Bats, climate, and air microorganisms in a Romanian cave

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ABSTRACT
Monthly surveys of bat dynamics, temperature, and humidity were performed for the duration of one year in a Romanian cave (Măgurici Cave). *Myotis myotis/Myotis blythii*, *Miniopterus schreibersii*, *Rhinolophus ferrumequinum*, and *R. hipposideros* were observed. The locations of the bat colonies in the cave are dependent on the temperature, humidity, on the wall structure, and underground ventilation. The air microorganisms present are in direct relation with the location of the bat colonies, showing a pronounced air infestation near the nursing colony during summer.

KEY WORDS
Bats,
cave climate,
air microorganisms.

RÉSUMÉ
Chauves-souris, climat et microorganismes de l'air dans une grotte de Roumanie.
La dynamique des chauves-souris, la température et l'humidité ont été relevées mensuellement, pendant une année, dans une grotte de Roumanie (Grotte de Măgurici). Les espèces trouvées dans cette grotte sont *Myotis myotis/Myotis blythii, Miniopterus schreibersii, Rhinolophus ferrumequinum* et *R. hipposideros*. La localisation des colonies dans la grotte dépend des facteurs physiques mentionnés, de la structure des murs, et de la ventilation souterraine. Il a été établi une relation entre la localisation des chauves-souris et les microorganismes de l'air, la contamination de l'air étant plus forte à proximité de la colonie de reproduction.

MOTS-CLÉS
Chauves-souris,
grotte,
climat,
microorganismes de l'air.

INTRODUCTION

The selection of the roosts used by bats depends on a specific combination of physical, chemical, and biological factors. Therefore, only a few categories of roost are used as permanent shelters by bats. In Romania, caves may serve as one of the most adequate roosting sites, especially in winter. Data recorded in two caves from Eastern ("Peştera de la Rarău") and South-Western ("Peştera lui Duţu") Romania indicated that *Myotis myotis* hibernated in great number at temperatures ranging between 2 °C and 5 °C and at 95% – 100% humidity (Valenciuc and Ion 1965). *Miniopterus schreibersii* and *Rhinolophus ferrumequinum*, however, chose warmer caves to hibernate in; temperatures beneath these colonies ranged between 7.0 °C and 8.8 °C, respectively (Barbu 1958). During summer, when the temperature measured inside the caves did not exceed 6 °C and 12 °C, respectively, the bats left these shelters.
Previous studies were based on punctual measurements. In order to obtain accurate information about the optimal climatic factors of the roosts, we undertook monthly measurements of the temperature and humidity in the entire length of a cave inhabited by bats.
The presence of bats can influence the microbiology of the air, especially in a cave habitat where the ventilation is reduced and bats often gather in big colonies. Therefore a microbiological examination of the Măgurici cave air was performed, to provide valuable information on the impact that bats have on the cave air. This study might prove useful for their protection.

MATERIAL AND METHODS

The studied cave, Măgurici Cave, is located in the Someşan Plateau (a limestone area in North-Western Romania, Transylvania) at 319 m altitude, being the only representative natural underground site important to bats activity in this region. The vegetation around the cave consists of potato and maize crops, meadows, plum orchards, and forests. The river Someş is also an excellent foraging habitat for bats.
The bats were observed and counted monthly during a one-year period (February 2001-February 2002) and identified at distance without catching them. However in some situations the exact size of nursery could not be observed directly due to the height of the gallery and in the ceiling pockets, where visibility was reduced. Therefore, the magnitude of the nursing colony was estimated based on the fresh guano quantities and the number of dead young.
Air temperature and relative humidity were registered monthly at the floor level, at 16 stations (arabic numerals, 1 to 15 in the cave and 0 – outside, Fig. 1), using an Assmann Psychrometer. In the proximity of the hibernating bat colonies, measurements were made with an electronic thermometer (Checktemp 1, Portugal).
Microorganisms from the air were measured seasonally through the gravitational fall-out of the germs attached to the solid air particles (Koch method). Five Petri plates each with specific colony medium were exposed for 15 minutes to the atmosphere of the cave, in two stations (Latin numerals, I and II) near the bat colonies (Fig. 1), in every season of the year. The mediums were agar standards for the total count of aerobic germs, Levine for the gram-negative bacteria, Chapmann for the staphylococci, Holmes for the streptococci, and Sabouraud for fungi. After medium exposure to the air microorganisms, the plates were incubated at 37 °C for 24 hours, except for the Sabouraud medium, which was incubated at 18-20 °C for 3-5 days.

