

# The male reproductive cycle of three species of Australian vespertilionid bat

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## Abstract

The male reproductive cycles of three species of vespertilionid bat (*Nyctophilus geoffroyi*, *N. major*, and *Vespadelus regulus*) are described. Descriptions are based on the degree of testicular and cauda epididymal development estimated from field captures, and for *N. geoffroyi*, on histological examination of fixed specimens and assays of their testicular testosterone concentrations. All species were found to undergo a dissociated pattern of reproduction; the activity of the testes and accessory glands of reproduction were asynchronous. Spermatogenesis occurred in summer/early autumn (December–March) and testes generally regressed before mating began in autumn (March–May). Spermatozoa were stored in the cauda epididymides during the mating period. Leydig cells of *N. geoffroyi* involuted prior to mating but the prostate gland remained enlarged and secretory during winter (June–August), regressing in late spring (November). Testicular testosterone concentrations were greatest when Leydig cells were maximally enlarged, and concentrations were low during the mating period. The reproductive cycle of all species apparently involved asynchrony between primary and secondary sexual function, which is typical of vespertilionid bats inhabiting temperate latitudes. As mating occurs when sperm stores cannot be replenished, selection for prudent sperm allocation may be expected.

**Key words:** vespertilionid bat, reproduction, testes, spermatogenesis, sperm storage

## INTRODUCTION

Gamete production, sex steroid production and mating behaviour are usually contemporary events in seasonally-breeding vertebrates (Crews, 1984). However, there are numerous exceptions to this general rule, particularly amongst microchiropteran bats (e.g. Gustafson, 1979; Oxberry, 1979; Racey, 1982). For example, female microchiropteran bats inhabiting temperate latitudes often mate during winter when their sex hormone levels are basal and gonadal activity is minimal (Crews, 1984), storing sperm until ovulation in spring (Racey, 1982; Hosken, O'Shea & Blackberry, 1996). However, the ability to store sperm over winter is not restricted to females, and in some rhinolophids and vespertilionids males also store sperm during hibernation (Racey, 1979, 1982).

Two broad reproductive patterns have been documented for male bats that hibernate (Gustafson, 1979): 1) males either complete their whole reproductive cycle

prior to hibernation (e.g. *Miniopterus*); or 2) seasonal torpor interrupts the male sexual cycle and male reproductive functions are dissociated (Wimsatt, 1960, 1969; Gustafson, 1979). In the second instance, typical of most vespertilionid bats that hibernate, temporal asynchrony exists between primary and secondary reproductive processes (Gustafson, 1979, 1987), spermatogenesis is renewed each spring after winter torpor, reaches a peak in summer/early autumn, before seminiferous activity plummets to a prepubertal state during autumn when mating begins (Racey, 1982; Gustafson, 1987). In contrast, the secondary reproductive glands are maximally enlarged after spermatogenesis has ceased, and remain hypertrophied and filled with secretion or spermatozoa during the overlapping hibernal and mating periods (Gustafson & Shemesh, 1976; Gustafson, 1979, 1987; Racey, 1982), and following spring arousal when testicular activity has recommenced, the secondary sex glands regress (Gustafson, 1979, 1987). In addition, there appears to be variation within the dissociated reproductive pattern relating to Leydig cell activity (Gustafson, 1979; Racey, 1982). The Leydig cells of New World species appear involuted during winter, while those of Old World species appear active

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(Gustafson, 1979; Racey, 1982). However, this apparent difference in cell activity may relate to latitudinal differences between studies, with bats at low latitudes able to complete a full gonadal cycle prior to hibernation (Racey, 1982).

While there is much speculation about the endocrine functions of Leydig cells in hibernating bats with a dissociated reproductive cycle, it appears that steroidogenesis of involuted cells is much reduced (Gustafson, 1979; Racey, 1982). However, endocrine studies have not been undertaken for the majority of species investigated (Gustafson, 1987; Singh & Krishna, 1996), but mating typically occurs when concentrations of circulating sex hormones are low (Crews, 1984). For example, male *Eptesicus fuscus* mate when gonadal testosterone levels are basal, and gonadectomy does not decrease male libido (Mendonça *et al.*, 1996). Such dissociated male reproductive function, including prolonged male sperm storage, is generally found in conjunction with prolonged female sperm storage (Gustafson, 1979; Oxberry, 1979; Racey, 1982).

We report here on the male reproductive cycle of three Australian vespertilionid bats, *Nyctophilus geoffroyi*, *N. major* and *Vespertilio regulus*. The lesser long-eared bat, *N. geoffroyi*, is a small ( $\approx 5.5$ – $8$  g) gleaning insectivore (Hosken, Bailey *et al.*, 1994). It is widely distributed and common throughout Australia (Maddock & Tidemann, 1995), roosting alone and entering torpor in the laboratory and field (Hosken, 1996; Hosken & Withers, *In press*). Much less is known about *N. major*. It is about twice the size of *N. geoffroyi* ( $\approx 11$ – $20$  g), widely distributed but rare throughout its range (Richards, 1991), and also enters torpor at low ambient temperatures (Hosken, 1997c). *Vespertilio regulus* has a more restricted distribution than either of the nyctophilines (Kitchener, Jones & Caputi, 1987). It is a small ( $\approx 4$ – $6$  g) insectivorous bat that has been the subject of several reproductive studies (e.g. Kitchener & Halse, 1978; Tidemann, 1993). Mating in this species begins during autumn and females store sperm throughout winter (Kitchener & Halse, 1978; Tidemann, 1993). Ovulation and fertilization occur at the end of winter, and a single young is born in early summer (Kitchener & Halse, 1978). In south-eastern Australia, spermatogenesis apparently peaks in January (mid-summer) and spermatozoa are stored in the caudae epididymides of mature males during winter (Tidemann, 1993). However, the reproductive cycle of males from Western Australia is undescribed.

