

Seasonal reproduction in the eastern rock elephant-shrew: Influenced by rainfall and ambient temperature?

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Abstract: Because environmental conditions vary seasonally in most regions, many small mammals reproduce at a specific time of the year to maximize their reproductive success. In the tropics and sub-tropics, the breeding season is usually determined by the extent of the dry and rainy seasons. We investigated the seasonality of reproduction in the eastern rock elephant-shrew (*Elephantulus myurus*), over a 12-month period and attempted to elucidate the factors which may influence seasonal reproduction in this endemic African mammal. *Elephantulus myurus* breeds seasonally during the warm and wet spring and summer months and cessation of breeding occurs during the cold and dry winter months of the southern hemisphere. Pregnant females were only collected from August through to January. Ovarian size and plasma progesterone started to increase a few months prior to the first rains, were highest in October and decreased thereafter. Follicular growth and corpora body numbers corresponded to this seasonal reproductive pattern. Testes and seminiferous tubule size and plasma testosterone concentration has already started to increase during the coldest months, two months prior to reproductive onset in females. We propose that seasonal reproduction evolved in *E. myurus* because of seasonally changing food availability brought about by severe seasonal changes in rainfall and ambient temperature. The direct effects of rainfall and ambient temperature on reproduction of *E. myurus* are ambiguous, and we discuss other environmental factors which may trigger reproductive onset in this species.

Keywords: *Elephantulus myurus*, Macroscelidea, Afrotheria, gonadal growth, rainy season, ambient temperature, food availability, southern hemisphere

Introduction

Reproduction is costly (Partridge and Harvey, 1985) and therefore, most mammals breed only during a specific time of the year when environmental conditions are favourable. However, many small mammals are opportunistic and may breed during harsh

conditions as long as prolonged or year-round breeding offsets the costs and ultimately maximizes reproductive success (Beer and MacLeod, 1961). In the tropics, many mammals breed throughout the year because the environmental conditions are more stable than those in temperate regions. Seasonal

reproduction has, however, also been reported and often occurs in conjunction with seasonal variation in rainfall (Delany, 1971).

Many different factors may influence and shape seasonal reproduction. Photoperiod, the seasonally changing day/night cycle, is most reliable at higher latitudes and is, therefore, mostly utilized by small mammals from temperate regions as a cue to predict events distant in time (Bradshaw and Holzapfel, 2007). Photoperiod is, however, also used by some sub-tropical rodents that occur in seasonally changing environments (Muteka, Chimimba and Bennett, 2006; Medger, Chimimba and Bennett, 2012). However, rainfall in conjunction with an increase in both food quantity and quality appear to be the major factors which control seasonal reproduction at sub-tropical and tropical latitudes, especially in habitats with distinct wet and dry seasons (Tinney, Bernard and White, 2001). Ambient temperature, although less investigated, has likewise been found to influence reproduction (Sicard et al., 1993).

Many temperate species, particularly *Peromyscus* species (Trainor et al., 2006), hamsters (Hegstrom and Breedlove, 1999) and voles (Kerbeshian, Bronson and Bellis, 1994), have been traditionally used to unravel the mechanisms of seasonal reproduction. On the other hand, the reproductive strategies of many tropical and sub-tropical mammals are still largely unknown (Bronson, 2009). Moreover, only few studies have been conducted on non-murid small mammals such as shrews, bats and the unique African elephant-shrews.

The elephant-shrews (family Macroscelidea) belong to the super-cohort Afrotheria, which also comprises aardvarks, golden moles, elephants, sirenians and hyraxes (Skinner and Chimimba, 2005). Elephant-shrews, also often referred to as sengis, have male and female reproductive systems that are distinct from those of other mammals (van der Horst and Gillman, 1941; van der Horst, 1951; Tripp,

1971; Woodall, 1995). Most elephant-shrew species investigated to date breed throughout the year although three species, the eastern rock elephant-shrew (*Elephantulus myurus*), the North African elephant-shrew (*E. rozeti*) and the bushveld elephant-shrew (*E. intufi*) have been found to breed seasonally (Neal, 1995). *Elephantulus myurus* occurs in Zimbabwe, Botswana, Mozambique and South Africa and appears to be the only *Elephantulus* species from south of the equator that displays a distinct breeding season throughout most of its range (van der Horst, 1946, 1954; Stoch, 1954; Woodall and Skinner, 1989). Although the breeding pattern of *E. myurus* has previously been investigated (van der Horst, 1946, 1954; Stoch, 1954; Woodall and Skinner, 1989), it is still unknown which factors govern seasonal reproduction in this elephant-shrew. This is further complicated as previous studies either pooled data from different localities and months or did not investigate reproduction over an entire calendar year. The current study, therefore, investigates seasonality of reproduction of *E. myurus* on a finer scale and over an entire year. In addition, we aimed to characterize environmental factors which may shape seasonal reproduction in *E. myurus* by recording ambient temperature and rainfall. In doing so, the present study represents the first attempt to understand the ecological significance and the evolution of seasonal reproduction in this elephant-shrew.

Materials and Methods

General

The study was carried out at Goro Game Reserve, Limpopo Province, South Africa (22°58'S, 22°57'S; 29°25'E, 29°24'E). Elephant-shrews were trapped in rocky outcrops with Sherman live traps (H. B. Sherman Traps, Inc. Tallahassee, Florida, U.S.A.) baited with a mixture of oats, peanut butter and fish at the end of every month from September 2007 until August 2008. A total of 59 females (five each month except September six and November three) and 57 males (five each month except September four and November three) were

captured over the entire study period. On the day of capture, all animals were weighed to the nearest 0.1 g using a digital balance (Scout Pro SPU123, Ohaus Corporation, Pine Brook, New Jersey, U.S.A.). During transportation and in the laboratory, animals were housed in polyurethane cages containing wood shavings and fed with Pronutro (high protein cereal; Pioneer Foods Ltd., Bokomo Foods, Cape Town, South Africa), canned dog food (Promeal Ltd., Dassenberg, South Africa) and grated apples or carrots. Fresh food and water were provided daily. Animals were collected under permit (permit number: CPM-333-00002) from the CITES and Permit Management Office, Department of Environmental Affairs, Limpopo Province. This study was approved by the animal ethics committee of the University of Pretoria (Ethics clearance number: EC028-07).

Monthly rainfall (mm) and ambient temperature (°C) were recorded throughout the study period. Rainfall data were provided by the Goro Game Reserve and two iButton digital temperature data loggers (Maxim Integrated Products, Dallas Semiconductor, U.S.A.) were used to measure ambient temperature to the nearest 0.01 °C. The iButtons were placed near the ground and protected from direct sunlight and measurements were taken every 2 h, from which the mean monthly ambient temperature was calculated.

All animals were euthanized with an overdose of halothane. The gonads of both males and females were dissected out and blood was obtained by exsanguinations from the heart. The blood was centrifuged at 500 *g* for 15 min and the blood plasma was stored at -35 °C until hormone analysis. The entire female reproductive tract was removed and any embryos or placental scars in the uterine horns were recorded. Females were also examined for milk in the mammary glands, indicating lactation. The gonads were fixed in Bouin's fluid for approximately 20 h and then stored in 70 % ethanol. Skulls were prepared using standard museum procedures for small mammals

(DeBlase and Martin, 2001) and were used to determine the relative age of an individual using the degree of maxillary molar tooth-wear and eruption (see "Relative age classes" section below).

Histology

After the removal of fat and connecting tissue, fixed ovaries and testes were weighed to the nearest 0.0001 g using a high precision scale (Ohaus Corp. Pine Brook, N.Y., U.S.A.) and the length and width were measured (mm) using a pair of digital callipers (SylvacOpto RS 232, Ultra Praezision Messzeuge GmbH, Germany). Gonadal length and width were used to calculate ovarian and testicular volume (mm³) by using the formula for the volume of an ellipsoid as follows: $V = 4/3 \pi ab^2$ where *a* represents half the maximum length and *b* half the maximum width (Woodall and Skinner, 1989). The gonads were dehydrated and embedded in wax and then cut into 7 µm thick sections with a rotary microtome (820 Spencer, American Optical, Scientific Instrument Division, Buffalo, N.Y., U.S.A.). While only a few sections were cut from the testes, each ovary was sectioned in its entirety. Sections were mounted on microscope slides and stained with Ehrlich's haematoxylin and counter-stained with eosin (Drury and Wallington, 1967). Round seminiferous tubules were photographed at a magnification of ×10 using a Moticam 1000 1.3 M Pixel USB 2.0 digital camera (Motic China Group, LTD., Xiamen, China) attached to a light microscope (Diaplan, Ernst Leitz Wetzlar GmbH, Germany). The programme Motic Images Plus 2.0ML (Motic China Group, LTD., Xiamen, China) was subsequently used to measure the diameter (µm) of 100 seminiferous tubules.

The ovarian sections were examined serially for stages of follicular growth with a light microscope (Vickers Instruments, U.K.) at magnifications of ×100, ×200 and ×400. Follicular stages, namely primordial, primary, secondary, tertiary and Graafian follicles as well as corpora hemorrhagica, corpora albicantia and corpora lutea, were identified and classified according to Bloom and Fawcett (1964). Primordial follicles

were counted in every 10th section only. For all other follicular stages, the total number was counted throughout each ovary. Numbers of corpora lutea, corpora hemorrhagica and corpora albicantia were combined for analyses (hereafter referred to as corpora bodies) to accommodate for small sample sizes.

