# Seasonal changes in reproductive development in male spiny mice (*Acomys spinosissimus*) from South Africa

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## Abstract

Seasonal reproduction is a common characteristic of many small mammals which inhabit seasonal environments in temperate regions, the sub-tropics as well as the tropics. It is important for an animal to reproduce during the most favourable time of the year to ensure the survival of the young and maximize reproductive success. In southern Africa, female spiny mice (*Acomys spinosissimus*) breed during the warm and wet spring and summer months, whereas the reproductive pattern of males is unknown although an opportunistic breeding pattern has been implicated. We investigated testes mass and volume, seminiferous tubule diameter, spermatogenesis and plasma testosterone concentrations in a South African population of male spiny mice on a 2-monthly basis over one year. Testes mass and volume started to increase in July/August and was high from September until December. Seminiferous tubule diameter and spermatogenesis increased during the same months. Plasma testosterone concentration was elevated from July/August to November/December. Development of the reproductive characteristics of male spiny mice was correlated with high rainfall and high ambient temperatures, but reproductive development had already started during the dry season and the coldest months. This shows that reproductive development in males may not be dependent on climatic conditions, and other factors, such as photoperiod, may trigger the onset of reproduction. The data, however, suggest that *A. spinosissimus* is a true seasonal breeder with reproduction confined to the spring and summer months in southern Africa.

Keywords: seasonal reproduction, spermatogenesis, testosterone, rainfall, southern hemisphere

## Introduction

In most parts of the world, especially temperate but also sub-tropical and tropical regions, the environment changes seasonally and with it also the energy available for most organisms. As a consequence, the large amount of energy required for reproduction in many mammals is only available for a short time of the year forcing these species to breed seasonally (Bronson and Heideman, 1994; Speakman, 2008). In the tropics of Africa and South America, for example, forest-dwelling rodents breed seasonally and seasonal variation in food abundance, particularly fruits in tropical forests, may well be the primary factor regulating reproduction (Dubost et al., 2005; Makundi et al., 2006). Many tropical but also sub-tropical regions experience considerable changes in rainfall throughout the year with one or two rainy seasons interrupted by partially severe dry periods (e.g., Africa; Nicholson, 1993). This rainfall leads to an increase in primary productivity and consequently higher food quantity and quality compared to the dry season and this relationship of rainfall and increased primary productivity has been suggested to be the ultimate factor regulating reproduction in such regions (Delany, 1972; Neal, 1986; Perrin, 1986). In contrast, ambient temperature appears to be a more important factor at temperate latitudes (Bronson, 1985; Nelson et al., 1989). Many mammals use the seasonally changing day-night cycle as a proximate cue to herald reproductive activation, which is especially effective in highly predictable environments (Bradshaw and Holzapfel, 2007).

In many large and often long-lived mammals, both males and females show reproductive activity for only a short period and are reproductively inactive during the remainder of the year (du Mond and Hutchison, 1967; Bronson, 1985). Small mammals, on the other hand, exhibit a wider range of reproductive adaptations and some may be reproductively active during winter when environmental conditions allow it (Beer and MacLeod, 1961). This opportunistic breeding strategy is typical for short-lived animals such as rodents, for which environmental conditions are often unpredictable as they seldom live for a year and maximum reproductive success can only be achieved by breeding at every available opportunity (Bronson and Perrigo, 1987). This results in variation in reproductive performance not just between species but also between populations and even within a population (Lynch et al., 1981; Neal, 1981; Prendergast et al., 2001). In South Africa, the four-striped field mouse (*Rhabdomys pumilio*) breeds opportunistically. In habitats with mild winters, this species breeds throughout the year whereas in harsh environments, females cease breeding, although males remain reproductively active year-round (Jackson and Bernard, 2006).

In contrast to temperate regions where the biology of most small mammal species is well-documented, the general and more so the reproductive biology of many tropical and sub-tropical species is unknown and in urgent need of investigation (Bronson, 2009). The present study was undertaken to unravel the reproductive biology of the spiny mouse (*Acomys spinosissimus*). The spiny mouse is a small, nocturnal rodent which inhabits rocky outcrops and riverine habitats where it finds shelter under boulders and rocks as well as in termite mounds (Sheppe, 1973; Pienaar et al., 1980; Fitzherbert et al., 2007). It has a widespread distribution across Africa, south of the equator, and is found in Tanzania, the Democratic Republic of Congo (DRC), Zambia, Malawi and in southern Africa in Mozambique, Botswana and South Africa (Skinner and Chimimba, 2005). The reproductive biology of the spiny mouse is almost entirely unknown although modest available data suggest that females reproduce seasonally in southern Africa (Smithers, 1971; Pienaar et al., 1980; Medger et al., 2010). Reproduction in male *A. spinosissimus* has not been documented and it is unknown if males breed seasonally.

The aim of the present study was to investigate the pattern of reproduction of male spiny mice from a summer rainfall region in southern Africa, by collecting animals on a monthly basis over one year and using morphological, histological and endocrinological methods to investigate male reproductive characteristics. In addition, the relative age of an individual was taken into account and monthly rainfall and ambient temperature of the habitat were recorded. In the present study, we propose the following two possible scenarios on the reproductive pattern of the male spiny mouse: 1) *Acomys spinosissimus* may be an opportunistic breeder with males showing reproductive activity throughout the year as has been found in *R. pumilio*; and 2) *Acomys spinosissimus* may be a seasonal breeder given that other rodent species found sympatrically with *A. spinosissimus* breed seasonally (Muteka et al., 2006a; Muteka et al., 2006b). The strong seasonal changes in rainfall and decreased food availability during the South African winter may result in a reduction of both testes size and circulating testosterone concentrations and ultimately, in a strong seasonal breeding pattern in male spiny mice.

