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# Seasonal reproduction in the female spiny mouse from South Africa

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**Keywords**
Muridae; follicular growth; ovary; rainfall; food availability; southern hemisphere; *Acomys spinosissimus*.

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## Abstract

Many mammal species reproduce seasonally because of annual fluctuations in temperature, rainfall and photoperiod in often nutritionally challenging habitats. The reproductive biology of many small southern African mammals is largely unknown and in critical need of study. We investigated the breeding pattern of the female spiny mouse (*Acomys spinosissimus*) from South Africa. We examined the ovarian development, follicular growth, circulating plasma progesterone concentrations and the reproductive status of wild-caught adult female spiny mice sampled over a 12-month period while also correcting for body mass and age. From these data, we conclude that female *A. spinosissimus* breed seasonally. The main breeding season of the spiny mouse is between September and January, with plasma progesterone concentrations being elevated, ovarian volume and primary, secondary, tertiary and Graafian follicle numbers as well as the corpora body number being the highest and pregnancies occurring during this period. Females were reproductively inactive from February through to August. The breeding season coincides with the onset of the rainy season in the habitat, which starts around September and ends in April. Rainfall, in association with an increase in primary productivity and hence higher food availability, might be the most important factor shaping reproduction in the female spiny mouse.

## Introduction

In seasonally changing environments, both temperate and tropical mammals often breed during the most favourable times of the year (Bronson, 1985). This ensures a higher survival and growth of the young and maximizes reproductive success, as reproduction is energetically costly (Gittleman & Thompson, 1988). In the tropical unstriped grass rat *Arvicanthis*, Neal (1981) observed a shift from continuous breeding in an aseasonal environment to a seasonal pattern of reproduction in an environment with seasonally changing rainfall combined with changing nutritional quality and quantity of food. These factors appear to be the primary cause for seasonal reproduction in numerous tropical and sub-tropical species of rodents (Perrin & Swanepoel, 1987; Makundi, Massawe & Mulungu, 2006; Muteka, Chimimba & Bennett, 2006*a*).

The effect of both quality and quantity of food and even of secondary plant compounds on reproduction has been studied in the laboratory and found to be of significance in regulating reproduction in a number of species (Batzli, 1986; Heideman, Deibler & York, 1998; Wube, Haim & Fares, 2009). Numerous other factors may affect reproduction such as water availability (Nelson, Gubernick & Blom, 1995), temperature (Nelson *et al*., 1989), social factors (Trainor *et al*., 2006), snow cover (Beer & MacLeod, 1961) and exercise (El-Bakry, Zahran & Bartness, 1998). Moreover, many mammals use the seasonally changing day–night cycle as a proximate cue to anticipate seasonal changes (Prendergast, Kriegsfeld & Nelson 2001). However, equatorial populations of many species do not respond to changes in photoperiod, while populations from higher latitudes are highly photoresponsive (Nunes *et al*., 2002). Photoperiod can interact with a number of other factors such as temperature and food availability to enhance reproductive responses (Nelson *et al*., 1992; Kriegsfeld, Trasy & Nelson, 2000).

The reproductive biology and environmental cues, in particular the affect of photoperiod, have been investigated in a large number of small mammals, but these studies have mainly been on species from the northern hemisphere such as hamsters (Prendergast *et al*., 2001), a number of *Peromyscus* species (Trainor *et al*., 2006) and voles (Stevenson, van Tets & Nay, 2009). The reproductive biology and life-history patterns of many southern African small mammals are still largely unknown and there is an increasing need for their investigation. The present study focuses on the reproductive seasonality of the spiny mouse, *Acomys spinosissimus*, from southern Africa.

The spiny mouse is a nocturnal, terrestrial rodent that occurs mainly in rocky habitats and is distinguished by thick and hard spine-like hair at the back which is characteristic of the genus (Sheppe, 1973; Pienaar, Rautenbach & de Graaff, 1980; Skinner & Chimimba, 2005). *Acomys spinosissimus* is

  

relatively widespread in Africa south of the equator and can be found in Tanzania, the Democratic Republic of Congo, Zambia, Malawi and in southern Africa (Mozambique, Botswana and the north-eastern parts of South Africa) (Skinner & Chimimba, 2005). The little data available suggest that this species breeds seasonally during the warm and wet summer months in Zambia (Sheppe, 1973) and southern Africa (Smithers, 1971; Pienaar *et al.*, 1980).