Since females of all three species store sperm (Kitchener & Halse, 1978; Tidemann, 1993; Hosken 1997d), we predicted that males would display asynchronous development of their primary and secondary sexual glands and store sperm over winter. Data from capture records obtained over three years, and for *N. geoffroyi*, data from histological examinations and testicular testosterone assays of fixed specimens, are presented and used to describe male reproductive cycles.

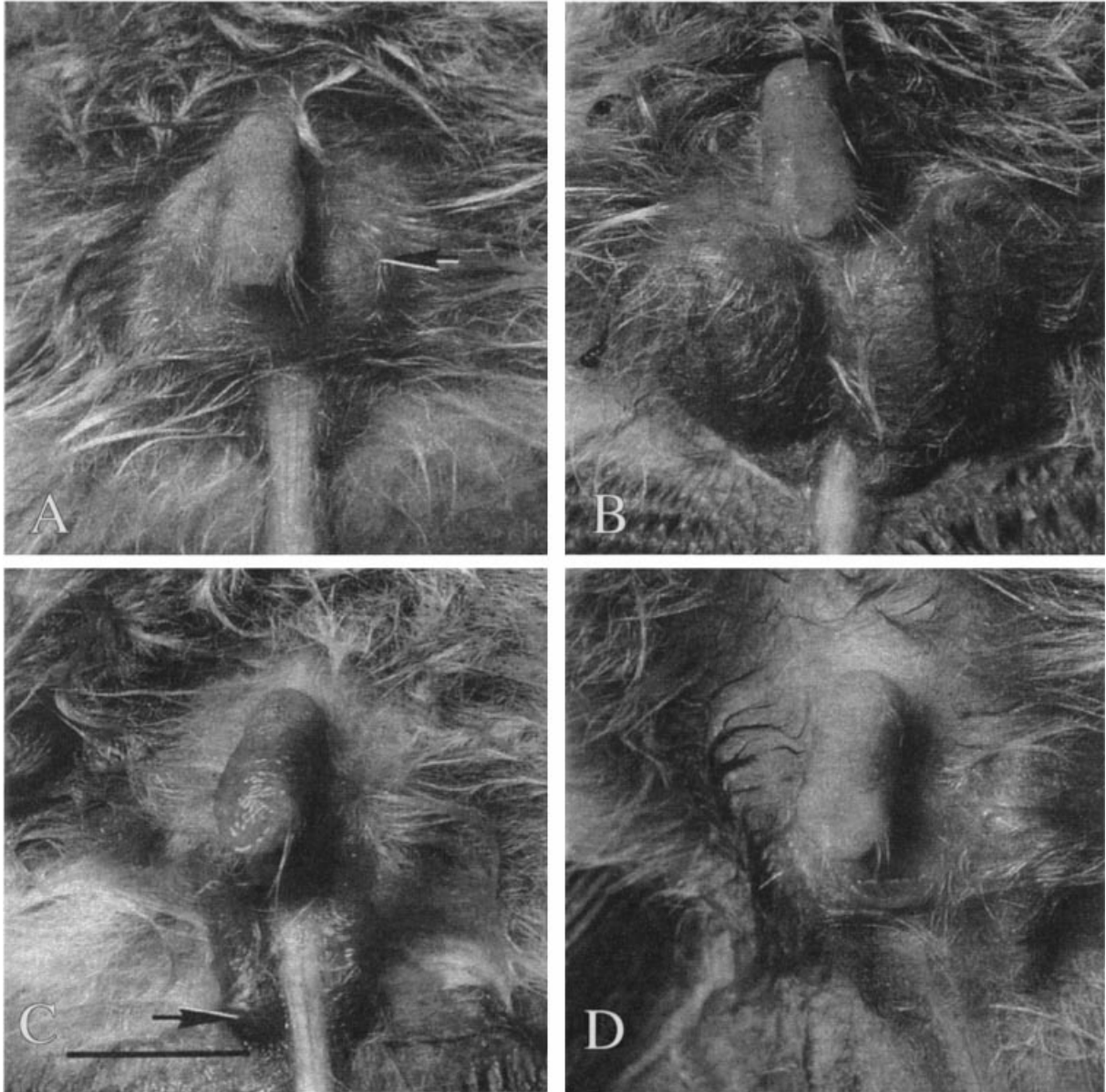
## MATERIALS AND METHODS

Bats included in this study were restricted to those caught in south-western Australia for reasons discussed elsewhere (Hosken, 1997b, d). Capture records were primarily obtained at the Harry Waring Marsupial Reserve ( $32^{\circ}15'S$ ,  $115^{\circ}50'E$ ) and Perrup Forest Research Station ( $34^{\circ}10'S$ ,  $116^{\circ}15'E$ ), using mist-nets and harp traps. Upon capture, bats were sexed and aged as adult or juvenile on the basis of wing epiphysial fusion, and at one site (HWMR) bats were wing-banded with bands supplied by the Australian Bird and Bat Banding Scheme. For males, the degree of testicular and epididymal enlargement was used as a measure of reproductive activity (Phillips & Inwards, 1985). The following classes were used: minor enlargement of the testes, testes at or near maximal enlargement, testes regressed or regressing and epididymides distended, or no obvious testicular or epididymal enlargement (Fig. 1). Bats were released after examination.

In addition to capture records, 24 fixed *N. geoffroyi* specimens captured from 1971 to 1996, were examined. Most specimens were obtained from The Western Australian Museum (Francis St. Perth, Western Australia), with additional specimens from N. L. McKenzie (Department of Conservation and Land Management, Woodvale, Western Australia) and from a small collection currently held at the Zoology Department of The University of Western Australia. All specimens had been fixed in 10% formalin and stored in 70% alcohol. At least two specimens were available for each month except June, August and October where none was available. These bats were aged as previously described.

Male reproductive tracts were removed. Measurement of testes using an Olympus 52H binocular light microscope fitted with a graticule indicated there were no consistent size differences between left and right testes or epididymides (Sign test  $0.75 \geq P \leq 0.65$ ). The left testis and epididymis were dissected free and extraneous fat was removed. The epididymis was separated from the testis, and the testis was blotted, dried and weighed to the nearest 0.001 g using a Sartorius 2434 mechanical balance. After weighing, left testes were prepared for testosterone assays. The left cauda epididymidis of each specimen was measured (as above), and the volume was calculated using the volume formula for a prolate spheroid:  $0.523 \times L \times W^2$  (Abbott & Hearn, 1978).