RESULTS

The most abundant bat species in the cave were *Myotis myotis* mixed with *M. blythii*, permanent inhabitants with nursing and hibernating colonies. The nursery of approximately hundreds to one thousand bats inhabited in the cave from April until the end of August and was located in the proximal part of cave (station 7), at 19-20 m height (Fig. 1). In winter, the colony of 108 (in

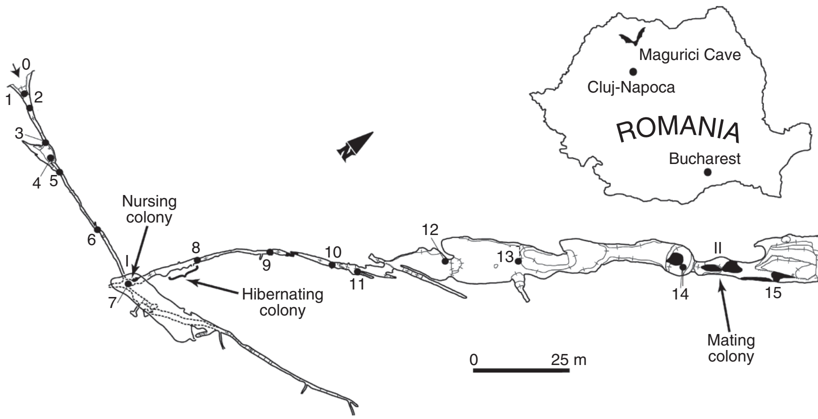


FIG. 1. — Map of the Măgurici Cave showing the location of the bat colonies. Arabic numerals indicate the stations where temperature and humidity measurements were recorded. Latin numerals indicate the stations for air microorganisms sampling.

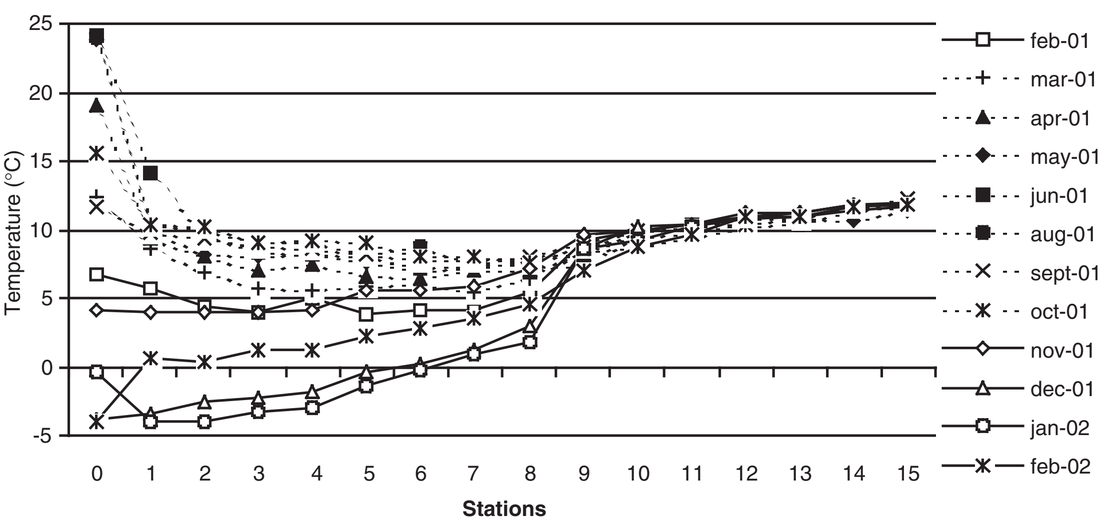


FIG. 2. — Temperature variations in Măgurici Cave from February 2001 to February 2002, outside the cave (point 0), and in fifteen stations situated along the cave (points 1-15). See also Fig.1.