Hormone analysis

Coat-a-count hormone kits (Siemens Medical Solutions Diagnostics, Los Angeles, U.S.A.) were used to measure progesterone and testosterone concentrations in the plasma of females and males, respectively. All assays were carried out according to the manufacturer's protocol. The slopes of a serial dilution curve, which was generated by serially diluting a sample from an individual with high hormone concentrations (i.e., pregnant female), and the calibration curve were tested for parallelism with a General Linear Model (GLM) after log-logit-transformation (Chard, 1978) to validate this method for *E. myurus*. The dilution percentages were used as covariates and the type of curve was employed as a random factor.

Plasma progesterone concentrations (ng/mL) were evaluated in 58 female *E. myurus* (December: $n = 4$, see above for n of all other months) throughout the 12-month period. There was no significant difference between the serial dilution curve of plasma progesterone and the calibration curve ($F_{1,5} = 0.01$; $n = 4$; $P = 0.93$), validating the assay for females. The intra-assay coefficient of variation for repeated determination of quality control was 11.2 % and the sensitivity of the assay was 0.11 ng/mL.

Plasma testosterone concentration (ng/dL) was determined for 56 male *E. myurus*, because no plasma could be obtained from one male from September ($n = 3$). The assay was validated for males as there was no significant difference between the serial dilution curve of plasma testosterone and the calibration curve ($F_{1,3} = 0.23$; $n = 3$; $P = 0.66$). The sensitivity of the assay was 40.09 ng/dL and the intra-assay

coefficients of variation were 3.6 % and 3.5 % and the inter-assay coefficient was 3.1 %.

Relative age classes

In order to be able to distinguish between young and old animals, all males and females were categorized into six relative age classes using tooth eruption patterns and the degree of maxillary tooth wear. For a description of how these six relative age classes were characterized, refer to the supplementary material.

Elephant-shrews reach their adult body size and sexual maturity before their permanent cheek teeth have erupted (Asher and Olbricht, 2009) and it is, therefore, very difficult to determine their reproductive status based on dentition. We performed a principal component analysis (PCA; see supplementary material) to determine if any of the relative age classes should be removed from the analysis due to their uncertain reproductive status. We therefore excluded relative age class (1) ($n = 1$ for each sex) from all analyses of body mass and reproductive variables of females and males (see supplementary material).

Data analysis

The number of individuals per relative age class was compared across the 12 months using a Chi-square test. All dependent variables were tested for normality and homogeneity of variances and were log-transformed where applicable. A Generalized Linear Model (GZLM) with a linear distribution was applied to assess differences in body mass across the 12 months, the two relative age classes and the sexes (factors). The means of the different variables, which were obtained twice per male or female (e.g., testicular and ovarian mass and volume and number per follicular stage), were calculated per individual.

Due to small sample sizes, all reproductive parameters of both males and females were analysed using GZLMs. For all GZLMs, months and relative age classes were used as factors while body mass was applied as a

covariate. If the dependent variable was found to be normally distributed, a linear distribution was used, whereas a gamma distribution with a log-link function was utilized if the variable was not normally distributed. Post-hoc least significant difference (LSD) tests followed all GZLMs. Akaike's Information Criterion (AIC) was used to verify the fit of the models for all GZLMs. The model with the smallest AIC and a $\Delta AIC > 2$ was selected as the best fitting (Burnham and Anderson, 2002). If a dependent variable was found to be significantly different between months, it was also investigated for a relationship with ambient temperature and rainfall by either a GLM or GZLM depending on the distribution of the data. Ambient temperature, rainfall and body mass were applied as covariates for these analyses. All statistical analyses were performed using the *Statistical Package for the Social Sciences (SPSS)* version 19.0 (SPSS Inc., Chicago, USA). The results are presented as mean \pm 1 standard error (SE) and were found to be significant at $P \leq 0.05$.

Results

Relative age classes

There was a significant difference in the numbers of individuals caught per relative age class across the 12 months ($\chi^2 = 121.74$; $df = 55$; $n = 116$; $P < 0.001$). Animals of relative age class (5) and to some extent age class (6) were caught throughout the year and in September and October only animals of these two age classes were collected (Figure 1). Animals of the relative age classes (1) to (4) were collected only during a few months of the year (Figure 1). Elephant-shrews of relative age class (4) were caught from February until August, individuals of relative age class (3) were caught from December until March and a few individuals of relative age class (2) were sampled in November, December and February (Figure 1). Only one male of relative age class (1) was collected in January and one female was collected in March (Figure 1). Very small and young animals were, however, caught but released again in October and December.

Small sample sizes necessitated the grouping of the age classes for subsequent analyses. According to the distribution of the age classes throughout the year, we concluded that relative age classes (5) and (6) were mature animals (hereafter referred to as relatively old individuals) and we combined these two age classes for further analyses. Similarly, relative age classes (2) to (4) were combined (hereafter referred to as relatively young individuals) as these individuals were most likely younger than one year. Relative age class (1) was removed from further analyses (see supplementary material).

Body mass

Elephantulus myurus did not show a sexual dimorphism in body mass (female: 51.9 ± 0.8 g ($n = 58$); male: 51.1 ± 0.7 g ($n = 56$); Wald $\chi^2 = 2.06$; $df = 1$; $P = 0.15$). There was also no difference in body mass between relatively young and old individuals (relatively young: 50.6 ± 0.8 g ($n = 41$); relatively old: 52.0 ± 0.6 g ($n = 73$); Wald $\chi^2 = 2.13$; $df = 1$; $P = 0.15$) and no interaction between months and sexes (Wald $\chi^2 = 12.82$; $df = 11$; $P = 0.31$) and between sexes and relative age classes (Wald $\chi^2 = 0.50$; $df = 1$; $P = 0.48$). In contrast, body mass was significantly different across the 12 months ($n = 114$; Wald $\chi^2 = 39.94$; $df = 11$; $P < 0.001$). There was also a significant difference in body mass between relatively young and old individuals throughout the 12-month period (Wald $\chi^2 = 17.17$; $df = 9$; $P < 0.05$) and a significant interaction between month, sex and relative age classes (Wald $\chi^2 = 21.06$; $df = 5$; $P = 0.001$). Body mass was lowest during July, but it increased subsequently, being significantly higher during September (LSD; $P < 0.02$). Elephant-shrews were heaviest from October until January (Figure 2). During November and December, when new relatively young individuals enter the population, they were found to be significantly lighter than relatively old individuals (LSD; $P \leq 0.02$; Figure 2). In addition, relatively young males were significantly lighter than relatively young females during November, December and January (LSD; $P < 0.04$) but this effect was not apparent when

pregnant females were excluded from analyses (LSD; $P > 0.12$).

Males

Testicular volume and mass showed a significant positive increase with increasing body mass (Table 1). Plasma testosterone concentration and seminiferous tubule diameter were not dependent on body mass (Table 1). Testicular volume and mass, seminiferous tubule diameter as well as plasma testosterone concentration were significantly different across the 12 months (Table 1). Volume and mass of the testes were lowest in April and increased significantly during May (LSD; $P = 0.02$), reaching their peak in August and decreasing significantly from October to December (LSD; $P < 0.01$) and January to March (LSD; $P < 0.001$; Figure 3A). Seminiferous tubule diameter was smallest in March and increased significantly from April to May (LSD; $P < 0.001$). It peaked in September and October and decreasing significantly towards November/December (LSD; $P < 0.001$) and January to February (LSD; $P = 0.04$; Figure 4). Although only very low levels of plasma testosterone were recorded overall, testosterone concentration was lowest from November/December until April and significantly increased between April and June (LSD; $P = 0.04$; Figure 3B). Plasma testosterone concentration was highest in July and August and declined significantly from October to November (LSD; $P < 0.04$; Figure 3B).

Relatively old males ($n = 36$) had significantly larger testicular mass (75.8 ± 3.4 g) and volume (62.8 ± 2.9 mm³) than those of relatively young males (mass: 44.2 ± 2.5 g; volume: 36.0 ± 2.2 mm³; $n = 20$; Table 1). In contrast, there was no significant difference in seminiferous tubule diameter and plasma testosterone concentration between relatively young and old males (Table 1). In addition, there was a significant interaction between months and relative age classes for seminiferous tubule diameter (Table 1), being significantly smaller in relatively young compared to relatively old males during November (LSD; $P = 0.03$; Figure 4).

We did not find a significant interaction between months and relative age classes for any of the other recorded parameters for males and the interactions were excluded from the models because ΔAIC was ≥ 6.6 .

Females

All pregnant females ($n = 17$) had two embryos each and those with placental scars ($n = 14$) had mostly two placental scars with the exception of one female that had four. Pregnant females were collected in every month from August until January. Females with placental scars were found throughout the year (Figure 5). Lactating females ($n = 7$) were caught in September and December through to March (Figure 5).