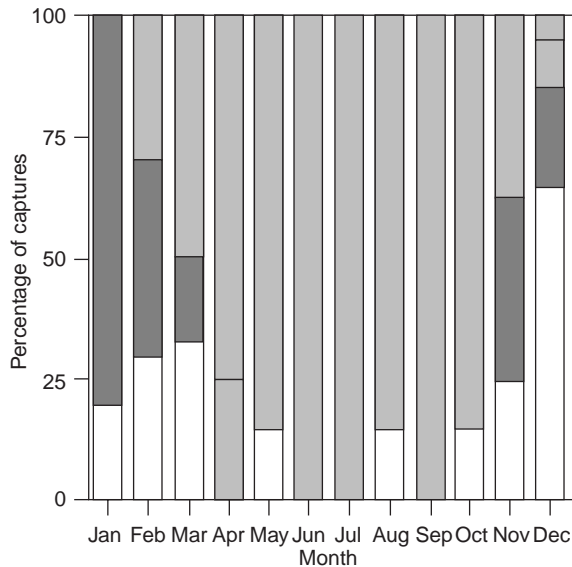
Testicular testosterone concentrations were determined using the methods of Martin *et al.* (1994) and Hötzel *et al.* (1995). Fixed and frozen sections of Merino ram testis were used initially to calibrate the assay. Samples of ram testis ( $\approx 1$  g) or whole bat testis (some fresh but mostly fixed) were weighed and homogenized in 5 ml of buffer (10 mmol phosphate  $\Gamma^{-1}$ , 1 mmol EDTA  $\Gamma^{-1}$ , 140 mmol NaCl  $\Gamma^{-1}$ , 15.4 mmol  $\text{NaN}_3$   $\Gamma^{-1}$ , pH 7.5). Two replicates at 0.1 ml and 0.5 ml of homogenates were extracted with ether. The tracer (0.1 ml containing 10 000 d.p.m. 1,2,6,7- $^3\text{H}$ ]testosterone; Amersham, Sydney, NSW, Australia) and the antibody (0.1 ml, diluted 1:70 000) were added, tubes were mixed and



**Fig. 1.** The seasonal variation in testes and epididymides size that was used to indicate male reproductive activity (shown here are long-eared bats, *Nyctophilus* spp.). (A) Minor testes enlargement (arrow), no epididymal distension. (B) Testes at or near maximal enlargement, no epididymal distension. (C) Testes regressed, cauda epididymis (arrow) distended. (D) No testicular or epididymal enlargement. Scale bar = 6 mm. (Adapted and reproduced from Phillips & Inwards (1985) with permission.)

incubated at 4 °C for 24 h. The antiserum (Rabbit 3), raised against testosterone-3-carboxymethyloximine-human serum albumin (CMO-HSA), was initially diluted to 1:1000 in gelatine-phosphate buffer, saturated with 10 mg/ml BSA, incubated at 37 °C for 2 h and centrifuged at 2000 g for 10 min before storage at – 20 °C. The reference preparation (4-androsten-17 $\beta$ -ol-3-one, 10  $\mu$ g/ml; Sigma Chemical Co., St. Louis, MO, U.S.A.) was dissolved in ethanol. Gamma globulin (0.1 ml normal rabbit serum [NRS] 1:400) and second antibody (0.1 ml diluted 1:10 in gelatine-phosphate buffer containing 1 mmol EDTA l<sup>-1</sup>) were added to the tubes, which were mixed and incubated overnight at 4 °C, before 1 ml cold gelatine-phosphate buffer was added to

all tubes except those used to determine total counts. Tubes were centrifuged at 4 °C at 2000 g for 30 min, the supernate was aspirated and redissolved in 0.5 ml 0.05 M HCl. The solution was dispensed into counting vials with 4 ml of toluene based scintillant and counted in a liquid scintillation counter. Cross-reactions were 100% with testosterone, 70% with dihydrotestosterone, 3.7% with androstenedione, and less than 0.05% with progesterone, oestradiol-17 $\beta$ , oestrone and oestriol. The assay's limit of detection was 0.12 ng/ml, and the within-assay coefficients of variation (mean  $\pm$  S.E.) were 7.85  $\pm$  0.4%, 7.25  $\pm$  0.75% and 9.3  $\pm$  0.2% for quality controls containing 5.15, 2.05 and 0.9 ng/ml, respectively. Results are presented as ng testis<sup>-1</sup> following Racey (1974).



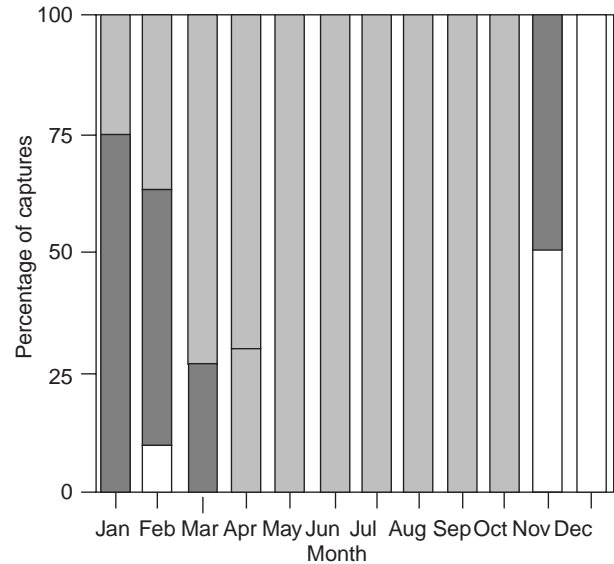
**Fig. 2.** Reproductive condition of male *N. geoffroyi* as revealed by bat captures: dark hatched bars, minor testes enlargement; vertically striped bars, testes at or near maximal enlargement; light hatched bars, testes regressed but cauda epididymides distended; white bars, no externally visible testicular or epididymal activity.

