about centralized regulation. Further, the preferences of cavers regarding training will likely vary from region to region, and also according to their reason for entering a cave, experience level, training, and other beliefs and practices. Despite these possibilities, the actual reasons for the lack of formal caving-skills training in the US are unknown.

Safety has been a foundational tenet of formalized caving in the United States since the creation of the National Speleological Society as an outgrowth of the Speleological Society of the District of Columbia. William J. Stephenson penned two articles (Stephenson, 1940, 1941) focusing directly on caving safety. The first article, published in 1940, begins, “With the Speleological Society yet in its first year and with interest in caves growing apace, it seems appropriate that one of the first problems to be dealt with is that of safety.” While the article contains discussions of caving approaches not commonly used today, it does make clear that skilled cavers of that time used techniques less risky than those of today. The article, however, does not discuss training. A similar article published one year later is more refined, specifically mentioning caving equipment selection and care, not rushing while moving through the cave, and being particularly cognizant of the two primary hazards in the cave, absence of light and slippery surfaces. Stephenson sets the stage for continued sharing of information on best practices in “methods, techniques, equipment, and exploring” by promising to print “any articles or notes any member sends in bearing on these points.” Nevertheless, a review of early issues of the NSS Bulletin and NSS News reveals an absence of discussion of actual caving-skills training.

However, at a local level in the US, training did become a focus early in the history of active caving. One example is the effort within the VPI Cave Club in Blacksburg, Virginia, to formalize key caving skills through a series of Training Bulletins that were developed by Anne Whittemore and others for that and other clubs to use in their training efforts (VAR, 2000; personal communication with Robert Hoke, 2012). In a May 1951 President’s Report, NSS President Charles E. Mohr wrote, “Orchids are due to the VPI Grotto for its splendid series of Training Bulletins…. Free copies are being mailed to other grottoes, and permission to copy part or all of them is given” (Mohr,
1951). The list of bulletins was given later in the same 1951 issue of the **NSS News**. The VPI Cave Club has had constant training offerings, primarily for its student members, since the early 1950s and formally since 1961. This may well be due to the establishment of best practices for safe caving described in the above bulletins. Establishment of and adherence to standard best practices is readily possible within a relatively small and cohesive organization like the VPI Cave Club. However, it is more of a challenge at the national level and would need to rely upon the structure of a larger organization and take into account regional preferences and caving conditions in order to be sustainable.

The US has seen attempts in the past to initiate formal, nationwide caving-skills training programs, including one that occurred in the 1950s and 1960s within the NSS. Beginning in 1958, the NSS Safety Committee (now the Safety and Techniques Committee) promoted a Qualified Caver and Qualified Leader training effort that included, among other things, demonstrating skills in knot-tying and belaying, carbide lamp operation and maintenance, and leading cave trips (Rane Curl, pers. comm.; Curl, 1958). A vote of the 1958 Congress of Grottos (NSS caving clubs) approved that the “National Safety Committee formalize a uniform-minimum safety-skill program” to be used by all in the NSS and to be implemented by individual grottos (Nicholas, 1958).

Training events were held from 1958 to 1960, and resulted in an increase in the numbers of cavers and caving trip leaders who participated in the safety-skills program in the Midwest, California, and Texas. In 1960, the plan was simplified and distributed nationally by the NSS to its local chapters. The program was used by some grottos, but did not experience widespread adoption. Reasons for this are believed to include the program’s reliance on volunteers and the opposition by some cavers to a national level program imposing uniform standards across the entire country (Rane Curl, pers. comm.).

Efforts to bring established caving skills to the broader membership of the NSS occurred on at least two subsequent occasions. In 1973, a vote was taken in the Congress of Grottos at the NSS Convention in Bloomington, Indiana, to determine the level of interest in certification (Devereaux, 1973). Positive interest in certification was expressed by a vote of 153 out of 159. However, the effort does not appear to have gone much further, as no evidence was found indicating a caver certification program was ever initiated within the NSS. There was likely opposition to certification over the next few years, as indicated by the following quote in the 1976 NSS Caver Training Commission Report (Ediger, 1976), “The idea that the Caver Training Manual is a precursor to caver certification should be extirpated from the mind of anyone harboring such a notion!” Notably, the Caver Training Commission was renamed the Caver Training Committee in 1984. No additional references were found about this Committee until 1996 when it was eliminated by an act of the NSS Board of Governors (NSS, 2012a).

Outside of the focus on caving skills and training for preventing injuries and fatalities, but still fully within the scope of caving training, is the National Cave Rescue Commission (NCRC). NCRC was established by an Act of the Board of Governors of the NSS in 1979 to, among other things, “prompt competence and cooperation among Society members, organizations, and related agencies in the field of cave rescue” (NSS, 2012a). The original wording has been replaced by the Charter of the National Cave Rescue Commission (NSS, 2003), which outlines the purposes, responsibilities, and management structure of the Commission, including training focused on cave rescue practices and procedures. Mirza (2003) summarizes the “mandates” in the charter as “to develop and teach [a] cave rescue curriculum,” “to act as the ‘voice’ of the NSS during cave rescues,” and “to develop working relationships with various governmental agencies as needed to help coordinate in the event of a rescue.”

In recent years, there have been multiple fatalities of individuals who were exploring caves for recreational purposes that invoked the mandates of the NCRC. In one case, two individuals died while attempting to climb a rope under a waterfall to retrieve a lost gear bag (Caulfield, 2011). In another, an individual crawled into a tight space from which he could not return (Farrell, 2009). Each of these deaths might have been avoided if the victims had taken formal caving-skills training. Since the US does not have such a program in place, the only option for reducing such fatalities would be through informal training in a caving club, which is sporadically offered throughout the US. Therefore, the continued presence of avoidable injuries and fatalities every year in US and UK caves is a valid reason for studying perceptions of caving skills training.

Mishaps in caves have significant emotional and psychological costs to the injured individual, families and friends, rescuers, and the communities; the actual costs in pain and suffering are immeasurable, and all injuries, illnesses, and fatalities in caves are to be avoided. However, with no intention of minimizing the significance of emotional impacts, it is possible to obtain an estimate of the economic loss from cave-related injuries and fatalities. Applying national average values (NSC, 2013), the average cost of an injury in 2011 US dollars is $8,300 for non-workplace-related injuries and the cost of an unexpected death outside of the workplace is $1.1 million. Applied to recreational caving, the value of $8,300 is likely too low, considering that injuries in caves require trained, equipped teams for extraction. A potential high-end value that could be used is around $54,000, which represents the average cost of employer expenses for a person getting hurt at work. Using the NSC cost estimates and data in *American Caving Accidents* (Stella-Watts et al., 2012), the costs of injuries and fatalities in US caves over the twenty-three-year period from 1986 through 2008 can be calculated to
range from $106 million to $147 million, using equivalent 2011 US dollars.

**MATERIALS AND METHODS**

An online, anonymous questionnaire was used for gathering information about the perceived value of formal caving-skills training. The Oakland University Institutional Review Board (IRB) for protection of human research participants reviewed and approved the questionnaire, participant recruiting approaches, and the associated consent forms.

The questionnaire included twenty-five items for gathering information about the perceived value of formal caving-skills training. *Formal caving-skills training* in this research is used to mean caving classes that are designed to teach a wide variety of people, that are scheduled, and that incorporate some measure of successful instruction. Four primary categories of information were requested from participants (Table 1). The first group of questions collected information on caving experience, membership in caving clubs or grottos, and primary caving region. The second group of questions collected information on the impact, or lack thereof, of educating people about caving skills and techniques. The third group investigated personal beliefs regarding the benefits or detriments of caving-skills training. The fourth group was intended to gauge perceptions of the value, or lack thereof, of caving-skills training. The fourth group was a single opportunity for respondents to write comments about caving training in their own words. The second and third categories contained questions intended to assess beliefs concerning past experience, as well as potential future outcomes. Responses from the online questionnaires were obtained anonymously and have no associated personal identifiers.

Responses from the online questionnaires were obtained anonymously and have no associated personal identifiers. Anyone who wished to complete the online surveys could do so. Responses were analyzed for those who visited caves and actually go caving.

Analyses of participant responses included determining the frequency of responses to particular questions and then reporting the outcomes. T-tests for independent means were used to determine whether differences of means were significant. Resulting values from t-tests include the t-statistic, which is related to the size difference between the means of two samples, and \( p \) value, which is a probability indication; if \( p < 0.05 \), there is a 95 percent probability that the difference is real for the populations being studied. One-way ANOVA were used for comparisons of means of more than two groups. This is a statistical procedure using \( F \)-ratio to determine whether the group means differ (Field, 2009). Filtering by categories was conducted to create blocks of participant feedback for comparison within question-response groups. Primary categories include experience, training level, and preferred caving region. Responses were categorized according to years of caving experience (Table 2). Within these categories, further subdivisions were made for type of training including formal, informal, and rescue.