In this study, we investigated the seasonality of reproduction in female *A. spinosissimus* by performing histology of the ovaries and monitoring the circulating concentrations of progesterone in the blood monthly from April 2007 until August 2008**.** We hypothesized that reproduction of the female spiny mouse is highly dependent on rainfall in combination with an increase in food availability and therefore, predicted that female *A. spinosissimus* would breed seasonally, with increased follicular growth and pregnancies occurring mainly during the warm and wet spring (September–November) and summer months (December–February), whereas ovaries would be regressed and progesterone concentrations would be low during the cold and dry winter months (June–August) of the southern hemisphere. We also investigated the influence of age on the reproductive biology of females of this species.

## Materials and methods

### General

Three to seven female *A. spinosissimus* were captured at the end of every month from April 2007 to August 2008 ($n = 67$ over the entire study period). All spiny mice were trapped overnight around the rocky outcrops of the Goro Game Reserve in the Soutpansberg region, Limpopo Province, South Africa (22°58′S, 22°57′S; 29°25′E, 29°24′E). Animals were collected under permit (CPM-333-00002) from the CITES and Permit Management Office, Department of Environmental Affairs, Limpopo Province. Specimens were trapped using Sherman live traps (H. B. Sherman Traps Inc., Tallahassee, FL, USA) baited with a mixture of peanut butter, oats and fish oil. The body mass (g) of each individual was recorded to the nearest 0.001 g using a digital balance (Scout Pro SPU123, Ohaus Corporation, Pine Brook, NJ, USA) immediately after capture and rounded to 0.1 g for subsequent analyses. Females were kept in polyurethane cages during transportation and in the laboratory. All cages were provided with wood shavings as bedding and paper towels for additional shelter. Water was provided *ad libitum* and mouse pellets, carrots and apples were provided as food. Monthly rainfall data were kindly provided by the Goro Game reserve. Ambient temperature was measured using two iButton digital temperature data loggers (Maxim Integrated Products, Dallas Semiconductor, Sunnyvale, CA, USA) for 2007 and 2008.

Animals were housed in the laboratory for at least 1 day for acclimatization before euthanasia by an overdose of halothane. Blood was collected by exsanguination from the heart and centrifuged at 500 ***g*** for 15 min. The plasma fraction was separated from blood cells and stored at −35 °C until hormone analysis. Female bodies were examined for swollen and elongated teats, which were used as an indication of lactation. Ovaries with uterine horns with foetuses were dissected out and the number of foetuses recorded. Ovaries were fixed in Bouin's fluid for *c.* 20 h before being rinsed and stored in 70% ethanol. Standard museum techniques (DeBlase & Martin, 2001) for small mammals were used to prepare skulls, which were subsequently used to determine the relative age of an individual using the degree of maxillary molar tooth-wear and eruption as defined under 'Relative age classes'.

### Histology

After the removal of all excess tissue, the length and the width (mm) of the fixed ovaries were measured using a pair of digital calipers (Sylvac Opto RS 232, Ultra Praezision Messzeuge GmbH, Glattbach, Germany). These dimensions were in turn used to calculate ovarian volume ($mm^3$) using the formula for the volume of an ellipsoid: $V = 4/3\,\pi ab^2$, where $a$ represents half the maximum length and $b$ represents half the maximum width (Woodall & Skinner, 1989). The volume was averaged for both ovaries per female. The two ovaries of every female were sequentially dehydrated in increasing concentrations of ethanol baths, embedded in a cube of paraffin wax and then serially sectioned at 5 $\mu$m widths in its totality with a rotary microtome (820 Spencer, American Optical, Scientific Instrument Division, Buffalo, NY, USA). The ovarian sections were mounted on microscope slides with gelatin as an adhesive and subsequently dried in an oven at 36 °C for 48 h. The sections were finally stained with Ehrlich's haemotoxylin and counter-stained with eosin (see Drury & Wallington, 1967). All ovarian sections were examined consecutively for stages of follicular growth under a light microscope (Vickers Instruments, UK) at magnifications of × 200 and × 400.

