

# Reproduction of the banana bat, *Neoromicia nanus*, in Mpumalanga Province, South Africa, with a discussion on sperm storage and latitudinal effects on reproductive strategies

M. van der Merwe\* & R.L. Stirnemann

Mammal Research Institute, Department of Zoology and Entomology, University of Pretoria, Pretoria, 0002 South Africa

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The banana bat, *Neoromicia nanus*, is characterized by seasonal monoestrous breeding. This study examined the reproductive timing and strategies used by *N. nanus* in the Province of Mpumalanga, South Africa. Male and female bats were histologically examined to determine their reproductive condition throughout the year. Spermatogenesis in the banana bat began in late September and spermatozoa were released into the caudae epididymides from late April to late July. Spermatozoa remained present in the caudae epididymides throughout August. Mating was initiated in May and ovulation and fertilization occurred at the beginning of August. Implantations first occurred during early August and parturition followed from late October to the end of November. Lactating females were found until mid January. In contrast to the same species in Malawi, it appears that in Mpumalanga no sperm storage takes place in females and occurs for only a short period in males. Reproductive timing was approximately one month earlier in Mpumalanga than in Malawi.

**Key words:** banana bats, embryology, *Neoromicia nanus*, reproduction.

## INTRODUCTION

The banana bat, *Neoromicia nanus*, is one of the smallest vespertilionid species, males weighing an average of 3.8 g (range: 3–5), females weighing 4.4 g (range: 3–6). The average forearm length for males is 31 mm (range: 29–38) and 32 mm (range: 30–34) for females (Taylor 2000). The banana bat has a reproductive cycle that is characterized by seasonal monoestrous breeding with births occurring mainly during the spring months. This species has an extensive geographical range extending throughout Africa in non-arid areas south of the Sahara (Bernard *et al.* 1997; Taylor 2000), making it an ideal candidate to study latitudinal reproductive variation.

The banana bat has previously been studied in KwaZulu Natal, South Africa (La Val & La Val 1997), Kenya (O'Shea 1980) and Malawi (Bernard *et al.* 1997; Happold & Happold 1990; 1996; 1998). However, no information has been collected on this species in Mpumalanga, South Africa, and the

only other comprehensive study on reproduction in this species, using histological techniques, was conducted in Malawi.

In Malawi, it has been reported that this species shows highly synchronous reproduction, but over a wider distribution, variation in reproductive timing has been reported (Bernard *et al.* 1997).

The most common reproductive delay mechanism described in bats is delayed fertilization and sperm storage (Oxberry 1979; Racey 1979), and occurs in members of the family Rhinolophidae and the subfamily Vespertilioninae of the family Vespertilionidae (Racey 1979). More recently, families other than the Vespertilionidae and Rhinolophidae in which storage of sperm occur has been identified (see Crichton 2000). Sperm storage can last several months in both males and females of some species (Racey 1979). Although sperm storage has been described for the banana bat in Malawi (Bernard *et al.* 1997) studies in Mpumalanga do not support sperm storage in female banana bats and sperm storage occur only

\*To whom correspondence should be addressed.  
E-mail: mvdmerwe@zoology.up.ac.za

for a short period in male banana bats (present study).

This study was undertaken to clarify the timing of reproductive events and to determine what reproductive strategies occur in the banana bat in Mpumalanga, South Africa. It also provides insight into the possible influence of latitudinal environmental factors on reproductive delay mechanisms such as sperm storage in this species.

### MATERIALS AND METHODS

Bats were collected from neighbouring commercial banana plantations in the Mpumalanga Lowveld, South Africa (25°30'S, 31°30'E) during 2003 and 2004. Annual rainfall in the area is approximately 116 mm, with a high in February of 68 mm. The wet season extends from October to April, the dry season from May to September which is also the coldest times of the year.

The sites were visited approximately monthly from January to December. Partially unrolled banana plant leaves (leaf tubes) were examined and the presence of bats was determined by looking into a leaf tube, or by pressing a leaf tube between the thumb and index finger and then pulling along the leaf from the base to the tip. In this manner bats were easily detected as little bumps in the leaf tubes. By gently squeezing the leaf tube from bottom to top the bats moved towards the opening and were collected in a net bag. All bat specimens were collected in 2003, with the exception of the June samples, which were collected in 2004. The number, sex and external reproductive condition of bats in each leaf tube were recorded to obtain information on social organization and reproductive behaviour.