**RESULTS**

Participants completed 135 questionnaires on perceptions of caving-skills training. Data were analyzed by experience, the kind of training the responder had, and preferred caving region. Respondents indicating the US and UK as their primary country for caving made up 98\% of all responses, and thus, regional analysis is focused on these groups. Of these, 98 responses were from North America; 35 were from the UK and Ireland. Of all responses, 73 percent reported the US being their primary country for caving, 25 percent reported caving in UK, and the remainder indicated Belize, Canada, Ireland, Slovenia, and Spain. Among responses from cavers indicating the US as their primary country for caving, 24\% listed the Southeast as most preferred region, 23\% the Virginias, 10\% Texas, and 9\% the Ohio Valley (Table 3). UK cavers listed Derbyshire and Yorkshire (63\%) and Wales (22\%), followed by Devon and Mendip (15\%), as their primary caving regions (Fig. 1). There were one or two responses

<table>
<thead>
<tr>
<th>Table 1. Primary categories on questionnaire.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Category</td>
</tr>
<tr>
<td>-----------------------------</td>
</tr>
<tr>
<td>Caving experience, training experience, membership in caving clubs or grottos, and primary caving regions</td>
</tr>
<tr>
<td>Impact, or lack thereof, of educating people about caving skills and techniques</td>
</tr>
<tr>
<td>Perceptions of the value, or lack thereof, of caving-skills training on reducing injuries and fatalities in caves</td>
</tr>
<tr>
<td>Freeform comments</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2. Experience level categories for data analysis.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coded Levels</td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
</tbody>
</table>
The participants tended to have quite a bit of experience: 60 percent of respondents had at least ten years of caving experience; 6.6 percent of all respondents reported having 0 to 2 years experience; 16 percent reported having 2 to 5 years experience; 12 percent had 5 to 10 years; 28.5 percent reported 10 to 20 years of experience and 36.5 percent reported having more than 20 years of caving experience. Generally, experienced cavers appeared to be more likely to respond to the survey than those with less experience. Of note, the most prevalent caving regions in the US (Southeast) and the UK (Yorkshire and Derbyshire) have respondents with the least amount of experience, although the averages are 10 and 12 years respectively (Figure 2). The high population densities and the many caves in these areas might explain this.

Overall, 95 percent of respondents reported having had some kind of training, formal, informal, or rescue. Among this group, informal training is most prevalent at just above 50 percent. Those with rescue training are fewer than 10 percent of the group, while those with formal training make up the remainder. As shown in Figure 3, informal training is more common in the UK, whereas formal training is more common in the preferred caving regions of the US. Rescue training, however, is common in the Western and Mid-Atlantic regions as well as the Virginias and Ohio Valley. Informal training is more common in the Southwest, Midwest, and Northeast regions.

Respondents to the questionnaire were categorized according to years of experience from 0 to 3, 3 to 5, 5 to 10, and greater than 10 (see Table 2). Analysis of the data indicates that having more experience does not necessarily

<table>
<thead>
<tr>
<th>Region</th>
<th>Percentage of NSS Members Residing in the Region</th>
<th>Percentage of Cavers Residing in the Region</th>
</tr>
</thead>
<tbody>
<tr>
<td>Northeast</td>
<td>7.3</td>
<td>5.0</td>
</tr>
<tr>
<td>Mid Atlantic</td>
<td>5.2</td>
<td>9.9</td>
</tr>
<tr>
<td>Virginias</td>
<td>23.0</td>
<td>9.2</td>
</tr>
<tr>
<td>Ohio Valley</td>
<td>9.4</td>
<td>10.4</td>
</tr>
<tr>
<td>Southeast</td>
<td>24.0</td>
<td>25.3</td>
</tr>
<tr>
<td>Midwest</td>
<td>4.2</td>
<td>11.3</td>
</tr>
<tr>
<td>Rockies</td>
<td>6.2</td>
<td>6.8</td>
</tr>
<tr>
<td>Texas</td>
<td>10.4</td>
<td>4.5</td>
</tr>
<tr>
<td>Southwest</td>
<td>5.2</td>
<td>4.4</td>
</tr>
<tr>
<td>Western</td>
<td>5.2</td>
<td>9.0</td>
</tr>
</tbody>
</table>

*NSS, 2012b, p. 56.

Table 3. US response and residence percentages by region.
Figure 2. Respondents' mean years of experience by preferred caving region.

Figure 3. Most prevalent type of training (informal, formal, or rescue) by region as reported by respondents in the US and UK.
mean cavers are more likely to have had formal caving-skills training: $F(3,124) = 0.26, p = 0.86$. However, statistical significance is obtained when looking at those with formal rescue training at an experience level greater than ten years: $F(3,126) = 6.25, p < 0.001$. Thus, respondents who have had cave rescue training are statistically more likely to be more experienced, and, vice versa, successful veteran cavers might accumulate more “experiential training equivalents” than would be provided through formal training. Subdividing by country, 53 percent of US respondents and 33 percent in UK report having had formal caving-skills training, not including cave rescue. When cave rescue is added, 61 percent of US respondents report having had formal caving-skills training, including rescue. When adding cave rescue, the percentage does not change among UK respondents.

The majority of respondents in the US (81%) agree that individuals entering caves without knowledge or skill are more likely to be injured or killed (see Table 4 for breakdown of responses by region). Respondents who have received formal training agree more (89%) than respondents who have received informal training (76%). However, only 70 percent of respondents in the UK agree with this statement, and there are no differences between those who have received formal and informal training.

Table 4. Survey question responses from the USA and from the UK by country and by region.

<table>
<thead>
<tr>
<th>Country/Region</th>
<th>Among Respondents with Formal Training (percent)</th>
<th>Among Respondents with Informal Training (percent)</th>
<th>Among All Respondents (percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>United States</td>
<td>89</td>
<td>76</td>
<td>81</td>
</tr>
<tr>
<td>Northeast</td>
<td></td>
<td>67</td>
<td></td>
</tr>
<tr>
<td>Mid Atlantic</td>
<td></td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>Virginia</td>
<td></td>
<td>81</td>
<td></td>
</tr>
<tr>
<td>Ohio Valley</td>
<td></td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Southeast</td>
<td></td>
<td>79</td>
<td></td>
</tr>
<tr>
<td>Midwest</td>
<td></td>
<td>67</td>
<td></td>
</tr>
<tr>
<td>Texas</td>
<td></td>
<td>78</td>
<td></td>
</tr>
<tr>
<td>Rocky Mountains</td>
<td></td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>Southwest</td>
<td></td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Western</td>
<td></td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>United Kingdom</td>
<td>67</td>
<td>67</td>
<td>70</td>
</tr>
<tr>
<td>Devon &amp; Mendip</td>
<td></td>
<td>67</td>
<td></td>
</tr>
<tr>
<td>Derbyshire &amp; Yorkshire</td>
<td></td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>Wales</td>
<td></td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

Confidence in caving is, of course, very important. However, actual outcomes can reveal the full impact of confidence training is critical, but it is interesting to note that 44 percent of respondents in the US report did not know where they can get caving-skills training. On the other hand, respondents from the UK overwhelmingly report being aware of caving skills training opportunities (83%), $t(121) = 4.14, p < 0.001$, but more people in the US would attend training if the courses were tailored to fit their needs than cavers in the UK, $t(120) = 2.25, p < 0.05$.

Confidence in caving is evident in Table 4. Survey question responses from the USA and from the UK by country and by region.
using training to prepare for cave exploration. Respondents report that formal training \((t(124) = 2.20, p < 0.05)\) and formal rescue training \((t(126) = 2.30, p < 0.01)\) have helped save their own or others lives while exploring caves. Furthermore, regardless of the type of training received, respondents report that without the knowledge and skill obtained from training, they believe there would be a high probability they could be injured or killed when they explore highly hazardous caves (69%). It is likely that an experienced caver would understand what activities and conditions are beyond normal comfort level and take steps to prepare for or avoid such situations.

Regarding potential future impact of training, respondents believe that improving knowledge and skill of caving will allow them to more safely explore caves, \(t(123) = 2.25, p < 0.05\). Furthermore, 62 percent of respondents believe there is a need for new and improved training programs. This prevalence, however, decreases slightly among cavers who have more than ten years of caving experience, where only 54 percent indicate a need. This may be because experienced cavers are more likely to turn to their more experienced peers, rather than put their trust in a training program.

**DISCUSSION**

It might be expected that the occurrences of injuries, illnesses, and fatalities in caves would influence people’s beliefs that there should be additional training beyond what is already being offered. The majority of all respondents in this study agree. However, among those who report having had caving-skills-related training, only those with rescue training come close to statistical significance in associating the presence of accidents and incidents with a need for more training. This is so because there is emphasis in cave rescue training on creating achievable outcomes, i.e., successfully rescuing a simulated injured caver, in order to enhance learning of the key objectives of the course. This likely creates a connection between the training environment (simulated cave rescues involving injuries, illnesses, and/or fatalities) and the successful demonstration of desired learning outcomes. Subsequently, this means that cave rescue trainees value training that positively addresses events associated with caving-related mishaps. The purposes of general caving-skills-related training are to successfully and confidently negotiate the cave environment, not to deal with injuries, illnesses, or fatalities. This further explains why there is no association observed in the data.

In the US, formal caving-skills training is more prevalent. However, most respondents in the UK report having had informal training. Participants’ qualitative comments provide some reasons for this. In the UK there is very little caver training for the new novice caver other than SRT. Nearly all the training is done at the club level and mainly through experience with novice trips being arranged.

Additional comments from respondents indicate that many are members of caving clubs affiliated with UK universities. In a study on culture in the United Kingdom, it has been suggested that there are rebellious and conscientious “post-materialistic” concerns among more educated sections of the UK population, whereas less educated and older portions of the population have more materialistic concerns (Majima and Savage, 2007). That is, they are less likely to comply with the constraints of structured organizations and prefer sports and endeavors that allow them to behave more freely. This ideology, assuming it is present within the university caving clubs, may provide some sort of explanation for the much lower prevalence of formal training in the UK, even though it is more readily available to UK cavers.

A number of respondents, both in the US and UK, express preference for informal caving-skills training, particularly because they are able to work directly with those who have more experience and knowledge, much like an apprentice-mentor teaching and learning model. Adult-style learning should focus on “discovery learning” where the individual is motivated to learn because of his desire to understand what he is learning (Knowles et al., 1998). Hands-on training is not necessarily incorporated into formal training; many respondents to the qualitative portion of this study suggested that caving training is preferred when it involves hands-on experiences in an informal setting. It has been suggested that new information is only imputed when there is an opportunity to reflect on experience consistent with the concept of “hands-on” learning or learning from practice (Bleakley, 2000), which again lends itself very well to the informal, apprentice-mentor learning model. We find it safest to incorporate an enthusiastic new caver into increasingly more difficult caving trips, and educate them on-site, as needed. A limited amount of vertical training and practice can be done above ground and prior to a trip, but nothing beats actual in-cave experience, especially when it comes to rigging ropes.