## Materials and Methods

All animals were collected from six sites along the rocky outcrops of the Goro Game Reserve, Limpopo Province, South Africa (22°58’S, 2 2°57’S; 29°25’E, 29°24’E) from April 2007 to October 2008. A total of 65 male spiny mice were caught with a monthly capture rate of between one and nine individuals. Small monthly sample sizes necessitated the evaluation of our data on a 2-monthly basis (hereafter referred to as

2-month intervals; i.e., January/February and March/April; for sample sizes see Figures 1a and 2). Animals were collected under permit number CPM-333-00002 from the CITES and Permit Management Office, Department of Environmental Affairs, Limpopo Province, South Africa. Spiny mice were trapped over-night with Sherman live traps (H. B. Sherman Traps, Inc. Tallahassee, Florida, U.S.A.) baited with a mixture of peanut butter, oats and sardines. Immediately after capture, each individual was weighed to the nearest 0.001 g using a digital balance (Scout Pro SPU123, Ohaus Corporation, Pine Brook, New Jersey, U.S.A.) and body mass was rounded to 0.1 g for subsequent analyses. Animals were kept in polyurethane cages provided with wood shavings for bedding and paper towelling for additional shelter during transportation and subsequently in the laboratory. They were fed with mouse pellets, carrots and apples and water was provided *ad libitum*. All male spiny mice were housed for at least one day but not more than three days in the laboratory before they were euthanized.

Animals were euthanized by an overdose of halothane and blood was collected immediately by exsanguination from the heart and centrifuged at 3000 rpm for 15 min. The plasma fraction was separated from blood cells and stored at -35 ℃ until hormone analysis. Testes were dissected out and fixed in Bouin's fluid for approximately 20 hrs before being rinsed and stored in 70 % ethanol. Standard museum techniques (DeBlase and 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 the "Relative age classes" section below. All animal procedures were approved by the animal ethics committee of the University of Pretoria, Pretoria, South Africa (Ethics clearance number A003-07).

Ambient temperature (°C) was recorded to the nearest 0.0625 °C by iButton digital temperature data loggers (DS1922L-F5, Maxim Integrated Products, Dallas Semiconductor, U.S.A.) every two hours from September 2007 until August 2008. The iButtons were located near the highest trapping site where they were placed near the ground and protected from direct sunlight. Monthly rainfall data (mm) from January 2006 until December 2008 were kindly provided by the Goro Game Reserve. In addition, the day length (h) for the $15^{th}$ of every month from September 2007 until August 2008 was calculated.

Histology

The length (mm) and the width (mm) of both testes per male were measured to the nearest 0.01 mm using a pair of digital callipers (Sylvac Opto RS 232, Ultra Praezision Messzeuge GmbH, Germany) after removal of fat and other tissues. These dimensions were in turn used to calculate testicular 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 half the maximum width (Woodall and Skinner, 1989). Testes were weighed to the nearest 0.0001 g using a high precision scale (Ohaus Corp. Pine Brook, New York, U.S.A.). Testicular mass and volume were averaged per male.

Testes were sequentially dehydrated in increasing concentrations of ethanol baths, embedded in a cube of paraffin wax and sectioned at 8 µm widths with a rotary microtome (820 Spencer, American Optical, Scientific Instrument Division, Buffalo, New York, U.S.A.). Sections were mounted on microscope slides with gelatin as an adhesive and subsequently dried in an oven at 36 °C for about 48 hrs. The sections were finally stained with Ehrlich's haemotoxylin and counter-stained with eosin (see Drury and Wallington, 1967). Testicular sections were checked for round seminiferous tubules with a light microscope (Diaplan, Ernst Leitz Wetzlar GmbH, Germany). Seminiferous tubules were then photographed at ×10 magnification with a digital camera (Moticam 1000 1.3 M Pixel USB 2.0, Motic China Group, LTD., Xiamen, China) attached to a microscope. The diameters of 100 seminiferous tubules (µm) were measured with Motic Images Plus 2.0ML (Motic China Group, LTD., Xiamen, China). In addition, spermatogenic activity in the seminiferous tubules was recorded.

Plasma testosterone analysis

A coat-a-count hormone kit (Siemens Medical Solutions Diagnostics, Los Angeles, USA) was used to determine plasma testosterone concentrations. The test was performed according to the guidelines of the manufacturer and hormone concentrations were calculated using a calibration curve. To validate the assay for *A. spinosissimus*, the slopes of a serial dilution curve and the calibration curve were tested for parallelism with a General Linear Model (GLM) after log-logit-transformation (Chard, 1978). The dilution percentages (1:1 to 1:4) were used as covariates and the type of curve was employed as a random factor.

Because of the small body mass of the spiny mice and the associated small blood volume, we could not obtain enough plasma for hormone analysis from all animals (V = 2 × 50 µl). Therefore, plasma testosterone concentration (ng/dL) was determined for 59 male *A. spinosissimus* over the whole study period (for 2-monthly sample sizes see Figure 3). There was no significant difference between the serial dilution curve of plasma testosterone and the calibration curve ($F_{1,3}$ = 5.87; $n$ = 3; $P$ =

0.09) and therefore, the testosterone assay could be validated for *A. spinosissimus*. The sensitivity of the assay was 39.71 ng/dL and the intra-assay coefficient of variation was 1.1 %.