Each month, five males and 5–8 females were collected for histological examination. Selected bats were sacrificed using Fluothane and reproductive organs were removed and stored in Bouins fixative for histological examination. After 16 hours in Bouins fixative they were placed in 70% alcohol for storage. One testis and one epididymis of each of the males were separated and cleaned of fatty tissue and weighed. The other testis and epididymis, as well as the female reproductive organs, were embedded in paraffin wax and later sectioned at 5  $\mu\text{m}$  and stained with Ehrlich's haematoxylin and counter-stained with eosin. Changes in male reproductive condition were determined by measuring the diameter of ten randomly selected epididymal and seminiferous tubules using an eyepiece micrometer.

Changes in female reproductive activity were assessed qualitatively by noting the presence or absence of primary follicles, secondary follicles, Graafian follicles, corpora lutea and the presence or absence of conceptuses. When present, the largest diameter of any secondary and Graafian follicles was measured for each specimen.

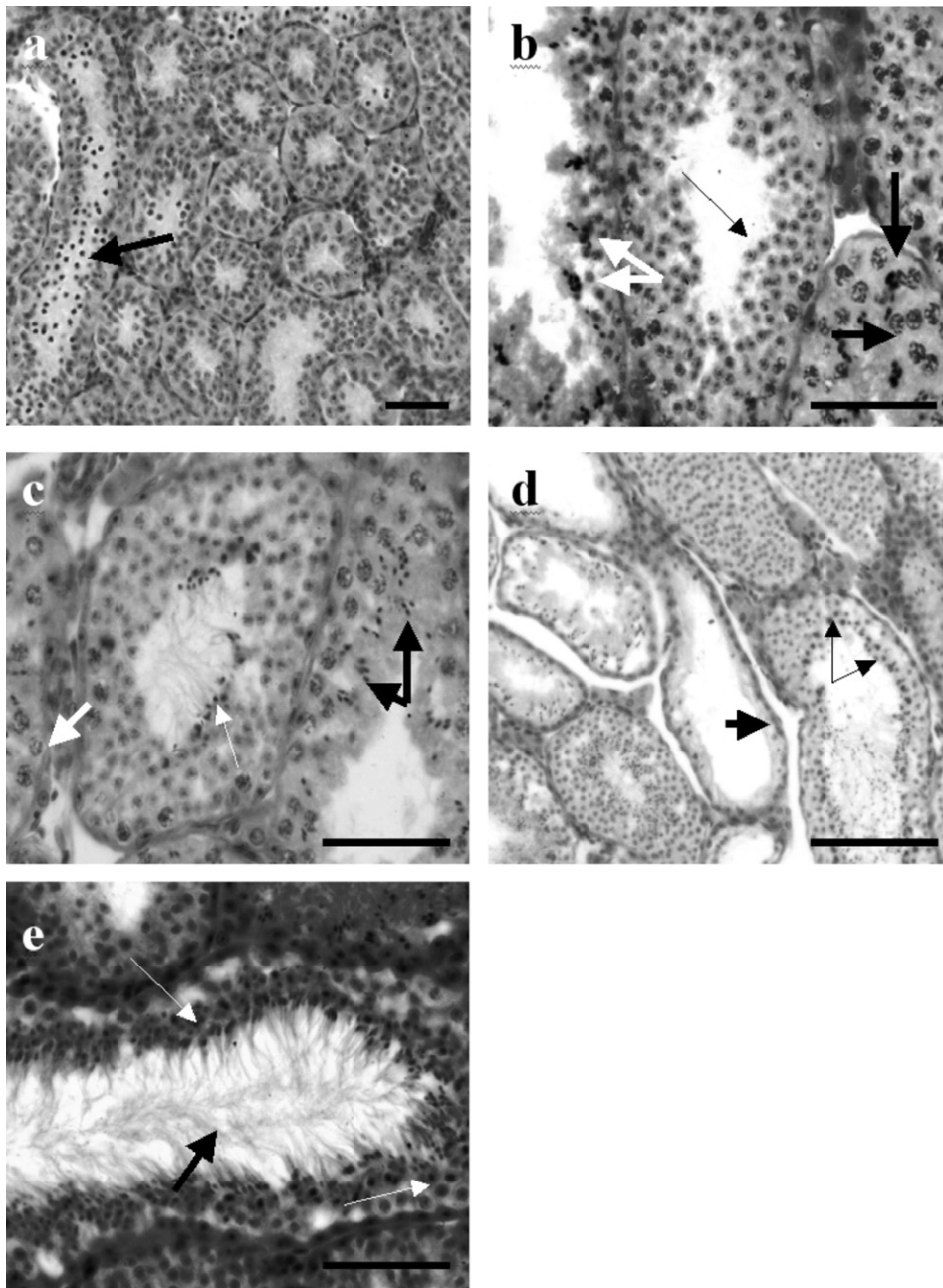
## RESULTS

### Male reproductive cycle

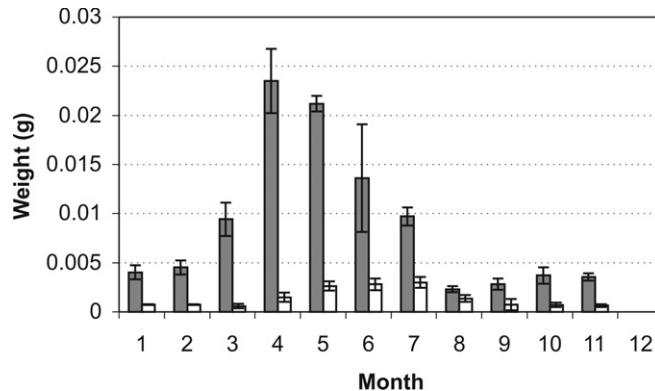
Spermatogenic activity in male *N. nanus* extended over a 10-month period from late September to late July. The first signs of mitotic activity were observed during late September, when Type A spermatogonia could be seen against the basement membrane in some of the seminiferous tubules. From November onwards there was a great increase in activity, with many of the seminiferous tubules full of Type B spermatogonia (Fig. 1a). By March, all males had numerous primary spermatocytes in the seminiferous tubules and the first meiotic divisions occurred from April onwards when primary spermatocytes gave rise to secondary spermatocytes (Fig. 1b). The second meiotic divisions followed soon after the first, with spermatids and the first signs of spermatozoa in the seminiferous tubules of two (40%) of the five males collected in April (Fig. 1b). Between late April and