Informal training is clearly desirable, popular, and apparently effective. However, respondents report that formal caving-skills training makes them more confident cavers than other forms of training. Research shows that confidence is associated with higher performance (Compere and Postlewaite, 2004). However, qualitative comments on the questionnaires provide cautions about becoming overconfident through formal caving-skills training. “Formal courses can teach skills and techniques—what they cannot provide is judgment and experience.”

Formal caving-skills training has the benefit of being designed with clear learning objectives and measures of learning outcomes. Informal training is likely more comfortable and tailored to the individual trainees’ learning paces and specific needs. Furthermore, because informal training is similar to apprentice-mentor models, it also provides the benefit of allowing trainees to reflect and synthesize what they’ve learned and practiced. It may be
possible to blend the benefits of these two approaches, wherein learning objectives and measures of learning outcomes are included within the apprentice-mentor model.

CONCLUSIONS

The cavers who participated in this study are experienced and trained, most through informal caving-skills training. Regionally, there are differences in experience level, training level, and perceptions among respondents. Of note, the two most popular caving areas in the US and UK, namely the southeast US and Yorkshire in the UK, have the least experienced responders, although average experience in each of these locations is 10 and 12 years, respectively. Respondents from a number of regional locations in the US have formal, cave-rescue-trained individuals as the highest percentage. In the UK, however, all caving regions have informally trained cavers as the highest percentages. Both formal and informal caving skills training have benefits. A blend of the two is likely the best approach for optimizing learning outcomes in caving-skills training.

ACKNOWLEDGEMENTS

The authors would like to thank Christopher Binding, Rachel Bosch, Jansen Cardy, Rane Curl, Amy Hinkle, Art Palmer, and Wm Shrewsbury for assistance in finding study participants, for contributing their expertise of caving cultures in the US and the UK, and reviewing drafts of this paper.

REFERENCES

Field, M.S., 2007, Risks to cavers and cave workers from exposures to low-level ionizing x radiation from 222Rn decay in caves: Journal of Cave and Karst Studies, v. 69, no. 1, p. 207–228.
Mohr, C.E., 1951, President’s annual report: NSS News, v. 9, no. 6, 5 p.
Nicholas, G., 1958, President’s column: NSS News, v. 16, no. 6, p. 51–52.
THE MINERALOGICAL STUDY OF THE GROTTA INFERIORE DI SANT’ANGELO (SOUTHERN ITALY)

MANUELA CATALANO1, ANDREA BLOISE2*, DOMENICO MIRIELLO2, CARMINE APOLLARO2, TERESA CRITELLI2, FRANCESCO MUTO2, ENZO CAZZANELLI1, AND EUGENIO BARRESE2

Abstract: In the present work, thirteen samples collected from the Grotta Inferiore di Sant’Angelo near the town of Cassano allo Jonio (Calabria region, southern Italy) were analyzed for their mineralogy. The Grotta Inferiore di Sant’Angelo is made up of sub-horizontal, interlinked galleries between 400 and 450 meters above sea level. The floor is littered with deposits such as bat-guano, gypsum, and many speleothems that also cover the walls. The samples were identified and characterized by X-ray powder diffraction, scanning electron microscopy with energy dispersive spectrometer, microthermometry, and micro-Raman spectroscopy. The ten primary minerals identified in this study belong to six different groups: carbonate, sulfate, apatite, oxide and hydroxide, halide, and silicate. Clay minerals and eight other detrital minerals were also found: enstatite, rutile, magnesite, pyrite, chrysotile, quartz, dolomite, and chlorite. Characterization of cave minerals could be useful to improve the knowledge of the relation between them and the lithology of the host rocks.

INTRODUCTION

Natural caves may give rise to complex minerogenetic processes controlled by particular conditions that change from one cave to the next (Hill and Forti, 1997). In-depth characterization of cave minerals may be the first step toward understanding the genetic processes of cave minerals and chemical deposits. However, to reach this ambitious goal, various aspects of the cave must be investigated, such as host rocks, cave sediments, and circulating fluids (Hill and Forti, 1997; Frau et al., 1998; Lattanzi et al., 1998). Some very different minerogenetical mechanisms may induce the deposition of crystalline and amorphous phases that are stable as long as environmental conditions remain constant. These phases may easily change if genetic conditions change (Benedetto et al., 1998; Forti et al., 1999, 2000, 2001). For this reason, caves where environmental conditions have changed are good environments in which various types of mineral phases can grow.

The presence of hypogenic caves (Galdenzi, 1997) made the karst area of Monte San Marco (Cassano allo Ionio) (Fig. 1) an ideal site in which to study cave mineralogy. The complex of the Sant’Angelo caves is the largest, and it is made up of three caves: Grotta Superiore di Sant’Angelo, Grotta Inferiore di Sant’Angelo, and Grotta Sopra la Grotte di Sant’Angelo. The Grotta Inferiore di Sant’Angelo was only explored a few decades ago, in 1951, by Orofino (1965). It was not until 1977–1979 that it was studied systematically by the Commissione Grotte Eugenio Boegan (Gasparo, 1979; Larocca, 1991). The cave extends about 1325 meters and is linked to other caves with natural entrances at various elevations on the slopes of Monte San Marco. It has been explored over the years and has revealed evidence of human habitation since the Neolithic and Bronze Ages (Tinè, 1964a, b; Malone, 2003).

Morphologically, the Grotta Inferiore di Sant’Angelo is made up of sub-horizontal, interlinked galleries between 400 and 450 meters above sea level. The floor is littered with deposits of heterogeneous alluvial material and filled with guano deposits, gypsum that was partially quarried in the past, and many speleothems that also cover the walls (Gasparo, 1979; Larocca, 1991). In contrast to the large number of papers on the speleological and geomorphological characterization of the Sant’Angelo caves (Gasparo, 1979; Larocca, 1991; Galdenzi, 1997), no previous papers report in detail the minerals of the Grotta Inferiore di Sant’Angelo. So the aim of this paper was the characterization of the minerals collected in the cave.

GEOLOGICAL SETTING

The study area is in the Cassano structural high, in particular in the dolomitic and calcareous Meso-Cenozoic successions outcropping in Monte San Marco (Fig. 1). The carbonate rocks are topped by alternating grey and varicolored shale and phyllite of Cretaceous age. The metamorphic rocks are overlapped by a thick clastic terrigenous succession of Pliocene-Pleistocene age (Fig. 2) made up of conglomerates, sand, and thick bodies of clay and silt. Knowledge of the geologic evolution of the Cassano structural high has been improved by data on the Quaternary tectonic and morphostructural phase. In

* Corresponding Author: andrea.bloise@unical.it
1 Dipartimento di Fisica, Università degli Studi della Calabria, Ponte P. Bucci cubo 31C, 87036 Arcavacata di Rende (CS), Italy
2 Dipartimento di Biologia, Ecologia e Scienze della Terra, Università degli Studi della Calabria, Ponte P. Bucci cubo 15B, 87036 Arcavacata di Rende (CS), Italy

Journal of Cave and Karst Studies, April 2014 • 51
particular, the area is characterized by large trascurrent and normal fault systems exposing the Mesozoic and Quaternary terrains. The fracture and joint systems in Monte San Marco are linked to the main northeast- and northwest-oriented fault systems (Schiattarella, 1998; Spina et al., 2009). Along the major fault, a large, deep cave system developed, probably during the Pleistocene uplift of the area (Fig. 2a). The first subsurface cavities were formed by dissolution of the limestone related to the rising of H₂S-rich water within the limestone (Fig. 2a). Tectonic activity in the major fault was responsible for exhumation of carbonate rocks and gave rise to aquifer circulation. Thermal springs represent the subsurface upwelling of the Sibari thermal system and sulfate waters (Apollaro et al., 2012). The chemical characteristics of the waters suggest that the oxidation of H₂S to sulfuric acid favored by the mixing between thermal water (Ca-SO₄ to Ca-SO₄-HCO₃ in composition) and fresh water (Ca-HCO₃ to Ca-Mg-HCO₃ (Apollaro et al., 2012) infiltrating along the major fault was the main source of aggressiveness and dissolution of limestone. The progressive fall in the piezometric level is due to gradual exhumation of the top and lateral aquicludes, represented by shale and phyllite displaced by major faults (Galdenzi, 1997) (Fig. 2b). During this stage, sulfate waters saturated carbonate rocks and caves, enabling the precipitation and growth of sulfate crystals. Unsaturated conditions were progressively established, due to erosion and exhumation of the hanging wall, with a consequent fall in the level of the thermal water that today lies at 230 to 270 m below the cave entrance (Fig. 2c).

**Sampling and Analytical Techniques**

Thirteen samples were collected along the main accessible galleries and rooms of the Grotta Inferiore di Sant’Angelo. Figure 3 is a map of the cave that shows the locations where the primary minerals were found in the cave. The cave samples were collected from filling deposits, fracture fillings, corrosion pockets, wall residues, puddles, floor detritus, clay-bearing carbonate speleothems, and fills, including those in fractures.

A detailed analysis of all samples by optical microscopy was performed to separate the mineralogical phases in each sample. The phases were analyzed by powder X-ray diffraction when the material was sufficient. Patterns were obtained on a Bruker D8 Advance X-ray diffractometer with CuKα radiation at 40 kV and 40 mA, monochromated with a graphite sample monochromator. Scans were collected in the range 3–66° 2θ, with a step interval of 0.02° 2θ and a step-counting time of 3 seconds. Analyses of clay minerals were carried out not only on untreated samples, but also after ethylene-glycol and thermal treatments. DIFFRACplus EVA software was used to identify the mineral phases in each X-ray powder spectrum, experimental peaks being compared with 2005 PDF2 reference patterns.

