

## Appendix 1

### *Tetradactylus breyeri* specimens examined

Abbreviations used: AJL = A.J.L. Lambiris Private Herpetological Collection, Hillcrest; LR = Lynn Raw Private Herpetological Collection, Pietermaritzburg; NM = Natal Museum, Pietermaritzburg; NMB = National Museum, Bloemfontein; NMZB-UM = Natural History Museum of Zimbabwe, Bulawayo; SAM = South African Museum, Cape Town; TM = Transvaal Museum, Pretoria; ZMA = Zoologisch Museum, Amsterdam.

The date of collection (when known) is indicated in parentheses after the museum catalogue number, followed by locality (including magisterial district) and quarter-degree locus code:

AJL 2751	(15 Feb. 1987), Dargle, near source of Umgeni (= Mgeni) River, Impendle district, KwaZulu-Natal (2929DB)
LR 1817	(Oct. 1982), Highmoor State Forest, Mooirivier district, KwaZulu-Natal (2929BC)
NM 593	(Dec. 1935), Umgeni Poort, headwaters of Umgeni (= Mgeni) River, vicinity of Le Sueur farm no. 15185, Impendle district, KwaZulu-Natal (2929BD)
NM 1204	near Greytown, Umvoti district, KwaZulu-Natal (2930BA)
NMB R1073	(26 Feb. 1978), Zwartkoppies farm no. 520, Frankfort district, Free State (2728AD)
NMB R6852-53	(21 Dec. 1992), Stratherrick farm no. 186, Sterkfontein Dam Nature Reserve, Harrismith district, Free State (2829AC)
NMZB-UM 3027	(8 Feb. 1908), 'Bushmans Cave' (precise location could not be traced), Witsieshoek, KwaZulu-Natal/Free State border (2828DB)
SAM ZR4220	(c. 1900), Rietvlei, near source of Umvoti (= Mvoti) River, Lions River district, KwaZulu-Natal (2930AB)
TM 682	(28 Mar. 1910), Weenen 'County', KwaZulu-Natal (precise location unknown)
TM 22674	(Apr. 1953), Cathedral Peak Forest Reserve, Bergville district, KwaZulu-Natal (2829CC)
TM 56692	(Feb. 1983), The Staircase, Long Tom Pass, Mauchsberg, Lydenburg district, Mpumalanga province (2530BA)
TM 56792	(Jul. 1983), Mount Sheba, Grootfontein farm no. 562KT, Pilgrim's Rest 2 district, Mpumalanga province (2430DC)
TM 78785	(13 Dec. 1993), De Kuilen farm no. 205JT, Sterkspruit Nature Reserve, Lydenburg district, Mpumalanga province (2530BA)
ZMA 10974	Holotype (1896), 'Transvaal'

### Additional records

Mount Sheba Nature Reserve, Pilgrim's Rest 2 district, Mpumalanga province (2430DC; sight records; Jacobsen 1988).

Hoedspruit farm no. 346JS, Middelburg district, Mpumalanga province (2529DA; sight record; Jacobsen 1989).

## Does calcium constrain reproductive activity in insectivorous bats? Some empirical evidence for Schreibers' long-fingered bat (*Miniopterus schreibersii*)

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Received 20 June 1996; accepted 13 September 1996

Insects are a poor source of dietary calcium and since they are seasonally abundant, it has been suggested that calcium availability may play a significant role in controlling the timing of reproduction in insectivorous bats. To assess the possible role of dietary calcium, we have measured bone calcium concentrations in female and male long-fingered bats (*Miniopterus schreibersii*) through a full reproductive cycle. The results indicate that winter was not a period of calcium stress and, therefore, that seasonal changes in insect abundance and dietary calcium availability are not a satisfactory explanation for the occurrence of delayed implantation in the long-fingered bat. Bone calcium concentrations of females did not differ significantly throughout pregnancy, indicating that the insects available in winter and early summer were sufficient to meet the calcium demands of pregnancy. Lactating females had the lowest bone calcium concentrations of all specimens, supporting the suggestion that lactation is indeed a period of severe calcium stress in aerial insectivores. We conclude that parturition is probably timed so that lactation coincides with the period of maximal insect abundance and corresponding dietary calcium availability.