May, spermatozoa were released into the epididymides (Fig. 1c) and this coincided with the decrease in testicular mass and simultaneous increase in epididymal mass from late April onwards (Fig. 2). There was also a decrease in the width of the seminiferous tubules, accompanied by an increase in the width of the epididymis tubule over the same period (Fig. 3). The testes continued to produce spermatozoa throughout May, June and July (Figs 2,3). Although spermatogenesis ceased in some seminiferous tubules in June and July (Fig. 1d), other seminiferous tubules continued to release spermatozoa into the epididymides (Fig. 1e). By August, the seminiferous tubules were depleted although spermatozoa were still present in the caudae epididymides of all males examined. Spermatozoa were therefore present and available in the epididymides for four months, from May to August.

### Female reproductive cycle

Females collected in March were in pro-oestrus. The endometrium was thin with a few short, straight uterine glands. The ovaries contained



**Fig. 1.** Section through testis during: **a**, November, showing Type B spermatogonia in many of the seminiferous tubules (thick arrow). **b**, During April primary spermatocytes (thick black arrows), secondary spermatocytes (thin black arrow) and spermatids (white arrows) are present in the seminiferous tubules. **c**, During May primary spermatocytes (thick white arrow), secondary spermatocytes, spermatids (black arrows) and spermatozoa (thin white arrow) are present in the seminiferous tubules. **d**, During June some of the seminiferous tubules became depleted, with only Sertoli cells lining the basement membrane (thick black arrow). In some seminiferous tubules spermiogenesis, is still active with lots of cytoplasmic residual bodies (thin black arrows). **e**, During July spermiogenesis, is still active taking place in many of the seminiferous tubules releasing spermatozoa into the epididymides. Cytoplasmic residual bodies are common (thin white arrows), while masses of spermatozoa are still present in some of the seminiferous tubules (black arrow). Scale bars = 50 µm.

