Foetal growth curves and seasonal breeding in the Natal clinging bat *Miniopterus schreibersi natalensis*

M. van der Merwe

Growth of the foetus of *Miniopterus schreibersi natalensis* is described. The gestation period in this subspecies is 240 days of which 120 days is the period of delayed implantation. Birth mass is taken as 2,7 g from measurements of near-term foetuses. Foetal growth follows a typical J-shaped curve during the last half of the gestation period. Young of *M. s. natalensis* are proportionately lighter at birth than many other mammals.
S. Afr. J. Zool. 14: 17–21 (1979)

Fetale groei in *Miniopterus schreibersi natalensis* word beskryf. Die dragtigheidsperiode in hierdie subspesies is 240 dae, waarvan 120 dae 'n periode van vertraagde implantering is. Geboortemassa is 2,7 g en is bereken uit gegewens verkry van fetusse net voor geboorte. Fetale groei volg 'n tipies J-vormige kurwe gedurende die laaste helfte van die dragtigheidsperiode. Die kleintjies van *M. s. natalensis* is proporsioneel ligter by geboorte as baie ander soogdiere.
S.-Afr. Tydskr. Dierk. 14: 17–21 (1979)

M. van der Merwe
Mammal Research Institute, University of Pretoria, Pretoria 0002

Studies on prenatal development normally require a knowledge of foetal age in order to follow up embryonic changes with age. Construction of foetal growth curves has therefore become an integral part of these studies. Unfortunately many studies are done on wild animals in their natural habitats, where close observation is not possible. Thus, the exact time of ovulation/copulation is not known, and if a pregnant female is collected, it is not known for how long she has been pregnant. The ovulation/copulation period of certain species is known to stretch over a few weeks. Therefore, because it is not known when during the ovulation/copulation period a female was fertilized, the age of the embryo/foetus she is bearing cannot be estimated accurately. Here the empiric equation of Huggett & Widdas (1951) has become very useful in the determination of foetal age. Unfortunately, however, a completely false picture may be obtained when this equation is used in certain mammals which deviate from the normal mammalian reproduction pattern. Such is the case in mammals where delayed implantation occurs (Frazer & Huggett 1974).

In the Chiroptera delayed implantation occurs only in the hibernating members of the genus *Miniopterus* (Wimsatt 1969, 1975) and it was found necessary to assume that $t_g - t_0$ covers the period from implantation onwards, rather than that pregnancy starts with fertilization (Frazer & Huggett 1974). In *Miniopterus schreibersi natalensis* (A. Smith, 1834) where delayed implantation occurs, it was however not found advisable to have t_0 coincide with the time of implantation as suggested by Frazer & Huggett (1974), for reasons to be discussed in the text.

The purposes of the present study are to construct growth curves for use in studying foetal development in this species, and to cover aspects of the reproduction pattern.

Methods

During 1968 and 1972–76, mostly during 1974, 178 foetuses were collected. Most foetuses were taken from females collected at Schurveberg Cave No. 1 (25°48'S, 28°01'E) situated on the southern Transvaal Highveld. Some pregnant females were also collected at two maternity caves, Sandspruit Cave No. 1 (24°37'S, 27°40'E) and Peppercorn's Cave (24°08'S, 29° 12'E), both situated in the northern Transvaal bushveld.

All foetuses were preserved in AFA, a mixture of ethyl alcohol (95 per cent), formalin (40 per cent), glacial acetic acid and distilled water in the ratio of 3:1:1:5. Measurements taken were body mass and crown–rump length.

Foetal age was determined by using an expression modified from the equation of Huggett & Widdas (1951) for mammalian foetal growth. This was done in order to have a general equation incorporating the period of delayed implantation. For convenience the gestation period in *M. s. natalensis* was divided into two parts, *viz* the retarded gestation period which covers all the events from conception until implantation, and the active gestation period which covers all the events from implantation until birth. The period of delayed implantation then coincides with the retarded gestation period and for all practical purposes most of the foetal development occurs during the active gestation period.