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It has been suggested that litter size in insectivorous bats may be constrained by calcium availability to a greater extent than by energetic considerations (Barclay 1994, 1995). Young are born at about 25% of adult mass, are weaned at 70–80% of adult mass and after weaning their bones have to withstand the stresses of flight (Barclay 1994, 1995). A number of previous studies have indirectly addressed the question of calcium demand during pregnancy and lactation in bats and have shown that they are periods of calcium stress (Kwiecinski, Krook & Wimsatt 1987a; Kwiecinski, Wimsatt & Krook 1987b; Studier, Viele & Sevicik 1991; Sevicik & Studier 1992; Studier, Sevicik, Keeler & Schenka 1994a; Studier, Sevicik, Ridley & Wilson 1994b). Insects are a particularly poor source of calcium (1–4 mg/g; Studier *et al.* 1994a) and since insects are seasonally abundant in temperate latitudes, it is reasonable to suggest that not only litter size but also the timing of reproduction may be constrained by calcium availability. We have investigated this hypothesis by measuring seasonal changes in bone calcium concentration of Schreibers' long-fingered bat. Bone calcium is in a constant state of flux and when dietary calcium does not meet the demands of, for example, pregnancy and lactation, calcium is resorbed from the bones. Changes in bone calcium therefore reflect the extent to which dietary calcium meets the calcium requirements of the bat. Schreibers' long-fingered bat is a small insectivorous species in which there is a period of delayed

implantation between copulation in late summer (April/May) and implantation in late winter (August; Bernard 1989). Parturition occurs in midsummer (December; Bernard 1989). Delayed implantation coincides with winter during which Schreibers' long-fingered bat enters periods of hibernation which are interspersed with periods of activity (Bernard & Bester 1988). It is established that mammals, including the little brown bat, *Myotis lucifugus*, undergo a progressive bone loss during hibernation (Mayer & Bernick 1958; Steinberg, Singh & Mitchell 1981; Whalen, Krook & Nunez 1972; Kwiecinski *et al.* 1987a). If this occurs in Schreibers' long-fingered bat during winter then shortage of dietary calcium during winter might be an alternative explanation for the cessation of reproductive activity at this time.

Eighty-three specimens of Schreibers' long-fingered bat (*Miniopterus schreibersii*) were collected from colonies near Grahamstown, South Africa (33°22'S, 26°32'E). Specimens were collected in March (before ovulation), May and August (during delayed implantation), November and December 1994 (midsummer; about 40 and 10 days respectively before birth), and in January 1995 (during lactation; Table 1). Both femurs were removed and cleaned of all muscle and connective tissue and the bones were digested using a dry ashing procedure (Duncan 1976). The calcium concentrations (in ppm) were measured by atomic absorption spectrophotometry and expressed as grams of calcium per gram of dry bone (Bernard, Kerley, Doubell & Davidson 1996). Mean bone calcium concentrations were compared using the students *t* test and ANOVA where applicable.

There was no statistically significant seasonal change in bone calcium concentration of males ( $P > 0.05$ ), and importantly, mean calcium concentration at the end of winter (August;  $322.5 \pm 27.8$ ) was not significantly lower than that measured at the end of the previous summer (March;  $325.2 \pm 31.2$ ;  $P > 0.05$ ; Table 1).

The first pregnant females were collected in May and there was no statistically significant change in bone calcium concentration of pregnant females from then until parturition in December ( $308.2 \pm 10.3$  &  $304.4 \pm 20.7$  respectively;  $P > 0.05$ ; Table 1). Bone calcium concentration of pregnant females was not significantly lower than that of non-pregnant, non-lactating females ( $P > 0.05$ ), and in November and December, bone calcium concentrations of pregnant females were not significantly lower than those of males in the same month ( $P > 0.05$ ; Table 1). We concluded from this that preg-

nancy has no significant effect on bone calcium concentrations. The bone calcium concentration of lactating females in January ( $270.9 \pm 10.2$  mg/g) was significantly lower than that measured in any other group of long-fingered bats ( $P < 0.001$ ).