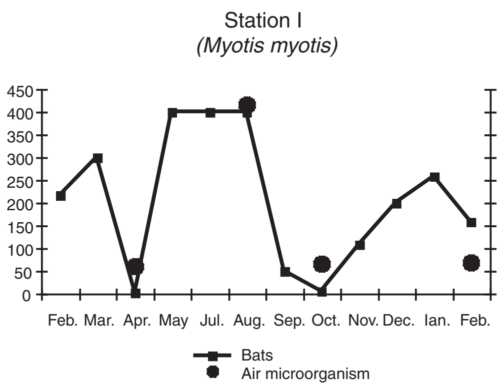
November) to 298 individuals (in February) occupied a lower gallery, 2-3 m high, with sinuous walls and ceiling (stations 7-8). *Miniopterus schreibersii* visited the cave in autumn for mating (Fig. 1), the maximum of individuals (*c.* 500) being recorded in September-October. *Rhinolophus ferrumequinum* and *R. hipposideros* were accidentally recorded in the winter, but only in small numbers.

Climatically, the cave has bidirectional ventilation in the disturbance meroclimatic zone (the first 100 meters from the entrance), descending to the floor level and ascending to the ceiling. The temperature and relative humidity recorded along the cave indicated a stability zone, with no air circulation, beyond the station 9 (Fig. 2).

In the proximity of the winter colony of *M. myotis*/*M. blythii* (stations 7-8), the mean

TABLE 1. — Mean values and standard deviation of temperature and humidity in the Măgurici Cave (Feb. 2001-Feb. 2002). In brackets are recorded the variations of these values determined as the differences between the maximal and the minimal values. Summer was considered from March through October, winter from November through February.

Parameters	Mean	Station 7	Station 8	Station 15
Temperature (°C)	Annual (12 samples)	5.62 ± 2.57 (7.0)	6.26 ± 2.16 (6.2)	11.78 ± 0.25 (0.8)
	Summer (7 samples)	7.18 ± 0.81 (2.5)	7.48 ± 0.54 (1.5)	11.75 ± 0.34 (0.8)
	Winter (5 samples)	2.90 ± 1.96 (4.8)	4.12 ± 2.01 (5.4)	11.80 ± 0.14 (0.4)
Humidity (%)	Annual (12 samples)	95.39 ± 4.64 (13.2)	92.34 ± 7.41 (12.8)	97.77 ± 1.29 (4.5)
	Summer (7 samples)	97.74 ± 2.89 (6.5)	95.22 ± 4.00 (9.2)	97.20 ± 1.13 (2.3)
	Winter (5 samples)	89.90 ± 2.21 (5.0)	85.60 ± 8.24 (20.0)	98.53 ± 1.03 (2.2)



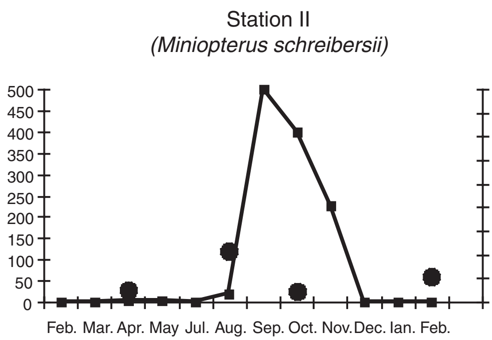


Fig. 3. — Correlation between the number of bats and air microorganisms in station I, located near the nursery of *M. myotis/M. blythii*, and station II located near the mating colony of *Miniopterus schreibersii*. Left y axis: number of bats; right y axis: air microorganisms (cfu/m^3).

temperature of the cave, measured at the floor level, varied between 2.9 °C and 4.1 °C. Near the hibernating bats and inside the colony, at 2-3 m height, the measured temperature was 6.3 °C. In summer, under the nursery, the mean temperature was 7.2 °C. In autumn, under the colony of *M. schreibersii* (station 15), the temperature recorded was constant at practically 11.8 °C (Table 1). The relative humidity had high values all year, ranging between 85.6% and 98.5%, the lowest values being measured in the winter.

