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Front cover: A ringtail captured on a remote camera at System's Key Cave in Great Basin National Park. See Gretchen Baker's article in this issue.

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D. D'Agostino, L. Beccarisi, M. Camassa, and P. Febbroriello – Microclimate and microbial characterization in the Zinzulusa show cave (South Italy) after switching to LED lighting. *Journal of Cave and Karst Studies*, v. 77, no. 3, p. 133–144, DOI: 10.4311/2014EX0123

MICROCLIMATE AND MICROBIAL CHARACTERIZATION IN THE ZINZULUSA SHOW CAVE (SOUTH ITALY) AFTER SWITCHING TO LED LIGHTING

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Abstract: Show caves are vulnerable environments whose conditions should be monitored over time. Many factors, such as visits by tourists and the installation of lights, can easily alter the equilibrium of the cave system. Grotta Zinzulusa is a show cave located on the southeastern coast of Italy. This cave is renowned for the richness of its faunal biodiversity, but little is known about its microclimate and about its microbial diversity in the form of lampenflora growth near the light sources. We investigated the photosynthetic microorganisms living in the Zinzulusa in regard to its microclimatic conditions one year after the change of the lighting system from tungsten to LED (light emitting diode) lamps. Most microbial activity was found in areas with vermiculations. We established four distinct communities based on macroscopic characteristics as a function of light intensity and wavelength. The observations were compared both with previous data collected in the last decade and with other caves reported in the literature. Results show that not much has changed within the first year after switching to a LED system with respect to the 1999 state. This research contributes to the knowledge of the environmental attributes and provides a baseline to check for changes in the lampenflora community over time in the Zinzulusa.

INTRODUCTION

Show caves provide important financial and educational opportunities for the increase in nature-based tourism (Hamilton-Smith, 2004). Opening caves to the public brings changes that alter the environment, such as construction of walking surfaces and infrastructure for electricity and water (Mulec, 2014). Recreational use of caves dates back to the early seventeenth century (Šebela and Turk, 2014). Nowadays, more than six hundred show caves are operating worldwide (Gillieson, 2011). The number of tourists visiting caves is continually increasing and can reach half a million visits per year in caves such as Grotte di Castellana in Italy and Han sur Lesse in Belgium (Parise, 2011).

The presence of large numbers of tourists can alter the natural microclimatic and environmental conditions of a cave, with consequences for the equilibrium of the system (Cigna, 2004). In particular, temperature and humidity can increase considerably within the cave due to the presence of tourists (Russell and MacLean, 2008). Moreover, human respiration can lead to an augmented CO_2 concentration that can promote speleothem corrosion (Baker and Genty, 1998). Visitors often contribute to the introduction of microorganisms and foreign species into caves (Gamble et al., 2000). The presence of these microorganisms creates management complications in many caves. Tourists also contribute to lint accumulation that supports the growth of bacteria on natural fibres, leading to discoloration or damage to speleothems. Other factors, such as the introduction of mud and dust, may adversely impact the equilibrium of hypogean caves.

In the artificially illuminated parts of caves, the development of heterotrophic biofilms (Jurado et al., 2009; Saiz-Jimenez et al., 2011) and phototrophic communities serving as primary producers (Bastian and Alabouvette, 2009) is common. This community, generally known as lampenflora, is usually composed of different microbes, eukaryote algae, cyanobacteria, bryophytes, mosses, and ferns. It adheres strongly to the substratum and deteriorates speleothems and other objects (Mulec, 2012). Nutrients and moisture levels are often sufficient to support its growth (Mulec et al., 2008). Rock surfaces, sediments, and artificial materials around lamps often become colonized by these phototrophs (Dobat, 1998a). Biomass fixed due to light energy, together with other organic matter brought by tourists on clothing and skin, becomes available to cave organisms. Light sources can contribute to a temperature increase that further supports their proliferation. The lighting system can alter the microclimate, favoring the growth of photosynthetic organisms, as happened in the Lascaux caves, France, where algal colonization damaged the cave paintings (Baker and Genty, 1998). Lampenflora is completely dependent on light, as the light saturation point of these species is quickly reached at the

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cave temperature (Dobat, 1998b). Moreover, some cyanobacteria and micro-algae can survive even at photon flux densities lower than their photosynthetic compensation point (Mulec and Kosi, 2009).

These phototrophic communities are inappropriate from an aesthetic point of view and cause degradation of colonized substrata, producing weak organic acids that can slowly corrode the speleothems. Until now, the most common treatments for controlling lampenflora growth included physical abrasion and bleach solutions. Hydrogen peroxide seems to be the most favorable biocidal among the tested chemicals (Faimon et al. 2003; Mulec and Kosi 2009). These methods can be replaced with a proper selection of time-limited illumination, reduction of light intensity and the use of lights whose spectrum does not support photosynthesis, such as light-emitting-diode (LED) systems (Olson, 2006 and references therein). In the Frozen Niagara section of the Mammoth cave, Olson (2006) monitored a narrow spectrum yellow LED (595 nm) with an intensity of 49.5 lux, corresponding to double the value recommended for white light by Aley (2004). After one year, a reduction of lampenflora growth was observed where previously a widespread algal cover was present (Toomey et al., 2009).

Some cave managers ignore light and tourist pollution until problems become very obvious and difficult to solve. Therefore, due to the high vulnerability of these environments, it is important to know their specific physical and biological attributes and constantly monitor their conditions. Remediation actions that are done without careful planning may not solve, or may even exacerbate, the problem. The installation of a new lighting system should be preceded by specific surveys. To minimize visitor impact, cave managers need to ensure a sustainable development of the cave system (Gillieson, 1996). Hence the introduction of a climatic and biological monitoring program should assist those involved in the adviser role to define and maintain a suitable environmental status in these systems (de Freitas and Littlejohn, 1987).

MATERIALS AND METHODS

Grotta Zinzulusa (Fig. 1a) is a karst cave facing the Adriatic Sea in the southeastern coast of Italy near the town of Castro, in the province of Lecce. Monitoring in Zinzulusa was aimed at investigating the microbial diversity established in the cave compared to its microclimatic conditions. With this aim, the following types of surveys were performed: microclimatic surveys to describe temperature and humidity inside the cave; analysis of the algal and microbiological communities checking their macroscopic characteristics; surveys to analyze the distribution of phototrophic organisms in response to light intensity; and microbiological surveys to investigate the composition of vermicular formations, as well as identifying bacteria and fungi. The research was carried out in the air-filled part of the cave (Fig. 2a) between March 2008 and December 2009. Additional data were used to evaluate the response of the cave environment to the new LED system that replaced tungsten incandescent lamps, among them microclimatic data available from December 2007 to March 2008 and microbiological data from 1999.

GROTTA ZINZULUSA

Grotta Zinzulusa is a renowned show cave located on the southeastern coast of Italy (Fig. 1a). It develops in a westerly direction for 156.5 m, at 11 m above sea level. The cave has a large entrance opening onto the Adriatic Sea (Fig. 1b) and is the result of a long karst erosive process during the Pleistocene. The cave was discovered in 1793, and it has been accessible to tourists since 1975. Grotta Zinzulusa is visited by about 130,000 people annually, and many facilities were built for tourists, including a passage excavated into the cliff to allow entrance into the cave (Ciccarese and Pesce, 1999). An internal walkway and a lighting system were installed between 1950 and 1957 to permit an easy visit in most of the air-filled part of the cave.

The cave can be divided into two parts, air-filled (Fig. 2a) and submerged (Onorato et al., 1999). The higher part can be itself divided into three parts. The first, from the entrance to the Crypt, is characterized by numerous speleothems (Fig. 1d) and a brackish pool, the Basin, that contains marine species (Toomey et al., 2009). The second area, from the Crypt to the Cathedral, contains fewer stalactites and stalagmites. In the final part, there is a 1.5 m deep freshwater pool, the Cocito, of cold brackish water in an area closed to visitors. The pool's water level often fluctuates with tidal changes due to the coastal location and the connection with the sea. This pool confirms the anchialine nature of the hydrological system. In this location, the rich diversity of fauna includes crustaceans, copepods, and blind fishes. The Cocito is a sump where the submerged part of the cave starts, and has been explored for about 120 m and is composed of many rooms connected by short passages. Unlike the entrance, which was subjected to marine erosive processes, the other parts were formed through a long karst process of carbonate rock dissolution (Parenzan, 1963).

More than sixty terrestrial and aquatic species have been identified in Zinzulusa. Among them are bats and anchialine species of ancient origin (Pesce, 2001). However, as in other caves (Fong, 2011), changes in the environment caused some animals, such as bats, to abandon their natural habitat. In 1999 the Karst Waters Institute included Grotta Zinzulusa in the top-ten list of the world endangered karst ecosystems (Belson, 1999). This was mainly due to tourist impact on natural formations, as well as pollution from urban water discharges that threatens the hypogean environment. Grotta Zinzulusa presents an abundance of vermicular clay-slime formations (Camassa and Febbroriello, 2003) whose origin is still unknown, spreading on the cave surfaces and the calcite speleothems (Fig. 2b). Various theories have been published about the origin of vermiculations

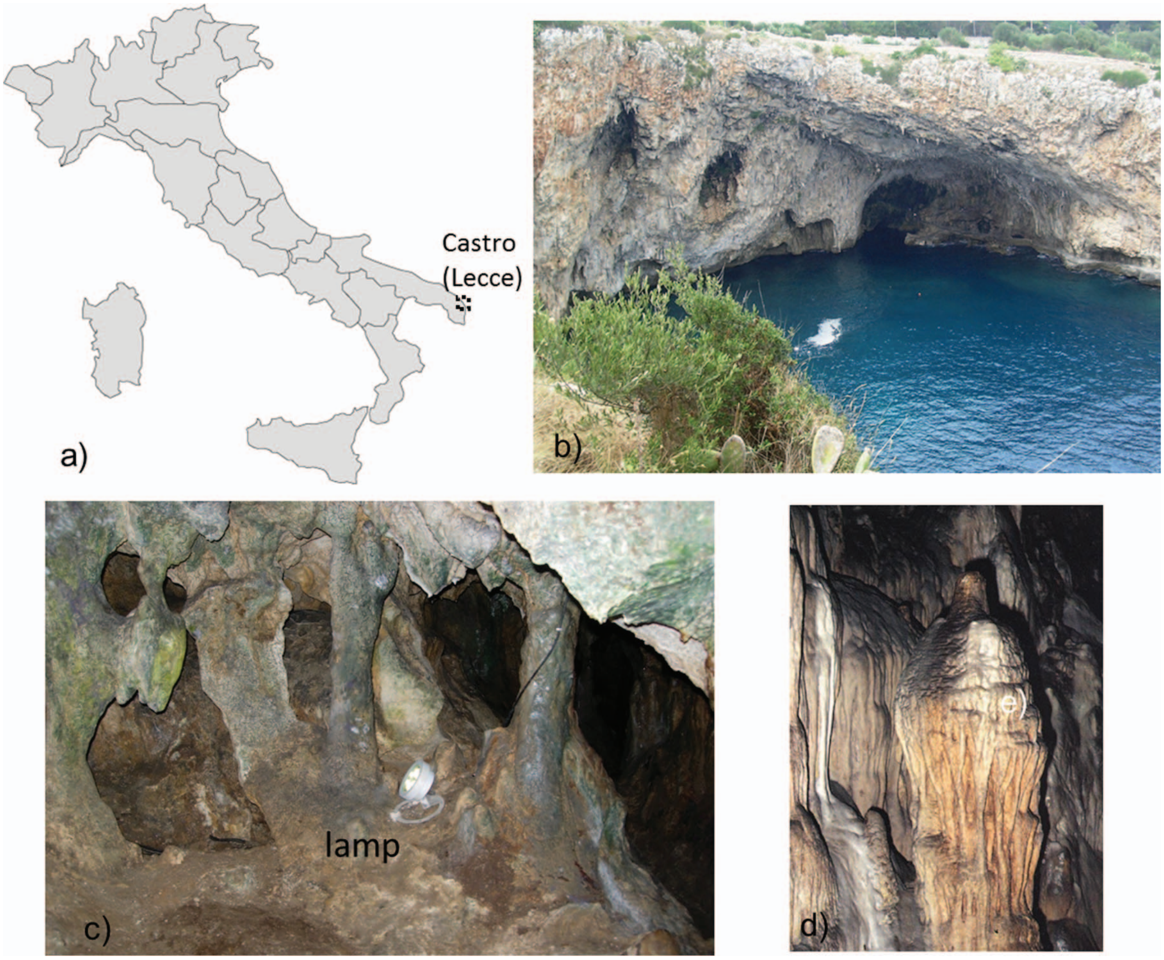

Figure 1. Grotta Zinzulusa, southeastern Italy: a) location. b) Natural entrance. c) Inside near the Crypt (Fig. 2a); a lamp is visible in the picture. d) Speleothems.

and the development of those formations on cave walls and speleothems (Nováková, 2009). Due to their biotic component, they represent a food source for microorganisms. Furthermore, the production of metabolic acids, especially lactic and succinic, is involved in the surface bioerosion of speleothems (Northup and Lavoie, 2001; Camassa, 2003) or litholitic processes (Mulec et al., 2007)

Lampenflora growth has been visible for a long time near the lights in Zinzulusa (Fig. 1c). To remove it, repeated applications of bleach were applied in the past, without investigating the relationship between microorganisms and lights. Grotta Zinzulusa has been examined for its faunal biodiversity (Pesce, 2001), but in the international literature there are no specific studies on its lampenflora and microbiological characteristics.

A LED system was installed in March 2008, consisting of spotlights with six to nine 1 watt LEDs without UV and IR spectral components. This system replaced tungsten incandescent lamps that emitted from 100 W to 200 W, increasing temperature and decreasing relative humidity. The new system was selected for various reasons: to have a lower impact on the microclimate, to reduce the lampenflora, to darken selected areas to benefit species having conservation issues, such as bats that were abundant between the Cathedral and Cocito (Lazzari, 1966), and to enhance the visibility for visitors inside the cave. Each diode furnishes a white light of maximum intensity of 45 lux, with narrow peaks in red (620–630 nm), green (515–530 nm) and yellow-amber (585–595 nm) wavelengths and minimal amounts of UV. This range of wavelengths is unfavorable for chlorophyll photosynthesis (Teramura and Sullivan, 1984). The lighting system is turned on during the visiting hours of the cave, which last for eight hours daily in summer and six hours during the other seasons.

THE MICROCLIMATE

In connection with the installation of the new lighting system, microclimatic monitoring was performed by the University of Salento, commissioned by the Castro municipality that provided permission to publish all the data. Data were automatically recorded every 15 minutes using ten data loggers (numbered 1–10 in Fig. 2a) provided with

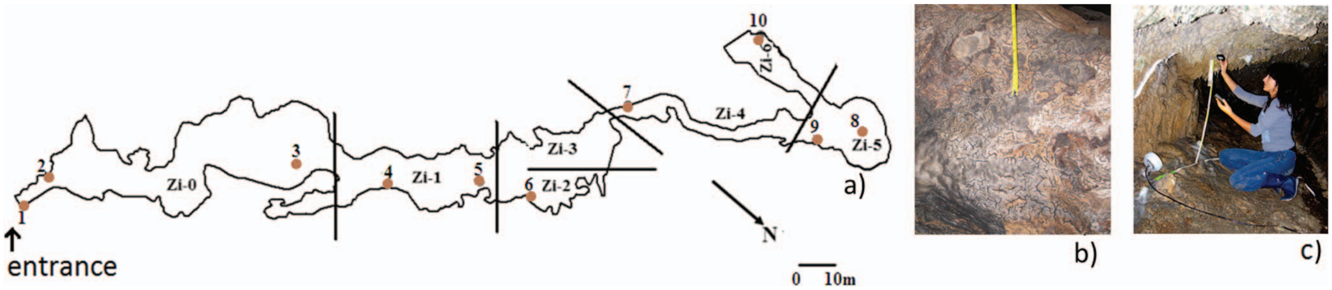

Figure 2. a) Plan map of the air-filled part of Grotta Zinzulusa, modified from Onorato et al. (1999), showing the points of the microclimatic survey performed by the University of Salento commissioned by the Castro municipality (1. Entrance Chamber, 2. Vestibule, 3. Pond, 4. Trap, 5. Canopy, 6. Waterfall, 7. Crypt, 8. Cathedral 1, 9. Cathedral 2, 10. Cocito), and the sampling areas for the microbiological surveys (Zi-0 through Zi-6) divided by lines. b) Vermicular formations in the cave. c) Collecting light intensity data.

sensors for temperature (range from −25 to +85 °C, accuracy ± 0.5 °C, resolution 0.01 °C) and relative humidity (range from 0 to 100%, accuracy ± 2%, resolution 0.5%). One data logger was placed outdoors to analyze the impact of the outside climate on the cave interior. The others were placed inside the cave, from the entrance (point 1 in Fig. 2a) up to the Cocito pool (point 10). These locations were selected to cover the air-filled part of the cave to analyze the microclimatic changes related to the increased use of the cave for tourism. The names of these locations originate from features in the cave. Starting from the entrance (Fig. 2a), the identified locations included: the Vestibule (point 2), the Pond (point 3), the Trap (point 4), the Canopy (point 5), the Waterfall (point 6), the Crypt (point 7), and the Cathedral (points 8 and 9).

PHOTOTROPHIC ORGANISMS IDENTIFICATION

The main types of communities that colonize the cave surfaces were identified near the light sources on the basis of their microscopic and macroscopic features. This approach was chosen in order to be easily reproduced by future cave management. It was assumed that the new lights were placed in the same locations as the previous installation. Samples were acquired in the monitoring sites shown in Figure 2a (sites Zi-1 to Zi-6). The phototrophic community types were identified on the basis of samples collected along transects that involve multiple lights. These were designed by randomly selecting six lamps at each site. At each sampling site, four samples were acquired at a distance of about 1 m from each lamp in different directions, north, south, east, and west. Samples were acquired by scraping off 2 cm^2 of surface at each sampling site and near the cave entrance. It is important to stress that the entrance is the only part of the cave that can be reached by solar radiation, therefore, a different flora is expected in this area. The presence of vermicular formations, the color and size of algal colonization, and the moisture content of the substrate were recorded for each sample. Each sample was collected using a disposable sterile spatula and stored separately in a sterilized plastic tube until its analysis in the laboratory one hour later. Samples were examined using a LEICA optical microscope (maximum magnification 1000×) to detect the presence of fungal mycelium, examine the morphology and frequency of the algal component, and distinguish between green algae and cyanobacteria. Algae were determined only at phyla taxonomic level. Bryophytes and pteridophytes were determined according to Pedrotti (2001) and Prelli and Boudrie (2001).

COMMUNITY DISTRIBUTION IN RESPONSE TO LIGHT INTENSITY

The distribution of the phototrophic community types previously identified was analyzed with respect to the light intensity measured with a luxmeter ISO-Tech ILM 350. Light intensity was acquired in a perpendicular direction with respect to the highest part of the cave in order to consider possible shadows cast in some parts of the transects. Data on light intensity were collected along thirty transects using the luxmeter. Each transect was designed to span two light sources. The transect length varied from 5 m (minimum distance between lights) to 15 m (maximum distance between lights). Each transect had its origin at one light source and covered the adjacent lateral surfaces up to the ceiling surface of the cave until its end in the next light source (Fig. 2c). Each transect was acquired in locations characterized by almost constant microclimatic conditions. The transects were designed in the Zi-2 through Zi-5 sites (Fig. 2a), where temperature remains almost stable over the year and relative humidity is over 90%. The community type, the height from the floor, the distance from the radiation source, and the intensity of the incident light were recorded every 30 cm along each transect.

COMPOSITION OF VERMICULAR FORMATIONS

Microbiological surveys were performed to investigate the composition of vermicular formations. Samples of vermicular formations and soil were collected in seven sites, labelled Zi-0 through Zi-6 in Fig. 2a, within the cave. The samples were collected using sterile equipment, such as disposable spatulas and fiber dracon swabs. An aliquot of the samples was introduced directly in liquid medium (tryptose broth), and another aliquot was sown directly onto solid malt agar, Sabouraud, and Triple Sugar Iron. Moreover, pool and dripping water samples were collected in the Zi-2 site using sterilized pipettes. Water samples were filtered using 47 mm millipore filter with 0.45 μm porosity. Standard methods were followed for the identification of bacteria and fungi (Bailey and Scott, 1974; Barnett and Hunter, 1998). It was carried out by the macroscopic examination of colonies (color, gloss, forms), by detection of the microscopic features of bacteria (Gram stain, presence of capsule, morphology, motility, size), and biochemical typing on Triple Sugar Iron in Methyl Red–liquid Voges Proskauer, nitrate liquid medium, phenylalanine agar, citrate agar, urea liquid medium.

A ZEISS UltraLux microscope and a ZEISS Axioscope microscope were used for the morphological analysis. A DCM500 digital imager was used to capture and store images. Fungi were identified on the basis of visible spore and hyphal morphology and color. Both classical (Bailey and Scott, 1974) and numerical methods (Holt, 1994; Goodall, 1966) were used for tests on each colony. Triple Sugar Iron (TSI), TSI gas, TSI butt, TSI slant, H_2S, Sellers, fluorescent slant, citrate, catalase, indole, methyl red, phenylalanine, urease, Voges Proskauer, nitrate and nitrite reduction, and oxidase reactions were used for bacterial typing and saved in a database for use in future tests. Fungi of genus *Geotrichum* were identified on the basis of visible rectangular arthrospores, in the germinative state (Bailey and Scott, 1974).

Numerical methods (Holt, 1994; Jaccard, 1912) optimally require a minimum of thirty tests to determine genus, and possibly as many as one hundred tests to determine species. The morphometric and biochemical data is generally compared to large databases of bacterial data to attempt automated identification by scoring each colony with the Jaccard index. However, in this case, the Sokal modification (Sneath and Sokal, 1973) to the Jaccard index, which ignores negative test results, is more suitable for biome comparison and study of metabolic processes within a biome (Goodall, 1966), as well as for mapping the flow of metabolites on cave surfaces. We carried out thirty tests to investigate the metabolic environment and to make a determination of similarity. Sokal scores were calculated from the morphometric and metabolic tests on the bacteria collected in the Zinzulusa in 1999 (Onorato et al., 1999) and to make a similarity comparison with 2009. Test results and images were stored in a database and processed to provide a comparison with other caves. The similarity index can provide a measure of change in metabolic profiles within the biomes caused by the changes made in illumination.

Results and Discussion

The Grotta Zinzulusa microclimate is consistent with other single-entrance caves, with large temperature and relative-humidity variations near the entrance and smaller variations internally. These variations depend on the position inside the cave and the season (Fig. 3). Only the first measurement sites are influenced by the outdoor exchanges, while starting from the Trap (point 4) the difference is lower and the temperature profile is more stable within the site during the monitoring period.

When the thermal inversion occurs, from November to May, the average cave temperature is 17.3 ± 2.6 °C, while the average external temperature is 14.1 ± 1.3 °C. In general, the thermal gradient between the cave and the exterior atmosphere is between 3 and 4 °C (Fig. 3b).

The maximum temperature gradient between the outside air and points in the cave occurs up to the Canopy (point 5 in Fig. 2a), approximately in the middle part of the cave. From the Trap (point 4 in Fig. 2a) to the Waterfall (point 6 in Fig. 2a) there is a gradient of at least 1 °C. After the Crypt (point 7 in Fig. 2a) no difference is found in both seasons (Fig. 3a).

Humidity within Grotta Zinzulusa tends to remain high at all the monitoring sites 1 to 10 in the cave, with values between 90% and 100%, showing small differences over time. The logger at point 1 recorded the most variation and the lowest relative humidity, with values around 80%. The saturation level is quite often reached inside the cave, and in general, an equilibrium is achieved between speleothems, ceiling areas, and surrounding air.

We now describe the outcomes related to the algal and micro-biological communities of Grotta Zinzulusa. The response of phototrophic organisms to light intensity revealed an abundant cryptogam flora present within 5 m from the entrance, where the light intensity and the solar radiation are reduced by about 80%. It primarily consists of lichens, bryophytes (mainly unidentified protonema and mosses such as *Rhynchostegiella tenella* and *Eucladium verticillatum*), gametophytes, and sporophytes of pteridophytes such as *Adiantum capillus-veneris. E. verticillatum* is responsible for the formation of organogenic concretions that grow from the dripping ceilings oriented towards the outside of the cave as phototropic growth. Bryophyte protonema and small lichens are only present occasionally, while algal communities consistently spread over artificially-lit surfaces. A dense presence of bacteria and fungi was detected.

Four phototrophic community types (Fig. 4 and Table 1) have been identified during the transects (Fig. 2c) carried out to analyze the response to light intensity. They are distinguished by macroscopic characteristics detectable with the naked eye, so this identification can be useful for rapidly detecting future changes in their distribution. From the entrance of the cave to the other locations, the relative frequency of the algal component increases. A major algal component can be observed passing from the community

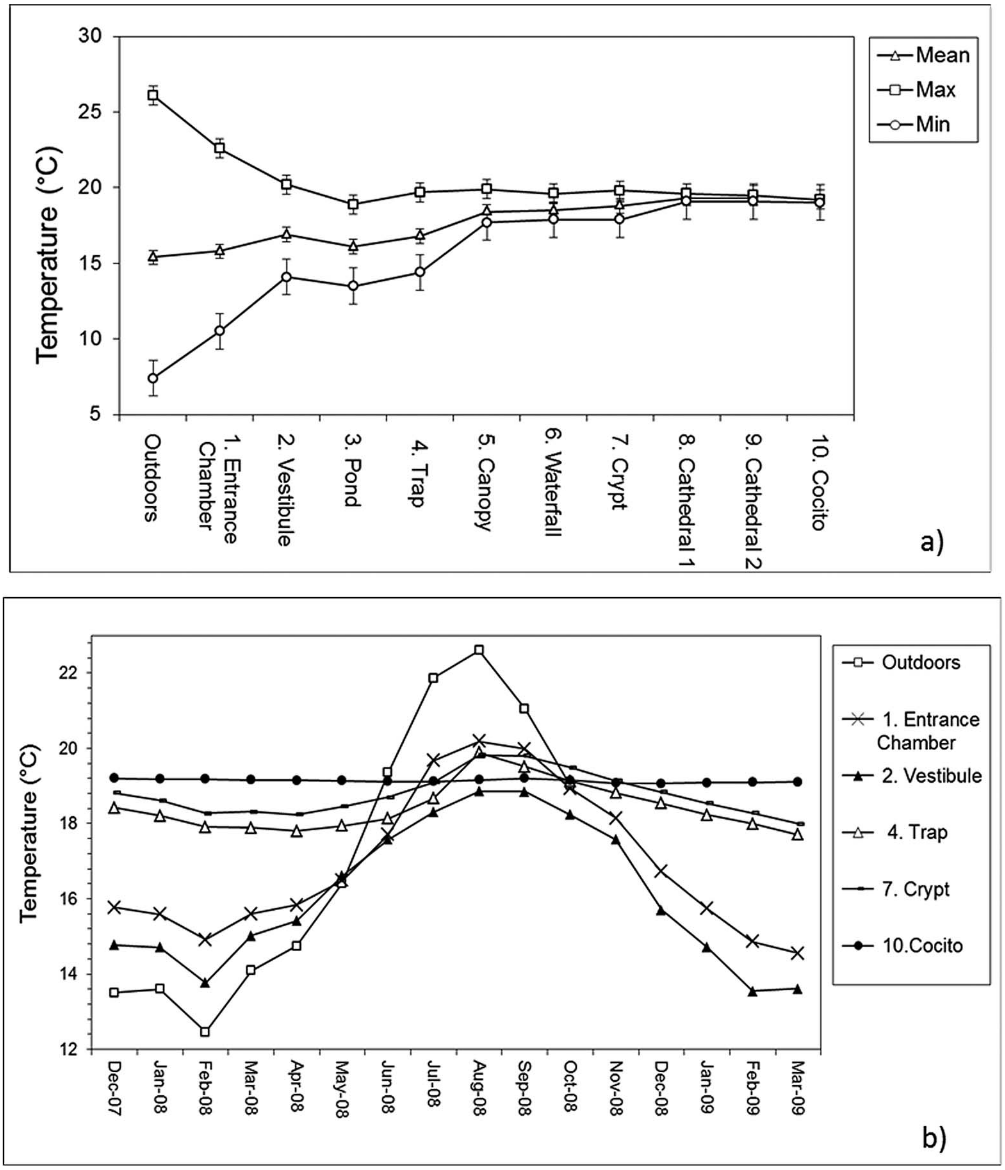

Figure 3. a) Average, maximum, and minimum air temperature (°C) in the period December 2007–December 2009 at the measurement points of Fig. 2a. b) Air temperature over the measurement period outside and at some named points inside the cave. The error bars indicate the uncertainty of the given values, they refer to the instrumental error of the measurements. Data collected by the University of Salento and provided with publishing permission by the Castro Municipality.

types 1 and 2 to the community types 3 and 4, in relation to the light gradient. Types 3 and 4 also respond selectively to the substrate wetting, and, in particular, the community type 4 requires a wet substrate.

The response to the light intensity is not monotonic. Fig. 5 shows the frequency distributions of the four community types versus the photon flux density (PPFD) obtained by combining data from all the transects. The community types are distributed selectively according to the light intensity around the lights. In particular, the community type 1 shows a peak at a flux of 0.3 μmol m^{-2} s^{-1}, while at other intensities, its presence is much lower. The community types 2 and 3 have their ecological optimum in the short interval between 0.1 and 0.3 μmol m^{-2} s^{-1}, and their presence is inhibited at both minor and major light intensities. The community type 4 is the most heliophilous, since it is distributed in a light range between 0.1 and 0.8 μmol m^{-2} s^{-1}. The community types 3 and 4 show algae growth at light intensity as low as 0.33 μmol m^{-2} s^{-1}, showing that algae in caves can survive at photon flux densities in the range of 0.5 to 1 μmol m^{-2} s^{-1}. Finally, it is worth noting that all community types have a reduced growth to light intensities higher than 0.8 μmol m^{-2} s^{-1}.

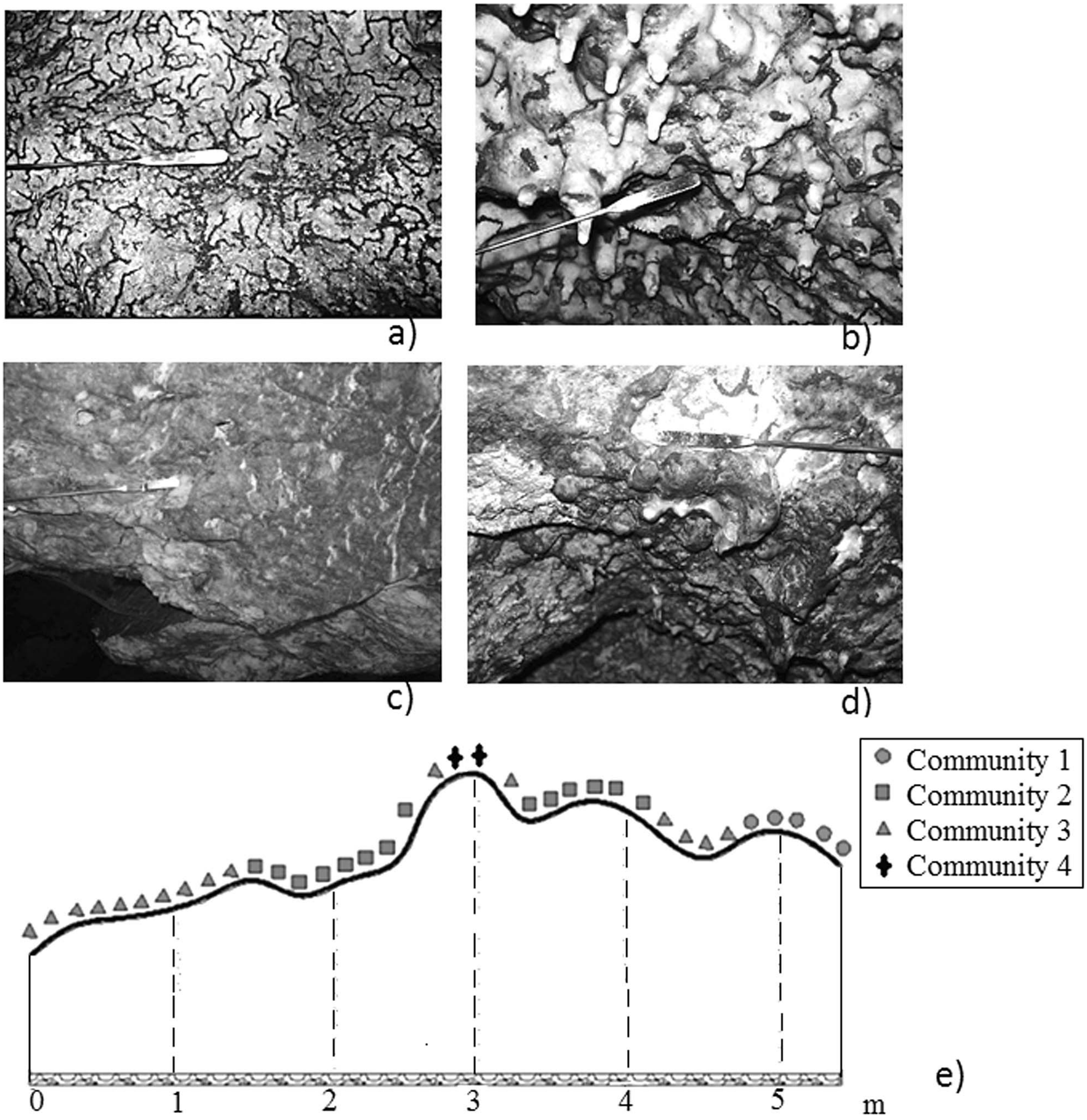

Figure 4. Community types (Table 1) living in Grotta Zinzulusa: a) type 1, b) type 2, c) type 3, d) type 4. e) A profile of of a 5-m transect two lamps, *h* the height measured from the cave floor.

Microscopic analysis of the vermicular formations has identified a biotic component mainly composed of bacteria and protozoa with a predominance of fungi (septate hyphae, spores). Moreover, elements of animal origin are found in the samples collected throughout the cave, including excreta and residuals of Isopoda, Diptera, and Lepidoptera scales. Lint has also been found in the collected material, and its origin is due to the tourist presence.

We identified *Geotrichum candidum* (Fig. 6), *Mucor troglophilus*, and *Aspergillus* sp. pl. in the vermicular formations. *Geotrichum* has cells which are fairly characteristic, with dimensions of 4 by 8 μm. It produces a thick mucus capable of aggregating clay particles (de Hoog et al., 1986). Biological activity of *Geotrichum* was detected by the production of the metabolic lactic and succinic acids. *Geotrichum* tends to develop linearly according to a dendritic pattern that suggests a role in the vermicular formations. Comparing the vermicular formation organisms with other cave collections and identification charts (Cornish-Bowden, 2012), an unsatisfactory classical identification profile has

Table 1. Main features of the phototrophic community types living in the lighted part of Grotta Zinzulusa.

Community type	Microscopic examination	Substrate	Macroscopic appearance
1	Abundant fungal mycelium and inorganic components. Algal cells almost absent.	Dry or slightly wet.	Vermicular formations. No green film.
2	Fungal mycelium and unicellular algae (*Cyanophytes*).	Dry or slightly wet.	Vermicular formation and bright green isolated stains.
3	Abundant fungal mycelium, unicellular and multicellular threadlike algae (*Cyanophytes)*. Clusters of small rounded algal cells (*Cyanobacteria*).	Dry or slightly wet.	Vermicular formations and bright green confluent stains.
4	Abundant *Cyanophytes.* Clusters of rounded algal cells (*Cyanobacteria*). Fungal mycelium relatively less frequent.	Wet.	Gelatinous and dark green films.

been obtained. Those fungi are capable of development via different types of interactions with other organisms by using their versatile metabolic pathways (Lazzari, 1966).

The bacterial taxa isolated in the samples are *Shigella* sp., *Campylobacter concisus, Enterobacter aerogenes, Enterobacter hafniae, Pseudomonas sutzeri, Pseudomonas aeruginosa, Ancalomicrobium adetum, Klebsiella pneumoniae, Moraxella lacunata, Moraxella osloensis, Yersinia* sp., *Providencia stuartii, Bacillus schlegelii, Bacillus polpilliae, Kingella kingie* (= *Moraxella kingie*), and *Psychrobacter phenylpyruvicus* (= *Moraxella phenylpyruvica*). They were almost uniformly distributed within the sampling sites.

Results of the computational comparison between the data of 1999 and 2009 reveal a Sokal index of 0.39, suggesting that some changes might have taken place between collections. Figure 7 and Table 2 present the metabolite information from the bacterial tests to identify the predominate chemistry in the different biomes. Table 2 shows that the large amount of nitrate observed at the Zi-4 site could be coupled to vegetal activity, but could also be related to light intensity. The table shows decreases in the later data in oxidase and Vogues-Proskauer (utilizing the butylene glycol pathway producing acetoin) and a reduction of motile organisms to 25% of 1999 levels. Also, there is no remaining urea metabolism

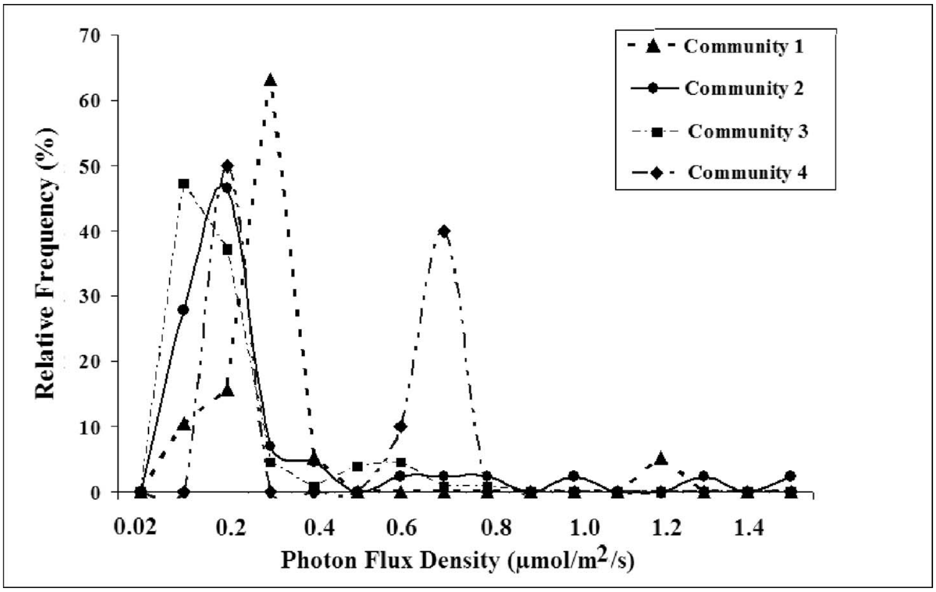

Figure 5. Relative frequency distribution of the four communities versus the light intensity, developed by combining data from all transects. Relative frequency is the percentage of the identified communities versus light intensity. The total of 100% is verified in the sum of the frequencies of each community (community 1, 2, 3, 4) with light intensity.