The identification and classification of follicles followed Bloom & Fawcett (1962). The total numbers of primary, secondary, tertiary and Graafian follicles as well as corpora lutea, corpora haemorrhagica and corpora albicans were counted over the entire ovary. Corpora lutea, corpora haemorrhagica and corpora albicans (hereafter referred to as the corpora bodies) were subsequently combined for further analyses to accommodate for small monthly sample sizes. Primordial follicles were counted in every tenth section through the entire ovary. The number of primordial follicles, therefore, only represents a sub-sample of the total number of primordial follicles in the entire ovary. For analysis, the mean numbers of follicles and corpora bodies were calculated for both ovaries in an individual to determine the relative number of follicles/corpora bodies per female. It was not possible to examine some of the ovarian sections because of the logistical problems encountered during the processing of the ovarian tissue ($n = 62$; for mean monthly sample sizes, see Table 4).

### Hormone analysis

Plasma progesterone concentrations were determined in female *A. spinosissimus* every month throughout the sampling period. Because of the small body weight and, therefore, a small blood volume of the spiny mouse, we could not obtain enough plasma for the hormone analysis from some individuals ($V = 2 \times 100\,\mu$L). Two individuals sampled in April and one individual sampled in July were, therefore, excluded from the analyses ($n = 64$). A coat-a-count progesterone kit (Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA) was used to determine plasma progesterone concentrations.

To validate the plasma progesterone concentration for *A. spinosissimus*, the slopes of a serial dilution curve and the calibration curve were tested for parallelism using a general linear model (GLM) after log–logit transformation (Chard, 1978). The dilution percentages were used as covariates and the type of curve was used as a random factor. There was no significant difference between the serial dilution curve of plasma progesterone with that of the calibration curve ($F_{1,11} = 0.13$; $P = 0.73$) and the assay could, therefore, be validated for *A. spinosissimus*. The intra-assay coefficient of variation for a repeated determination of quality control was 5.3% and the sensitivity of the assay was 0.36 nmol/L.

### Relative age classes

All females were aged using the degree of maxillary molar tooth wear and eruption. Each individual was initially placed in one of five tooth-wear classes, as described and illustrated by Dippenaar & Rautenbach (1986) for *A. spinosissimus*. None of our animals showed incompletely erupted cheek teeth (i.e. tooth-wear class 1 as defined by Dippenaar & Rautenbach, 1986) and thus, we only considered the four remaining consecutive tooth-wear classes recategorized here as follows: (1) Tooth-wear class 1 – minimal wear; (2) Tooth-wear class 2 – obvious wear; (3) Tooth-wear class 3 – extensive wear; (4) Tooth-wear class 4 – severe wear. We can, therefore, be certain that we do not have subadult animals in our sample (some of the females with tooth-wear class 1 were found to be pregnant). To accommodate for the small monthly sample sizes, we combined tooth-wear classes 1 and 2 and tooth-wear classes 3 and 4 into two broad tooth-wear classes comprising relatively young and relatively old individuals, respectively.

### Data analysis

Data for the same months over the 2-year sampling period were combined into one universal 12-month dataset for all subsequent analyses. Body mass was log-transformed and compared between the relative age classes using a *t*-test. A GLM was applied to assess differences in the log-transformed ovarian volume and the square root-transformed data of primordial and primary follicles for the two relative age class groupings and over the 12-month period. Differences in plasma progesterone concentration and numbers of secondary, tertiary and Graafian follicles as well as the number of corpora bodies for the two relative age class groupings and over the 12 months were evaluated using a generalized linear model (GZLM). Relative age class and month of the year were used as factors and body mass was used as a covariate for all GLM and GZLM analyses. Adjusted $R^2$ (GLM) or Akaike's information criterion (GZLM) were applied as a measure of fit for the model. A Tukey's *post hoc* test and a least significant difference test (LSD) followed every GLM and GZLM, respectively. In case of a relationship between a dependent variable and body mass, Pearson's correlation was performed on parametric data, while Spearman's correlation was used for nonparametric data. All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 17.0 (Polar Engineering and Consulting 1993–2007).

## Results

The rainy season lasted from September until April, with the highest rainfall in December, while the dry season started in May and terminated in August. The temperatures were highest from September until March, with mean monthly temperatures above 20 °C and February being the warmest month (23.5 °C). Mean monthly temperatures below 20 °C were recorded between April and August, and July was the coldest month (16.2 °C).