The remainder of the reproductive tracts (i.e. minus left testes and epididymides) were prepared for histological examination. Tissue was dehydrated in a graded alcohol series, cleared in xylene, wax embedded and serially sectioned at 6  $\mu\text{m}$ . Sections were slide mounted and stained in Harris's haematoxylin and counter stained with eosin (Humason, 1979). For each specimen, 10 seminiferous tubules were randomly selected from the right testis (e.g. Jolly & Blackshaw, 1987; Churchill, 1995), and the following measurements made: tubule diameter, lumen diameter and width of the seminiferous epithelium. The seminiferous tubules, cauda epididymidis and accessory sex glands (prostate and seminal vesicles) were examined for the presence of spermatozoa and/or secretory products, respectively. From the prostate, 10 acini were examined and acinar diameter and epithelial thickness measured. Accessory gland acini and seminiferous tubules to be measured were randomly selected by overlaying a grid and selecting coordinates from a random number generator. Tubules or acini that intersected the coordinates were measured. All measurements were made using an Olympus BX50 binocular microscope fitted with a Pulnix TMC-76 camera which conveyed images to a PC running BIOSCAN<sup>©</sup> OPTIMUS<sup>TM</sup> software. Program calibration was checked before measuring each specimen.

## RESULTS

### Capture records

During the three-year study period, 258 male bats were captured (84 *N. geoffroyi*, 46 *N. major* and 128 *V.*

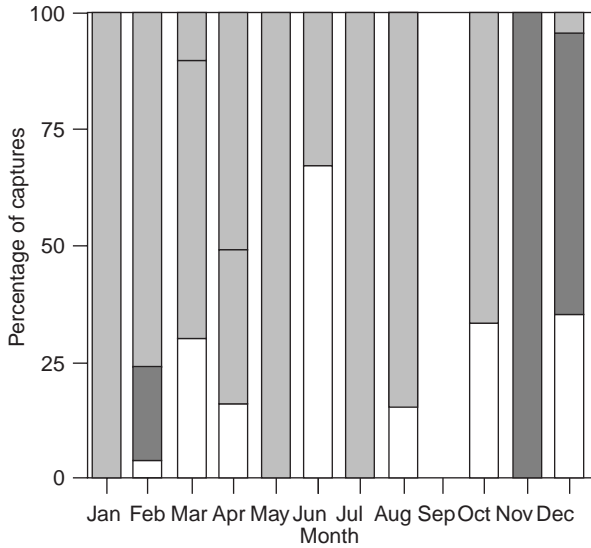


**Fig. 3.** Reproductive condition of male *N. major* as revealed by bat captures: dark hatched bars, minor testes enlargement; vertically striped bars, testes at or near maximal enlargement; light hatched bars, testes regressed but cauda epididymides distended; white bars, no externally visible testicular or epididymal activity.

*regulus*). For each species, there were no statistical differences in reproductive activity between years (factorial ANOVA; year and year by month interactions were highly insignificant, F-values <0.3, P-values >0.9), so data could be pooled across years and are presented here by month.

Testicular activity in *N. geoffroyi* was renewed in late spring (November), when about 25% of captured males ( $n=2$  of 8) showed signs of minor enlargement of the testes (Fig. 2). By March (early autumn), testicular activity was well underway with 50% of captured *N. geoffroyi* ( $n=6$  of 12) having maximally enlarged testes (Fig. 2). From March onward, the proportion of bats with enlarged testes steadily declined, until May (late autumn) when no males showed signs of testes enlargement ( $n=7$ ), but most had engorged epididymides ( $n=6$  of 7) (Fig. 2). During most months, a small proportion of the captured males showed no sign of reproductive activity (Fig. 2). Interestingly, a relatively large number of non-reproductive males were captured in March ( $n=4$ ) when many males had maximally enlarged testes. The non-reproductive males were not obviously juvenile (as indicated by the complete fusion of their digital epiphysial joints) but may have been recently fledged young of the year.

A similar pattern of reproductive activity was found for *N. major* (Fig. 3). Testicular activity was first detected in November (late spring), when one of two captured males displayed minor testes enlargement. Peak testes enlargement occurred in March (early autumn) ( $n=8$  of 11 bats), and by May (late autumn) no captured *N. major* had enlarged testes. From May until October, all captives had enlarged



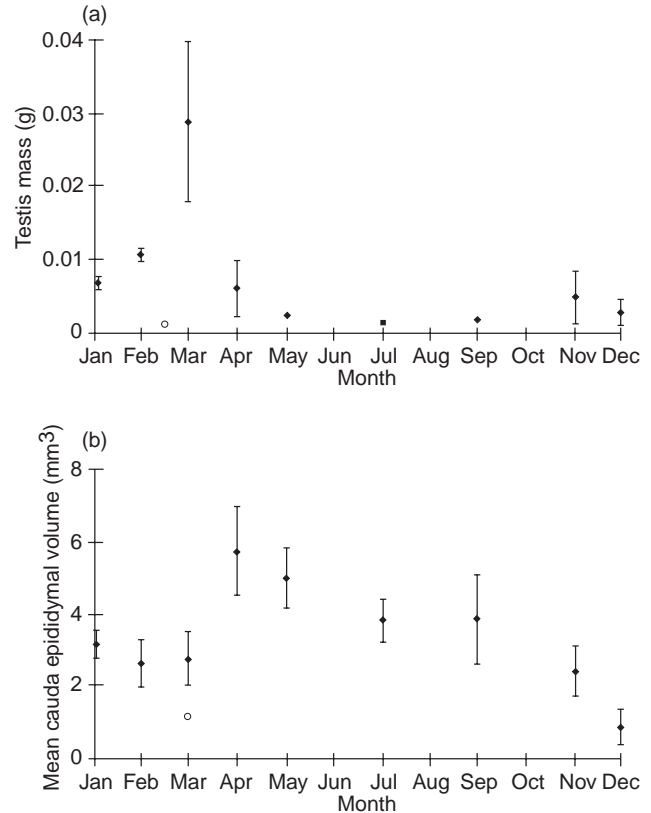
**Fig. 4.** Reproductive condition of male *V. regulus* as revealed by bat captures: dark hatched bars, minor testes enlargement; vertically striped bars, testes at or near maximal enlargement; light hatched bars, testes regressed but cauda epididymides distended; white bars, no externally visible testicular or epididymal activity.

cauda epididymides and regressed testes ( $n = 13$ ) (Fig. 3).