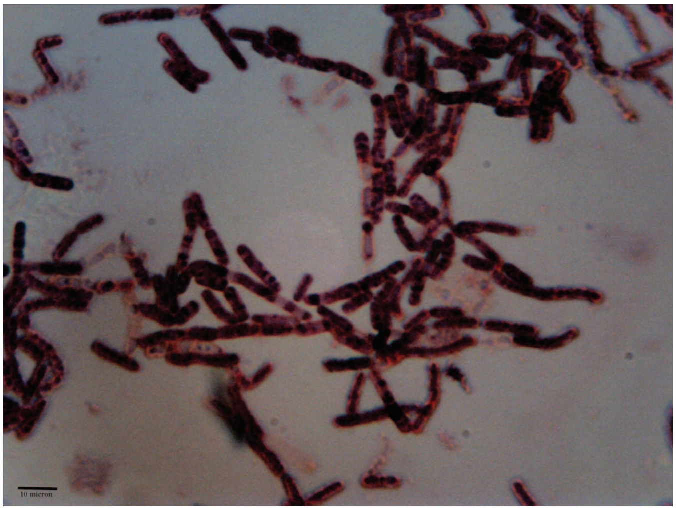

Figure 6. ***Geotrichum candidum*** **(Gram stain, scale bar is 10 μm).**

by vermiculation bacteria, possibly due to the change to LED lighting. Evidence of nitrogen fixation from nitrate is seen in low-light areas even though, according to Dunstan et al. (1982) and de Freitas and Schmekal (2003), this activity should be inhibited in darkness. It is also interesting to observe that very little nitrite reduction to N_2 is taking place (Table 2 and Fig. 7), supporting the hypothesis of nitrite consumption by vegetation or fungi rather than by bacteria.

In the light range between 0.1 and 0.3 μmol m^{-2} s^{-1}, which is the ecological optimum of the community types 2 and 3, the maximum level of metabolic activity is observed (Fig. 7). Of course, while there seems to be some correlation between photon flux and sugar metabolism (Fig. 7), there could be several other factors at play. More study is needed in this direction. Moreover, there was a doubling of citrate utilization, which might indicate mutations in *E. coli* (Lenski, 2000), and indole production, which reinforces the need to remediate the sewage influx problem with Zinzulusa.

A comparison of the microbiological component of Grotta Zinzulusa with the microbiological component of other caves is depicted in Figure 8. Among the tested caves, the most dissimilar caves, corresponding to score values close to zero, were the closed-to-visitors Horsethief Cave, Wyoming, (Sokal score 0.015) and a cave explored in Canada with spherical ceiling pendants having *Geotrichum* sp. These caves are rather dry caves compared to Zinzulusa. On the other end of the scale, Snaggletooth Cave, a partially restricted cave in New York State, scored highest at 0.47; a value justified by the fact that this cave is subject to seepage of treated sewage. The remainder are mostly caves with influx consisting of woodland drainage, all of which represent animal activity in bacterial content.

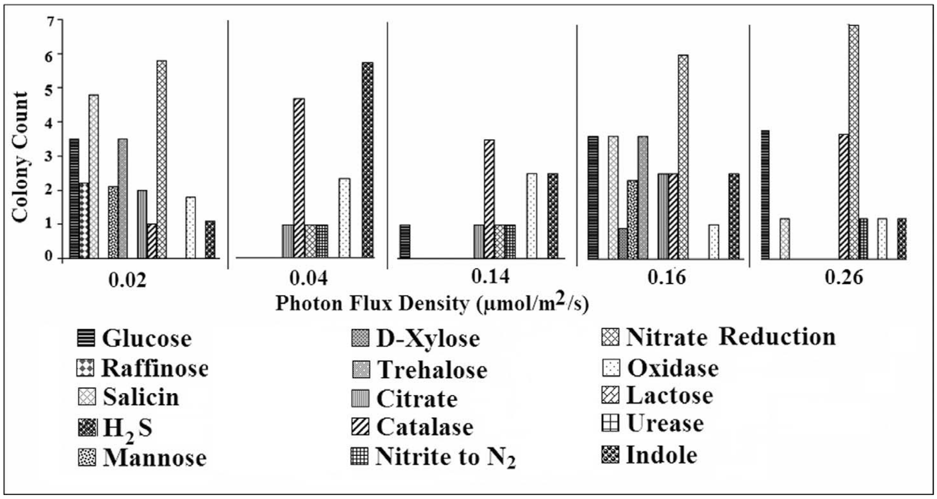

Figure 7. Metabolic activity of the bacterial component in the community types in Grotta Zinzulusa versus the light intensity.

Table 2. Metabolic characteristics of the bacterial component of the samples collected during the surveys of 1999 (Camassa and Febbroriello, 2003) and 2009.

Metabolome	# 1999	% POS 1999	% NEG 1999	# 2009	% POS 2009	% NEG 2009
TSI GAS	14	0	100	33	0	100
pH in V-P broth <6	14	14.3	85.7	36	38.9	61.1
Utilization of citrate	14	14.3	85.7	28	25	75
Gram Positive (young cultures)	7	42.8	57.1	23	52.2	47.8
Catalase	10	50	50	27	55.6	44.4
Indole	14	0	100	28	28.6	71.4
Motility	7	42.8	57.1	8	12.5	87.5
Oxidase	6	83.3	16.7	27	44.5	55.5
Phenylalanine deaminase	6	0	100	20	0	100
TSI	14	57.1	42.8	33	51.5	48.5
TSI BUTT	6	0	100	16	0	100
TSI H2S	14	0	100	33	0	100
TSI SLANT	8	0	100	16	0	100
Urease	14	7.2	92.8	20	0	100
Voges Proskauer	13	7.6	92.3	35	0	100
Nitrate reduction	14	64.3	35.7	36	75	25
TSI GAS	14	0	100	33	0	100

Many factors can favor a long-term colonization, among them a high relative humidity, relatively stable temperature, a water pH close to neutrality, and other features influencing the attachment of bacteria, such as micro-roughness, substratum chemistry and pH, and fluid-dynamics. As an example, a large presence of bacteria is observed in the closed-to-visitors Lascaux Cave (Cunningham et al., 1995) and in the Maltravieso (Arroyo et al., 1997) and Altamira Caves (Laiz et al., 1999), where cyanobacteria and faecal indicators appeared in abundance, especially in dripping waters (Northup et al., 2003). Even though these measurements have helped to identify caves with metabolic similarity, the generally low scores clearly suggest that every cave environment is unique and worth a detailed study and protection.

CONCLUSIONS

This paper represents the first assessment of the microclimatic and microbial characterization of Grotta Zinzulusa in southeastern Italy. We performed surveys to analyze both the distribution of the phototrophic organisms in response to light intensity and the microbiological component of vermicular formations. The outcomes of this research contribute to the knowledge of lampenflora contamination after lighting replacement by LEDs. The environmental monitoring performed in the cave revealed that the light sources contribute to the development of a complex system of communities inside the cave.

The replaced tungsten lamps were not suitable for cave preservation, as they increased temperature and decreased humidity. The new LED system has a smaller impact on the cave microclimate, emits light of natural appearance, consumes less electricity, and has no IR spectrum. Another advantage is to have a lower irradiance with high illuminance values that create contrast with the illuminated objects. Tourist attendance is contributing to the introduction of live microorganisms that can persist in the cave environment, such as fungi and bacteria. To prevent the introduction of microbes, a disinfection barrier can be installed outside the cave. Among the different species, photosynthetic algae appeared to be dependent on light intensity, but in the presence of available nutrients, an unsuitable light quality for lampenflora growth can slow their proliferation slightly, but only within a short period of time.

The study assessed the presence and identification of four principal phototrophic communities with a preference for low light intensities. The complex lampenflora algae is ubiquitous. Furthermore, a dense diffusion of microbial forms, mainly represented by *Geotrichum*, was found spreading in the cave, with the production of metabolic lactic and succinic acids that are responsible for superficial bioerosion. To reduce lampenflora growth, the lighting regime should be restricted. Specific actions could be taken to control the diffusion of communities inside the cave. For example, the open hours of the cave or the hours during which the lights are turned on could be reduced. A limit of people entering the cave or further spatial restrictions could be also established.

The results of this study are intended to be used to support the development of a rigorous monitoring program for the on-going management of Grotta Zinzulusa. A long-term program is needed to monitor lampenflora growth, the microclimate, and environmental. These results can be a basis for the establishment of guidelines for the use of the cave system as a natural asset, including maximum number of tourists and frequency of visits. They might also serve as a starting point for future investigations, as well as for comparisons with other caves.

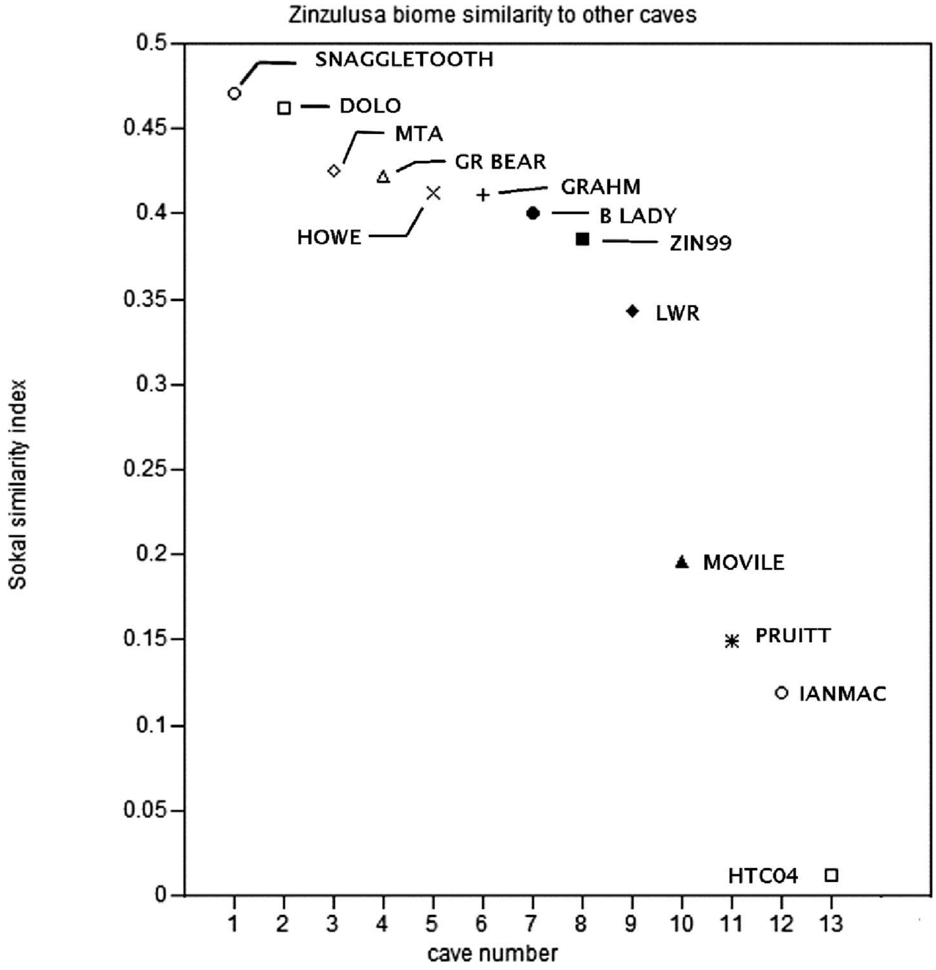

Figure 8. Comparison of Sokal index of similarity with other caves: Snaggletooth Cave (SNAGGLETOOTH), NY, USA, treated sewer seepage; Dolo Cave (DOLO), MA, USA, woodland surface stream insurgence; Mount Anthony Cave (MTA), VT, USA, woodland surface stream insurgence; Growling Bear (GR BEAR) Cave, MA, USA, underground spring; Howe Caverns, NY, USA, tourist cave; Grahm Mountain Cave (GRAHM), NY, USA, bat hibernaculum (B LADY), CT, USA, woodland swamp drainage; Zinzulusa, 1999 samples (ZIN99); Lake of the White Roses (LWR), Lechuguilla Cave, NM, USA, sulfur cycle with minimum surface water influx; Movile Cave (MOVILE), Romania, H_2S, underwater samples; Buford Pruitt (PRUITT) cave samples, FL, USA, rarely entered; Ian McKenzie (IANMAC) cave samples, Canada, dry mountain cave ceiling pendants, restricted access; Horsethief Cave (HTC04), WY, USA, mountainous cave area.

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K.J. Vanderwolf, D. Malloch, and D.F. McAlpine – Fungi associated with over-wintering tricolored bats, *Perimyotis subflavus*, in a white-nose syndrome region of eastern Canada. *Journal of Cave and Karst Studies*, v. 77, no. 3, p. 145–151, DOI: 10.4311/2015MB0122

FUNGI ASSOCIATED WITH OVER-WINTERING TRICOLORED BATS, *PERIMYOTIS SUBFLAVUS*, IN A WHITE-NOSE SYNDROME REGION OF EASTERN CANADA

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Abstract: The tricolored bat (*Perimyotis subflavus*) is threatened by white-nose syndrome (WNS), a fungal disease caused by *Pseudogymnoascus destructans* (*Pd*) and was recently ranked as endangered under the Canadian Species-at-Risk Act. There have been few prior studies on the fungi associated with over-wintering bats. Such information is important in assessing overall fungal diversity within the cave habitat, in determining the ecological role that bats may play as dispersers of fungi, and in the identification of fungal species potentially antagonistic to *Pd*. We swabbed twenty-two *P. subflavus* over-wintering in caves and mines in New Brunswick, Canada, in 2012 and 2013. This produced 408 isolates comprising 60 taxa in 49 fungal genera with an average of 10.2 ± 3.9SD fungal taxa recorded per bat. We found fungal assemblages on *P. subflavus* (post-WNS) very similar to those we cultured previously from *Myotis spp*. (pre-WNS) at the same sites. We suggest that the variation in fungal assemblages observed from site-to-site on hibernating *P. subflavus* is largely due to environmental and ecological characteristics of individual caves, rather than the presence of *Pd* or roosting habits.

Introduction

The tricolored bat (*Perimyotis subflavus*) is considered one of the most common and widely distributed species of bats in eastern North America (Briggler and Prather, 2003). However, the species is threatened by white-nose syndrome (WNS), a disease of hibernating bats caused by the fungus *Pseudogymnoascus destructans* (*Pd*) that was first observed in 2006 in Albany, New York (Lorch et al., 2011). *Perimyotis subflavus* has suffered mortality of up to 100% in multiple caves, with an average of 76% mortality in the northeastern-state hibernacula surveyed (Turner et al., 2011). Cumulative declines for the species in overall regional abundance from its peak levels to 2011 have been estimated at 34% (Ingersoll et al., 2013).

In Canada *P. subflavus* occurs in southern Ontario, Quebec, New Brunswick, and Nova Scotia (van Zyll de Jong, 1985), the northern limit of the species range where it is considered rare to uncommon (Hitchcock, 1965; Forbes et al., 2010; Mainguy et al., 2011). The arrival of WNS has placed the Canadian population of *P. subflavus* at particular risk, and as of December 2014, the species has been ranked as endangered under the Canadian Species-at-Risk Act.

There have been few studies of fungi associated with over-wintering bats, but with the advent of WNS there has been increasing interest in bat- and cave-associated fungi (Johnson et al., 2013; Lorch et al., 2013a; Vanderwolf et al., 2013). Such information is important in assessing overall fungal diversity within the cave habitat, in determining the ecological role that bats may play as dispersers of fungi, and in the identification of fungal species potentially antagonistic to *Pd*. Our earlier study in Maritime Canada, carried out prior to *Pd* arrival, determined that assemblages of ectomycota cultured from over-wintering *Myotis lucifugus* and *M. septentrionalis* (hereafter *Myotis* spp.) were relatively diverse (>100 species) (Vanderwolf et al., 2013). *Myotis* spp. are widespread in eastern Canada with hundreds to thousands of individuals over-wintering together in hibernacula, while *Perimyotis subflavus* are relatively rare (<10 individuals/hibernaculum) and usually roost singly (Vanderwolf et al., 2012). It has been suggested that roosting alone may slow the transmission of *Pd* (Langwig et al., 2012). If roosting habits affect the diversity of fungi on hibernating bats, we hypothesize that the fungal assemblage on *P. subflavus* may differ from *Myotis* spp. within the same hibernaculum. Here we report on an investigation of the fungi associated with over-wintering *P. subflavus* carried out in 2012–2013 in a WNS-positive region of eastern Canada. Our sample interval is especially noteworthy because it straddles the period from the first detection of *Pd* on *P. subflavus* in Canada in 2011 to the apparent extirpation of *P. subflavus* from hibernacula in New Brunswick due to WNS.

Methods

The number of *Perimyotis subflavus* over-wintering in caves and mines in New Brunswick, Canada, were recorded during regular surveys as described in Vanderwolf et al. (2012). Data on physical characteristics of study sites, including location, length, and temperatures, can be found in Vanderwolf et al. (2012). Where *P. subflavus* was present,

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numbers at hibernacula ranged from one to seven per site. With the exception of a single *P. subflavus* removed from each site for WNS confirmation by histology and sequencing at the Canadian Wildlife Health Cooperative, live bats were assessed in the field for the presence of characteristic *Pseudogymnoascus destructans* fungal growth by visual inspection of exposed skin surfaces only. However, lack of visible *Pd* growth does not equate to the absence of WNS (Verant et al., 2014). Since all *P. subflavus* observed in New Brunswick roosted singly, generally 1 to 2 meters from the hibernaculum floor, we had access to all *P. subflavus* observed. While our assessment method minimized disturbance to hibernating bats, it prevented determination of sex.

Perimyotis subflavus individuals were swabbed for fungi February–March 2012 and March–April 2013 using methods identical to those reported in Vanderwolf et al. (2013). All *P. subflavus* encountered during these two hibernation periods were sampled (n=22). None of the fifteen *P. subflavus* sampled in 2012 showed visible signs of *Pd* growth, while four of the six *P. subflavus* sampled in 2013 had *Pd* growth based on visual inspection. Swabs were taken with a sterile, dry, cotton-tipped applicator from the dorsal fur or skin of live bats; the term skin meaning one or more of the face, ears, patagium, or uropatagium, with sampling dependent on which skin surfaces were accessible. Bats were swabbed while they were roosting and were not removed from cave walls. Swabs were cultured on either dextrose-peptone-yeast extract (DPYA) agar (Papavizas and Davey, 1959) or Sabouraud-Dextrose (SAB) agar, both of which contained the antibiotics chlortetracycline and streptomycin. Four swabs were taken using all combinations of fur or skin on either SAB or DPYA from each *P. subflavus*, except where swabbing was terminated with one and three swabs for two bats that awoke during swabbing. A new applicator was used for each swab. After swabbing, the applicator was immediately streaked across an agar surface in a petri plate. Dilution streaks were completed in the hibernaculum within 3 h of the initial streak, after which plates were sealed in situ with parafilm (Pechiney Plastic Packaging, Chicago, IL).

Samples were incubated, inverted, in the dark at 7 °C in a low temperature incubator to approximate the hibernaculum environment and target fungi adapted to cave microclimates. The average winter temperature in the dark zone of New Brunswick hibernacula is 5.1 ± 1.1 °C, with winter defined as 1 November–30 April (Vanderwolf et al., 2012). Samples were monitored over four months until no new cultures had appeared for three weeks on a plate or the plate had become overgrown with hyphae. Once fungi began growing on the plates, each distinct colony was subcultured to a new plate. DPYA without oxgall and sodium propionate was used for maintaining pure cultures.

Identifications were carried out by comparing the micro- and macromorphological characteristics of the microfungi to those traits appearing in the taxonomic literature and compendia (Domsch et al., 2007; Seifert et al., 2011). We also had access to reference collections of cultures from *Myotis* spp. identified previously using a mix of sequencing and morphological features (Vanderwolf et al., 2013). Permanent cultures of fungi reported here are vouchered in the University of Alberta Microfungus Collection and Herbarium (UAMH 11335, 11725, 11730, 11731), and desiccant-dried samples are housed in the New Brunswick Museum (NBM# F–04824–04839, 04841–04843, 04871–04882, 04916–04941, 04949–04951, 04961). After testing for normality, a 2-sample t-test was used to compare the number of fungal taxa on individual *P. subflavus* that did and did not culture positive for *Pd*. Since the data were not normally distributed, a Mann-Whitney test was used to compare the number of fungal isolates recovered from fur versus skin and DPYA versus SAB. Minitab statistical software was used for all tests.

We followed the protocol of the United States Fish and Wildlife Service for minimizing the spread of WNS during all visits to caves (revised decontamination protocol: June 25, 2012. Available online http://www.whitenosesyndrome.org/resource/revised-decontamination-protocol-june-25-2012). Necessary permits were obtained from the New Brunswick Department of Natural Resources.

RESULTS

WNS was first observed in New Brunswick in Berryton Cave in March 2011, at which time a single dead *Perimyotis subflavus* was collected among thousands of dead *Myotis* spp. This bat was subsequently confirmed *Pseudogymnoascus destructans* and WNS-positive, the first such detection for *P. subflavus* in Canada (S. McBurney, Canadian Wildlife Health Cooperative, pers. comm.). Thereafter, we did not encounter *P. subflavus* with visible *Pd* growth until December 2012, although *P. subflavus* were observed roosting near *Myotis* spp. with visible *Pd* growth during this interval. *P. subflavus* has not been observed at any of our study sites since December 2013.

Pd was cultured from three *P. subflavus* in February–March 2012, though visible fungal growth was not seen on *P. subflavus* until 2013. *Pd* was cultured from 100% of *P. subflavus* with visible *Pd* growth (n=4) and from 27.8% of *P. subflavus* without visible *Pd* growth (5 of 18 bats). However, failure to culture *Pd* from a bat does not demonstrate that *Pd* was absent. Sixteen of the eighteen *P. subflavus* sampled without visible *Pd* growth, including the five that cultured *Pd*-positive, were located in hibernacula with *Myotis* spp. that had visible *Pd* growth. *Pd* was isolated at similar frequencies from fur (n=10) and skin (n=13) swabs and on DPYA (n=13) and SAB (n=10) media.

Fungi were successfully cultured from all bats and from 73 of 79 swabs (92.4%), producing 408 isolates. An average of 10.2 ± 3.9SD fungal taxa were recorded per bat (n=22, range 2–22; Table 1). *Perimyotis subflavus* that cultured positive for *Pd* had 12.1 ± 4.3SD fungal taxa per bat (n=9 bats) compared with 8.9 ± 3.2SD for *P. subflavus* that cultured negative (n=13 bats). There was no significant difference in the number of fungal taxa cultured between *Pd*-negative and *Pd*-positive bats ($T_{1,13}= -1.89$, P=0.082).

During this study sixty taxa in forty-nine fungal genera were isolated, plus nine sterile fungal morphs (Table 1). Twenty six (43.3%) fungal taxa were found on only a single *P. subflavus*. The most common taxa isolated were *Leuconeurospora* spp. (detected on 86.4% of the twenty-two bats sampled), *Cephalotrichum stemonitis* (68.2%), *Humicola* cf. UAMH 11595 (63.6%), *Pseudogymnoascus pannorum sensu lato* (63.6%), *Penicillium* spp. (54.5%), *Wardomyces* spp. (54.5%), *Trichosporon* spp. (50.0%), and *Pseudogymnoascus destructans* (40.9%). The number of isolates recovered for each fungal taxon was not significantly different between fur and skin swabs ($W_{1,60}=3999$, P=0.194) or DPYA and SAB media ($W_{1,60}=3878.5$, P=0.503), although fur and DPYA tended to yield greater fungal diversity (182 isolates on fur, 157 on skin; 176 on DPYA, and 163 on SAB).

DISCUSSION

The diversity of fungi isolated from *P. subflavus* is similar to that isolated from *Myotis* spp. during previous investigations at the same sites (Vanderwolf et al., 2013). In 2010 (pre-WNS), fifty-two fungal taxa in thirty-eight genera were isolated from *Myotis* spp. (n=20) in Markhamville Mine and Glebe Mine, the principal sites that we found *P. subflavus* selected for over-wintering. In comparison, fifty-five fungal taxa in forty-two genera were isolated from *P. subflavus* (n=19) at these two sites post-WNS. Six of the eight most common fungal taxa isolated from *P. subflavus* post-WNS, as well as many of the rarer fungi, were identical to those cultured from *Myotis* spp. pre-WNS at these two sites. The number of fungal taxa isolated per bat was also similar. In 2010, an average of 8.3 ± 3.7 and 8.5 ± 1.7 fungal taxa per *Myotis* spp. were isolated from Glebe Mine (n=10 bats) and Markhamville Mine (n=10 bats) respectively (Vanderwolf et al., 2013). This compares to an average of 10.2 ± 2.2 and 11.2 ± 4.7 fungal taxa per *P. subflavus* isolated from Glebe Mine (n=9 bats) and Markhamville Mine (n=10 bats) respectively when combining 2012 and 2013 data. The average number of fungal taxa per bat is not significantly different between *Myotis* spp. and *P. subflavus* either in Glebe Mine ($T_{1,14}=1.38$, P=0.188) or Markhamville Mine ($T_{1,11}=1.72$, P=0.114).

Perimyotis subflavus characteristically roost alone during hibernation, although clusters of two to four have been reported (Briggler and Prather, 2003; Vincent and Whitaker, 2007). It has been suggested that this roosting habit may slow the transmission of WNS and may help explain why *P. subflavus* has lower mortality rates from WNS than *M. lucifugus*, which often roost together (Turner et al., 2011; Ingersoll et al., 2013). In New Brunswick, *P. subflavus* did show a delay in the development of visible *Pd* growth and ensuing mortality relative to *Myotis* spp. (Vanderwolf et al., unpublished). However, once *Pd* becomes widespread on cave walls and in cave sediment (Lorch et al., 2013b;), substrate to bat transmission may negate any protection that roosting alone provides.

Although the transmission of *Pd* may be density-dependent, at least initially (Langwig et al., 2012), this does not appear to be the case with other fungi found on bats. We found over-wintering *P. subflavus* harbor a similar diversity of fungi compared to colonial *Myotis* spp. Aside from *Pd*, *Trichophyton redellii* is the only fungus identified to date that grows on cave-hibernating bats (Lorch et al., 2015). However, McAlpine et al. (2015) have recently reported of unidentified ascomycetes growing on big brown bats (*Eptesicus fuscus*) over-wintering in buildings in New Brunswick. The genus *Trichophyton* appears to be rare on bats in our study area, as we only isolated one *Trichophyton* sp. culture from among eighty hibernating *Myotis* spp. sampled in 2010 (Vanderwolf et al., 2013), and we detected no *Trichophyton* sp. isolates in the current study. Lorch et al. (2015) cultured samples on SAB at 7 °C, so it is likely we would have detected *Trichophyton redellii* if present. It is unknown if the transmission of *Trichophyton redellii* is density-dependent.

As with hibernating *Myotis* spp. (Vanderwolf et al., 2013), over-wintering *P. subflavus* seem to acquire spores from their environment, both prior to and during hibernation. It is likely that bats play a role in the dispersal of fungal spores, although there is virtually no data on this aspect of fungal ecology. Sources of spores within Glebe Mine and Markhamville Mine, the main over-wintering sites for *P. subflavus* in New Brunswick, include old mine timbers and mammal dung, both of which exhibit visible growth of Basidiomycota and Ascomycota. It is likely that fungi such as *Cephalotrichum stemonitis* and *Leuconeurospora polypaeciloides* have some association with mammal dung (unpubl. data). These species are particularly abundant on bats at sites where mammal dung is present (Vanderwolf et al., 2013).

The mean number of fungal taxa per bat in Markhamville Mine was noticeably higher in 2013 (15.3 ± 5.9, n=3) compared to 2012 (9.4 ± 3.0, n=7). The trend is not statistically significant; if it is real, the cause is unclear. Some fungal species in oligotrophic caves will proliferate opportunistically when presented with a food source (Cubbon, 1976). No bat carcasses were observed in the mine 2012–2013, but raccoon (*Procyon lotor*) activity in the mine, as evidenced by the presence of dung, seemed higher in 2013 compared to 2012. Based on visual sightings, the occasional carcass, and dung, raccoons often shelter in Markhamville Mine during the winter, and their droppings support luxuriant fungal growth. This may increase spore density in the mine, potentially leading to an increase in

Table 1. The number of *Perimyotis subflavus* sampled for fungi, the total and mean number of fungal taxa isolated, and the number of individual *P. subflavus* from which specific fungal taxa were cultured in each hibernaculum in each year. Mark= Markhamville Mine.

Fungal Taxa	Mark 2012	Mark 2013	Glebe 2012	Glebe 2013	Harbell 2012	White 2010	Dalling 2013	Total # of Bats
Number of bats sampled	7	3	7	2	1	1	1	22
Number of fungal taxa isolated	34	23	32	12	2	8	13	n/a
Mean number of fungal taxa/ bat ±SD	9.4 ± 3.0	15.3 ± 5.9	10.9 ± 2.0	8.0 ± 1.4	n/a	n/a	n/a	n/a
Ascomycota								
Acremonium sp.	0	0	1	0	1	0	0	2
Arthroderma sp.	0	1	0	0	0	0	0	1
Arthroderma silverae Currah, S.P. Abbott & Sigler	2	0	0	0	0	0	0	2
Arthrographis sp.	1	0	1	0	0	0	0	2
Auxarthron cf. californiense G.F. Orr & Kuehn	1	0	0	0	0	0	0	1
Cephalotrichum stemonitis (Pers.) Link	5	3	6	1	0	0	0	15
Chrysosporium spp.	5	1	0	0	0	0	0	6
Cladosporium spp.	2	0	1	0	0	1	0	4
Clonostachys sp.	0	0	1	0	0	0	0	1
cf. *Cryomyces* sp.	0	1	0	0	0	0	0	1
Diplococcium sp.	1	0	0	0	0	0	0	1
Eremomyces sp.	0	0	1	0	0	0	0	1
Fusarium sp.	0	0	0	0	0	0	1	1
Hormographiella sp.	0	0	0	0	0	0	1	1
Humicola sp.	0	2	0	0	0	0	0	2
Humicola cf. UAMH 11595	3	3	6	1	0	1	0	14
cf. Hyphozyma sp.	0	0	0	0	0	0	1	1
Isaria farinosa (Holmsk.) Fr.	1	0	1	0	0	0	0	2
Leuconeurospora polypaeciloides Malloch, Sigler & Hambleton	3	3	6	2	0	1	1	16
L. capsici (J.F.H. Beyma) Malloch, Sigler & Hambleton	2	0	0	0	0	0	1	3
Mammaria sp.	1	0	0	0	0	0	0	1
Microascus caviariformis Malloch & Hubart	0	0	0	0	0	0	1	1
Myceliophthora sp.	1	2	0	0	0	0	0	3
Myxotrichum sp.	0	0	0	0	0	1	0	1
Oidiodendron truncatum G.L. Barron	0	0	1	0	0	0	0	1
Paecilomyces sp.	1	0	0	0	0	1	0	2
Penicillium spp.	5	3	1	0	0	0	1	10
P. expansum Link	1	0	0	0	0	0	0	1
P. solitum Westling	1	0	0	0	0	0	0	1
Phaeotrichum sp.	0	0	1	0	0	0	0	1
P. hystricinum Cain & M.E. Barr	0	0	5	0	0	0	0	5
Phoma sp.	0	0	1	0	0	0	0	1

Table 1. Continued.

Fungal Taxa	Mark 2012	Mark 2013	Glebe 2012	Glebe 2013	Harbell 2012	White 2010	Dalling 2013	Total # of Bats
Preussia sp.	0	0	4	1	0	0	0	5
P. funiculata (Preuss) Fuckel	0	0	1	0	0	0	0	1
Pseudogymnoascus destructans (Blehert & Gargas) Minnis & D.L. Lindner	1	3	2	2	0	0	1	9
P. pannorum sensu lato (Link) Minnis & D.L. Lindner	6	3	2	1	0	1	1	14
P. roseus Raillo	0	0	0	0	0	0	1	1
Scopulariopsis cf. candida Vuill.	0	1	0	0	0	0	0	1
Scytalidium sp.	0	0	1	0	0	0	0	1
Thelebolus crustaceus (Fuckel) Kimbr.	1	3	0	0	0	0	0	4
Tolypocladium inflatum W. Gams	1	0	0	0	0	0	0	1
Trichoderma sp.	0	2	0	0	0	1	0	3
Trichosporiella sp.	1	3	3	0	0	0	1	8
Thysanophora penicillioides (Roum.) W.B. Kendr.	0	0	0	1	0	0	0	1
Wardomyces sp.	1	1	0	0	0	0	0	2
W. humicola Hennebert & G.L. Barron	1	0	1	0	0	0	0	2
W. inflatus (Marchal) Hennebert	0	0	6	2	0	0	0	8
Zopfiella pleuropora Malloch & Cain	0	0	2	0	0	0	0	2
unidentified ascomycete	1	0	0	0	0	0	0	1
Basidiomycota								
Asterotremella sp.	1	1	1	1	0	0	0	4
Baeospora sp.	0	0	5	0	0	0	0	5
Cystofilobasidium sp.	1	2	1	0	0	0	0	4
Hypholoma sp.	0	0	3	0	0	0	0	3
Trichosporon sp.	3	3	0	1	0	0	0	7
T. dulcitum (Berkhout) Weijman	2	1	1	0	0	0	0	4
unidentified Basidiomycete	1	1	6	1	0	0	0	9
Zygomycota								
Thamnidium elegans Link	0	0	1	0	0	0	0	1
Mortierella sp.	3	0	0	2	0	0	1	6
Mucor sp.	1	3	1	0	0	0	0	5
unidentified yeast	1	0	0	0	0	0	0	1
Sterile	2	1	3	0	1	1	1	9

Note: n/a = not applicable.

fungal assemblage diversity on hibernating bats. In contrast, both *Myotis* spp. and *P. subflavus* in Harbell's Cave yield low diversity of fungi in general, possibly because the passage floor encloses a fast flowing stream that prevents the accumulation of exposed sediment or soil in the cave that might harbor fungi (Vanderwolf et al., 2013). It appears that fungal assemblages on over-wintering bats can vary in response to local factors, both within sites from year-to-year and between sites. These factors include cave morphology, differences in cave fauna, and probably other factors as yet unknown.

The only previous study of fungi associated with *P. subflavus* was conducted by Johnson et al. (2013) in Illinois in April–May 2010 and Indiana in June 2011. Johnson et al. (2013) reported twenty-three fungal genera from *P. subflavus*, with 4.83 ± 2.04SD (n=6 bats) and 7.25 ± 4.57SD (n=4 bats) fungal genera per bat in two WNS-negative Illinois caves, and 2.2 ± 1.64SD fungal genera per bat (n=5 bats) in a single WNS-positive Indiana cave. Although we found a similarly low number of fungal taxa per bat in Harbell's Cave, our low sample size (n=1 bat), and the negative *Pd* status for both the cave and the bat make comparison with the Indiana site investigated by Johnson et al. (2013) inadvisable. However, the fungal taxa isolated from *P. subflavus* by Johnson et al. (2013) include widespread genera, such as *Cladosporium*, *Penicillium*, *Mortierella*, *Mucor*, *Trichosporon*, and *Pseudogymnoascus pannorum* sensu lato, which we also isolated from *Myotis* spp. and *P. subflavus* in New Brunswick (Vanderwolf et al., 2013).

Johnson et al. (2013) attributed the low number of fungal genera on WNS-positive *P. subflavus* in Indiana to the presence of *Pd*. In contrast, we cultured a diverse assemblage of fungi from *Pd*-positive *P. subflavus*. We suggest that since bats in Indiana were captured during flight outside the hibernation period using a harp trap and were subsequently bagged and handled, that this may have influenced the fungal diversity encountered on *P. subflavus* in Indiana, rather than any interactions with *Pd*. It has been our observation that bats will often groom upon waking and prior to flight, which may also remove some fungal spores. Probably more importantly, and as we show above, environmental and ecological characteristics of individual caves may influence the fungal assemblages that can be cultured from hibernating bats at specific hibernacula.

The diversity of cold-tolerant fungi cultured from bats in this study is similar to that found in sediments from other caves in North America (Lorch et al., 2013a; Zhang et al., 2014), further emphasizing that the fungal assemblage on hibernating bats reflects the assemblage found in the surrounding environment. Ascomycota dominate, particularly *Penicillium* spp. and *Pseudogymnoascus pannorum* s.l., and these fungi appear to be adapted to cave conditions. A subgroup of dominant cosmopolitan fungal genera are usually found in studies of cave fungi, accompanied by a diversity of rare fungi (Vanderwolf et al., 2013; Zhang et al., 2014). The high proportions of fungal taxa found singly suggest that the actual diversity of fungi in caves is much higher than detected (present study; Vanderwolf et al., 2013; Zhang et al., 2014). Undoubtedly, additional diversity would be discovered with the use of a greater variety of media.