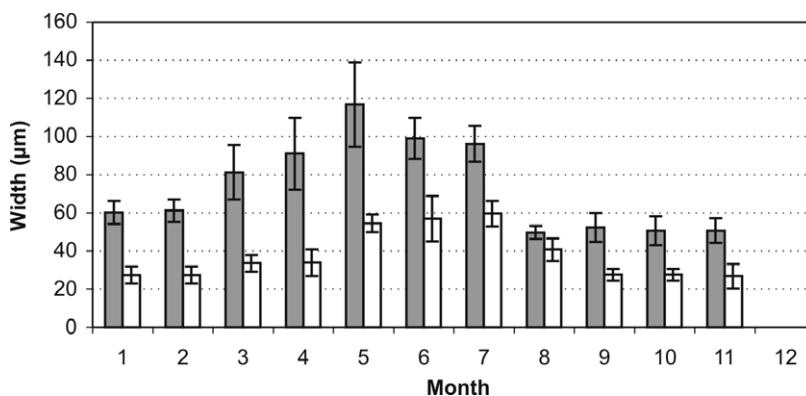


**Fig. 2.** Mean (■) testis and (□) epididymis mass ( $\pm$ S.D.) in male *Neoromicia nanus*. The months of January to December are numbered 1 to 12.

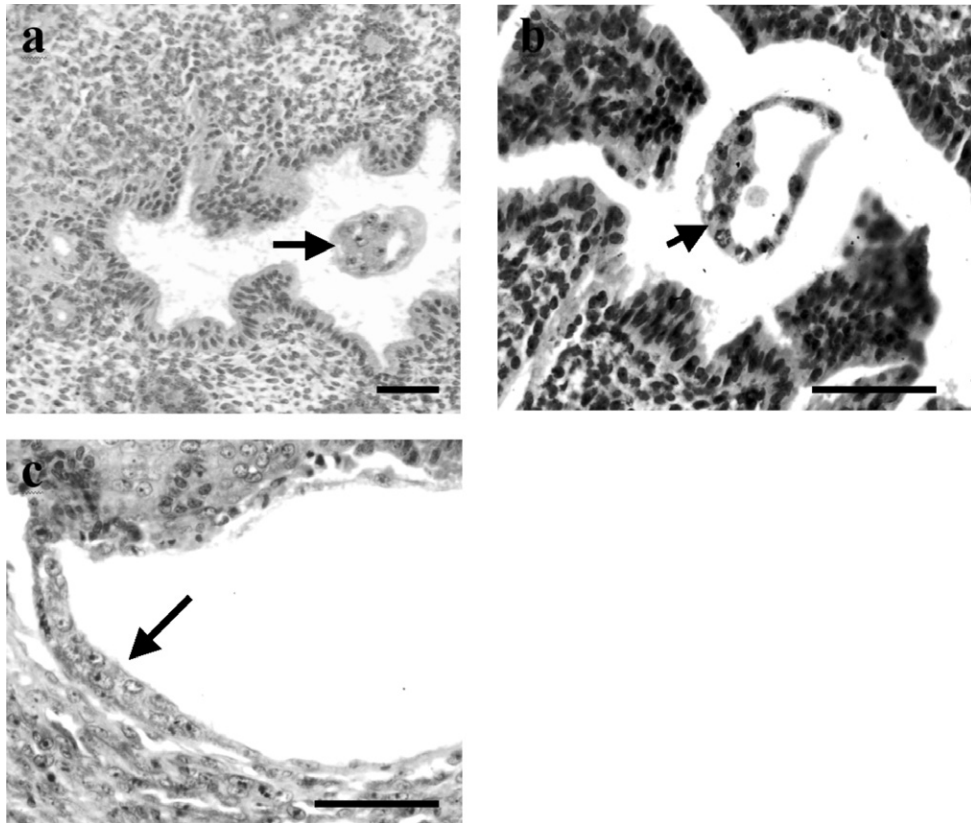
developing primary and secondary follicles that were beginning to become tertiary. The mean diameter of the secondary follicles was  $141.31 \mu\text{m}$  (S.D.  $\pm 40.45$ ). Polymorphonuclear leucocytes were found in the uterine lumen of five (83%) of the six females sampled. In April, all five females sampled had tertiary follicles with one female having large Graafian follicles (one in one ovary and two in the opposite ovary). Polymorphonuclear leucocytes were found in the uterine lumen of two of the five females. By May, most females had tertiary follicles and polymorphonuclear leucocytes were present in all the female tracts. Sperm heads were found in two of the seven females sampled. In June five (83%) of the six females sampled had low numbers of spermatozoa at the uterotubal junction area of the reproductive tract. Furthermore, the females with spermatozoa in the reproductive tract also had polymorphonuclear leucocytes in the uterine lumen. Graafian follicles (one in each ovary) were present in the ovaries of

all the females. In July, Graafian follicles (one in each ovary) were present in the ovaries of all six females collected and only three had spermatozoa in the reproductive tract. The mean size of the Graafian follicles was  $265.34 \mu\text{m}$  (S.D.  $\pm 55.32$ ). By mid-August, all eight females examined had ovulated and in seven (87.5%) implantation of the embryos had begun. The two youngest embryos were collected on 15 August. One of these was at a late morula stage where the blastocyst cavity was just beginning to form, while the other was a young unilaminar blastocyst (Fig. 4a,b).