The specific foetal growth velocity a for *M. s. natalensis* was determined from the following equation, in which the age of the foetus t is always greater than the time of implantation y,

$$W^{1/3} = a[(t - y) - (t_g - y)f]$$

where $W^{1/3}$ is the cube root of the foetal mass (in grams), $(t - y)$ is the age of the foetus from the time of implantation, t_g is the true gestation period (retarded gestation period + active gestation period), $(t_g - y)$ is the active gestation period from the time of implantation until birth, and f represents arbitrary values given by Huggett & Widdas (1951) for calculating t_0 values for mammals with various gestation periods.

With a gestation period $t_g = 240$ days, the time of implantation y = 120 days after conception and an average birth mass of 2,7 g, the specific foetal growth velocity a for *M. s. natalensis* was established at 0,0145 using f = 0,2 for mammals with a gestation period of 100–400 days (Huggett & Widdas 1951).

With the specific foetal growth velocity a known, the age in days t of a foetus with known body mass can be determined with the expression

$$t = W^{1/3}/a + y + (t_g - y)f$$

which in the case of *M. s. natalensis* will be

$$t = W^{1/3}/0,0145 + 120 + (240 - 120)0,2$$
$$= W^{1/3}/0,0145 + 144$$

The gestation period of 240 days was calculated by noting the time of the first ovulations/conceptions (first week of March) and the first parturitions (last week of October), and by comparing the peak of ovulations/conceptions with that of parturition. The time of implantation (120 days after conception) was calculated by comparing the time when the first ovulations/conceptions occurred with the time when the first implantations were recorded, as based on histological material. In all cases where a mean mass was determined the limits of the population mean were also determined. These figures are quoted as a + b, which means that it can be assumed with 95% confidence that the true mean of the population lies in the interval (a − b; a + b). In addition the range is given in each case.

Results

In *M. s. natalensis* the average mass at birth was calculated from near-term foetuses and not from neonates, because the average mass of near-term foetuses was slightly higher than that of neonates. The average mass of 25 near-term foetuses was 2,7 ± 0,1 g (range 2,4-3,1 g). No significant difference in body mass was found between the sexes of these near-term foetuses (t = 0,19; P > 0,05).

Eighteen neonates still attached to their mothers, of which eight had moist and soft umbilical cords and placentae attached, were collected at Sandspruit Cave No. 1 on 21 November 1974. They had an average mass of 2,5 ± 0,2 g (range 2,1-2,8 g) which is 0,2 g lower than that of foetuses just before birth. The age of these neonates was estimated to be from a few hours to one day post partum. It was suspected that this decrease in mass continues for a day or two, stabilizes, and then starts to increase. It appeared that the neonates only regained birth mass on the second or third day post partum. On 28 October 1975 at commencement of parturition only 65 juveniles were found in a single small cluster in the maternity chamber of Sandspruit Cave No. 1. These were the first juveniles born during the 1975 parturition period and none of them could have been older than two or three days. Of these, 17 were collected at random and their average mass was 2,7 ± 0,2 g (range 1,9-3,5 g), the same as that of foetuses before birth.

The crown-rump length and mass are plotted against age of foetuses in Figs. 1 and 2. On account of their small size and because all foetuses are not folded in exactly the same manner in the uterus, crown-rump lengths varied too much to be used as a criterion for age determinations. The most
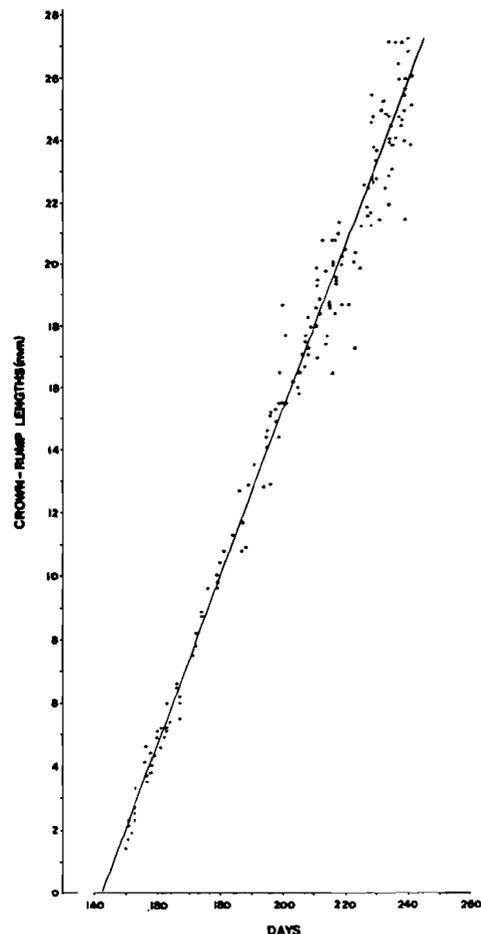