The climate of the study area is strongly seasonal with hot summers and cool winters. Rainfall is unpredictable and falls throughout the year with most rain during summer. Mean minimum daily temperature falls below 10°C between May and September (Bernard & Tsita 1995). The relationship between temperature, rainfall and insect abundance is established (Rautenbach, Kemp & Scholtz 1988) and, although not quantified for the study area, a marked decrease in both insect abundance and activity has been noted in winter (Bernard unpublished observation). In spite of the seasonal climate, long-fingered bats at 33°S remain active for most of winter and only hibernate during particularly cold spells (Bernard & Bester 1988). It is probably due to this continued activity that neither male nor female long-fingered bats showed a decrease in bone calcium concentration during winter. We conclude that at 33°S winter does not represent a period of calcium stress. By contrast, in temperate North America, hibernation in the little brown bat is a period of calcium stress and bone demineralization (Kwiecinski 1982; Kwiecinski *et al.* 1987a). Clearly the severity of winter will influence insect abundance, the duration of hibernation and the pattern of bone demineralization. Thus at 33°S the occurrence of spermatogenesis and copulation prior to winter, and the period of delayed implantation during winter, cannot be explained in relation to calcium demand.

Bone calcium concentrations of males and pregnant females did not change significantly from August to November, suggesting that insect abundance in early summer is sufficient to balance the increased calcium demands of post-implantation pregnancy. However, there is some evidence (Table 1) that the last month of pregnancy was a period of elevated calcium demand that could not be met from the diet and which was supplemented by some bone demineralization. The significant decrease in bone calcium concentration during lactation supports the widely held belief that lactation is a time of calcium stress. These results are remarkably similar to those for the little brown bat (*Myotis lucifugus*) from temperate North America which indicate that after arousal from winter hibernation there is a period of bone accretion which continues through summer in the males. In females, bone resorption begins in late pregnancy and is accelerated during lactation which is thought to represent a greater period of calcium stress than does winter (Kwiecinski *et al.* 1987a, b). The pattern of faecal calcium in the big brown bat (*Eptesicus fuscus*) indicates that females are probably in negative calcium balance from the onset of pregnancy to the end of lactation (Studier *et al.* 1991; 1994a). These results are corroborated by changes in the curvature of the forearm of the big brown bat during pregnancy (Sevick & Studier 1992).

In conclusion, our results for Schreibers' long-fingered bat suggest that at 33°S winter was not a period of calcium stress and that calcium shortage cannot explain why copulation occurred before winter or why implantation was delayed during winter. It is however likely that calcium availability has played a major role in the evolution of the timing of birth and

**Table 1** Mean bone calcium concentrations (mg of calcium per gram of dry bone) of male and female long-fingered bats

Month	Mean bone calcium conc $\pm$ SD (N)	
	Male	Non-pregnant or lactating female
March	325.2 $\pm$ 31.2 (11)	331.7 $\pm$ 24.8 (4)
May	326.0 $\pm$ 12.1 (3)	308.2 $\pm$ 10.3(7)
August	322.5 $\pm$ 27.8 (10)	317.7 $\pm$ 26.3 (10)
November	341.4 $\pm$ 20.7 (10)	343.4 $\pm$ 39.7 (4)
December	332.2 $\pm$ 23.3 (4)	304.4 $\pm$ 20.7 (8)
January	343.8 $\pm$ 11.9 (6)	270.9 $\pm$ 10.2 (6)

lactation since it is probable that the high calcium demands of late pregnancy and lactation could only be met in summer. With the time of parturition fixed by energy and calcium constraints, spermatogenesis and copulation occurred at the end of the previous summer when males were probably in their best condition. This separation of spermatogenesis, copulation and ovulation from developmental pregnancy necessitated the evolution of a reproductive delay which both lengthened pregnancy so that births occurred in mid-summer, and ensured that the energetic demands of reproductive activity in winter were minimal.

### Acknowledgements

We thank Rhodes University and the FRD for financial assistance.

