Seasonal reproduction in the Arabian spiny mouse, *Acomys dimidiatus* (Rodentia: Muridae) from Saudi Arabia: the role of rainfall and temperature

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Abstract

In deserts where unpredictable rainfall arises and a large variation in temperature occurs, this poses severe energetic and water related constraints on reproduction in small mammals and makes the use of photoperiod as cue for reproduction unreliable. In the present study, the gonad morphometrics, gonadal histology and endocrine profiles of the Arabian spiny mouse (*Acomys dimidiatus*) were studied over 12 consecutive months in a field population from western Saudi Arabia in an attempt to assess the seasonality of reproduction and gain insights into the environmental cues that may trigger reproduction in this species. Although sperm

was present throughout the year, most reproductive parameters exhibited a seasonal pattern with a minimum in winter in both sexes and pregnancies were observed from spring to autumn. Whereas testes volume, the number of corpora lutea and progesterone levels were correlated with temperature, all other reproductive parameters were correlated with rainfall. Thus, it appears that *A. dimidiatus* ceases to breed during winter and uses rainfall as an environmental cue to initiate reproduction in the study area. However, the species appears to have the ability to reproduce throughout the year, if favourable conditions arise.

Key words: Reproduction, *Acomys*, seasonality, gonads, hormones, Saudi Arabia, environmental cues

1. Introduction

Deserts are characterized by broad daily and seasonal changes in temperatures, minimal primary productivity and low yearly rainfall, which is characteristically sporadic and unpredictable (Schmidt-Nielsen, 1964; Prakash and Ghosh, 1975; Predavec, 1994). Small mammals occurring in these regions must tightly regulate both their water and energy balance to ensure survival (Bozinovic and Gallardo, 2006; Schwimmer and Haim, 2009). The harsh conditions associated with arid environments may constrain reproduction in rodents to periods of opportunity. Desert adapted mammals usually reproduce during a period which maximises the growth and survival of their offspring (Schneider, 2004; Bronson, 2009; Schwimmer and Haim, 2009).

Reproduction in small mammals may be regulated by a number of environmental variables such as photoperiod, rainfall, the availability of food and dietary salinity (Bronson and Heideman, 1994; Wube, Haim and Fares, 2009). Photoperiod is a dominant proximate cue commonly used by small mammals to inform the animal that favourable conditions for

reproduction are approaching (Bronson and Heideman, 1994; Prendergast, Kriegsfeld and Nelson, 2001; Bronson, 2009). This is particularly true of organisms occurring at higher latitudes where seasonal variation in food availability is fairly predictable. However, in semi-arid and arid regions that are characterised by their erratic climate, food and water availability, reliance solely on photoperiodic cues is likely to result in a mismatch between reproductive investment and favourable environmental conditions (Noy-Meier, 1973; Bronson, 1985, 2009). Instead several other environmental cues, including rainfall, temperature and food availability, have been suggested to override or fine-tune photoperiodic cues to ensure successful reproduction in unpredictable habitats such as deserts (Bronson & Heideman, 1994; El-Bakry, Zahran and Bartness, 1998; Shanas and Haim, 2004).

An increase in water availability as a result of rainfall has been suggested to be a reliable cue for the initiation of reproductive activity in deserts (Sicard et al., 1993; Bronson, 2009; Bukovetzky et al., 2012). Indeed this cue has been implicated for a number of species inhabiting arid habitats (Khammar and Brudieux, 1987; Medger, Chimimba and Bennett, 2010, 2012a; Sarli et al., 2015). Rainfall may be used as an indicator for primary production in arid environment and affect both the quantity and quality of food (Louw and Seely, 1982; Nelson and Desjardins, 1987). Alternatively, lack of rainfall may result in increases of salinity in plant tissues and in turn the food salinity can be used as cue for the regulation of reproduction in desert rodents (Wube et al., 2009; Bukovetzky et al., 2012).

The muroid rodent genus *Acomys* comprises 19 species (Wilson and Reeder, 2005). The genus is characterized by the hard spine-like hairs found on the back of the pelage. Most species of *Acomys* inhabit arid habitats in the Middle East and northern, eastern and southern Africa (Wilson and Reeder, 2005). A great diversity of reproductive patterns has been reported for members of the genus *Acomys* ranging from seasonal to aseasonal breeding (Happold, 1966; Neal, 1984; Delany and Farook, 1989; Fleming and Nicolson, 2002; Medger

et al., 2010, 2012a). Several studies have been undertaken on species of *Acomys* in countries that border with a peak in reproduction during the months with the highest rainfall (Al-Khalili and Delany, 1986; Kivanç et al., 2013). Kivanç et al. (2013) found that *A. cilicicus* from Turkey has an extended breeding period lasting from February to November. They further proposed that if suitable weather conditions and food availability were optimal, reproduction could occur throughout the year.