Relative age classes

All males were aged using the degree of maxillary molar tooth-wear and eruption. Each individual was initially placed into one of five tooth-wear classes as described and illustrated by Dippenaar and Rautenbach (1986) for *A. spinosissimus*. None of our animals showed incompletely erupted cheek teeth (i.e., tooth-wear class 1 as defined by Dippenaar and Rautenbach, 1986) and to accommodate for the small monthly sample sizes, we re-defined the remaining four tooth-wear classes into relatively young and relatively old individuals as described by Medger *et al.* (2010).

Data analyses

We first analysed body mass and all reproductive variables over an entire year, for which the data were combined for the two-year sampling period resulting in one universal dataset comprising six 2-month intervals. To also account for differences between 2007 and 2008, we performed a second analysis for body mass and each reproductive variable which only included the months when animals where sampled in both years; namely March/April until September/October. Male body mass was analysed by GLMs. As testicular mass and volume, seminiferous tubule diameter and plasma testosterone concentration were not normally distributed even after log-transformation, all reproductive variables were analysed using Generalized Linear Models (GZLM) with a gamma distribution and log-link function and body mass as a covariate. At first, body mass and all reproductive variables were investigated for differences between the six 2-month intervals with month as a fixed factor. From these results, we identified the months that represent either the breeding or non-breeding season. Because of the small sample sizes for each relative age class per 2-month interval, all variables were re-analyzed with season and relative age class as fixed factors. Year and month were applied as fixed factors to compare body mass and reproductive characteristics between 2007 and 2008. Post-hoc least significant difference (LSD) tests were carried out when significant differences were found and the Akaike Information Criterion (AIC) was used to assess the fit of the models. Effects of ambient temperature and rainfall on all reproductive variables were analysed with Spearman's rank correlation. All statistical analyses were performed using the *Statistical Package for the Social Sciences (SPSS)* Statistics version 19.0 (SPSS Inc., Chicago, USA). The results herein are presented as mean ± 1 standard error (SE) and significance was assumed at $P \leq 0.05$.

## Results

<u>Reproductive variables</u>

Testicular mass and volume, diameter of seminiferous tubules and plasma testosterone concentration were significantly different between the six 2-month intervals (Wald $\chi^2 > 83.59$; $df = 5$; $P < 0.001$). Mass and volume and seminiferous tubule diameters started to increase significantly from May/June to July/August (LSD: $P < 0.001$), were largest from September until December and then significantly decreased again towards January/February (LSD: $P \leq 0.001$; Figures 1 and 2a). In comparison to any other month, these three reproductive variables were significantly smaller from March until June (LSD: $P < 0.001$; Figures 1 and 2a). Furthermore, there was no spermatogenic activity evident in the seminiferous tubules from February until June. From July until January, corresponding with the increased testes and seminiferous tubule sizes, spermatogenic activity was observed (Figures 2b and c). Plasma testosterone concentration was significantly larger from July until December compared to the rest of the year (LSD: $P < 0.05$; Figure 3).

The previous analyses indicated that the months July until December span the breeding season and January until June the non-breeding season of male *A. spinosissimus.* For further analyses of relative age class effects on reproductive variables, the data were combined accordingly. All reproductive variables were significantly larger during the breeding than the non-breeding season as expected from the earlier analyses (Wald $\chi^2 \geq 56.55$; $df = 1$; $P < 0.001$). However, testicular volume and mass, seminiferous tubule diameter and plasma testosterone concentration did not differ significantly between relatively old and relatively young males (Wald $\chi^2 \leq 0.12$; $df = 1$; $P \geq 0.73$). There was no significant interaction between the seasons and the relative age classes for any of the reproductive variables (Wald $\chi^2 \leq 2.59$; $df = 1$; $P \geq 0.11$).

When we investigated differences between the years 2007 and 2008 for the months March/April until September/October, we found that there was no significant difference in the monthly changes of testicular mass and volume and seminiferous tubule diameter (Wald $\chi^2 \leq 4.91$; $df = 3$; $P \geq 0.18$) as well as plasma testosterone concentration (Wald $\chi^2 = 4.69$; $df = 2$; $P = 0.10$). For further analyses of testicular mass and volume, the interaction of year and month was removed because AIC was smaller without the factor interaction (mass: 334.40; volume: 323.82) than with it (mass: 338.10; volume: 329.11). The interaction factor was left in the model for further analyses of the two other reproductive variables. Testicular mass and volume were not significantly different between 2007 and 2008, whereas seminiferous tubule diameter and plasma testosterone concentration were larger during 2007 than 2008

(Table 1). *Table 1 to appear here* We also observed a significant difference between the four 2-month intervals for all reproductive variables analysed as expected from analyses over the entire year (Table 1). Testicular mass and volume, seminiferous tubule diameter and testosterone concentration increased significantly from May/June to July/August (LSD: $P < 0.05$). In addition, testicular mass and seminiferous tubule diameter were significantly raised in May/June compared to March/April (LSD: $P < 0.05$) and these two variables plus testicular volume were significantly larger in September/October than July/ August (LSD: $P \leq 0.05$).

Testicular mass and volume and seminiferous tubule diameters increased with increasing body mass for all GZLMs (Wald $\chi^2 \geq 8.40$; $df = 1$; $P \leq 0.004$). Plasma testosterone concentration was not correlated with body mass for neither GZLM (Wald $\chi^2 \leq 1.91$; $df = 1$; $P \geq 0.17$).

