

## Ammonia volatilization in a Mexican bat cave ecosystem

DONALD A. MCFARLANE<sup>1</sup> RAYMOND C. KEELER<sup>2</sup> & HIROSHI MIZUTANI<sup>3</sup>

<sup>1</sup> Joint Science Department, The Claremont Colleges, 925 N. Mills Ave, Claremont CA 91711-5916, USA; <sup>2</sup> 22354 N. 64th Drive, Glendale AZ 85310, USA; <sup>3</sup> Department of Basic Sciences, Faculty of Science and Engineering, Senshu University of Ishinomaki, Shinmito 1, Minamisakai, Ishinomaki, Miyagi 986, Japan

Received 29 July 1994; accepted 2 March 1995

**Abstract.** Direct measurements of gaseous ammonia in the atmosphere of a dry bat cave containing several million insectivorous bats revealed a peak concentration of 1779 ppm (0.96 mg/dm<sup>3</sup>). Observations indicate that the origin of the gaseous ammonia is rapid microbial decomposition of bat urea, not chitinous guano. Modelling of ammonia distribution and diffusion indicates that ammonia production at the Cueva del Tigre is ~257 g NH<sub>3</sub>/day, equivalent to the decomposition of ~454 g urea/day. Ammonia production is also characterized by significant isotopic fractionation favoring isotopically light ( $\delta^{15}\text{N}$  depleted) ammonia.

**Key words:** ammonia, bats, caves, guano, stable isotopes

### Introduction

The use of stable isotope signatures to illuminate aspects of contemporary and paleoecology has developed into a major field of endeavor in recent years (Peterson & Fry 1987). In particular,  $^{15}\text{N}/^{14}\text{N}$  ratios have proven useful in studies of paleodiet and food chain analysis (DeNiro 1987; Mizutani & Wada 1988). More recently, Mizutani et al. (1992a, 1992b) have demonstrated the utility of fossil and subfossil bat guano deposits as paleoclimatic and geochemical records extending back at least into the early Holocene. Unfortunately, the interpretation of the  $\delta^{15}\text{N}$  record has been complicated by a lack of information on the complex biogeochemistry of nitrogen in bat guano ecosystems.

In a series of studies, Mizutani and colleagues have demonstrated that  $\delta^{15}\text{N}$  levels are greatly enhanced in the soils of seabird rookeries, an effect attributed in large part to the volatilization of isotopically light ammonia (Mizutani 1984; Mizutani et al. 1985a; Mizutani et al. 1985b). Bouwmeester et al. (1985) have also reported on the significance of ammonia volatilization in the nitrogen budget of urea-fertilized agricultural soils. The demonstration of the source, rate, and isotopic fractionation effects of ammonia volatilization

in bat guano deposits is clearly a necessary first step in deciphering the paleoecological record preserved in these unique resources.

Caves hosting large colonies of insectivorous bats receive a very significant input of organic nitrogen, primarily as urea and insect chitin. Limited exchange of cave air with the epigeal atmosphere can allow significant concentrations of gaseous ammonia to develop (Constantine 1988). A small number of caves in the southwestern United States and northern Mexico support populations of Mexican Free Tailed Bats, *Tadarida brasiliensis*, that range from  $10^5$  to  $10^7$  individuals. Ammonia concentrations in these caves can reach levels high enough to bleach the fur of the resident bats (Constantine 1958) and may exceed National Institute of Occupational Safety and Health maximum permitted human exposures by a factor of 30 or more. In this paper we report on the dynamics of ammonia volatilization in the Cueva del Tigre, an important *T. brasiliensis* roost in northern Sonora, Mexico.

## Methods

The Cueva del Tigre is a voluminous, 170 m long limestone cave (not volcanic as reported by Mitchell 1964) located 38 kms SSE of Carbo, Sonora, Mexico (29.58 N, 110.86 W). The cave is used as a roost by at least seven species of bat (Mitchell 1964), including the U.S. Federally Endangered Long-Nosed Bat, *Leptonycteris nivalis* (population  $\sim 10^2$ – $10^3$  in May 1992) and a very large population of *T. brasiliensis* (population  $\sim 2 \times 10^6$  in August 1991; Moreno-Valdez, personal communication 1993). Work was conducted at the cave 23rd–24th May 1992.