Fig. 1 Crown-rump lengths of *M.s. natalensis* foetuses plotted against age in days.
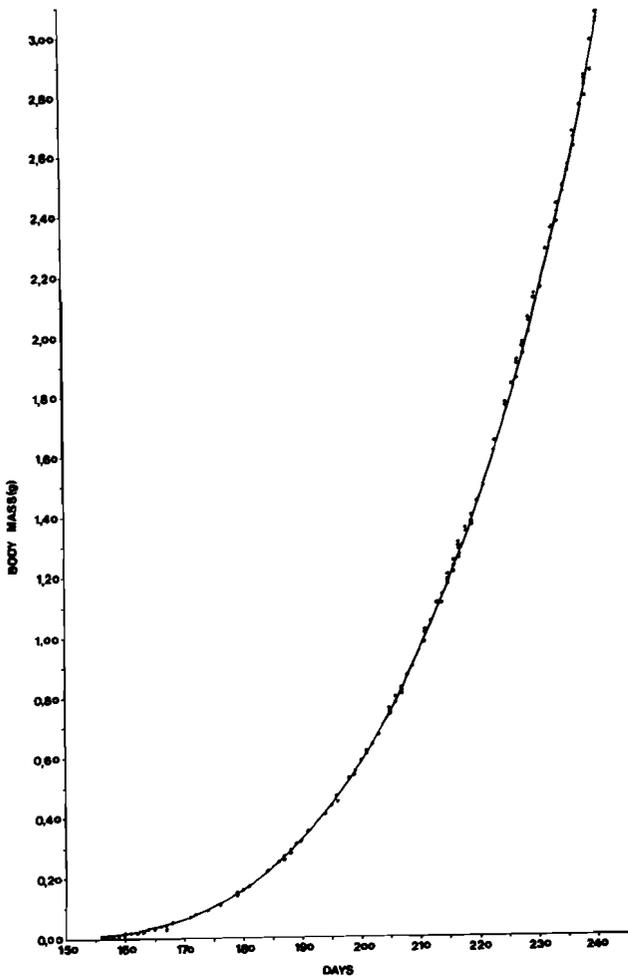
Fig. 2 Body mass of *M. s. natalensis* foetuses plotted against age in days.

reliable criterion was found to be the body mass of the foetuses. The increase of mass with age followed the typical J-shaped curve (Fig. 2). The cube root of foetal mass is plotted against age in Fig. 3, and was found to be most convenient for the determination of foetal age. The graphs appear to be fairly reliable, although their accuracy cannot be checked because there were no foetuses of known age. The earliest embryos at the primitive streak stage of

development were collected on 11 July 1974, indicating an age of 128 days prenatal life. The average age of all foetuses examined when the limb buds just started to show was 154 days, estimated from the cube root graph for foetal mass (Fig. 3). This indicates that the limb buds only started to appear at about 26 days after the primitive streak stage. This seems reasonable because if implantation occurs 120 days post coitum, it takes 34 days from implantation to the limb bud stage. From the limb bud stage to birth it then takes 86 days, indicating that the foetal stage of the 'active gestation period' accounts for 71,7 per cent of the total period from implantation to birth, while development from implantation up to the limb bud stage accounts for 28,4 per cent of the active gestation period. The foetal period (in terms of the active gestation period) in *M. s. natalensis* is of the same order as in large mammals. In the sheep it accounts for 77 per cent of the total gestation period and in the bovines for 84 per cent (Green & Winters 1945).