Body mass

Male body mass was normally distributed and homoscedastic (Levene's test: $F_{11,53} = 1.69$; $P = 0.10$) warranting analysis with a GLM. Mean body mass of male spiny mice was 19.1 ± 0.4 g for the entire study. Body mass was significantly different between the six 2-month intervals ($F_{5,59} = 4.09$; $P = 0.003$). Male *A. spinosissimus* were significantly heavier during September/October (LSD: $P = 0.001$; 21.6 ± 0.9 g) than during March/April (17.4 ± 0.7 g) and May/June (17.7 ± 0.6 g). As a result, the males were significantly heavier during the breeding season (20.5 ± 0.5 g) compared to the non-breeding season (19.0 ± 0.6 g; $F_{1,61} = 4.00$; $P = 0.05$). In addition, relatively old males were significantly heavier (21.4 ± 0.7 g) than relatively young males (18.1 ± 0.4 g; $F_{1,61} = 11.20$; $P = 0.001$). There was no significant interaction between season and relative age class ($F_{1,61} = 0.14$; $P = 0.71$). If body mass was compared between 2007 and 2008 for four 2-month intervals from March until October, it was shown to be significantly larger during 2007 (22.0 ± 0.8 g) than 2008 (17.1 ± 0.4 g; $F_{1,32}$ = 20.60; $P$ < 0.001). However, body mass was similar in all months analysed ($F_{3,32}$ = 2.33; $P$ = 0.10) and there was no significant effect of year on body mass per month ($F_{3,32}$ = 4.84; $P$ = 0.47).

Weather data

The weather of the study area was characteristic of the summer rainfall area in southern Africa. The rainy season typically started in either September (2007) or October (2006 and 2008) and stopped in March (2006) or April (2007 and 2008) with December being the month with the highest rainfall (146 mm in December 2007; Figure 4). During the winter of the southern hemisphere, from May until August, no rainfall was recorded in any of the years during the study. Ambient temperatures were highest from September until March with mean monthly temperatures above 20 ℃ and February being the warmest month (23.5 ± 0.2 ℃). Mean monthly temperatures below 20 ℃ were recorded between April and August, and July was the coldest month (16.2 ± 0.2 ℃). Figure 4 shows monthly rainfall, ambient temperature and day length from September 2007 until August 2008. *Figure 4 to appear here*

Testicular mass and volume and seminiferous tubule diameter were previously found to be dependent on body mass (see GZLM's) and therefore, these variables were corrected for body mass for all correlation analyses. Testicular mass and volume as well as seminiferous tubule diameter increased significantly with an increase in ambient temperature ($\rho$ > 0.28; $n$ = 58; $P$ < 0.03) and rainfall ($\rho$ > 0.56; $n$ = 65; $P$ < 0.001). Although plasma testosterone concentration increased with greater rainfall ($\rho$ = 0.39; $n$ = 59; $P$ = 0.002), it was not significantly correlated with ambient temperature ($\rho$ = 0.04; $n$ = 55; $P$ = 0.76).

## Discussion

The present data on the reproductive characteristics of male spiny mice over a 12-month period demonstrate that *A. spinosissimus* is a strongly seasonal breeder in South Africa. The volume and mass of the testes were reduced from January/February until May/June and were largest from September until December. The increase in testes size was considerable with for example, testes mass increasing 16-fold from a minimum of 14.9 ± 4.0 mg in March/April to a maximum of 235.7 ± 12.0 mg in September/October. Seminiferous tubule diameter and spermatogenesis followed a similar pattern. Plasma testosterone concentration was elevated for a shorter period and only increased in July/August and remained high until November/December. For the remainder of the year, plasma testosterone concentrations were consistently low. Recrudescence of reproductive development in male spiny mice occurred at the end of winter and peaked during the southern hemisphere spring and summer. Reproductive characteristics were highly correlated with rainfall and ambient temperature, being larger during periods of high rainfall and ambient temperatures; however, testes, seminiferous tubule diameters and testosterone concentrations already started to increase about two months (July/August) prior to the start of the rainy season (September).

The pattern of breeding exhibited by male *A. spinosissimus* investigated in the present study is similar to that found in female spiny mice (Medger et al., 2010). However, reproductive development of male spiny mice appears to be 1.5 to 2 months prior to that of recrudescence in the females with spermatogenesis already occurring in either July or August in males, but corpora lutea of ovulation and pregnancy were only recorded in September in females (see Medger et al., 2010). This appears to be a common pattern in strongly seasonally breeding rodents with reproductive readiness of males occurring approximately two months before activation in females in a number of small mammals (Kenagy and Bartholomew, 1985; Perrin and Swanepoel, 1987; Muteka et al., 2006b). It is speculated that early reproductive development of males may increase their potential to secure a mate since they will have already reached their maximum reproductive potential when females start to be reproductively active.

There was no difference in testes size, seminiferous tubule diameter, or testosterone concentration between relatively young and relatively old males. This implies that all males caught during the study were mature although relatively young males weighed less than relatively old males. We also found that all reproductive variables increased during the same months in the two years of the study showing a consistent seasonal reproductive pattern in both years. Nonetheless, all reproductive variables were larger during 2007 than 2008. This may be explained by better environmental and ultimately breeding conditions for *A. spinosissimus* during 2007 compared to 2008, which is supported by higher rainfall in 2007 (208 mm) compared to 2008 (124 mm) for the period January to October. In addition, the different sampling periods may also have contributed to these results because spiny mice were sampled in November and December in 2007 but not 2008, a time when reproductive variables increased.