Photoresponsiveness of the reproductive axis has been shown for some, but not other *Acomys* spp. and effects may differ between the sexes or depend on the parameters measured. In mesic adapted *A. cahirinus* (annual rainfall ≤ 700mm) testes mass and volume as well as uterine volume were significantly larger during long-day (LD) compared to short-day (SD) conditions in the laboratory, but did not affect the number of oestrus cycles or the spermatogenic index (Wube, Haim and Fares, 2008b). Similarly, all reproductive parameters in mesic adapted male *A. spinosissimus* were significantly elevated during LD compared to SD (Medger, Chimimba and Bennett, 2012b). In contrast, the spermatogenic index was the only one of these reproductive parameters affected by photoperiod in xeric adapted (annual rainfall ≤ 47mm) A. russatus (El-Bakry et al., 1998; Wube et al., 2008b). These findings are in accordance with the hypothesis that environmental cues other than photoperiod become more important as indicators of favourable conditions for reproduction in desert environments (Bronson, 2009; Schwimmer and Haim, 2009).

Apart from rainfall, dietary salinity may be an indicator of water availability. Indeed increases in dietary salinity have been shown to result in reductions in spermatogenic index and uterine as well as ovarian mass, but not testes mass in *A. russatus* during LD (Shanas and Haim, 2004; Wube et al., 2009). In contrast, no effect of dietary salinity on testes mass, testosterone levels or ovary mass was observed during LD in *A. cahirinus* while testes mass was reduced during SD (Wube et al., 2009; Bukovetzky et al., 2012). These studies suggest

that species of *Acomys* that live in arid habitats may pursue an opportunistic strategy and use cues indicating water availability (e.g. rainfall or dietary salinity) rather than photoperiod to initiate reproduction allowing quick responses to the erratic rainfall patterns in such habitats. However, this is largely based on laboratory studies and only limited data is available from wild populations for any of the species in this genus.

To date, little research has been reported on the patterns of reproduction in rodents from Saudi Arabia (Al-Khalili and Delany, 1986; Henry and Dubost, 2012; Sarli et al., 2015). The present study focuses on the reproductive seasonality of the Arabian spiny mouse, *Acomys dimidiatus*, from the western region of the Arabian Peninsula. *A dimidiatus* has previously been considered a sub-species of *A. cahirinus*, but recent research has suggested it should be elevated to full specific status (Volobouev, Gautun and Tranier, 1996; Volobouev et al., 2007). We have therefore adopted the name *A. dimidiatus* for our study species here and in accordance with a recent genetic assessment we consider it distinct from *A. cahirinus* (Bray et al., 2013). *A dimidiatus* is a nocturnal rodent which occurs in rocky habitats and is commonly found in the arid and semi-arid regions from the Sinai Peninsula of Egypt, across the Middle East with southern Iran and southern Pakistan being the extremes of its distributional range (Harrison and Bates, 1991; Alagaili et al., 2014). Al-Khalili and Delany (1986) undertook a study on a population of *A. dimidiatus* from June 1979 to July 1981 in south-western Saudi Arabia in an area with moderate aridity (annual rainfall 356mm), reporting reproductive activity from late winter until autumn. From their findings it was suggested that *A. dimidiatus* may be a seasonal breeder with opportunistic tendencies allowing them to produce young throughout the year if conditions are favourable. However, this work lacked quantification through examining the histology of the gonads, and monthly trapping to more accurately determine the breeding strategy and identify the possible cues used by this species to trigger the onset of reproduction.

In this study we undertook a detailed analysis of the reproductive biology of *A. dimidiatus* from Saudi Arabia by observing the monthly circulating concentrations of progesterone and testosterone in the plasma of females and males, respectively. In addition, we examined the morphology and detailed histology of the gonads of both sexes over an entire calendar year. We hypothesised that (1) reproduction would be initiated by rainfall. If this was indeed the case, we predicted that (2) in contrast to the population of south-western Saudi Arabia (Al-Khalili and Delany, 1986), *A. dimidiatus* in the study area would exhibit reproductive quiescence during the dry cold and dry autumn and winter months due to the low amount of rainfall.

2. Materials and methods

2.1 Study site and animal capture

Animals were collected from the National Wildlife Research Centre located 42 km south of Taif in Saudi Arabia (N21°15.164', E40°42.984'). The reserve is 250ha of desert ecosystem comprised of annual grasses and small herbaceous plants interspersed with rocky outcrops, small trees and small wadis. The geology comprises a fairly flat sandy/ gravel desert which is on the north eastern Asir foothills. There are a few gneiss inselbergs and dry wadis The dominant grasses are *Cyanodon dactylon*, *Eragrostis pappasa* and *Stipagastris obtusa*. The shrubs and trees in the area include *Acacia ehrenbergiana, Vachellia tortilis, Salsola spinescens* and *Lycium shawii* (Asmode, 1989)..