The cave was surveyed using standard compass and tape techniques (Ellis 1976). Cave temperatures and humidities were measured to  $\pm 0.05$  at  $\sim 1.2$  m above floor level using standard instruments. Airflow was assessed with a Sper Digital Anemometer (nominal accuracy 0.1 m/s) and by observation of candle smoke. Guano moisture content was measured by oven drying at 60 °C for 24 hrs. Atmospheric ammonia was sampled quantitatively by passing 1.2 dm<sup>3</sup> of cave air through 6M H<sub>2</sub>SO<sub>4</sub>. Bulk ammonia samples for isotopic study were collected by running cave air through 6M H<sub>2</sub>SO<sub>4</sub> for 15 hrs using a battery operated aquarium pump. Sampling stations were selected to represent significant changes in cave morphology, and are mapped in Fig. 1b. After return to the laboratory, samples were assayed for ammonia content by the phenate method (Greenberg et al. 1985). Estimates of 50 ppm were made at two additional locations, this being the lower limit of human olfactory detection. Isoclines of ammonia concentration were plotted by linear interpolation (Fig. 1b). Nitrogen isotope ratios were determined using procedures described in Mizutani et al. (1992). Workers were protected

*Table 1.* Atmospheric ammonia concentrations and microclimate data of Cueva del Tigre sampling stations.

Station	NH <sub>3</sub> (ppm)	Temperature	Relative humidity
1		32	13.5
2		31.5	14.0
3	50		
4	1266	32.5	
5	1456	30.5	>90
6	50	30.8	14.0
7		30.9	48.1
8		30.2	48.0
9		32.0	81.0
10		34.1	79.8
11	1412	32.5	83.5
12	1779	31.0	90.5
13	1367		

during fieldwork with industrial respirators fitted with ammonia absorption cartridges (Lab Safety Supply Inc, Janesville, WI).

## Results

Cave survey statistics indicate that the cave is 170 m long with a vertical range of 41 m. The cave consists of two units, the lower cave ('Ammonia Hall', the 'Sauna Room' and 'Lepto Lair') and the upper cave ('Vulture Dome') which are ecologically isolated by the daylight and air circulation which pervades the Entrance Chamber (Fig. 1a). Air temperature was remarkably constant with respect to sampling location in the upper and lower caves, averaging  $31.6^{\circ}\text{C} \pm 1.13$ , whereas relative humidity increased with distance from the entrance and density of roosting bats from 13.5% to 90.5% (Table 1). Surface *T. brasiliensis* guano collected from the 'Sauna Room' contained 7.46% water by weight. Atmospheric ammonia concentrations ranged from 1338 ppm to 1779 ppm at the sampling locations, which compares favorably with a maximum concentration of 1850 ppm measured by Mitchell (1964) in August 1962.

Ammonia concentrations showed a marked increase along the length of 'Ammonia Hall' and down into the 'Sauna Room'. Concentrations peaked at the bottom of the 'Sauna Room', corresponding to the maximum observed