Ovarian volume and mass showed a significant positive increase with body mass, although plasma progesterone concentration was not related to body mass (Table 2). Ovarian mass and volume as well as plasma progesterone concentration were significantly different across the 12 months, but not between relatively young and old females (Table 2). None of these variables were significantly different between the relative age classes in any month (Table 2) and $\Delta AIC \geq 11.2$, warranting removal of the interactions from the models. The lowest ovarian mass and volume were recorded in April followed by a significant increase from June to July (LSD; $P < 0.02$; Figure 6A). Ovarian mass and volume reached their maximum in October (Figure 6A). Both measurements decreased thereafter and were significantly smaller in November compared to October (LSD; $P < 0.05$; Figure 6A). A significant rise in plasma progesterone concentration was observed from July to August (LSD; $P = 0.001$) and August to October (LSD; $P = 0.04$; Figure 6B). After a peak in plasma progesterone concentration in October, it decreased significantly towards November (LSD; $P = 0.003$) and from November to December (LSD; $P = 0.04$; Figure 6B). Plasma progesterone concentration was lowest from December until July (Figure 6B).

The number of corpora bodies increased significantly with increasing body mass (Table 2). None of the follicle types was affected by body mass (Table 2). Except for the number of primordial follicles, which did not differ across the 12 months, all other follicle types and corpora bodies were significantly different (Table 2). However, primordial follicle numbers were significantly different between the relative age classes (Table 2) with the ovaries of relatively young females containing more primordial follicles (4133.5 ± 426.2 ; $n = 21$) than those of relatively old females (2438.5 ± 303.4 ; $n = 37$). None of the other follicle types was significantly different between the relative age classes (Table 2), but relatively old females (28.4 ± 4.4 ; $n = 21$) had significantly more corpora bodies than relatively young females (3.7 ± 1.8 ; $n = 37$; Table 2). In addition, there was no significant interaction between months and relative age classes for primordial, primary and Graafian follicles (Table 2) and the interaction was excluded from these models because of a $\Delta AIC \geq 7.4$. Secondary and tertiary follicles as well as corpora bodies showed an interaction between months and relative age classes (Table 2).

Monthly comparisons showed that most primary follicles were found in May and from then on the number of these follicles decreased progressively towards December, being significantly different between May and December (LSD; $P = 0.005$; Table 3). Primary follicle numbers were similar from December and January to April the following year and significantly different from May (LSD; $P \leq 0.009$; Table 3). The number of secondary, tertiary and Graafian follicles reached their maximum one month later than the previous follicular stage, with the number of secondary follicles peaking in July, tertiary follicle numbers peaking in August and Graafian follicles being highest in September (Table 3). In comparison to July, secondary follicle numbers were significantly reduced in December and March/April (LSD; $P \leq 0.01$) with their lowest number in April (Table 3). At the peak of secondary follicle numbers in July,

we observed significantly more secondary follicles in relatively old than in relatively young females (LSD; $P = 0.02$). The number of tertiary follicles was significantly higher from August until October in comparison to February until May (LSD; $P \leq 0.05$) when it was lowest (Table 3). There were no Graafian follicles present from February until July and the highest Graafian follicle numbers were recorded in August and in September/October of the previous year, which were significantly different to the numbers found in December (LSD; $P \leq 0.05$; Table 3). No corpora bodies were found from March until July. The number of corpora bodies started to increase significantly from July to August (LSD; $P < 0.001$) and September to October (LSD; $P = 0.03$), were highest in November and then decreased significantly towards December (LSD; $P = 0.01$) and from December to January (LSD; $P < 0.001$; Table 3). When they start to enter the population during December, January and February, relatively young females had significantly fewer corpora bodies than relatively old females (LSD; $P < 0.03$), suggesting possible reduced reproductive capacity in these younger female elephant-shrews during this period.

Weather data

The rainy season in the study area began in September and lasted until April with December having the highest rainfall (146 mm). From May until August no rain was recorded with these months spanning the dry season. Mean monthly ambient temperatures $> 20^\circ\text{C}$ were recorded from September to March with February being the warmest month ($23.5 \pm 0.2^\circ\text{C}$). The coldest period of the year was from April until August with temperatures being $< 20^\circ\text{C}$ and July was the coldest month ($16.2 \pm 0.2^\circ\text{C}$). To allow a comparison between weather and reproductive data, an insert showing monthly rainfall and ambient temperature is included in Figures 3 and 6.

Body mass was not significantly affected by either ambient temperature or rainfall ($F_{1,111} \leq 1.15$; $P \geq 0.29$). A decrease in ambient temperature was related to an increase in

testicular volume and mass as well as seminiferous tubule diameter ($F_{1,52} \geq 10.47$; $P \leq 0.002$). Interestingly however, there was a positive relationship between these variables and rainfall ($F_{1,52} \geq 5.94$; $P < 0.02$). Plasma testosterone concentration was negatively affected by both ambient temperature and rainfall (Wald $\chi^2 \geq 8.81$; $df = 1$; $P \leq 0.003$).

Surprisingly, in comparison to males, reproduction of females seemed to be less affected by ambient temperature and rainfall. There was no significant relationship of ambient temperature and rainfall with ovarian volume ($F_{1,54} \leq 0.05$; $P \geq 0.31$) and mass (Wald $\chi^2 \leq 0.71$; $df = 1$; $P \geq 0.40$) as well as plasma progesterone concentration (Wald $\chi^2 \leq 0.20$; $df = 1$; $P \geq 0.66$). In addition, none of the follicle types or the corpora bodies were significantly affected by rainfall (secondary follicles: $F_{1,54} = 0.19$; $P = 0.67$; others: Wald $\chi^2 \leq 2.27$; $df = 1$; $P \geq 0.13$). The number of primary and tertiary follicles decreased with increasing ambient temperature (Wald $\chi^2 \leq 0.20$; $df = 1$; $P \geq 0.66$). There was, however, no significant relationship between ambient temperature and secondary ($F_{1,54} = 1.73$; $P = 0.19$) and Graafian follicles (Wald $\chi^2 = 0.17$; $df = 1$; $P = 0.68$) or corpora bodies (Wald $\chi^2 = 0.24$; $df = 1$; $P = 0.62$).

Discussion

The present study confirmed that *E. myurus* is a seasonal breeder with breeding being predominantly confined to the warm and wet spring and summer months in South Africa. The breeding season was shown to begin in August and to end in January, the time during which pregnant females were found. Lactating females were, however, collected up until March. The significant increase in ovarian size and progesterone concentrations from June to August indicates that the reproductive system altered drastically during this period. In addition, the occurrence of corpora lutea at the end of August, but not in July, indicates that ovulation and fertilization must have occurred at the beginning of August rather than in July. In extensive histological studies, van der Horst

(1954) similarly observed that *E. myurus* comes out of anoestrus in July. We found that reproductive activity in females was very high from August until October and ovarian size and progesterone concentrations were highest in October, whereafter both declined rapidly. The end of the breeding season was reached at the summer/autumn interface, which was marked by lower progesterone concentrations and lower numbers of tertiary and Graafian follicles as well as corpora bodies in February and March compared to the previous months. A number of studies have established a longer breeding season for *E. myurus* and pregnant females were collected until March (van der Horst, 1954 - South Africa; Woodall and Skinner, 1989 - South Africa and Botswana) and even April (Smithers, 1971 - Botswana).

Males started to prepare for the breeding season several months prior to females. Testes size and seminiferous tubule diameters increased significantly in May, two months prior to females emerging from anoestrus. The peak breeding season for males was found to be from August to October, after which the size of the testes, seminiferous tubule diameter and testosterone concentration dropped significantly. Woodall and Skinner (1989) found a similar pattern for testes volume and seminiferous tubule diameter in *E. myurus*, although they established that the peak in male breeding season occurred two months later than in the present study. In addition, in an earlier study, no reduction in testes size and seminiferous tubule diameter was encountered, while the size and activity of the accessory glands varied with the seasons (Stoch, 1954). However, both studies combined data from different months, which may have led to different results.

Moreover, the distribution of young and old *E. myurus* across the year supports the results of the reproductive parameters and emphasises the notion that this species breeds seasonally. While relatively old animals (relative age class (5)) were caught throughout the year,

an influx of new and very young animals was observed from October until March. These very small animals, which appeared to have just been weaned, were caught in October, but during our trapping regime were released again and it is probable that they would have been placed in an age class prior to relative age class (1). It is likely that the first birth occurred in September as this is also the month when lactating females were found for the first time. Since the young are precocial and probably released early into the population, this may explain the occurrence of relatively large numbers of very young individuals in October. It is interesting that the captured individuals become progressively "older" from October onwards as animals of relative age class (2) were caught initially in November and those of relative age classes (3) and (4) were collected in December and February, respectively. Moreover, at the beginning of the breeding season, the population is relatively old and it is possible that not many of these animals survive until the end of the season. Individuals of relative age class (6) possessed completely worn teeth, which would make feeding difficult and might provide an explanation for the disappearance of these older animals from the population from April until June. The combined evidence suggests that the population of *E. myurus* turns over about once a year and most individuals may only breed during one breeding season. Some young individuals were, however, already pregnant or showed signs of previous pregnancies during December and January and it is possible that these may breed during a second season (see also van der Horst, 1954).