The period from implantation to the primitive streak stage in *M. s. natalensis* was estimated at eight days. Therefore, during the active gestation period, development from implantation to the primitive streak stage accounts for 6,7 per cent; from the primitive streak stage to the limb bud stage 21,7 per cent, and from the limb bud stage to birth (i.e. the foetal period) for 71,7 per cent of the time. Therefore, each period is roughly one third of the following stage. However, when the true gestation period (i.e. retarded plus active gestation period) is taken into consideration, these values are half the active gestation period values. The period of the zygote occupies by far the greatest time (50 per cent), and the percentages of the other three stages are respectively 3,4, 10,8 and 35,8 per cent of the gestation time.

As the retarded gestation period in this study was taken from fertilization to implantation, and incorporated in the general expression to estimate the real age of a foetus, the actual date of ovulation and conception could be estimated. This was possible because the exact dates on which the foetuses were collected were known. Therefore by extrapolating the age of a foetus in days (determined with the general expression) from the date of collection, the time of
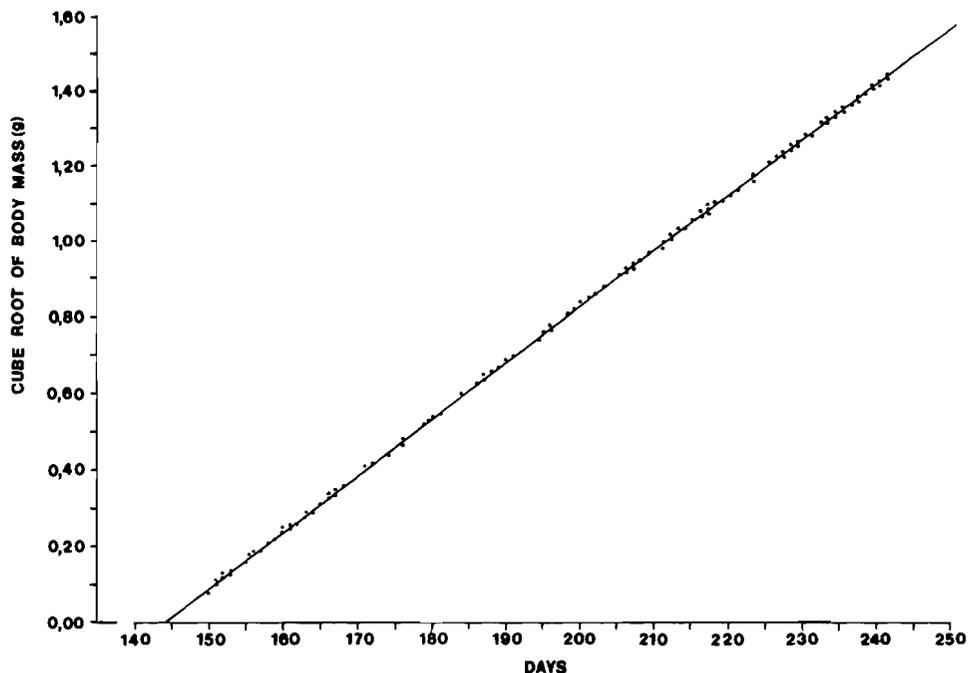