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M. Tian and S. Huang – Two new species of cavernicolous trechines from southern China karst (Coleoptera: Carabidae: Trechinae). *Journal of Cave and Karst Studies*, v. 77, no. 3, p. 152–159, DOI: 10.4311/2015LSC0107

TWO NEW SPECIES OF CAVERNICOLOUS TRECHINES FROM SOUTHERN CHINA KARST (COLEOPTERA: CARABIDAE: TRECHINAE)

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Abstract: Two new species of subterranean trechine beetles are described and illustrated: *Sinaphaenops mochongensis* n. sp. from a limestone cave called Yueliang Dong, in Duyun, southern Guizhou, and *Luoxiaotrechus yini* n. sp. from Shuilian Dong, in Lianhua, western Jiangxi. A key to all species of the genus *Sinaphaenops* Uéno et Wang, 1991 is provided.

Introduction

As the largest karstic area in the world (Waltham, 2009), southern China is also one of the hottest of the four world hotspots for cave biodiversity, and the only one in the subtropics (Deharveng, 2012). For example, the trechine cave beetles, typical representatives of terrestrial troglobitic animals, in southern China have undergone impressive radiations and exhibit extreme morphological adaptations to subterranean habitats (Uéno and Wang, 1991; Deuve, 1993, 2002; Deuve et al., 1999; Tian et al., 2014). More than one hundred species, included in thirty-three genera, of cavernicolous and eyeless trechines live in this region (Tian, 2008, 2014; Tian and Clarke, 2012; Tian and Yin, 2013; Tian and Huang, 2014; Tian and Luo, 2015).

Sinaphaenops Uéno et Wang, 1991 is the first and a highly modified troglobitic trechine genus reported from China. It was set up to contain *S. mirabilissimus* Uéno et Wang, 1991 from a limestone cave in the Maolan karst of southern Guizhou. Several years later, Magrini (1997) described *Thaumastaphaenops pulcherrimus* from a cave in the Ziyun karst. Uéno and Ran (1998) added two new species into *Sinaphaenops*; both are very close to *S. mirabilissimus* and are living also in the Maolan karst. Then Uéno (2002) described three new species of this genus from caves in Sandu, Pingtang, and Ziyun counties. In the same paper, he treated *Thaumastaphaenops* as a junior synonym of *Sinaphaenops* and transferred *T. pulcherrimus* to *Sinaphaenops*. Deuve and Tian (2014) described a new subgenus based on *Sinaphaenops (Dongaphaenops) xuxiakei*, a species from westernmost Guizhou. They confirmed the valid status of *Thaumastaphaenops* as a subgenus of *Sinaphaenops*. To date, the genus *Sinaphaenops* is composed of eight species in three subgenera, all endemic to Guizhou Province, except *Sinaphaenops (s. str.) wangorum* Uéno et Ran, 1998, which also occurs in the Mulun karst of northern Guangxi Zhuang Autonomous Region (Tian et al., 2010). In recent years, several visits to Yueliang Dong, a limestone cave located in Mochong karst, Duyun, southern Guizhou, led to the discovery of two female trechine specimens that represent a new *Sinaphaenops (s. str.)* species.

The semi-aphaenopsian trechine genus *Luoxiaotrechus* was proposed by Tian and Yin (2013) for a species (*L. deuvei*

Figure 1. Habitus of *Sinaphaenops mochongensis* n. sp., holotype, female.

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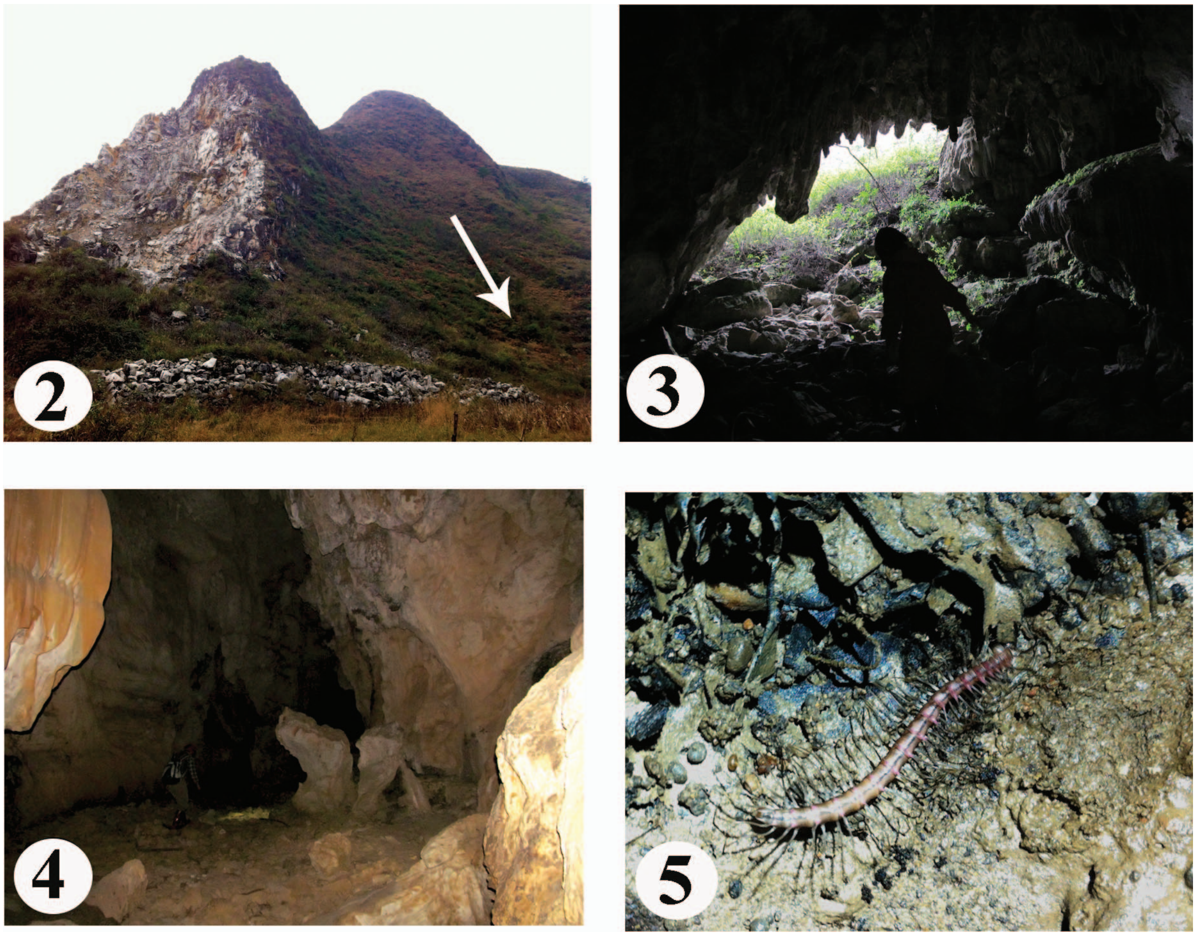
Table 1. Key to species of the genus *Sinaphaenops* Uéno et Wang, 1991.

Number	Characteristics
1	Right mandible tridentate, elytra without preapical pore, protibiae with a longitudinal sulcus (subgenus *Sinaphaenops* Uéno et Wang) 2
	Right mandible bidentate, elytra with preapical pore, protibiae without a longitudinal sulcus. 8
2	Body stout, neck constriction comparatively wide (Fig. 6). 3
	Body slender, neck constriction distinctly narrowed (Fig. 7) (*mirabilissimus* species group). 6
3	Three dorsal pores present on 3rd elytral stria (*trisetiger* species group) 4
	Two dorsal pores present on 3rd elytral stria (*orthogenys* species group). 5
4	1st pore of humeral group of the marginal umbilicate pores at behind the level between 2nd and 3rd . . . . *S. (S.) bidraconis* Uéno, 2002
	1st pore of humeral group of the marginal umbilicate pores at before the level of 2nd pore . . . . *S. (S.) trisetiger* Uéno, 2002
5	Body stouter, head with three pairs of supraorbital pores, neck constriction indistinct, hind pronotal angles rectangular. . . . . *S. (S.) mochingensis* n. sp.
	Body slenderer, head with two pairs of supraorbital pores, neck constriction distinct, hind pronotal angles rounded . . . . *S. (S.) orthogenys* Uéno, 2002
6	Posterior supraorbital seta absent (in Maolan karst) or present (in Mulun karst), aedeagus slenderer, but apical lobe broader. . . . . *S. (S.) wangrum* Uéno et Ran, 1998
	Posterior supraorbital seta present, aedeagus stouter, but apical lobe narrower 7
7	Head thinner, lateral margin of elytra visible from above . . . . *S. (S.) gracilior* Uéno et Ran, 1998
	Head thicker, lateral margin of elytra invisible from above. . . . . *S. (S.) mirabilissimus* Uéno et Wang, 1991
8	Pronotum with two pairs of latero-marginal setae, 1st pore of the humeral group of the marginal umbilicate series located much behind 3rd, both pores of the middle set markedly posteriad located, about 1/4 from apex of elytra, protarsi in male with only protarsomere 1 dilated, and inermous (subgenus *Dongaphaenops* Deuve et Tian). . . . . *S. (D.) xuxiakei* Deuve et Tian, 2014
	Pronotum without latero-marginal setae, 1st pore of the humeral group of the umbilicate marginal series located at same level with 3rd both pores of the middle set located at about middle of elytra, protarsi in male with both protarsomeres 1 and 2 dilated, and inermous (subgenus *Thaumastaphaenops* Magrini, Vanni et Zanon). . . . . *S. (T.) pulcherrimus* Magrini, Vanni et Zanon, 1997



Figures 2–5. Yueliang Dong, type locality of *Sinaphaenops mochongensis* n. sp. (2. the site of entrance, indicated by arrowhead; 3. entrance; 4. passage; 5. a dragon millipede *Desmoxytes* sp. in cave).

Tian et Yin, 2013) from a cave in the Youxian karst, eastern Hunan Province. Thanks to Dr. Ziwei Yin, School of Life Science, Shanghai Normal University, we received several interesting specimens that are very similar to *L. deuvei* but were collected from a cave in Lianhua County, western Jiangxi Province, about 20 km from Haitang Dong, the type locality of *L. deuvei*. Further investigations confirmed that it is a new species of *Luoxiaotrechus*, and the second subterranean trechine species known from Jiangxi Province, following *Jiangxiaphaenops longiceps* Uéno et Clarke, 2007.

MATERIAL AND METHODS

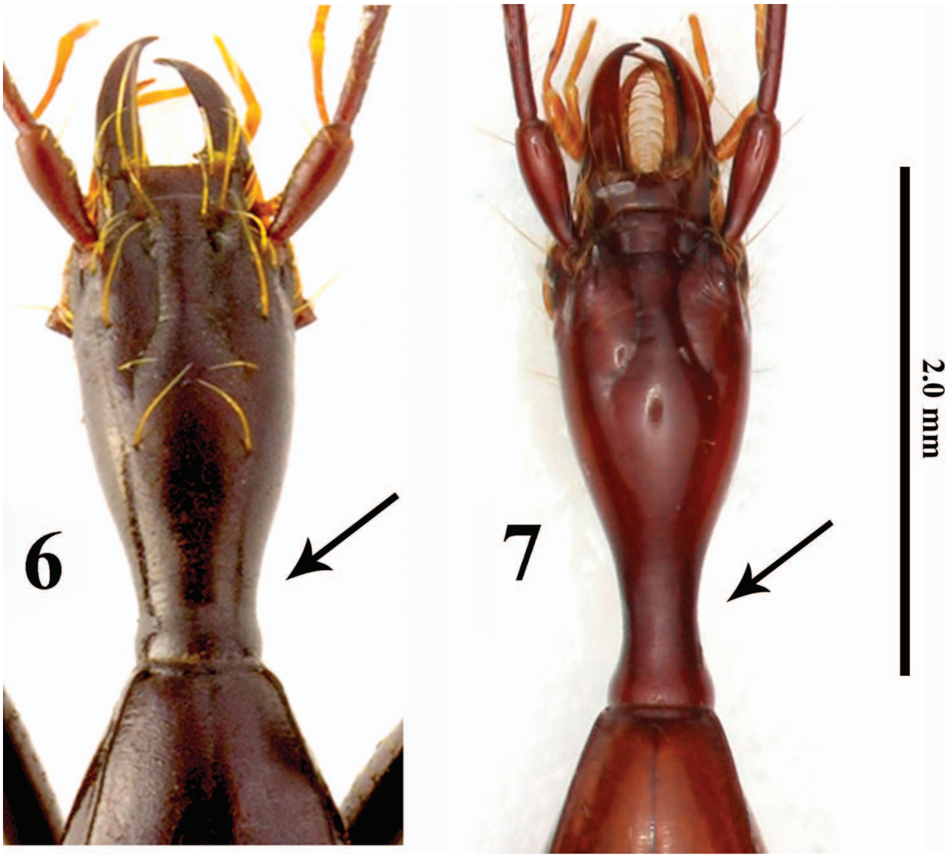
The beetles were collected by hand or by using an aspirator and kept in 55% ethanol before study. Dissections and observations were made under a Leica MZ75 microscope. Dissected genital pieces, including the median lobe and parameres of aedeagus, were glued on small paper cards and pinned under the specimen. Digital pictures were taken using a Canon EOS 40D camera, and then processed by means of Adobe Photoshop CS5 software. Length of body is measured from apex of right mandible (in opened position) to elytral apex.

Abbreviations of other measurements used in the text are:

HLm:	length of head including mandibles, from apex of right mandible to occipital suture;
HLl:	length of head excluding mandibles, from front of labrum to occipital suture;
HW:	maximum width of head;
PL:	length of pronotum, along the median line;
PW:	maximum width of pronotum;
PtW:	maximum width of prothorax;
PfW:	width of pronotum at front;
PbW:	width of pronotum at base;
EL:	length of elytra, from base of scutellum to elytral apex;
EW:	maximum width of combined elytra.

Abbreviations for the specimens' depositories are:

IOZ:	National Museum of Zoology, Institute of Zoology, Chinese Academy of Sciences, Beijing;
MNHN:	Muséum National d'Histoire Naturelle, Paris;
SCAU:	South China Agricultural University, Guangzhou;
SHNU:	Shanghai Normal University, Shanghai;
ZUBM:	Biological Museum of Zhongshan University, Guangzhou.



Figures 6–7. Head of *Sinaphaenops* species (6. *S. mochongensis* n. sp.; 7. *S. wangorum*).

TAXONOMY

SINAPHAENOPS MOCHONGENSIS N. SP.

Holotype. female, Guizhou: Qiannan: Duyun: Mochong: Yueliang Dong, 26°02′36.24″ N, 106°26′39.46″ E, 901 m, 29-I-2011, leg. Mingyi Tian; paratype: 1 female, same cave, 25-VIII-2014, leg. Mingyi Tian. All are deposited in SCAU.

Description. Length: 8.1–8.2 mm; width: 1.1–1.2 mm. Habitus as in Figure 1.

Large sized, dark brown in holotype, except elytra, tarsi, and palps which are pale or reddish brown; reddish brown in the paratype; body moderately shiny, impunctate; head with a few hairs on genae, glabrous on frons and vertex, prothorax and elytra glabrous; microsculptural engraved meshes transverse striate on head and pronotum, moderately transverse on elytra.

Head much longer than wide, HLm/HW=2.91, HLl/HW =2.08; slightly stouter than in *S. orthogenys* Uéno, 2002, frontal impressions longer, ending point closer to posterior supraorbital pores than to anterior ones; three pairs of supraorbital setae present, the median pair slightly shorter than others in holotype, but as long as in the paratype, and close to anterior ones; widest at about middle, neck constriction indistinct, a little more than half width of head, without hourglass-shaped part; labial suture well defined, submentum 10-setose, mentum bisetose; ligula adnated with paraglossae, with two long setae apically, and 10-setose subapically; apex slightly protruding; sparsely setose on ventral head, pair of suborbital setae present at a little before the constricted part; antennae slightly shorter than in *S. orthogenys*.

Prothorax much shorter than head, PL/HLm=0.61, PL/HLl=0.86; widest at a little behind middle, without lateral marginal setae; propleura distinctly expanded and visible from above, PtW/PW=1.18; pronotum slightly wider than head, PW/HW=1.17; prothorax strongly wider than head, PtW/HW=1.38; base distinctly wider than front, PbW/PfW=1.54.

Elytra slenderer than in *S. orthogenys*, elongate ovate, quite similar in *S. bidraconis* Uéno, 2002, but humeral part more pronounced; elytra as long as head (including mandibles) plus pronotum, EL/(HLl+PL)=1.22; much wider than prothorax, EW/PtW=1.88; much longer than wide, EL/EW=1.82; widest at about middle, and nearly parallel-sided; two dorsal pores present on the site of third stria, at about 1/5 and 2/5 of elytra from base respectively; preapical pore absent; chaetotaxal pattern of the marginal umbilicate pores similar in *S. orthogenys*, but first pore of the humeral group just behind the level of anterior dorsal pore, and fourth pore behind level of posterior dorsal pore.

Figure 8. Habitus of *Luoxiaotrechus yini* n. sp., paratype, male.

Prosternum sparsely setose; meso- and metasterna unsetose, abdominal ventrites setose, setae shorter than those on prosterna; epipleura smooth and unsetose; ventrite VII 6-setose, each of IV-VI bisetose.

Male. Unknown.

Remarks. *Sinaphaenops mochongensis* n. sp. is close to *S. orthogenys* from the Sandu karst, southern Guizhou, but easily distinguished from the latter by: three pairs of supraorbital pores present on head (two pairs in *S. orthogenys*); neck constriction wider (narrower in *S. orthogenys*); fore body stouter, but with slenderer elytra than in *S. orthogenys*. A key to genus *Sinaphaenops* is shown in Table 1.

Etymology. Refers to Mochong karst, the type locality of this new species.

Distribution. China (southern Guizhou). Known only from the type locality, a limestone cave called Yueliang (=*moon*, in Chinese) Dong (Figs. 2–5). The cave is 157 m long, 315 m wide, and 68 m high, with a seasonal underground river inside (Chen, 1997). The beetles were found and collected on wall of the cave in an area about 6080 m from the entrance. The first author has visited Yueliang Dong four times since 2010, but failed to find more beetles. Nobody knows what will happen to Yueliang Dong and its fauna in near future, because several caves in the Mochong karst have been destroyed by a large cement factory.

Luoxiaotrechus yini n. sp.

Holotype. male, China: Jiangxi: Pingxiang: Lianghua: Gaotan: cave Shuilian Dong, 27°25′00″ N, 113°58′23″ E, 520 m, 17-X-2013, leg. Mingyi Tian, Weixin Liu, Haomin Yin, and Yanjing Wang, in SCAU. Paratypes: 11 males, 3 females, ibid, in SCAU; 2 males, same cave, 24-VII-2013, leg. Ziwei Yin, Xiaobin Song and Yiming Yu; 3 males, 3 females, same cave, 29-X-2013, leg. Zhong Peng and Jiawei Shen, in SCAU, SHNU, IOZB, ZUBM and MNHN, respectively.

Description. Length: 6.3–7.8 mm (mean 6.9 mm), width: 1.5–1.9 mm (mean 1.7 mm). Habitus as in Figure 8. Very similar to *Luoxiaotrechus deuvei* Tian et Yin, 2013.

Medium sized, light dark brown, but lighter in two individuals (immature); head glabrous and smooth on frons and vertex, pronotum with dense and long setae on disc, elytra covered with short and sparse setae, ventral side of head and thorax sparsely setose.

Head much longer than wide, HLm/HW=1.86–1.88, HLl/HW=1.28–1.38; labial suture developed, mentum bisetose, submentum 4 to 7-setose, mental tooth bifid.

Prothorax much shorter than head (including mandibles), PL/HLm=0.72–0.75, or almost as long as head (excluding mandibles), PL/HLl=0.98–1.10; propleura invisible from above; pronotum slightly wider than head, PW/HW=1.17–1.22; base distinctly narrower than front, PbW/PfW=0.77–0.82.

Elytra longer than head (including mandibles) plus pronotum, EL/(HLm+PL)=1.17; EL/(HLl+PL)=1.21–1.27; much wider than pronotum, EW/PtW=1.67–1.77; much longer than wide, EL/EW=1.84–2.00; chaetotaxal pattern similar in *L. deuvei*.

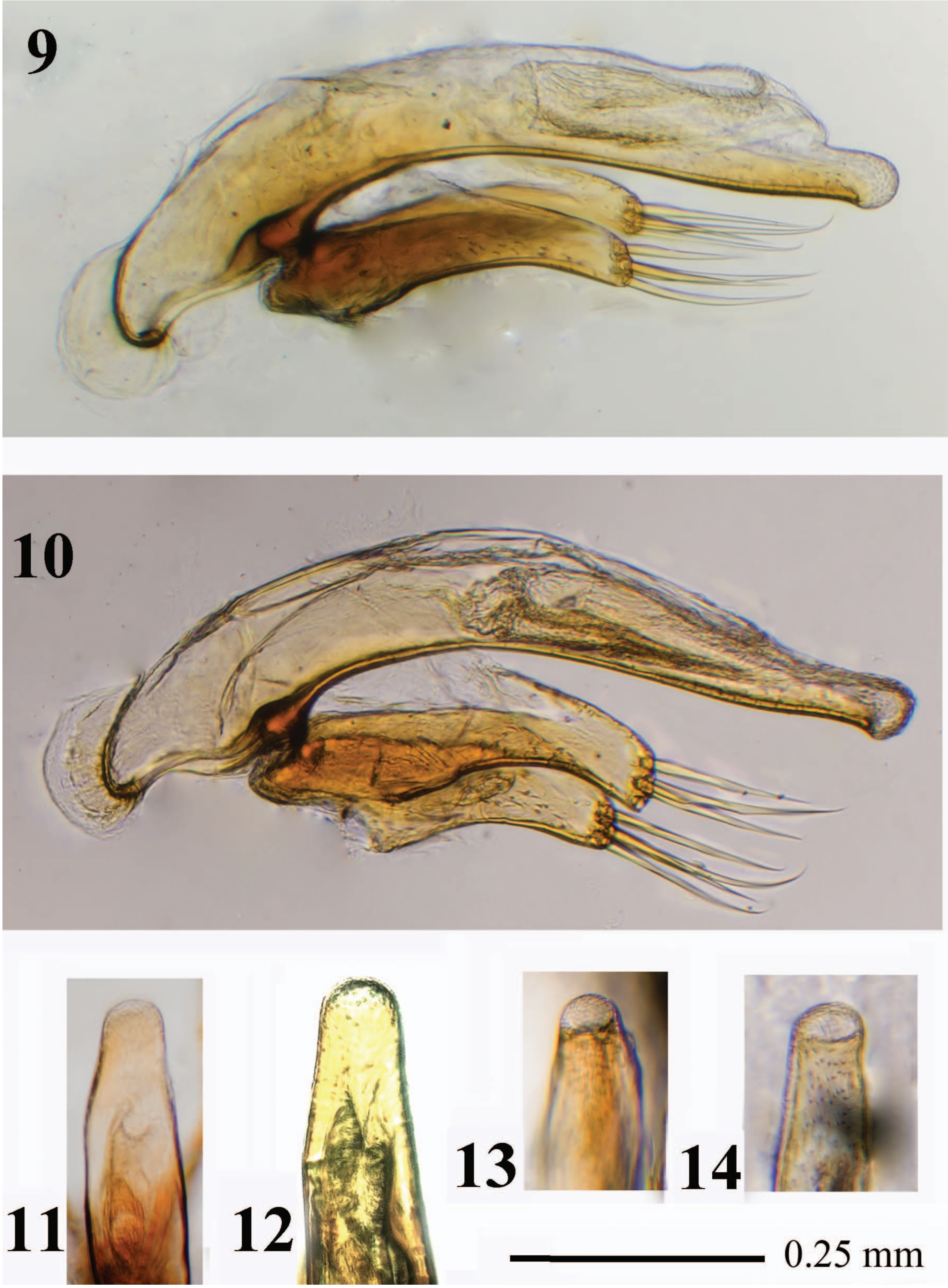
Male genitalia (Figs. 9, 11, 13): Similar in *L. deuvei* (Figs. 10, 12, 14), but shorter, and slightly stouter than the latter, with apical lamella a little narrower in dorsal view and smaller opening hole in ventral view; right parameres with four long setae at apex, while the left with three to four.

Remarks. Allied to *L. deuvei* Tian et Yin, 2013, but recognized by the pronotum slightly narrower, less expanded laterally, slightly sinuate before hind angles (strongly sinuate in *L. deuvei*), base almost straight (distinctly convex medially in *L. deuvei*), hind angles sharper; and male genitalia shorter.

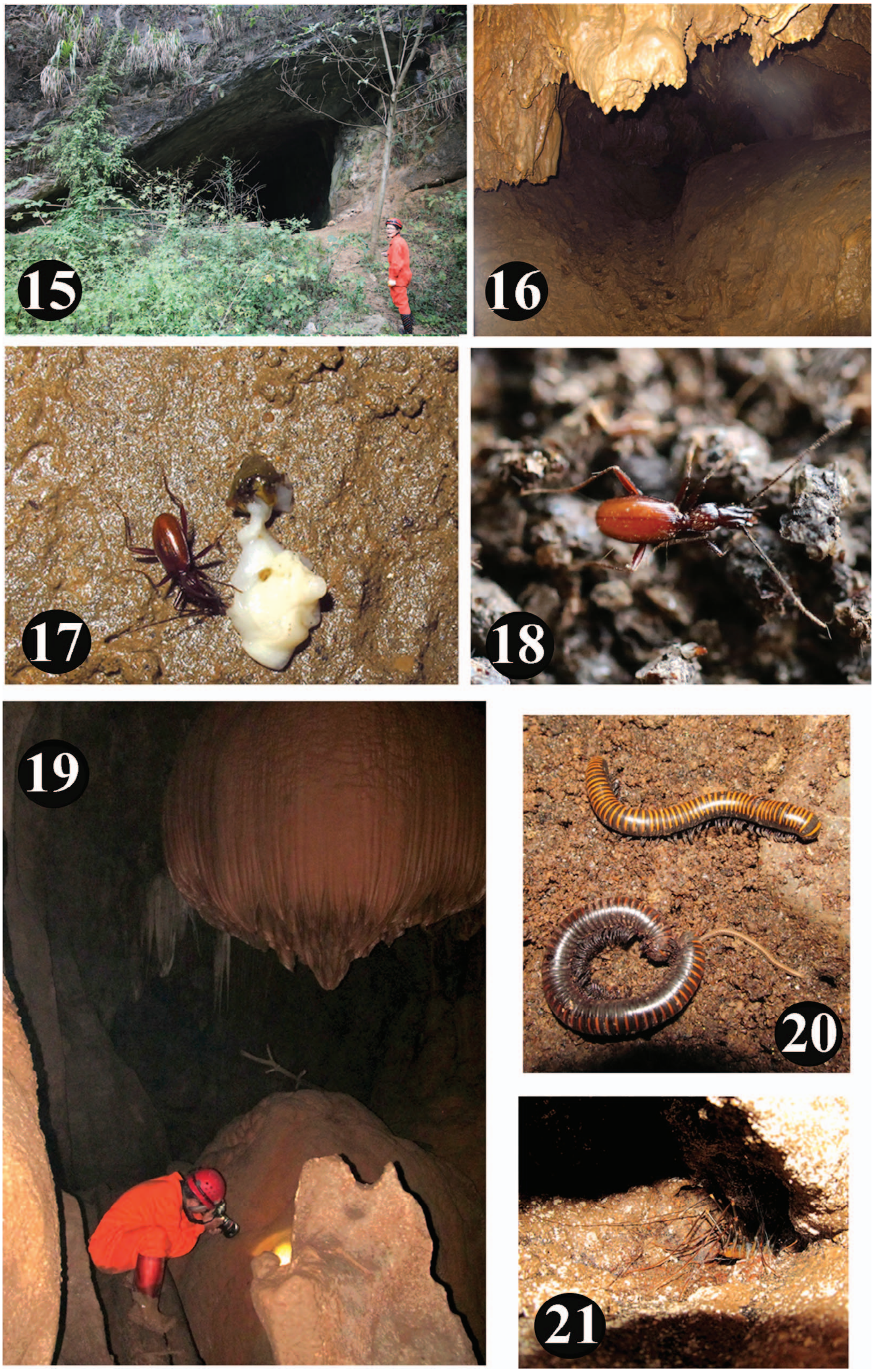
Etymology. Dedicated to Dr. Ziwei Yin, Shanghai Normal University, a specialist of Pselaphidae.

Distribution. China (Jiangxi). It is the first record of a Luoxiaotrechus occurring in Jiangxi Province, eastern China.

Shuilian Dong, the type locality, is a beautiful cave and contains interesting fauna (Figs. 15–21). According to the native people it is several kilometers long, but exact length remains unknown. It opens on the side of a mountain near



Figures 9–14. Male genitalia of *Luoxiaotrechus* species (9, 11, and 13: *L. yini* n. sp.; 10, 12, and 14: *L. deuvei*; 9, 10: median lobe and parameres, lateral view; 11, 12: apical lobe, dorsal view; 13, 14: apical lobe, ventral view).



Figures 15–22. Shuilian Dong, type locality of *Luoxiaotrechus yini* n. sp. (15. entrance; 16 and 19: passage and beautiful deposits, showing where the beetles were collected; 17, 18: beetles of *L. yini* n. sp. in cave. 20: millipedes in cave; 21: a *Scutigera* centipede in the cave.

the village Gaotan, with a quite large entrance that is about 5 m high. It is muddy inside the cave, and the floor is covered with a lot of cattle dung and bat guano. There is an underground river inside the cave. The trechine beetles were collected on the ground, under stones, or on the wall, in the dark zone at about 50 to 80 m from the entrance.

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M. Peñuela-Salgado and J. Pérez-Torres – Environmental and spatial characteristics that affect roost use by Seba's short-tailed bat (*Carollia Perspicillata*) in a Colombian cave. *Journal of Cave and Karst Studies*, v. 77, no. 3, p. 160–164, DOI: 10.4311/2015LSC0105

ENVIRONMENTAL AND SPATIAL CHARACTERISTICS THAT AFFECT ROOST USE BY SEBA'S SHORT-TAILED BAT (*CAROLLIA PERSPICILLATA*) IN A COLOMBIAN CAVE

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Abstract: Bats use roost sites to reduce energy costs, for reproduction, and to protect themselves from harsh weather conditions and predators. The roosts differ in the environmental and spatial features that may be used by bats during roost selection, but the relationship between the types of roosts and the physical and spatial characteristics remain poorly understood. We studied the characteristics of roost sites used by *Carollia perspicillata* in a cave with an area of approximately 600 m^2 located in a remnant of tropical dry forest in Colombia.

A total of 156 roost sites were sampled. Temperature, relative humidity, distance of the roost site from the cave entrance, relative location within the cave, the roost-site height, and the roost-site area were correlated with each other by canonical correlation analysis. A total of seven types of roost were identified, Cavity, Protrusion, Crack-with-Protrusions, Cavity-with-Protrusions, Cavity-with-Crack-and-Protrusions, Stalactite, and Rock-Structure. Protrusions had the highest frequency (64) and was preferred, along with Crack-with-Protrusions (32), over Rocky-Structure (9) and Stalactite (4), and these were not positively related to the measured characteristics. The Cavity-with-Protrusions, Cavity-with-Crack-and-Protrusions, and Cavity types were not used by *Carollia perspicillata*. The results indicate that the selection of the roost sites is addressed by the tendency of lower values, environmental characteristics, and spatial measures.

INTRODUCTION

Roost site is one of the most important limiting factors for bats, as they spend more than half their lives in roost-based activities (O'Keefe et al., 2009). Roosting sites may be in caves, mines, rocky crevices, cavities in stems, among leaves, and in human constructions. Bats search for roosts that allow them to protect themselves from climate variations or predators (Fleming et al., 1998) and that also favor their reproduction and increase their survival and that of their offspring (Kurta, 1985; O'Keefe et al., 2009).

The environmental and spatial characteristics of the roosts affect social structure, residence time, energy balance, and intensity of intra- and interspecific relationships (Sedgeley and O'Donnell, 1999). Indeed, environmental characteristics directly affect metabolic rate and energy balance (O'Donnell and Sedgeley, 2006), whereas spatial features can influence parasitism (Altringham, 1988), predation (O'Keefe et al., 2009), competition (Dechman and Kerth, 2008), and the risk of offspring falling from the roost during rearing (Sedgeley, 2001). So understanding the characteristics that bats use during roost-site selection allows an improved understanding of the functional responses to changes in habitat and conditions and their importance in the conservation of bat populations.

Caves are the most common roost sites used by bats (O'Keefe et al., 2009). Several authors have stressed the importance of caves for bat conservation at the regional and local levels (Muñoz-Saba et al., 2007). Caves can shelter numerous bat species whose abundance varies from a few tens to thousands of individuals (Arita, 1993). In temperate zones, many species of insectivorous bats use the caves as permanent roost sites (McCracken and Wilkinson, 2000). In tropical regions, fruit bats frequently roost in caves (Kunz, 1982) such as Olhos d'Água Cave in Brazil and Las Vegas Cave in México, both containing thirteen species, the highest number known (Medellín and López-Forment, 1985; Trajano and Gimenez 1998). Colombia has an extensive system of underground environments of which an estimated 25% have been mapped (Muñoz-Saba et al., 2007). Seba's short-tailed bat *Carollia perspicillata* and the common vampire bat *Desmodus rotundus* are the species most frequently recorded using caves in different regions of the country (Muñoz-Saba et al., 2007), and Macaregua Cave, our study site, is the richest, with ten species found (Pérez-Torres et al., 2015).

Roosting sites like caves may be selected when they provide a suitable combination of characteristics for roosting (Kunz, 1982; Graham, 1988; Arita, 1996; Aguirre et al., 2003). For some species of bats, roost selection criteria can depend on sex, maturity status, and pregnancy status of females (Sedgeley and O'Donnell, 2004; Willis et al., 2006;

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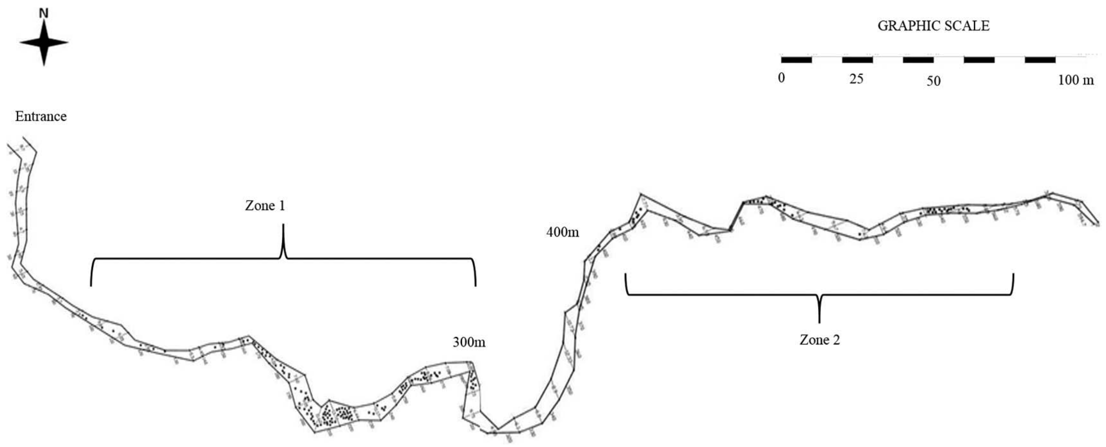

Figure 1. Macaregua cave, Curití, Colombia.

Perry et al., 2007). However, it remains unclear which features are used to select a specific type of roost in underground environments. In this study, we attempt to relate physical (temperature, relative humidity) and spatial characteristics (cave entrance distance, height of the roost, relative location, total area) of roost sites used by *Carollia perspicillata* with types of roosts present in a karst cave in a tropical dry forest ecosystem in Colombia.

Materials and Methods

The Macaregua Cave (6°39′36.2″ N and 73°6′32.3″ W) (Fig. 1) is located in Las Vueltas, Curití municipality, Department of Santander in the northeastern region of Colombia at an elevation of 1566 m. This cave, not altered by man, has two galleries, one dry (80 m) and one wet (approximately 600 m), at least six chambers, and a watercourse for the first 300 m (Pérez-Torres et al., 2015). The ceiling heights range from 0.22 to 7.3 m, and the passage widths range from 0.6 to 13.72 m. The cave has ancient lithostratigraphic units that appear similar to stalactites, but with a metamorphic-igneous gneiss origin; migmatites, schists, and small granodiorite intrutions are also present (Mejia, 2008).

This study was performed in the dry season, between January 16, 2011 and February 8, 2011, which is the reproductive period of this species of bat. Throughout the parts of the 600 m of wet gallery used by *Carollia perspicillata* 156 sites were cataloged. Each roost site was classified according to the structure of the roof of the cave and taking into account the criteria by Unesco and FAO (1972). Roosts were classified into one of seven types, Cavity, Protrusion, Crack-with-Protrusions, Cavity-with-Protrusions, Cavity-with-Crack-and-Protrusions, Stalactite, and Rocky-Structure, the last of which includes those not in any other class, and temperature and humidity measurements were taken at each visit.

At each roost site the roof temperature (62 ± 1 °C) was measured using a Fluke infrared thermometer 62 with laser pointer, and relative humidity was measured below each roost site using a thermohygrometer Hanna HI 93640 (± 2%) in both the morning (0700–1000 h) and afternoon (1500–1800 h); readings were then averaged to produce daily mean values for each type of roost (Table 1). Light was not considered in the study because the cave from 100 m is in complete darkness.

At each roost site roost distance to the entrance of the cave was measured using a decameter. Relative location of the roost site within the width of the passage, as the distance from the right wall divided by the width, and the height of the roost site from the cave floor were measured directly. The area of the passage at each roost site was estimated by photographic analysis using ImageJ software (Table 1).

Roost types associated with physical location and environmental and spatial characteristics were subjected to canonical correspondence analysis using CANOCO v4.5 (Braak and Smilauer, 2002). Monte Carlo procedures were used to determine if there was a linear relationship between the variables and types of roost (index $p < 0.05$ = Rho).We evaluated which roost sites were preferred, used, or avoided. For this, the software used was HaviStat v2.2. The program applied several indexes based on the observed frequency and the potential use (area available) of the types of roost found. (Montenegro et al., 2014).