The results here are presented as mean ± SD. The total monthly sample sizes and sample sizes per relative age class in addition to the mean monthly body masses are presented in Table 1. Collectively, old females were significantly heavier than young females ($t_{65} = 4.38$; $P < 0.001$; young individuals: 18.7 ± 3.7 g; old individuals: 23.3 ± 3.6 g). Pregnant females were detected from September through to January, while no pregnant females were encountered from February until August and females were found to have two

**Table 1** Monthly sample sizes (*n*), sample sizes for two relative age classes (young and old individuals) and mean body mass (g) and body mass ranges of female *Acomys spinosissimus* from South Africa sampled over 12 months in 2007 and 2008

| | | Relative age class | | Body mass (g) | |
|---|---|---|---|---|---|
| | *n* | Young | Old | Mean ± SD | Range |
| January | 5 | 3 | 2 | 23.5 ± 2.5 | 20–26 |
| February | 7 | 6 | 1 | 20.7 ± 3.7 | 15–25 |
| March | 7 | 7 | 0 | 18.1 ± 3.3 | 16–20 |
| April | 8 | 6 | 2 | 18.2 ± 2.0 | 15–24 |
| May | 6 | 6 | 0 | 15.7 ± 2.5 | 14–19 |
| June | 4 | 3 | 1 | 16.6 ± 2.1 | 14–20 |
| July | 6 | 6 | 0 | 16.5 ± 1.7 | 14–20 |
| August | 5 | 4 | 1 | 17.5 ± 3.4 | 16–20 |
| September | 4 | 1 | 3 | 22.6 ± 3.1 | 19–27 |
| October | 6 | 2 | 4 | 22.3 ± 7.7 | 19–26 |
| November | 3 | 2 | 1 | 23.1 ± 2.6 | 16–31 |
| December | 6 | 5 | 1 | 25.4 ± 2.6 | 22–28 |
| Total | 67 | 51 | 16 | 19.8 ± 4.2 | |

**Table 2** Number of females with embryos, average number of embryos and number of lactating females for each month of *Acomys spinosissimus* from South Africa sampled over 12 months in 2007 and 2008

| | Summer | | Autumn | | | Winter | | | Spring | | | Summer |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | January | February | March | April | May | June | July | August | September | October | November | December |
| Females with embryos | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 4 | 2 | 3 |
| Average number of embryos | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 3.5 | 3.5 | 3.3 |
| Lactating females | 3 | 6 | 4 | 1 | 0 | 1 | 0 | 0 | 0 | 6 | 2 | 6 |

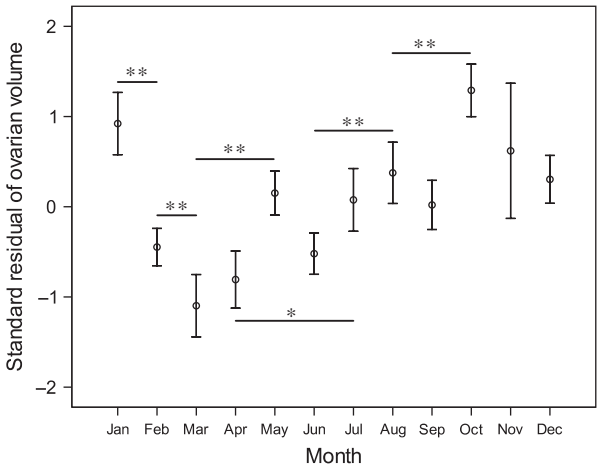


**Fig. 1** Standard residuals for ovarian volume ($mm^3$) by body mass (g) of *Acomys spinosissimus* from South Africa averaged over 12 months in 2007 and 2008. Values are mean ± 1 SE. Statistical significance: $^*P<0.05$; $^{**}P<0.01$.

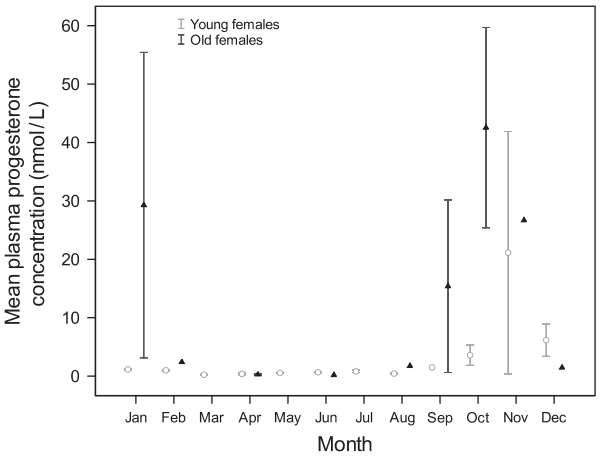


**Fig. 2** Monthly plasma progesterone concentration (nmol/L) of female *Acomys spinosissimus* from South Africa averaged over 12 months in 2007 and 2008. Plasma progesterone is displayed separately for relatively young and relatively old individuals. Values are mean ± 1 SE.

to four embryos (Table 2). Evidence of lactation was observed in some female *A. spinosissimus* from October until April (Table 2).