Capture data revealed a slightly more complicated reproductive pattern for *V. regulus*. January (mid-summer) was the month with the greatest proportional capture of males with enlarged testes (Fig. 4;  $n = 8$  of 8), slightly earlier than for the nyctophilines [note that February was when the maximum number of *V. regulus* with enlarged testis were captured ( $n = 42$  of 55 males)]. However, as for the *Nyctophilus* spp., some *V. regulus* individuals had maximally enlarged testes as late as April (mid-autumn) ( $n = 2$  of 6 bats) (Fig. 4). Between May and August, most captured *V. regulus* had regressed testes and enlarged caudae epididymides ( $n = 7$  of 9 bats). Minor testes enlargement was first detected in October (mid-spring) ( $n = 2$  of 3 bats), again about one month before the nyctophilines. In most months male *V. regulus* showing no sign of reproductive activity were captured (Fig. 4). Once again, these bats were not obviously juvenile but may have been young of the year.

**Mass of the testis and epididymis of preserved *N. geoffroyi***

Data on testis mass obtained from fixed *N. geoffroyi* are in accordance with capture data (Fig. 5a). Testis mass was greatest and also most variable during March ( $n = 5$ ), with testes weighing about five times more than in mid-winter (Fig. 5a, 6A) (March testis mass values were significantly greater than those for April till December; ANOVA Fisher's PLSD all comparisons  $P < 0.05$ ). Between March and April, testis mass returns to early summer values and from May to September the

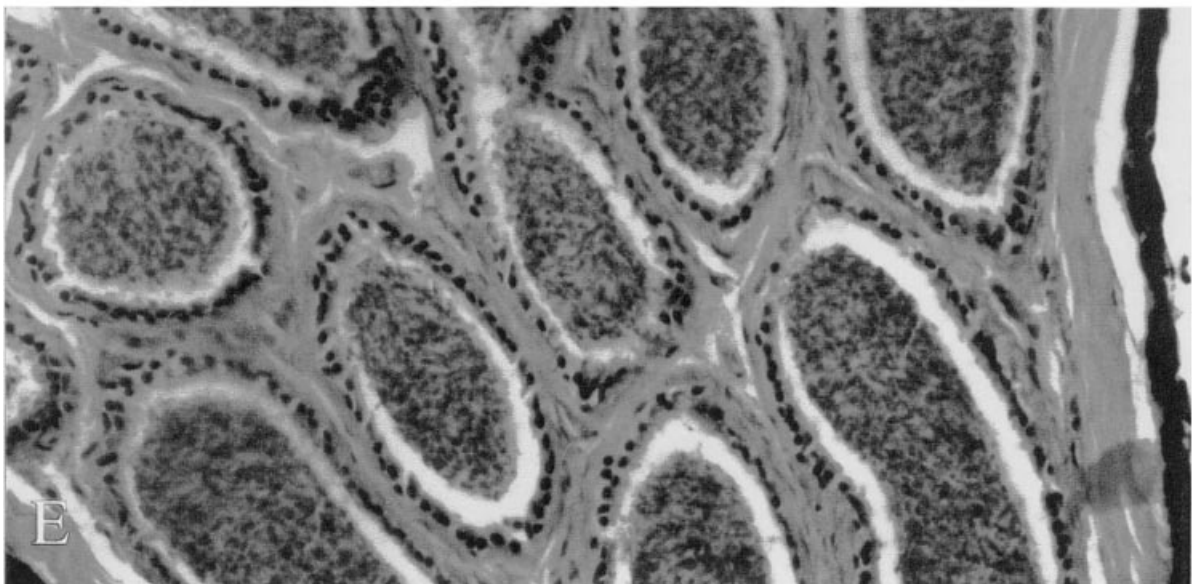
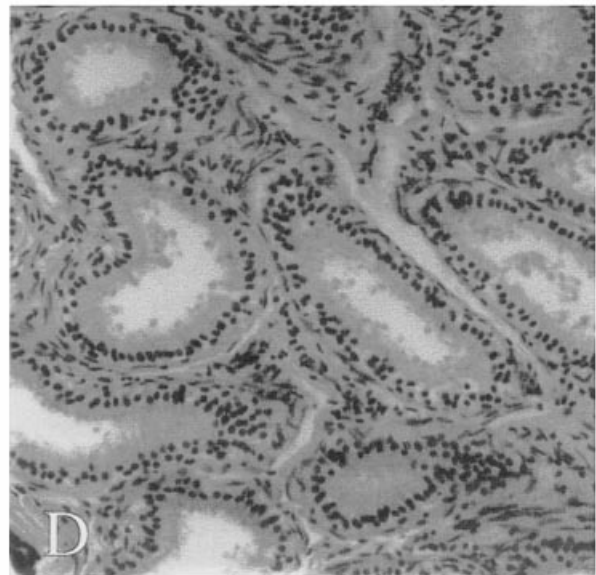
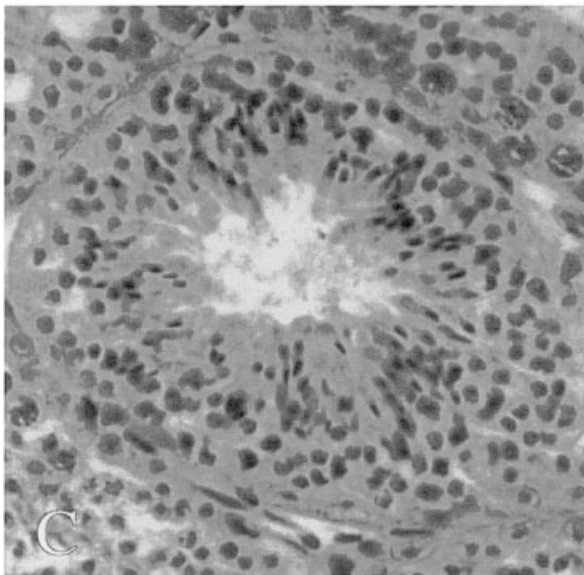
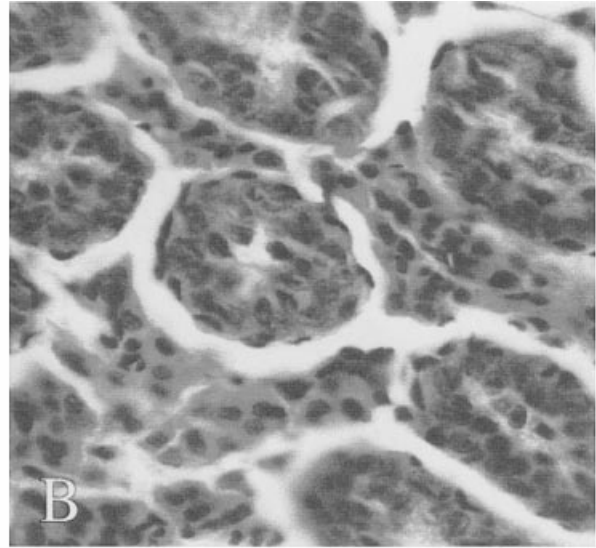
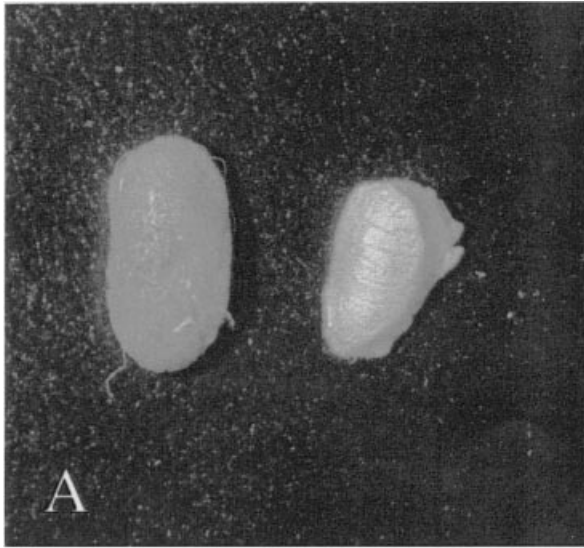