Chemical composition and morphology were investigated by energy-dispersive spectroscopy with a field-emission...
scanning electron microscope (FEI Quanta 200). Microthermometry of fluid inclusions was conducted on a Linkam THMSG 600 heating and freezing stage coupled with a microscope equipped with 40\(\times\) or 100\(\times\) objectives. The stages were calibrated by a set of synthetic fluid inclusions with an estimated accuracy of about \(\pm 0.1^\circ{\text{C}}\) at the triple point of CO\(_2\) (\(-56.6^\circ{\text{C}}\)), at the triple point of H\(_2\)O (0.015 \(^\circ{\text{C}}\)), and at the critical temperature of H\(_2\)O (374 \(^\circ{\text{C}}\)). The heating and cooling rate used was 0.1 \(^\circ{\text{C}}\) min\(^{-1}\). Spectroscopic investigations were performed by a Raman microprobe Jobin-Yvon Labram (spectral resolution ~2 cm\(^{-1}\)) equipped with a CCD detector and a He-Ne laser (632.8-nm emission wavelength).

**RESULTS**

The mineralogical study of the samples revealed ten primary minerals (Table 1); the most abundant groups were carbonates, phosphates, and sulfates. In addition to the primary minerals, enstatite, rutile, magnesite, pyrite, chrysotile, quartz, dolomite, clinochlore, and clay minerals also were found in some samples, but they are not of cave origin (Fig. 4).

**PRIMARY MINERAL**

**Carbonates**

Calcite (CaCO\(_3\)) was the most abundant mineral found in the cave, in view of the fact that the speleothems in the cave are mainly of calcitic composition. Calcite crystals are a few microns in size when growing in lanceolate aggregates (Fig. 5a), classic rhombohedral crystals (Fig. 5b), and radial aggregates of needle-like crystals diverging from the same nucleus ending in rounded surfaces, and they reached about 3 mm in the case of botryoidal crystals (Fig. 5c). Calcite crystals were detected in flowstones, stalagmites, and stalactites and in concretions that cover almost all the walls. The growth of calcite was controlled by evaporation and variations in CO\(_2\) concentrations in groundwater previously in contact with carbonate rocks (Holland et al., 1964; White 1976).

**Sulfates**

Gypsum (CaSO\(_4\)-2H\(_2\)O) was a very common mineral, present in all thirteen samples and showing colors from milky to semi-transparent dark grey. It exhibits various morphologies: granular masses growing over corrosion pockets in the limestones of the cave walls (Fig. 5d); prismatic gypsum crystals (Fig. 5e), sometimes characterized by parallel intergrowth (Fig. 5f); lanceolate (Fig. 5g); sub-euhedral aggregate (Fig. 5h); and lamellar (Fig. 5i). Barite (BaSO\(_4\)) occurs as globular concretions or aggregates of fibrous and lamellar crystals about 3 \(\mu\)m in length.

In agreement with Galdenzi (1997), abundant gypsum deposits were formed as the result of oxidation of H\(_2\)S-rich water during the cave origin, also confirmed by barite genesis. Gypsum crusts precipitated on the walls of the cave probably originated from water that had partially dissolved gypsum in the evaporite levels in the carbonate rocks.

**Phosphates**

The general name of apatite group has been given to hydroxylapatite, carbonate-hydroxylapatite, carbonate-fluorapatite, and fluorapatite, because it was almost impossible to discriminate among these phosphates by stereo-microscopy or EDS microprobe analysis. The resulting morphology of these crystals of biogenetic origin in our samples is extremely variable and unusual. The largest amount occurs as a thin crust growing on a carbon-rich phase that has a spongy appearance (Fig. 6a), perfect cylindrical morphology (Fig. 6b), and as microcrystals that sometimes give rise to small spheres of 10 \(\mu\)m diameter (Fig. 6c) that may be insulated or aggregated to form thin crusts. Organic matter, verified by energy-dispersive spectroscopy, may have acted as nuclei for the crystals shown in Figure 6c. In some cases, scanning electron microscopy shows aggregates of close subparallel fibers and radial aggregates of fibrous crystals (Figs. 6d, 6e). As the literature shows, phosphates come from the reaction...
Figure 3. Plan of Grotta Inferiore di Sant’Angelo showing the location of secondary deposits sampled and the mineral occurrences observed.

Figure 4. Scanning electron microscope images of minerals from Grotta Inferiore di Sant’Angelo: (a) subeuhedral dolomite crystals, surrounded by aggregates of calcite crystals; (b) subeuhedral quartz crystal; (c) rutile crystal; (d) chrysotile fiber; (e) elongated prismatic crystals of enstatite; (f) subeuhedral magnesite crystals.

*Journal of Cave and Karst Studies, April 2014*
Table 1. Minerals of the Grotto Inferiore di Sant’Angelo.

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Identification Techniques</th>
<th>Chemical Formula</th>
<th>Group</th>
<th>Morphology</th>
<th>Size, max / min (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary Minerals</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gypsum</td>
<td>XRPD, EDS/SEM</td>
<td>CaSO₄·2H₂O</td>
<td>Sulphates</td>
<td>Lamellar, massive, lanceolate</td>
<td>2000 / 1</td>
</tr>
<tr>
<td>Barite</td>
<td>EDS/SEM</td>
<td>BaSO₄</td>
<td>Sulphates</td>
<td>Anhedral</td>
<td>5 / 3</td>
</tr>
<tr>
<td>Calcite</td>
<td>XRPD, EDS/SEM</td>
<td>CaCO₃</td>
<td>Carbonates</td>
<td>Lamellar, tabular, needle-like</td>
<td>3000 / 1</td>
</tr>
<tr>
<td>Goethite</td>
<td>EDS/SEM</td>
<td>FeO·OH</td>
<td>Hydroxide</td>
<td>Fibrous</td>
<td>10 / 2</td>
</tr>
<tr>
<td>Hematite</td>
<td>EDS/SEM</td>
<td>Fe₂O₃</td>
<td>Oxides</td>
<td>Iron pencil, mammillary, botroydal</td>
<td>20 / 5</td>
</tr>
<tr>
<td>Iron hydroxide</td>
<td>EDS/SEM</td>
<td>FeO·OH·ₙH₂O</td>
<td>Hydroxide</td>
<td>Massive</td>
<td>...</td>
</tr>
<tr>
<td>Sylvite</td>
<td>EDS/SEM</td>
<td>KCl</td>
<td>Halide</td>
<td>Sub-euhedral</td>
<td>2 / 8</td>
</tr>
<tr>
<td>Halite</td>
<td>EDS/SEM</td>
<td>NaCl</td>
<td>Halide</td>
<td>Anhedral</td>
<td>12 / 2</td>
</tr>
<tr>
<td>Sauconite</td>
<td>XRPD</td>
<td>Na₀.₃Zn₃(SiAl)₄O₁₀(OH)₂·₄H₂O</td>
<td>Clay</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Apatite</td>
<td>XRPD, EDS/SEM</td>
<td>Ca₅(PO₄)³[F, OH, Cl]</td>
<td>Apatite</td>
<td>Cylindrical, massive, fibrous, spherical</td>
<td>50 / 0.5</td>
</tr>
<tr>
<td><strong>Detrital Minerals</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dolomite</td>
<td>XRPD, EDS/SEM</td>
<td>CaMg(CO₃)₂</td>
<td>Carbonates</td>
<td>Massive, granular</td>
<td>3000 / 1</td>
</tr>
<tr>
<td>Rutile</td>
<td>EDS/SEM</td>
<td>TiO₂</td>
<td>Oxides</td>
<td>Prismatic</td>
<td>200 / 15</td>
</tr>
<tr>
<td>Quartz</td>
<td>EDS/SEM</td>
<td>SiO₂</td>
<td>Silicates</td>
<td>Sub-euhedral</td>
<td>20 / 30</td>
</tr>
<tr>
<td>Pyrite</td>
<td>EDS/SEM</td>
<td>FeS₂</td>
<td>Sulphur</td>
<td>...</td>
<td>10 / 2</td>
</tr>
<tr>
<td>Magnesite</td>
<td>EDS/SEM</td>
<td>MgCO₃</td>
<td>Carbonates</td>
<td>Anhedral</td>
<td>40 / 10</td>
</tr>
<tr>
<td>Enstatite</td>
<td>EDS/SEM</td>
<td>MgSiO₄</td>
<td>Silicates</td>
<td>Prismatic</td>
<td>40 / 20</td>
</tr>
<tr>
<td>Chrysotile</td>
<td>XRPD</td>
<td>Mg₃Si₂O₅(OH)₄</td>
<td>Serpentine</td>
<td>Fibrous</td>
<td>15 / 5</td>
</tr>
<tr>
<td>Montmorillonite</td>
<td>XRPD</td>
<td>(Na, Ca)₀.₃(Al, Mg)₂Si₄O₁₀(OH)₂·ₙH₂O</td>
<td>Clay</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Sauconite</td>
<td>XRPD</td>
<td>Na₀.₃Zn₃(SiAl)₄O₁₀(OH)₂·₄H₂O</td>
<td>Clay</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Phlogopite</td>
<td>XRPD</td>
<td>KMg₃(Si₃Al)O₁₀(F, OH)₂</td>
<td>Clay</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Clinohlore</td>
<td>XRPD</td>
<td>(Mg, Fe)₂Al(Si₃Al)O₁₀(OH)₆</td>
<td>Clay</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

Note: XRPD = X-ray powder diffraction. EDS = Energy dispersive spectroscopy. SEM = Scanning electron microscopy.
between calcite speleothems and phosphoric solutions derived from guano.

