

Reproductive patterns in the Baluchistan gerbil, *Gerbillus nanus* (Rodentia: Muridae), from western Saudi Arabia: the role of rainfall and temperature

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Abstract

There is little information pertaining to the reproductive biology of the Baluchistan gerbil (*Gerbillus nanus*) despite a broad distribution throughout the Middle East. In the current study, body mass, reproductive-tract morphometrics plus gonadal histology and endocrine profiles of males and females were studied over 12 consecutive months in a field population of Baluchistan gerbils from the western region of Saudi Arabia in an attempt to gain insights into a) the environmental *zeitgeber(s)* that correlate with reproduction as well as b) to assess if reproduction is seasonal or aseasonal in this species. With the exception of testosterone all male reproductive indicators varied seasonally and were lower during winter than the rest of the year and most increased with rainfall. In contrast, ovarian mass and volume as well as immature follicle counts showed no seasonal patterns and were independent of rainfall or temperature. First pregnancies were observed in late spring coinciding with the increased progesterone concentrations and peaked in summer. This was accompanied by seasonal peaks in the number of Graafian follicles and corpora lutea in females and these parameters were significantly correlated with temperature, but not rainfall. In the Arabian Peninsula male, but not female Baluchistan gerbils show seasonal recrudescence and this appears to be correlated largely to rainfall.

Key words: Reproduction, rodent, seasonality, gonads, hormones, Saudi Arabia

Introduction

Reproduction in small mammals usually occurs at a time which maximises the growth and survival of the offspring (Fitzgerald and McManus, 2000). Classically mammals occurring in temperate and polar latitudes have a defined seasonal component to reproduction avoiding hostile conditions when the young are unlikely to survive (Nelson et al., 1992). Seasonal breeders successfully mate only during particular times of the year. These periods allow for the birth of offspring at a time which is ideal for the survival of the young (Prendergast, 2005).

A seasonal change in photoperiod is the one of the most classic of environmental cues used for timing reproduction at higher latitudes (El-Bakry et al, 1998, Medger et al, 2011, Trainor et al, 2006). However, photoperiod does not play such an important cue in desert and semi-desert environments where the correlation between photoperiod and food availability is often not so clear cut (Henry and Dubost, 2012, Breed and Leigh, 2011). Deserts experience extreme ranges in daily and seasonal ambient temperatures, with little or no rainfall, intense solar radiation and have a minimal primary productivity (Schmidt-Nielsen, 1964). These habitats have sparse vegetation and the soil substrate is exposed to the full rays of the sun and rainfall is often unpredictable. Consequently, mammals occupying these regions are prone to desiccation and must regulate both their water balance and energy tightly (Macfarlane, 1968). Similarly, reproduction in desert ecosystems may be difficult to achieve because of the scarceness and unpredictability of rainfall, extreme daily and seasonal temperature ranges and the associated general lack of available food resources (Reichman and Graff, 1973). As a consequence successful reproduction usually transpires only when food resources and water coincide with favourable temperatures to provide optimal conditions for mating and the maintenance of pregnancies (Bronson, 1988). For example, the Spinifex hopping mouse, (*Notomys alexis*) is

widely distributed in the deserts of central and western Australia. They show marked boom/bust patterns in population density as a result of sporadic rainfall that triggers reproductive activity probably in response to changes in food availability (Predavec, 1994). In this species reproductive activity may occur in all seasons but there is evidence for this to be more likely in spring and early summer (Breed 1992, Breed and Leigh, 2011). In *Spinifex* hopping mice, males maintain reproductive function throughout the year while females are more sensitive to environmental cues and corpora lutea as well as pregnancies are usually only observed after rainfall (Breed, 1992, Breed and Leigh, 2011). Mammals occurring in desert environments may be partially activated by photoperiod, but fully activated when combined with other *zeitgebers* such as food, a drop in dietary salinity, precipitation or secondary plant compounds which ensure the optimal season is utilized (Pravec, 1994, Reichman, 1975 and Wube, et al., 2009). Both the quality and quantity of available food is important for reproduction due to its high energetic costs (Bronson, 1988, Speakman, 2008).

Recrudescence of reproduction with the onset of rainfall has been found to arise in many species of rodents in desert regions of the world for example, *Arvicanthis niloticus* in the Sudan (Happold, 1966), *Gerbillus gerbillus* and *Psammomys obesus* in the Sahara (Amiratet et al., 1977), *Gerbillus dayurus* in the Negev (Shenbrot et al., 1977) and *Gerbillus pyramidium* and *Gerbillus anersoni* in Egypt (Soliman and Mohallal, 2009). Dietary salinity of food may act as an important cue to the body to stop reproduction when salinity is high or to initiate the direction of energy towards reproduction when they are low, as has been found in golden spiny mice (*Acomys russatus*) in the deserts of the Middle East and deer mice (*Peromyscus maniculatus*) from North America (Nelson and Desjardins 1987, Wube, 2009). However, there is still a paucity of information pertaining to reproductive cycles in desert rodents, particularly from the

Arabian Peninsula. Many small mammals, and in particular gerbils (subfamily Gerbillinae), are active in the cooler parts of the day which usually is at dusk through to dawn and as a consequence are nocturnal in habits (Alagaili *et al.* 2012; 2013). The subfamily comprises more than 100 species in Africa and Asia all of which are adapted to arid environments (Nowak & Wilson, 1999). Gerbils are fossorial and spend the daylight hours in their burrows which afford protection from the heat of the day. Gerbils are also physiologically adapted to living in the deserts of the world and their kidneys have long Loops of Henle for water reabsorption (Schmidt-Nielsen, 1961, Diaz, 1991).

The Mongolian gerbil, *Meriones unguiculatus* which is distributed in semi arid steppes and desert grasslands is a strongly seasonal breeder which cues its reproduction into photoperiod, with recrudescence of reproduction occurring in spring and ending in autumn (Liu *et al.*, 2009). Interestingly in the desert gerbil, *Gerbillus gerbillus* from Algeria, reproductive activation in males, as monitored by increasing androgen levels, appears to coincide with an increase in both temperature as well as photoperiod (Khammar and Bridieux, 1987).

To date there has been little research conducted on the gerbils from the Arabian Peninsula. Henry and Dubost (2012) studied Cheesman's gerbil, *Gerbillus cheesmani* from the southwestern part of Saudi Arabia and found it to cue its reproduction with precipitation. Indeed reproduction is highly seasonal with both males and females synchronizing their reproduction to the short period of rainfall, rapidly producing offspring that can benefit from the short periods of food abundance. These findings are in contrast to those of the Egyptian greater gerbil, *Gerbillus pyramidium* and Anderson's lesser Egyptian gerbil, *Gerbillus andersoni* where reproductive recrudescence in males is initiated prior that to that of females (Solima and Mohallal, 2009).