Results

The average monthly temperature of the cave varies between 23.2 and 27.3 °C, whereas relative humidity varies between 84.5 and 97.2%. *Carollia perspicillata* individuals are found along the wet passage except between 300 and 400 m from the cave entrance, which is an area in which insectivorous bats (*Mormoops megalophylla* and *Natalus tumidirostris*) roost. This section divides the wet section occupied by *C. perspicillata* into two zones, zone 1 from 100 to 300 meters and zone 2 from 400 to 530 m.

Table 1. Types of available roosts in the part of Macaregua Cave where *Carollia perspicillata* bats roost and average environmental and spacial parameters.

	Number			Environmental		Roost Details		
Type of Roost	Total	Zone 1	Zone 2	Temperature, °C	Relative Humidity, %	Cave Entrance Distance to Roost, m	Height of Roost Above Cave Floor, m	Cross-Sectional Area, m^2
Protrusion	64	47	17	25.64 ± 0.86	92.00 ± 3.63	308.22 ± 134.19	2.99 ± 0.63	0.46 ± 0.19
Crack with Protrusions	32	19	13	25.99 ± 0.99	93.03 ± 2.87	338.47 ± 132.27	3.14 ± 0.57	0.46 ± 0.15
Cavity with Protrusions	25	19	6	25.67 ± 1.18	95.05 ± 3.62	276.29 ± 119.23	3.57 ± 0.59	0.45 ± 0.20
Cavity with Cracks and Protrusions	16	14	2	25.57 ± 0.50	93.08 ± 0.83	247.33 ± 87.95	4.01 ± 0.44	0.36 ± 0.13
Rocky Structure	9	9	0	24.70 ± 0.73	88.43 ± 3.72	200.50 ± 42.18	2.79 ± 0.78	0.24 ± 0.19
Cavity	6	6	0	24.58 ± 0.73	88.34 ± 3.51	176.46 ± 52.78	3.30 ± 0.70	0.26 ± 0.26
Stalactite	4	4	0	24.52 ± 0.82	90.05 ± 0.82	142.84 ± 34.99	2.24 ± 0.29	0.06 ± 0.02

Seven types of roost (Fig. 2) were located in those zones of the cave. Between 80 and 300 m from the cave entrance (zone 1) 118 roost sites were identified, whereas between 400 and 530 m (zone 2) 38 roost sites were identified. The frequency of different types of roost differed significantly within the cave environment ($\chi^2 = 119.2$, df = 6, $n = 156$, $p < 0.001$). Protrusion ($n = 64$) and Crack-with-Protrusions ($n = 32$) were the most frequent types of roost (Table 1). Rock-Structure, Cavity, and Stalactite roost sites were present only in zone 1. Other types of roost were found throughout the cave.

The difference between the temperature data taken in the morning and in the afternoon was not significant ($W = -1.6$; $n = 156$; $p = 0.112$), but between the morning

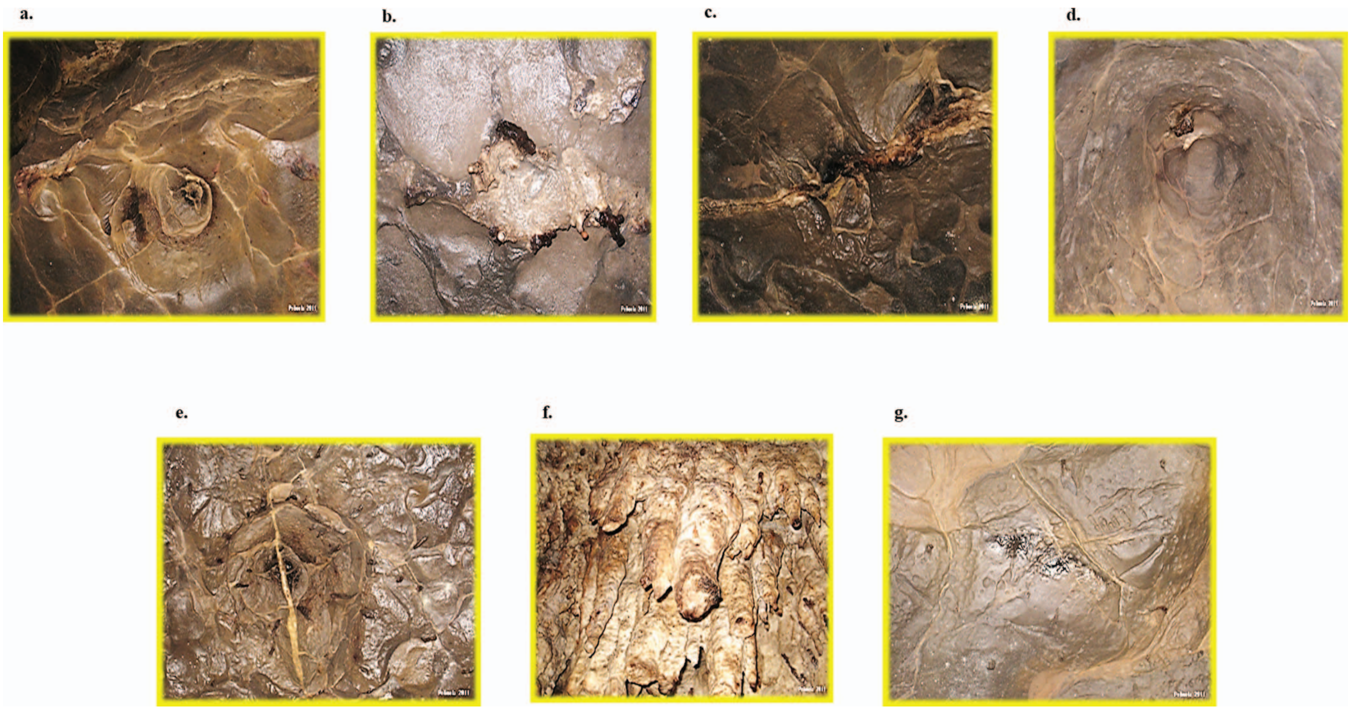

Figure 2. Examples of types of roost found in the cave: a. Cavity; b. Protrusion; c. Crack-with-Protrusion; d. Cavity-with-Protrusions; e. Cavity-with-Cracks-and-Protrusions; f. Stalactite; g. Rocky-Structure.

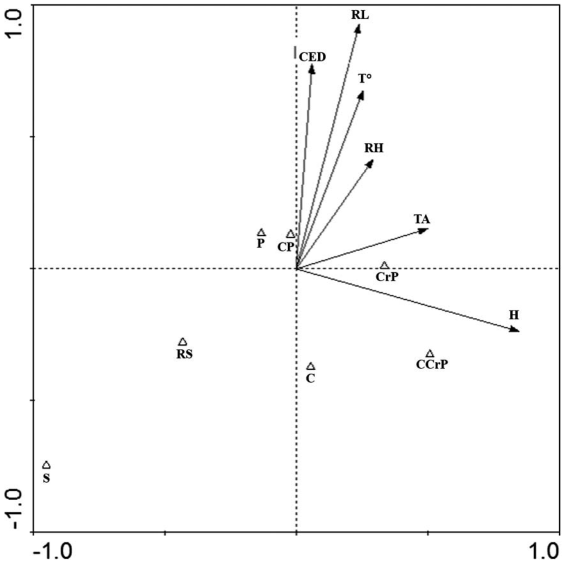

Figure 3. Canonical correlation analysis ordination of the types of roost with environmental and spatial characteristics. Triangles represent the types of roost: Cavity (C), Protrusion (P), Crack-with-Protrusions (CrP), Cavity-with-Protrusions (CP), Cavity-with-Cracks-and-Protrusions (CCrP), Stalactite (S), and Rock structure (RS). Vectors represent the environmental and spatial variables of the roosts: Temperature (T°), relative humidity (RH), cave entrance distance from roost (CED), height of the roost above the floor (H), relative location (fraction of passage width that is distance from right wall, RL), and total passage area (TA) (Table 1). The orientation of the arrow and the size represents the association, direction, and strength between environmental and spatial variables and ordination axes. The first and second axis explains 62% and 45.3%, respectively, and the total value of the organization itself was 0.768.

and afternoon relative-humidity data significant differences were found ($W = -3.3$; $n = 156$; $p = 0.001$).

Protrusion was the most common type of roost observed (0.41), with Cracks-with-Protrusions next (0.21); Rock-Structure (0.07) and Stalactite (0.04). Stalactite was the type of roost most used (Manly and Thomas, 1993: $10.6 > 1$ prefers) and Rock Structure was the second one (Manly and Thomas, 1993: $2.4 >$ prefers). The Cavity-with-Protrusions (0.16), Cavity-with-Crack-and-Protrusions (0.11) and Cavity (0.04) were not used by *Carollia perspicillata.*

Environmental (temperature, relative humidity) and spatial (distance to cave entrance, roost height, roost relative location, available area) variables were associated with the different types of roost (Table 1). Cumulative variance in the types of roost was: axis 1: 50.1%, axis 2: 76.8%, axis 3: 90.5%, axis 4: 97.3%, respectively (Fig. 3). The large values in the first two axes indicate that the measured variables are significantly explained by the types of roost. The rock structure was inversely related to the measured characteristics, so the roosts with low or medium values are those grouped under that type of roost.

Discussion

Roost selection by *Carollia perspicillata* was significantly correlated with temperature and Stalactite was the type of roost site most preferred followed by Rock Structure. Species such as *Sturnira lilium*, *C. perspicillata,* and *Artibeus lituratus* select roosts with high altitude in the cave passage (Ortiz -Ramirez et al., 2006). Temperature was a major factor for selection of the types of roost in our study (Table 1), as observed previously (Snoyman and Brown, 2011). In other studies, the temperature is the most important aspect (Vonhof and Barclay, 1996; Fleming et al., 1998; Avila-Flores and Medellin, 2004), and in some others,it is not so important (Ortiz-Ramirez et al., 2006).

Macaregua is a warm cave, and its temperature range (23.25 to 27.28 °C) coincided with previous findings that indicate that Emballonuridae, Mormoopidae, Phyllostomidae, and Natalidae bat families prefer warm roosting sites (Avila-Flores and Medellin, 2004; Broders and Forbes, 2004). Selection of warmer roosting sites is important because it reduces the need to invest energy resources in body-temperature homeostasis while sleeping (McNab, 1982).

Spatial characteristics of roost sites can influence heat dissipation and facilitate bat-flight maneuvers in the case of disturbance (Kurta, 1985). The disturbance associated with the entry and exit of the cave (Clark et al., 1996) and the space within the roost site limits the number of bats that use it. Roost structure can also affect social organization (Kunz, 1982) and the formation of groups for thermoregulation (Kurta, 1985), whereas microclimate features of roost sites (Bell et al., 1986) and microstructural heterogeneity favors the establishment of multi-species bat assemblages in caves (Brunet and Medellin, 2001).

Future studies could examine the role of gas concentration on roost site selection, as it is clear that temperature and humidity may vary with changes in the local gas concentrations, particularly the accumulation of ammonia in the atmosphere (McFarlane et al., 1995). This is of particular relevance in caves where changes in the gaseous composition of the atmosphere can correlate with local increases in temperature of >3 °C (Baker and Genty, 1998). The role of reproductive status and sex-based differences in roost-site selection are also likely to provide valuable information for promoting bat conservation programs involving cave habitats in the Neotropical region.

Conclusion

Roost selection by *Carollia perspicillata* involves a combination of physical, spatial, and environmental variables. Stalactite was the type of roost site preferred, and temperature was the major environmental factor.

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J. Lenart – Morphological patterns of crevice-type caves in sedimentary rocks of the Outer Western Carpathians (Czech Republic).
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MORPHOLOGICAL PATTERNS OF CREVICE-TYPE CAVES IN SEDIMENTARY ROCKS OF THE OUTER WESTERN CARPATHIANS (CZECH REPUBLIC)

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Abstract: I evaluate crevice-type caves in the Outer Western Carpathians from the morphological point of view at different scales, from the overall arrangement of cave passages within the massif, to their shape and finally to the micro-morphological features of their walls and ceilings, using examples from the Czech part of the Carpathians. The cave passages are typically parallel to and follow the strike of the slope, but the passages of relatively large caves are typically fan-like arranged. Vertically, the passages are organized at particular levels governed by lithology. They can be staircase-shifted with each other. The gradual opening of the uppermost level may lead to deformation of the surface terrain, however, this terrain can be entirely flat, which gives evidence of the gravitational widening of the caves from the inside of the massif. In cross-section, the passages are A, V, or H letter shaped, according to the type of gravitational movement leading to their development. The various micro-morphological features that originate from sedimentology, tectonics, gravitational processes, and mechanical and chemical weathering sometimes mark the exposed walls and ceilings.

INTRODUCTION

Crevice-type caves (CTCs) are one of many types of pseudokarst caves (Bella and Gaál, 2013). They were defined for the first time by Vítek (1983). These caves are formed during the evolution of slope deformations and consist of a system of accessible gravitationally widened joints that form the cave passages. It means that these caves are formed as products of gravitational processes without any role of karstification during their evolution. For comparison, Gutiérrez et al. (2014) summarizes the role of the karst processes before and during the dilation and propagation of joints and clefts in soluble rocks.

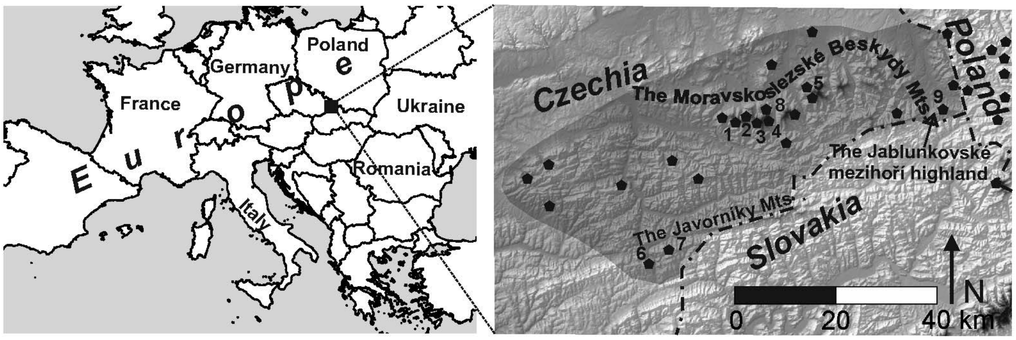
The development of the caves is described and widely discussed in Margielewski and Urban (2003), Pánek et al. (2010) and Lenart et al. (2014). CTCs occur in various rock types, such as clastic rocks (Winkelhöfer, 1975; Vítek, 1983; Wagner et al., 1990; Pánek et al., 2010; Lenart et al., 2014), limestones (Pánek et al., 2009), chalk (Rodet, 1983), basalts (Gaál and Gaál, 1995), granites (Finlayson, 1986), gneisses (Demek and Kopecký, 1999), and others. Although CTCs occur worldwide, there is a lack of investigations evaluating the morphology of these caves. The caves commonly accompany landslides of various types: topples, translational, and rotational slides and lateral spreads (according to Varnes, 1978), both deep-seated (according to Hutchinson, 1988) and shallow (according to Margielewski, 2009) types.

CTCs are very often developed in incoherent sedimentary flysch deposits, e.g., in sandstones and conglomerates with intercalations of shales such as siltstones and claystones (Wagner et al., 1990; Margielewski and Urban, 2003; Urban and Margielewski, 2013; Lenart et al., 2014).

This paper evaluates and reviews CTCs from the morphological point of view, based on examples from the flysch belt of the Czech portion of the Western Flysch Carpathians (Fig. 1). I extend the investigations of Margielewski and Urban (2003), Pánek et al. (2010), Urban and Margielewski (2013), and Lenart et al. (2014). I analyze morphological peculiarities of CTCs within gravitationally affected flysch slopes. Using the detailed morphological and structural data from CTCs in the Czech section of the Outer Flysch Carpathians, this study aims to describe and evaluate morphological features and patterns of CTCs at four different scales: (i) overall horizontal arrangement of the passages, (ii) overall vertical arrangement of the passages, (iii) morphology of the particular passages, and (iv) micro-morphology of the walls and ceilings of the caves.

STUDY AREA

Crevice-type caves were investigated in the Czech portion of the Outer Flysch Carpathians (Fig. 1), which is strongly affected by mass movements of various types. The study area has local relief up to 600 m and is affected by numerous deep-seated gravitational slope deformations, mainly by structurally predisposed translational and rotational landslides (Krejčí et al., 2004; Pánek et al., 2011). The Moravskoslezské Beskydy mountains (highest peak Lysá hora, 1323 m a.s.l.) and the Javorníky mountains (Velký Javorník: 1071 m a.s.l.) are the most distinct geomorphic units of that area.



CTCs	overall	small	medium	long
count	109	92	11	6
percentage	100	84	10	6

	distribution of the crevice-type caves			
CTCs	overall	small	medium	long
identified caves	56	41	9	6
parallel	44	31	7	6
% of parallel	78	76	78	100
non-parallel	12	10	2	0
% of non-parallel	22	24	22	0
not identified	53	51	2	0

Figure 1. Studied area in the Czech section of Outer Flysch Carpathians (shaded). Black dots indicate places with crevice-type cave occurrence, with the longest and the deepest caves labeled: 1, Salajka Cave; 2, Cyrilka Cave; 3, Čertova Díra Cave; 4, Kněhyňská Jeskyně Cave; 5, Velká Ondrášova Jeskyně Cave; 6, Naděje Cave. Other caves mentioned in the text: 7, Velryba Cave; 8, Kyklop Cave; 9, Na Girové I Cave. Tables give total number of caves in each size category and numbers and percentages of caves arranged parallel and non-parallel to the strike of the slope.

The Outer Flysch Carpathians are composed primarily of Mesozoic (Late Jurassic) to Paleogene/Neogene (early Miocene) rocks. Several nappes were folded and thrusted onto the foredeep in a northerly direction during the lower and middle Miocene alpine phases. The strata predominantly consist of thick-bedded, medium-grained sandstones or coarse-grained sandstones to conglomerates with content of mica, glauconite, and calcareous cement, frequently interrupted by thin-bedded intercalations of shales, such as claystones and siltstones, with a predominant illite and kaolinite content. During the Miocene, the flysch massif was strongly disrupted by joints and faults trending in several directions (E-W, N-S, NW-SE, NE-SW and WNW-ESE). The most important is the NW-SE direction, which corresponds to the direction of regional faults. All geological characteristics are taken from Menčík et al. (1983), Pánek and Duras (2002), Krejčí et al. (2002), Margielewski and Urban (2003), Krejčí et al. (2004), and Pánek et al. (2011).

Mountain ridges typically are monoclinal ridges and cuestas, structural ridges formed by more resistant strata, and anticlinal and synclinal ridges. The northern slopes are often steep and correspond to the frontal parts of beds, whereas the southern slopes are long and gentle, following the dip, ranging from SW to SE, of the bedding planes (e.g., Menčík et al., 1983; Krejčí et al., 2004).

CTCs are considered as important geomorphic elements of gravitationally deformed slopes. To date, more than one hundred CTCs are known in the study area. Figure 1 shows the distribution of the most remarkable examples localized in the outer part of the Czech Flysch Carpathians. The majority of the 109 investigated CTCs (84 %) are categorized as small caves up to 30 m in length, and 10 % are categorized as medium caves, with length between 30 and 100 m and up to 30 m in depth. The remaining part (6 %) is represented by long caves, longer than 100 m or deeper than 30 m. The longest one, the Cyrilka Cave, reaches 535 m and the deepest is the Kněhyňská jeskyně, with the bottom at 57.5 m of depth (Wagner et al., 1990).

METHODS

To study the overall horizontal arrangement of the passages, I used the older maps of crevice-type caves published mostly by Wagner et al. (1990). These maps were updated and revised using a DISTO Leica A3 laser rangefinder improved with a DistoX component (Heeb, 2008). Maps were compared with surface topography that was measured to high precision with a geodetic total station (Lenart et al., 2014). Overall, the vertical arrangements of the passages were identified on the basis of cave cross-sections. The morphological character of each particular passage was determined on the basis of its detailed cross-sections combined with the dip directions of walls and ceilings. In terms of the strike characteristics of the sedimentary beds, the joints and faults were classified as L (longitudinal), T (transversal), and D1+D2 (two diagonal systems), a classification according to Mastella et al., 1997, based on the relation between the dip direction of rock layer and the direction of joint set. The detailed micro-morphological features were observed directly.

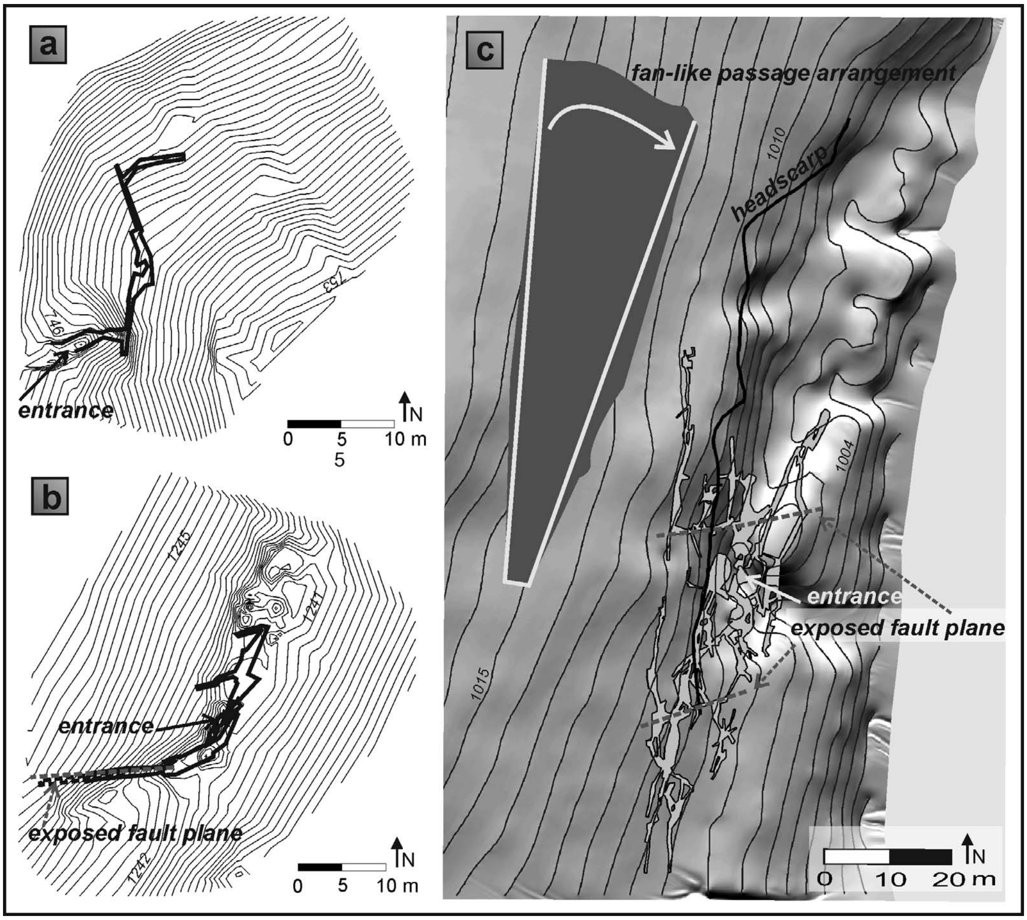


Figure 2. Examples of different arrangement of the passages in crevice-type caves with or without surface manifestations: a. CTC formed by passages that follow the strike of the slope. Note the limited topographical manifestation (Velryba Cave, the Javorníky Mts.). b. CTC with secondary passages diagonally or transversally oriented toward the main passage. Note the strong correlation with topography (Kyklop Cave, the Moravskoslezské Beskydy Mts.). c. Complicated CTC with fan-like arrangement of the passages due to the laterally uneven gravitational movement of rock blocks. Some parts do not correlate with the surface features (Cyrilka Cave, the Moravskoslezské Beskydy Mts.).

MORPHOLOGY

HORIZONTAL ARRANGEMENT OF CAVE PASSAGES

Crevice-type caves consist of a system of narrow and mutually parallel accessible crevices formed by gravitationally widened joints, the orientation of which is the most important feature controlling the direction of individual cave passages. In many cases, the small CTCs, up to 30 m in length, are formed by several accessible crevices that follow the strike of the slope (Fig. 2a). In the case of the medium-length CTCs, the main passages follow the strike of the slope and connecting passages are diagonal or transverse to them (Fig. 2b), according to the directions of joint sets. In some cases, these secondary passages follow the strike-slip fault planes (Figs. 2b and 3a). The long caves are formed by a system of mutually parallel passages along the strike of the slope that are connected by diagonal and transverse passages developed along the strike-slip faults or within the highly disrupted rock blocks (Fig. 2c). Figure 1 shows the numbers and percentages of identified caves with passages arranged generally parallel to the strike of the slope in each category.

The passages of the caves with lengths of more than approximately one hundred meters are typically fan-like arranged (Fig. 2c). This can result, first, due to an intersection of joint sets in two or more main directions (Fig. 3d) or, second, from the laterally uneven gravitational movement of rock blocks (Fig. 2c), so that each side of the fan moved differently and one side is now opened more than the other. In the first case, the largest cave rooms and passages are formed in and around the point of intersection of the main directions of joint sets (Fig. 3d). These passages are usually the most dissected and disturbed. In the second case, the widest cave passages are formed in the area of the largest opening of the fan (Fig. 2c), and these passages often pass into a talus or boulder cave (after Vítek, 1983).

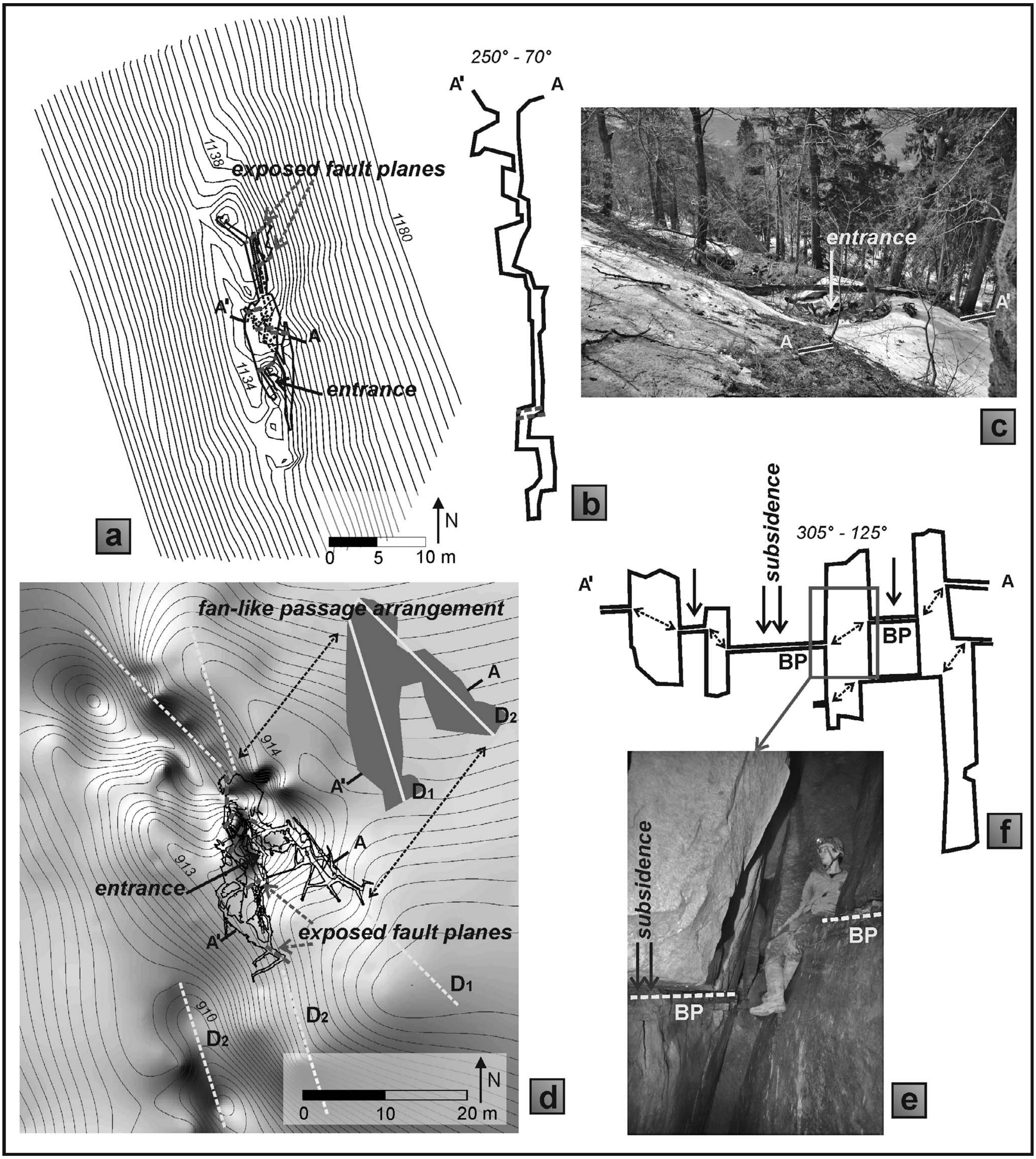


Figure 3. Examples of different horizontal and vertical arrangements of the passages. a. crevice-type cave with strong surface manifestations is developed along the faults passing through the massif (Čertova Díra Cave, the Moravskoslezské Beskydy Mts.). b. The cross-section of the same cave. The lowermost passages are terminated as inaccessible joints, and the vast chambers are in the upper levels. c. Surface trench connected with the development of the Čertova Díra Cave in parts a. and b.; photo by the author. d. Fan-like arrangement of passages in a complex multilevel cave due to an intersection of two main sets of joints (Velká Ondrášova Cave, the Moravskoslezské Beskydy Mts.). A-A′, the location of the cross-section in part f.; D 1 and D2, the orientation of the joint sets. e. The subsidence of rock blocks within the lower level of the same cave; BP bedding plane. Photo by the author. f. Idealized cross-section of the Velká Ondrášova Jeskyně Cave in parts d. and e. The inner rock blocks are the most subsided. The dashed arrows connect the disrupted bedding planes.

These parts are often disrupted by collapses, and the walls and ceilings are mostly crushed. The directions of joint sets here do not correlate with the prevalent directions measured elsewhere, and the arrangement of the passages is rather chaotic. Toward the tip of the fan, the passages become more compact, undisturbed, and narrow (Fig. 2c). In the study area, five out of the six long caves have fan-like passage arrangement.

VERTICAL ARRANGEMENT OF CAVE PASSAGES

Generally, 96 % of all investigated crevice-type caves reach the maximum depth of 16 m, and only four caves are from 30 to 57 m deep. At the cross-section perpendicular to the strike of the slope, the CTCs are composed of narrow and high passages, which may form levels that are connected together by pits elongated in the direction of the main joint set or by narrow openings. The vertical extent of levels is governed by the bedding planes. However, in the case of strictly vertically developed caves, there are only pseudo-levels formed by boulders and debris wedged between the walls.

Due to the lithological conditions within the massif, the levels typically can be staircase-shifted relative to each other. Generally, the lowermost passages are very narrow (~ 0.1 m) and are terminated by inaccessible joints (Fig. 3b). Conversely, in the upper levels, the passages are much wider (~1 m), are regularly shaped, accessible, and, in some places, widened into spacious rooms (Figs. 3b and 4d). The ceilings are commonly formed from the lower bedding planes. The uppermost levels are formed between the particular disrupted and horizontally or vertically rotated rock blocks (Fig. 4f). These levels are often affected by rock collapses, and the ceilings are formed from the wedged boulders or disturbed rock debris, with small openings between particular blocks.

All fifteen medium and long caves have upper level passages wider than the lower levels. In some cases, the progressive opening of the uppermost levels leads to the deformation of the ground surface and to the formation of surface trenches, sinkholes, or collapsed pits (Fig. 3a,c). However, the terrain above ten out of the fifteen medium and long caves is undisturbed and flat (Fig. 2a). This is evidence of the development of widening of the caves gradually increasing from depth to the surface.

There are two caves with huge floating inner rock blocks in the study area. In the large cave systems (length 100 m or depth 10 m) the staircase arrangement of levels is disorganized by huge floating inner rock blocks (~10 m^3), which sag 0.1 to 1 m into the widened underground space (Fig. 3e,f). During this vertical subsidence, these blocks were horizontally and vertically rotated. These blocks are usually situated within the cave systems, but even can reach the ground surface.

MORPHOLOGY OF THE PARTICULAR CAVE PASSAGES

The individual passages are the gravitationally widened joints, the accessible crevices. They are regularly shaped and high (~ 0.5 to 15 m) but relatively narrow (~ 0.5 to 1 m). Their main directions commonly follow the strike of the slope, while a few passages follow different joint systems.

In cross-section, the passages are A, V, or H shaped, according to the type of gravitational movement that leads to their development (Figs. 4a,b,c). Their bottoms are covered by a mixture of mud, sand, and debris or by larger collapsed rock blocks. The walls are straight, flat, inclined 70° to 90°, and are formed by a single rock bed or by a sequence of thinner beds with exposed bedding planes (Fig. 4d). Rarely, the passages are curved, probably following en echelon joints; an example is the 5 m long curved passage in Salajka Cave.

The ceilings are formed by the lower bedding planes of the overlying beds, which can be flat or jointed (Fig. 4e), or by wedged boulders with narrow intervening openings (Fig. 4f). There are usually very thin (~1.0 cm) plastic layers of siltstones or claystones observable between the overhead bedding plane and the wall, separating the sandstone beds (Fig. 4e).

MICRO-MORPHOLOGY OF THE WALLS AND CEILINGS

The rock surfaces within the caves sometimes expose micro-morphological features that originate from the depositional stage, tectonics, and secondary processes of gravity and weathering.

The sandstone beds are preserved as graded bedding with Bouma sequences, and occasionally with thin layers of fossilized plant detritus. The ceilings of the passages are often formed by exposed lower bedding planes, which can be shaped by load (groove) casts (Fig. 5a), flute marks (Fig. 5b), burrows, or markings. The oval depressions found in Cyrilka Cave on the upper bedding plane are probably tool marks (Fig. 6f).

The cave walls are formed by the joint surfaces. Their shape is controlled by the tectonics. The rose diagram in Figure 4g shows the directions of 4557 joints measured in the caves, combined with the directions of faults (arrows).

Fault tectonics can cause the gradual opening of joints perpendicular to the strike-slip faults and parallel to the normal faults. In several caves, some of the fault surfaces are now exposed on the walls (Fig. 5c). These surfaces are covered by slickensides (dip ~ 0° to 15° or 80° to 90°; Fig. 5c,d). In the faults, the slickenfibers and slickolites can occur on the exposed walls (Fig. 5d,g). High pressure within the fault zone can cause the formation of tectonic breccia (Fig. 5f) and mylonitic quartz (Fig. 5e). The sense of tectonic strains is expressed by hackle marks on the exposed walls (Fig. 5h). The small-scale pinnate structures, which indicate the shear sense, might be visible on rock blocks subjected to extreme compression (Fig. 5j). Rarely, the plumose structures are preserved on the joint planes (Fig. 5i).

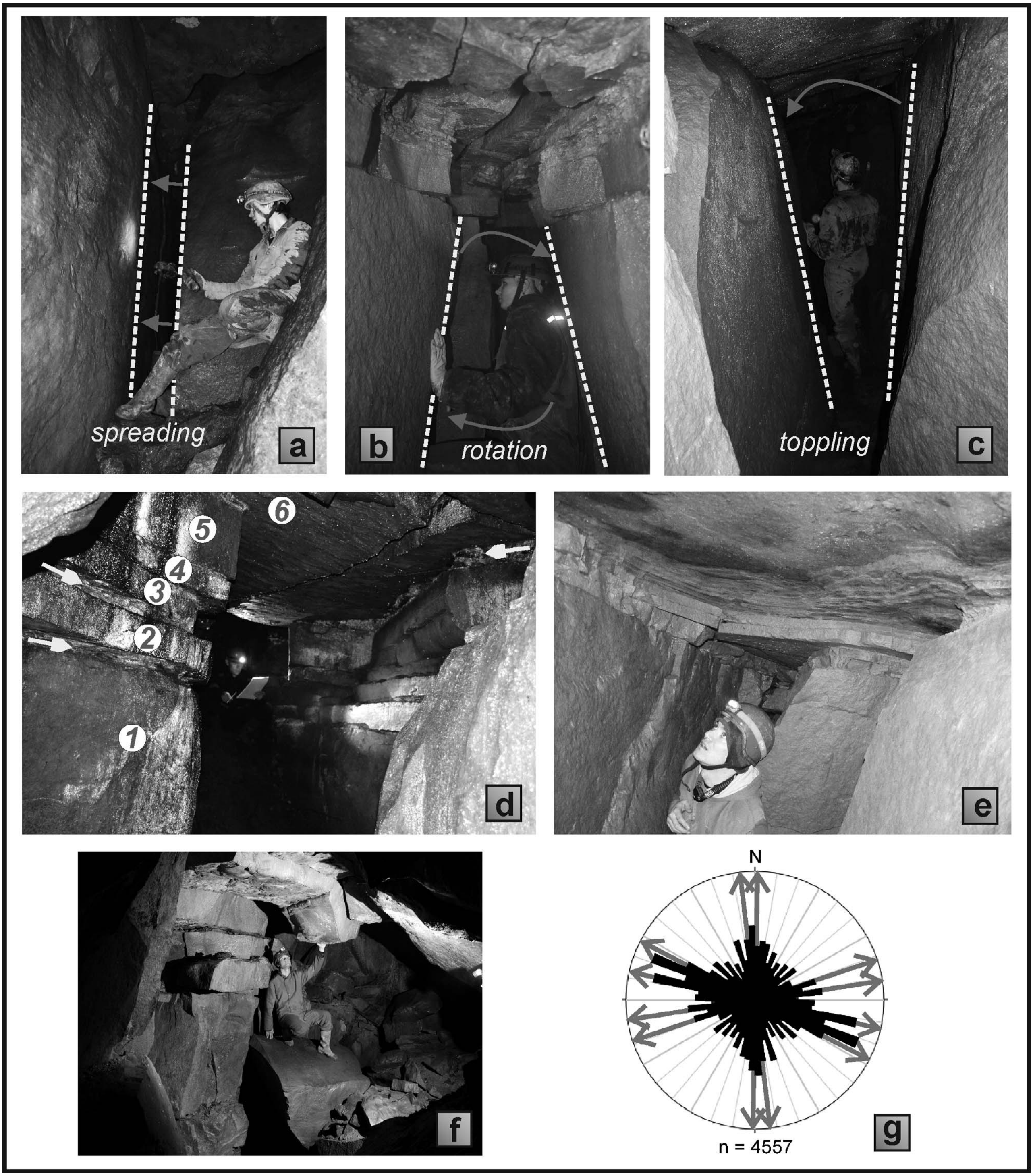


Figure 4. Morphology of the cave passages: a. H letter shaped passage caused by spreading-type movement (Velká Ondrášova Cave, the Moravskoslezské Beskydy Mts.). b. A letter shaped passage caused by rotational movement (Salajka Cave, the Moravskoslezské Beskydy Mts.). c. V letter shaped passage caused by toppling (Cyrilka Cave, the Moravskoslezské Beskydy Mts.). d. An example of typical walls formed by the exposed thick sandstone beds (numbers) and thin siltstone or claystone layers (arrows) Čertova Díra Cave, the Moravskoslezské Beskydy Mts.). e. Exposed flat ceiling formed by the base of a bed in Cyrilka Cave (the Moravskoslezské Beskydy Mts.) with plastic layers of siltstones or claystones between the overhead bedding plane and the wall. f. A ceiling formed by wedged boulders. Note the morphology of the walls shaped by the sandstone beds (the upper level in Kněhyňská Cave, the Moravskoslezské Beskydy Mts.). g. Rose diagram of 4557 measurements of joints within the caves, with arrows indicating the directions of faults taken from the geological maps.