Oxides and Hydroxides

Iron oxide and hydroxide minerals form from the oxidation of pyrite or other iron-bearing sources (White et al., 1985; White 1988). Hematite (Fe₂O₃) often occurs as overgrowth on gypsum crystals developed as mammillary-botryoidal aggregates (Fig. 7a) and with a platy habit (Fig. 7b). Two Fe-hydroxides phases were detected, massive amorphous iron hydroxide accumulations (Fig. 7c) and goethite (FeO·OH) with small fibrous tufts and small

Table 2. Minerals identified in clay subsample a before and after treating with ethylene glycol and subsample b before and after heating to 450 °C.

<table>
<thead>
<tr>
<th>Subsample a</th>
<th></th>
<th>Subsample b</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>Quartz</td>
<td>Quartz</td>
<td>Quartz</td>
<td>Phlogopite</td>
</tr>
<tr>
<td>Calcite</td>
<td>Calcite</td>
<td>Calcite</td>
<td>Dolomite</td>
</tr>
<tr>
<td>Dolomite</td>
<td>Dolomite</td>
<td>Dolomite</td>
<td>Phlogopite</td>
</tr>
<tr>
<td>Clinochlore</td>
<td>Clinochlore</td>
<td>Clinochlore</td>
<td>Sauconite</td>
</tr>
<tr>
<td>Clinochlore</td>
<td>Clinochlore</td>
<td>Clinochlore</td>
<td>Montmorillonite</td>
</tr>
</tbody>
</table>

56 • Journal of Cave and Karst Studies, April 2014
spherical aggregates growing over gypsum and calcite crystals (Fig. 7d). According to Fischer and Schwertmann (1975), although hematite and goethite phases are found together, they are not related genetically by a simple hydration-dehydration type of reaction, but were formed separately from a common source of iron. Environmental conditions play an important role in determining which of the two forms is produced (Schwertmann, 1970). Generally, higher temperatures and excess of moisture favor hematite; higher organic carbon and lower pH favor goethite.

**Halides**

Halite and sylvite (Fig. 7e), belonging to the evaporite mineral group, were also detected. They were deposited...
inside fractures in the gypsum crystals and on their surfaces. A thin film of halite (Fig. 7f) and in some cases small cubic crystals of sylvite (Fig. 7f) about 5 μm in size coated the gypsum. The deposition of these evaporite minerals over the gypsum occurred during a period of strong evaporation when the cave was no longer flooded by meteoric water.

**DETRITAL MINERALS**

Dolomite [CaMg(CO₃)₂] occurs as aggregates of opaque, milky grains, sometimes massive, or large subhedral crystals (Fig. 4a). Crystal size ranges between 1 μm and 3000 μm, depending on whether they are single crystals or aggregates. The origin of the dolomite is probably not related to the cave environment, because of the complex conditions that dolomite requires for its formation (Bosellini, 1991; Alonso-Zarza and Martínez-Peñé, 2008) that are unlike those found in the Grotta Inferiore di Sant’Angelo. Probably dolomite was derived from the surrounding carbonate rocks.

Small pyrite (FeS₂) crystals, about 10 μm in average size, were detected rarely, probably because most had been oxidized to iron oxides. Rare subhedral quartz (SiO₂) (Fig. 4b), found on the cave floor ranges in size from 20 to 30 μm (Table 1).

Pyrite, quartz, and rutile (TiO₂), the latter identified only in two samples (Fig. 4c), are residual minerals in the weathering deposits formed on conglomerates in the area surrounding the cave and then introduced into the cave by streams or seeping waters.

Chrysotile [Mg₃Si₂O₅(OH)₄] of detrital origin showed the classic fibrous morphology up to 15-μm long (Fig. 4d). Enstatite (MgSiO₃) was very rare, and it was identified only in one sample, with crystals of about 20 μm and elongated prismatic habit (Fig. 4e). Both chrysotile and enstatite are also residual minerals derived from conglomerates. Magnesite (MgCO₃) as subhedral crystals of about 20 μm (Fig. 4f) was found in only a few samples of detrital floor deposits. Magnesite was also occasionally present as microcrystalline, porous masses, white in color. It probably originates through metasomatism of the carbonate rock.

**Clay Minerals**

One clay sample representative of the three samples collected from the puddles was divided into two parts and prepared for analysis by X-ray powder diffraction. The phases from untreated subsamples a and b were quartz, dolomite, calcite, clinochlore [(Mg,Fe)₅Al(Si₃Al)O₁₀(OH)₈] and phlogopite [(KMg₃(Si₃Al)O₁₀(F,OH)₂] (Table 2).

Powder X-ray diffraction patterns from the untreated clay sample a (Fig. 8b) show the presence of (001) basal reflections at 14 Å that could represent either montmorillonite [(Na,Ca)_0.3(Al,Mg)_2Si₄O₁₀(OH)₂·nH₂O; JCPDS Card No. 3-0016] or clinochlore (JCPDS Card No. 12-0243) phases. To discriminate between them, the sample was heated to 450 °C, after which the (001) basal reflection increases in intensity in the case of clinochlore (Deer et al., 1982). Comparison between the diffraction patterns before (Fig. 8, b) and after (Fig. 8, b1) heating confirmed that the (001) basal reflection belongs to the clinochlore phase because of its increase in intensity. The (002) reflection does not disappear, as predicted by Villieras et al. (1994),
but decreases in intensity, probably because structural transformation was not complete at 450 °C.

Montmorillonite (JCPDS Card No. 12-0219) and sauconite ([Na$_{0.3}$Zn$_3$(SiAl)$_4$O$_{10}$(OH)$_2$·4H$_2$O]; JCPDS Card No. 8-0255; smectite-group mineral) were found in powder diffraction patterns after saturating sample a (Fig. 9, a) with ethylene glycol at 60 °C for 24 hours (Fig. 9, a1).

According to Polyak and Güven (2000a) understanding the origin of cave clays requires extensive studies, and the authigenesis of clay minerals is very hard to demonstrate. Cave sediments of detrital origin usually contain more than one clay mineral. Indeed the silicate clays observed in our samples consist of several clay phases, phlogopite, montmorillonite, sauconite, and clinochlore. The first three were probably formed through weathering of feldspars and micas derived from phyllites and conglomerates and were subsequently introduced into the cave by streams or seeping waters. The trioctahedral smectite (sauconite) found in clay-bearing carbonate speleothems could be of authigenic origin, in agreement with Polyak and Güven (2000b). Clinochlore among the clay minerals is of detrital origin, being derived from the rocks outcropping in the area around the cave.

**STUDY OF GYPSUM INCLUSIONS**

Detailed examinations were performed on the inclusions observed in gypsum crystals. Photomicrographs illustrate the shape of the inclusions. Their size is variable, as is their shape. The inclusions show euhedral shapes, rectangular to pseudo-hexagonal, reflecting sometimes the shape of the host mineral. Their size is between 100 µm (Fig. 10a) and 236 µm (Fig. 10b). Raman spectroscopy studies show that the inclusion contents are solid. Indeed, the spectra obtained analyzing both the surface of the gypsum crystal and the inclusions within this crystal are the same. For the gypsum, bands were observed at 407 cm$^{-1}$, 492 cm$^{-1}$, 617 cm$^{-1}$, 1008 cm$^{-1}$, 1141 cm$^{-1}$, and 3402 cm$^{-1}$ (Fig. 11). This is in agreement with Frezzotti et al. (2012), and all can be also assigned to the inclusion that, for this reason, is considered as gypsum. Although both inclusion and bulk crystal are gypsum, there are some important differences. As recorded by spectra obtained by Z-scan, the ratio between water bands at 3402 cm$^{-1}$ and 3506 cm$^{-1}$ changes from the top to the bottom of the crystal. In particular, the ratio is 1 inside the inclusion, while above and below it, the ratio of 3402 cm$^{-1}$ and 3506 cm$^{-1}$ is always greater than 1. This change in ratio can be explained by assuming that the inclusion and the crystal in which it is contained are oriented in two different directions and thus precipitated at different times. In fact, a test rotating the samples with respect to the direction of light polarization was performed and demonstrated how the different orientation of the crystals affects the intensity ratio between the various Raman bands.

Spectra obtained from the inclusion at a temperature of about −40 °C, didn’t show peaks typical of ice, confirming that there probably is not liquid water in the inclusion, within the limit of detection of the Raman technique. Moreover, the inclusion does not change when cooled below −180 °C by a stream of liquid nitrogen.

![Figure 9. Powder X-ray diffraction patterns from (a) untreated clay sample and (a1) clay after ethylene-glycol treatment.](imageURL)
Only the very weak band at 3235 cm\(^{-1}\) recorded at room temperature of about 20 °C overlaps the typical spectrum of liquid water. Nevertheless, this band is sometimes also reported in spectra obtained from gypsum crystals free of inclusions and even observed in the present investigation in sample regions without liquid water. It can be assigned to a Fermi resonance of the stretching mode with an overtone of the symmetric bending vibration, both belonging to the water molecules in the gypsum crystal structure. The presence or absence of a very weak peak at 3235 cm\(^{-1}\) in different points of the same crystal can be due to a different orientation of it.

**CONCLUSION**

The primary minerals belonging to carbonates, sulfates, oxides and hydroxide, halides, phosphates, and silicates groups from the Grotta Inferiore di Sant’Angelo result from the interactions of cave atmosphere or water with the karst rocks and the nearby metamorphic (phyllite) and sedimentary (conglomerates) rocks. Calcite was the most abundant mineral and is very common in limestone caves, in view of the fact that the concretions (i.e., flowstones, stalagmites, and stalactites) in the cave are mainly of calcitic composition. The method of precipitation of relevant gypsum (sulfate) deposits in the cave is related to a particular water chemistry and the mineralogical characteristics of the wall rocks; indeed, sulfate waters saturated carbonate rocks and caves, enabling the precipitation and growth of sulfate crystals. Data from gypsum inclusions prove that gypsum originated in two different steps of deposition and that the secondary generation of gypsum englobed the previous one during its growth. The occurrences of hematite and goethite give valuable information about the environments in which they formed. Generally, higher temperatures and excess of moisture favor hematite, while higher organic carbon and lower pH favor goethite. Phosphates in the cave are scattered, and they occur in sections of the cave where bat guano supplied phosphate ions.