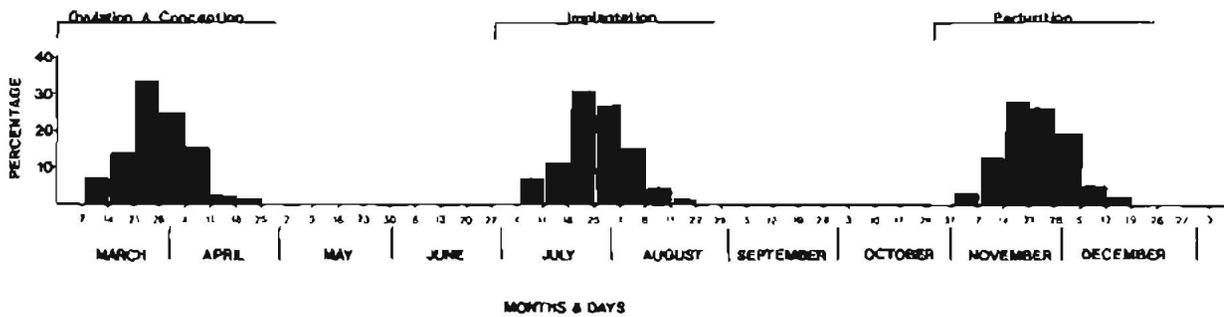Fig. 3 Cube root of foetal mass plotted against age for *M. s. natalensis* foetuses.

Fig. 4 Estimated dates and percentages of ovulations/conceptions, implantations and parturitions for foetuses of *M. s. natalensis*.

conception was established. It is believed that conception normally occurs within one to two days after ovulation. Therefore, the possible date of ovulation could be worked out by adding one or two days to the age of a foetus, and extrapolating it from the time of collection. Because ovulations and conceptions occurred over more than $1\frac{1}{2}$ months the age of foetuses from any collection (random sampling) varied considerably ($1\frac{1}{2}$ months or more). Therefore, the age and time of conception of each foetus was determined individually. With the time of conception known for each foetus, the time of implantation and birth for each foetus was determined by adding 120 days (for implantation) and 240 days (for birth) respectively to the time of conception. These data are summarized in Fig. 4. From this it can be seen that the majority of ovulations and conceptions occurred around the end of March and the beginning of April. In some individuals ovulation and conception had already occurred during the first week of March. The percentage of such early conceptions was, however, very low (less than 1 per cent), thus the chance of collecting such foetuses was slight. Of the 178 foetuses examined one was conceived on 7 March 1974, indicating ovulation in that female on about 5 March. However, in three uteri from individuals collected on 6 March 1974 the situation varied, with the youngest conceptus a zygote (Fig. 5) in the fallopian tube, and the oldest conceptus a young morula (Fig. 6) already located in the right uterine lumen. This shows that ovulations occurred during the first week of March. The same then accounts for implantation (not observed but estimated) and parturition, where less than one per cent of the implantations and parturitions may be expected a week

earlier. Although this could only be estimated for the implantations, it was actually observed for parturition. On two visits to Sandspruit Cave No. 1 (27 October 1968 and on 28 October 1975) 100 and 65 neonates were estimated respectively in small juvenile clusters. It is difficult to observe the last conceptions as a very few may occur as late as May. Of the foetuses examined one was conceived on 9 May 1974, indicating ovulation on about 7 May. Implantation in this foetus could have occurred as late as 6 September 1974 and it could have been born as late as 13 January 1975. This is not surprising, as two or three neonates were occasionally found as late as January, where they were always obvious (being pink and naked) among the other juveniles which were already fully covered with juvenile fur. The sectioned uterus of a female collected on 5 September 1974 revealed a blastocyst that was implanting, indicating that the foetus would only have been born about 14 January 1975. The peak of parturitions, however, was towards the end of November (Fig. 4).

Neonatal mass was found to be 27 per cent of the maternal mass. The mass of females and their own young directly after birth was not measured, and the figure of 27 per cent was obtained from the mean mass of unrelated females and young. Although not the best way to do it, this method gave a fair idea of the relationship that could be expected between neonatal and maternal mass. Mean mass of females taken in two ways was of the same order. The mean mass of 18 lactating females collected on 21 November 1974 (i.e. roughly two-thirds through the parturition period) at Sandspruit Cave No. 1 was $10,16 \pm 0,40$ g (range 9,81-10,68 g). Secondly, the mean neonatal mass at
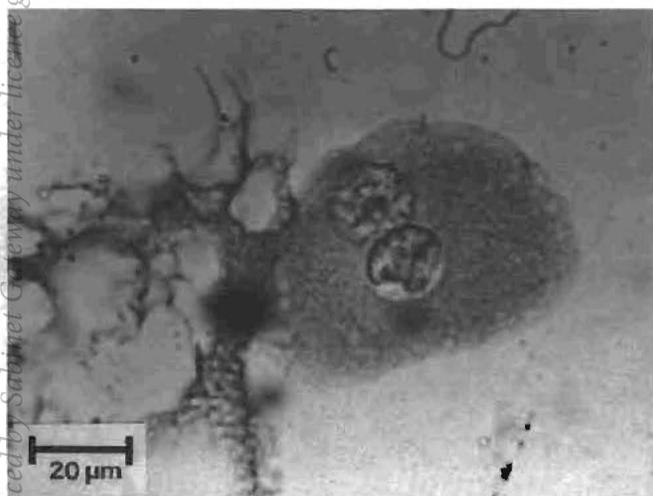