Ovarian volume was significantly different across the 12 months ($F_{11,53} = 9.28$; $P<0.001$), but it did not differ significantly between the relative age classes ($F_{11,53} = 0.17$; $P = 0.68$). The standard residuals for ovarian volume were calculated to account for the relationship between ovarian volume and body mass and a Tukey's test was performed on these. The ovarian volume was significantly higher in October than in February, March and April (Tukey's test; $P<0.01$), and it decreased significantly from January to March (Tukey's test; $P = 0.002$; Fig. 1). There was no significant interaction between the month of the year and relative age classes for the ovarian volume ($F_{8,45} = 0.75$; $P = 0.65$). There was a significant relationship between ovarian volume and female body mass ($F_{1,53} = 5.27$; $P = 0.02$). Ovarian volume increased with increasing body mass ($r_{67} = 0.68$; $r^2 = 0.46$; $P<0.001$).

There was a significant difference in plasma progesterone across the 12 months (Wald $\chi^2 = 82.64$; d.f. = 11; $P<0.001$) and an interaction between the month of the year and relative age classes (Wald $\chi^2 = 44.19$; d.f. = 8; $P<0.001$). There was, however, no significant difference in the plasma progesterone concentration between the relative age classes alone (Wald $\chi^2 = 0.62$; d.f. = 1; $P = 0.43$). LSD tests demonstrated a significant increase in the progesterone concentration from the levels found in February to August (inclusive) to October ($P<0.05$); furthermore, plasma progesterone concentration decreased significantly from October to December and from January to March ($P<0.05$; Fig. 2). In September, old females were found to have higher plasma progesterone levels than the young females, whereas in December, the opposite was observed (LSD; $P \leq 0.05$; Fig. 2). However, this effect should be interpreted in light of the small sample sizes, as mainly relatively old pregnant females were collected in September, while predominantly relatively young pregnant females were collected in December. Plasma progesterone concentration was statistically dependent on body mass (Wald $\chi^2 = 5.56$; d.f. = 1; $P = 0.02$).

None of the follicle types or corpora bodies demonstrated an interaction between month of the year and relative age classes (Table 3). Body mass significantly influenced the numbers of primordial ($F_{1,48} = 8.11$; $P<0.01$) as well as Graafian follicles (Wald $\chi^2 = 4.00$; d.f. = 1; $P<0.05$), but not the number of primary ($F_{1,48} = 1.42$; $P = 0.29$), secondary and tertiary follicles and corpora bodies (Wald $\chi^2<2.56$; d.f. = 1; $P>0.11$). The numbers of primordial, primary and secondary follicles were significantly different between relative age classes, while the number of tertiary and Graafian

**Table 3** Results for numbers of primordial, primary, secondary, tertiary and Graafian follicles, and corpora bodies for *Acomys spinosissimus* sampled over a 12-month period in 2007 and 2008, between age classes and the interaction between month and age classes established by the general (*F*-value) and generalized linear model (Wald $\chi^2$)

| | Months | | | Age classes | | | Months × age classes | | |
|---|---|---|---|---|---|---|---|---|---|
| | *F* or Wald $\chi^2$ | d.f. | *P* | *F* or Wald $^2$ | d.f. | *P* | *F* or Wald $\chi^2$ | d.f. | *P* |
| Primordial follicles | 1.38 | 11,48 | 0.21 | 7.15 | 1,48 | **0.01** | 0.84 | 7,41 | 0.56 |
| Primary follicles | 7.12 | 11,48 | < **0.001** | 7.20 | 1,48 | **0.01** | 1.16 | 7,41 | 0.34 |
| Secondary follicles[a] | 63.08 | 11 | < **0.001** | 7.28 | 1 | **0.007** | 6.50 | 7 | 0.48 |
| Tertiary follicles[a] | 77.39 | 11 | < **0.001** | 0.52 | 1 | 0.47 | 5.79 | 7 | 0.56 |
| Graafian follicles[a] | 57.71 | 11 | < **0.001** | 2.47 | 1 | 0.12 | 7.03 | 7 | 0.43 |
| Corpora bodies[a] | 155.50 | 11 | < **0.001** | 0.13 | 1 | 0.71 | 9.03 | 7 | 0.25 |

[a]Variables that were evaluated by generalized linear model
Significant *P*-values are highlighted in bold.