In southern Africa, the bushveld gerbil, *Gerbilliscus leucogaster*, is reported to be seasonal in its breeding, although the length of the breeding season varies with geographic distribution and is often associated with rainfall pattern e.g South Africa (Rautenbach, 1982, Perrin and Swanepoel, 1987) and Zimbabwe (Neal, 1991). Yet in other regions, Botswana (Smithers, 1983) and Namibia (Griffin, 1990) aseasonal patterns of breeding were recorded.

The Baluchistan gerbil (*Gerbillus nanus*) is a small terrestrial, nocturnal rodent of around 20g found in desert, semi-desert and arable land. It commonly occurs in wadis (valleys), oases, Sebkhass edges and sandy clay pans where there is deep soil and abundant vegetation (Harrison and Bates, 1991). It is relatively widespread in the Arabian Peninsula, the Middle East and throughout the region to northwest of India (Harrison and Bates, 1991). Baluchistan gerbils have also been recorded in the western part of the Sahara and Sahel region in Morocco, Tunisia and from Mauritania to northern Mali, as well as from Niger to the south of Chad. Despite its wide distributional range, currently, there is no reported information on the breeding patterns of *G. Nanus* (Harrison and Bates, 1991).

In this study, the pattern of reproduction in *G. nanus* from Saudi Arabia was monitored by measuring the seasonal concentrations of progesterone and testosterone in the plasma of females and males, respectively, and by examining the gross morphology and detailed histology of their gonads over an entire calendar year. We hypothesized that reproduction in the Baluchistan gerbil would be highly dependent on rainfall, which in turn dictates food availability. We further predicted that *G. Nanus* would cue reproductive recrudescence of gonadal activity and elevation in reproductive hormones mainly during the warm and wet spring and summer months (April–July), while ovaries and testes would regress and progesterone and testosterone concentrations would be lower during the cold and dry winter months (November to February). We finally

predicted that, as with Cheesman's gerbil, the males and females would synchronize reproductive activation so as to maximize reproductive success in the short period of rainfall and increased food availability.

Materials and methods

Study site and animal capture

Sampling took place at the 250ha National Wildlife Research Centre located 42 km east of Taif (N 21° 15.164', E 40°41.984') in Saudi Arabia. The reserve is a desert ecosystem comprising annual grasses, small herbaceous plants, small trees, rocky outcrops and small wadis. The soil is dominantly deep sandy soil with clay pans. Rodents were captured on a monthly basis using 31 x 12.5 x 12.5cm small mammal live traps which were set between December 2011 and November 2012. Live traps were not of a specific brand and were purchased at a local store in Taif.

Trapping began on the first day of every month and continued until the 15th day, or when the sample size had been reached. A maximum of 10 females and 10 males were captured per month. However in December 12 males were captured and processed. Thirty traps were set up in a grid formation (10 x 3 x 3 traps) in sandy areas, with approximately 12 metres between each trap. Every 3-5 days the traps were moved to a new site. Trapping sites were chosen at random. The traps were baited with peanut butter with either flat bread or apple. Each trap was opened in the evening between 16h00 and 17h00 and then checked at dawn, after which they were closed and any captured animals removed. Individuals captured were transported in their traps from the trapping site back to the laboratory for further processing. The trap was subsequently placed back in its original location when traps were opened in the evening. All non-focal study species were released at the capture site.

Dissections

On the day of capture animals were weighed to the nearest 0.01g using a digital scale (Scout Pro SPU123, Ohaus Corporation, Pine Brook, New Jersey, U.S.A.). The animals were then euthanized by placing the rodent in a jar with an overdose of ether. Once the animal had expired, whole blood was obtained by exsanguinations of the heart using a heparin lined 1.0cc syringe with a 25g x 5/8" needle. The blood was then transferred to an eppendorf tube and centrifuged at 3000rpm for 15 minutes. Plasma was collected and stored at -20 °C for later hormonal analysis.

Histology

The reproductive organs were removed and placed into Bouin's fixative and remained in the solution for 20 hours. After being fixed in Bouin's fixative for this period, the gonads were taken out of the fixative and placed in 70% ethanol. Excess fat and connective tissue was removed and the gonads were weighed to the nearest 0.001g (Ohaus Corp. Pine Brook, N.Y., U.S.A.). Gonad mass was converted to milligrams and corrected for body mass by dividing the gonad mass (mg) by the body mass (g) for each individual. The maximum length and width of the gonads were recorded using Vernier callipers (Mitutoyo American Corporation, Aurora, Illinois, USA). The measurements for the paired gonads were averaged. From these data the average gonadal volume was determined using the equation for the volume of an ellipsoid $V = 4/3 \pi ab^2$, where a is one half maximum length and b is one half maximum width (Woodall and Skinner, 1989).

Gonads were then put through a dehydration process with a series of ethanol baths of increasing concentrations (70%, 80%, 90%, 96%, and 100%) and finally embedded into wax. Random sections from the testes were cut at 7 μ m using a rotary microtome (820 Spencer,

American Optical, Scientific Instrument Division, Buffalo, N.Y., U.S.A.), while the whole of the ovary was cut with a thickness of 6 μm . All cuts were mounted onto slides with gelatine in consecutive order and then dried for 48 hours in an oven at 36°C. Once dry the slides were stained with haematoxylin and eosin. Testicular sections were examined for round seminiferous tubules with a light microscope (Diaplan, Ernst LeitzWetzlar GmbH, Germany) and photographed at a magnification of $\times 10$ with a digital camera (Moticam 1000 1.3 M Pixel USB 2.0; Motic China Group, LTD., Xiamen, P.R. China) attached to the microscope. The programme Motic Images Plus 2.0ML (Motic China Group, LTD., Xiamen, P.R. China) was used to measure the diameter of 50 seminiferous tubules from each testis. The functional state of the ovaries were assessed by examining and counting the number of follicles and corpora lutea in all serial sections throughout both ovaries of each female under a light microscope at magnifications of $\times 100$, $\times 200$ and $\times 400$. The follicular stages were classified as primordial follicles, primary follicles, secondary follicle, tertiary follicles, Graafian follicles and corpora lutea, according to Bloom and Fawcett (1964). Primordial follicles were counted only every tenth cut on a slide, while all other follicle stages were counted throughout the entirety of each ovary.