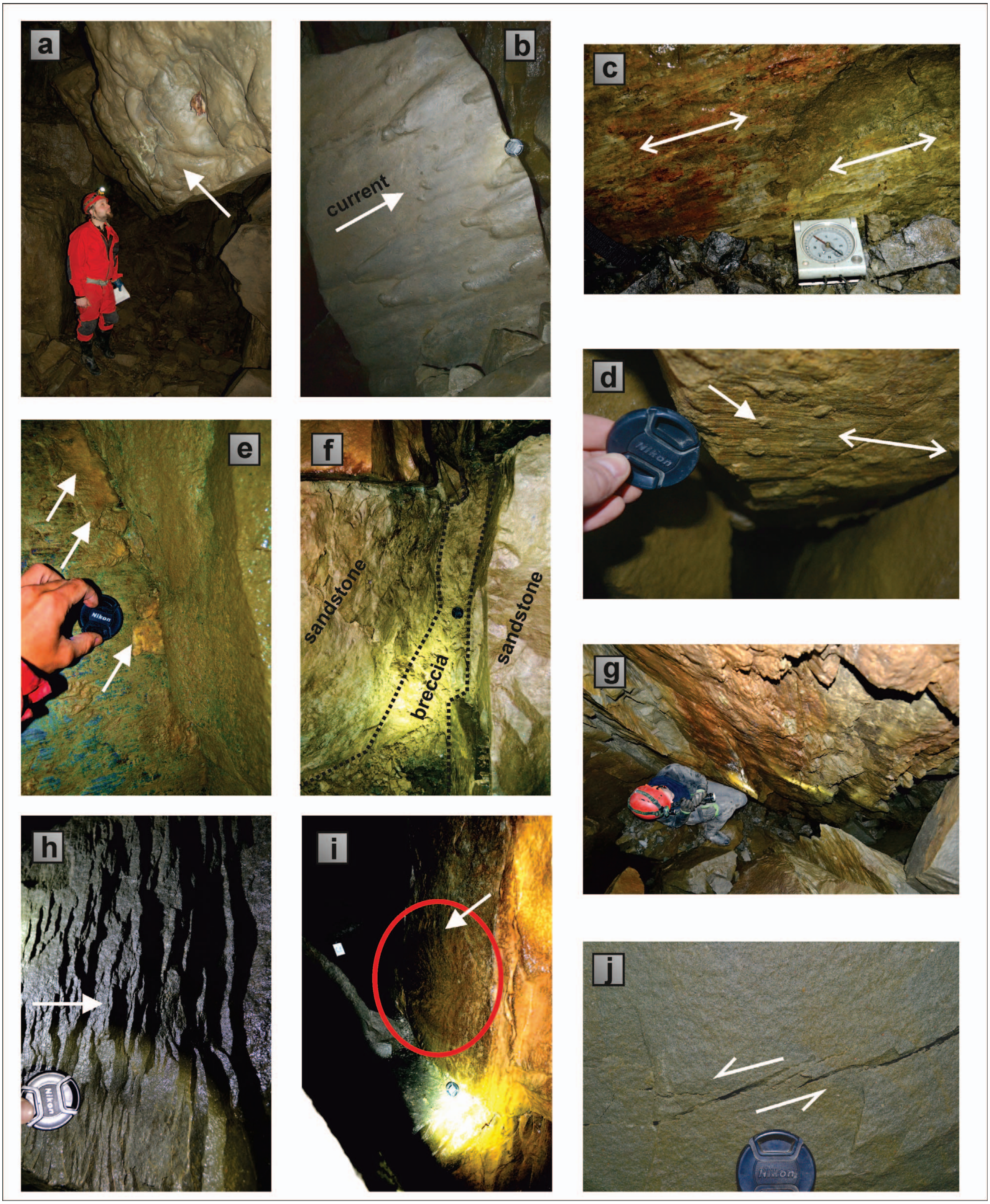


Figure 5. Micro-morphology of walls and ceilings of some crevice-type caves: a. Load casts. b. Flute marks; white arrow shows the current direction. c. Fault surface with marked direction of slickensides. d. Slickensides (direction indicated by double arrow) with slickenfibers (simple arrow). e. Mylonitic quartzite. f. Tectonic breccia infilling a crack, g. Slickolites. h. Hackle marks. i. Plumose structure with ripple marks (white arrow). j. Pinnate fractures indicating shear stress. a. Velká Ondrášova Cave (the Moravskoslezské Beskydy Mts.). b,. f., g., h., i., j. Kněhyňská Cave (the Moravskoslezské Beskydy Mts.). c., e. Čertova Díra Cave (the Moravskoslezské Beskydy Mts.); d. Cyrilka Cave (the Moravskoslezské Beskydy Mts.).

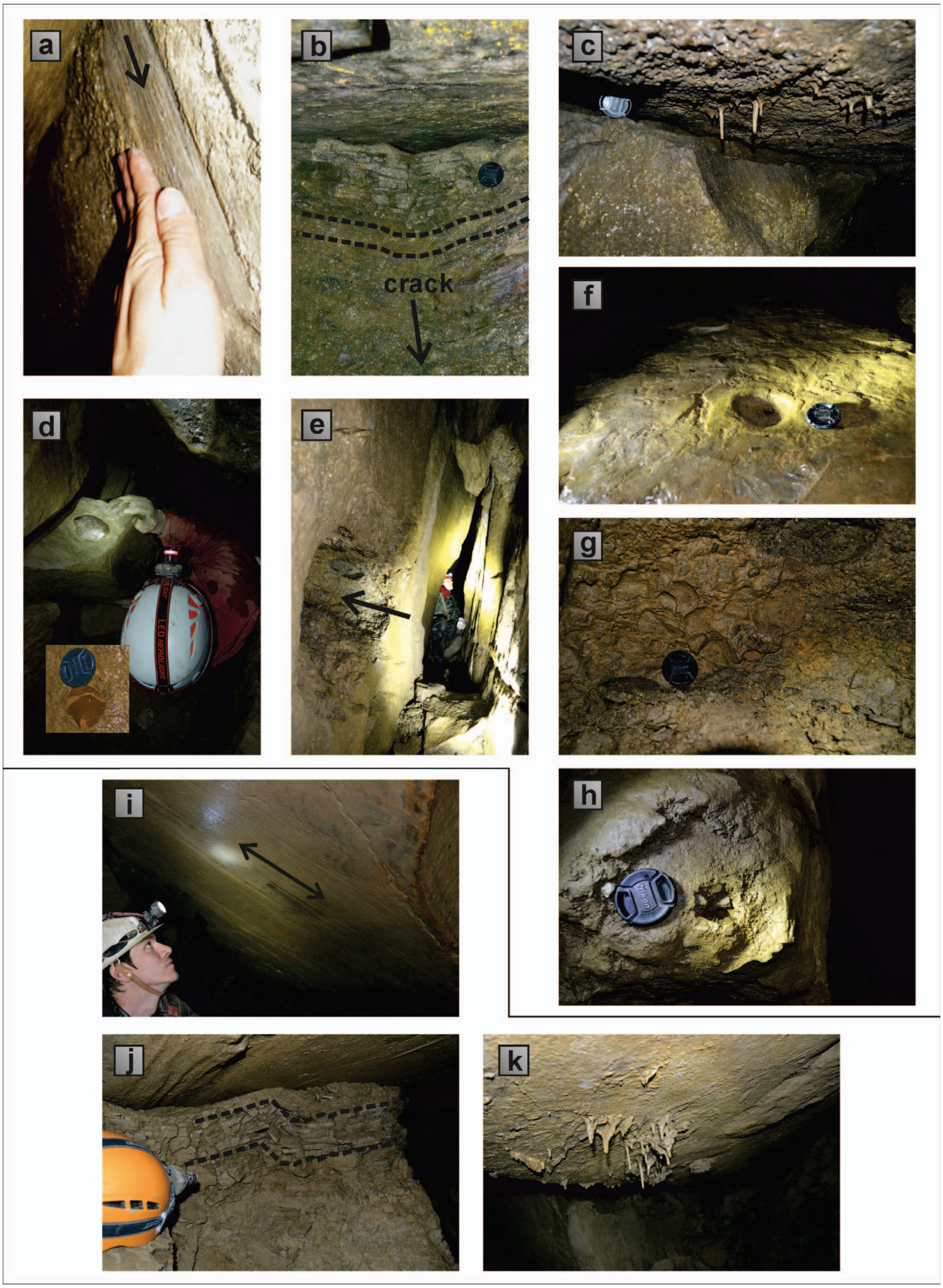


Figure 6. Micro-morphology of walls and ceilings of some crevice-type caves: a. Gravitationally caused striae; black arrow shows the direction of movement. b. Fold-like structure induced by the subsidence of shale layer into the gravitationally widened crack below the picture. c. Soda-straw-like stalactites. d. Ironstone balls. e. Holes from eroded coarse-grained layer sections. f. Tool marks (small-scale impact craters in the top of bed). g. Erosional surface after fallen out clasts. h. Holes in the solid rock due to dripping water. i. Consequent slickensides on the bottom of bed. j. Gravitationally induced fold. k. Soda-straw-like stalactites with mud content. a. Na Girové I Cave (the Jablunkovské mezihoří highland); b. Kněhyňská Cave (the Moravskoslezské Beskydy Mts.). c. d. inset, e., f., g., h. Cyrilka Cave (the Moravskosleszké Beskydy Mts.). d. Velká Ondrášova Cave (the Moravskoslezské Beskydy Mts.). i., Wiślańska Cave (the Beskid Śląski Mts., Poland, outside the study region); j., k. Pod Spišskou Cave (the Levočské vrchy Mts. Slovakia, outside the study region).

Recent gravitational processes, such as rock collapses, caused the formation of fresh erosive surfaces and striae on the walls (length: ~ 10 cm; Fig. 6a). The plastic siltstones and claystones are occasionally gravitationally folded. This arises either from the sinking of sandstone beds up to several meters thick into the thin (up to 0.2 m), plastic shale layers that are then pushed away and folded, or from subsidence of the shale layers into the gravitationally widened cracks (Fig. 6b).

Some parts of caves are modified by mechanical and chemical erosion. Due to presence of a variety of compositions of the beds in sedimentary flysch deposits, the weathering of exposed rocks within the passages is irregular. This is visible in the cave walls, where some sequences of beds, or only some sections, erode faster than others. This is especially true for the conglomerate or coarse-grained sandstone layers (Fig. 4d). In the walls, there are holes with erosion surfaces, formed from eroded coarse-grained layer sections (Fig. 6e,g) or intraclasts. The erosion sporadically exposes ferrolites, such as ironstone balls (Fig. 6d) or limestones and exotic blocks. Holes around 2 or 3 cm in diameter produced by dripping water are formed in the unconsolidated sediments and in rigid rock (Fig. 6h).

The solid walls are covered by thin (~0.1 mm) layers of clayey mud that originate from the weathered siltstones and claystones. On well-preserved walls, this mud is sometimes formed into a small mud flowstones. Clayey mud, in conjunction with the calcareous cement from the sandstones, can form small sinter films or fragile soda-straw-like stalactites (length: ~ 1 to 10 cm; Fig. 6c) that have been described from four places within two caves in the study area (Wagner et al., 1990). Mineral iron coatings occasionally, though rarely, cover the walls.

Discussion

In contrast with karst caves, which are formed in connection with the slope movement evolution under the influence of karst dissolution (Gutiérrez et al., 2014), the crevice-type caves are products of gravitational processes without any dissolution.

Although the CTCs occur in various types of rocks, the examples in flysch sediments have many peculiarities. In the sense of the overall horizontal arrangement of the passages, their organization is controlled by the gravitational development of the landslide body. The formation of cave passages between blocks is conditioned by the discontinuities in the rock mass, lithological boundaries and bedding planes, while the directions of passages typically follow faults and joints. The gravitational processes tend to create passages typically parallel to the strike of the slope (Figs. 2 and 3). This arrangement has been observed at many other crevice-cave sites (Wagner et al., 1990; Demek and Kopecký, 1999; Margielewski and Urban, 2003; Majerníčková et al., 2005; Margielewski et al., 2007) and is caused by the lithological properties of rocks, tectonics, and morphometry of relief. The passages can also form parallel to the sloping ridge, as in the case of the Diabla Dziura w Bukowcu Cave (Polish Outer Carpathians; Margielewski and Urban, 2004).

Recent measurements show that the dilatation rate of the joints reaches ~ 0.01 mm/year (Petro et al., 2011; Klimeš et al., 2012). Baroň et al. (2003) measured dilatation rates as high as 0.01 to 1 mm/month. Although the movements within the crevices are measurable, we still do not know whether the caves were formed by slow movements or during sudden short events, such as catastrophic landslides. Apparently, the regular network of sub-parallel passages is established during the progressive movement of particular rock blocks, but sudden movement cannot be excluded.

Along with the continuous release of the massif, the joints that are diagonal and perpendicular to the slope are also widened. The zone of joint widening is often laterally limited by the strike-slip faults (Figs. 2 and 3). Within the landslide body, the sliding movements and release of rocks are unevenly distributed. Recently measured vertical and horizontal rotations of the rock blocks (Petro et al., 2011), together with the uneven movements within the bulk of the landslide body, cause the irregular opening of the passages that is typical for most of the caves. This long-lasting evolution leads to the formation of the fan-like arrangement of the passages observed in plan view (Figs. 2c and 3d). A similar arrangement may develop when the widening of joints occurs in more than one direction; these directions may intersect to form the resulting arrangement. The fan-like feature is recognized at many other crevice-cave sites (Margielewski and Urban, 2004; Majerníčková et al., 2005; Margielewski et al., 2007; Szura, 2010; Lenart et al., 2014). In the case of the largest CTCs, there can be more than one fan-like zone compounded and connected together (Margielewski et al., 2007).

The cave passage network is limited and directed by the lithological boundaries (bedding planes) and joints. Along these discontinuities, the vertical levels are typically staircase-shifted (Fig. 3e). This shifting is known from other localities in the Polish and Slovak flysch Carpathians (Margielewski and Urban, 2003, 2004; Imrich et al., 2007). Particular levels usually show small and narrow connections (Fig. 3b). According to the main gravitational processes forming the cave, the width of the passages can change significantly from level to level. Although the lowermost passages are usually very narrow and the uppermost passages are wide, there are exceptions, where the widest passages are formed at greater depth (Imrich et al., 2007; Lenart et al., 2014). This might be caused by the character of the movements and also by the lithology. In the case of horizontally fan-like arranged passages ordered at several levels, the innermost rock blocks, which are wedged between the passages of the fan, typically have subsided into the space left by the gravitational release of the massif. This feature is also manifested in the vertical cross-section (Fig. 3f; Lenart et al., 2014). At the cross-section along the strike of the slope, the levels are not strictly horizontal along the profile. The benches of the passages often descend or ascend into the massif (Margielewski and Urban, 2004; Margielewski et al., 2007).

Regarding the morphology of the passages, the A, V, or H letter shape of the cross-sections is also typical for CTCs described from other localities (Self, 1985; Margielewski and Urban, 2004; Majerníčková et al., 2005). The specific shape is a result of the particular movement of two adjacent rock blocks (Lenart et al., 2014). The walls are usually vertically dissected by the particular beds, but they may also be flat, formed in a single thick bed (e.g., Imrich et al., 2007), or even curved (e.g., Margielewski and Urban, 2003).

Although the CTCs are not as rich in microforms as the classic karst caves, such features can be found at many cave localities. The most common microforms are Riedel shear structures on the walls. More unusual are hackle marks (ribbed or fringe structures), which are also known from other CTCs (e.g., Margielewski and Urban, 2004). I registered the pinnate structures in the Jaskyňa Pod Spišskou, in the Levočské vrchy Mts., Inner Flysch Carpathians, Slovakia, where the tectonically predisposed slickensides and fault surfaces along the fault zones occur on the exposed walls. In the Jaskinia Wiślańska, in the Beskid Śląski Mts., Poland, these slickensides also occur on the exposed lower bedding planes (Fig. 6i,) and thus, might be gravitationally predisposed. Margielewski et al. (2008) described spectacular gravitationally induced folds in the plastic shale layers from the Jaskinia Miecharska, in the Beskid Śląski Mts., Poland. Gravitationally induced folds, as well as holes formed from eroded intraclasts, groove casts, and small soda-straw-like stalactites up to 10 to 12 cm in length (Imrich et al., 2007, Majerníčková et al., 2005; Fig. 6k) are known from the Jaskyňa Pod Spišskou, in the Levočské vrchy Mts., Inner Flysch Carpathians, Slovakia (Fig. 6j). Those stalactites are the most similar to those observed in this study.

Various types of other speleothems (stalactites, helictites, draperies, flowstone sheetings, moonmilk, crusts, microstromatolites, mud flowstones, etc.) are described from the Polish part of the Flysch Carpathians (Urban et al., 2007; Margielewski et al., 2008; Gradziński et al., 2010). Margielewski and Urban (2004) describe the iron mineral coatings on the exposed walls. Other speleothems described by Urban et al. (2007) include small calcite stalactities, moonmilk, and gypsum-calcite crusts or stalactites formed by amorphous substances with the participation of silica. Gradziński et al. (2010) describe the role of trapping and binding of siliciclastic detrital particles in microstromatolites by bacteria.

Majerníčková et al. (2005) found the carbonized remains of plants in the rocks forming the walls. Margielewski et al. (2008) describe various fluvial forms, such as bedforms, sandy mud sediments and ferruginous coatings in the streambed, from the Jaskinia Miecharska cave, the Beskid Śląski Mts., Poland.

CONCLUSIONS

Some of the observations concerning crevice-type caves in flysch deposits have been published earlier by previous scholars. This paper is a recapitulation of these observations in the Czech portion of the Outer Flysch Carpathians.

Although the crevice-type caves seem to be composed of chaotically organized underground passages of free space between rock blocks, the results of investigations suggest certain rules in the resulting arrangement, composition, and geometry of the passages. I investigated crevice-type caves in the area of Czech Outer Carpathians from the morphological point of view. According to the results, the main passages are typically parallel to each other and to the strike of the slope. The passages of the large caves are typically fan-like arranged due to an intersection of joint sets or to the nonuniform gravitational movement of the landslide body, where one side is opened more than the other. The passages become more undisrupted and narrow toward the tip of the fan.

Vertically, the passages are organized into levels, strictly governed by lithology, and connected together by elongated, but narrow, openings. Due to the lithological conditions within the massif, these levels can be typically staircase shifted. Toward the ground surface, there is usually detectable growth of the breakage of the massif and the amount of displacement of joints increases; the uppermost levels are often formed among open rock blocks. The gradual opening of the uppermost level may lead to the deformation of superficial terrain by trenches or sinkholes. The terrain above the caves may also be flat, which is evidence of the gravitational widening of the caves from within the massif. In the large caves, the arrangement of the levels is chaotic due to subsided rock blocks.

In cross-section, the passages are A, V, or H letter shaped, according to the type of gravitational movement leading to their development. The exposed rock surfaces are sometimes marked by various micro-morphological features originating from the sedimentary structures, tectonics, gravitational processes, and mechanical and chemical weathering processes.

Although the crevice-type caves are not as actively studied as karst caves, they represent a specific, and often unexpectedly frequent, phenomenon in terrain affected by slope deformations. The investigations show that the evolution of crevice-type caves is controlled by a true landslide behavior. Finally, this behavior drives the resulting morphology of the caves.

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AIRBORNE BACTERIA AND FUNGI IN A COAL MINE IN POLAND

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Abstract: We determined the species composition and the concentration of colony forming units of airborne bacteria and fungi in a coal mine in Poland. We sampled at six locations in a working shaft at about 500-600 m below ground level. Air samples were collected between 6 and 9 a.m. using the impact method onto Potato Dextrose Agar and TSA. The volume of air filtered for each coalmine sample was 50 L. We found 11 fungal species, with *Penicillium meleagrinum* and *P. notatum* as the most common. Six bacterial genera were identified, with *Micrococcus* spp. as the most common. There were no pathogenic fungi or bacteria identified. The concentration of fungal spores and bacteria in the coal mine air is not a direct hazard to mine workers.

Introduction

Bioaerosols are ubiquitous in indoor air and may be potentially negative to human health. Bioaerosols or organic dust may include pathogenic or non-pathogenic live or dead bacteria and fungi, viruses, high molecular weight allergens, bacterial endotoxins, mycotoxins, peptidoglycans, $\beta(1\rightarrow3)$-glucans, pollen, and plant fibers. Bioaerosols are transmitted by the airborne droplets or dust through the skin, mucous membranes, and respiratory tract and rarely get into animals and humans orally (European Agency for Safety and Health at Work, 2000; Douwes et al., 2003; Górny 2004). Air contaminated with bioaerosols can be an important source of infection for humans. Depending on the composition of bioaerosols, they can cause simple irritation or ailments, allergic reactions, light or serious infections, and toxic reactions (Douwes et al., 2003; Gamboa et al. 1996; Górny 2004). Therefore, the concentrations of individual components of bioareosols measured by CFU of bacteria and fungi is one of the indicators of indoor air pollution (Douwes et al., 2003; European Agency for Safety and Health at Work, 2000; Drenda, 2012).

Working conditions in coal mines are hard, due to the depths of the mines, which can be up to 1000 m, high temperatures reaching over 30 °C, and relative humidities of 70 to 100% (Drenda, 2012). These conditions, plus the presence of organic substances, create a fairly favorable growth environment for microscopic fungi, which can be found on any organic material used in the mining process, especially on timber, all types of organic waste, as well as on insulators, machinery tires, or other rubber surfaces (Piontek and Bednar 2010). The miners going down to work bring fungi into the mine. The research conducted by Pusz et al. (2014) in a copper mine has shown that the number of colony forming units (CFU) is dependent on the kind of work done and presence of organic material.

The level of bioaerosols found underground in coal mine shafts was high, containing a great number of spores spread through the shafts by the ventilation system. Frequent air changes, which are characteristic in the microclimate of the mine, result in significant variations of the number of CFU present in the air samples. Some of the airborne fungal species (*Aspergillus* spp., *Penicillium* spp., *Cladosporium* spp.) that occur in the mine excavations and galleries can possibly cause individual allergies and fungal infections in some exposed workers (Gamboa et al. 1996; Obtułowicz 2006; Cabral 2010).

The official limits of indoor airborne bacterial and fungal spores concentrations used in Poland as safety standards (Polish Committee for Measurements and Quality Standards 1989a, 1989b) do not apply to mine excavations or galleries. Most species of the fungi spores found in the coal mine air samples and on organic material, such as timber safety structures built in the galleries, can produce mycotoxins that can cause different diseases when introduced into the human body (Rusca et al. 2008).

In Poland, there are no legal regulations that would allow for a reliable assessment of the microbiological quality of air (Tsapko, et al. 2011). The reference point is the European Agency for Safety and Health at Work (2000) and the proposed limit values given in the literature (e.g., Górny 2004, 2010). The aim of our research was to determine which species of airborne bacteria and fungi occur in the coal mine and to identify their CFU concentration.

Materials and Methods

This study was performed in May 2014 in the coal mine KWK Murcki-Staszic, owned by Katowicki Holding Węglowy S.A. The coal mine is located in Katowice, Upper Silesia, south Poland (Fig. 1). The mine under its present name was formed on 1 January 2010 as the result of merger of the oldest, KWK Murcki, and one of the youngest, KWK Staszic, mines in Upper Silesia. The mine underlies more than 67 km^2. Extraction is carried out at five levels from

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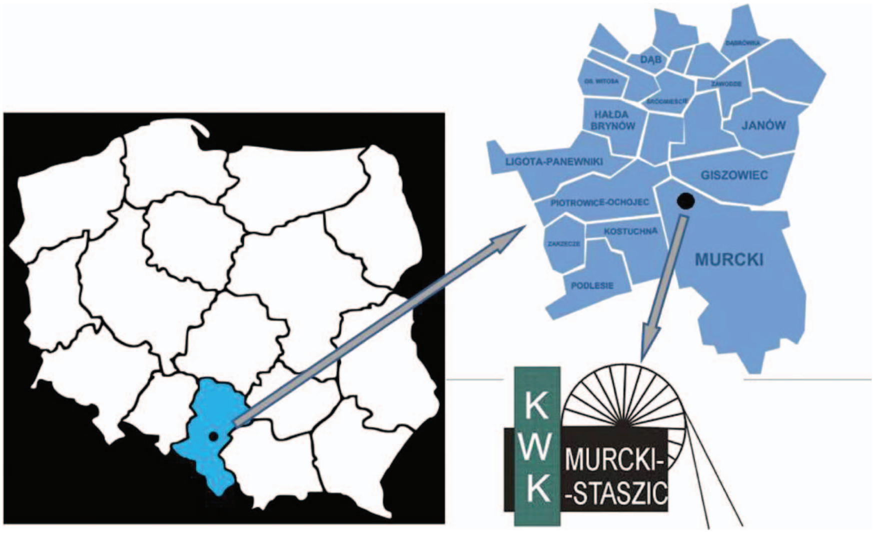

Figure 1. Location of the KWK Murcki-Staszic coal mine in Poland.

416 to 900 m below ground level. Daily production of the mine is an average of 23,000 tons. The mine's coal resources are estimated at about 50 years of extraction.

Air measurements were taken at six sampling points in the working shafts at depths of 500 and 600 meters below ground level. The sample locations are referred to as *shaft insert*, the bridge at the mouth of the mineshaft from which the crew enters the mine cage to go down to the mining level; *shaft bottom*, an underground excavation at the mining level of 500 m directly below the mine shaft, the place where the crew leaves the mine cage; *shaft collar*, a ventilation shaft located a few hundred meters from the main shaft through which the exhaust air leaves the mine; *miner's meeting place*, a small chamber located a few hundred meters from the shaft bottom where workers gather before starting work, from which the miners' brigades move forwards to their workplaces; *working face*, the surface at the end of the mining corridor about 1000 m from the shaft bottom where the mining work is advancing, with a machine and a crew of about ten men and a very high dust level; *station to operate machines*, a miners' work station located in the middle of the corridor with a conveyor belt that transports the excavated material.

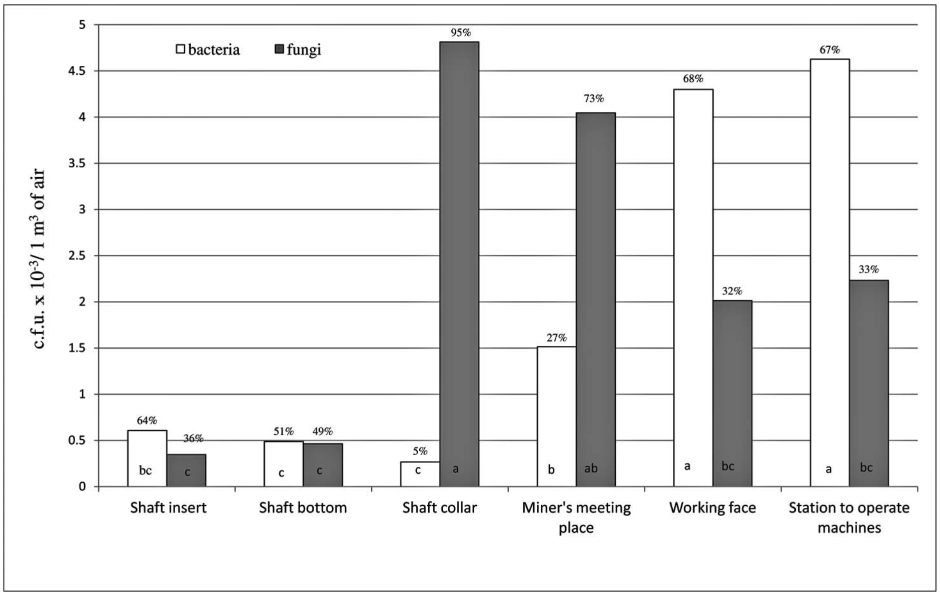

Figure 2. Measured concentrations of bacteria and fungi in the air at the samples sites. Percentages are of the total microorganisms at the site. Bars marked with the same small letter a, b, or c did not differ significantly ($p < 0.05$) by the Tukey test.

Table 1. The total concentrations of microorganisms in the air by sample location.

Elements of Bioaerosol	Shaft Inset	Shaft Bottom	Shaft Collar	Miner's Meeting Place	Working Face	Station to Operate Machines
The total number of fungi (CFU/m^3 of air)	347	463	4813	4047	2013	2233
The total number of bacteria (CFU/m^3 of air)	607	487	267	1513	4300	4627
The number of bacteria and fungi together	954	950	5080	5560	6313	6860

The samples were collected between 6:00 and 9:00 a.m., when 200 miners were working in the mine. We used the impact method and the Air Ideal 3P sampler to analyze the fungal load using PDA (Potato Dextrose Agar) plates manufactured by Biocorp. For the isolation of bacteria, TSA medium was used (Trypcase-Soy Agar by bioMerieux, France). In order to eliminate fungi and yeast from the bacteria samples, 30 g/mL of nystatin was added to the TSA medium (Polfa, Kraków).

The volume of each collected coal mine-air sample was 50 L, 100 L, or 150 L. The sampling at each site was performed three times, and the impactor was held at 1.5 m above the floor. The PDA plates were incubated for 7 days at room temperature (22 °C). The TSA plates were incubated for 72 hours at 37 °C. After incubation the number of visible colonies was determined, and the fungi were identified to species according to their morphology. Pure cultures of bacteria were obtained by reductive isolation and then diagnosed by Gram staining, spore staining, catalase test, and API tests (BioMerieux, France). The number of colony forming units (CFU) per 1000 L (1 m^3) of air was determined by $X = (a \times 1000)/V$, where a is the total number of colonies grown on three plates and V is the collected air volume of 150 liters (50 L for each plate).

The resulting concentrations of microorganisms per 1 m^3 of air (CFU/m^3) were compared with the guidelines developed by the Team of Experts of Biological Factors (ZECB) (Górny, 2004 and 2010). The results obtained from the total numbers of bacteria and fungi (CFU/m^3) were also subjected to analysis of variance using the Tukey test ($p < 0.05$) using Statistica for Windows v. 5.1.

RESULTS

The numbers of bacteria and fungi present in the tested air are shown in Figure 2 and Table 1. The presence of bacteria and fungi at the sampling points varied. The number of bacteria developed in the range of 0.27 to 4.63×10^3 CFU/m^3 of air, and fungi from 0.35 to 4.81×10^3 CFU/m^3 of air. The highest concentration of bacteria (above 4.3×10^3 CFU/m^3) was observed at underground depths of 500 to 600 m, at the station to operate machines and the working face. At these sample points bacteria were the predominant microflora in the bioaerosol (∼ 68%). The lowest number of bacteria (0.27×10^3 CFU/m^3) were isolated from the shaft collar. However, at this point the air was contaminated with fungi (4.8×10^3 CFU/m^3); they accounted for 95% of all microorganisms. Similarly, a high number of fungi in the air was observed at the miner's meeting place (above 4.0×10^3 CFU/m^3). The lowest counts of both groups of microorganisms were observed in the bioaerosol in the shaft insert and shaft bottom. The number of fungi and bacteria in these samples did not exceed 6.1×10^2 CFU/m^3 of air. These measuring points are in open spaces, which usually have a

Table 2. Concentrations in CFU/m^3 of types of bacteria in the air at sampled sites, with percentages of all bacteria at the site in parentheses.

Species	Shaft Inset	Shaft Bottom	Shaft Collar	Miner's Meeting Place	Working Face	Station to Operate Machines
Micrococcus spp.	287 (47)	227 (47)	127 (47)	840 (56)	2780 (65)	3187 (70)
M. luteus	67 (11)	60 (12)	53 (20)	340 (22)	660 (15)	360 (8)
Gram-positive cocci	nd	nd	nd	73 (< 5)	60 (1)	140 (3)
Staphylococcus spp.	93 (15)	67 (< 14)	20 (7)	27 (2)	180 (4)	280 (6)
S. epidermidis	7 (1)	7 (1)	20 (2)	33 (2)	167 (< 4)	80 (< 2)
S. equorum	13 (2)	nd	nd	nd	nd	nd
S. hominis	nd	nd	nd	20 (1)	127 (< 3)	153 (3)
S. saprophyticus	40 (< 7)	33 (< 7)	7 (2)	73 (< 5)	200 (< 5)	227 (5)
S. xylosus	nd	nd	nd	13 (< 1)	93 (2)	107 (2)
Gram-negative rods	73 (12)	40 (8)	47 (17)	40 (< 3)	nd	13 (< 1)
Bacillus spp.	27 (4)	20 (4)	7 (2)	40 (< 3)	nd	7 (< 1)
Actinomycetes	nd	33 (< 7)	nd	13 (< 1)	33 (< 1)	73 (< 2)

Note: nd = not detected.

Table 3. Concentrations in CFU/m^3 of types of fungi in the air at sampled sites, with percentages of all fungi at the site in parentheses.

Species	Shaft Inset	Shaft Bottom	Shaft Collar	Miner's Meeting Place	Working Face	Station to Operate Machines
Aspergillus niger Tiegh.	7 (2)	nd	nd	nd	nd	nd
Aspergillus versicolor (Vuill.) Tirab.	nd	nd	nd	33 (< 1)	30 (1)	113 (5)
Cladosporium cladosporioides (Fresen) G.A. de Vries	nd	13 (3)	nd	nd	nd	nd
Mucor flavus Schrank	nd	nd	nd	nd	7 (< 1)	nd
Mucor hiemalis Wehmer	10 (3)	nd	nd	nd	nd	23 (1)
Penicillium chrysogenum Thom	nd	57 (12)	nd	nd	nd	nd
Penicillium meleagrinum Biourge	60 (17)	230 (50)	2890 (60)	687 (17)	1943 (97)	1740 (78)
Penicillium notatum Westling	150 (43)	43 (9)	1887 (39)	3317 (82)	33 (< 2)	313 (14)
Talaromyces funiculosus (Thom) Samson, N. Yilmaz, Firsvad & Seifert	120 (35)	50 (11)	37 (< 1)	10 (< 1)	nd	nd
Trichoderma hamatum (Bonard.) Bainier	nd	nd	nd	nd	nd	43 (2)
Trichoderma harzianum Rifai	nd	70 (15)	nd	nd	nd	nd

Note: nd = not detected.

smaller amount of microorganisms. Contributions of individual genera and species of bacteria in the tested air are shown in Table 2. The dominant group of bacteria (from 49% to 80%) were Gram-positive cocci, especially of the genus *Micrococcus* spp. We also isolated staphylococci, the most numerous of those species being *Staphylococcus saprophyticus* and *S. epidermidis.*

Actinobacteria were also present in the coal-mine samples, with their greatest concentration observed in the station to operate machines (7.3 × 10^1 CFU/m^3). However, the frequency of their occurrence in comparison to the total number of bacteria was very low. Their share in the total number of bacteria did not exceed 2%. We did not identify pathogenic Gram positive or Gram negative bacteria in the air.

The contributions of individual species of fungi in the air of the mine are shown in Table 3. The largest share of fungi were of the genus *Penicillium*, especially *P. notatum* and *P. meleagrinum*.

DISCUSSION

The presence of microorganisms in underground halls and corridors is correlated with the number of users, the relative humidity, and the amount of dust particles. The number of bacteria in such areas can be up to several thousand per m^3 of air (Błaszczyk, 2010, p. 357–369). It is assumed that the total number of bacteria and fungi combined in manufacturing and industrial areas should not exceed 1 × 10^7 CFU/m^3 of air. Recommendations of the Zespoół Ekspertoów ds. Czynnikoów Biologicznych (Team of Experts of Biological Factors) was used as the norm for mesophilic bacteria of 1 × 10^5 CFU/m^3, for gram-negative 2 × 10^4 CFU/m^3, and for total fungi 5 × 10^4 CFU/m^3 (Górny 2004, 2010). Measured concentrations of microorganisms at all of the measuring points were within acceptable values according to Górny (2010).

None of the examined sites exceeded normative values for Gram-negative bacteria. This type of bacteria was isolated at less than 1 × 10^2 CFU/m^3 of air in the production halls. The standard is 1 × 10^3 CFU/m^3 according to Górny (2010). *Actinomycetes* are bacteria commonly present in soil. They are also detected in air samples and are common environmental pollutants in production and housing spaces. Occurrence of them indoors is associated with increased humidity (Frączek and Kozdrój, 2013). The highest concentration of those airborne microorganisms was recorded in the station to operate machines (7.3 × 10^1 CFU/m^3). According to the recommendations of the PN-89 Z-04111/02 and presented in it, the scale of actinobacterial air pollution at this location was moderate (Górny 2010). Frączek and Kozdrój (2013) conduced similar experiments in a subterranean spa located in the former salt-mine of Bochnia. They report the average concentrations of actinobacteria underground ranged from 0 to almost 4 × 10^1 CFU/m^3 throughout the year.