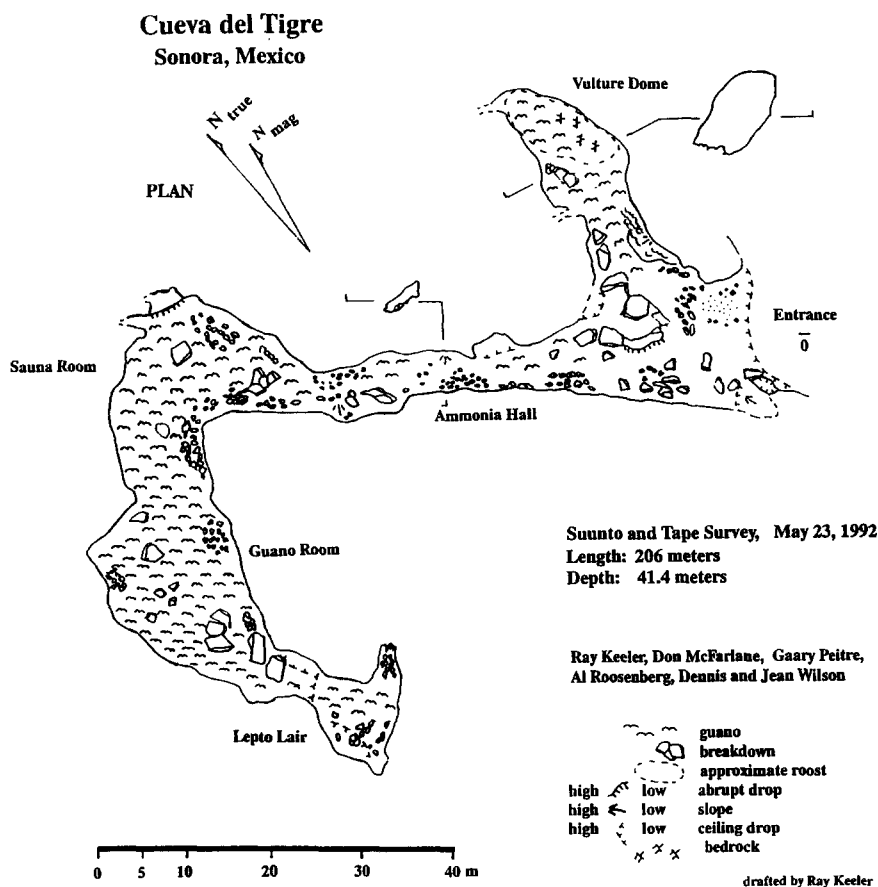


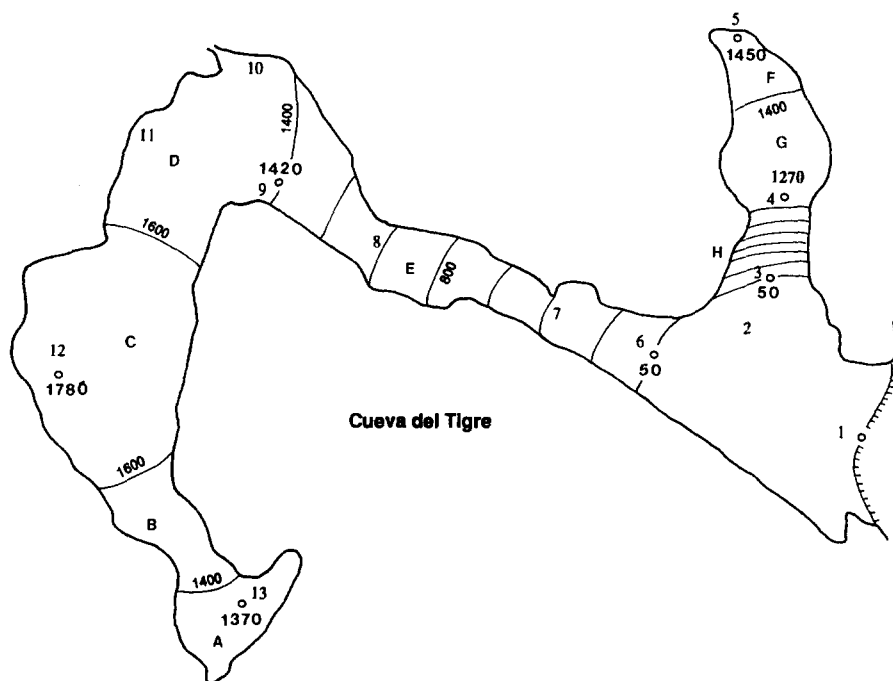
Fig. 1a. Plan view of Cueva del Tigre. Sonora Mexico.

density of *T. brasiliensis*, and were lowest in the 'Lepto Lair' where *T. brasiliensis* is replaced by much smaller numbers of *Leptoncyteris nivalis*. Ammonia production in the steeply ascending 'Vulture Dome' area is independent of that in the lower cave. Table 2 lists ammonia concentrations and masses for eight contiguous areas of the cave (Fig. 1b), with an estimated total mass of 5932 g NH<sub>3</sub>, calculated from the measured concentrations at ~1.2 m above floor level and the surveyed areas and cross sections of the passages. Since cave height in Cueva del Tigre is typically ~2.5 m, we assume that our mid-level sampling stations average any vertical stratification in atmospheric ammonia concentration.

Fresh guano (chitin) being deposited in Cueva del Tigre was characterized by a  $\delta^{15}\text{N}_{\text{air}}$  of 14.0 ppt, whereas two assays of atmospheric ammonia collected in the cave gave  $\delta^{15}\text{N}_{\text{air}}$  values of 4.7 and 4.6, respectively.

*Table 2.* Calculated volumes and ammonia masses for sections of the Cueva del Tigre, May 1992.

Cave zone	Mean NH <sub>3</sub> (ppm)	NH <sub>3</sub> mg/ dm <sup>3</sup> air	Air volume, dm <sup>3</sup> × 10 <sup>3</sup>	Mass NH <sub>3</sub> grams
A	1370	0.932	153	142
B	1500	1.020	209	213
C	1690	1/149	3113	3576
D	1500	1.020	517	527
E	725	0.493	673	332
F	1425	0.969	330	320
G	1300	0.884	756	668
H	625	0.425	362	154



*Fig. 1b.* Plan view of Cueva del Tigre showing sampling stations numbers (Table 1), point ammonia concentrations (open circles, bold face numerals) and ammonia concentration isoclines (interval: 200 ppm) for 23–24 May 1992. Letter designations refer to cave zones in Table 2.