Several authors suggest that rainfall, and the related increase in primary productivity and ultimately increase in food quantity and quality, is the primary cause for seasonal reproduction in many tropical and sub-tropical rodents (Delany, 1972; Neal, 1986; Perrin, 1986). In southern Africa, a relationship between seasonal rainfall and reproduction has been observed in the bushveld gerbil (*Gerbilliscus leucogaster*) (Perrin and Swanepoel, 1987), the Namaqua rock mouse (*Micaelamys namaquensis* (formerly *Aethomys namaquensis*; Skinner and Chimimba, 2005)) (Muteka et al., 2006b) and the Tete veld rat (*Aethomys ineptus*) (Muteka et al., 2006a). A study on reproduction in female *A. spinosissimus* (Medger et al., 2010) proposed that rainfall may also be the primary cause for seasonal reproduction in this species. However, reproduction in male *A. spinosissimus* commenced well before the onset of the rains and during the months with the lowest ambient temperatures, intimating that male recrudescence of reproduction may be less affected by weather conditions than in females. In contrast to opportunistic breeders such as *R. pumilio* (Jackson and Bernard, 2006), males of strongly seasonal species appear to cease reproduction similarly to females, as breeding opportunities are doubtful outside of the breeding season and maintaining reproductive activity is unlikely to be advantageous (Bronson and Heideman, 1994; Taggart et al., 2005). The same may hold for *A. spinosissimus*, in that males discontinue breeding activities during the time that females are not reproductively active, although environmental conditions may allow male reproduction. Reproductive regression in males occurs during autumn and part of winter implying that *A. spinosissimus* is a distinctly seasonal breeder rather than an opportunistic breeder in southern Africa.

As rainfall may not be the primary trigger for the onset of seasonal reproduction in male spiny mice, other factors, such as photoperiod and temperature, may be utilized by males to time reproduction. Ambient temperature was correlated with most reproductive variables except testosterone concentration in male spiny mice, but is also unlikely to be a cue that triggers the onset of reproduction in males because reproductive development in males commenced during the coldest month of the year. On the other hand, changes in photoperiod are constant between years and are, thus, especially useful for timing reproductive events in predictable environments (Bradshaw and Holzapfel, 2007) such as the habitat of *A. spinosissimus* where the start and extent of the rainy season is relatively consistent between years as was the case in this study. In addition, rodents which occur sympatrically with *A. spinosissimus* have been found to respond to changing photoperiods (Muteka et al., 2006c) and males of the golden spiny mouse (*Acomys russatus*) showed reduced spermatogenesis under short-day photoperiods (Wube et al., 2008). Photoperiod may, therefore, be the most likely environmental factor that is used by males to trigger reproductive development, and there are indications that male *A. spinosissimus* are reproductively photoresponsive (Medger, 2010; Medger et al., 2012).

Members of the genus *Acomys*, which have a wide distribution throughout Africa, southern Europe, south-west Asia and Pakistan (Musser and Carleton, 2005), exhibit a wide range in reproductive patterns. *Acomys russatus*, which occurs in strongly seasonal habitats, appears to be a strong seasonal breeder as is *A. spinosissimus* (Shargal et al., 2000). In contrast, *A. subspinosus*, which is endemic to the Western Cape Province of South Africa, breeds opportunistically, but unlike *A. spinosissimus*, this species inhabits a winter rainfall area and flowering *Protea humiflora* is used as a food source in winter (Fleming and Nicolson, 2002). The common spiny mouse (*Acomys cahirinus*) and the eastern spiny mouse (*Acomys dimidiatus*), both from highly variable habitats, may also be opportunistic breeders (Happold, 1966; Shargal et al., 2000; Al-Khalili and Delany, 1986). This shows that species of *Acomys* tend to be seasonal breeders in seasonal habitats, whereas they are aseasonal breeders in habitats with less seasonal changes in resource availability. Surprisingly however, Neal (1983) observed year-round breeding in *A. percivali* and *A. wilsoni* from a seasonal rainfall area in Kenya.

In conclusion, male *A. spinosissimus* breed seasonally with the onset of reproductive development at the end of winter and peak in reproduction during spring and the beginning of summer in South Africa. The peak in reproduction coincides with the rainy season and reproduction in females, but reproductive development in males starts prior to the onset in females and during the driest and coldest month of the year. This suggests that reproduction in male *A. spinosissimus* may not depend on climatic conditions, but other factors may regulate reproduction in males. The present data suggest that *A. spinosissimus* is a true seasonal breeder in South Africa with the breeding season corresponding well with the warm and wet spring and summer months. Since males were not reproductively active during the remainder of the year during the present study, an opportunistic breeding pattern is improbable for this species.