Hormone analyses

Plasma samples were assayed for testosterone using Coat-a-Count Testosterone kits (Diagnostic Products Corporation). Cross reactivity of the antibody was 16% with 1-ketotestosterone –one, <5% with dihydrotestosterone and 19-hydroxyandrostendione and 1% with aldosterone, androstendione, cortisol, corticosterone, oestrone, methyltestosterone and progesterone. The assay was validated for plasma testosterone in *G. nanus* by testing the slope of the curve

produced using serial dilutions of unextracted plasma obtained from a male with high testosterone concentrations (over the range 1:1 – 1:16) against the standard curve. After logit-log transformation of the data (Chard, 1987) the slopes of the lines were compared and found not to be significantly different from the reference preparation (ANOVA: $F_{1,4} = 1.244$, $P = 0.302$). The sensitivity of the assay was 20ng/l. Intra-assay coefficient of variation for a quality control was 4.9%.

For the assessment of female progesterone levels, plasma samples were assayed using Coat-a-Count Progesterone kits (Diagnostic Products Corporation). The antiserum is highly specific for progesterone. Cross reactivity of the antibody was <0.5%, with the exception of 17 α -hydroxyprogesterone (3.4%), 11-deoxycorticosterone (2.4%), 5 β -pregnan-3, 20-dione (3.2%), and 5 α -pregnan-3,20-dione (9%). The assay was validated for plasma of *G. nanus* by testing the slope of the curve produced using serial dilutions of unextracted plasma obtained from a female with high progesterone concentrations (over the range 1:1 – 1:16) against the standard curve. After logit-log transformation of the data (Chard, 1987) the slopes of the lines were compared and found not to differ significantly from the reference preparation (ANOVA: $F_{1,4} = 0.077$, $P = 0.787$). The sensitivity of the assay was 0.36 nmol/l. Intra assay coefficient of variation for a quality control was 2.35%. The research was approved by the Animal Ethics Committee, University of Pretoria (ECO46-11) and the Saudi Wildlife Authority.

Data Analysis

Due to low sample numbers in some months (see results section) the data for the 12 month period was lumped into four seasons: winter (December-February), spring (March-May), summer (June-August) and autumn (September-November). Only individuals with a body mass

above 15g were considered in our analyses. After a log transformation the body mass data were normally distributed (Kolmogorov-Smirnov (KS) test: $D_{142} = 0.078, P = 0.200$). We explored the effects of sex and season on body mass using a general linear model (GLM) that included sex and season as well as the interaction between sex and season. None of the reproductive parameters measured for males (i.e. testicular mass and volume, seminiferous tubule diameter and testosterone levels) was normally distributed (KS test: $P < 0.0001$ for all) and transformations were unsuccessful. Consequently, the effect of season on these parameters was evaluated employing Kruskal-Wallis (KW) tests. Posthoc comparisons were carried out with Mann-Whitney-U (MWU) tests. Bonferroni corrections for repeated tests were used to adjust the P-values for these tests ($P = 0.017$). Ovarian mass, secondary and tertiary follicles were normally distributed (KS test: $P = 0.200$, for all). In addition, the number of primordial and primary follicles conformed with the requirements for a parametric data distribution after being log-transformed. Consequently, the effect of season on these parameters was explored using a one-way ANOVA with season as independent variable. Least significant difference (LSD) *post-hoc* tests were carried out for significant tests. The number of Graafian follicles and corpora lutea was not normally distributed and transformations proved unsuccessful. Consequently, the effects of season on these follicle types was analysed as indicated above for male reproductive parameters. In addition, we explicitly analysed the impact of rainfall and temperature on reproductive parameters. Daily rainfall and temperature data were provided by the National Wildlife Research Centre at Taif. From this, total rainfall and mean temperature for each season was calculated. To test for the effect of rainfall and temperature, respectively, GLMs were used with total seasonal rainfall and mean seasonal temperature as independent variables. Generalized linear models (GZLM) fitted with a Poisson distribution and a log-link function were employed

for all non-normal dependent variables. Seasonal rainfall and temperature averages, alongside body mass were used as covariates for all models.

All statistical analyses were implemented using the IBM Statistical Package for the Social Sciences (SPSS) Statistics version 21.0 (2012). The results herein are presented as mean \pm standard error (SE) and were found to be significant at $P = 0.05$.

Results

Climate data

The cumulative rainfall for the study period was 61.72 mm. Rainfall was recorded between April and August (Fig. 1). Peak rainfall occurred in April (31.75 mm). Temperatures on average reached above 27°C (range 15.9-38.6) from May to September, with July (29.92°C \pm 4.15; range 19-38.6) being the warmest month (Figure 1). The coldest months were from December to March with December having the lowest monthly mean temperature of (16.63°C \pm 5.2; range 15 to 27).

Body Mass

A total of 79 male and 63 female Baluchistan gerbils were captured over the 12 month period at Taif (Table 1). Body mass did not differ significantly between females (20.28 \pm 2.7g) and males (20.50 \pm 2.3g; $F = 0.020$, $df = 1$, $P = 0.887$). In contrast, body mass differed significantly between seasons ($F = 3.848$, $df = 3$, $P = 0.011$, Figure 2). Posthoc analyses revealed that it was significantly lower in winter compared to spring (LSD: $P = 0.001$) and summer (LSD: $P = 0.025$)

but did not differ between any of the other seasons ($P \geq 0.160$). The interaction between sex and season was not significant ($F = 0.412$, $df = 3$, $P = 0.745$).

Males

Testicular mass was significantly different between seasons (KW test: $Z = 33.180$, $df = 3$, $P < 0.0001$). It was significantly lower in winter than during the other three seasons ($P \leq 0.001$; Figure 3a). In addition, testicular mass was significantly lower in spring compared to summer ($U = 242.00$, $P = 0.017$). None of the remaining pairwise comparisons was significant ($P \geq 0.407$). The GZLM showed that testis mass increased significantly with temperature (coefficient: 0.118 ± 0.022 , Wald $\chi^2 = 30.627$, $df = 1$, $P < 0.0001$) but not rainfall (Wald $\chi^2 = 0.688$, $df = 1$, $P = 0.407$).

Testicular volume was significantly different between seasons (KW test: $Z = 34.223$, $df = 3$, $P < 0.0001$). Mean testicular volume was significantly lower in winter compared to the other three seasons (< 0.0001 for all comparisons; Figure 3b). Significant differences were not found between spring, summer or autumn after Bonferroni adjustments ($P \geq 0.032$). The GZLM showed that testes volume increased significantly with rainfall (coefficient: 0.015 ± 0.004 , Wald $\chi^2 = 11.880$, $df = 1$, $P = 0.001$) and temperature (coefficient: 0.051 ± 0.014 , Wald $\chi^2 = 14.295$, $df = 1$, $P < 0.0001$).