### References

- BARCLAY, R.M.R. 1994. Constraints on reproduction by flying vertebrates: energy or calcium. *Am. Nat.* 144: 1021–1031.
- BARCLAY, R.M.R. 1995. Does calcium or energy availability constrain reproduction by bats? In: Ecology, evolution and behaviour of bats, (eds.) P.A. Racey & S.M. Swift. *Symp. zool. Soc. Lond.* 67: 245–258.
- BERNARD, R.T.F. 1989. The adaptive significance of reproductive delay phenomena in some South African bats. *Mammal Rev.* 19: 27–34.
- BERNARD, R.T.F. & BESTER, G.J. 1988. Roost selection by the long-fingered bat, *Miniopterus schreibersii*, is influenced by reproductive condition, and roost microclimate and condition. *S. Afr. J. Sci.* 84: 921–922.
- BERNARD, R.T.F. & TSITA, J.N. 1995. Seasonally monoestrous reproduction in the molossid bat, *Tadarida aegyptiaca* from low temperate latitudes (33°S) in South Africa. *S. Afr. J. Zool.* 30: 18–22.
- BERNARD, R.T.F., KERLEY, G.I.H., DOUBELL, T. & DAVISON, A. 1996. Reproduction in the round-eared elephant shrew (*Macroscelides proboscideus*) in the southern Karoo, South Africa. *J. Zool., Lond.* 240: 233–243.
- DUNCAN, L. 1976. Clinical analysis by atomic absorption. pp.46. Varian Techtron Pty Ltd, Springvale, Australia.
- KWIECINSKI, G.G. 1982. Bone remodelling and its regulation in *Myotis lucifugus*. *Bat Res. News* 23: 75.
- KWIECINSKI, G.G., KROOK, L. & WIMSATT, W.A. 1987a. Annual skeletal changes in the little brown bat, *Myotis lucifugus lucifugus*, with particular reference to pregnancy and lactation. *Am. J. Anat.* 178: 410–420.
- KWIECINSKI, G.G., WIMSATT, W.A. & KROOK, L. 1987b. Morphology of thyroid C-cells and parathyroid glands in summer-active little brown bats, *Myotis lucifugus lucifugus*, with particular reference to pregnancy and lactation. *Am. J. Anat.* 178: 421–427.
- MAYER, W.V. & BERNICK, S. 1958. A comparative study of the dentin, periodontium and alveolar bone of warm and awake and hibernating ground squirrels, *Spermophilus undulatus*. *Anat. Rec.* 131: 580.
- RAUTENBACH, I.L., KEMP, A.C. & SCHOLTZ, C.H. 1988. Fluctuations in the availability of arthropods correlated with microchiropteran and avian predator activities. *Koedoe* 31: 77–90.
- SEVICK, S.H. & STUDIER, E.H. 1992. Change in forearm curvature throughout summer in female big brown bats. *J. Mammal.* 73: 866–870.
- STEINBERG, B., SINGH, I.J. & MITCHELL, O.G. 1981. The effects of cold stress, hibernation, and prolonged inactivity on bone dynamics in the golden hamster, *Mesocricetus auratus*. *J. Morphol.* 167: 43–51.
- STUDIER, E.H., VIELE, D.P. & SEVICK, S.H. 1991. Nutritional implications for nitrogen and mineral budgets from analysis of guano of the big brown bat *Eptesicus fuscus* (Chiroptera: Vespertilionidae). *Comp. Biochem. Physiol. A.* 100: 1035–1039.
- STUDIER, E.H., SEVICK, S.H., KEELER, J.O. & SCHENCK, R.A. 1994a. Nutrient levels in guano from maternity colonies of big brown bats. *J. Mammal.* 75: 71–83.
- STUDIER, E.H., SEVICK, S.H., RIDLEY, D.M. & WILSON, D.E. 1994b. Mineral and nitrogen concentrations in feces of some neotropical bats. *J. Mammal.* 75: 674–680.
- WHALEN, J.P., KROOK, L. & NUNEZ, E.A. 1972. A radiographic histological study of bone in the active and hibernating bat (*Myotis lucifugus*). *Anat. Rec.* 172: 97–108.