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Table 1. Generalized linear models exploring differences in testicular mass (mg) and volume ($mm^3$), seminiferous tubule diameter (µm) and plasma testosterone concentration (ng/dL) of male spiny mice (*Acomys spinosissimus*) between 2007 and 2008 and between four 2-month intervals from March/April to September/October. Means ± SE of each variable are shown for 2007 and 2008 and sample sizes for each year are indicated in parentheses. Significant *P*-values are highlighted in bold.

| | Year | | | | | Month | | |
|---|---|---|---|---|---|---|---|---|
| | Wald $\chi^2$ | *df* | *P* | 2007 | 2008 | Wald $\chi^2$ | *df* | *P* |
| Testicular mass (mg) | 1.66 | 1 | 0.20 | 164.0 ± 28.0 (13) | 38.7 ± 11.1 (27) | 177.15 | 3 | **< 0.001** |
| Testicular volume ($mm^3$) | 3.56 | 1 | 0.06 | 49.7 ± 6.9 (13) | 34.3 ± 3.4 (27) | 149.51 | 3 | **< 0.001** |
| Diameter of seminiferous tubules (µm) | 18.99 | 1 | **< 0.001** | 145.4 ± 7.0 (13) | 110.8 ± 3.5 (27) | 192.37 | 3 | **< 0.001** |
| Plasma testosterone concentration (ng/dL) | 7.22 | 1 | **< 0.01** | 410.5 ± 177.1 (9) | 72.9 ± 15.6 (27) | 23.61 | 3 | **< 0.001** |

## Figure legends

Figure 1. Box-plots showing differences in testicular mass (mg) (a) and volume ($mm^3$) (b) of spiny mice (*Acomys spinosissimus*) from South Africa in 2-month intervals for an entire year. Sample sizes for each 2-month interval are included in a.

Figure 2. Box-plot of seminiferous tubule diameter (µm) of spiny mice (*Acomys spinosissimus*) from South Africa shown for two months combined over one year (a) and histological sections of seminiferous tubules photographed at ×16 magnification of spiny mice caught in April (b) and September (c). Arrows indicate spermatozoa (c) and 2-monthly sample sizes are shown.

Figure 3. Plasma testosterone concentration (ng/dL) of male spiny mice (*Acomys spinosissimus*) from South Africa compared between six 2-month intervals from January/February until November/December using a box-plot. *S*ample sizes per 2-month interval are shown.

Figure 4. Monthly mean ambient temperature (℃) and total monthly rainfall (mm) in the Goro Game Reserve, Limpopo Province, South Africa from September 2007 until August 2008. In addition, day length for the 15th of each month is illustrated.

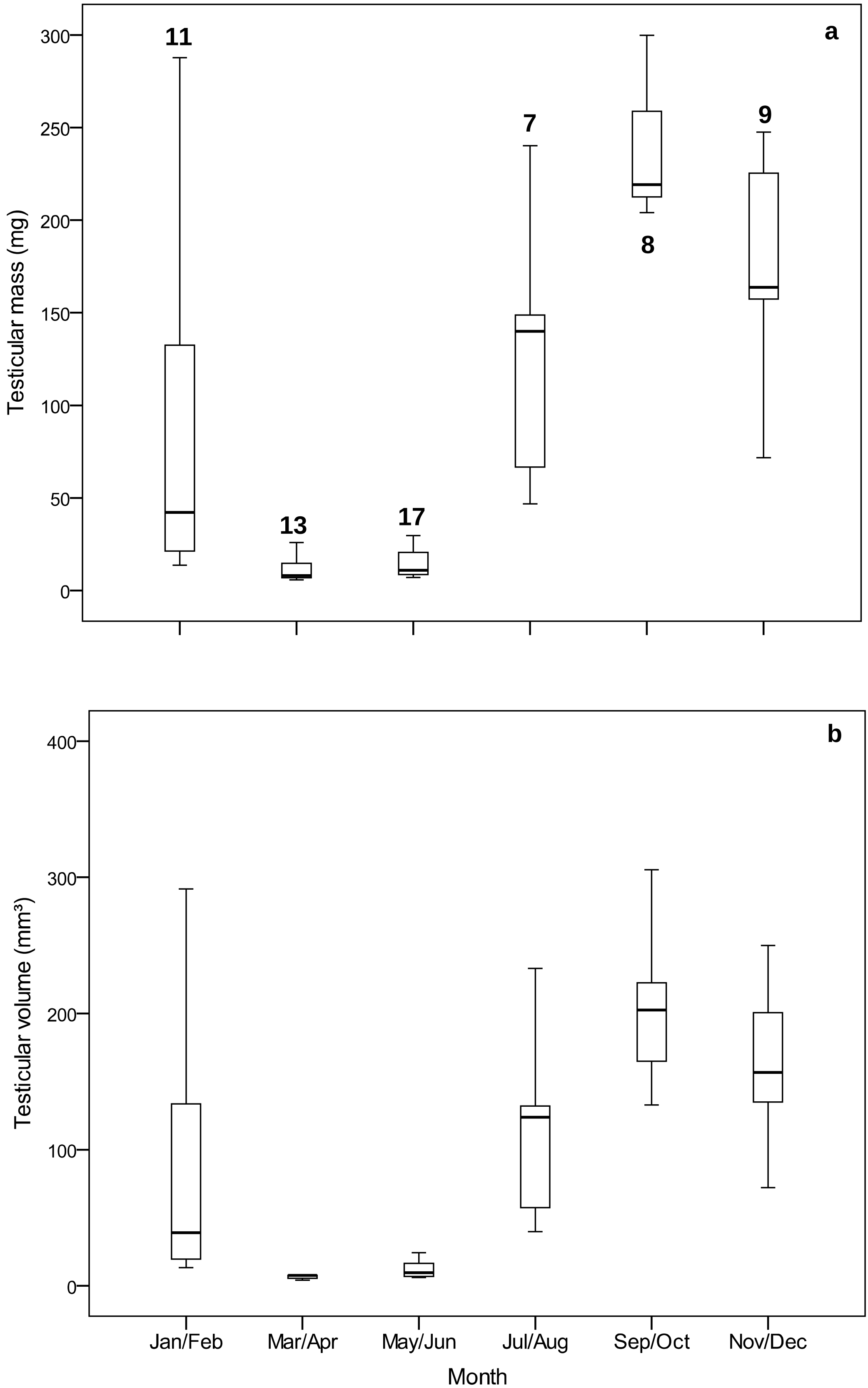

a
Testicular mass (mg)
300
250
200
150
100
50
0
11
13
17
7
8
9
b
Testicular volume (mm³)
400
300
200
100
0
Jan/Feb
Mar/Apr
May/Jun
Jul/Aug
Sep/Oct
Nov/Dec
Month

Figure 1.