The presence of halite and sylvite over the gypsum is related to a period of strong evaporation when the cave

---

**Figure 10.** Photomicrographs illustrating two different sizes of solid inclusions present in gypsum crystals.

**Figure 11.** Representative Raman spectrum of a gypsum crystal.

60 • *Journal of Cave and Karst Studies*, April 2014
was no longer flooded by meteoric water. Finally, triocahedral smectite (sauconite) found in clay-bearing carbonate speleothems could be of authigenic origin. Clay minerals (phlogopite, montmorillonite and sauconite) and the detrital minerals enstatite, rutile, magnesite, pyrite, chrysotile, quartz, dolomite, and chlinochlor originated from the surrounding carbonate rocks and conglomerates.

This study presents the first data on minerals in the Grotta Inferiore di Sant’Angelo cave. Mineralogical studies in this cave cannot be considered complete. An in-depth investigation of the cave samples may reveal other mineralogical species and increase knowledge about minerogenetic changes in the cave over time.

REFERENCES


Orofino, F., 1965, Primo elenco catastale delle grotte della Calabria, Notiziario Circolo Speleologica Romano, no. 11, p. 15–42.


A NEW THREAT TO GROUNDWATER ECOSYSTEMS:
FIRST OCCURRENCES OF THE INVASIVE CRAYFISH
PROCAMBARUS CLARKII (GIRARD, 1852) IN
EUROPEAN CAVES

GIUSEPPE MAZZA1*, ANA SOFIA P.S. REBOLEIRA2,3, FERNANDO GONÇALVES2, LAURA AQUILONI1, ALBERTO F. INGHILESI1, DANIELE SPIGOLI1, FABIO STOCH2, STEFANO TAITI1, FRANCESCA GHERARDI1, AND ELENA TRICARICO1

Abstract: The American red swamp crayfish, Procambarus clarkii, is today the alien species most widespread in European water bodies. This invasive crayfish was found for the first time in some caves of Europe, specifically in Portugal and Italy. The presence of P. clarkii in caves is noteworthy, representing a new threat for the groundwater ecosystems due to the possible negative impacts on the native communities.

INTRODUCTION

The subterranean environment hosts unique biological communities of remarkable diversity that, however, are still understudied in large parts of Europe (Gibert and Culver, 2009). Groundwater biodiversity has attracted much attention in recent years for two important aspects: the distinctiveness of its fauna, whose composition is highly different from that of surface freshwater fauna, and its narrow endemism (Deharveng et al., 2009). This particular habitat is vulnerable to local extinctions caused by anthropogenic impacts such as groundwater pollution, water abstraction, and habitat deterioration (Danielopol et al., 2003). Biological invasions, one of the most significant components of human-induced environmental change, could also imperil this environment (Lodge et al., 2000; Sala et al., 2000). Specifically, invasive species exert particularly heavy impacts in inland waters (Sala et al., 2000), where they threaten biodiversity, leading to extinction of indigenous species (Lodge, 2001; Gherardi, 2007) and altering the structure and function of ecosystems (Mack and D’Antonio, 1998; Strayer, 1999). Invasive species colonizing the subterranean environment by way of rivers have already been reported in Europe, e.g., the Asiatic clam Corbicula fulminea (Müller, 1774) in France (Callot-Girardi et al., 2012) and the New Zealand mudsnail Potamopyrgus antipodarum (Gray, 1843) in Italy (Bodon et al., 2009).

One of the most successful and best known invasive species of aquatic ecosystems in Europe is the North American red swamp crayfish Procambarus clarkii (Girard, 1852), now widely distributed in the world due to its biological features such as plastic life cycle, high fecundity, ability to rapidly disperse in the habitat, and tolerance to environmental extremes (Gherardi, 2006). This successful invader, that can cause a large variety of impacts (see Savini et al., 2010), was first introduced in Portugal in the 1970s and then in Italy in the 1980s, and it has now colonized most of the surface freshwater systems of both countries (Correia, 2003; Aquiloni et al., 2010). Here we report the first occurrences of this invasive crayfish in some cave waters of Portugal and Italy and briefly discuss the implications of this discovery for this unique and complex aquatic environment.

MATERIALS AND METHODS

Procambarus clarkii was collected in July 2007 in the Sicó massif (Anços spring: 39°58′42.41″N 8°34′22.67″W) and observed since August 2007 in the Estremenhos massif (Olhos de Água do Alvilea: 39°26′44.25″N 8°42′43.63″W), both caves being located in central Portugal. It was also collected in September 2011, October 2012, and February 2013 in pools inside of the adjoining Grotta del Leone, Grotta del Lago, Buca della croce di Agnano n. 2, Buca dell’acqua presso la Buca di Agnano, and Buca dei Ladri in the Monti Pisani area: 43°44′17.7″N 10°28′24.8″E, municipality of San Giuliano Terme, Pisa Province, Tuscany Region, Central Italy (Fig. 1). The Portuguese caves are located in two karst massifs and are up to 110-m deep and 700-m long. In Italy, Monte Pisano includes a group of hills, rich in karst caves and quarries, extending from northwest to southeast between the Lucca and Pisa plains in northwestern Tuscany.

In Portugal, specimens were collected by active search with dip-nets, while in Italy also by baited traps left...
overnight. In Italy, sampling also was conducted in the nearby epigean channel by both methods. For each crayfish, sex was determined and the length of the cephalothorax (CL), from the tip of the rostrum to the posterior edge of the carapace, was measured using a caliper with 0.1 mm precision. The number of individuals partially or totally depigmented was recorded.

The associated aquatic fauna was also sampled and recorded using dip-nets and small baited traps. Portuguese material is deposited at the Museu Nacional de História Natural, Lisbon, and the Italian samples at the Department of Biology, Florence.

During the surveys, the following water parameters were recorded with a multiparameter sonde: temperature, pH, dissolved oxygen, conductivity, and NH$^+$4. Frequency data were analyzed by G test with Williams correction (statistic G). Differences in CL between the sexes were compared by t-test for independent samples (statistic t).

RESULTS

Since 2007, six specimens of Procambarus clarkii have been observed in the groundwater of the Anços and Alviela springs in Portugal. Both caves are active resurgences, although the Rio Anços may dry up in the peak of summer. The individuals found inside the caves are totally depigmented, and one male was collected at 40-m depth in the Anços spring (CL = 33.1 mm; Fig. 2). Pigmented adults of the species are abundant (two individuals per meter) in the corresponding surface streams.

In Italy, a total of fifteen specimens of P. clarkii were collected from Grotta del Leone (one female: CL = 34 mm), Grotta del Lago (two males: CL = 33.9±0.05 mm and one female: CL = 23.1 mm), Buca della croce di Agnano n. 2 (one male: CL = 26.4 mm and three females: CL = 24±5.5 mm), Buca dell’acqua presso la Buca di Agnano (five males: CL = 23.5±2.1 mm and one female: CL = 33.7 mm) and Buca dei Ladri (one male: CL = 3.88 mm). About 53% of the crayfish had pereopods and cephalothorax bluish-whitish in color. The pools inside the caves were also inhabited by several species of Crustacea, the most abundant invertebrates in groundwater (Botoșâneanu, 1986), such as many species of stygobiotic copepods of the genera Acanthocyclops and Diacyclops and several specimens of amphipods belonging to Salentinella angelieri pisana Ruffo, 1953 (Salentinellidae, endemic to Monti Pisani karstic massif), Niphargus sp. group speciae Schellenberg, 1936, and Niphargus sp. prope stefanellii Ruffo and Vigna-Taglianti, 1968. Both Niphargidae are probably species new to science, according to F. Stoch and J.F. Flot. In Grotta del Lago, we also found three adult specimens of the European eel Anguilla anguilla (Linnaeus, 1758).

In the nearby epigean channel we collected fifty-six individuals of P. clarkii with a balanced sex-ratio (males = 32; females = 24; G = 1.14, df = 1, p > 0.1) and a similar mean size (CL males = 35.7±2.2 mm; CL females = 39.1±2.4 mm; t = 1.03, df = 54, p = 0.31). All the crayfish were pigmented. The invasive mosquitofish Gambusia holbrooki Girard, 1859, the crucian carp Carassius carassius (Linnaeus, 1758) and the black bullhead Ameiurus melas (Rafinesque, 1820) were also abundant in this channel. Concerning the water parameters, the Portuguese and Italian caves had similar temperatures (nearly constant during the year), pH, conductivity, and NH$^+$4. Only the epigean channel showed a wide range of temperatures (Table 1).