Fig. 5 Zygote where karyogenesis is already initiated. This section is eccentric through the zygote in order to show the two nuclei separating (Bouin, 5 μm, Mallory).
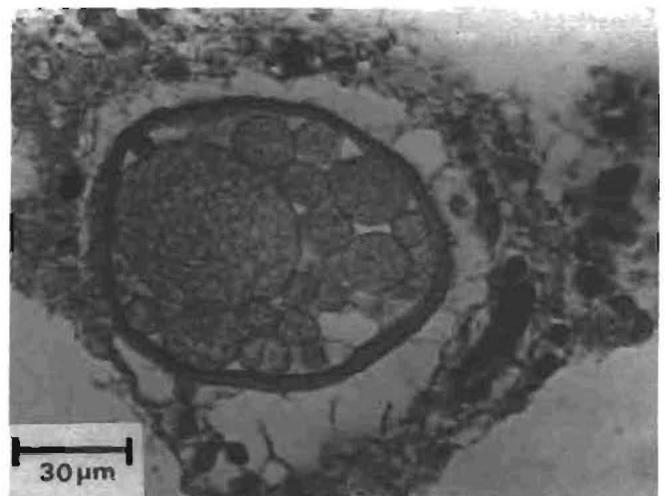

Fig. 6 Young morula already in dextral uterine lumen. It had originated from the sinistral ovary (Bouin, 5 μm, Mallory).

birth (2,7 g) was subtracted from the mass of each of eight full-term females caught at Schurveberg Cave No. 1 on 22 October 1974, to give their approximate mass after parturition, and the mean was 10,18 ± 0,45 g (range 9,38-10,89 g). From the calculated mean mass of non-pregnant females the ratio of neonatal to maternal mass was 26,57 and 26,52 per cent respectively.

Discussion

In the hibernating members of the genus *Miniopterus* in temperate regions there is a characteristic period of retarded embryonic development (Wimsatt 1969), which in all cases is presumably the period of delayed implantation. In *M. s. natalensis* on the southern Transvaal Highveld, the period of delayed implantation (retarded gestation period) appears to be fairly constant at four months. During this period there is no obvious increase in size and it is doubtful whether any significant increase in mass occurs during the unimplanted stage. It was therefore assumed that for all practical purposes growth and development take place only during the active gestation period, i.e. from implantation onwards. However, the delayed implantation period has been incorporated in a general equation, derived from that of Huggett & Widdas (1951), which proved adequate for the determination of foetal age in this species. The foetal growth velocity for *M. s. natalensis* was found to be 0,0145, which falls in the range 0,01-0,09 given for Chiroptera by Frazer & Huggett (1974). These authors mentioned the problems encountered where delayed implantation occurs, and that it would be better to calculate specific growth rates from the time of implantation rather than from the time of conception.