## Discussion

Ammonia volatilization could, in principle, result from the microbial conversion of urea, or chitin (used here in the sense of arthropod exoskeletal

material, i.e. the n-glutasamine matrix together with proteinaceous, waxy and fatty functional components) or both. However research at Eagle Creek Cave, Arizona, has shown that *T. brasiliensis* guano chitin decomposes very slowly under dry conditions, with no systematic  $\delta^{15}\text{N}$  shift over 30 years (Mizutani et al. 1992a). In contrast, ammonia volatilization at Eagle Creek Cave responds rapidly to the seasonal movement of bats, rising to high levels within weeks of the bats' arrival and falling to <50 ppm with a few weeks of their departure. The process of ammonia volatilization was expected to favor isotopically light  $^{14}\text{N}$  relative to  $^{15}\text{N}$ , which was found to be the case at Cueva del Tigre. Previous work by Mizutani et al. (1992a; 1992b) has shown that the subfossil and fossil bat guanos which remain after early diagenetic ammonia volatilization are not correspondingly enriched with respect to  $\delta^{15}\text{N}$ . We therefore conclude that significant ammonia volatilization results from the decomposition of bat urea only and does not involve the chitinous component of the guano, at least in very dry caves. The estimated instantaneous mass of ammonia in the Cueva del Tigre on 23–24th May 1992 was 5.9 kg, equivalent to the conversion of 10.4 kg of urea.

The mass of  $\text{NH}_3$  in the Cueva del Tigre represents a balance between rates of production and loss. Production is a function of bat population size, which varies on an annual timescale but is considered to be stable on a timescale of a month during the breeding season (May through August). Loss of ammonia might occur through aqueous dissolution, oxidation by nitrifying bacteria, exchange of cave air with the epigeal atmosphere, or diffusion between the cave and epigeal atmospheres. We reject aqueous solution as a significant factor at Cueva del Tigre because free water occurs only in a small area of the 'Lepto Lair' as a thin film of condensate on the cave wall. Lowered ammonia concentrations in the 'Lepto Lair' were probably the result of both dissolution into the condensate and substantially reduced *in situ* production consistent with the absence of *T. brasiliensis* in this area. Bacterial oxidation is also unlikely to be significant, since none of the suite of biospeleogenic nitrate minerals (Hill & Forti 1986) are present on the cave wall or guano surface, even in areas where the humidity is low enough for these deliquescent species to crystallize (<50% RH for nitrocalcite or <60% RH for nitrammite; Hill & Forti 1986). We attempted to measure air flow in 'Ammonia Hall' by anemometer and candle smoke, but could detect none. Thus, although we cannot reject the possibility that the nightly exodus of bats drives some air exchange, temperature measurements taken before and after this event suggest the effect, if present, must be small. We conclude that diffusion of ammonia between the hypogean and epigeal atmospheres is the principle limitation on the accumulation of ammonia in the cave.

Diffusion of ammonia from the atmosphere of the lower cave is likely to be restricted by the length and small cross-sectional area of 'Ammonia Hall'. The rate of ammonia diffusion,  $R_{\text{NH}_3}$ , along a cave passage of cross sectional area  $A$  and length  $L$  is given by:

$$R_{\text{NH}_3} = K_{\text{NH}_3} A \left( \frac{C_{\text{high}} - C_{\text{low}}}{L} \right) \quad (1)$$

where  $C_{\text{high}}$  and  $C_{\text{low}}$  are the ammonia concentrations at the ends of the passage and  $K$  is the diffusion constant for the gas. Based on molecular weight differences between ammonia, carbon dioxide and oxygen ( $K_{\text{CO}_2} = 0.139$ ;  $K_{\text{O}_2} = 0.129$ ; Weast & Astle 1982) we derive an estimate of  $K_{\text{NH}_3} = 0.23$  at  $0^\circ\text{C}$ .

Ammonia concentrations fall from  $0.96 \text{ mg/dm}^3$  (1400 ppm) to  $0.34 \text{ mg/dm}^3$  (50 ppm) over the 30 m length of 'Ammonia Hall', which has a minimal cross-sectional area of  $4 \times 10^4 \text{ cm}^2$ . These values lead us to predict a diffusion rate of  $2.98 \text{ mg NH}_3/\text{sec}$  at  $30^\circ\text{C}$ , or  $257 \text{ g NH}_3/\text{day}$ . This corresponds to the complete decomposition of 454 g of urea per day.