The seminiferous tubule diameter varied significantly between seasons (KW test: $Z = 32.731$, $df = 3$, $P < 0.0001$). Post-hoc tests revealed that it was significantly smaller in winter compared to the other seasons ($P < 0.0001$ for all comparisons; Figure 3c). No significant differences were found between spring, summer and autumn ($P \geq 0.081$). The GZLM showed that rainfall (coefficient: 0.007 ± 0.002 , Wald $\chi^2 = 8.895$, $df = 1$, $P = 0.003$) and temperature

(coefficient: 0.032 ± 0.008 , Wald $\chi^2 = 17.834$, $df = 1$, $P < 0.0001$) both had a significant positive influence on the diameter of the seminiferous tubules.

No significant differences between seasons were found in plasma testosterone concentrations (KW test: $Z = 6.96$, $df = 3$, $P = 0.073$, Figure 3d). However, the GZLM showed that testosterone levels increased significantly with rainfall (coefficient: 0.019 ± 0.006 , Wald $\chi^2 = 10.509$, $df = 1$, $P = 0.001$) but not temperature (Wald $\chi^2 = 2.867$, $df = 1$, $P = 0.09$). The percentage of males carrying sperm was reduced in winter compared to the other three seasons (Figure 4).

Females

Ovarian mass was significantly different between seasons (KW test: $Z = 12.557$, $df = 3$, $P = 0.006$). Ovarian mass was significantly lower in winter than in summer and autumn ($P \leq 0.007$, Figure 5a). No significant differences were found between the other pairwise comparisons ($P \geq 0.054$). The GZLM indicated that neither rainfall (Wald $\chi^2 = 2.551$, $df = 1$, $P = 0.110$) nor temperature (Wald $\chi^2 = 1.165$, $df = 1$, $P = 0.281$) had a significant impact on ovarian mass.

The ovarian volume did not differ significantly between seasons ($F = 2.259$, $df = 3$, $p = 0.091$, Figure 5b). The GLM showed that ovarian volume was not significantly affected by either rainfall (Wald $\chi^2 = 3.113$, $df = 1$, $P = 0.078$) or temperature (Wald $\chi^2 = 0.001$, $df = 1$, $P = 0.975$).

The number of early-stage follicles (primordial through to tertiary) did not differ significantly between the four seasons (primordial: $F = 0.702$, $P = 0.555$; primary: $F = 0.480$, $P = 0.698$; secondary: $F = 0.170$, $P = 0.916$; tertiary: $F = 1.698$, $df = 3$, $P = 0.179$, Table 2).

Similarly, neither rainfall ($P \geq 0.145$) nor temperature ($P \geq 0.152$) did significantly affect the number of any of the early-stage follicle counts.

In contrast, the number of Graafian follicles differed significantly between seasons (KW test: $Z = 16.548$, $df = 3$, $P = 0.001$). The number of Graafian follicles increased significantly between winter and spring (MWU: $U = 227$, $P = 0.006$) and winter and summer ($U = 176.5$, $P < 0.0001$) with counts being lowest in winter (Fig 5c). No other significant differences were found between remaining seasons after Bonferonni corrections ($P \geq 0.022$). The GLM showed that the number of Graafian follicles increased significantly with temperature (Wald $\chi^2 = 8.276$, $df = 1$, $P = 0.004$). In contrast, rainfall had no impact on the number of Graafian follicles (Wald $\chi^2 = 1.646$, $df = 1$, $P = 0.199$).

Corpora lutea were also found to differ significantly between seasons (KW test: $Z = 23.262$, $df = 3$, $P < 0.0001$). The number of corpora lutea was significantly lower in winter compared to summer ($U = 178$, $P < 0.0001$) and autumn ($U = 165$, $P = 0.010$) and in spring compared to summer ($U = 101.5$, $P = 0.013$, Figure 5c). No other pairwise comparisons were significant after Bonferonni corrections ($P \geq 0.022$). The GLM showed that temperature was significantly positively correlated to the number of corpora lutea (Wald $\chi^2 = 28.582$, $df = 1$, $P < 0.0001$) but rainfall was not a significant factor (Wald $\chi^2 = 0.031$, $df = 1$, $P = 0.860$).

Plasma progesterone concentrations differed significantly between seasons (KW test: $Z = 9.827$, $df = 3$, $P = 0.020$). A significant increase in progesterone concentration was observed from winter to spring ($U = 75$, $P = 0.003$, Figure 5d). In contrast, no significant differences were found between any other seasons ($P \geq 0.068$). The General Linear Model showed that neither rainfall (Wald $\chi^2 = 0.003$, $df = 1$, $P = 0.954$) nor temperature had a significant positive influence on progesterone variation (Wald $\chi^2 = 0.000$, $df = 1$, $P = 0.984$). Body mass was not a significant

predictor for any of the reproductive measurements ($P \geq 0.138$). The majority of pregnant females were collected during summer (Figure 6).

Discussion

Reproduction is an energetically costly process during which organisms prepare their gonads for reproductive processes to ensure maximum survival of their offspring (Bronson and Heideman, 1994). In males, recrudescence of gonadal activity occurs with an increase in testicular mass and volume, as well as an increase in the seminiferous tubule diameters and the production of sperm (Medger et al., 2011). This can happen over a much shorter period of time compared to those changes in females. In females, reproductive activation requires the onset of follicular development with periods of ovulation to maximize the chances of pregnancy on encountering a male and being mated (Medger et al., 2011). Synchronization of reproduction in small mammals is brought about by favourable environmental conditions (ultimate factors) that result in the rapid growth and maximal survival of offspring (Ims, 1990). Many environmental *zeitgebers* trigger reproduction, these include photoperiod in the higher latitudes, temperature in sub-tropical and tropical areas and rainfall in desert regions which are the most important abiotic factors bringing about the onset of reproductive activation (Jameson, 1988). The process of reproduction requires significant investment of energy and consequently birthing is found to peak during times when food and water are in sufficient quantities; and this usually results in desert environments when sufficient rainfall has occurred. Food availability, is a major driver of annual periods of reproduction in small mammals (Bronson and Heideman, 1994).

The current study shows that the Baluchistan gerbil, *Gerbillus nanus*, from the foothills of the Hijaz Mountains of Saudi Arabia appears to be a seasonal breeder and adds to the limited

literature currently available about reproduction of small mammals in desert ecosystems.

Breeding was initiated in the latter part of the spring (March through to May) and concluded in late summer suggesting that despite the lower rainfall during summer the sufficient water must have been retained to support live vegetation that provides the energy and nutrients for such long periods of reproduction.