**Fig. 5.** (a) Plot of mean ( $\pm$  S.E.) monthly variation in left testis mass for fixed *N. geoffroyi* males.  $\blacklozenge$  = adult males,  $\circ$  = a juvenile male. (b) Plot of mean ( $\pm$  S.E.) monthly variation in left cauda epididymal volume for fixed *N. geoffroyi* males.  $\blacklozenge$  = adult males,  $\circ$  = a juvenile male.

testes of all specimens were small ( $n = 7$ ). In November (late spring), testis mass appeared to increase ( $n = 3$ ) (Fig. 5a), although the increase was not significant compared with winter values. Note, however, some captured bats had minor testes enlargement during November (Fig. 2). In contrast to testis mass, epididymal volume peaked in April (mid-autumn) [April values were significantly greater than those for February, March, November and December (ANOVA Fisher's PLSD all comparisons  $P < 0.02$ ) with a tendency to be larger than January ( $P = 0.084$ )], was still high from May to September, when testis mass was at its nadir, and was lowest in December (early summer) (Fig. 5b).

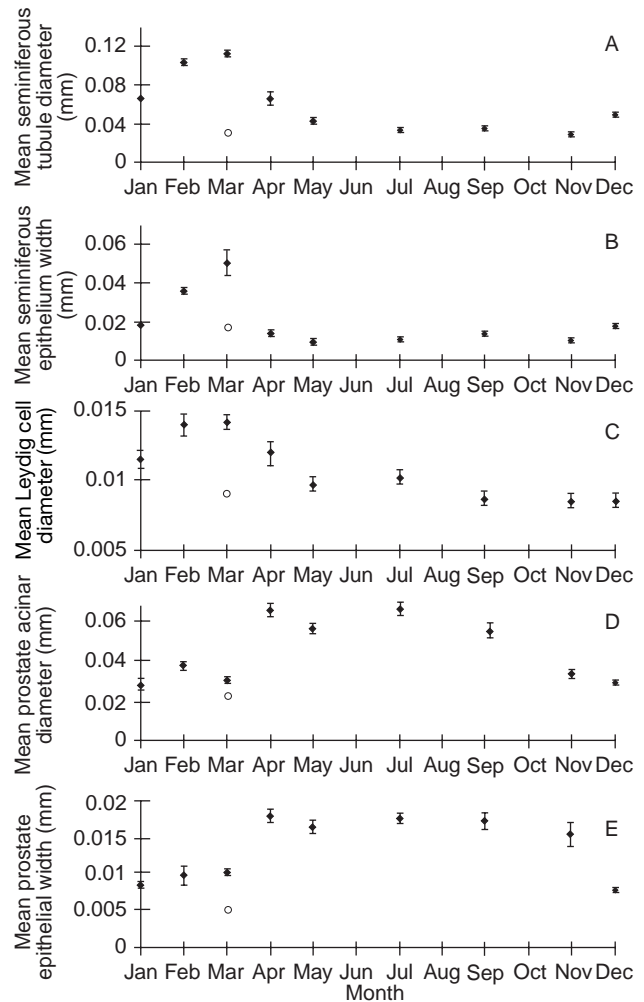
**Histology of fixed *N. geoffroyi* reproductive organs**

Histological examination of fixed material confirmed the reproductive pattern apparent from other data. The mean diameter of seminiferous tubules increased from November to a peak in March (Fig. 7A), and then decreased until May when it was approximately the same as for a juvenile male. Tubule diameter stayed at this small size until November. During January and February, spermatogonial division began, and in