Microorganisms commonly found in the air are natural components of the bacterial flora of the skin and mucous membranes. Responses of individuals to mine exposures to bioaerosols depend in their individual sensitivities, including immunity, time of exposure, or dose of microorganisms introduced into the body. They can cause immunopathogenic reactions (Szczuka et al., 2013). Inhalation of bioaerosols, depending on the composition, exposure time, and sensitivity of the human immune system, can cause respiratory disorders such as allergic rhinitis, asthma, allergic alveolitis, bronchitis, chronic pulmonary insufficiency, tuberculosis, sinusitis, or conjunctivitis (Gąska-Jędruch and Dudzińska, 2009; Prasanth et al., 2011). Piontek and Bednar (2010) claim that *Penicillium* spp., *Aspergillus* spp., and other frequently isolated fungi, if present in sufficiently large quantities, can damage the mining machinery, timber structures, or cables, and as a consequence, result in serious accidents.

Unlike other working environments, there is little research on the concentration of bacteria and fungi in the halls of underground coal mines. Previous work describes exposure to bioaerosols in the chambers of the Wieliczka salt mine and copper mine in Lubin. Pusz et al. (2014) studied the occurrence of air-borne fungi in three copper-mining shafts: Bolesław, Lubin Zachodni (Lubin West shaft), and Lubin Główny (Lubin Main shaft) in the Lubin mining site, property of KGHM Polska Miedź S.A. They found twenty-seven fungal species, the most numerous being *Penicillium notatum*, *P. urticae*, and *Aspergillus flavus*. The population of fungi varied considerably among the copper mines' shafts or shaft parts. The maximum concentrations ranged from 5.06×10^3 CFU/m^3 of air in Lubin Main shaft to 2.15×10^4 CFU/m^3 in Bolesław shaft. The largest concentration of spores was observed in the part of mine where the timber was stored (2.15×10^4 CFU/m^3). The KWK Murcki-Staszic coal mine was also dominated by fungi of the genus Penicillium (*P. notatum* and *P. meleagrinum*), but their concentration in the air was much lower. That could be caused by a smaller amount of organic matter present in the mine and a much lower prevailing temperature in the coal mine. In the studied mine, timber was not stored underground, and the place where the highest concentration of fungi recorded was the shaft collar (4.81×10^3 CFU/m^3). Ogórek (2012) examined air quality in a gold mine in Złoty Stok (Poland), which turned out to host several fungal species, with *Trichoderma harzianum*, *P. expansum*, and *Botrytis cinerea* being the most numerous. *Aspergillus* and *Penicillium* are capable of producing mycotoxins, which are hazardous to human health (Cabral, 2010), and the spore concentration of those fungi in the gold mine examined by Ogórek (2012) could be a health threat to the miners working in some parts of it. Again the highest concentration of spores was present in the parts of mine abundant in timber.

Prasanth et al. (2011), in his work on a lignite opencast mine in India, describes the occurrence of fungi. They recorded a total of 520 colonies of fungi with an average of 260 CFU/m^3 of air. The isolated colonies were classified into 24 species belonging to 13 genera of fungi. Among the species recorded, *Aspergillus niger* was dominant in the atmosphere, with 82.5 CFU/m^3 of air and *Penicillium restrictum* (21.5 CFU/m^3 of air). Spore concentration was much lower than in the case of KWK Murcki-Staszic and species composition was more varied, which could be due to the different characters of open and underground mines. The air indoors is more polluted than outdoors. The degree of contamination depends on the premises, number of people, ventilation, and outdoor-air properties.

Much more research is concerned with the quality of air in caves than in active mines because of the frequency of tourist visits, which makes it necessary to know about possible potentially pathogenic microorganisms in caves, determine their reservoirs, and inform the public about the consequences of such visits (Jurado et al., 2010). The concentration of spores and bacteria in natural caves are usually much lower than in the mines. For example, in a Niedźwiedzia Cave in the Sudety moutain, which is often visited by tourists, Ogórek et al. (2014a) reported the concentration of spores between 123 and 214 CFU/m^3, while the most commonly isolated fungi were *Cladosporium herbarium* and *Rhizopus stolonifer*. Similar results were obtained by Ogórek et al. (2014b) examining the air in an artificial underground complex named Włodarz, located inside the massif of Włodarz, within the Owl Mountains, Lower Silesia, Poland. Between 65.5 and 1003 colony-forming units of fungi per m^3 of air were isolated from the air sampled in the adit, and the most common fungus isolated from the air outside and inside the adit was *Cladosporium cladosporioides*, followed by *C. herbarum* at one location in the adit.

On the other hand, Pusz et al. (2015) isolated from the newly discovered cave Jarkowicka, visited so far by few people, 22 species of fungi, whose concentration in the air, depending on the measure point, ranged from 76 to 200 CFU/m^3. *Cladosporium cladosporioides* was the fungus most frequently isolated. It amounted to almost 75% of airborne spores inside the cave. *Alternaria alternata* spores were also relatively frequent in the cave, making up 12% at the entrance and 9% in the middle section. The reason for such large differences in species composition and concentration of fungi in bioaerosols may be differences in air temperature, relative humidity, and the presence or absence of organic matter in each area.

Conclusions

The number of fungi and bacteria isolated from the tested air is not a threat to the health of people working in the coal mine KWK Murcki-Staszic. The microbiological quality of indoor underground air is connected, in this case, primarily with the effectiveness of the ventilation system. Modernization of the existing ventilation system and the introduction of modern, better equipment can improve aerosanitary conditions in the underground mine.

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H.E. Ridley, J.U.L. Baldini, K.M. Prufer, I.W. Walczak, and S.F.M. Breitenbach – High-resolution monitoring of Yok Balum Cave, Belize: An investigation of seasonal ventilation regimes and the atmospheric and drip-flow response to a local earthquake. *Journal of Cave and Karst Studies*, v. 77, no. 3, p. 183–199, DOI: 10.4311/2014ES0117

HIGH-RESOLUTION MONITORING OF YOK BALUM CAVE, BELIZE: AN INVESTIGATION OF SEASONAL VENTILATION REGIMES AND THE ATMOSPHERIC AND DRIP-FLOW RESPONSE TO A LOCAL EARTHQUAKE

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Abstract: The nature of cave ventilation is of interest to cavers, speleologists, and paleoclimatologists working with stalagmites. Because cave ventilation systematics may change over the growth span of a stalagmite, understanding what factors affect them is critical for determining events that may have affected climate proxies within the stalagmite. Similarly, understanding how the hydrology of the drips feeding a stalagmite evolves through time is key to building robust records of paleoclimate, particularly because stalagmite records have become critical archives of climate change information of the last 500,000 years. Here we present data from an extensive, on-going monitoring effort at Yok Balum Cave, Belize, initiated in 2011, that characterizes high-resolution ventilation dynamics at this site. Clear seasonal ventilation regimes exist, driven by thermally induced inside-outside air density differences. The winter regime is dominated by air inflow into the cave, decreased drawdown from the epikarst into the cave, and a limited diurnal signal. Conversely, summer ventilation is dominated by air outflow from the cave, greater CO_2 drawdown and drip water degassing, and a strong diurnal signal. Active monitoring during a large (M7.4) earthquake in November 2012 provides a unique opportunity to assess the response of the cave atmosphere and hydrology to substantial seismic activity. Cave atmosphere dynamics and hydrology are found to be highly resilient to seismic activity, with no observable disturbance occurring around the earthquake, despite there being considerable evidence of physical disruption in the cave. Monitoring included different kinds of drips, and the earthquake affected none of the monitored drip types. This suggests that stalagmite-derived paleoclimate records are not affected by seismic activity, except in extreme cases where the stalagmite or conjugate stalactite is damaged or reoriented.

Introduction

Characterizing caves in terms of their unique ventilation processes is important, as it has a first-order control on atmosphere composition, can potentially lead to seasonal bias in speleothem growth; and consequently, has significant implications when interpreting paleoclimate proxy signals from speleothems (Kowalczk and Froelich, 2010; Baldini, 2010; Sanchez-Canete et al., 2013). Cave atmosphere data are also useful when characterizing cave ecosystems (Oh and Kim, 2011; De Freitas et al., 1982) and assessing the suitability of caves for industry and tourism (De Freitas et al., 1982; Smithson, 1991; Dueñas et al., 1999; Dueñas et al., 2011; Virk et al., 1997). Estimation of cave ventilation is possible directly via anemometers, indirectly via measurement of levels of radon gas (^{222}Rn) (Kowalczk and Froelich, 2010; Hakl et al., 1997; Faimon et al., 2006; Oh and Kim, 2011) and other tracer gases (De Freitas et al., 1982), or by studies of air density contrasts and thermal patterns within the cave (Faimon et al., 2012; Smithson, 1991; Sanchez-Canete et al., 2013). The importance of understanding specific cave ventilation mechanisms has been well highlighted in recent studies (Kowalczk and Froelich, 2010; Cowan et al., 2013; Mattey et al., 2010; Baker et al., 2014), as the distinct nature of ventilation in individual caves can negate general assumptions regarding the seasonality of carbonate precipitation. For example, Mattey et al. (2010) identified unusual seasonal ventilation regimes in New St. Michaels Cave, Gibraltar, where the summer season was typified by low cave-air pCO_2. This proved important when linking seasonal regimes to calcite fabric, paired annual laminae, and stable isotope and trace element variability, and highlighted the importance of understanding particular cave environments. Studies like this become increasingly important as speleothem-based paleoclimate

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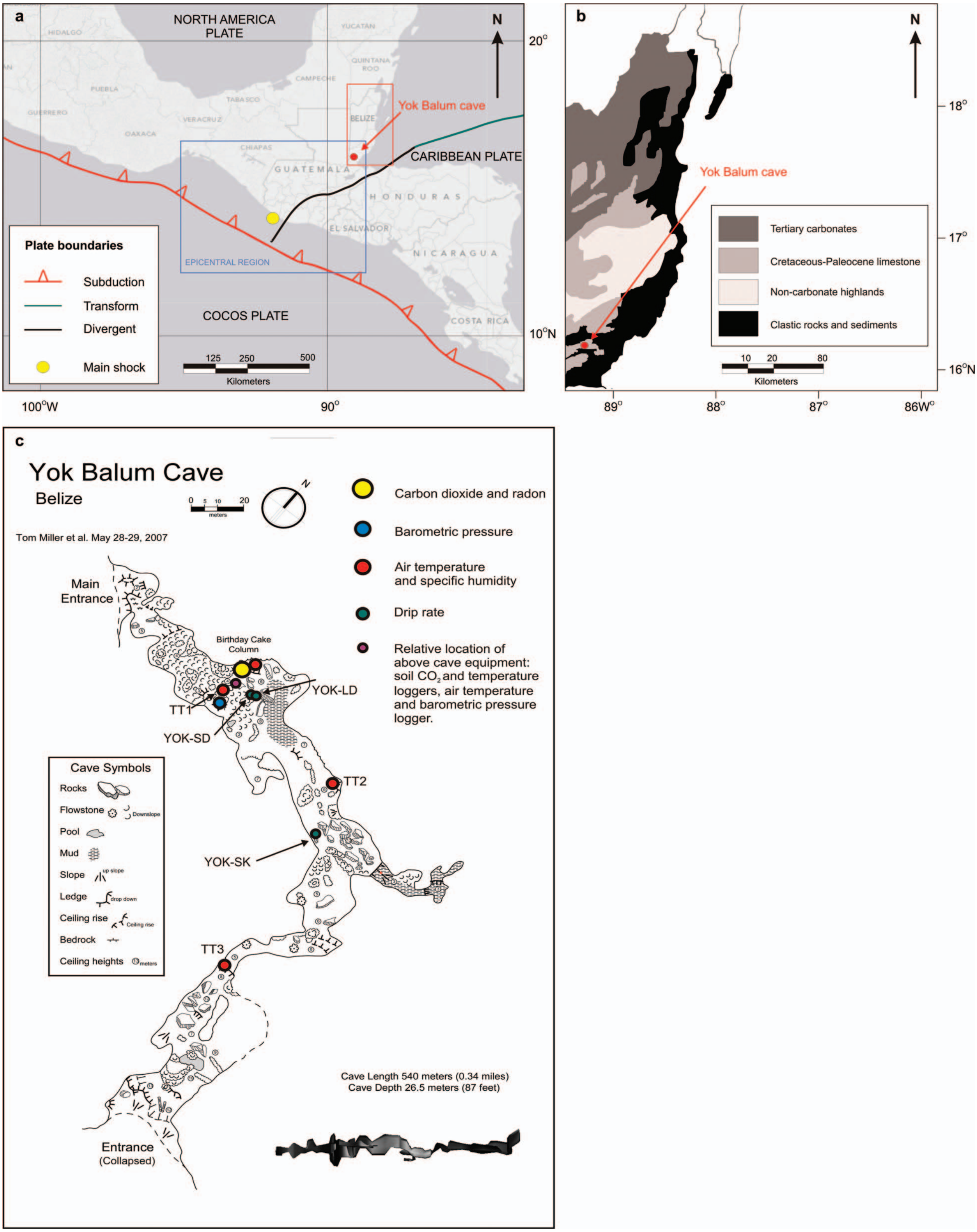

Figure 1. (a) Tectonic setting of Belize region, including tectonic boundaries (adapted from the U. S. Geological Survey). Estimated epicenter (yellow dot) and epicentral region (blue rectangle) of 7th November 2012 earthquake. Red box identifies Belize, Yok Balum Cave (red dot) and the area covered in (b), which is a geological schematic of Belize (adapted from Miller, 1996) with the location of Yok Balum Cave (red dot). (c) Map of Yok Balum Cave with equipment locations and drip sites monitored in this study.

research continues to develop higher-resolution records that are resolved to a seasonal or sub-seasonal level.

Caves in seismically active regions can display considerable evidence of past seismic activity, such as broken speleothems, speleothem growth anomalies and deformation, displacement, and rock-fall events (Becker et al., 2006; Gilli, 1999; Gilli and Serface, 1999; Gilli and Delange, 2001). A limited number of studies have attempted to quantify the effect seismic activity may have on the atmosphere in a karst cave (Sebela et al., 2010; Virk et al., 1997), particularly with regard to CO_2 variability. Such information is pertinent when interpreting proxy paleoclimate evidence from speleothems in caves that may have been subject to substantial tectonic activity, as seismic activity affects cave ^{222}Rn and CO_2 levels through pro-/co-seismic degassing and increased influx to the cave (Sebela et al., 2010; Wu et al., 2003; Virk et al., 1997; Menichetti, 2013). Crushing of material during seismic activity increases the rock's permeability for ^{222}Rn gas and CO_2, leading to higher in-cave concentrations. Cave-air pCO_2 levels exert a strong control on carbonate-precipitation rates (Baldini, 2010; Kowalczk et al., 2008; Palmer, 2007; Banner et al., 2007); and therefore, substantial crustal degassing has the potential to stagnate speleothem growth, particularly in deep, poorly ventilated passages. This can complicate paleoclimate-proxy interpretations from speleothems for weeks to years, depending on site-specific ventilation regimes. ^{222}Rn is a radioactive yet inert tracer gas frequently used to assess cave ventilation (Kowalczk and Froelich, 2010; Oh and Kim, 2011), but it can also pose a health risk in confined, poorly ventilated caves (Field, 2007; Virk et al., 1997); therefore, its relation to seismic activity warrants assessment, particularly in caves used for commercial or tourism purposes. It is also largely unknown how seismic activity may affect karst hydrology and stalagmite-drip regimes. Changes in the hydrological regime feeding a stalagmite can affect speleothem growth rates and the transmission of geochemical signals from overlying climate to the speleothem carbonate; consequently, changes in hydrology can have important implications when interpreting paleoclimate-proxy data in speleothems.

This study presents high-resolution cave monitoring data from Yok Balum Cave, Belize. These data provide detailed information regarding seasonal cave ventilation mechanisms by demonstrating cave pCO_2 and air density relationships and examination of thermal gradients as evidence of internal-external air exchange. An understanding of the subtle seasonally variable fluxes of cave air CO_2 allows improved interpretations from not only Yok Balum, but also other tropical sites. Additionally, active monitoring during a large (M7.4) earthquake in November 2012 provides a unique opportunity to assess the response of cave atmosphere and hydrology to substantial seismic activity.

Study Site

Yok Balum Cave (Mopan Mayan for Jaguar Paw Cave) is located in the Toledo District of southern Belize, approximately 3 km south of the modern Mopan Maya village of Santa Cruz (16°12′30″ N, 89°4′24″ W; 366 m above sea level) (Fig. 1a). The cave is developed within the tectonically uplifted Cretaceous Campur Limestone formation that originated from massive limestone deposition around the granite intrusions composing the Maya Mountains to the north (Miller, 1996; Kennett et al., 2012). The cave is one of several that occur in a southwest-to-northeast-trending limestone-karst ridge (Fig. 1b), and its formation was likely associated with the vertical flow of chemically aggressive allogenic water originating on the highlands of the Maya Mountains (Miller, 1996), although no stream exits within the cave today. Yok Balum extends approximately 540 m as a main trunk passage from a small opening in the west, the main entrance, to a larger, more elevated opening to the south, the second entrance (Fig. 1c). The second entrance resulted from cave collapse probably associated with tectonic activity. U-series dating of the base of a stalagmite growing on a breakdown block associated with the creation of the second entrance dates the collapse at a minimum of 44,000 ± 3300 years BP. There is also considerable evidence of seismic activity within the cave, including large faulted flowstones and displaced speleothems. U-series dating of carbonate precipitated within a faulted flowstone provided a date of 26,400 ± 170 years BP.

The western coast of Central America displays relatively high seismic-hazard potential due to the subduction of the Cocos Plate beneath the North American and Caribbean Plates (Fig. 1a). A divergent boundary exists between the North American and Caribbean Plates approximately 100 km south of the southern Belize border. The dominant source of seismic activity felt in southern Belize, however, results from intermediate depth earthquakes occurring within the subducted Cocos Plate.

Monitoring Instrumentation

Tropical environments provide a challenging environment for electronic monitoring instrumentation, especially for long term monitoring studies in remote areas. In this study, the threat of malfunction due to high humidity and water was minimized by keeping non-waterproof equipment in airtight boxes and sealed plastic bags with a silica desiccant where applicable. Above-cave soil temperature was recorded hourly using a Tiny tag temperature logger buried at a 0.4 m depth. Cave-air CO_2 was monitored every three hours between April 2011 and January 2013 (with a four month break from June 2012 to October 2012 due to equipment failure) using a Vaisala Carbocap carbon-dioxide probe DMP343 (± 3 ppmv + 1% of reading) linked to a Vaisala MI70 indicator and powered by two Duracell MN918 lantern batteries. A Radon Scout Plus, powered by two D-cell batteries and a four-D-cell external battery pack was

Table 1. Summary of equipment used in this study, including equipment accuracy, sampling interval, and additional comments.

Parameter	Equipment	Accuracy	Monitoring Interval	Comment
Cave Air CO_2	Vaisala CARBOCAP Carbon Dioxide GMP343 Probe with Vaisala MI70 Indicator (plus two Duracell MN918 Lantern batteries)	± 3 ppmv ± 1% of reading	Three-Hourly	Tested upon installation.
Temperature	Tinytag Logger	± 0.1°C	Hourly	Data compared to Barotoll temperature data.
^{222}Rn	Radon Scout Plus (plus 4 × D-cell External battery pack)	⋯	Three-Hourly	Ten days of data discarded after data download due to anomalously high ^{222}Rn counts after opening the box.
Pressure and Temperature	Barotroll	± 0.1%	Hourly	Data compared to Tinytag temperature data.
Rainfall	Pluvimate Rain Gauge	± 0.01 mm	Hourly	Located at Santa Cruz village.
Drip Rate	Stalagmate Drip Logger	⋯	Hourly	Tested upon installation.

set up next to the within-cave CO_2 logger to measure radon fluctuations every three hours for the same time interval. The Radon Scout, being extremely sensitive to moisture, was kept in a watertight box. This resulted in a muted radon measurement, because fewer α particles reached the alpha counter. For qualitative assessment of ^{222}Rn fluctuations this was not considered an issue. However, ^{222}Rn values peaked following a data download when the logger was removed from the box. To account for this, ten days of data were removed after each data download to allow ^{222}Rn values to return to normal levels. Combined Barotroll pressure and temperature loggers were installed both inside and above the length of the cave to measure hourly barometric pressure and temperature (precision ± 0.1% and ± 0.1°C). Tinytag temperature loggers were placed in transect along the cave to measure hourly temperature. Stalagmate automated drip-loggers recorded hourly rates of drips feeding three stalagmites of potential paleoclimate interest. Data were downloaded and the equipment maintained every four months. A summary of all monitoring equipment is shown in Table 1. The location of all equipment and monitored stalagmites is shown in Figure 1c.

CAVE VENTILATION

Cave ventilation (air exchange with the outside atmosphere) has a first-order control on cave atmosphere composition and is dependent on a number of factors, including fluctuations in temperature and pressure, cave geometry, and susceptibility to external winds (Cowan et al., 2013; Bourges et al., 2001; Spötl et al., 2005; Baldini et al., 2006; Denis et al., 2005; Kowalczk and Froelich, 2010). Pflitsch and Piasecki (2003) classify cave passages in terms of air movement as being dynamic, transitional, or static. However, the static state is very rarely observed, aside from in deep passages (Pflitsch and Piaseki, 2003; Przylibski and Ciezkowski, 1999). Convective air circulation, driven by internal versus external air density differences, is a dominant ventilation mechanism in caves with more than one entrance at different elevations (Gregoric et al., 2013; Kowalczk and Froelich, 2010; Wigley, 1967; Badino, 2010). In tropical caves, where cave air temperatures do not vary significantly on seasonal timescales, air density differences will be predominantly controlled by surface temperature and barometric-pressure variations (Fairchild et al., 2006). Air density responds primarily to temperature (Faimon et al., 2012; Gregoric et al., 2013; Gregoric et al., 2011) and to a lesser extent pressure and humidity as expressed in Equation (1) below (after Kowalczk (2009)):

$$\rho_{\mathrm{air}} = \frac{P}{R_d T_{\mathrm{v}}} \tag{1}$$

where R_{d} is the universal gas constant and P is barometric pressure in mb. T_{v} is virtual temperature, calculated via Equation (2), in which both T and dew point T_{d} are in K.

$$T_{\mathrm{v}} = \frac{T + 237.15}{1 - 0.379\left(\frac{6.11 \times 10^{\frac{7.5\,T_{\mathrm{d}}}{237.3+T_{\mathrm{d}}}}}{P}\right)} \tag{2}$$

If cave pCO_2 is more than an order of magnitude greater than that of the free atmosphere, T_{v} is affected. This can lead to errors of up to 9°C when calculating cave T_{v} (Sanchez-Canete et al., 2013), and consequently, errors in air density calculations. At Yok Balum the maximum recorded pCO_2 is 770 ppm and the summertime mean is ~ 500 ppm. This is less than an order of magnitude greater

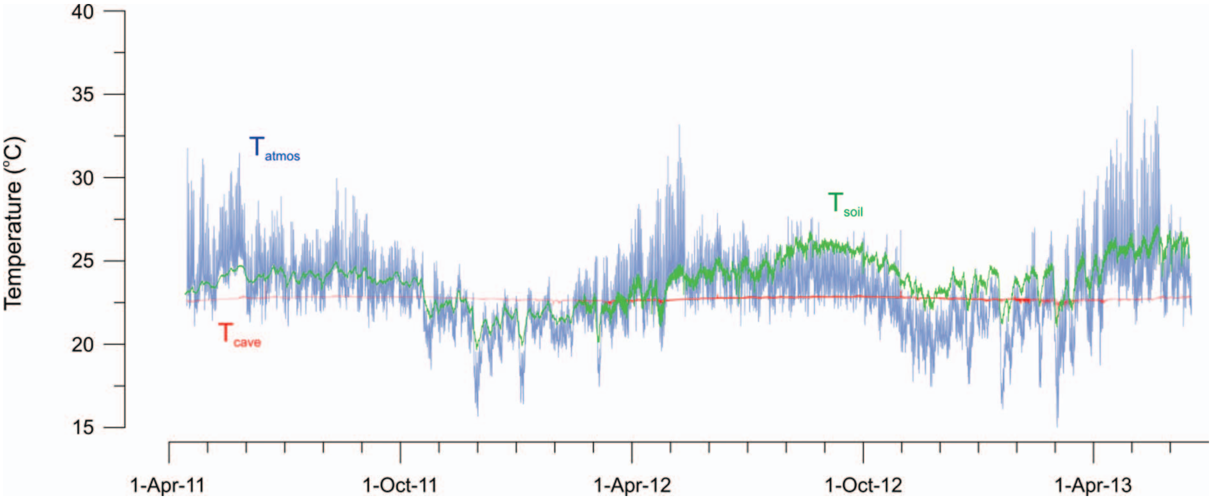

Figure 2. Time series for 26.5 months of hourly cave temperature (T_{cave}) (using the Barotroll logger), soil temperature (T_{soil}) (measured using a TinyTag), and outside air temperature (T_{atmos}) (using the Barotroll logger).

than the free atmosphere; and therefore, this CO_2 exerts a negligible effect on cave air density.

Typically, during the winter months external air temperature will be cooler than that of the cave and a positive air density difference will dominate, i.e, external air will be denser, although a diurnal signal will also exist. Alternatively, during the summer typically warmer external temperatures will result in largely negative air density differences. Local weather may result in short-lived reversals in cave/atmosphere air density differences. The particular ventilation influence of seasonal air density differences between cave and free atmosphere is governed by the cave geometry (e.g., passage orientation and size), the distance from cave entrances, and total cave volume (Batiot-Guilhe et al., 2007; Cowan et al., 2013).

RESULTS AND DISCUSSION OF VENTILATION

The diurnal and seasonal patterns of airflow at Yok Balum are a direct response to a thermally induced disequilibrium in air density between the cave and outside air, similar to other caves (De Freitas et al., 1982; Kowalczk and Froelich, 2010). Within-cave temperature is nearly constant at 22.4°C (± 0.5°C) year-round, although a low-amplitude diurnal signal is present. Within-cave temperature is equivalent to the average yearly external temperature (Fig. 2) and is likely a result of moderation of outside temperatures by the epikarst. External air temperature can affect cave air $p\mathrm{CO}_2$ by both inducing density-driven ventilation associated with inside-outside air density differences (De Freitas et al., 1982) (Fig. 3a) and by causing higher soil $p\mathrm{CO}_2$ by stimulating biological activity in the soil zone (Baldini et al., 2008; Bond-Lamberty and Thomson, 2010; Hess and White, 1993; Murthy et al., 2003; Sherwin and Baldini, 2011).

The simple structure of Yok Balum Cave, with two entrances at either end of a single main trunk passage, results in a well-ventilated dynamic cave system, evidenced by the low annual mean CO_2 values (461ppm) (Fig. 3b). However, CO_2 displays clear seasonal trends in both mean concentration and variability. Summer (April–October) is characterized by higher mean $p\mathrm{CO}_2$ (~ 500 ppm) and high temporal variability (standard deviation of 72.5 ppmv) whereas winter (November–March) has lower $p\mathrm{CO}_2$ (~ 420 ppm) and displays lower temporal variability (standard deviation 24.3 ppmv). Here, we use the theory of entropy of curves to highlight the differences between summer and winter ventilation. Entropy (E) is a measure of variance within a dataset. It is described as the mean cumulative sum of absolute first differences of a time or spatial derivative (Denis et al., 2005; Denis and Crémoux, 2002), or specifically, in this case, the average change in $p\mathrm{CO}_2$ values at 3-hour intervals. Higher entropy values indicate a greater change in subsequent $p\mathrm{CO}_2$ measurements; and therefore, an indication of variance within subsets of the dataset. CO_2 displays entropy of approximately 430 during the summer and 150 during the winter (Fig. 3b), indicating that the variance is nearly three times greater during the summer months. These trends in $p\mathrm{CO}_2$ mean values and variance are controlled by seasonal CO_2 flux into the cave and ventilation, most likely controlled by external temperatures and infiltrating rainfall. The following sections will use high-resolution-monitoring data to describe the seasonal ventilation regimes occurring in Yok Balum. It should be noted that the summer and winter seasons are not synonymous with the wet and dry seasons.

SUMMER REGIME

Air density differences between the cave and the free atmosphere, controlled predominantly by external

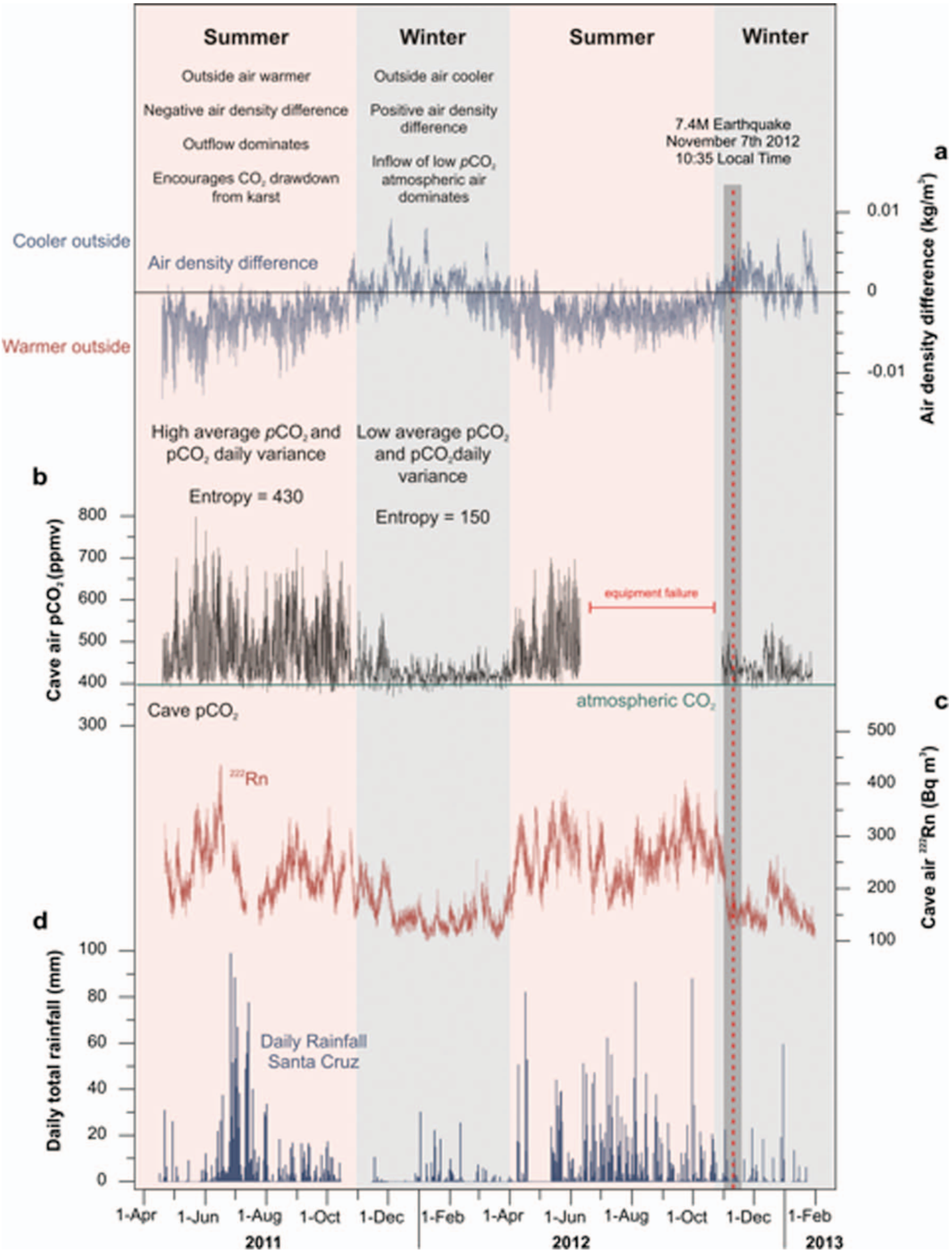

Figure 3. Seasonal regimes and dominant characteristics of (a) hourly inside versus outside air density difference. (b) Three-hourly cave pCO_2 and summer (April through October) and winter (December through March) pCO_2 entropy values. Horizontal line at about 400 ppmv is CO_2 content of outside air, presumed constant. Three hourly ^{222}Rn (c) and daily rainfall at Santa Cruz (d). November 7 earthquake indicated by the red dashed line and surrounding two-week period by the gray-shaded section.

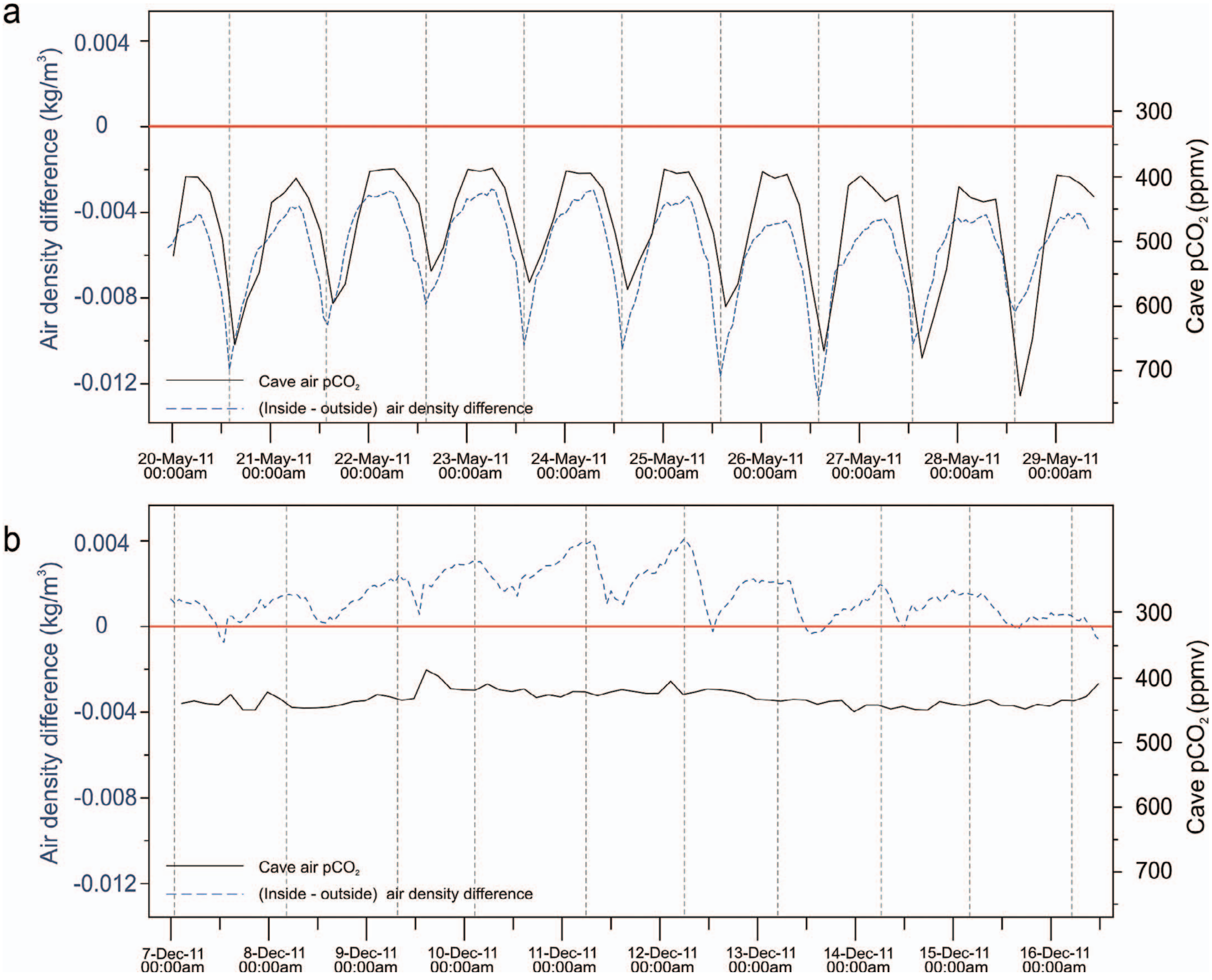

Figure 4. Hourly density difference between cave and outside air and cave pCO$_2$ over (a) December 7–16, 2011 (winter) and (b) May 20–39, 2011 (summer).

temperature, drives the summer season diurnal ventilation regime. Outside air temperatures (T_{atmos}) are higher on average than that inside the cave (T_{cave}) producing an almost constant negative air density difference (Fig. 3b). In a typical one-entrance cave system this could cause severe season-long stagnation and consequently very high pCO$_2$ as the cooler, denser cave air becomes trapped at the lowest point of elevation in the cave (Cowan et al., 2013; Spötl et al., 2005). At Yok Balum complete stagnation does not occur because the dual-entrance system provides for density-driven flow from the more elevated southern entrance to the lower western entrance.