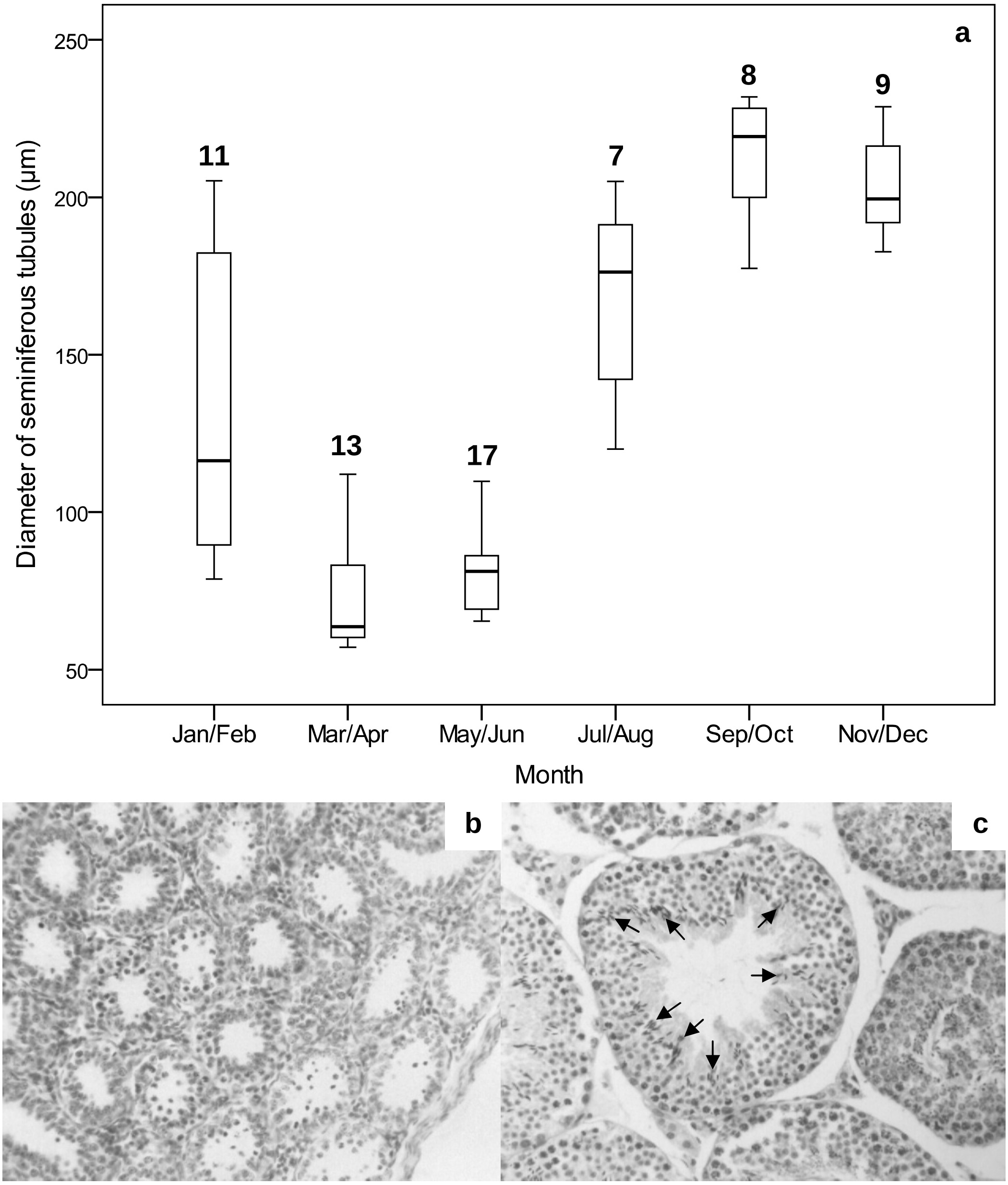
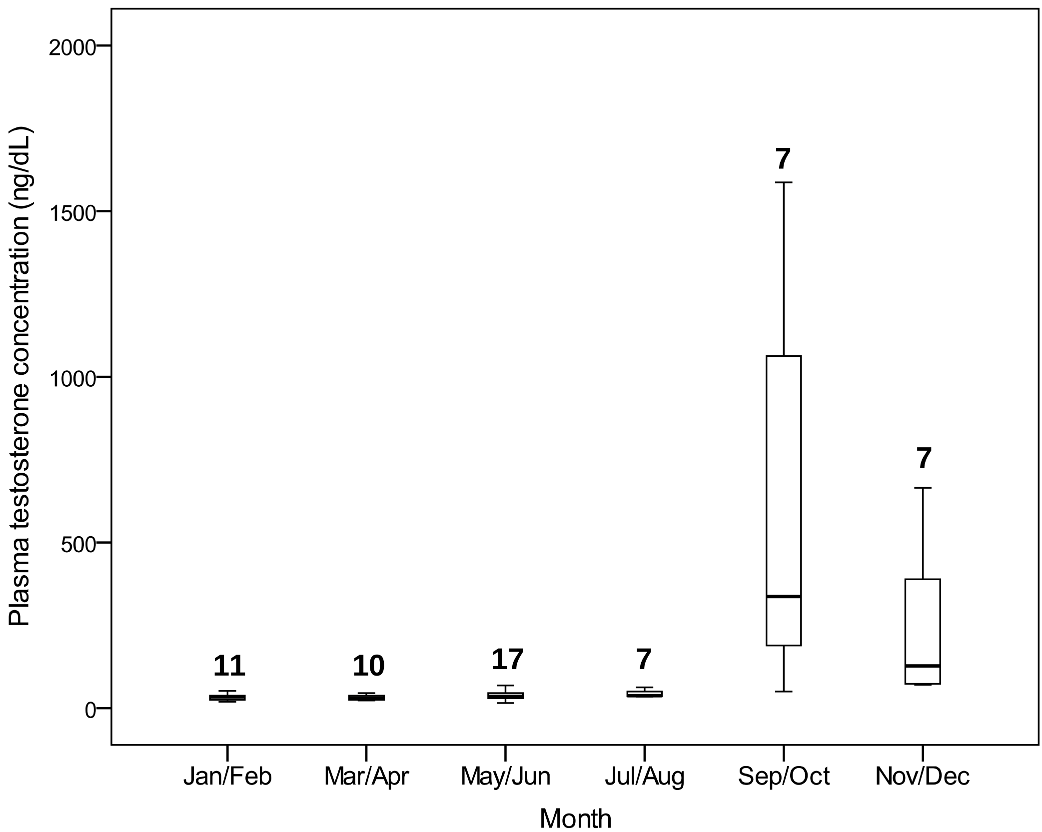