Testicular recrudescence arose in males at this study site mirrored rainfall patterns and in the spring some morphological reproductive parameters of males (testes volume but not mass, seminiferous tubule diameter) increased significantly with rainfall and concomitantly there was evidence for an upregulation of spermatogenesis. At the same time testes mass and volume as well as seminiferous tubule diameter mirrored the seasonal pattern of temperature. In contrast, testosterone concentrations did not vary significantly with season although rainfall, but not temperature, did significantly affect testosterone concentrations. The peak in testosterone concentration observed in spring is probably brought about by increased reproductive activity in the form of mate solicitation and mating during this period. These observations appear to largely confirm our prediction that reproduction in the study species is correlated with rainfall although increases in temperature may contribute as well. Given the divergent patterns of rainfall and temperature from summer onwards this may indicate that rainfall acts as an all or nothing switch and could account for the correlations of some of the parameters measured with temperature. The lack of strong correlations between some of our measures and the climate variables considered may partially be attributable to masking effects due to pooling data across several months for our data analyses. This meant that our analyses was unable to pick up population responses to single climate events (e.g. single extreme rainfall event). These hypotheses should be addressed in future studies. The patterns of male reproductive activity in the study species appears to differ

markedly from that observed in other desert rodents such as *Spinifex* hopping mice where males show little variation in testes size and sperm counts throughout the year (Breed 1992). However, *N. alexis* is unusual among murine rodents in that their relative testes is markedly smaller in than that of similarly sized murid rodents (Breed 1992, Breed & Leigh 2011). The generally small testes in this species may limit the potential for seasonal fluctuation in size and to date no hormonal measurements are available for this species. Also, *Spinifex* hopping mice are unusual in that they show marked boom/bust cycles (Predavec 1994) that may require that are not commonly encountered among desert rodents (Breed & Leigh 2011).

Neither ovarian mass nor volume exhibited seasonal patterns and similar to the immature follicle numbers they were not affected significantly by either rainfall or temperature. This suggests that females, unlike males, may maintain fully functioning gonads irrespective of the climatic conditions and hence they appear unresponsive to these environmental cues.

For females such a strategy may be favourable in unpredictable environments such as many desert habitats since recrudescence of reproductive organs is often energetically costly. In addition, this process often requires a longer period of time than in males (Prendergast, 2001). In a desert habitat this may mean that undue delays may result in unfavourable conditions for offspring at the time of their birth. Maintaining reproductive function, however, ensures that females are ready to mate as soon as males have responded to the appropriate triggers (i.e. in spring) and pregnancies did arise by the end of the spring season that was accompanied by a seasonal peak in progesterone concentrations. However, the greatest numbers of pregnant females was recorded during the summer. As a result of this breeding activity seasonal peaks in the number of Graafian follicles and corpora lutea of ovulation and pregnancy were present in the ovaries of females. This would also account for the correlation of these follicle types with

temperature, but not rainfall. Superficially, this reproductive pattern appears to be divergent from that of other desert murids such as the *Spinifex* hopping mouse. However, since only data on corpora lutea, pregnancies and lactation have been collected for *N. alexis* while no information is available regarding the seasonal patterns of other follicle types or progesterone (Breed 1992, Breed & Leigh 2011) a lack of seasonal fluctuations in these measures cannot be entirely excluded for *N. alexis* and they may in fact show very similar features to the study species.

Lactating females, identified by elongated nipples were observed only from the summer until early in the autumn (J. Sarli pers. obs.). This indicates that parturition in females may have arisen in the late summer; a three week gestation period and subsequent four week period of lactation in *Gerbillus* spp. is consistent with this assumption (Nowak & Wilson, 1999). Indeed, there are many studies in reproductive biology which show that males undergo reproductive recrudescence before that of females (e.g. Henry and Dubost, 2012; Christian, 1979). Successful breeding and offspring production occurred in the summer and early autumn. The cessation of reproductive activity during unfavourable winter conditions indicated by male, but to a lesser extent female reproductive measures is a common pattern among small mammals (Nelson and Desjardins 1987, Wube 2009). Small mammals that have high surface area to volume ratios such as *G. nanus* divert energy from non-essential process such as reproduction to help ensure immune functions, thermoregulation and cellular maintenance during the winter months to increase their chances of survival in the more inclement months (Klein and Nelson, 1999). That a similar mechanism is likely to explain the patterns observed in *G. nanus* is supported by the significantly lower body mass observed in winter. Although this may partially be accounted for by the recruitment of juveniles in the study population, the lack of body mass effects on reproductive parameters supports the idea that low food availability combined with high

thermoregulatory needs are likely to be a more important constraint to reproduction during winter months.

Water is the resource in shortest supply in desert environments, the amount of rainfall and its timing directly affects the growth and sprouting of seeds in a desert. Food availability is significant for breeding animals since reproduction is costly and the synchronization of breeding with rainfall and food availability has been confirmed in a number of rodent species (Neal, 1984; Predavec, 1994; Soliman and Mohallal, 2009). Male and female *G. nanus* initiate breeding in close synchrony with the onset of the rainy season and rising temperatures. Therefore the main environmental factor influencing reproductive seasonality of this small mammal in desert regions appears to be rainfall and temperature. The breeding pattern of *G. nanus* is similar to that of Cheesman's gerbil. Henry and Dubost (2012) report that the Cheesman's gerbil from the Saja Umm Ar-Rimth region of Saudi Arabia cues its reproduction with rainfall. Seasonal patterns of breeding have been recorded in a number of other desert rodents species ranging from North Sudan to the Western Algerian Sahara (Happold 1966, Amirat et al., 1977). In contrast, rodents from other desert regions for example the Namib, Sinai, Egypt, the Negev and the Tahr breed throughout most of the year (Reichen, 1973, Sinai et al., 2003, Tripathi 2005, Krug, 2007, Soliman and Mohallal, 2009). These differences between our findings and those of the studies highlighted above may be explained by differences in the climate at these localities; for example in our study rainfall is sporadic and unpredictable. In these other localities where breeding occurs for longer periods it is possible that increased temperatures and/or a prolonged rainy season may provide for longer periods of green vegetation which is available for reproduction.

In conclusion, *Gerbillus nanus* reproduces seasonally throughout the warm, wet spring and summer months following the onset of the first rains in Taif, Saudi Arabia. The most

important factors influencing reproduction in this small rodent appears to be rainfall in males and temperature in females. While males undergo reproductive recrudescence females maintain their reproductive function throughout the year, possible due to different gonadal response times between the sexes. As with the Egyptian greater gerbil and Anderson's lesser Egyptian gerbil breeding is synchronized with rainfall (Soliman and Mohallal, 2009). However, unlike Cheesman's gerbil where male and female cue into rainfall and synchronise reproduction (Henry and Dubost, 2012), in the Baluchistan gerbil, males are activated by rainfall while females appear to be in reproductive readiness for much of the year in Taif, Saudi Arabia.