DISCUSSION

To the best of our knowledge, these are the first documented occurrences of the invasive crayfish Procambarus clarkii in European caves. In Europe, crayfish occasionally found in subterranean habitats have been almost unknown or rarely reported, and only identified native species, such as Austropotamobius torrentium (Schrank, 1803) (Koutrakis et al., 2005). The only large...
A new threat to groundwater ecosystems: first occurrences of the invasive crayfish *Procambarus clarkii* (Girard, 1852) in European caves

## Table 1. Water parameter ranges recorded in the Portuguese and Italian study areas.

<table>
<thead>
<tr>
<th>Sites</th>
<th>Temperature, °C</th>
<th>pH</th>
<th>Oxygen, mg L(^{-1})</th>
<th>Conductivity, (\mu S , cm^{-1})</th>
<th>(\text{NH}_4^+), mg L(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anços</td>
<td>15.8–16.4</td>
<td>7</td>
<td>8.5–9.5</td>
<td>497–540</td>
<td>0</td>
</tr>
<tr>
<td>Alviela</td>
<td>15.6–17.5</td>
<td>7</td>
<td>7.6</td>
<td>443–508</td>
<td>0</td>
</tr>
<tr>
<td>Epigean channel</td>
<td>11–31</td>
<td>6</td>
<td>5.4</td>
<td>482–500</td>
<td>0.4</td>
</tr>
<tr>
<td>Grotta del Leone</td>
<td>16–17</td>
<td>6</td>
<td>4.3</td>
<td>524–540</td>
<td>0</td>
</tr>
<tr>
<td>Grotta del Lago</td>
<td>14.5–17.7</td>
<td>6</td>
<td>4.3</td>
<td>560–585</td>
<td>0</td>
</tr>
<tr>
<td>Buca della croce di Agnano n. 2</td>
<td>12–19</td>
<td>7</td>
<td>1.4–2.5</td>
<td>695–730</td>
<td>0.6</td>
</tr>
<tr>
<td>Buca dell’acqua presso la Buca</td>
<td>15–23</td>
<td>6.5</td>
<td>3.9</td>
<td>503–515</td>
<td>0</td>
</tr>
<tr>
<td>di Agnano</td>
<td>14.5–18</td>
<td>6.5</td>
<td>5</td>
<td>498–516</td>
<td>0</td>
</tr>
</tbody>
</table>

A stygobiotic decapod in Europe is represented by the blind prawn genus *Typhlocaris* Calman, 1909, present in Italy (Apulia) with the species *T. salentina* Caroli, 1923. The family Cambaridae is represented in the stygobiotic American fauna with four genera, three of which have epigean members as well (*Procambarus* Ortmann, 1905, *Orconectes* Cope, 1872, and *Cambarus* Erichson, 1846: Hobbs et al. 1977; Hobbs, 1988). For example, in Florida, thirteen of the fifty-two known species and subspecies are troglobites, while in southern Indiana six species or subspecies inhabit the subterranean caves in the karst region (Hobbs, 1988; Hogger, 1988). There are also cases among the Cambaridae of stigoxenic (largely confined to surface water) and stygophilic (able to spend part of their life in ground waters but without or with limited specialization to subterranean life) species. Specifically, *P. clarkii* has been found in caves of Texas (Hobbs et al., 1977), probably due to the displacement of populations living in epigean environments. In Portugal, the individuals found in ground waters were totally depigmented, while in Italy pigmentation of cave-dwelling individuals of *P. clarki* occurs. The presence of mostly pigmented crayfish inside the Italian caves could indicate a more recent invasion; an external source of carotenoids, which can occur in caves providing a source of food for crayfish as mentioned in Hogger (1988) and in Koutrakis et al. (2005), even if it is probably not abundant, as evidenced by the presence of bluish individuals; or movement from the epigean channel to the caves and vice versa, due to the locomotory activity of crayfish. The individuals found in Monti Pisani area can be undoubtedly classified as stygoxic, while specimens from Portugal show a certain degree of stygobization. In both areas, the caves showed similar water conditions favorable for the species; only the oxygen differed substantially among sites, but *P. clarkii* can tolerate a wide range of oxygen content, being capable of air breathing (Huner, 2002).

The presence of *Procambarus clarkii* in caves is noteworthy, due to the possible negative impacts on native communities. This species is recognized as one of the most invasive crayfish in Europe (Tricarico et al., 2010) and, thanks to its plasticity, it can prey on many endemic cave species, since it feeds on diverse items in proportion to their availability. The European groundwater fauna comprises more than 1,800 stygobitic species (Stoch and Galassi, 2010), most of them endemic to restricted areas or single karstic massifs in southern countries. At least sixty-seven groundwater-dwelling species are endemic to Portugal (Reboleira et al., 2011, 2013), and *P. clarkii* could be an important threat to some of these species, such as asellid isopods like *Proasellus lusitanicus* (Frade, 1938), as well as amphipods belonging to the genus *Pseudoniphargus* Chevreux, 1901. Groundwater biodiversity is even greater in Italy, with more than 320 stygobiotic species reported in recent catalogues (Ruffo and Stoch, 2006, and Fauna Europaea Web Service, 2013), around 300 of them being endemic. In the karstic area where it was found, *P. clarkii* can feed on the amphipods of the genus *Niphargus* (two species yet to be described) and *Salentinella angeli* *pisana*, all endemics to the restricted area of Monti Pisani. However, in Italy, the eels in the pools could control the population of *P. clarkii*; indeed *Anguilla anguilla* actively preys on crayfish juveniles (Aquiloni et al., 2010).

Further studies are necessary to investigate more deeply the status of this species in caves and to assess how the species arrived and the possible impacts on this particular habitat.

**Acknowledgements**

Authors are grateful to Gruppo Speleologico CAI, Pisa, and in particular to P. Mannucci, G. Stasolla, and D. Abiuso (University of Florence) for field assistance. We express our gratitude to A. Correia (Museu Nacional de História Natural) for species identifications and to SAGA Historía Natural) for species identifications and to SAGA and NEUA cave divers J. Neves, L. Neves, J. Morrison, M. Lançã, R. Pinheiro, and M. Lopes and to J. Meynié, for the first observations and collections of *P. clarkii* in caves of Portugal and also to DIR-SPE for support on cave diving.

**References**

Aquiloni, L., Brusconi, S., Cecchinelli, E., Tricarico, E., Mazza, G., Paglianti, A., and Gherardi, F., 2010, Biological control of invasive populations of crayfish: the European eel (*Anguilla anguilla*) as a


BOOK REVIEW

Water in Karst: Management, Vulnerability, and Restoration


This is the latest in a line of books on karst waters; the author is a very prolific writer of groundwater texts who specializes in karst hydrogeology. As many karst professionals will no doubt note, for many years, karst science was all but ignored in the United States, but that is no longer the case. This book represents a timely addition to the many new karst books being rolled out by publishers because so many individuals are now working in the field of karst hydrogeology.

The book is divided into three parts: Karst Hydrogeology and Hydrology, Management of Water in Karst, and Vulnerability and Restoration of Water in Karst. Chapter 1, Karst Aquifers, is over two hundred pages long and provides a very comprehensive overview of basic karst hydrology and hydrogeology. Even though chapter 1 is an overview, it is worth reading even by experienced karst professionals because of the wealth of information it contains. For example, those individuals familiar with the concept of epikarst are likely to find interesting and controversial perspectives on the topic. Chapters 2, Flow Measurements and Analysis, and 3, Drainage Areas in Karst, are more practically oriented than is Chapter 1, and will be beneficial to practitioners involved in site investigations where discharge measurements, tracer testing, and geophysical methods are appropriate.

Part 2 includes six chapters on various aspects of water management, ranging from basic management principles and education to modeling methods, flooding or droughts, groundwater extraction, and engineering of karst aquifers and springs. Sustainability, a current trend in hydrogeology and other environmental sciences, is briefly addressed. More useful to many might be the discussions on government regulations, such as Groundwater Rule and GroundWater Under Direct Influence of surface water (GWUDI), that are so pervasive throughout the United States and elsewhere (e.g., Canada). This reviewer has been involved in sites where these regulations were important both at the federal and state level, and it is critical that professional groundwater scientists and engineers understand them from the perspective of both general hydrogeology and karst hydrogeology. Groundwater exploitation and aquifer management are familiar to hydrogeologists, although the discussions of capture of spring flow and of surface dams will be new to many.

Part 3 includes just two chapters, vulnerability and restoration. Most karst professionals are very aware that karst aquifers are generally more vulnerable to contamination than are other aquifer types, because of their rapid flow and lack of filtration. This section of the book provides a solid foundation for professionals working at vulnerable or contaminated sites where aquifer protection or restoration must be accomplished. Those familiar with contaminated karst aquifers will note that not a lot is presented regarding aquifer restoration, especially for karst aquifers contaminated with dense nonaqueous-phase liquids, because the technology for DNAPL removal is still relatively limited, especially for karst aquifers. Still, the chapter on restoration is very strong despite the necessary limitations.

This book is chock-full of figures and photos from all over the world. In fact, nearly every page includes either a figure or a photo that complements the text and enhances the reader’s understanding. The center of the book includes thirty-six high-quality color plates. This book is no substitute for formal education and experience, but it will go a long way toward educating those unfamiliar with karst hydrology and hydrogeology and serve as a refresher for those who are experienced.

Reviewed by Malcolm S. Field, U.S. Environmental Protection Agency, 1200 Pennsylvania Ave., NW, Washington, DC 20460-0001 (field.malcolm@epa.gov).
BOOK REVIEW

Speleothem Science: From Process to Past Environments


This is the first book to summarize in depth the concepts and methods of analyzing speleothems for their climatic record. Prior literature on the subject, though abundant, consists mainly of journal articles and occasional symposium volumes. This is such a rapidly growing field that it is a wonder that two of its most active researchers have been able to produce a book as detailed and comprehensive as this. Many speleologists find this an uncomfortable topic because it involves the harvesting of decorative cave features, often by researchers who give little thought to conservation. But one reason to welcome this book is that its authors are karst scientists who promote conservation through judicious field practices and meticulous archiving.

A brief review of the book’s topics can barely scratch the surface: (1) The cave environment and speleothem growth, the concept of environmental systems, and systems analysis. Two new terms are coined: The *speleothem factory* refers to the system that supplies the raw ingredients for speleothems via the atmosphere, soil, and epikarst and transfers them to the cave environment. The *speleothem incubator* consists of aspects of the cave environment that regulate their growth, such as local variations in cave air, water flow, and chemistry. (2) The karst setting, including carbonate sedimentology and petrography, karst geology, and speleogenesis. (3) Surface conditions: climates, soils, and vegetation. (4) Physics of mass transfer, fluid flow, cave atmospheres, thermal processes, and evolution of dripwater along its flow paths. (5) Inorganic water chemistry, including stable isotopes. (6) Biogeochemistry and its influence on speleothems. (7) Speleothem shapes, patterns, crystal structures, and growth rates. (8) Speleothem growth and patterns, isotopic fractionation; (9) Sampling techniques and lab preparation. (10) Dating methods. (11) Interpretation of paleoenvironments, calibration techniques, validation of proxies. (12) Summary of results and relation to global climate patterns and orbital forcing. (13) An appendix on proper archiving of samples and the data obtained from them.