When the blastocyst was ready to implant, it was completely surrounded by uterine endometrium and the unilaminar blastocyst's inner cell mass had already formed a disc (Fig. 4c). In all cases, the blastodisc was orientated antimesometrially and the invasion of the maternal endometrium by the trophoblast was also antimesometrial. From August onwards all females were visibly pregnant. Parturition began at the end of October,



**Fig. 3.** Mean (■) seminiferous and (□) epididymis tubule width ( $\pm$ S.D.) in male *Neoromicia nanus*. The months of January to December are numbered 1 to 12.



**Fig. 4.** Sections through uteri. **a**, Late morula (arrow) in the uterine lumen with blastocyst cavity beginning to form. **b**, Young blastocyst (arrow) in the uterine lumen. The endoderm has not yet formed. **c**, Blastocyst at the blastodisc stage (arrow). Scale bars = 50  $\mu$ m.

approximately 11 weeks after fertilization.

Of a total of 182 females examined on 1 November, 9.7% were lactating, indicating parturition during late October, 80.6% were pregnant and 9.7% were not pregnant but not lactating. By 6 December, 82.0% were lactating, none were pregnant and 18.0% were not pregnant ( $n = 90$ ). This indicates close synchronization of the breeding season, with ovulation and fertilization occurring over a five week period (end July to end August), and parturition occurring over a five week period (end October to end November). On 15 January only 6.2% of the 64 females examined were still lactating.

#### DISCUSSION

The first signs of spermatozoa in the seminiferous tubules of banana bats collected from the Mpumalanga Province, South Africa, were seen in late April. Spermatogenesis was completed by the end of July and spermatozoa were first found in the

reproductive tract of the females in May and early stages of implantation were first seen in August. It is expected that ovulation occurred from late July to August, as the two youngest embryos collected on 15 August were a late morula and young blastocyst in the uterine lumens of two females (Fig. 4a,b). These embryos must have resulted from fertilizations that occurred during the beginning of August since the estimated time to reach the uterine lumen in vespertilionids is 5–7 days (Badwaik & Rasweiler 2000).

#### Sperm storage

In males, the caudae epididymides and vasa deferens (Racey 1979) and in some cases the testes (Krutzsch 1975; Krutzsch & Crichton 1986) act as sperm storages. In females, sperm storage occurs in three main areas; the uterus, the uterotubal junction and the oviduct (see Racey 1979). In Mpumalanga, it is predicted that banana bat copulations occur from late April to August



since these bats do not appear to hibernate, and a drastic increase in the mass of the epididymis was not observed (Fig. 2), suggesting frequent loss of spermatozoa due to copulation. Although it is expected that these bats enter daily torpor they, however, wake immediately when disturbed and would rapidly crawl deeper into the leaf tubes or when near the entrance fly out. This phenomenon was found on all visits to the banana plantations including the cooler months May to July and is in contrast to the situation found by Bernard *et al.* (1997) where males and females were very torpid during the day in the cool dry season. In Mpumalanga only small amounts of spermatozoa were retained in the female at the uterotubal junction area which is also in contrast with the findings of Bernard *et al.* (1997) who reported that large amounts of spermatozoa were found in the upper end of the uterine horns and the uterotubal junction. In the Mpumalanga bats the amount of sperm present in the females did not increase from May to July, suggesting a possible loss of spermatozoa. It has long been argued that for some bat species additional copulation during the hibernation period and even after arousal in the spring may be necessary to assure fertilization, because the initial spermatozoa may either lose their capacity to fertilize or be expelled from the female reproductive tract (Guthrie 1933; Guthrie & Jeffers 1938; Krutzsch 1975). Guthrie (1933) mentioned that muscular activity result in the expulsion of uterine contents into the vagina and to the outside and further that there is a reduction of sperm due to the phagocytic behaviour of the leucocytes. It thus seems that activities such as flying around may result in the emptying of the sperm and other contents from the female tract, and that repeated winter foraging might each time empty the tract of sperm (Krutzsch 1975). Racey (1975) also referred to factors that may be involved in the removal of spermatozoa from their storage organs, such as muscular contractions, enzymatic dissolution, phagocytosis by leucocytes or epithelial cells, or the active entry of sperm into the epithelial and subepithelial tissues. According to Crichton (2000) leucocytes are responsible for the removal of dead spermatozoa prior to and following ovulation while Mori & Uchida (1980) mentioned that while spermatozoa in the uterotubal junction appeared normal, those remaining in the uterus degenerated and were engulfed by a massive invasion of polymorphonuclear leucocytes. According to Racey (1975) bat species that store spermatozoa in the female tract