Apart from *E. myurus*, only two other elephant-shrew species have been reported to breed seasonally: *E. rozeti*, the only elephant-shrew species that occurs in northern Africa (Séguignes, 1989), and *E. intufi* from southern Africa (Skinner and Chimimba, 2005). *Elephantulus rozeti* exhibits a distinct breeding season from January until August and thus largely during the spring and summer months of the northern hemisphere (Séguignes, 1989).

Although *E. intufi* appears to breed seasonally in southern Africa, it has been suggested that this species breeds year-round in Namibia (reviewed in Skinner and Chimimba, 2005). It is therefore possible that *E. myurus* and *E. rozeti* are the only elephant-shrews which breed seasonally throughout their range. In contrast, most other elephant-shrews such as the rufous elephant-shrew (*E. rufescens*) from Kenya, the short-snouted elephant-shrew (*E. brachyrhynchus*) from Zimbabwe and Tanzania and the round-eared elephant-shrew (*Macroscelides proboscideus*) from South Africa, breed throughout the year although all species occur in seasonal habitats (Neal, 1982; Leirs et al., 1995; Neal, 1995; Bernard et al., 1996). Nonetheless, *E. brachyrhynchus* and *M. proboscideus* display a reduction in pregnancies during the southern African winter (Neal, 1995; Bernard et al., 1996). Because of the non-overlapping southern and northern latitude distributions of *E. myurus* and *E. rozeti*, respectively, these species provide a unique opportunity to investigate the environmental factors that lead to seasonal reproduction, which may also allow further insights into the evolution of seasonal reproduction in these unique African mammals.

Neal (1995) suggested that reproduction in *Elephantulus* shifts from seasonal at high latitudes towards aseasonality at lower latitudes. This has been frequently observed in mammals (Bronson, 1985) and possibly explains the extended breeding season of *E. myurus* found in Botswana (Smithers, 1971; Woodall and Skinner, 1989) and the aseasonal reproduction of *E. rufescens* (Neal, 1982), *E. intufi* and *E. brachyrhynchus* closer to the equator (Leirs et al., 1995; Neal, 1995; Skinner and Chimimba, 2005). However, factors that affect the timing of the breeding season in *E. myurus* are unknown, although some conclusions can be drawn from studies on other species of elephant-shrew. Neal (1995) suggested that the increase in insect abundance during the warm and wet period might be a possible reason for seasonal reproduction in *Elephantulus*. Although body mass was found to be relatively stable during

the year, we observed a significant decrease in body mass of both males and females just prior to the beginning of the breeding season in August, which is consistent with the importance of food availability during reproduction of *E. myurus*. As food availability is probably drastically reduced at the end of the dry and cold southern winter, elephant-shrews may not be able to offset the high energetic demands (gonadal growth and courtship) at the beginning of the breeding season by increasing food intake.

Food availability and quality, the main factors that drive seasonal reproduction in tropical as well as southern African mammals (Delany, 1972; Perrin, 1986), are most likely also influencing seasonal reproduction in *E. myurus*. Although plant growth and insect abundance are dependent on rainfall (Wolda, 1978), the direct effects of rainfall and ambient temperature on the reproduction of *E. myurus* are ambiguous. Rainfall was correlated with an increase in testes size and testosterone concentrations and may therefore affect reproduction of male *E. myurus*. However, an effect of rainfall on female reproduction could not be detected. In addition, ambient temperature did not affect female reproductive organs, but appeared to have a negative effect on the testes. As male *E. myurus* begin reproductive growth in winter, much earlier than females, a decrease in temperature may well trigger reproductive onset in males.

Both males and females initiate breeding before the start of the rainy season in September and females are pregnant already one month before the first rainfall. Consequently, time of birth and weaning of the young coincides with the rainy season. The greater food availability during the rainy season may be crucial for the female to endure the high energetic demands of lactation and may also facilitate the survival of the independent young. Pregnancy is less dependent on food availability compared with lactation, which is more energetically demanding (Speakman, 2008). Cumming and Bernard (1997) suggested that

similar mechanisms explain the timing of birth in insect-eating bats (Microchiroptera), which occurs commonly one month before peak rainfall. Furthermore, early pregnancies may enable the young, which were born early in the breeding season, to breed during the season of their birth. This early reproduction may be essential for the survival of an animal with a short life span such as *E. myurus*.

Although the end of the breeding season of *E. myurus* varies by a number of months between studies, there appears to be less variation in the onset of breeding. This suggests that there might be one or more factors that regulate the initiation of the reproductive season in this species. As rainfall and ambient temperature, especially in females, are unlikely candidates, other factors may control reproduction of *E. myurus*. Bernard *et al.* (1996) suggested that the higher efficiency of calcium assimilation from fresh green plants at the beginning of the rainy season might be the main trigger for an increase in pregnancies of the omnivorous *M. proboscideus*. Plant compounds or other features in the diet may be used as cues for the onset of reproduction by *E. myurus*. Furthermore, Neal (1995) suggested that photoperiod is the most likely factor that triggers reproduction in both *E. myurus* and *E. rozeti* since both species occur in highly predictable environments and start breeding just after the winter solstice. However, a recent study suggests that *E. myurus* might not be reproductively photoresponsive (Medger, 2010).

In conclusion, *E. myurus* is a seasonal breeder, which reproduces during the warm and wet spring and summer months and is reproductively inactive during most of the cold and dry autumn and winter months of South Africa. We suggest that food quantity and quality are the main factors that drive seasonal reproduction in *E. myurus*. Although rainfall is likely affecting seasonal food availability, it does not appear to have a direct effect on the reproduction of female *E. myurus*, but both rainfall and ambient temperature may affect

male reproduction. In addition, a number of other environmental factors, such as plant compounds, may also be responsible for shaping reproduction in this elephant-shrew and may trigger the onset of reproduction in August. The habitat of *E. myurus* is ecologically challenging and seasonal reproduction may have evolved to enable this species to cope with these severe seasonal changes.

Acknowledgments

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Tables

Table 1. Generalized linear models investigating differences in testicular volume (mm³) and mass (mg), seminiferous tubule diameter (μm) and plasma testosterone concentration (ng/dL) of male eastern rock elephant-shrews (*Elephantulus myurus*) across 12 months and between relatively young and old individuals (age class). Indicated are also sample sizes (*n*) and effects of body mass and results for the interactions of month and relative age classes are shown where applicable and significant differences are indicated in bold.

	<i>n</i>	Month			Age class			Month x age class			Body mass		
		Wald χ^2	<i>df</i>	<i>P</i>	Wald χ^2	<i>df</i>	<i>P</i>	Wald χ^2	<i>df</i>	<i>P</i>	Wald χ^2	<i>df</i>	<i>P</i>
Testicular volume (mm ³)	56	118.02	11	< 0.001	6.39	1	0.01	-	-	-	13.91	1	< 0.001
Testicular mass (mg)	56	128.63	11	< 0.001	5.31	1	0.02	-	-	-	20.65	1	< 0.001
Seminiferous tubule diameter (μm)	56	223.50	11	< 0.001	0.05	1	0.82	16.62	6	0.01	0.74	1	0.39
Plasma testosterone concentration (ng/dL)	55	139.62	11	< 0.001	3.49	1	0.06	-	-	-	1.37	1	0.24

Table 2. Generalized linear models investigating differences in ovarian volume (mm³) and mass (mg), plasma progesterone concentration (ng/mL), primordial, primary, secondary, tertiary and Graafian follicle numbers as well as numbers of corpora bodies (corpora lutea, corpora hemorrhagica and corpora albicantia combined) over 12 months and between relatively young and old (age classes) female eastern rock elephant-shrews (*Elephantulus myurus*). Shown are also sample sizes (*n*), effects of body mass and interactions between month and age class where applicable and significant differences are indicated in bold.

	<i>n</i>	Month			Age class			Month x age class			Body mass		
		Wald χ^2	<i>df</i>	<i>P</i>	Wald χ^2	<i>df</i>	<i>P</i>	Wald χ^2	<i>df</i>	<i>P</i>	Wald χ^2	<i>df</i>	<i>P</i>
Ovarian volume (mm ³)	58	128.69	11	< 0.001	0.12	1	0.73	-	-	-	8.20	1	0.004
Ovarian mass (mg)	58	91.30	11	< 0.001	0.03	1	0.85	-	-	-	5.07	1	0.02
Plasma progesterone concentration (ng/mL)	57	159.36	11	< 0.001	0.05	1	0.82	-	-	-	3.44	1	0.06
Primordial follicles	58	13.30	11	0.27	8.21	1	0.004	-	-	-	0.008	1	0.93
Primary follicles	58	25.62	11	0.007	1.20	1	0.27	-	-	-	0.47	1	0.15
Secondary follicles	58	40.72	11	< 0.001	0.72	1	0.40	18.75	8	0.02	1.19	1	0.28
Tertiary follicles	58	113.31	11	< 0.001	0.01	1	0.91	30.55	8	< 0.001	2.18	1	0.14
Graafian follicles	58	97.57	11	< 0.001	0.04	1	0.85	-	-	-	0.16	1	0.69
Corpora bodies	58	7088.13	11	< 0.001	54.98	1	< 0.001	188.49	8	< 0.001	5.82	1	0.02

Table 3. Number of primordial, primary, secondary, tertiary and Graafian follicles and corpora bodies (corpora lutea, corpora hemorrhagica and corpora albicantia combined) and standard errors (*SE*) of the eastern rock elephant-shrew (*Elephantulus myurus*) from South Africa over 12 months between 2007 and 2008.