February and March, spermatids underwent maturation (Fig. 6C). However, there was variation between specimens. Spermatozoa were found in the lumen of some seminiferous tubules (on average less than one/tubule section) in one May specimen. However, this male was not spermatogenically active and from April to December, the seminiferous tubules were characterized by a thin layer of Sertoli cells and few inactive spermatogonia (Fig 7B). This is seen in a plot of monthly seminiferous epithelial width, where the March peak and subsequent plummet in spermatogenic activity is apparent (Fig. 7B). Changes in Leydig cell diameter showed a similar pattern, with increased size from December to a February/March peak. Cells then involuted and were small during autumn and winter (Fig. 7C). Conversely, mean diameter of the prostate acinar was not elevated until April, after testicular regression (Fig. 7D). Prostate glands remained secretorally active until about September, before involuting in late spring/early summer (November/December) (Fig. 7E). Spermatozoa were first detected in the cauda epididymides in February (one specimen), although none was found in five March specimens (Fig. 6D). By April, all specimens had large numbers of spermatozoa in their cauda epididymides, and this did not change until November, when only about 25% of epididymal tubules contained sperm. Thus storage appeared to last about 8 months. Stored sperm formed extensive, densely packed masses within the epididymal lumen, showing no specific orientation with respect to the epithelial lining (Fig. 6E). No sperm were found in the cauda epididymides of January specimens or in a juvenile male.

◀ **Fig. 6.** Testicular and epididymal extremes during the reproductive cycle of *N. geoffroyi*. (A) Maximally enlarged testis from a specimen captured in March (left) compared with that of an April specimen. The testis on the left is 5 mm long. (B) Thin section through the testis of a specimen captured in January, stained with Harris's haematoxylin and counter-stained with eosin. Note the thin seminiferous epithelium, small tubule diameter plus the lack of spermatogenic activity. ( $\times 450$ ) (C) Thin section through the testis of a specimen captured in March, stained with Harris's haematoxylin and counter-stained with eosin. Note the thick seminiferous epithelium, large tubule diameter and various stages of spermatogenesis. ( $\times 530$ ) (D) Thin section through the cauda epididymis of a specimen captured in March, stained with Harris's haematoxylin and counter-stained with eosin. Note the lack of spermatozoa within the lumen of tubules. ( $\times 200$ ) (E) Thin section through the cauda epididymis of a specimen captured in April, stained with Harris's haematoxylin and counter-stained with eosin. Note the densely packed spermatozoa within the lumen of tubules. ( $\times 320$ )



**Fig. 7.** Plot of mean ( $\pm$  S.E.) monthly variation in: (A) seminiferous tubule diameter; (B) seminiferous epithelial width; (C) Leydig cell diameter; (D) prostate acinar diameter; and (E) prostate epithelial width for fixed *N. geoffroyi* males. ◆ = adult males, ○ = a juvenile male.

**Testosterone**

Testosterone concentrations were always lower in fixed compared with fresh tissue (both in bat and ram testis). As a result, comparisons presented here are for fixed tissue only. However, there was no consistent relationship between the duration of storage and testosterone concentrations (in months with two specimens available [Table 1] specimens that had been fixed for longer had lower testosterone levels in four of six cases, in months with three specimens available [Table 1] there was no consistent relationship between duration of preservation and testosterone level). Adult testicular testosterone concentrations appeared to peak in March and were basal from about May to December (the December mean, 0.08 ng testis<sup>-1</sup>, was only slightly greater than that obtained for a juvenile male, 0.04 ng testis<sup>-1</sup>) (Table 1). However, ANOVA revealed that March concentrations were only significantly different from September and

**Table 1.** Testicular testosterone concentration [mean (range) ng testis<sup>-1</sup>] obtained from fixed *N. geoffroyi* specimens. For March, November and December three specimens were used, while for all other months two specimens were available

Month	Jan.	Feb.	Mar.	Apr.	May	Jul.	Sep.	Nov.	Dec.
*Conc.	0.216 (0.18–0.25)	0.135 (0.13–0.14)	0.254 (0.11–0.4)	0.185 (0.13–0.24)	0.08 (0.04–0.12)	0.16 (0.14–0.18)	0.12 (0.10–0.14)	0.14 (0.03–0.24)	0.08 (0.04–0.14)

\* = Testosterone concentration

December (Fisher's PLSD  $P < 0.04$  in both comparisons), although a trend for a difference (Fisher's PLSD  $P < 0.18$  and  $> 0.07$ ) was noted between March values and those from all other months except January, February and April.

## DISCUSSION

The changes in testicular development (capture and histological data) and testis mass clearly indicate that spermatogenesis only occurred in late summer and early autumn for *N. geoffroyi*. As with many other histological studies (see Gustafson, 1979), maximum testes size was associated with maximal spermatogenic activity. Therefore, testes size was a reliable indicator of spermatogenic activity. Based on this, the testicular development of captured *N. major* and *V. regulus* indicated that spermatogenesis occurred only during summer and early autumn. Thus, the species studied here resemble many northern temperate zone vespertilionid and rhinolophid bats [e.g. *Myotis* spp. (Miller, 1942); *Rhinolophus hipposideros* (Gaisler, 1966); *Nyctalus noctule* (Racey, 1974); *Pipistrellus pipistrellus* (Racey & Tam, 1974)]; they undergo seasonal spermatogenesis during summer and autumn, and spermatogenic activity ceases before winter torpor. Furthermore, this spermatogenic pattern has been documented for other Australian bats [e.g. *N. gouldi* (Phillips & Inwards, 1985); *Vespadelus* spp. (Tidemann, 1993)], although the timing of events varies between studies. For example, peak spermatogenesis occurs during January for *N. gouldi* in eastern Australia (Phillips & Inwards 1985), about two months before its close relatives *N. geoffroyi* and *N. major* in this study. The difference is probably due to the cooler, shorter summers around Canberra (the site of Phillips & Inwards study) relative to the present study, since photoperiod and temperature may influence male reproductive cycles (e.g. Beasley & Zucker, 1984). However, the timing of the testicular cycle of *V. regulus* is similar to that in eastern Australia (e.g. Tidemann, 1993).