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Figure legends

Figure 1: Climatic data recorded for National Wildlife Research Centre at Taif, Saudi Arabia during the period of study. The solid line indicates mean monthly temperature while the dashed and dotted line indicate minimum and maximum monthly temperatures, respectively.

Figure 2: Seasonal variation in male body mass of the Baluchistan gerbil, *Gerbillus nanus* at the National Wildlife Research Centre at Taif, Saudi Arabia.

Figure 3: Seasonal variation of reproductive parameters (mean \pm SE) of male Baluchistan gerbils, *Gerbillus nanus* at the National Wildlife Research Centre at Taif, Saudi Arabia. Showing a, relative testicular mass (mg/g), b, testicular volume (mm³), c, seminiferous tubule length (μ m), d, plasma testosterone concentration (ng/ml).

Figure 4: The percentage of male Baluchistan gerbils, *Gerbillus nanus* at the National Wildlife Research Centre at Taif, Saudi Arabia with sperm present in the seminiferous tubules for each season.

Figure 5: Seasonal variation of reproductive parameters (mean \pm SE) of female Baluchistan gerbils, *Gerbillus nanus* at the National Wildlife Research Centre at Taif, Saudi Arabia. Showing a, relative ovary mass (mg/g), b, ovarian volume (mm³), c, Graafian follicle and corpora lutea counts, d, progesterone testosterone concentration (ng/ml).

Figure 6: The percentage of pregnant female Baluchistan gerbils, *Gerbillus nanus* collected at the National Wildlife Research Centre at Taif, Saudi Arabia.