The results of the microbiological investigations of the cave air are shown in Table 2. The total aerobic germ count varied between 10^2 and 10^4 cfu/m^3 of air. The gram-negative bacteria and staphylococci ranged between 0 and 10^2 cfu/m^3 and the values of streptococci and fungi between 5×10 and 10^3 cfu/m^3 of air. For all microorganisms, the maximal values were measured in summer, near the nurseries (Fig 3).

DISCUSSION

The five most frequent bat species (*Myotis myotis/Myotis blythii*, *Miniopterus schreibersii*, *Rhinolophus ferrumequinum*, and *R. hipposideros*) in Romania, especially in Transylvania, are recorded in the Măgurici Cave. The nursing

TABLE 2. — Air microorganisms in Măgurici Cave (**TAG**, total count of aerobic germs; **G**, Gram negative bacteria; **SP**, staphylococci; **ST**, streptococci; **cfu/m³**, colony forming units/m³ of air).

Season	Station	TAG (cfu/m³)	G⁻ (cfu/m³)	SP (cfu/m³)	ST (cfu/m³)	Fungi (cfu/m³)
Spring	I	890	0	0	524	1084
(April)	II	210	0	52	157	576
Summer	I	11317	209	104	1152	3929
(August)	II	1833	52	0	1152	1152
Autumn	I	628	52	0	104	1834
(October)	II	314	0	0	52	524
Winter	I	996	0	0	943	1205
(February)	II	262	0	0	157	1676

colony of *Myotis myotis/Myotis blythii* found in this cave is medium-sized for the country, where this species usually forms nurseries that gather 3000-5000 individuals (Dumitrescu *et al.* 1962-1963a; Nagy *et al.* 2000). As only three hundred individuals were found here during the winter, this cave was not one of the more important hibernacula. For example, Valenciuc and Ion (1965) noted a large winter colony of 6500-7500 *M. myotis* in "Peştera de la Rarău" Cave and more recently a minimum of 2941 individuals of *M. myotis* were found hibernating in a cave from Padurea Craiului Mountains (Szodoray-Paradi and Szanto 1998).

Although karst represents only 1.94% of the Romanian territory (Onac and Cocean 1996), 6800 caves were recorded in 1981 (Goran 1982), and their number reaches up to 13000 now (Goran pers. comm.).

In summer, bats prefer warm caves for nursing. *M. myotis* can often bear temperatures of 40 °C (Zahn 1995), although generally in nurseries avoid temperatures higher than 30 °C (Zahn and Henatsch 1998). When the temperature lowers to 20 °C-25 °C, *M. myotis* clusters tightly, using social thermoregulation. Heidinger *et al.* (1989) correlated the ambient temperature with cluster density and found ca. 400 females/m² at 15 °C and 20/m² at 35 °C. In the Romanian caves such high temperatures are exceptional, and nurseries in caves form big clusters with hundreds to thousands of individuals. For example, in 1952 in "Peştera de la Mânăstirea Bistriţa" Cave, a mixed nursery of ca. 15000 *M. myotis* and *Miniopterus schreibersii* was observed by Dumitrescu *et al.* (1955). The ambient temperature of *M. myotis* nurseries, recorded by the same authors, ranged between 8 °C and 14 °C in the studied caves (Dumitrescu *et al.* 1962-1963b). In Măgurici Cave, the maternity colony was in a higher section than the hibernating colony, and we assume that females and newborns roosted at a higher ambient temperature than the mean of 7.2 °C that was measured at the floor level.