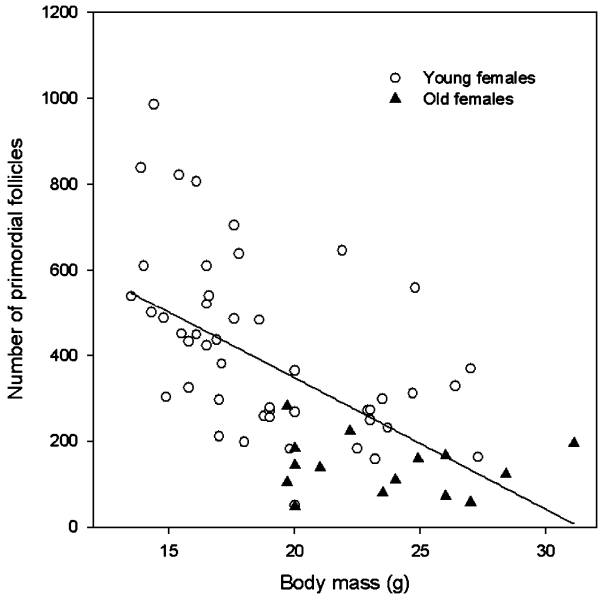


**Fig. 3** The relationship between the number of primordial follicles and body mass (g) of female *Acomys spinosissimus* from South Africa. Values are presented separately for relatively young and relatively old females.

follicles as well as corpora bodies were not significantly different between relative age classes (Table 3).

Except for primordial follicles, the numbers of all other follicles and corpora bodies were significantly different between the 12 months (Table 3). The number of primordial follicles decreased with increasing body mass ($r_{62} = -0.63$; $r^2 = 0.40$; $P < 0.001$) and relatively young females had significantly more primordial follicles than relatively older females (young individuals: $413.5 \pm 204.0$; old individuals: $138.9 \pm 64.9$; Fig. 3).

The number of primary follicles was significantly higher from October to January compared with February to May (Tukey's test; $P < 0.05$) and increased significantly between May and June (Tukey's test; $P < 0.05$; Table 4). The number of secondary follicles was lowest in April and highest in October (Table 4). The number of primary and secondary follicles was higher in relatively young females than in relatively older females (primary/young: $33.8 \pm 20.0$, primary/old: $28.2 \pm 14.1$ and secondary/young: $2.6 \pm 2.6$, secondary/old: $2.1 \pm 2.3$). There was a significant difference in the number of tertiary follicles from April to June compared with July to January (LSD; $P < 0.05$), with no tertiary follicles being recorded in April and the highest number being recorded in January. The number of tertiary follicles decreased significantly from January to February (LSD; $P < 0.05$) and increased significantly from June to July (LSD; $P < 0.05$; Table 4). The number of Graafian follicles was positively correlated with body mass ($\rho_{62} = 0.46$; $\rho^2 = 0.21$; $P < 0.001$). No Graafian follicles were recorded from March to June and most Graafian follicles were found in December (Table 4). We did not find any corpora bodies between May and August and the numbers were significantly lower in March and April compared with September through to January (LSD; $P < 0.01$), while most corpora bodies were recorded in January (Table 4).

## Discussion

The present study confirms and extends previous evidence that female *A. spinosissimus* breed seasonally, with the onset of reproduction at the beginning of the rainy season in September and the last pregnancies in January. The breeding season reaches its peak in October when the highest incidence of pregnancies, largest ovarian volume and highest plasma progesterone concentrations were recorded. Plasma progesterone concentrations were very low from February until August, which coincided with an absence of gravid females in our data. Lactating females were, however, found from October until April and no evidence of lactation was observed from May until September with one exception in June. The numbers of primary, secondary, tertiary and Graafian follicles as well as corpora bodies correspond with the seasonal reproduction of *A. spinosissimus*. The numbers of these follicles and corpora bodies were highest from September/October until January and lowest during March/April and May and corpora bodies were only again found in September.