Figure 1

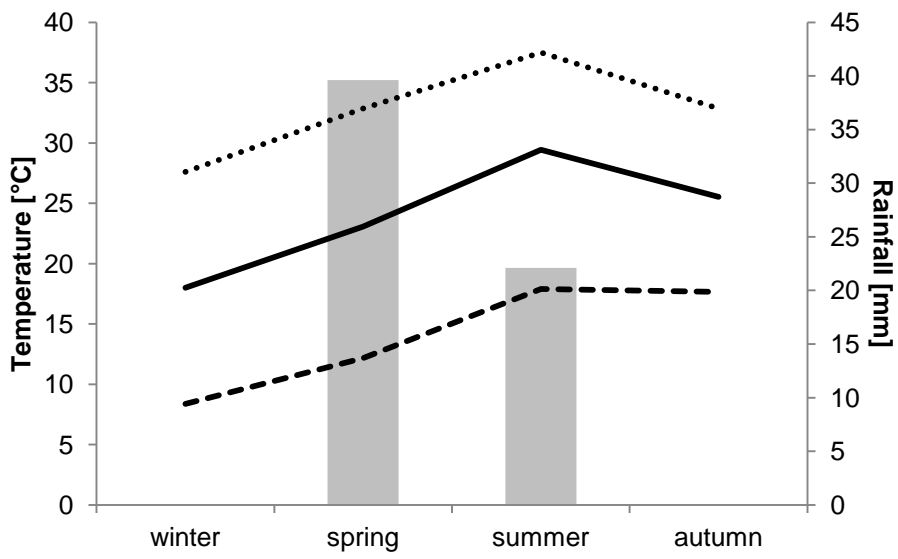


Figure 2

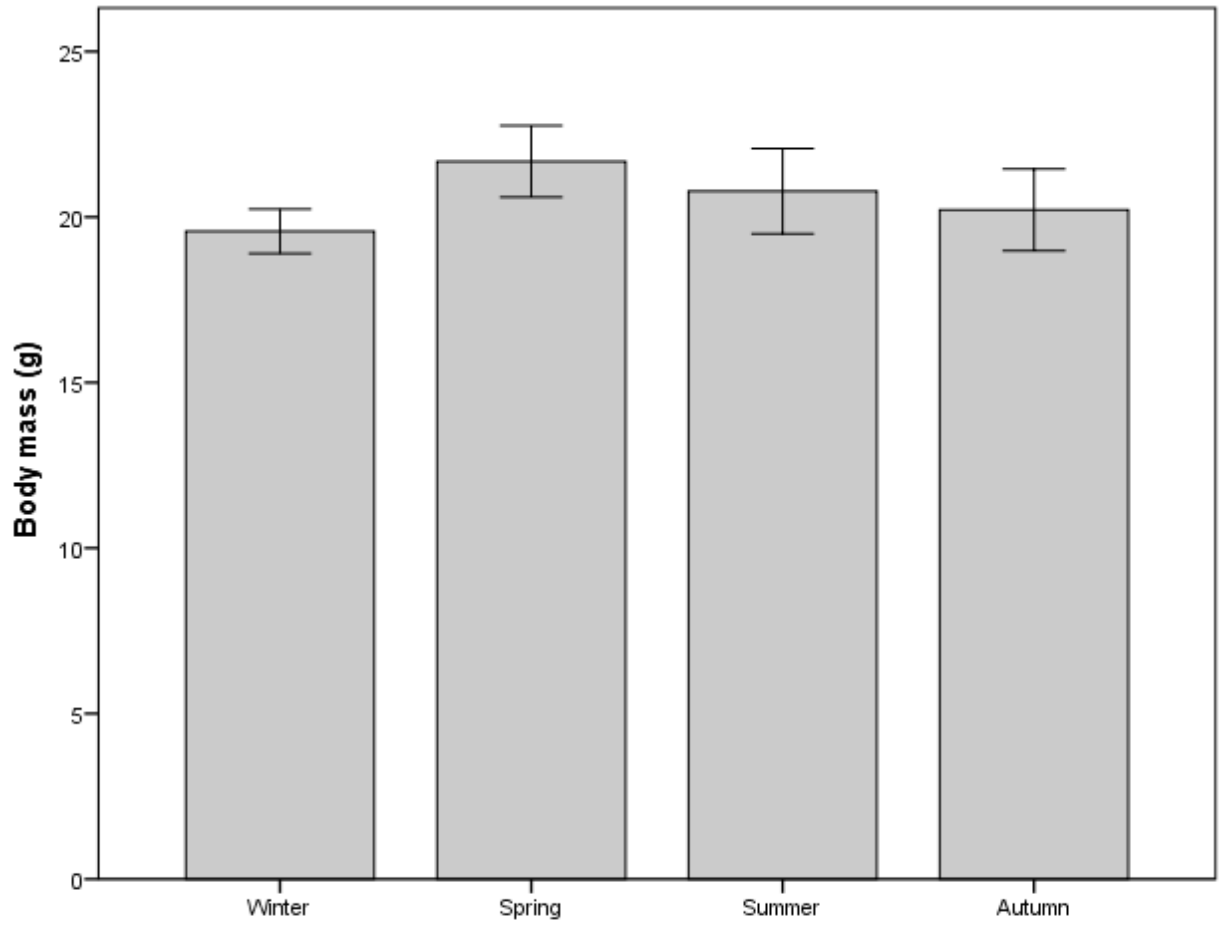


Figure 3

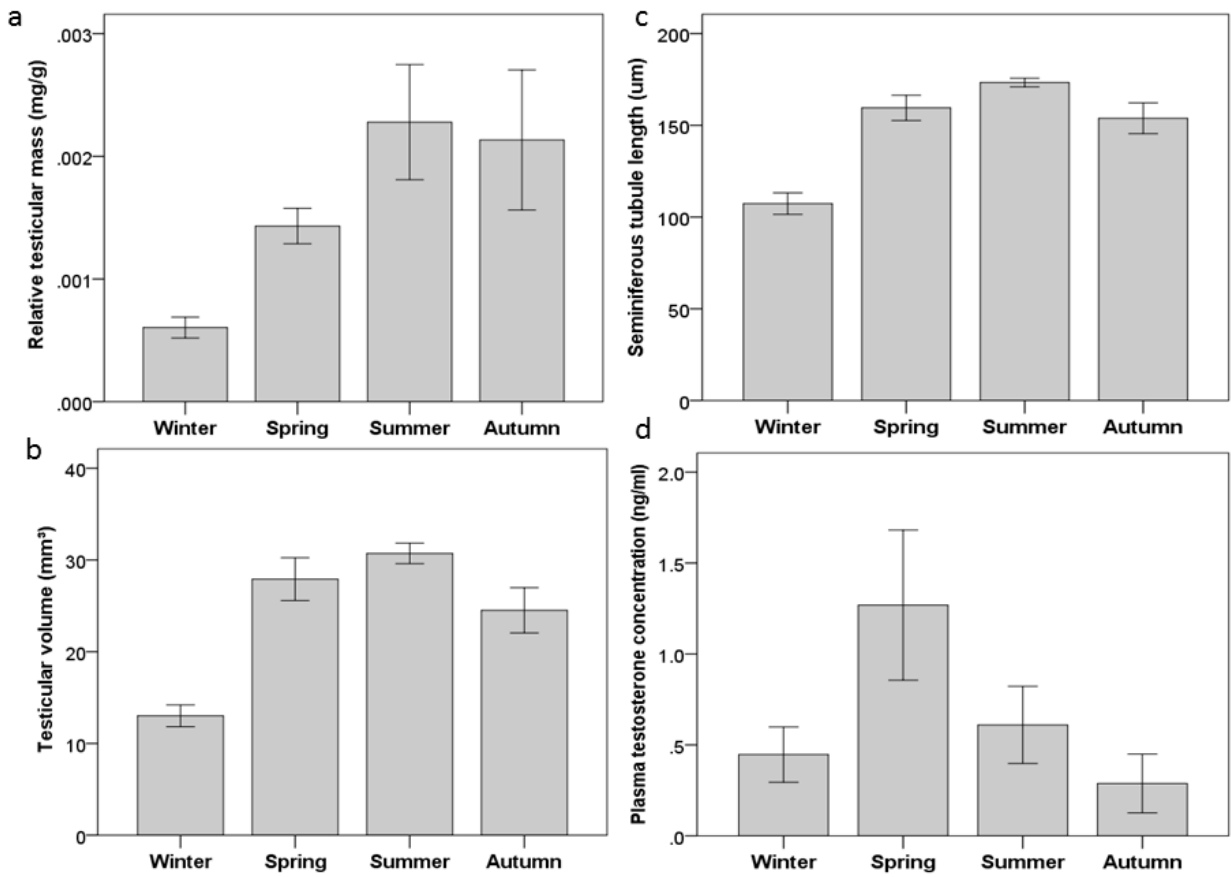


Figure 4

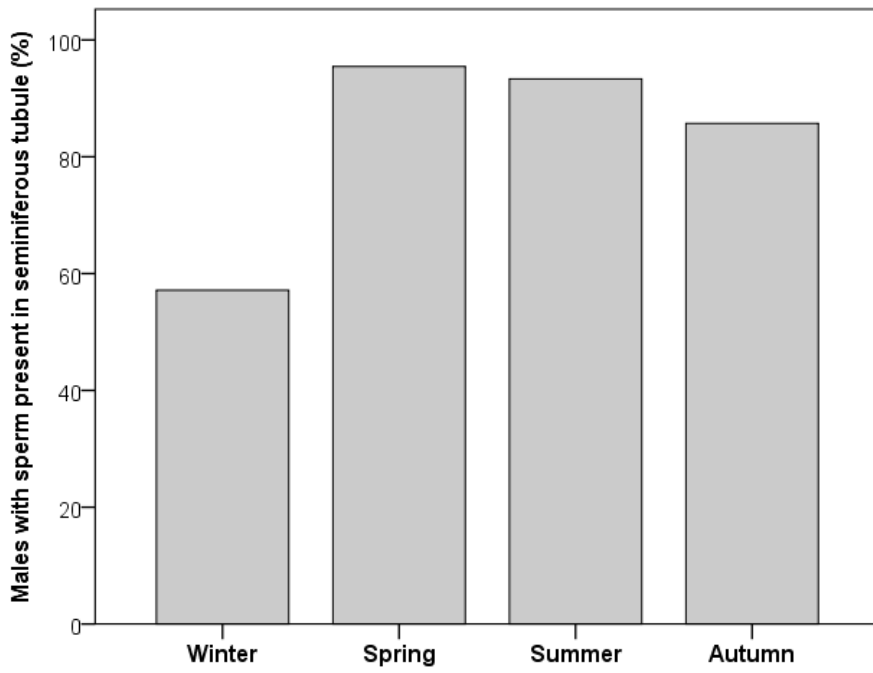


Figure 5

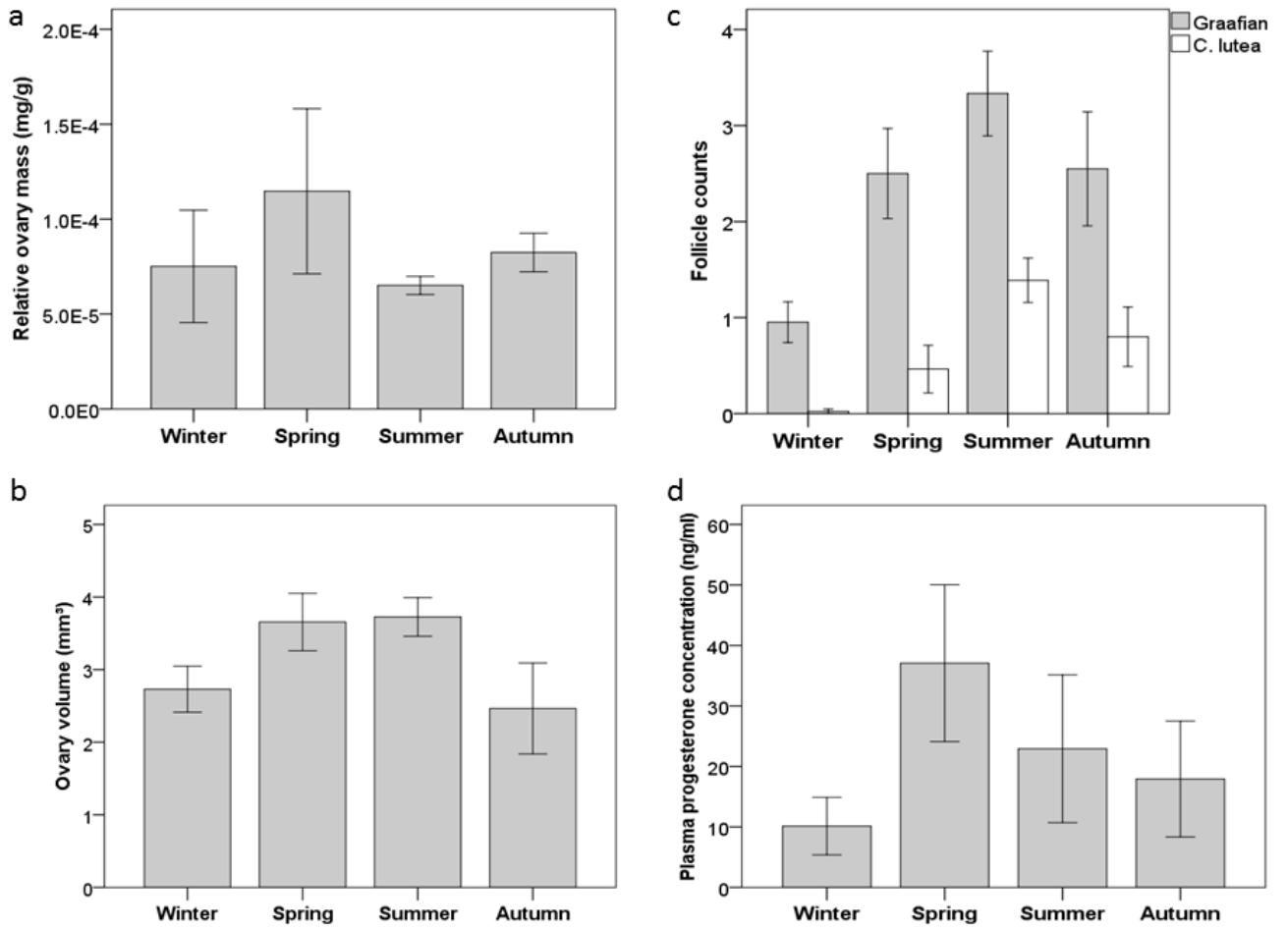


Figure 6

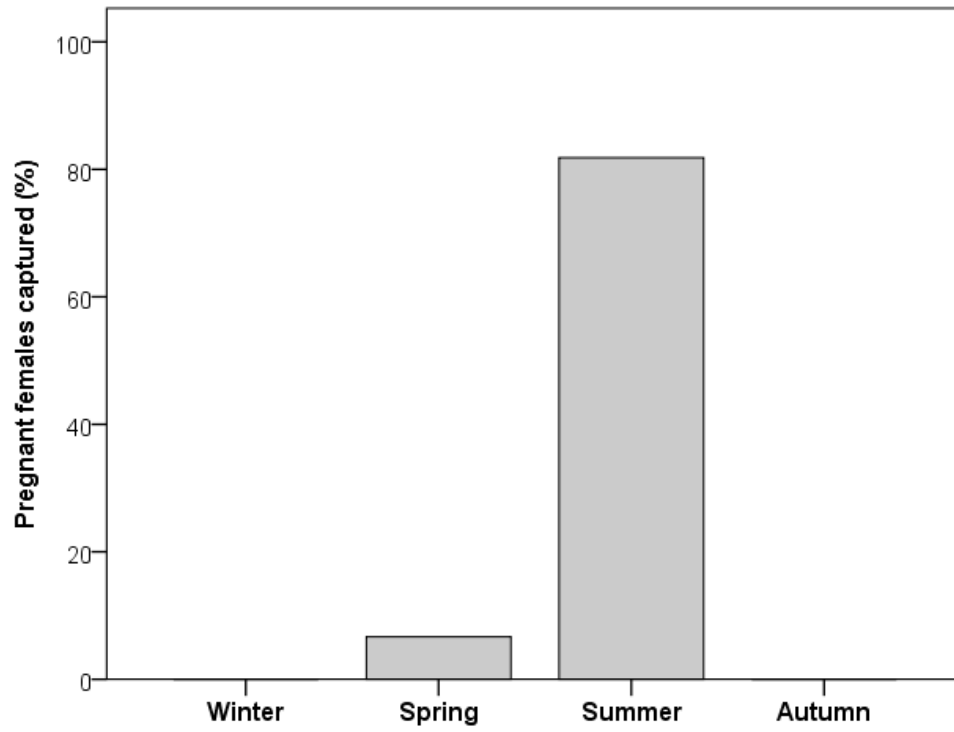


Table 1. Numbers of *Gerbillus nanus* captured on a monthly basis at the National Wildlife Research Centre at Taif, Saudi Arabia from December 2011 until November 2012.

	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.
Males	12	7	9	9	8	5	6	6	3	1	5	8
Females	10	9	6	8	5	2	5	2	4	4	3	5

Table 2. Number of primordial, primary, secondary and tertiary follicles across seasons for *Gerbillus nanus* at the National Wildlife Research Centre at Taif, Saudi Arabia between December 2011 and November 2012.

	N	Primordial	Primary	Secondary	Tertiary
winter	21	362.38 (± 33.33)	36.17 (± 3.50)	35.19 (± 2.74)	3.74 (± 0.78)
spring	14	457.18 (± 60.36)	32.32 (± 1.99)	35.54 (± 2.28)	5.36 (± 0.67)
summer	9	385.39 (± 74.29)	35.83 (± 3.10)	38.29 (± 4.12)	6.22 (± 0.55)
autumn	10	358.65 (± 49.64)	38.95 (± 3.79)	36.15 (± 3.20)	5.15 (± 1.09)

Highlights

Baluchistan gerbil in Saudi Arabia is a seasonal breeder
Males correlate recrudescence of reproduction to rainfall
First pregnancies in spring and peak in late summer
Females correlate breeding to temperature