The authors clarify the different approaches for speleothem analysis according to the range of dates involved. Great attention is given to interpreting climate records over the past 500 years, which may at first seem strange because written records are already available for that time. But this overlap is crucial for calibrating speleothem data with real observations before they can be extrapolated to earlier climates or, once climatic patterns are better understood, into the future. Records from various climatic zones have now been calibrated against proxies such as the coral oxygen isotope record, tree rings, and cores of ocean sediment and glacial ice. There is strong emphasis on the Holocene because of its rich speleothem record and application to archeology. Because well-preserved ancient examples are rare and scattered, Pleistocene and older records receive relatively little coverage. This is a great challenge for the future in extending speleochronology into the realms of tectonic, sedimentary, and geomorphic history and paleokarst. Suggestions and caveats are offered for expanding this aspect of the field. Certain popular cave-dating methods, such as the use of cosmogenic radionuclides in cave sediment and argon dating of alunite generated by sulfuric acid, lie outside the scope of the book.

Technical subjects and laboratory techniques are covered in detail, and readers are guided through the maze with helpful chapter introductions, abundant sidebars, and lists of important questions. Guest authors have been enlisted to fill in relevant details such as speleogenesis. There are fifty pages of cited publications, as well as sixteen unnumbered pages of color photos and diagrams. Figures and tables in the book can be downloaded at www.wiley.com/go/fairchild/speleothem.
This is essential reading for any new or prospective researcher in the field and a valuable resource even for those at the most advanced levels. Even those with only a casual interest in the field can gain a great deal of insight. The field is expanding so rapidly that the authors needed to complete the book quickly, while assimilating the ever-growing torrent of background material. Certain diagrams and terminology could use more clarification, and a few topics are described in more detail than necessary (e.g., petrology of carbonate rocks); but these are insignificant points in view of the overwhelming service this book provides. It will help those who genuinely need guidance and, one hopes, deter those who intend only to dabble. Those who consider caves to be clandestine and isolated places may be interested to learn how far speleology can be applied toward understanding the outside world.

Reviewed by Arthur N. Palmer and Margaret V. Palmer, 619 Winney Hill Road, Oneonta, NY 13820 (palmeran@oneonta.edu).
GUIDE TO AUTHORS

The Journal of Cave and Karst Studies is a multidisciplinary journal devoted to cave and karst research. The Journal is seeking original, unpublished manuscripts concerning the scientific study of caves or other karst features. Authors do not need to be members of the National Speleological Society, but preference is given to manuscripts of importance to North American speleology.

LANGUAGES: The Journal of Cave and Karst Studies uses American-style English as its standard language and spelling style, with the exception of allowing a second abstract in another language when room allows. In the case of proper names, the Journal tries to accommodate other spellings and punctuation styles. In cases where the Editor-in-Chief finds it appropriate to use non-English words outside of proper names (generally where no equivalent English word exists), the Journal italicizes them. However, the common abbreviations i.e., e.g., et al., and etc. should appear in roman text. Authors are encouraged to write for our combined professional and amateur readerships.

CONTENT: Each paper will contain a title with the authors’ names and addresses, an abstract, and the text of the paper, including a summary or conclusions section. Acknowledgments and references follow the text.

ABSTRACTS: An abstract stating the essential points and results must accompany all articles. An abstract is a summary, not a promise of what topics are covered in the paper.

STYLE: The Journal consults The Chicago Manual of Style on most general style issues.

REFERENCES: In the text, references to previously published work should be followed by the relevant author’s name and date (and page number, when appropriate) in parentheses. All cited references are alphabetical at the end of the manuscript with senior author’s last name first, followed by date of publication, title, publisher, volume, and page numbers. Geological Society of America format should be used (see http://www.geosociety.org/pubs/geoguid5.1.htm). Please do not abbreviate periodical titles. Web references are acceptable when deemed appropriate. The references should follow the style of: Author (or publisher), year, Webpage title: Publisher (if a specific author is available), full URL (e.g., http://www.usgs.gov/citguide.html) and date when the web site was accessed in brackets; for example [accessed July 16, 2002]. If there are specific authors given, use their name and list the responsible organization as publisher. Because of the ephemeral nature of websites, please provide the specific date. Citations within the text should read: (Author, Year).

SUBMISSION: Effective February 2011, all manuscripts are to be submitted via Peertrack, a web-based system for online submission. The web address is http://www.edmgr.com/jcks. Instructions are provided at that address. At your first visit, you will be prompted to establish a login and password, after which you will enter information about your manuscript (e.g., authors and addresses, manuscript title, abstract, etc.). You will then enter your manuscript, tables, and figure files separately or all together as part of the manuscript. Manuscript files can be uploaded as DOC, WPD, RTF, TXT, or LaTeX. A DOC template with additional manuscript specifications may be downloaded. (Note: LaTeX files should not use any unusual style files; a LaTeX template and BibTeX file for the Journal may be downloaded or obtained from the Editor-in-Chief.) Table files can be uploaded as DOC, WPD, RTF, TXT, or LaTeX files, and figure files can be uploaded as TIFF, EPS, AI, or CDR files. Alternatively, authors may submit manuscripts as PDF or HTML files, but if the manuscript is accepted for publication, the manuscript will need to be submitted as one of the accepted file types listed above. Manuscripts must be typed, double spaced, and single-sided. Manuscripts should be no longer than 6,000 words plus tables and figures, but exceptions are permitted on a case-by-case basis. Authors of accepted papers exceeding this limit may have to pay a current page charge for the extra pages unless decided otherwise by the Editor-in-Chief. Extensive supporting data will be placed on the Journal’s website with a paper copy placed in the NSS archives and library. The data that are used within a paper must be made available. Authors may be required to provide supporting data in a fundamental format, such as ASCII for text data or comma-delimited ASCII for tabular data.

DISCUSSIONS: Critical discussions of papers previously published in the Journal are welcome. Authors will be given an opportunity to reply. Discussions and replies must be limited to a maximum of 1000 words and discussions will be subject to review before publication. Discussions must be within 6 months after the original article appears.

MEASUREMENTS: All measurements will be in Systeme Internationale (metric) except when quoting historical references. Other units will be allowed where necessary if placed in parentheses and following the SI units.

FIGURES: Figures and lettering must be neat and legible. Figure captions should be on a separate sheet of paper and not within the figure. Figures should be numbered in sequence and referred to in the text by inserting (Fig. x). Most figures will be reduced, hence the lettering should be large. Photographs must be sharp and high contrast. Color will generally only be printed at author’s expense.


COPYRIGHT AND AUTHOR’S RESPONSIBILITIES: It is the author’s responsibility to clear any copyright or acknowledgement matters concerning text, tables, or figures used. Authors should also ensure adequate attention to sensitive or legal issues such as land owner and land manager concerns or policies.

PROCESS: All submitted manuscripts are sent out to at least two experts in the field. Reviewed manuscripts are then returned to the author for consideration of the referees’ remarks and revision, where appropriate. Revised manuscripts are returned to the appropriate Associate Editor who then recommends acceptance or rejection. The Editor-in-Chief makes final decisions regarding publication. Upon acceptance, the senior author will send one set of PDF proofs for review. Examine the current issue for more information about the format used.

ELECTRONIC FILES: The Journal is printed at high resolution. Illustrations must be a minimum of 300 dpi for acceptance.
Journal of Cave and Karst Studies

Volume 76  Number 1  April 2014

Article
Biology and Ecology of Bat Cave, Grand Canyon National Park, Arizona
Robert B. Pope

Article
Microhabitat Influences the Occurrence of Airborne Fungi in Copper Mine in Poland
Wojciech Pusz, Włodzimierz Kita, and Ryszard Weber

Article
Millipedes (Diplopoda) from Caves of Portugal
Ana Sofia P.S. Reboreira and Henrik Enghoff

Article
Assessment of Forward Osmosis as a Possible Mitigation Strategy for Urine Management During Extended Cave Exploration
Catherine H. Borow, Warren J. Stiles, Joshua C. Stevenson, and Katherine E. Cabanillas

Article
Ecotourism in the State Forest Karst of Puerto Rico
Andrea Hall and Mick Day

Article
Perceptions and Prevalence of Caving-Skills Training in the United States and the United Kingdom
Aaron J. Bird, Melissa Sawa, and Mike Wiles

Article
The Mineralogical Study of the Grotta Inferiore di Sant’Angelo (Southern Italy)
Manuela Catalano, Andrea Bloise, Domenico Mirtiello, Carmine Apollaro, Teresa Critelli, Francesco Muto, Enzo Cazzanelli, and Eugenio Barrese

Article
A New Threat to Groundwater Ecosystems: First Occurrences of the Invasive Crayfish Procambarus clarkii (Girard, 1852) in European Caves
Giuseppe Mucchi, Ana Sofia P.S. Reboreira, Fernando Gonçalves, Laura Aquiloni, Alberto E. Inghilei, Daniele Spigoli, Fabio Stoch, Stefano Taiti, Francesco Gherardi, and Elena Tricarico

Book Review
Water in Karst: Management, Vulnerability, and Restoration

Book Review
Speleothem Science: From Process to Past Environments

Journal of Cave and Karst Studies Distribution Changes

During the November 9, 2013, Board of Governors meeting, the BOG voted to change the Journal to electronic distribution for all levels of membership beginning with the April 2014 issue. Upon publication, electronic files (as PDFs) for each issue will be available for immediate viewing and download through the Member Portal on www.caves.org. For those individuals that wish to continue to receive the Journal in a printed format, it will be available by subscription for an additional fee. Online subscription and payment options will be made available through the website in the near future. Until then, you can arrange to receive a print subscription of the Journal by contacting the NSS office at (256) 852-1300.