The spiny mice were captured on a monthly basis using 31 x 12.5 x 12.5cm small mammal wire live traps from December 2011 to November 2012. We aimed to capture 10 individuals of each sex to allow meaningful statistical analyses on a monthly basis. Consequently, trapping was terminated once this goal was achieved. However, low

population densities of the study species meant that this was not possible during all capture months and trapping was restricted to the first half of each month. Thirty locally manufactured traps were placed in a single line around the rocky outcrops with 12 paces between each trap. Every 3-5 days the traps were moved to a new set of rocky outcrops or moved around the same cluster of rocks. Trapping sites were chosen at random and traps were baited with peanut butter and either flat bread or apple. Each trap was set in the evening between 16h00 and 18h00, checked at dawn and subsequently closed after any captured animals were removed. All non-focal study species were released at the capture site. Captured individuals were transferred to the laboratory for further processing.

2.2 Dissections

Captured animals were weighed to the nearest 0.01g using a digital scale (Scout Pro SPU123, Ohaus Corporation, Pine Brook, New York, U.S.A.). The individuals were then euthanized by giving the animal an overdose of ether in a glass jar. Once expired, whole blood was extracted with a heparin lined 1.0cc syringe with a 25g x 5/8" needle through exsanguinations of the heart. The blood was then placed in an Eppendorf tube and centrifuged at 3000rpm for 15 minutes. A 200μl Gilson pipette set to 100μl was used to collect plasma and placed into an Eppendorf to be stored at -20 °C for later hormonal analysis. The reproductive organs were dissected out and placed into Bouin's fixative and were removed 20 hours later, rinsed and placed in 70% ethanol.

The research was approved by the Animal Ethics Committee, University of Pretoria (ECO46-11) and by the Saudi Wildlife Authority.

2.3 Histology

Excess fat and connective tissue was cut off and the organs were weighed to the nearest 0.001g on a scale (Ohaus Corp. Pine Brook, N.Y., U.S.A.). Gonad (testes for males and ovaries for females) mass was corrected for body mass by dividing the gonad mass (mg) by the body mass (g) for each individual (relative gonad mass). The maximum length and width of the gonads were recorded using digital callipers (Mitutoyo American Corporation, Aurora, Illinois, USA). The measurements for the paired gonads were averaged and used to determine the average gonadal volume 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 underwent a process of dehydration with a series of ethanol baths of increasing concentrations (70%, 80%, 90%, 96%, and 100%) and xylene, after which they were embedded in wax. Sections were randomly taken from the testes using a rotary microtome set to cut at 7μm (820 Spencer, American Optical, Scientific Instrument Division, Buffalo, N.Y., U.S.A.), whereas the entire ovary was cut with a thickness of 6μm. All cuts were mounted with gelatin onto slides 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. Testes sections were examined for round seminiferous tubules with a light microscope (Diaplan, Ernst Leitz Wetzlar GmbH, Germany) and photographed at a magnification of x10 with a digital camera (Moticam 1000 1.3 M Pixel USB 2.0; Motic China Group, LTD., Xiamen, P.R. China) connected to the microscope. The diameter of 50 seminiferous tubules from each testis was measured using the program Motic Images Plus 2.0ML (Motic China Group, LTD., Xiamen, P.R. China). The reproductive status 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 ×100, ×200 and ×400. The follicular

stages were classified according to Bloom and Fawcett (1964), as primordial follicles, primary follicles, secondary follicles, tertiary follicles, Graafian follicles and corpora lutea. Primordial follicles were counted in every tenth section only, while all other follicle stages were counted throughout the entirety of each ovary as described in Medger et al. (2010).

2.4 Hormone analyses

Testosterone for *A. dimidiatus* was assayed from blood plasma samples using Coat-a-Count Testosterone kits (Diagnostic Products Corporation). Cross reactivity of the antibody was <5% with dihydrotestosterone and 19-hydroxyandrostendione and 1% with aldosterone, androstendione, cortisol, corticosterone, oestrone, methyltestosterone and progesterone. The plasma testosterone assay was validated in *A. dimidiatus* by undertaking tests for parallelism. After log-logit transformation of the data (Chard, 1978) the slopes of the lines were compared and found not to be significantly different from the reference preparation (ANOVA: $F < 0.001$, $p = 0.992$).The sensitivity of the assay was 20ng/l. Intra assay coefficient of variation for *A. dimidiatus* was 8.8%.