The involution of the Leydig cells of *N. geoffroyi* prior to winter is consistent with what has been termed the 'Myotis pattern' of Leydig cell activity (Gustafson, 1979). All the bat species studied here were captured at latitudes between approximately 32° and 36°S. Involution of the Leydig cells of *N. geoffroyi* before winter is consistent with Racey's (1982) hypothesis that different patterns of Leydig cell activity in sperm-storing bats relate to the time available to complete a full gonadal

cycle prior to winter torpor (i.e. patterns of Leydig cell involution vary with latitude); bats from similar northern latitudes display a similar pattern [e.g. *Myotis* spp. at 38°N (Miller, 1942); *Pipistrellus hesperus* at 32°N (Kruttsch, 1975)]. The limited testicular testosterone data available are in accordance with the Leydig cell data; testosterone levels are apparently elevated from about January till April, with a peak in March. A larger sample size would clarify this, but as with other studies (e.g. Gustafson, 1987), Leydig cell morphology appears to be a reasonable indicator of changes in cellular activity and testosterone production levels. Moreover, mating occurs when gonadal testosterone levels are low (Hosken, 1997d, 1998), as reported for other hibernating bats (e.g. *Vespadelus vulturinus*, Tidemann, 1993; *Eptesicus fuscus*, Mendonça *et al.*, 1996). Thus it appears that testosterone concentration thresholds differ for different reproductive function, as noted elsewhere (e.g. Racey, 1982).

In addition to testicular changes, the activity of *N. geoffroyi* accessory sex glands also typifies vespertilionid bats inhabiting temperate latitudes. Prostate gland and epididymal activity peaked after spermatogenesis was terminated, was maintained throughout winter, and both regressed in spring. Thus accessory sexual glands and caudae epididymides were enlarged during the autumn/winter mating period. However, *N. geoffroyi* and many other bats conclude spermatogenesis before mating commences (e.g. Gustafson, 1979; Racey, 1982; Tidemann, 1993; Hosken, 1997d, 1998). The same appears to be true for *V. regulus* in Western Australia (and has been noted in eastern Australia; Tidemann, 1993); mating begins in April (Kitchener & Halse, 1978), about 2–3 months after the peak in the number of individuals captured with maximum testes enlargement in the present study. For *N. major*, however, spermatozoa have been found in the female reproductive tract in late March (Hosken, 1997b, d), when the testes of captured males were maximally enlarged (Fig. 3), but copulation in captive bats occurs as late as June (Hosken, 1997b). Moreover, most copulations probably occur after spermatogenesis has ceased for all three species; this is the general pattern for most hibernating vespertilionid and rhinolophid bats (e.g. Gustafson, 1979). It is therefore apparent that many bats mate when sperm supplies cannot be renewed. Furthermore, mating causes measurable decreases in the size of epididymal sperm stores (Gustafson, 1979).

In mammals, large numbers of inseminated sperm are typically required for successful fertilization

(Dewsbury, 1982), with sperm number influencing male reproductive success in many ways (Møller, 1991). For example, sperm number affects the probability of fertilization *per se* (e.g. Gibson & Jewell, 1982; Bedford, Rodger & Breed, 1984) and also the probability of fertilization under conditions of sperm competition (e.g. Parker, 1970; Martin *et al.*, 1974; Martin & Dziuk, 1977; Stockley *et al.*, 1996). However, because sperm are costly to produce (Dewsbury, 1982), and their supply may limit male reproductive success (e.g. Nakamura & Kramer, 1982; Birkhead, 1991; Shapiro & Giraldeau, 1996), males are predicted to allocate their ejaculates prudently (Dewsbury 1982; Parker, 1982, 1990a, b). This may be especially true when sperm replenishment is a lengthy process (e.g. the bats of this study). The way sperm are allocated among ejaculates depends on many factors including the likelihood of finding additional mates, search costs, the mating history of males and females, and the mechanism and risk of sperm competition (Dewsbury, 1982; Parker, 1990a, b). For example, males may tailor their ejaculates to the perceived risk of sperm competition (e.g. insects, Gage, 1991; Simmons *et al.*, 1993; birds, Birkhead & Møller, 1992; primates, Møller, 1988; Baker & Bellis, 1989), and strategies that maximize male reproductive success vary with the socio-sexual situation (e.g. Dewsbury, 1982). However, no data on sperm allocation are available for bats. Moreover, the degree to which they are actually sperm-limited, which will depend on ejaculate volume, the size of the male store, and mating frequency, is unknown, but some male *N. geoffroyi* can successfully fertilize at least five females (Hosken, 1998). Furthermore, epididymal sperm stores of free-living bats are often not significantly reduced until well after females become pregnant (e.g. this study; Racey & Tam, 1974) and in other mammals, male potency is estimated to show no decline until epididymal sperm counts decrease by 90% (Searle & Beechey, 1974). So male bats may not be sperm limited *per se*.

It has been suggested that bats sustain male accessory gland activity during winter because of the energetic costs associated with involution (Gustafson, 1979). However, it is equally likely that, because of prolonged female receptivity (and/or the chance of finding uniseminated females late in their elongated oestrus), selection has favoured male sperm storage. The energetic costs of spermatogenesis during winter are probably prohibitive, although sperm production during torpor occurs for at least one species (Anand Kumar, 1965; and see Racey, 1982). That spermatogenesis typically occurs only when food is abundant (Gustafson, 1979) suggests the costs are substantial, and maintenance of gonadal tissue for small mammals (<100 g) is estimated to be up to 5–10% of basal metabolic rate (Kenagy & Trombulak, 1986). Furthermore, maintaining high testosterone levels may also be energetically expensive (Emerson & Hess, 1996). Thus male sperm storage during winter but not spermatogenesis is favoured.

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