In winter, bats elect hibernacula with a cooler climate, due to the conformation of the cave or to the particularities of ventilation (Meyer 1971), where *M. myotis* shows a great tolerance for variable temperatures during hibernation. In the Romanian caves, these temperatures ranged from –2 °C to 9,5 °C (Dumitrescu *et al.* 1962-1963a). These values are similar with the ambient temperature recorded in different hibernacula (caves, cellars and galleries) from South-West-Germany: –2.5 °C to 9.0 °C (Nagel and Nagel 1991). In Măgurici Cave, the mean of the hibernation temperatures for *M. myotis/Myotis blythii* (2.9 °C-4.1 °C) was higher than the lowest temperature recorded for this species, and therefore the colony did not move during hibernation. Both winter colony and nursery of *M. myotis/Myotis blythii* chose a place with an optimal climate, situated in the ventilated sector of the cave, right in front of the thermal stability zone.

Miniopterus schreibersii and *Rhinolophus* spp. prefer hibernacula with higher temperatures: 7 °C-10 °C (Barbu 1958; Pandurska 1993), or 8 °C-11 °C (Dumitrescu *et al.* 1967). Therefore, *Miniopterus schreibersii* selected the warmer place in the temperature stable section of the cave, only for a short time in autumn. In the North-Western part of Romania, big colonies of *R. ferrumequinum* (up to 852 individuals) were reported hibernating in caves at an ambient temperature varying between 8.6 °C and 9.4 °C (Szodoray-Paradi and Szanto 1998). Occasionally few isolated individuals of *R. ferrumequinum* and *R. hipposideros* were found at temperatures lower than 5 °C (Szodoray-Paradi and Szanto 1998) as in Măgurici Cave.

The relative humidity, 85.6%-89.9%, was somewhat lower that the values 90.0%-100.0% recorded by Dumitrescu *et al*. (1962-1963a, 1967), but fell in the range 74.0%-95.6% reported by Szodoray-Paradi and Szanto (1998).

The soil, as well as humans and animals which eliminate germs through their secretions, excretions and dejections are the main sources of air microorganisms (Drăghici 1982). Inside the caves, the air is usually "cleaner" when compared with the exterior atmosphere. The few germs found in the cave are brought in the air circulation from outside, by trogloxene and troglophile animals, and by tourists (Manolache 2001). Due to the high humidity, in caves the mass of aerosols is greater, and the fall-out speed of particles is more elevated than outside. Thus, the results of microbiological determinations depend on the season, on the distance from the entrance and on the humidity. The most infested air (with total aerobic germs) was near the nursing colony of *M. myotis*. The microbiological values noticed in summer under the nurseries are comparable with those recorded in the Central Park from the city of Cluj-Napoca (TAG = 629-10479 cfu/m^3; G$^-$ = 0-786 cfu/m^3; SP = 0-1402 cfu/m^3; ST = 314-1572 cfu/m^3; Fungi = 550-109970 cfu/m^3). In spring, autumn, and winter, values were similar to the air microbiology at the mountain resort of Păltiniș (TAG = 366 cfu/m^3; G – = 0 cfu/m^3; SP = 0 cfu/m^3; ST = 0 cfu/m^3; Fungi = 2907 cfu/m^3) (Drăghici 1982), particularly at station II where bats roosted for a short time. The amount of air microorganisms in the investigated cave seems to be correlated with the intensity of the foraging activity (outside the cave), the size of the nursery and probably the number of dead young.

This contamination of the air with microorganisms may cause risks to animals and man (Hartung 1994). About 20% of the infectious diseases are transmitted by air and in the temperate zone these diseases are the most frequent (Manescu *et al.* 1993). One of the best methods to diminish the number of germs in the air is ventilation (Manescu *et al.* 1993). It is known that bats choose for hibernation the ventilated parts of the caves, for a cooler climate, but it is also possible that ventilation play an important role in the maintenance of a healthy air.

CONCLUSION

A great number of caves in Romania are good shelters for winter colonies of bats and also for their nurseries. Due to the different wall morphology, humidity and especially ambient temperature the Măgurici Cave is an important underground site for the bat populations of the Someșan Plateau. The cave ventilation might play an important role in the prevention of the air contamination and can contribute to the health of bat populations. Pursuant to the importance of this site for bats, Măgurici Cave is proposed to be included in the list of caves protected by law.

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