Follicle		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Primordial	Mean	2004.6	3947.8	4070.1	3425.4	4178.6	3245.3	3535.0	3506.00	1603.2	1963.5	2865.7	2187.4
	<i>SE</i>	618.8	901.8	919.8	1462.8	410.5	859.6	738.9	1230.9	483.7	518.1	1202.6.	755.3
Primary	Mean	132.1	137.2	133.0	130.8	269.7	262.0	221.9	216.3	182.2	174.4	185.7	132.2
	<i>SE</i>	20.1	12.4	28.5	35.3	29.6	33.1	25.4	39.8	37.2	52.3	49.8	23.2
Secondary	Mean	56.1	72.7	36.9	35.0	64.4	82.0	106.0	98.3	62.9	56.3	61.2	54.6
	<i>SE</i>	8.1	2.6	4.5	6.8	12.5	6.2	18.3	25.1	10.1	8.4	20.3	13.0
Tertiary	Mean	11.6	0.3	3.8	0.1	3.0	7.1	13.6	53.3	31.3	39.9	26.2	11.1
	<i>SE</i>	11.2	0.3	2.2	0.1	2.9	3.7	5.2	22.6	7.9	4.5	3.6	6.8
Graafian	Mean	2.4	0	0	0	0	0	0	13.6	20.4	16.4	4.8	0.5
	<i>SE</i>	2.4	0	0	0	0	0	0	7.0	12.9	7.8	4.3	0.5
Corpora bodies	Mean	20.6	10.1	0.3	0	0	0	0	32.0	50.7	38.8	72.8	19.3
	<i>SE</i>	11.0	10.1	0.3	0	0	0	0	5.2	7.1	2.7	8.5	7.9

Figures

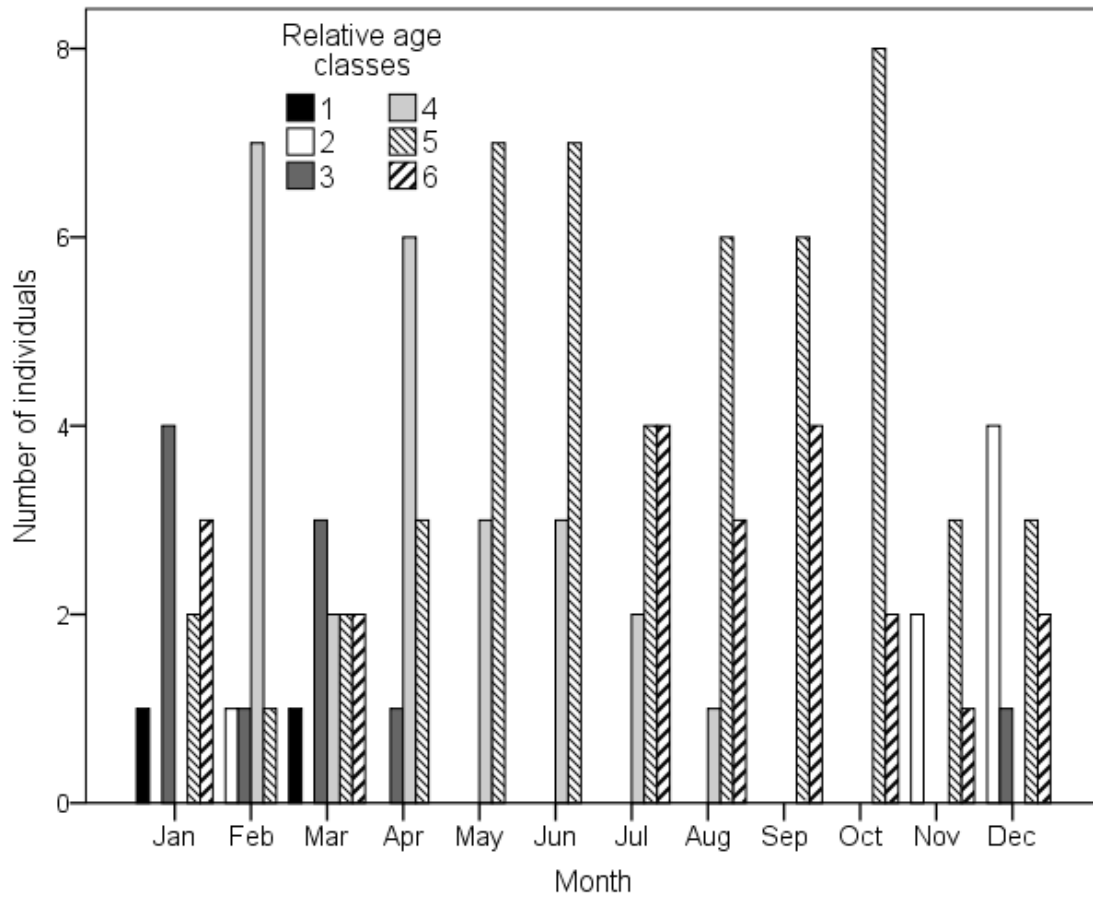


Figure 1. Sample sizes of the eastern rock elephant-shrew (*Elephantulus myurus*) from South Africa per relative age class presented separately for every month over a 12-month study period. Relative age classes are shown from (1) the relatively youngest to (6) the relatively oldest animals.

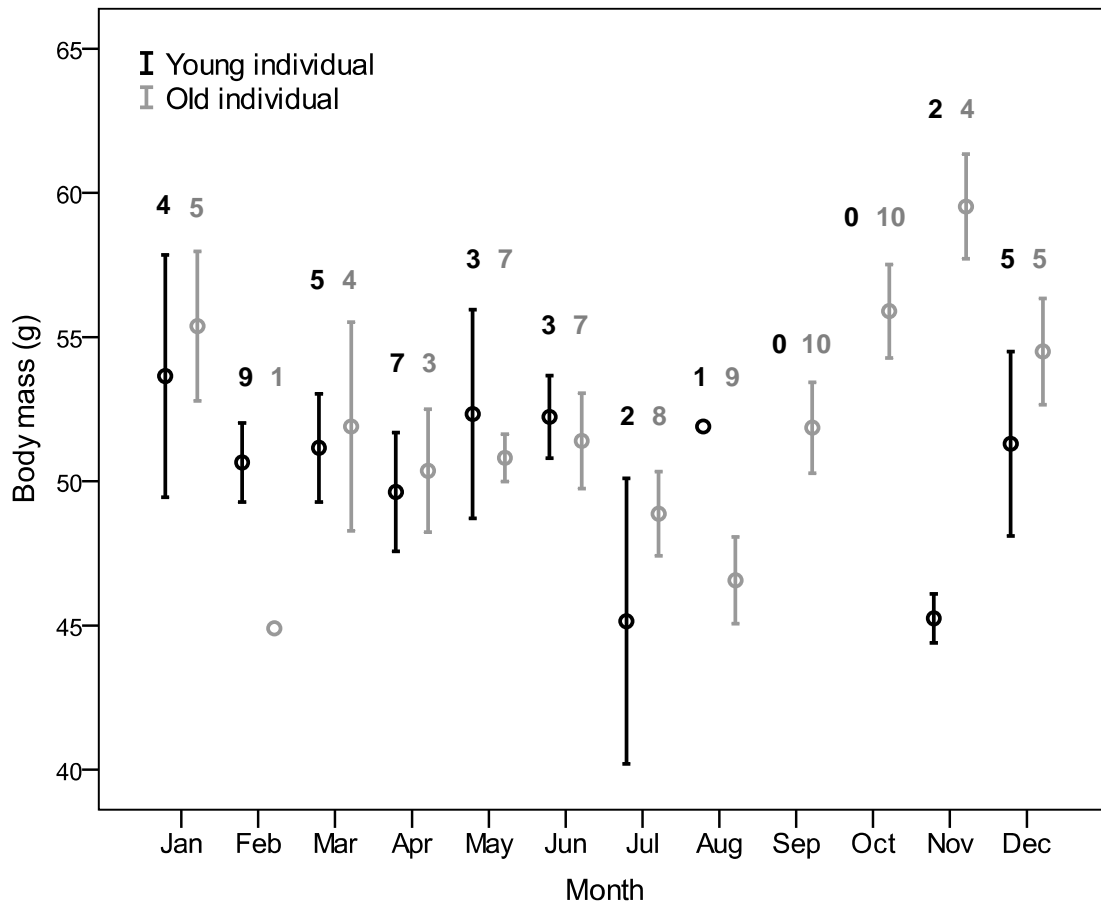


Figure 2. Monthly body mass (g) \pm standard error of relatively young and relatively old eastern rock elephant-shrews (*Elephantulus myurus*) from South Africa over a 12-months period. Monthly sample size for relatively young (black) and old individuals (grey) are indicated.