In this study the derived equation used was found to be more reliable for the determination of foetal ages than by having t_0 coinciding with the time of implantation as suggested by Frazer & Huggett (1974). This was especially the case with foetuses in the earlier stages of foetal development. We have established that ovulations in this species are initiated at the beginning of March, and terminated during the last half of April. Therefore, because the oldest foetuses of any sample will be the product of the earliest conceptions of that breeding season, the oldest foetuses collected on any particular date during this study should have originated from conceptions that occurred during the first half or two-thirds of March. However, by having t_0 coinciding with the time of implantation (i.e. $t_0 = 120$ for *M. s. natalensis*) the age of foetuses during the earlier foetal development stages proved too young. Here the biggest foetuses were found to have resulted from conceptions that occurred between the end of March and beginning of April, whereas it should have fallen within the first half or two-thirds of March. During the later stages of foetal development the differences in age calculated by both methods under discussion decreased progressively as the foetus grew bigger (or older), e.g. the body mass of a very near full-term foetus collected on 17 November 1972 was 2,66 g. The age of that foetus is 239,5 days when calculated with the derived equation, while it is 239,4 days when t_0 coincides with the time of implantation. However, during early foetal developmental stages significant differences between the two methods emerged. For example: the oldest foetus collected on 15 September 1976 had a body mass of 0,17 g. With the derived equation its age was calculated at 181,8

days, indicating conception on 17 March 1976. By having t_0 coinciding with the time of implantation the age of this specific foetus was calculated at 167,3 days with conception on 1 April 1976, which was too late in the season for a foetus of that size.

In *M. s. natalensis* a significant drop in body mass was found in neonates, even a few hours post partum. This may be a common phenomenon in mammals and the effect may be greater in very small mammals. Therefore, if birth mass can not be obtained at the time of parturition it may be advisable to take the mass of near-term foetuses. Reasons for the drop in body mass directly after birth have been given by some authors. Minot (1891, 1907) mentioned that there is a lessening of the power of growth immediately after birth, which is caused by the physiological shock from which the organism suffers as a consequence of being born. Hammond & Marshall (1958) mentioned the loss of water by evaporation shortly after birth as another factor. Slight dehydration after birth is believed to be the main factor causing this drop in mass in *M. s. natalensis*.

Racey (1972), using the expression of Leitch *et al.* (1959) which relates maternal and neonatal mass, found that pipistrelle young are proportionately smaller at birth than those of many other mammals. Using the same expression $N = 0,5408 \, M^{0,8323}$ (where M = mass of the mother and N = mass of the young) on *M. s. natalensis* where average maternal mass was taken at 10,16 g and average neonatal mass as 2,7 g, the predicted neonatal mass is 3,72 g or 36,6 per cent. This is 10 per cent higher than the observed figure which also indicates the proportionally lighter mass of neonatal *M. s. natalensis*.

Acknowledgements

References

FRAZER, J.F.D. & HUGGETT, A. St G. 1974. Species variations in the foetal growth rates of eutherian mammals. *J. Zool. Lond.* 174: 481-509.

GREEN, W.W. & WINTERS, L.M. 1945. Prenatal development of the sheep. *Univ. Minn. Agr. Exp. Sta. Tech. Bull.* 169: 1-36.

HAMMOND, J. & MARSHALL, F.H.A. 1958. The life-cycle. In: *Marshall's physiology of reproduction*, 2: 793-846, ed. Sparkes, A.S. London. Longmans.

HUGGETT, A. St G. & WIDDAS, W.F. 1951. The relationship between mammalian foetal weight and conception age. *J. Physiol.* 144: 306-317.

LEITCH, I., HYTTEN, F.E. & BILLEWICZ, H.Z. 1959. The maternal and neonatal weights of some mammalia. *Proc. zool. Soc. Lond.* 133: 11-28.

MINOT, C.S. 1891. Senescence and rejuvenation. I. On the weight of guinea-pigs. *J. Physiol.* 12: 7-153.

MINOT, C.S. 1907. The problem of age, growth and death. *Pop. Sci. Mon.* 71: 193-216.

RACEY, P.A. 1972. Aspects of reproduction in some heterothermic bats. Ph.D. thesis, St. Bartholomew's Hospital Medical College, London.

WIMSATT, W.A. 1969. Some interrelations of reproduction and hibernation in mammals. *Symp. Soc. exp. Biol.* 23: 511-549.

WIMSATT, W.A. 1975. Some comparative aspects of implantation. *Biol. Reprod.* 12: 1-40.