Noting the semi-quantitative nature of available methods of censusing very large populations of *T. brasiliensis* (c.f. the discussion of order-of-magnitude errors in published estimates of the population at Eagle Creek Cave, Arizona in McFarlane & Keeler 1991) we assume a *T. brasiliensis* population for the lower cave of 2 million individuals, consistent with the whole-cave estimates of 3 million (May 1962) and 4 million (August 1962) by Mitchell (1964), and 2 million (August 1991) by Moreno-Valdez (personal communication). We thus derive an estimate of  $0.2 \text{ mg/urea/day/bat}$  decomposed in the cave in May 1992, consistent with estimates of urinary output for neotropical insectivorous bats provided by Studier & Wilson (1983).

Ammonia volatilization is an important biogeochemical pathway in dry bat cave ecosystems, accounting for the removal of most, if not all of the urea-derived nitrogen. At a maximum roosting density of 1500 adult *T. brasiliensis* per square meter (McCracken & Gustin 1987), this could amount to as much as  $0.3 \text{ g/m}^2/\text{day}$ . Observations in the Cueva del Tigre and Eagle Creek Cave ecosystems suggest that the loss of isotopically light ammonia gas does not involve the chitinous component of bat guano and is probably not a significant influence on the  $\delta^{15}\text{N}$  record of sub-fossil and fossil (chitinous) guano deposits. The biogeochemical pathways of chitin-derived nitrogen remain to be investigated.

## Acknowledgements

We thank Gary Petrie and the members of the Central Arizona Grotto of the National Speleological Society for assistance with the fieldwork and J. P. Ledant for useful comments on the manuscript. The Keck Fund of the Joint Science Department, the Claremont Colleges provided partial support for this work.

## References

- Bouwmeester RJB, Vlek PLB & Stumpe JM (1985) Effects of environmental factors on ammonia volatilization from a urea-fertilized soil. *Journal Soil Soc. America* 49: 376–381
- Constantine DG (1988) Health precautions for bat workers. In: Kunz TH (Ed) *Ecological and Behavioral Methods for the Study of Bats*. Smithsonian Institution Press, Washington DC
- DeNiro MJ (1987) Stable isotopy and archaeology. *Am. Sci.* 75: 182–191
- Ellis BM (1976) *Cave Surveying*. British Cave Research Association, Bridgewater. 80 pp
- Greenberg AE, Trussel RR & Clesceri LS (Eds) (1985) *Standard Methods for the Examination of Water and Wastewater*. 11th edition. American Public-Health Association, Washington, DC
- Hill CA & Forti P (1986) *Cave Mineral of the World*. National Speleological Society, Huntsville
- McCracken GF & Gustin MK (1987) Batmom's daily nightmare. *Nat. Hist.* 96: 66–73
- McFarlane DA & Keeler RC (1991) A proxy population record for the Mexican Free-Tailed Bat at Eagle Creek Cave, Arizona. In: *Proceedings of the Symposium of Managing Wildlife in the Southwest* (pp 100–106). Wildlife Society, Tucson
- Mitchell HA (1964) Investigations of the cave atmosphere of a Mexican bat colony. *Journal of Mammalogy* 45: 568–577
- Mizutani H (1984) A chemical study on seabird rookeries – biogeochemical approach. *Marine Sci. Monthly* 16: 226–230
- Mizutani H & Wada E (1988) Nitrogen and carbon isotope ratios in seabird rookeries and their ecological implications. *Ecology* 69: 340–349
- Mizutani H, Kabaya Y & Wada E (1985a) High performance liquid chromatographic isolation of uric acid from soil for isotopic determination. *J. Chromatogr.* 331: 371–381
- Mizutani H, Kabaya Y & Wada E (1985b) Ammonia volatilization and high  $^{15}\text{N}/^{14}\text{N}$  ratio in a penguin rookery in Antarctica. *Geochem. J.* 19: 323–327
- Mizutani H, McFarlane DA & Kabaya Y (1992a) Nitrogen and carbon isotope study of a bat guano core from Eagle Creek Cave, Arizona, USA. *Mass Spectroscopy* 40: 57–65
- Mizutani H, McFarlane DA & Kabaya Y (1992b) Carbon and nitrogen isotopic signatures of bat guanos as a record of past environments. *Mass Spectroscopy* 40: 67–82
- Peterson BJ & Fry B (1987) Stable isotopes in ecosystem studies. *Ann. Rev. Ecol. Syst.* 18: 293–320
- Studier EH & Wilson DE (1983) Natural urine concentrations and composition in neotropical bats. *Comp. Biochem. Physiol.* 75A: 509–515
- Weast RC & Astle MJ (1982) *CRC Handbook of Chemistry and Physics*. 63rd Ed. CRC Press. Boca Raton, Florida