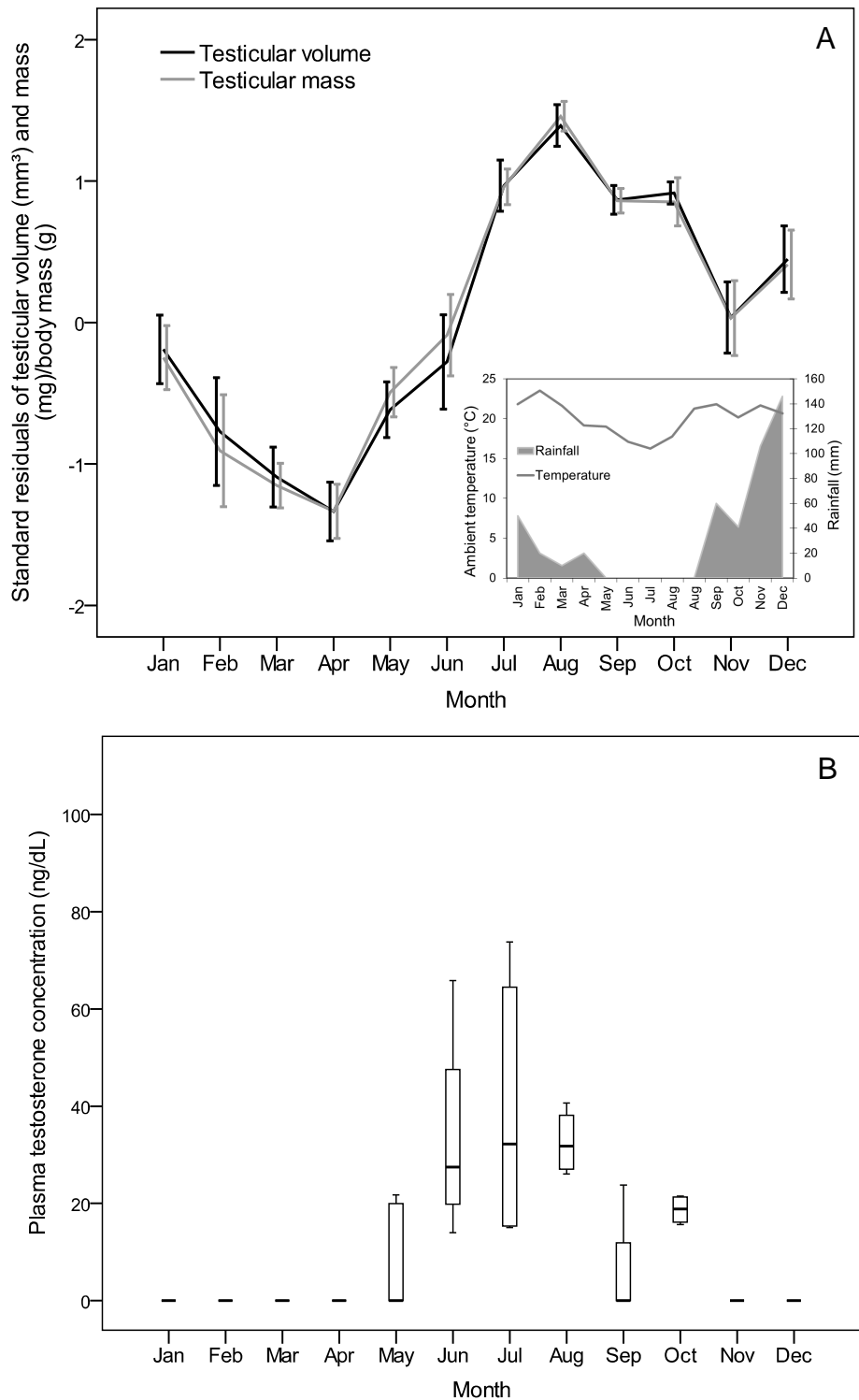


Figure 3. Mean standardized residuals of testicular volume (mm³) and mass (mg) by body mass \pm 1 standard error (A) and box-plot illustrating monthly plasma testosterone concentrations (ng/dL) (B) of male eastern rock elephant-shrews (*Elephantulus myurus*) from South Africa presented over a 12-month study period. Insert in A represents total rainfall (mm) and mean monthly ambient temperature ($^{\circ}$ C) over the same period.

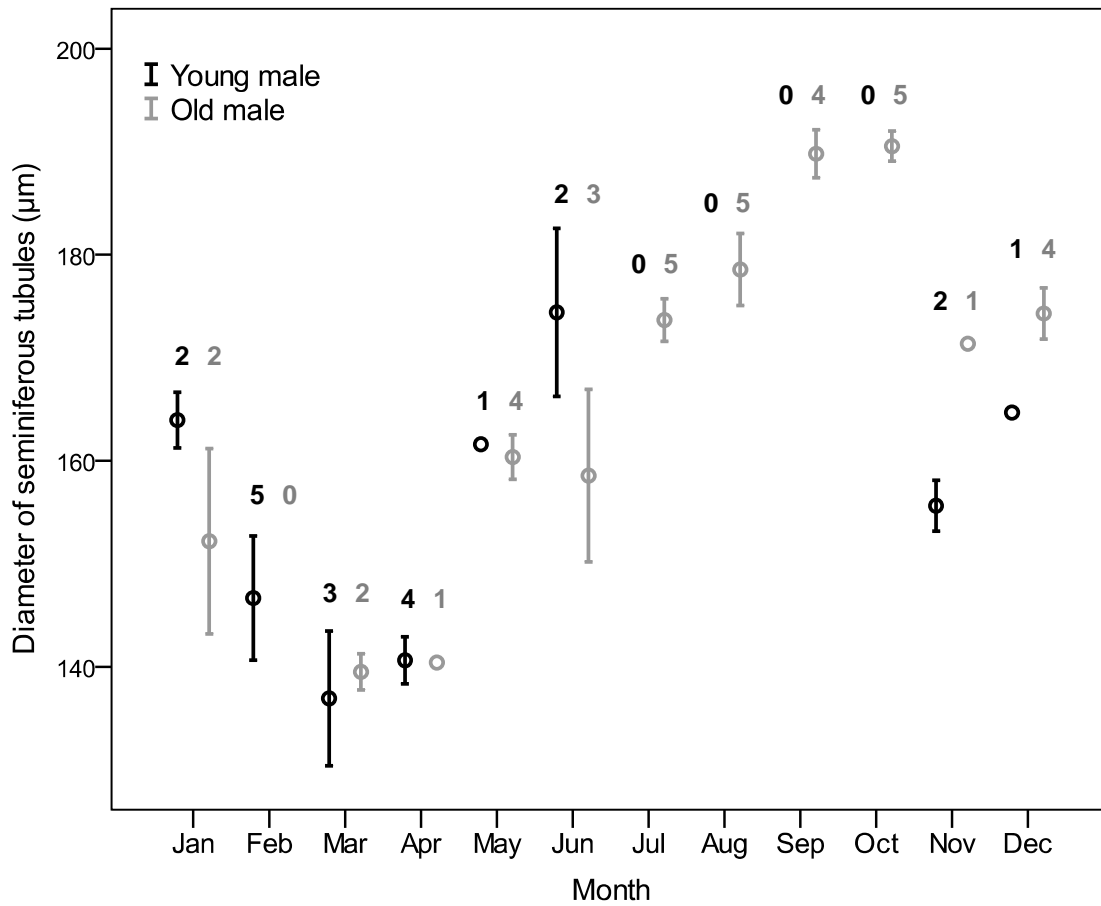


Figure 4. Seminiferous tubule diameter (μm) \pm standard error per month of relatively young and relatively old eastern rock elephant-shrews (*Elephantulus myurus*) from South Africa sampled over a 12-month period. Shown are also monthly sample sizes for relatively young (black) and relatively old males (grey).