are remarkably free of leucocytes. This was not the case with the banana bats in Mpumalanga where polymorphonuclear leucocytes were found throughout the mating period. The presence of sperm heads together with leucocytes in some females suggests that sperm break down occurred in the afore mentioned method.

Since about 98% of all parous females examined were either pregnant or lactating it is expected that spermatozoa should be present in the majority of parous females examined during the period May to July, if sperm storage occurred. However, three out of six (50%) parous females examined in July contained no sperm in the reproductive tract, strongly suggesting a lack of sperm storage. In addition, if sperm storage occurred in banana bat females in Mpumalanga, an increase in the amount of spermatozoa in the female tract would be expected over the three month period from May to July due to regular copulations. Copulations during the hibernation period are not uncommon amongst bats (Gustafson 1979; Tidemann 1982; Van der Merwe & Rautenbach 1990; Feldhamer *et al.* 1999; Vaughn *et al.* 2000).

In Mpumalanga, male banana bats are capable of copulating with females over a period of approximately four months (late April to late August). In the banana bat it is questionable whether it can be regarded as true sperm storage when spermatogenesis is actively taking place and sperm is constantly released into the epididymides (Figs 2,3). No obvious increase in epididymidal sperm was found over the three month period from May to July. Because epididymal sperm did not increase conspicuously from May to July, a continuous replacement of spermatozoa is suggested as a result of regular copulations. In the rusty pipistrelle there is a simultaneous increase in the size of the epididymides with a decline in the size of the testes, so that by the time the testes become completely involuted, the epididymides have increased about three-fold in size (Van der Merwe & Rautenbach 1990). The only possible period of sperm storage in the male banana bat was in the month of August when the testes were completely involuted and the epididymides were full of spermatozoa. This is necessary to fertilize ova from ovulations that occurred from late July to late August. Though it could be argued that the females may store spermatozoa, any sperm storage would be of no significance because of the regular copulations which take place during the four months May to August. Furthermore, if sperm storage

does take place it is likely to be an evolutionary trait that is retained in the female but is no longer of any adaptive value to the banana bat in Mpumalanga.

### Latitudinal differences

Different latitudes have varying energetic constraints which may provide a selective pressure for variation in reproductive strategies. It is now widely accepted that species cue into predictable seasonal changes to optimize reproduction (Racey 1982; Heideman 1995; Cumming & Bernard 1997; Wikelski *et al.* 2000). The timing of bat reproduction could also potentially be affected by other factors unrelated to seasonal weather conditions, including seasonal changes in the ease of obtaining food, in competition and in predatory pressures (Cumming & Bernard 1997; Heideman & Utzurrum 2003).

Latitudinal climatic variation can affect the timing of reproduction over latitudinal distributions. The reproductive periodicity evident in many bat species reflects seasonal variations in food supply (Racey 1982). Bats from temperate latitudes give birth only during the summer months (Oxberry 1979), when insect food supply is at a peak due to the high rainfall (Taylor 1963; Pirlot 1967). In the Kruger National Park (Mpumalanga) the highest monthly availability of aerial arthropods corresponds with the warm summer rainy season (Rautenbach *et al.* 1988).

The reproductive strategies observed in the Banana bat in Mpumalanga appear to be different to those in Malawi where Bernard *et al.* (1997) reported sperm storage in both males and females of this species. Timing of reproductive events in Malawi was also about one month later than in Mpumalanga. The timing of rainfall and temperature does not seem to explain the variation in timing and strategies observed in the banana bat, since the wet and dry seasons in Malawi correspond to those in Mpumalanga. The only possible explanation for these differences is that the timing of mating and birth, length of gestation and reproductive strategies varies with latitude as has been noted in a number of bat species (see Racey 1982; Van der Merwe 1980).

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