Outside air temperature begins to rise around 0600 and reaches a maximum in the early afternoon. At this point cave/atmosphere air density difference is greatest and air outflow is at a maximum (Fig. 4a). As the air density difference increases during this period, outflow occurs at both entrances; CO$_2$ concentrations will simultaneously increase as high pCO$_2$ air is drawn out of the overlying epikarst and soil zones (Fig. 5a). During the day, biological activity in the soil will also be at a diurnal maximum, producing higher soil pCO$_2$. By late afternoon the cave/atmosphere air density difference begins to decrease and the volume of outflowing air decreases, reducing CO$_2$ drawdown from the epikarst. Outflow at the lower main entrance weakens or ceases completely. As the cave/atmosphere air density difference reaches a minimum, around 0100, cave air pCO$_2$ reaches minimal values. This is most likely due to minimized CO$_2$ drawdown and inflow of low-pCO$_2$ atmospheric air from the second entrance if T_{cave} reaches or surpasses T_{atmos}, flushing through the cave from the second entrance to the lower main entrance (Fig. 5b). If the

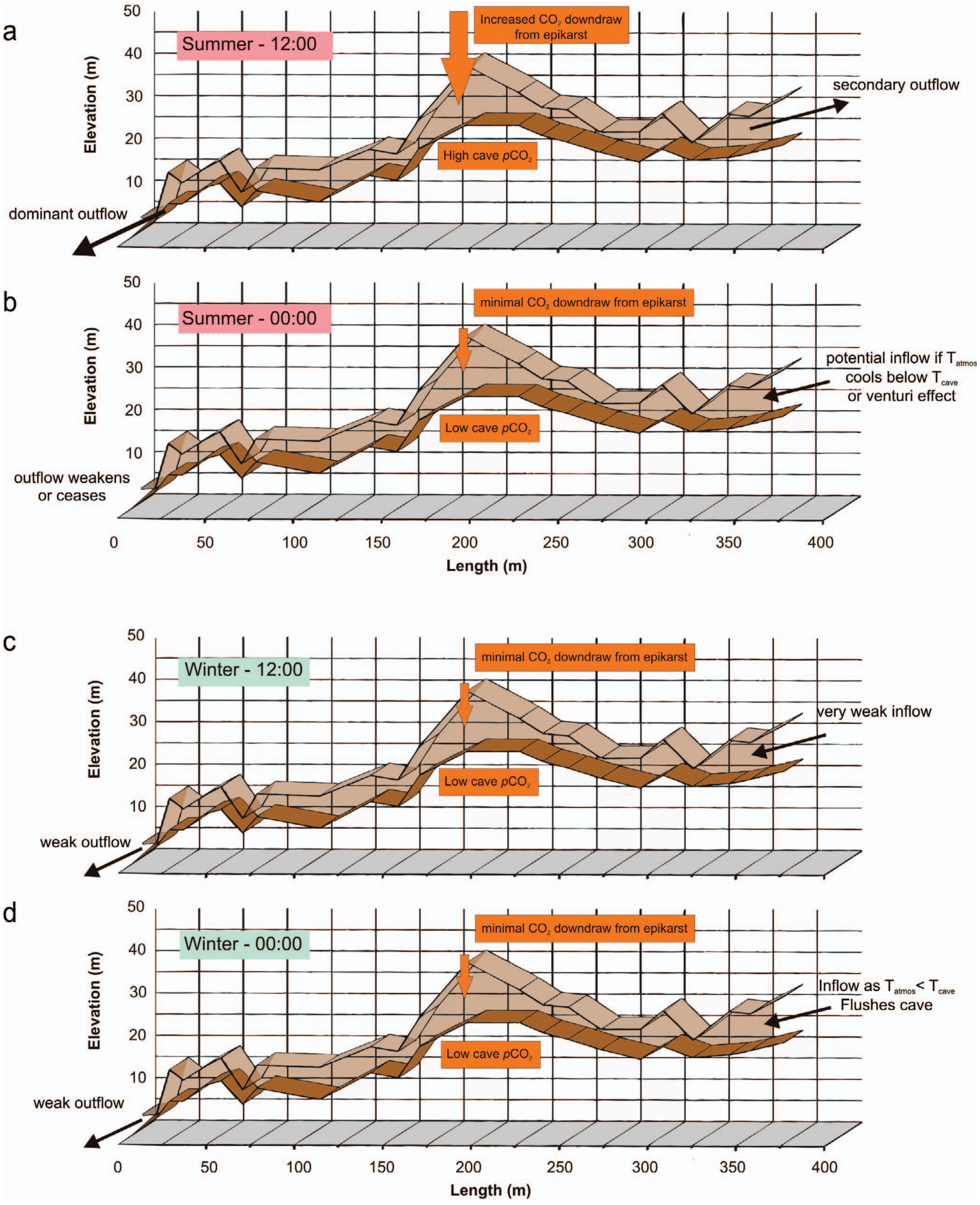

Figure 5. Yok Balum long profile with schematic of theorised primary air flows and CO_2 flux during the summer at noon (a) and midnight (b). Schematic of theorised primary air flows and CO_2 flux during the winter at 1200 (c) and 0000 (d).

outside air density remains considerably higher than that of the cave, then $p\mathrm{CO}_2$ may remain elevated, but will decrease somewhat due to decreased CO_2 drawdown from the epikarst, lower soil activity, and some air movement driven by the venturi effect (Fig. 5b). Areas closest to the entrances can be expected to undergo the most ventilation, particularly at the second entrance, which is larger. Increased water through-flow during the wet season is undoubtedly an additional driver of higher average summer $p\mathrm{CO}_2$, as it increases dissolved CO_2 transport to the cave, increasing degassing, and consequently, producing higher cave $p\mathrm{CO}_2$.

WINTER REGIME

During the winter season, outside air temperatures are generally cooler than those inside the cave, producing a positive air density difference and a ventilation regime dominated by inflow. Ventilation is therefore more continuous than during the summer.

Maximum air density difference occurs at night (around 0300) when T_{atmos} is at a minimum (Fig. 4b). Cooler outside air flushes into the cave, predominantly through the more elevated second entrance, but also from the main entrance. Cave-air $p\mathrm{CO}_2$ will therefore approximate that of the external atmosphere. Outside air temperatures begin to rise at about 0600 and reaches a maximum at about 1400, as in the summer season. However, as the outside temperature increases, it approaches that of the cave air, reducing the air density difference to near zero, or to negative values if T_{cave} surpasses T_{atmos}. This reduces air inflow to the cave, and if a negative air density difference occurs, then outflow may occur during this time (Fig. 5c). This variation of air density difference over a threshold value results in a daily ventilation regime whereby the cave inhales during the day and exhales at night. The inhalation during the day draws low-$p\mathrm{CO}_2$ air into the cave, flushing the cave and keeping $p\mathrm{CO}_2$ values similar to atmospheric levels (Fig. 5d). Any weak exhalation at night continues effective air turnover and maintains low $p\mathrm{CO}_2$ concentrations. Again, it is the areas close to the entrances that will undergo the most rigorous air turnover.

A combination of inflow-dominated ventilation and less CO_2 from drip-water degassing keeps winter cave-air $p\mathrm{CO}_2$ at near atmospheric levels. A less distinct diurnal regime is observed in CO_2 and air density difference variability. During the summer, increased water through-flow, strong air outflow and large CO_2 drawdown increase average $p\mathrm{CO}_2$ and daily variability.

TEMPERATURE OBSERVATIONS

Hourly temperature data are used as an indicator of air movement to determine seasonal modes of ventilation and to understand exactly how air moves through the cave. We use Tinytag (TT) temperature loggers at different sites to assess thermal variability. TT3, a temperature logger located about 50 m from the second entrance, shows more variance than TTI, located about 50 m from the main entrance, and TT2, located in the midsection of the cave about 100 m from the main entrance and about 140 m from the second entrance (Fig. 6e). TT2 displays the least variance and most moderated temperature (Fig. 6b). Entropy (see section 5) can be illustrated graphically through time as a cumulative curve, the slope of which indicates variability within the dataset. Entropy curves are calculated for each temperature dataset from the three loggers (Fig. 6a), thus facilitating comparison of their variance with time. TT3 displays the greatest variability over the whole time series, suggesting that this region of the cave is most strongly coupled with external air temperatures via air exchange. During the summer months TT3 increases by 0.4°C, as air in this region responds to warmer external temperatures. TT2 is stable through the same period, and TT1 displays an increase similar to that of TT3, but of only 0.3°C. This thermal variability decreasing with distance from a cave entrance is in accordance with a traditional cave temperature models (Wigley, 1967) and previous thermal profile studies of caves (Sanchez-Canete et al., 2013; De Freitas et al., 1982).

During the winter, TT3 displays greater variance than the other two loggers, again indicating that this section of the cave is more closely coupled to the outside air during winter than summer (Fig. 6). This is consistent with the ventilation mechanism described in the previous section, where inflow of cooler atmospheric air dominates the winter ventilation regime, simultaneously lowering long-term cave-air temperature in this area of the cave and mimicking the diurnal external temperature cycle in the cave. TT2 remains the least variable, due to its location in the midsection of the cave. TT1 decreases, indicating that cooler atmospheric air flows in, but that ventilation at the main entrance is less rigorous than at the second entrance. Furthermore, short-lived decreases in temperature recorded by TT1 (and to a lesser extent in TT2) are in anti-phase with TT3. This could be an indication of air entering at the main entrance and flushing through the cave, forcing warmer air from the less dynamic mid-section of the cave through to the second entrance, where it is recorded as a small increase in temperature at TT3. This thermal-pulse process would also operate in reverse, with cooler air entering at the second entrance and forcing air through the cave to the main entrance.

Data collected through high-resolution temperature experiments, conducted over two 14-hour intervals in June and late October 2012, are used to characterize ventilation on short time scales. A transect of three temperature loggers placed in the cave recorded temperature every 10 seconds to capture very short-term thermal fluctuations overnight, from 1800 to 0800. Failure of one of the loggers during the June experiment limits the number of loggers to two, but does not affect data interpretation for this project. During the June experiment (Fig 8) the two temperature loggers, TT5 and TT7, record essentially static temperature, supporting the idea that air density driven outflow dominates during this season. During the logging interval, the cave air density difference does not drop below zero and so

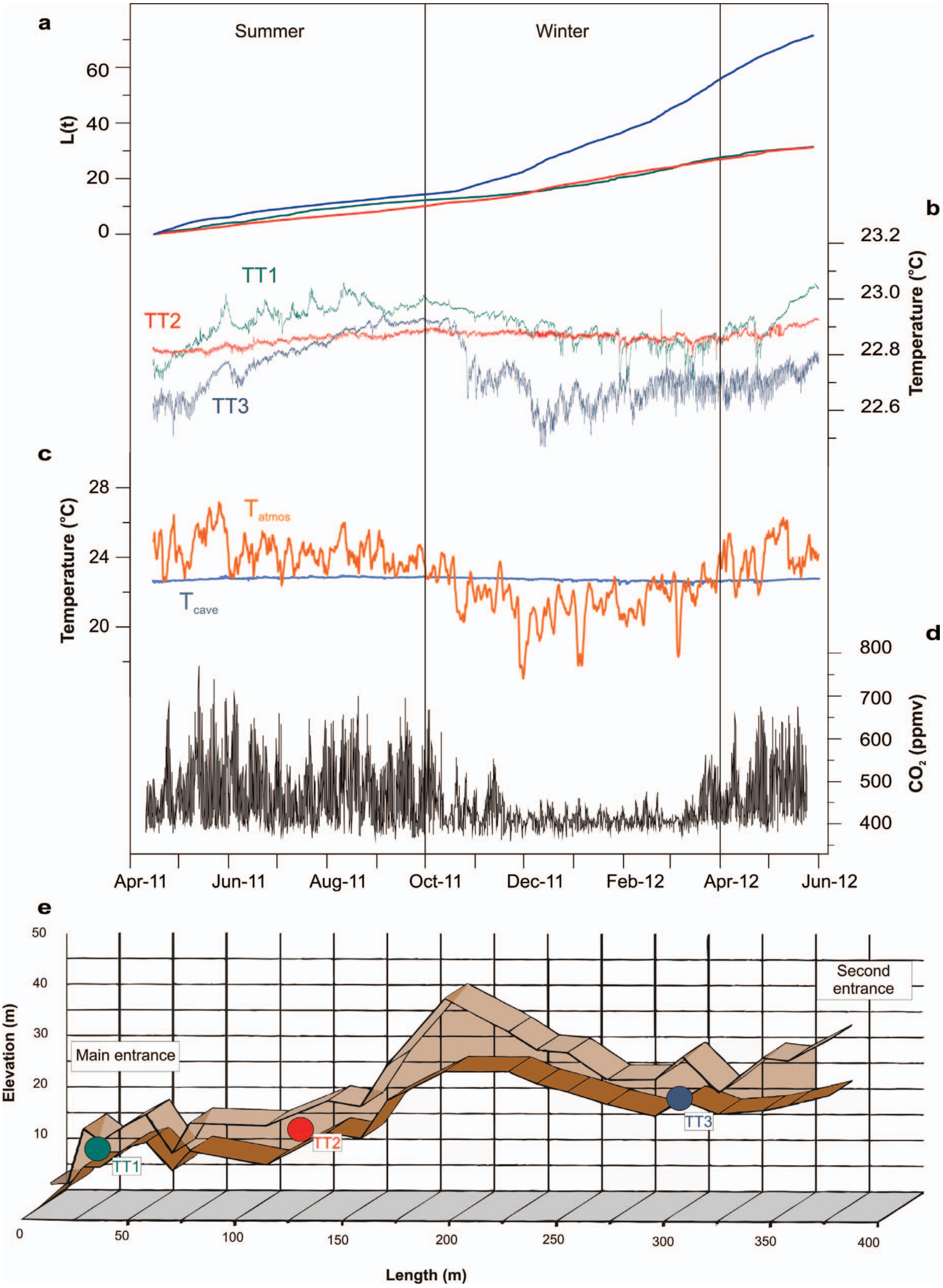

Figure 6. Time series for 14 months of (a) variability L(t) of three temperature time-series expressed as entropy (cumulative sum of the absolute first differences) against time, (b) hourly temperature from the three temperature loggers in the cave, (c) hourly record of temperatures inside (TT2) and outside the cave, (d) three hourly cave air pCO$_2$, and (e) location of temperature loggers within the cave.

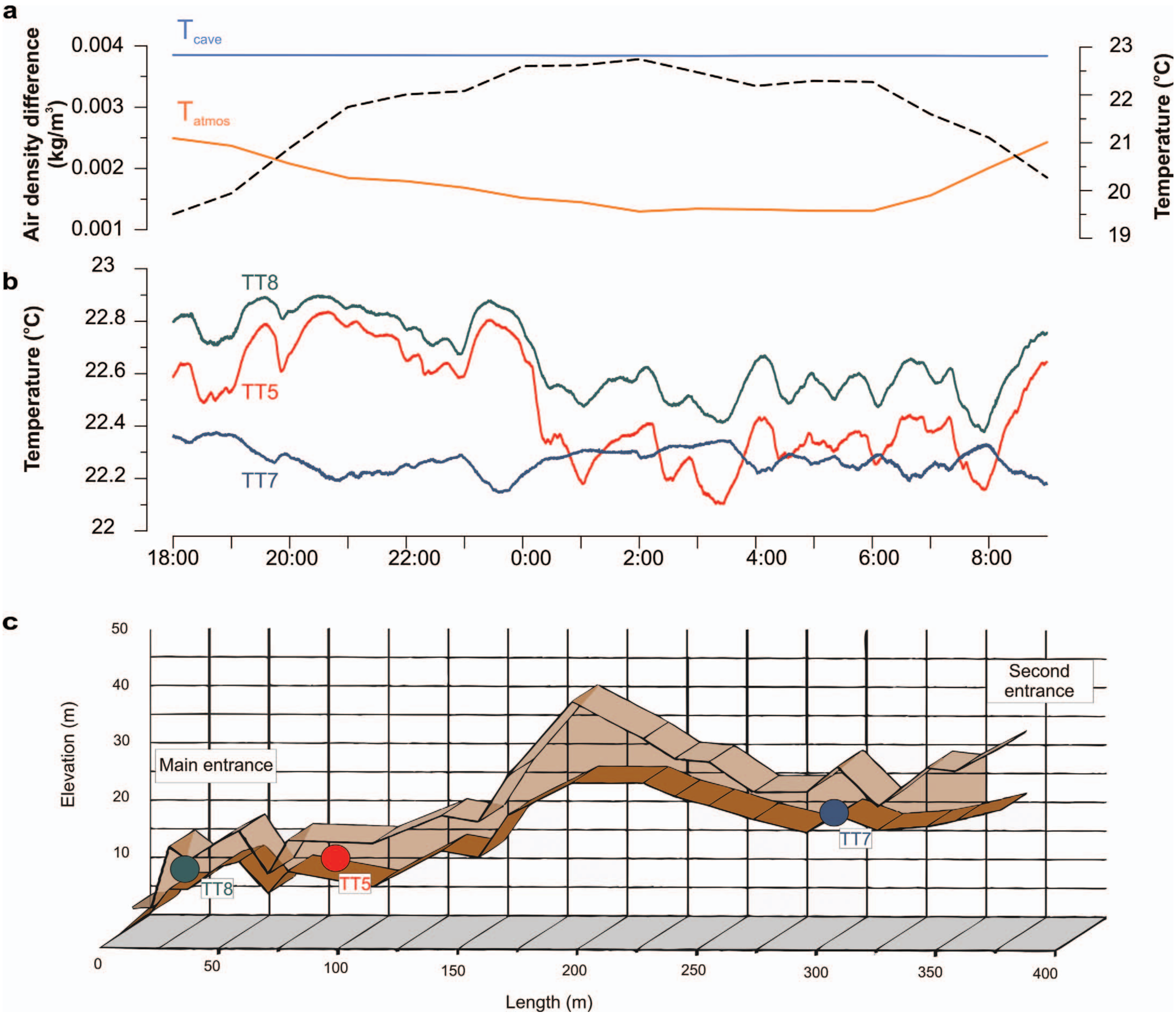

Figure 7. High-resolution winter experiment. Fourteen-hour time series of (a) hourly cave temperature, external air temperature, and internal/external air density difference and (b) 10-second temperature measurements of TT8, TT5, and TT7. (c) shows location of TT8, TT5, and TT7 in the cave.

inflow does not occur. During the late-October experiment, thermal variance at all three sites is much greater (Fig. 7). TT5 and TT8 record more thermal variability than TT7, suggesting that air inflow close to the main entrance is less persistent. TT5 and TT7 both record cooler temperatures than TT8, presumably due to their proximity to a cave entrance. TT8 and TT5 track each other, roughly in accordance with cave/atmosphere air density difference and are weakly in anti-phase with TT7. This is similar to what we see in the longer-term record (Fig. 6), where cooler external air enters the main entrance and forces air along the main passage, which is recorded as a pulse of warmer deep-cave air at the second entrance. It would appear from this high-resolution time series that this occurs in both directions. The limited temporal timeframe of these two experiments hinders making firm conclusions about the diurnal movement of air at Yok Balum Cave, but it is encouraging that the results acquired are in agreement with the longer, hourly-resolution time series.

NOVEMBER 7 EARTHQUAKE

On November 7, 2012, at 1635 (UTC; 1035 local time) a 7.4 magnitude earthquake struck off the coast of Guatemala (Fig. 1a). The epicentre was estimated to be at a depth of 24.0

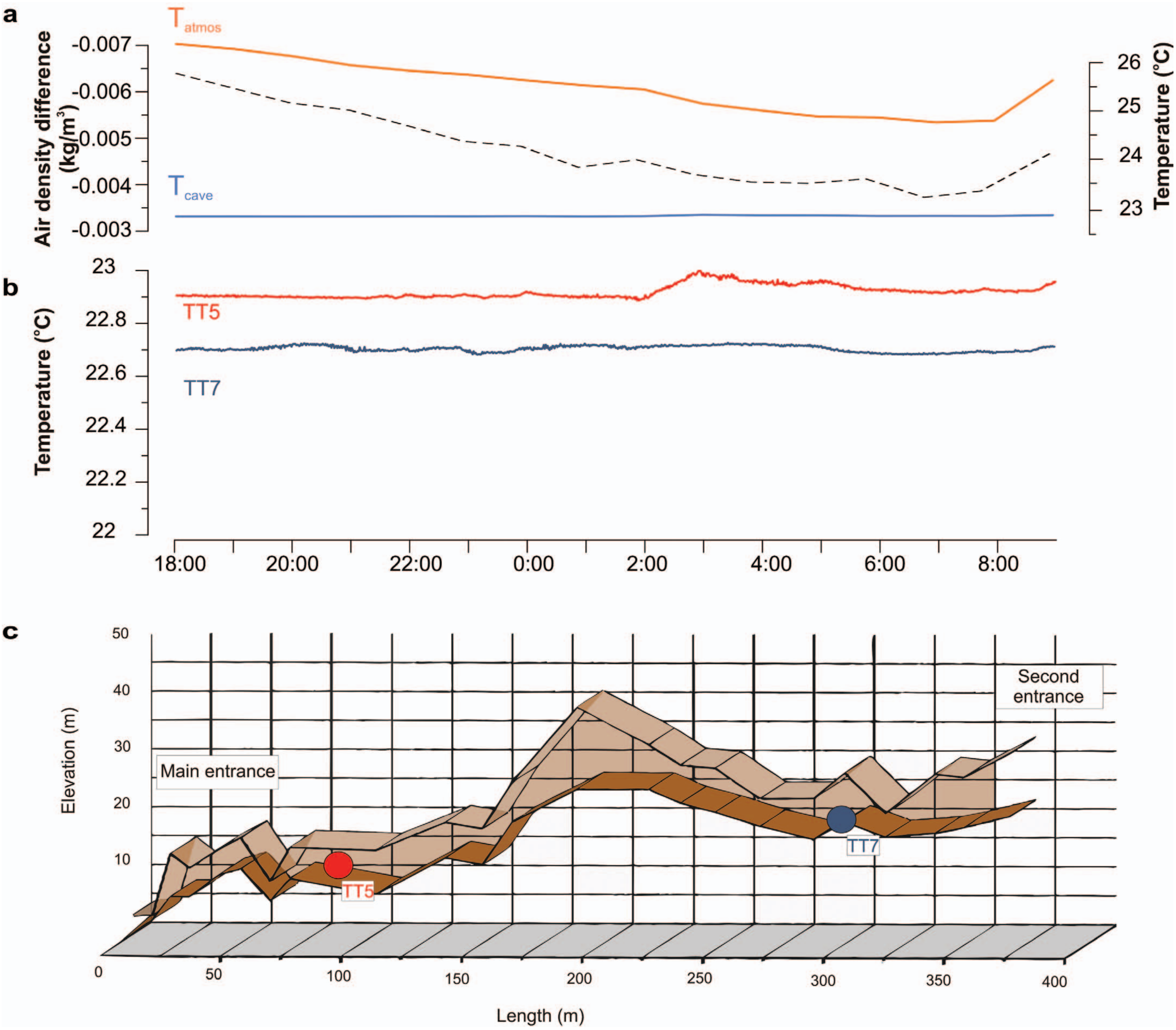

Figure 8. High-resolution summer experiment. Fourteen-hour time series of (a) hourly cave temperature (from Tinytag loggers), external air temperature, and calculated internal/external air density difference and (b) 10-second temperature measurements of TT5 and TT7. (c) shows location of TT5 and TT7 in the cave.

km and occurred as a result of thrust faulting on or near the subduction zone of the Cocos plate and the overlying Caribbean and North American plates. Tremors were felt in parts of Belize, and villagers from Santa Cruz village, 5 km from Yok Balum Cave, reported feeling the tremors. According to United States Geological Survey estimates, this shock would result in a seismic hazard, measured in peak ground acceleration at the cave site of 1.6 to 2.4 m s^{-2}. A field crew returned to the cave in January 2013 to find large fallen blocks at the cave's main entrance and numerous displaced and freshly broken stalagmites and stalactites within the cave. Reasonable evidence therefore suggests that the cave was subject to seismic activity on or around November 7, 2012. There are only a handful of published studies reporting earthquake damage to caves (Gilli, 1999; Gilli and Delange, 2001; Renault, 1970), and so direct monitoring observations of the effect of seismic activity are pertinent to the science of speleology, in general, and have implications for reconstructing climate from cave deposits. It will be particularly useful to determine how environmental variables that affect speleothem growth and carbonate deposition, such as drip hydrology and cave ventilation, may be affected be seismic activity. For example, if seismic activity causes considerable water re-routing, we might expect subsequent changes in speleothem growth for days to months, which can confuse a climate record. Similarly, atypical speleothem growth or carbonate isotopes could be produced from significant seismically induced changes in cave $p\mathrm{CO}_2$.

Cave Atmosphere Response

No clear change occurred in cave-air $p\mathrm{CO}_2$ or $^{222}\mathrm{Rn}$ during or for the week following the earthquake (Fig. 9). Two $^{222}\mathrm{Rn}$ peaks occur around the earthquake (Fig. 9a) and although fracture dilation induced by seismic activity may cause such peaks in cave-atmosphere $^{222}\mathrm{Rn}$, these peaks are not significant, in terms of magnitude or duration, when the entire $^{222}\mathrm{Rn}$ dataset is considered (Fig. 3c). A sharp increase in both $^{222}\mathrm{Rn}$ and CO_2 occurs on December 20 (Fig. 3 b and c) but given the short half-life of radon (3.8 days) it is extremely unlikely that this is a delayed signal of the November 7 event. This increase could be explained instead by a coincident decrease in air density difference associated with a moderate rainfall event that temporarily reduced air inflow to the cave and resulted in a short-lived increase in CO_2 and $^{222}\mathrm{Rn}$.

A significant change in cave atmosphere may not have been observed due to the seasonal timing of the earthquake. As previously observed, cave ventilation during the winter is dominated by inflow, which acts to keep $p\mathrm{CO}_2$ levels low. Seismic activity of a similar magnitude occurring during the summer season, when outflow is dominant, may lead to a discernible increase in cave air $^{222}\mathrm{Rn}$ and CO_2. Potentially a clearer influence could be observed in a less well ventilated cave. Prior to the collapse and opening of the second entrance at Yok Balum, ventilation would have been less effective and cave air $p\mathrm{CO}_2$ and $^{222}\mathrm{Rn}$ higher. Considerable seismic activity at that time may have created uncharacteristically high $p\mathrm{CO}_2$ and $^{222}\mathrm{Rn}$ values as a result of limited ventilation and should be a consideration when studying speleothems from Yok Balum deposited prior to the collapse of the second entrance, when ventilation would have been restricted. Similarly, in any cave where ventilation is less efficient it may be pertinent to assess the impact of seismic activity on cave-atmospheric composition, particularly when speleothems from the cave are being considered for palaeoclimate reconstruction.

Hydrological Response

Three drip loggers were deployed during the November 2012 earthquake: YOK-SK, YOK-SD, and YOK-LD. Of these drips, two (YOK-LD and YOK-SD) were static in nature (Smart and Friederich, 1987; Baker et al., 1997), because they displayed low drip rates and low variability (Fig. 10a), indicative of a diffuse-flow-dominated hydrology. YOK-SK is classified as a seasonal drip (Baker et al., 1997), because it responds to local rainfall events and seasonal climate variability (Fig. 10a), suggesting that a fracture flow pathway is activated once a threshold rainfall rate or epikarst saturation is achieved. YOK-SK responds to local rainfall with a lag time of less than 6 days, but displays greater variability during the wet season, when the epikarst and soil are closer to saturation.

None of these three loggers recorded any clear drip response to the seismic activity on November 7 (Fig. 10b). YOK-LD and YOK-SD, the two static drips, show no response, suggesting that diffuse flow regimes are not affected by seismic activity of substantial magnitude. Similarly, YOK-SK, which at the time of the earthquake was displaying a peak in drip rate in response to rainfall events in the preceding days, shows no response outside what would be expected from the longer time series. These data suggest that preferential flow routes are not necessarily altered by seismic activity of this nature. This observation is of significant value for speleothem-based paleoclimate studies, as it suggests that seismic activity does not affect hydrological flow pathways or alter carbonate geochemistry.

Conclusions

Yok Balum is an extremely well ventilated cave system that displays distinct seasonal ventilation regimes, consistent with changes in air density differences between the cave and outside atmosphere. The winter regime is dominated by air inflow, low $p\mathrm{CO}_2$, and lower epikarstic drawdown and CO_2 flux into the cave. Conversely, air outflow, high epikarstic CO_2 drawdown, increased drip-water degassing, and a strong diurnal signal dominates the summer regime. Based on air-temperature changes, the degree of air exchange increases from the center of the cave to the entrances, and the second entrance experiences greater air exchange than the main entrance, presumably due to its size. By looking at thermal fluctuations of cave air on a ten-second timescale, the direction of air movement is identified during summer and winter nights, respectively, and both entrances are found to display active dual-directional connections to the free atmosphere. The three datasets presented here: long term three-hour CO_2, hourly temperature, and the two high-resolution studies all help to build a comprehensive understanding of ventilation at Yok Balum Cave. This will be pertinent as on-going paleoclimate research at this cave. Continued monitoring will help to discern inter-annual fluctuations and identify long-term links between cave $p\mathrm{CO}_2$ and local climate.

Cave $p\mathrm{CO}_2$ and $^{222}\mathrm{Rn}$ did not show any discernable response to the November 7 earthquake. Likewise, none of the three drips displayed any discernible hydrological response to the earthquake, suggesting that seismic activity, even of considerable magnitude, has minimal hydrological repercussions at Yok Balum and is insufficient to result in perturbations in speleothem petrographical or geochemical records. It is noteworthy that the three loggers recorded two end members of standard drip types, from highly

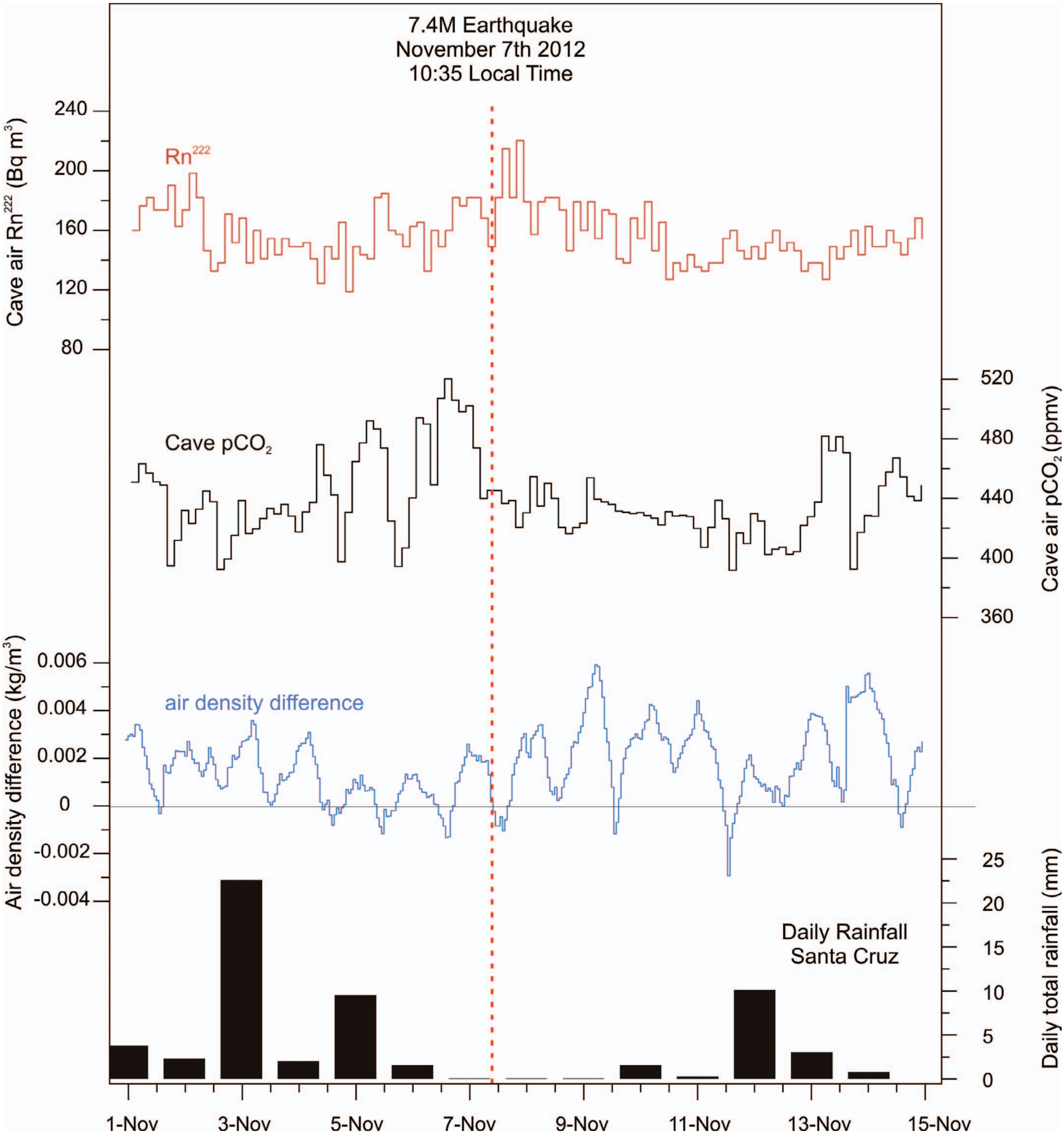

Figure 9. Traces of (a) cave-air Rn^{222}, (b) CO$_2$, (c) calculated inside cave versus outside air density difference, and (d) daily rainfall at Santa Cruz village during a 15-day period surrounding the November 7 earthquake (red dashed line).

diffuse, slow, and static drip rates (YOK-LD and YOK-SD), to highly variable and relatively fast drip rates (YOK-SK). This suggests that intermediate drip types would probably be similarly unaffected by seismic activity of a similar magnitude. The primary effect seismic activity may have on a speleothem record is by altering the growth-axis position or orientation, rather than a direct disruption of the overlying hydrology. This could appear as a hiatus or sudden shift in isotope values if the growth axis movement was not accounted for during milling.

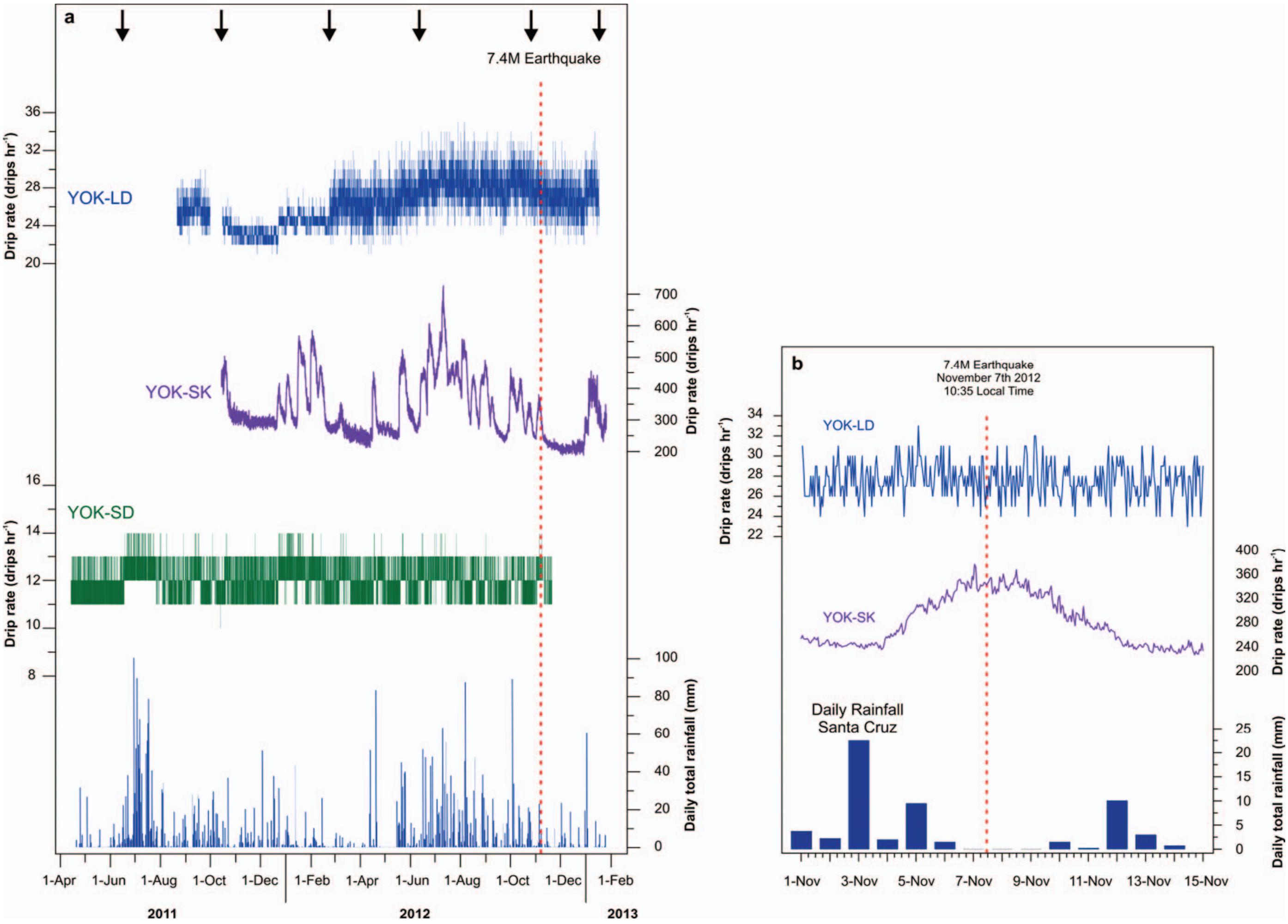

Figure 10. (a) Time series for 22 months of drip regimes of YOK-LD, YOK-SK, and YOK-SD against Santa Cruz daily rainfall; note different scales. November 7 earthquake indicated by red dashed line. Black arrows indicate visits to the cave. b) YOK-LD and YOK-SK drip- rates and Santa Cruz daily rainfall from 1 through 15 November, with time of earthquake indicated by red dashed line.

This study provides real-time data on the effect of seismic activity on cave hydrology and atmosphere. In seismically active regions, determining this site-specific response is a desirable outcome of cave monitoring studies designed to aid speleothem paleoclimate proxy interpretation. These data provide encouraging evidence that seismic activity of this level does not have implications for speleothem paleoclimate proxy interpretations from caves with similar ventilation dynamics as Yok Balum.

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QUANTIFYING WILDLIFE USE OF CAVE ENTRANCES USING REMOTE CAMERA TRAPS

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Abstract: Digital infrared remote camera traps were placed at the entrance of twelve caves in Great Basin National Park, Nevada during the summer of 2013 to assess the wildlife use of cave entrances. The use of caves by surface wildlife is a major nutrient source for cave organisms that spend their entire lives underground. Cave entrances varied in size (0.9 to 50 m^2), cave length (10 to 1000 m), surface habitat (riparian versus pinyon/juniper), and management approach (gated versus no gate). Data from eight cave entrances are presented, with four other entrances removed from the analysis due to equipment failure. The cameras were deployed for a total of 372 trap days, with an average of 46.5 days per cave (range 28 to 62). The cameras captured 632 trap events, with separate events defined as more than an hour apart for the same species. Of the seventeen taxa documented, the most abundant species photographed were mice, chipmunks, humans, woodrats, and squirrels. Other species observed in cave entrances were cottontail rabbits, bats, skunks, foxes, insects, birds, and domestic dogs. Wildlife entered and exited caves most frequently between 1800 and 0600. Very little information has been previously documented about fauna using cave entrances, and this non-invasive, repeatable technique can help managers learn more about the dominant species using the entrance and twilight areas of the caves they manage, as well as peak use times.