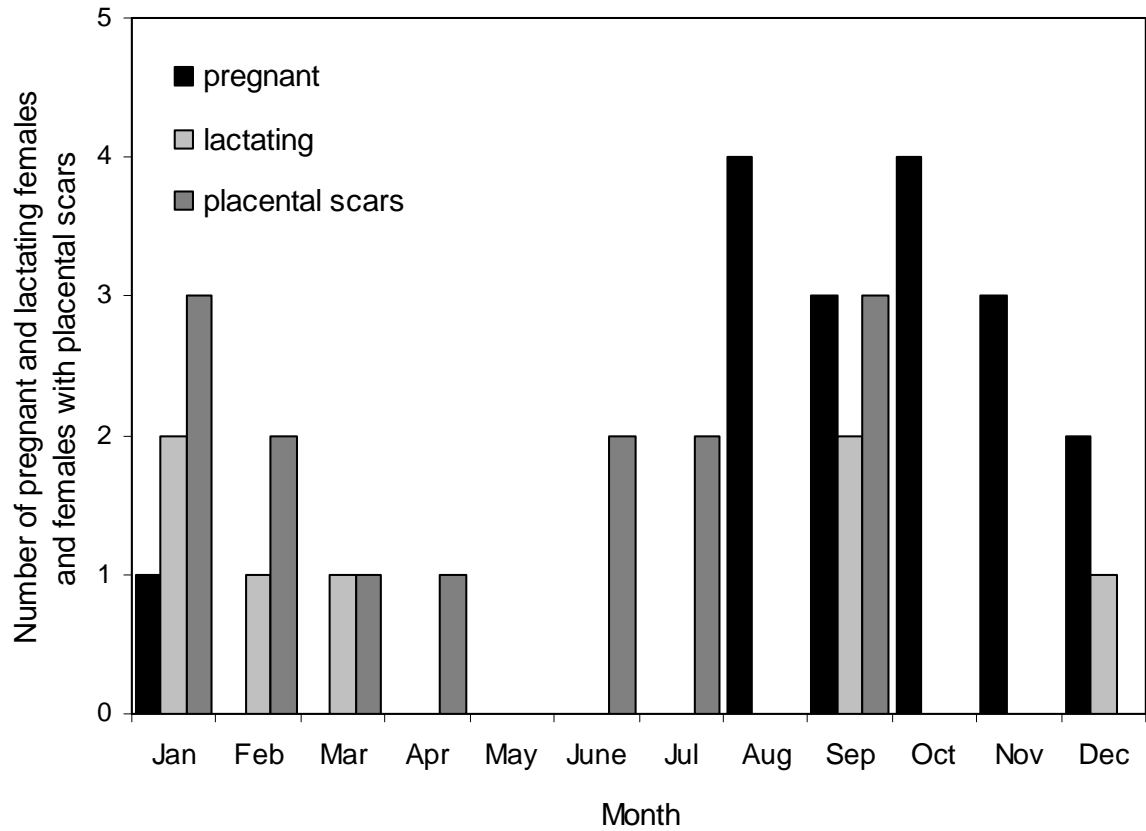


Figure 5. Number of pregnant and lactating females and females with placental scars of the eastern rock elephant-shrew (*Elephantulus myurus*) from South Africa presented over a 12-months period.

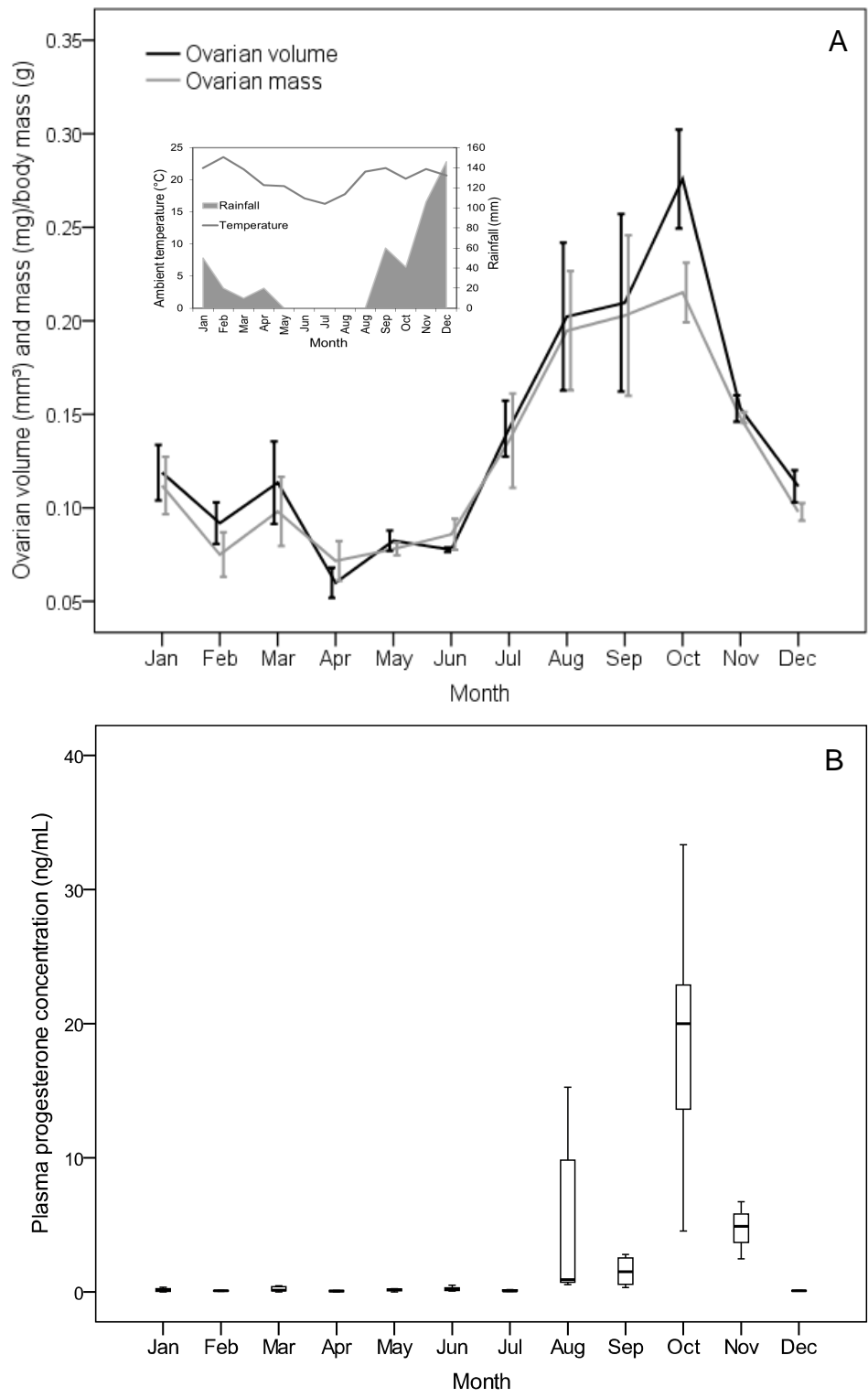


Figure 6. Ovarian volume (mm³) and mass (mg) by body mass (g) ± 1 standard error (A) and box-plot of monthly plasma progesterone concentrations (ng/mL) (B) of female eastern rock elephant-shrews (*Elephantulus myurus*) from South Africa over a 12-month study period. The insert illustrates total rainfall (mm) and mean monthly ambient temperature (°C) over the same 12 months.

Supplementary material

1. Characterization of the relative age classes

In an attempt to distinguish between young and old animals, all males and females were separated in relative age classes using the eruption patterns and the degree of maxillary tooth wear. Only the premolar and molar on the right side of the maxilla were analyzed for the degree of tooth wear and eruption. The following six relative age classes could be characterized by this method:

(1) Age class 1:

All premolars completely erupted and a cavity present where M^1 is about to appear. No signs of tooth wear and cusps are sharp with very deep grooves on the surface (Figure 1A).

(2) Age class 2:

M^1 completely erupted and a cavity, where the M^2 is about to emerge, is present. Very little sign of tooth wear evident and the cusps are pointed with deep grooves in between. Deciduous dentition is still present (Figure 1B).

(3) Age class 3:

All individuals exhibit a change from deciduous to permanent dentition. Deciduous teeth are loosely attached to the emerging permanent teeth or have fallen out, in which case, the new permanent teeth are present underneath, emerging from the cavity. All teeth are fully erupted (Figure 1C).

(4) Age class 4:

Minimal tooth wear is evident with the grooves between cusps being slightly rounded but still narrow. Premolar and molar cusps are sharp showing little signs of wear (Figure 1D).

(5) Age class 5:

Obvious premolar and molar tooth wear apparent. Grooves between cusps are obviously worn and curved out and some dentine is exposed. Cusps with some signs of wear being more rounded than sharp but still distinct (Figure 1E).

(6) Age class 6:

All teeth are severely worn and completely deformed. Teeth are entirely smooth with no discernible grooves on the surface. Only remnants of cusps on the side of the teeth are visible or the cusps are almost indistinguishable from the rest of the tooth. Large amount of dentine is present on the premolar and molar surfaces (Figure 1F).