**Table 4** Monthly sample sizes (*n*), mean numbers and standard errors (SE) for primary, secondary, tertiary and Graafian follicles and corpora bodies (corpora lutea, corpora albicans and corpora haemorrhagica combined) of *Acomys spinosissimus* from South Africa over 12 months in 2007 and 2008

| Follicle | January | February | March | April | May | June | July | August | September | October | November | December |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *n* | 5 | 7 | 6 | 8 | 4 | 3 | 5 | 5 | 4 | 6 | 3 | 6 |
| Primary | | | | | | | | | | | | |
| Mean | 37.5 | 20.1 | 20.8 | 13.6 | 17.8 | 32.5 | 39.6 | 39.8 | 31.9 | 47.6 | 41 | 58 |
| SE | 4.7 | 2.1 | 4 | 3.7 | 3.3 | 0.5 | 8.3 | 8 | 5.1 | 8.2 | 10.6 | 9.3 |
| Secondary | | | | | | | | | | | | |
| Mean | 2.4 | 2 | 1.1 | 0.9 | 1.1 | 2.2 | 1.6 | 1.7 | 1.3 | 6 | 4 | 5.1 |
| SE | 0.8 | 1 | 0.5 | 0.6 | 0.1 | 0.2 | 0.3 | 0.6 | 0.5 | 1.6 | 1.2 | 1 |
| Tertiary | | | | | | | | | | | | |
| Mean | 4.9 | 2 | 0.7 | 0 | 0.1 | 0.3 | 2 | 3.5 | 2.8 | 3.5 | 2.8 | 2.8 |
| SE | 1.1 | 0.7 | 0.6 | 0 | 0.1 | 0.3 | 0.5 | 0.4 | 1.2 | 1.2 | 1.7 | 0.6 |
| Graafian | | | | | | | | | | | | |
| Mean | 0.9 | 0.4 | 0 | 0 | 0 | 0 | 1.1 | 0.6 | 0.8 | 1.3 | 1.5 | 1.6 |
| SE | 0.3 | 0.3 | 0 | 0 | 0 | 0 | 0.4 | 0.2 | 0.3 | 0.5 | 0.8 | 0.2 |
| Corpora bodies | | | | | | | | | | | | |
| Mean | 2.7 | 1.6 | 0.2 | 0.2 | 0 | 0 | 0 | 0 | 1 | 2 | 1.5 | 2.6 |
| SE | 0.3 | 0.3 | 0.2 | 0.2 | 0 | 0 | 0 | 0 | 0.4 | 0.1 | 0.3 | 0.4 |

Our data on seasonality of reproduction correspond partly with the results found by Sheppe (1973) for *A. spinosissimus* in Zambia and Smithers (1971) in Botswana. Both studies demonstrated a breeding season for *A. spinosissimus* during the warm and wet summer months; however, both recorded pregnant females until March and April. This difference with our results might be explained by the more northerly populations examined by Sheppe (1973) and Smithers (1971), where a prolonged reproductive season may be achieved through increased temperatures and/or prolonged rainy seasons and longer vegetation periods. In addition, we collected lactating females until April, indicating that females might have been giving birth up until early April and it is probable that we just did not collect pregnant females during these months although there were pregnant females in the population. The significant decrease of ovarian volume, primary, tertiary and Graafian follicle numbers as well as corpora bodies from January to February/March, however, indicates a decline in reproductive activity after January. We used swollen teats as an indicator of lactation, which may have been misleading, because they still might have been pronounced although the females may not have been lactating anymore.

The seasonal reproductive pattern of female *A. spinosissimus* is similar to that found for another *Acomys* species north of the equator. *Acomys minous* from Crete breeds during the northern hemisphere spring (March–May) and summer months (June–August) (Dieterlen, 1978). In contrast, *Acomys percivali* and *Acomys wilsoni* from central Kenya breed throughout the year, although they occur in a strongly seasonal habitat with two distinct rainy seasons (Neal, 1983). Interestingly, *Acomys subspinosus* from the Western Cape Province of South Africa, the closest geographical congener of *A. spinosissimus*, is an opportunistic breeder that is able to reproduce during the winter months (June–August) by utilizing the flowering *Protea humiflora* as a food source (Fleming & Nicolson, 2002).