INTRODUCTION

Cave entrances provide important habitat to wildlife. Cave entrances can offer a thermal and moisture refuge from above-ground conditions, particularly in desert areas where summer daytime and winter nighttime temperatures can vary more than 30 °C from the relatively steady temperature found underground (Cigna, 2004). Cave entrances provide shelter for a wide variety of vertebrate and invertebrate species that may not use deeper cave environments. They serve as food caching locations for many animals, including mice. In the American West, they frequently provide suitable habitat for nesting cave swallows (*Petrochelidon fulva*) as well as woodrats (*Neotoma* spp.). Over 44,000 caves are known within the United States (Culver et al., 1999). Many caves have an interesting and varied fauna, including many species that are cave obligates. Despite knowledge of what lives in caves, few studies have focused on wildlife use of the transition zone between above and below ground, especially in desert environments (Winkler and Adams, 1972; Strong, 2006; Strong, 2010).

Two main types of wildlife, cave accidentals and trogloxenes, use cave entrances. The facultative use of cave entrances by cave accidentals like beetles and lizards, and the regular use by trogloxenes, such as bats and cave crickets, are critical to cave ecosystems. These wildlife species introduce energy into the nutrient-poor environments in the form of scat, nesting materials, and occasionally carcasses. These deposits provide a nutrient source for troglophiles, species that can complete their entire life cycle in the cave or in a similar habitat aboveground, and troglobites, cave-adapted organisms that never leave caves (Barr and Holsinger, 1985). Therefore, to better understand the nutrient flow into the cave, it is logical to study the use of the cave by cave accidentals and trogloxenes at their point of entrance.

Remote camera trapping, or using remote cameras to take photographs of animals, is a non-invasive technique to study wildlife use. This technique can capture rare and elusive species, monitor animal behavior, and document predation (Kucera and Barrett, 2011). Camera traps can also sample locations where it would be uncomfortable for a person to stay for long. Cameras work both day and night, so diurnal, nocturnal, and crepuscular animals are sampled. In addition, multiple species can be studied at the same time. Studies using remote cameras in various habitats such as forests, shrublands, and riparian areas have been conducted for decades (Kucera and Barrett, 2011), but they have not been reported for cave entrances.

The primary objective of this study is to fill an information gap about which wildlife species use cave entrances, when they use them, and to what extent other variables may influence usage. In particular, I focused on entrance usage by time of day, vegetative habitat above ground, entrance size, and cave gate presence or absence. This information can help managers better understand the role cave entrances play for surface wildlife, as well as better comprehend the role surface wildlife has on nutrient input into caves.

METHODS

The study area is located in Great Basin National Park in east-central Nevada (Fig. 1), part of the Basin and Range

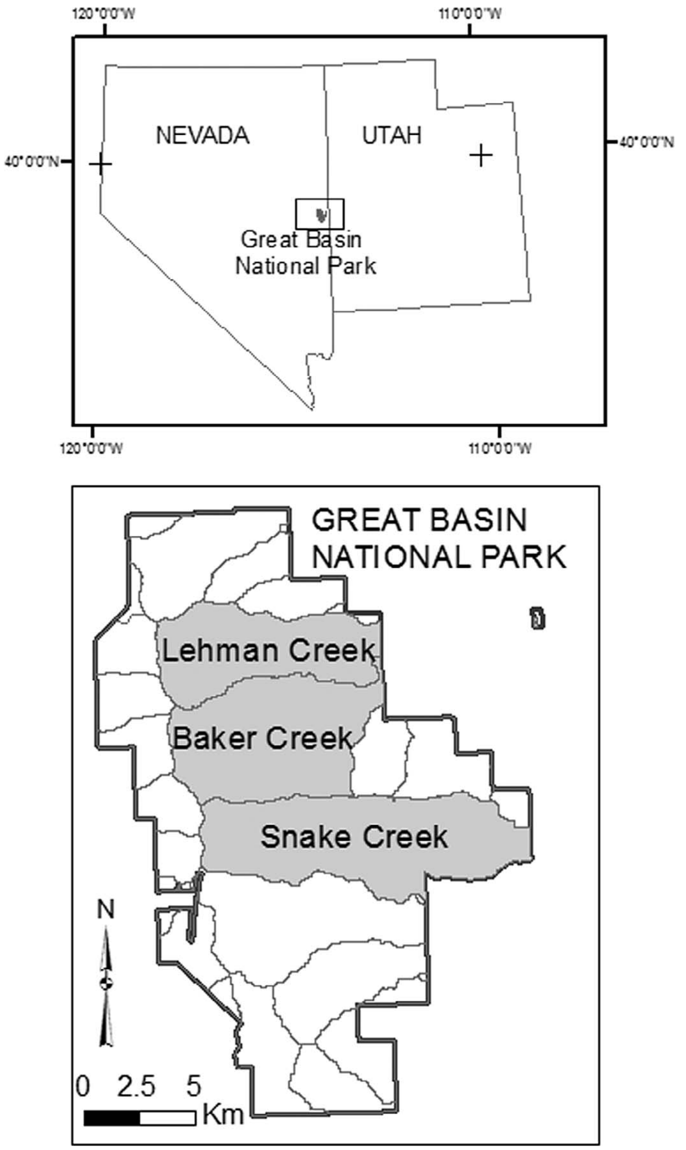

Figure 1. General location of Great Basin National Park, White Pine County, Nevada, and location of study watersheds (Lehman, Baker, and Snake) within the park.

Province. The park encompasses much of the southern Snake Range, which is considered a metamorphic core complex of Proterozoic to Middle Cambrian age. About 40% of the surface rock at the park consists of karst, primarily Middle Cambrian to Ordovician limestones (Hose and Blake, 1976; Miller et al., 1987; Graham, 2014). Elevations in the park range from 1,600 to over 3,900 m. The climate is typical of the high desert, with summer highs exceeding 30 °C and winter lows below –20 °C at the park headquarters, located at 2,070 m. Annual precipitation at this elevation averages 33 cm, with precipitation increasing and temperatures decreasing as one rises in elevation (Elliott et al., 2006; Reinemann et al., 2011).

Twelve caves were selected in the Lehman, Baker, and Snake Creek watersheds based on their accessibility, within a ten-minute walk, and size, at least 12 m in length. The caves were located at elevations from 2,020 to 2,235 m in Middle Cambrian Pole Canyon limestone in the Baker and Lehman watersheds and in the Notch Peak limestone in the Snake watershed. Nearby habitat consisted of pinyon pine (*Pinus monophylla*) and Utah juniper (*Juniperus osteosperma*) woodlands or riparian habitat dominated by water birch (*Betula occidentalis*). Half the caves were gated, and half were not gated. Entrance areas ranged from 0.9 m^2 to 50 m^2. Lengths of the caves ranged from 10 m to 1000 m, with a mean length of 250 m. Data were successfully obtained from eight of the caves (Table 1).

Four models of infrared-triggered wildlife cameras were installed at the caves: Cuddeback Expert (n = 1), Cuddeback NoFlash (n = 2), Reconyx PC 90 HO (n = 2), and Reconyx PC 85 (n = 2). The Cuddeback cameras took one photo per trigger, whereas the Reconyx cameras took one to three photos per trigger. Cameras were mounted with bungee cords or bailing wire to rocks and signs or were propped up with rocks where appropriate flat surfaces were present. Cameras were aimed at or across the cave entrance, but not pointed directly into the rising or setting sun. No lures or bait were used for this study.

The total number of photographs taken, events, and trap days of effort were summarized after subtracting the days

Table 1. Caves selected for remote camera study in Great Basin National Park, White Pine County, Nevada.

Cave Name	Entrance size (in m; width × height)	Gated?	Length, m	Elevation, m	Habitat type	Watershed
Fox Skull Cave	3 × 2	No	60	2020	Pinyon/Juniper	Snake
Ice Cave	2 × 3; 2 × 4[a]	No[b]	900	2150	Riparian	Baker
Lower Pictograph Cave	10 × 5	No	12	2140	Riparian	Baker
Model Cave	2 × 1	Yes	590	2080	Riparian	Baker
Root Cave	1 × 1.5	Yes	120	2090	Pinyon/Juniper	Lehman
System's Key Cave	0.5 × 0.5	Yes[c]	300	2120	Riparian	Baker
Three Hole Cave	1 × 1, 1 × 1.2[a]	No	10	2120	Pinyon/Juniper	Baker
Wheeler's Deep	2 × 3	Yes	1000	2150	Pinyon/Juniper	Baker

[a] Ice Cave and Three Hole Cave both have two entrances, and cameras were alternated between the entrances.

[b] Ice Cave is gated, but the cameras were placed at the ungated section of the cave.

[c] System's Key is gated, and the camera was placed at the gate and later above the gate, which showed a wider variety of animal life.

Table 2. Number of trap events for different wildlife taxa at each selected cave in Great Basin National Park, White Pine County, Nevada.

Common Name	Scientific Name	Fox Skull Cave	Ice Cave	Lower Pictograph Cave	Model Cave	Root Cave	System's Key Cave	Three Hole Cave	Wheeler's Deep Cave	Total Events	Number of Caves
Mouse	*Peromyscus* cf. *maniculatus*	8				19	61	69	6	163	5
Cliff chipmunk	*Tamias dorsalis*	5	12	2	1	11	3	2	33	69	8
Desert woodrat	*Neotoma lepidus*	9	43		7				6	65	4
Human	*Homo sapiens*		1	63				1		65	3
Rock squirrel	*Otospermophilus variegatus*	12		25				5	2	44	4
Bat	unknown				4		15	1	1	21	4
Rock wren	*Salpinctes obsoletus*		1				6	6		13	3
Western spotted skunk	*Spilogale gracilis*	1	1	1			1	8		12	5
Mountain cottontail	*Sylvilagus nuttallii*	7						3		10	2
Gray fox	*Urocyon cinereoargenteus*					6				6	1
Beetle	unknown	3								3	1
Fly	unknown			1	1				1	3	3
Western fence lizard	*Sceloporus occidentalis*							3		3	1
Dog	*Canis lupis familiaris*			2						2	1
Ringtail	*Bassaricus astutus*						1	1		2	2
Black-chinned hummingbird	*Archilochus alexandri*							1		1	1
Cliff Swallow	*Hirundo pyrrhonota*			1						1	1
Unknown		39	9	24	14	10	20	15	18	149	8
Total Events		84	67	119	27	46	107	115	67	632	
Number of species		7	5	7	4	3	6	10	6		
Number of Trap Days		62	53	56	39	24	59	51	28		

Figure 2. A ringtail (*Bassaricus astutus*) outside of System's Key Cave at Great Basin National Park, White Pine County, Nevada.

that the camera malfunctioned, most commonly due to battery failure. Animals that could not be distinguished as individuals and that were captured within one hour of each other were considered to be the same event. After one hour, they were arbitrarily considered to be a new photographic event. All photos were examined at least two times, with wildlife-biologist consultation as needed to reach the lowest taxonomic level feasible, usually species. Microsoft Excel (v. 2007) and Minitab Statistical Software version 14 (www.minitab.com) were used to perform data analyses. Values are presented as means ± standard deviation.

Results

Cameras were deployed at various caves from May 30 to September 20, 2013 (Table 2). During the 113-day study period, the cameras recorded 372 trap days, with 46.5 ± 14.4 days of effort per cave (range of 28 to 62) for eight caves. Cameras at four caves (Lehman Annex, Upper Pictograph, Snake, and Squirrel Springs) malfunctioned, and their data were not included in the analysis. Seventy-six percent of the trap events had identifiable taxa, to species level except for some mice, bats, and invertebrates.

Camera traps documented a minimum of seventeen taxa at the cave entrances. The most common animals captured by the cameras were mice, with 163 trap events (Table 2; includes scientific names). These were followed by cliff chipmunks, humans, desert woodrats, rock squirrels, and bats. Several species were only captured ten to fifteen times, including western spotted skunks, mountain cottontails, and rock wrens. Gray foxes were only captured six times, all at one cave. Ringtails (Fig. 2) were even more elusive, being recorded only twice.

Some taxa were more widespread across cave entrances than others. Chipmunks were found at all eight cave entrances, and mice were found at five of the eight cave entrances. Squirrels, desert woodrats, birds, and bats were recorded at four cave entrances. However, four taxa were found at only one or two cave entrances. The number of taxa per cave entrance varied from three at Root Cave to ten at Three Hole Cave, with a mean of 6.0 ± 2.1 per cave. Species accumulation curves are shown in Figure 3.

The number of trap events per cave ranged from 27 at Model Cave to 119 at Lower Pictograph Cave, an average of 79 ± 33 trap events per cave. To compensate for the varying efforts at the caves, the trap events per camera day were calculated, with a resulting catch of 1.7 ± 0.6 trap events per camera day, with a range of 0.7 trap events per camera day at Model Cave to 2.4 trap events per camera day at Wheeler's Deep Cave.

At the three caves equipped with Reconxy 85 cameras that took three photos for each trigger, the first photo identified the species 73% of the time, while the second photo accounted for 5% and the third photo for 2% of the identifications. The second and third photos did not add to species richness. In 20% of the trap events, the species was not identifiable or was absent from the photos.

Sixty-four percent of trap events occurred during the twelve-hour period between 1800 and 0600 (Fig. 4). Some wildlife species showed distinct preference for particular time periods (Fig. 5). Animals most active at night were bats, mice, skunks, and ringtails. Animals most active during the day were chipmunks, humans, birds, and squirrels. Although woodrats were primarily nocturnal, at Three Hole Cave they showed a surprising tendency to be active during daylight hours as well. Cottontails (Fig. 6) were captured at all hours of the day and night except from 0900 to 1300.

Although ungated caves had a higher number of trap events ($n = 413$) than gated caves ($n = 248$), the difference between the medians was not significant ($p = 0.113$), using a Mann-Whitney U test. Likewise, the difference between pinyon/juniper and riparian habitats was not significant using a Mann-Whitney U test. A regression did not show the length of cave ($p = 0.197$) or cave entrance size $p = 0.260$) to significantly predict different numbers of wildlife. However, some taxa were more common with certain conditions (Fig. 7). Ungated caves accounted for all human visits, as well as 95% of squirrel and 80% of woodrat trap events. Ninety-five percent of bats and 70% of chipmunks were seen at gated caves. No taxa showed a preference for cave entrances in pinyon/juniper areas, but 99% of human, 95% of bat, 86% of woodrat, and 74% of chipmunk trap events were found at caves with entrances in riparian areas. The four longer caves (>250 m) accounted for 95% of bat and 70% of chipmunk trap events, while 100% of human, 91% of squirrel, and 80% of woodrat trap events were at shorter caves.

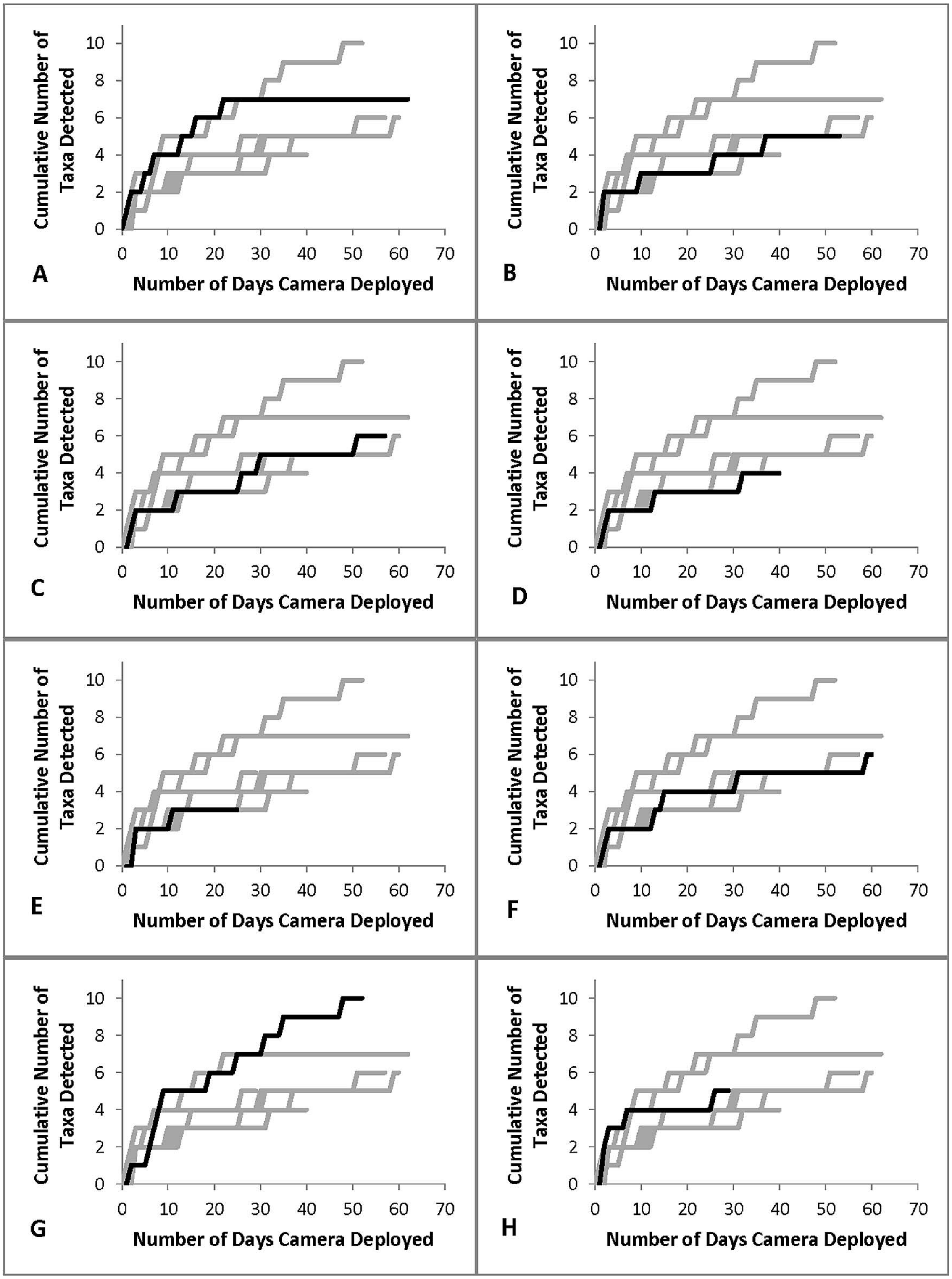

Figure 3. Species accumulation curves from wildlife camera events for each of the eight caves studied at Great Basin National Park, White Pine County, Nevada. A-Fox Skull Cave, B-Ice Cave, C-Lower Pictograph Cave, D-Model Cave, E-Root Cave, F-System's Key Cave, G-Three Hole Cave, H-Wheeler's Deep Cave. Each cave is shown in black, with the other seven caves in gray for context.

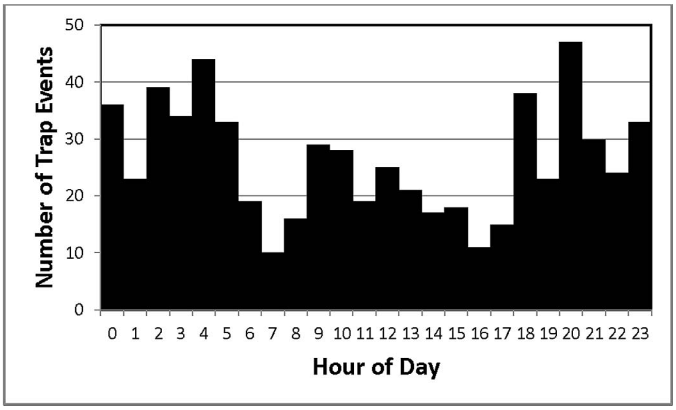

Figure 4. Total number of wildlife camera trap events pooled by hour of day for all eight caves sampled in Great Basin National Park, White Pine County, Nevada.

Discussion

Previous information about overall wildlife use of cave entrances is extremely limited (Winkler and Adams, 1972; Strong, 2006; Strong, 2010), but this low-cost, low-personnel-effort provided a baseline of summer use for multiple cave entrances. In addition to informing managers as to what animals use the cave entrances, the camera traps also allowed a glimpse into the peak times of use and showed habitat preferences for some taxa.

No species were captured that had not been previously documented in the park in other habitats. However, this study was the first to document the use of park cave entrances by several species, including western fence lizard, gray fox, domesticated dog, ringtail, western spotted skunk, rock squirrel, rock wren, black-chinned hummingbird, and cliff swallow. Previous studies (Desert Research Institute, 1968; Stark, 1969; Krejca and Taylor, 2003; Taylor et al., 2008) noted use of park caves by taxa also found in this study: bats, humans, cliff chipmunks, mountain cottontails, woodrats, mice, beetles, and flies. This study expanded the number of caves and the seasons of use by these taxa. The previous studies also found kit fox, black-tailed jackrabbit, broad-tailed hummingbird, and canyon wren use of caves. Taylor et al. (2008) noted that in their biological inventories of 15 caves in Great Basin National Park, evidence of woodrats was found in nearly every cave, but no live specimens were ever seen. This study photographed desert woodrats at half of the caves studied. With regards to small mice, previous studies (Desert Research Institute, 1968; Stark, 1969; Krejca and Taylor, 2003; Taylor et al., 2008) documented them in three caves in the Lehman Creek watershed (Fig. 1): Lehman, Lehman Annex, and Root Caves. Taylor et al. (2008) stated that they were likely facultative trogloxenes but not easily observed. This study had 163 total events showing mice, but most of those were at night, which may account for the difficulty of finding them during standard biological inventories conducted during the day.

I was unable to detect a significant effect of entrance size, length of cave, habitat, and presence or absence of a gate on cave entrance utilization by overall wildlife. However, certain taxa appeared to have preferences. Due to the low sample size of eight caves, it may be easy to oversimplify. For example, 95% of bats were photographed at gated, long caves in riparian areas, but looking more closely at the data, 71% of all bats seen were at just one cave (System's Key Cave). Although it is not surprising that bats were found in the longer caves, it is unknown why chipmunks would prefer entrances of long caves. While several abundant taxa showed an inclination to certain cave entrance characteristics, mice had no preferences for gated/ungated, habitat, or length of cave.

The percentage of unknown events per cave varied from just over one tenth of the events (13.0 and 13.5% for Three Hole Cave and Ice Cave, respectively) to around half of the events (46.4 and 51.9% for Fox Skull Cave and Model Cave, respectively). These differences may reflect different sensitivities of the kinds of cameras, the presence of wildlife that vary in detectability, changes in lighting, triggers due to movement of vegetation due to wind, or differences in appropriateness of positioning of the cameras. In any case, the use of different types of cameras at different cave entrances was a constraint on interpreting the results (Kelly and Holub, 2008). However, Hughson et al. (2010) found that even using the same model of camera, detection probability can vary greatly. Although some cameras can be programmed to take multiple photos per trigger, only an additional 5% of wildlife species were captured in this study by the second photo and 2% by the third photo. The value of this additional identification should be weighed against the amount of time needed to sort through a much larger data set. The second and third photos did not add additional taxa for any of the caves.

Many camera trap studies focus on one size of target, such as medium- or large-sized mammals (e.g., Swann and Perkins, 2013). This study analyzed all sizes, and the cameras performed well in this regard. Nevertheless, it should be noted that due to camera limitations, particularly in the passive infrared detection capabilities, some classes of wildlife such as cold-blooded vertebrates are likely underrepresented in this survey. Invertebrates like beetles, flies, and cave crickets, which may be an important nutrient source to the cave (Taylor et al., 2005), are only rarely captured by remote cameras. One way to compensate for this could be to use both time- and motion-triggered settings. With an average of just 1.7 trap events per day per cave, setting time-triggered settings could greatly augment the number of photos to analyze.

Wildlife were observed using cave entrances for a variety of reasons. At Root Cave, photos of a mouse at the cave entrance were closely followed by that of a gray fox (Fig. 8), suggesting the fox may have been hunting. At

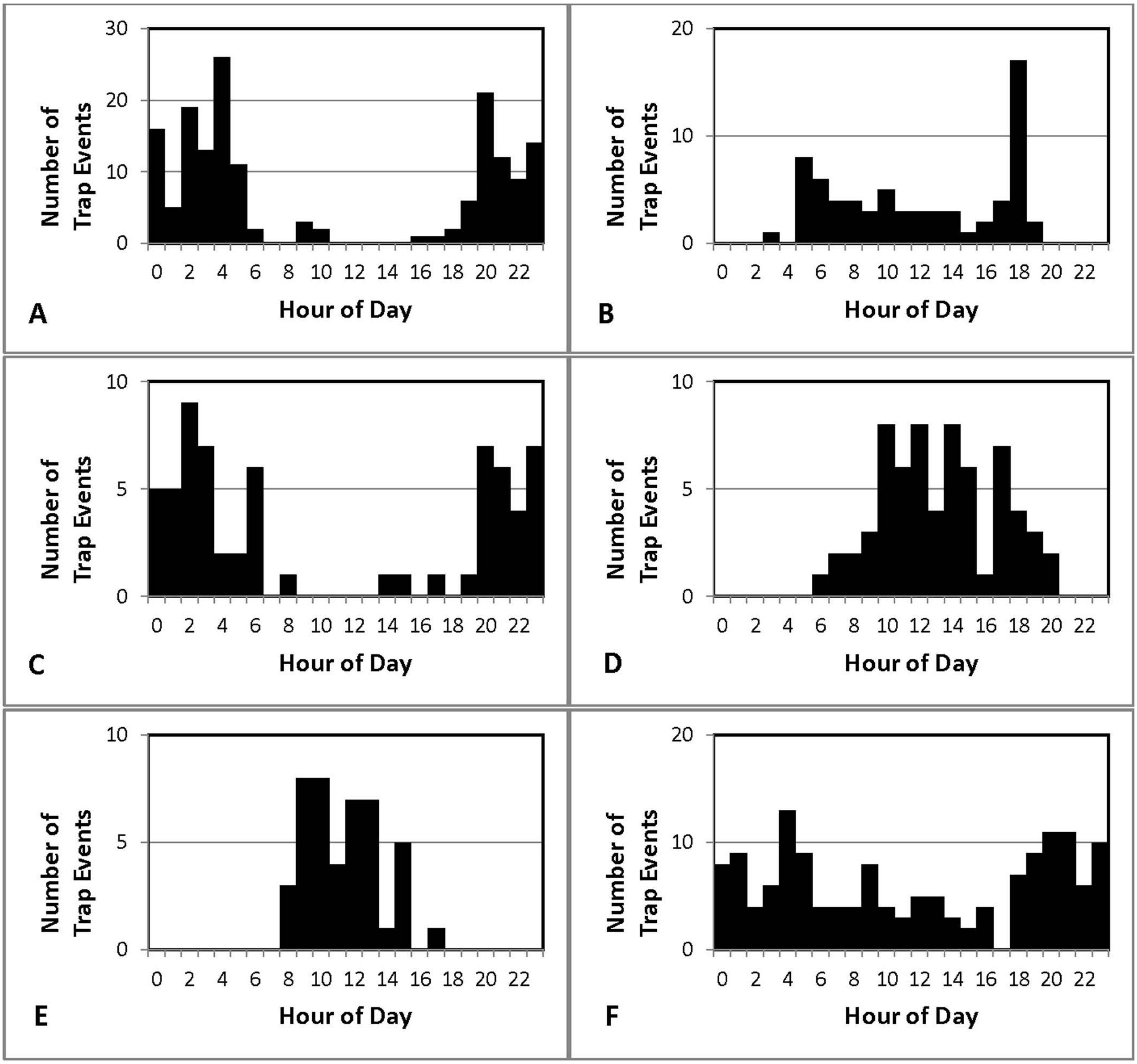

Figure 5. Total number of wildlife camera trap events by taxa pooled by hour of day for all eight caves sampled in Great Basin National Park, White Pine County, Nevada. Shown in order of abundance, except for unknown: A-mouse (*Peromyscus* cf. *maniculatus*), B-cliff chipmunk (*Tamias dorsalis*), C-desert woodrat (*Neotoma lepidus*), D-human (*Homo sapiens*), E-rock squirrel (*Otospermophilus variegatus*), F-unknown trigger.

System's Key Cave, a mouse was photographed eating a fly. The Three Hole Cave entrances may be a resting location for skunks, given their coming and going at intervals. Desert woodrats and small mice are clearly using cave entrances as a home and may enter and leave the caves multiple times per day. For this reason, using camera traps to estimate abundance is not advisable, as the same individuals are likely photographed more than once, and in some cases many more times than once. Nevertheless, the camera traps can be valuable for documenting wildlife interactions with each other and their environment that help augment the understanding of cave ecology (Baker et al., 2014).

With the exception of Upper Pictograph Cave, this study did not target any caves with known bat maternity colonies. This study found bat use of four caves with previous documented bat use, did not find bats in three caves with previous documented bat use, and did not find bat use in one cave with no previous bat use documented (unpublished

Figure 6. A mountain cottontail (*Sylvilagus nuttallii*) photographed with a wildlife camera at the entrance of Fox Skull Cave, Great Basin National Park, White Pine County, Nevada.

bat data for Great Basin National Park). This study augmented previous bat data by adding to the months of observed bat use for two caves. The times of bat outflight and inflight varied. The bats were captured primarily in gated caves. It is possible that bats may be flying fast enough that they escaped capture by the camera traps except in caves that have gates, where the bats may be flying more slowly to navigate the bars of the gate. Hirakawa (2005) used a pencil eraser connected to a line to attract bats searching for prey and to slow them sufficiently for camera traps, However, a non-photographic approach such as an infrared beam counter or acoustic loggers may be more appropriate (Wilson, 2000; MacSwiney et al., 2008; Blumstein et al., 2011).

The photographs indicate that temporal-niche partitioning is occurring at some cave entrances. Mice and woodrats were most abundant from 1900 to 0700, with only 6% of either species seen from 0700 to 1900. Meanwhile, chipmunks and humans were predominant from 0600 to 2000, with only 13% and 3% respectively from 2000 to 0600. Squirrels showed an even narrower range of use, with all captured from 0800 to 1500. Trap events classified as unknown occurred fairly consistently across the 24 hours, further adding credence that camera positioning may be a large reason for those unknowns. Other remote camera studies have documented temporal-niche partitioning, but have focused on medium-sized mammals (Fedriani et al., 2000, Almeida Jácomo et al., 2004). Lower Pictograph Cave, the only cave with high human visitation in this study, is readily visible to human visitors to the park, as it has a large entrance and is located right next to a publicly accessible road. All other caves in this study are less accessible and less visible to casual park visitors. Human visitation to Lower Pictograph Cave clearly showed humans visiting the cave entrance to observe and photograph the pictographs (Fig. 9). This human visitation to Lower Pictograph Cave did not entirely preclude wildlife use, although the cave entrance had fewer species than nearby Three Hole or System's Key caves.

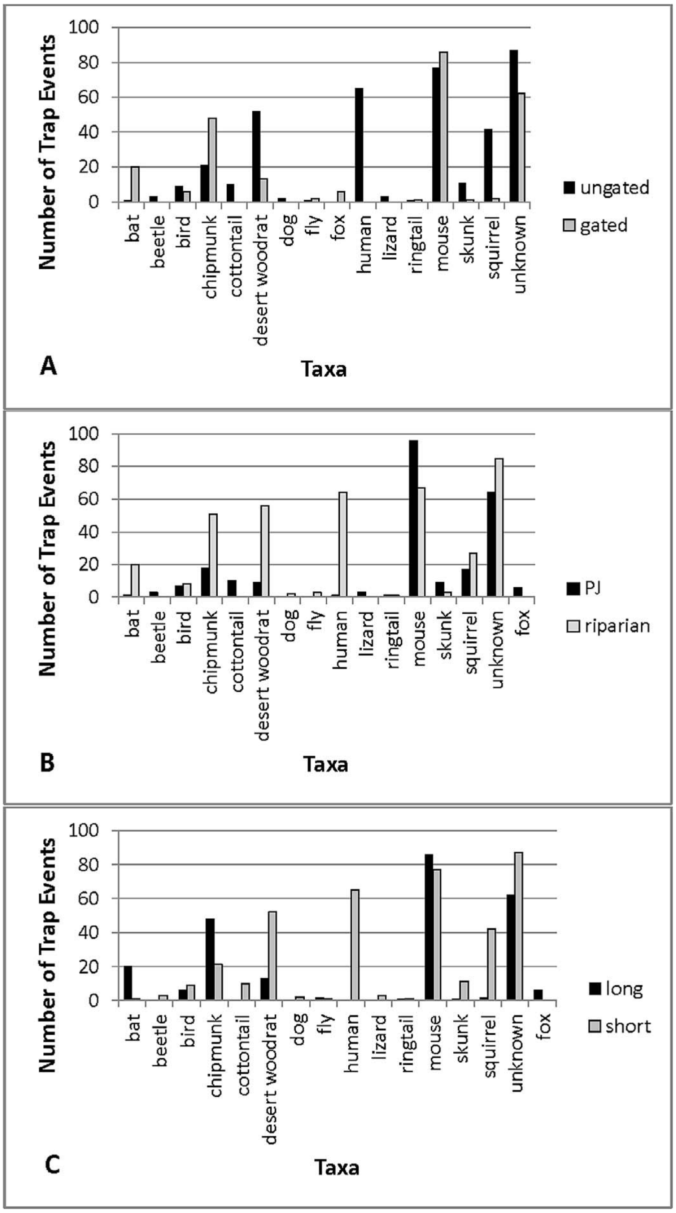

Figure 7. Number of wildlife camera trap events pooled for all caves by species in Great Basin National Park, White Pine County, Nevada. A—gated vs. ungated caves, B—pinyon/juniper vs. riparian habitat, C—long (>100 m) vs. short (<100 m) caves.

Some taxa expected to appear in this study, such as mountain lions and bobcats (Rickart and Robson, 2008), did not show up at any of the camera traps. Other camera-trap studies have shown that at least one thousand trap

Figure 8. This wildlife camera photo of a gray fox (*Urocyon cinereoargenteus*) was immediately preceded by a photograph of a mouse at the same location, suggesting the fox was hunting at the entrance to Root Cave, Great Basin National Park, White Pine County, Nevada.

Figure 9. A human visitor photographing the pictographs at the cave entrance to Lower Pictograph Cave, Great Basin National Park, White Pine County, Nevada.

days are needed in an area to infer that a species is absent (Carbone et al., 2001). In addition, for some species, remote photography may not be the best tool; for example, Harrison (2006) found a dog trained to find bobcat scats was superior in New Mexico. Park staff have noted mountain lion tracks entering a cave in the winter (M. Horner, pers. comm.). In addition, in the winter some species hibernate (e.g., western fence lizard) or migrate (e.g., hummingbirds), so it is likely that different fauna are using the cave entrances at different seasons, and conducting seasonal inventories would provide a fuller picture of wildlife use of cave entrances.

Determining the ideal length of time to conduct a similar cave-entrance study is important for future study designs and efficient research-resource allocation. An asymptote of species accumulation was only found with Fox Skull Cave, with seven species reached at 22 days and no additional species added through the 62 day monitoring period (Fig. 3). The other seven caves did not reach an asymptote, including the three caves with over 50 days of sampling. System's Key Cave appeared to have an asymptote with five species at day 30, but then at day 58 a spotted skunk appeared. This long period of accumulation is different from that seen by Silveira et al. (2003), who found that species richness increased for about 34 days when they used camera traps in grasslands of central Brazil. However, Tobler et al. (2008) estimated the need of 400 to 500 camera days for a camera-trap array to capture the most common medium- to large-sized mammals in rainforests, and 2,000 days to get nearly all. Rovero et al. (2013) recommend 1,000 to 2,000 trap-days to get 60 to 70% of the species. Clearly longer camera-trap studies at cave entrances are needed to determine the ideal time needed to capture species richness.

Some caves studied in the Baker Creek drainage are located within 65 to 800 m of each other, yet the camera traps did not record the same species. For example, skunks were found only at System's Key Cave, Three Hole Cave, and Ice Cave, but not at Lower Pictograph Cave or Wheeler's Deep Cave. This suggests that some species are more particular about the cave habitat they use. Future studies could incorporate more physical measurements such as air temperature and relative humidity, as well as topography, access, or amount of human use in conjunction with the camera traps. The inclusion of these covariates could assist in conducting occupancy estimation, if desired (O'Connell and Bailey, 2011).

CONCLUSIONS

Very little information has been documented about what fauna use cave entrances. This non-invasive technique using camera traps can help managers learn about the dominant species using the entrance and twilight areas of the caves they manage, as well as supplement cave bioinventories. Although the cost of camera trapping may initially be high if new equipment must be purchased, it is preferred over track surveys and direct counts for rapid faunal assessments of mammals (Silveira et al., 2003). Remote cameras can often be borrowed from wildlife or law enforcement programs. Using remote cameras at cave entrances is easily repeatable, which would allow for monitoring to determine changes in a measure such as species richness over time.

The use of camera traps to do this inventory of eight cave entrances in summer at Great Basin National Park worked well, with some caveats that serve as recommendations for others who would like to use this technique for inventorying wildlife at cave entrances in their area:

1. Use the same make and model of camera if possible at all cave entrances;
2. Use cameras with both infrared and motion detection to improve trapping rates;
3. Three photos per trigger result in 7% more identifications, but three times more photos to process, adding little to the understanding of site usage by wildlife;
4. Install cameras for a minimum of 60 days at each cave entrance to capture most of the wildlife species using it; longer is better;
5. Measure covariates, as listed above, if trying to account for why species may or may not be present at a particular cave entrance; and
6. Sample during different seasons and for subsequent years to obtain a stronger dataset.

Camera traps are a useful tool for conducting wildlife inventories of cave entrances. They can be broadly applied to caves throughout the world.

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