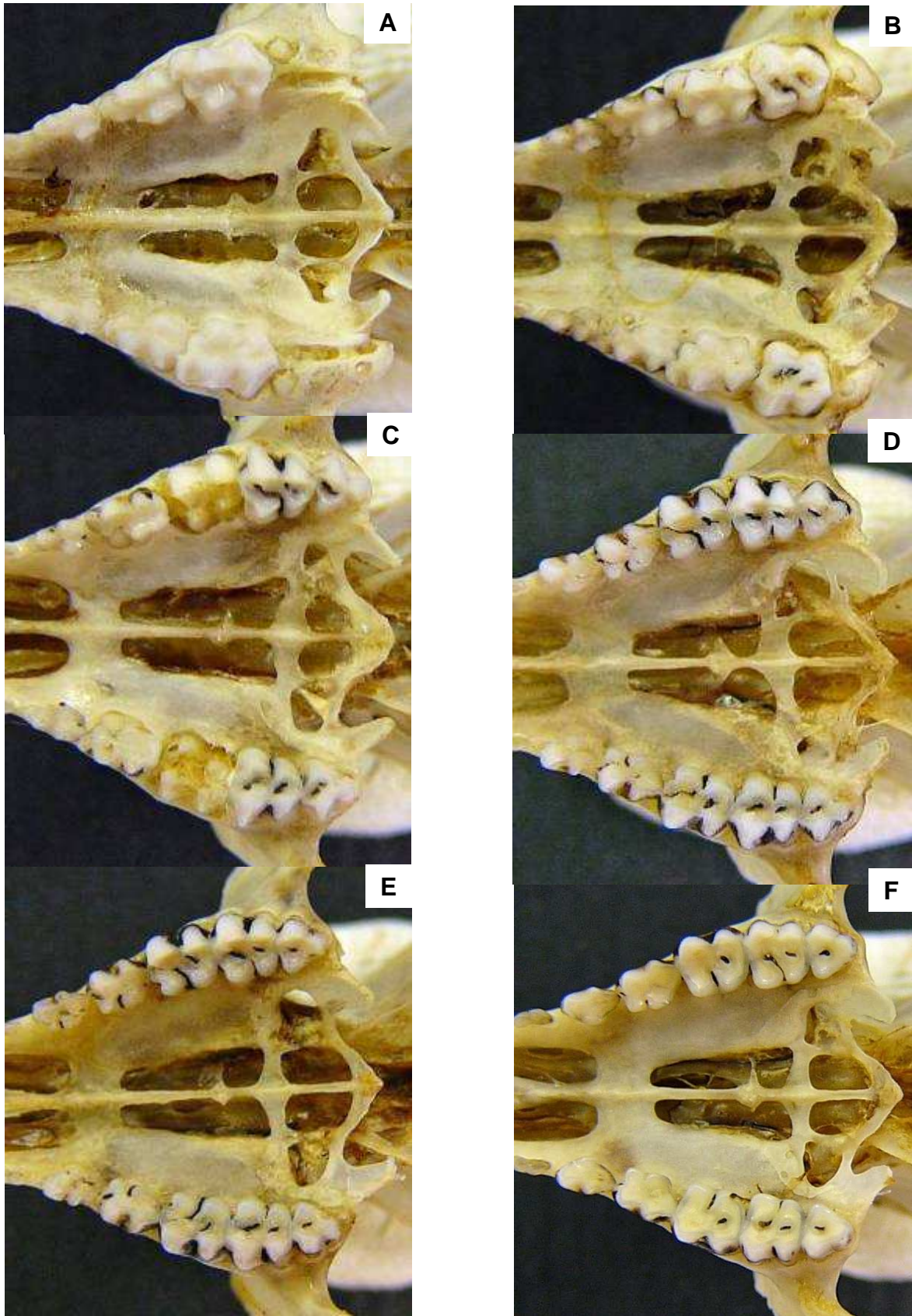


Figure 1. Relative age classes of the Eastern rock elephant-shrew (*Elephantulus myurus*) from South Africa categorized by maxillary molar tooth wear and eruption.

2. Principal component analysis

A Principal Component Analysis (PCA) on the reproductive variables and body mass of *E. myurus* was performed to determine if some of the relative age classes have to be excluded from the analysis because of their uncertain reproductive status in comparison to individuals of the other relative age classes. The PCA was performed separately for male and female *E. myurus* resulting in the analysis of five variables for males and ten for females (see Table 1). The principal component (PC) scores of the first two PC axes explained most of the variation of the total variance (Table 1). The derived scatter-plots from these two PC-axes showed that most of the six relative age classes were grouped together in both males and females. Nevertheless, relative age class (1) was separated from all other relative age classes in both sexes (Figure 2) suggesting the uncertainty of the reproductive status of this relative age class with reference to dentition. Because individuals of this relative age class may represent juveniles, they were excluded from all subsequent analyses.

Table 1. Principal component analysis (PCA) of five variables measured for male and ten variables measured for female Eastern rock elephant-shrews (*Elephantulus myurus*). % trace is presented separately for males and females.

Sex	Variable	Component	
		1	2
Males	Body mass (g)	0.35	0.91
	Testicular volume (mm ³)	0.96	-0.01
	Testicular mass (mg)	0.97	0.002
	Seminiferous tubule diameter (µm)	0.86	-0.26
	Testosterone (nmol/l)	0.35	-0.24
	% trace	57.0%	19.2%
Females	Body mass (g)	0.44	-0.58
	Ovarian mass (mg)	0.95	0.11
	Ovarian volume (mm ³)	0.96	0.10
	Progesterone (nmol/L)	0.89	0.08
	Primordial follicle	-0.29	0.74
	Primary follicle	0.18	0.81
	Secondary follicle	0.09	0.78
	Tertiary follicle	0.74	0.07
	Graafian follicle	0.76	0.14
	Corpora bodies	0.71	-0.27
% trace	45.7%	22.8%	

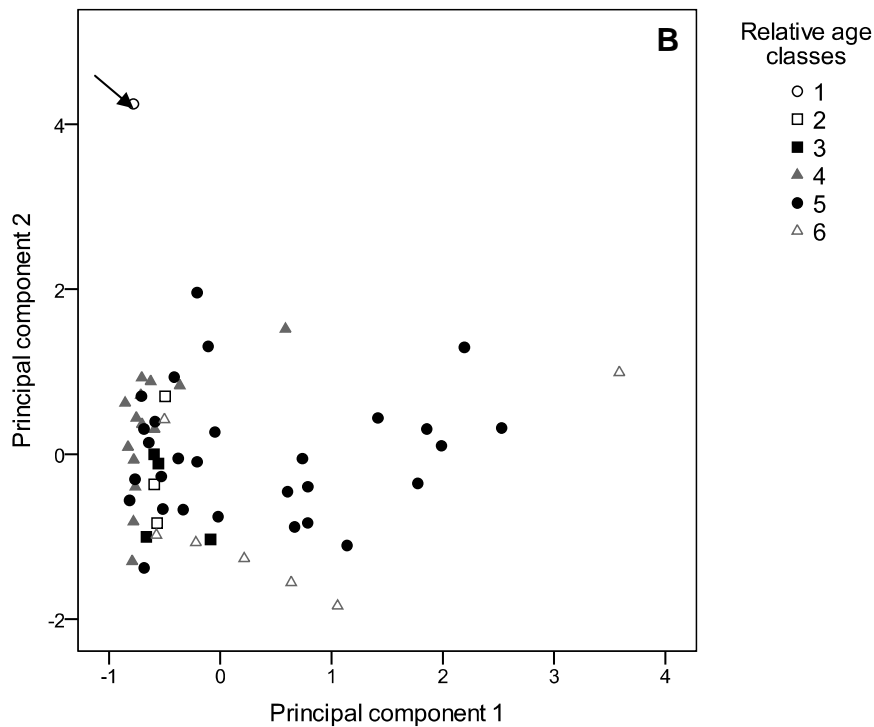
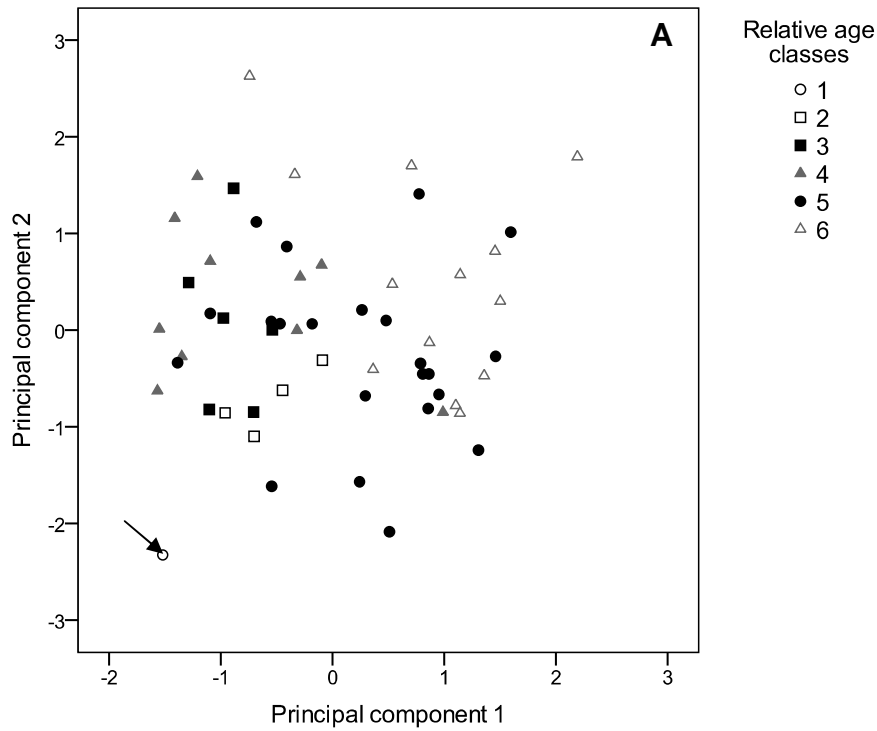


Figure 2. Scatterplots of the two principal component scores generated by principal component analysis from five male (A) and ten female (B) reproductive parameters of the Eastern rock elephant-shrew (*Elephantulus myurus*) from South Africa. The scores are presented separately for six relative age classes. Relative age class (1) is indicated with an arrow in both graphs.