The females initiated breeding exactly in coincidence with the onset of the rainy season, and therefore, the main environmental factor influencing the reproductive seasonality of *A. spinosissimus* in South Africa appears to be rainfall, probably combined with the increase in primary productivity and higher food quantity and quality. Many small tropical and sub-tropical mammals reproduce at the time of the rainy season and the time of the highest abundance of food resources. In the French Guiana rainforests, two large rodent species, the acouchy (*Myoprocta exilis*) and the agouti (*Dasyprocta leporine*), reproduce during the time when most fruits are available, which is linked to rainfall even in a rainforest (Dubost, Henry & Comizolli, 2005). Neal (1977) established the importance of rainfall for reproduction in the Natal multimammate mouse (*Mastomys natalensis*) from Uganda. However, food abundance can also influence reproduction independently of rainfall (Neal, 1991). In South Africa, seasonal rainfall in combination with food abundance has been found to influence the breeding of a number of rodent species. Indeed, the Namaqua rock mouse *Micaelamys namaquensis* (Muteka *et al.*, 2006*a*) as well as the bushveld gerbil *Tatera leucogaster* (Perrin & Swanepoel, 1987) breed during the rainy summer months of the northern parts of South Africa.

As rainfall in concert with food quality and quantity might be the main factors regulating seasonal reproduction in *A. spinosissimus*, other factors such as temperature and photoperiod might also be important. *Acomys spinosissimus* is a very small rodent, with the females having a mean body mass of $19.8 \pm 4.2$ g. Therefore, the low environmental temperatures of below 20 °C recorded from April to August might place high energetic constraints on this small mammal, which, in turn, might prevent it from breeding during the winter period when more energy is needed for

thermogenesis, cellular maintenance and immune functions (Klein & Nelson, 1999). In the Drakensberg Mountains of the KwaZulu-Natal Province, South Africa, some rodent populations at higher altitudes start breeding later than in lower altitudes due to lower temperatures, although reproduction is otherwise dependent on the rainy season and the subsequent increase in food availability (Rowe-Rowe & Meester, 1982). Furthermore, photoperiod has been reported to influence breeding in South African rodent species (Muteka, Chimimba & Bennett, 2006*b*) and there are initial indications that male *A. spinosissimus* may be reproductively photoresponsive (K. Medger, unpubl. data).

Primordial follicle number was the only variable recorded that did not differ throughout the year. However, primordial follicle numbers were higher in relatively younger than in relatively older females and decreased with increasing body mass, which might be a result of older females being heavier than younger females. In female humans, monkeys and rodents, it has been reported that the numbers of germ cells decrease substantially with female age and new follicles are only recruited from an initial follicle pool that proliferates around the time of birth (Peters, Levy & Crone, 1962; Nozaki, Yamashita & Shimizu, 1997; Broekmans, Soules & Fauser, 2009). The gradual decrease in fertility with the shrinking follicle pool finally results in menopause or reproductive senescence. Briston-Gould *et al.* (2006) developed models to explain the phenomenon of follicle number decrease in female mammals and compared these to findings in primordial, primary and secondary follicle number reduction in mice. They found that a fixed pool model, meaning all germ cells are present at the time of birth and no new ones are recruited afterwards, best explains the observed decrease of primordial, primary and secondary follicle numbers. This may also explain the slight effect that age has on the primary and secondary follicles in our study. Therefore, as no new primordial follicles are recruited in the course of the year, no monthly differences in primordial follicle numbers should be observed, even within a seasonally breeding mammal species.

Female *A. spinosissimus* reproduce seasonally during the warm and wet summer months in South Africa. The most important factor influencing reproduction in this small rodent appears to be rainfall that brings about a concomitant increase in food quality and quantity. A number of other factors such as temperature and photoperiod are additional factors that may influence seasonal reproduction, both on their own and in combination with rainfall and food availability.

This study has raised a number of questions as to the potential cues that may be influencing seasonal reproduction in *A. spinosissimus*, and to obtain a profound understanding of seasonality in this rodent and other small mammals from tropical and sub-tropical regions, much more information is required. Although *A. spinosissimus* seems to be common and is listed as a species of Least Concern in terms of conservation in the *Red Data Book of the Mammals of South Africa* (Friedmann & Daly, 2004), this species is not very common throughout its range and is restricted to mountainous habitats. The fragmented habitat (Friedmann & Daly, 2004), anthropogenic influences and deficient knowledge on its general biology and ecology might pose future threats. Thus, it is important to learn as much as possible about this species to be able to avoid future threats to its survival, especially in the southern African subregion.

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