a
b
c
Diameter of seminiferous tubules (µm)
250
200
150
100
50
11
13
17
7
8
9
Jan/Feb
Mar/Apr
May/Jun
Jul/Aug
Sep/Oct
Nov/Dec
Month

Figure 2.



Plasma testosterone concentration (ng/dL)
2000
1500
1000
500
0
11
10
17
7
7
7
Jan/Feb
Mar/Apr
May/Jun
Jul/Aug
Sep/Oct
Nov/Dec
Month


Figure 3.

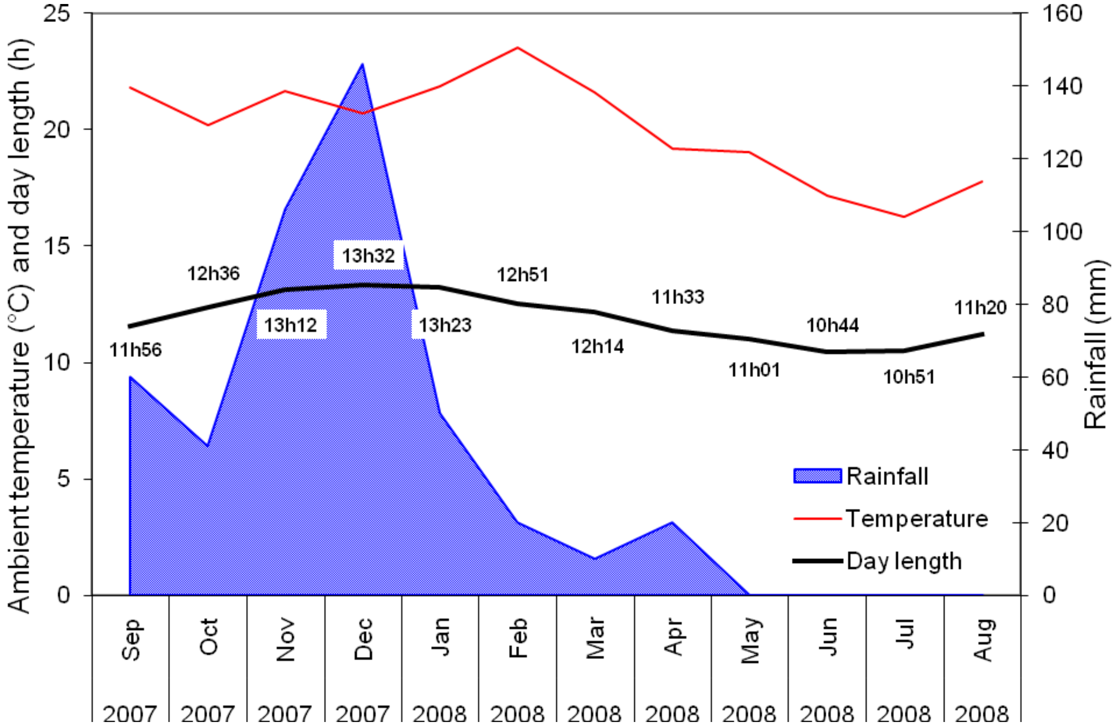

Ambient temperature (°C) and day length (h)
Rainfall (mm)
25
20
15
10
5
0
160
140
120
100
80
60
40
20
0
11h56
12h36
13h12
13h32
13h23
12h51
12h14
11h33
11h01
10h44
10h51
11h20
Rainfall
Temperature
Day length
Sep 2007
Oct 2007
Nov 2007
Dec 2007
Jan 2008
Feb 2008
Mar 2008
Apr 2008
May 2008
Jun 2008
Jul 2008
Aug 2008

Figure 4.