Blood plasma progesterone levels for *A. dimidiatus* females were determined 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 17α-hydroxyprogesterone (3.4%), 11-deoxycorticosterone (2.4%), 5β-pregnan-3, 20-dione (3.2%), and 5α-pregnan-3, 20-dione (9%). The plasma progesterone assay was validated by tests for parallelism. After log-logit transformation of the data (Chard, 1978) the slopes of the lines were compared and found not to differ significantly from the reference preparation; (ANOVA: $F < 0.001$, $p = 0.989$). The sensitivity of the assay was 0.36nmol/l and the intra assay coefficient of variation was 1.5%.

2.5 Data Analysis

      Due to the low number of animals captured in some months for analyses, we pooled data across seasons into four intervals of equal length starting with the month when captures were initiated: winter (December-February), spring (March-May), summer (June-August) and autumn (September-November). Only the data for testes volume, the number of primordial and primary follicles satisfied the criteria for parametric data (Kolmogorov-Smirnov (KS) test: $p = 0.200$), while a log-transformation produced a normal distribution in the body mass data. Consequently, we employed a general linear model (GLM) to analyse the effects of season on log body mass, relative testes mass, the number of primordial and primary follicles with season as independent variables. Additionally, we included sex as well as the interaction between sex and season as independent variables in the GLM for log body mass. Since the remaining reproductive parameters were not normally distributed and transformations were unsuccessful (KS test $p \leq 0.100$) we employed generalized linear models with a Gamma-distribution and a log-link function and season as independent variable for these parameters. Least significance difference (LSD) was used for post-hoc comparisons. To evaluate possible effects of temperature and/or rainfall on the reproductive parameters measured we repeated all models using mean seasonal temperature and cumulative rainfall for each season added as covariates. Initially, we included body mass as covariate for all parameters in the models. However, with the exception of ovarian volume the inclusion of the variable did not affect the results and we will hence with the exception of ovarian volume report the results without the variable body mass. All statistical analyses were implemented using the SPSS version 21.0. Results are presented as mean $\pm$ standard error (SE) and were found to be significant at $p \leq$ 0.05.

3. Results

3.1 Climate

During the study period rainfall peaked in April and was entirely absent from September to March as well as in June (Table 1). Thus, the entire rainfall fell during spring and summer and with a total of less than 62 mm was below the long-term annual average of ?? for the study area (Ref?). Peak temperatures around 29°C occurred between June and September while the lowest mean temperature of 16.6°C was recorded in December (Table 1).

Table 1. Number of *Acomys dimidiatus* captured on per season at the National Wildlife Research Centre at Taif, Saudi Arabia during the study period. Numbers in brackets indicated the number of pregnant females.

	Month	Females	Males	Mean temperature [°C]	Cumulative rainfall [mm]
Winter	December	9	8	16.6	0
	January	9	9	19.0	0
	February	5	8	18.3	0
		23 (0)	**25**	**18.0**	**0**
Spring	March	5	6	20.1	0
	April	5	8	21.9	31.8
	May	5	4	27.2	7.9
		15 (2)	**18**	**23.1**	**39.7**
Summer	June	5	5	28.8	0
	July	5	2	29.9	12.7
	August	5	6	29,6	9.4
		15 (4)	**13**	**29,4**	**22.1**
Autumn	September	4	5	29.6	0
	October	3	6	26.0	0
	November	3	6	21.1	0
		10 (1)	**17**	**25.5**	**0**

3.2 Body mass

In total 73 males and 63 females *A. dimidiatus* were captured during the study period (Table 1). Body mass did not differ significantly between males (35.7 ± 1.0g) and females (36.4 ± 0.7g, χ^2=0.013, df=1, p=0.911). However, body mass varied significantly between seasons (χ^2=9.690, df=3, p=0.021). It was significantly higher in summer compared to all other seasons (p≤0.038), but did not differ significantly between any of the other seasons (p≥0.397). Furthermore, the interaction between sex and season was significant (χ2=13.8676, df=3, p=0.003). Post hoc analyses showed that body mass was significantly greater for males than females in winter (LSD: p=0.001, Fig. 1), but none of the other seasons (p≥0.120). Furthermore, among females body mass was lower in winter compared to summer (LSD: p<0.0001) and autumn (LSD: p=0.017, Fig. 1). In addition, it was lower in spring compared to summer (LSD: p=0.013). No other pairwise comparison was significant for females (p≥0.121). For males body mass was significantly greater in winter compared to autumn (LSD: p<0.048) but did not differ between any of the other seasons (p≥0.083).

Fig. 1 Seasonal variation in body mass (mean ± SE) of *A. dimidiatus* at the National Wildlife Research Centre at Taif, Saudi Arabia.
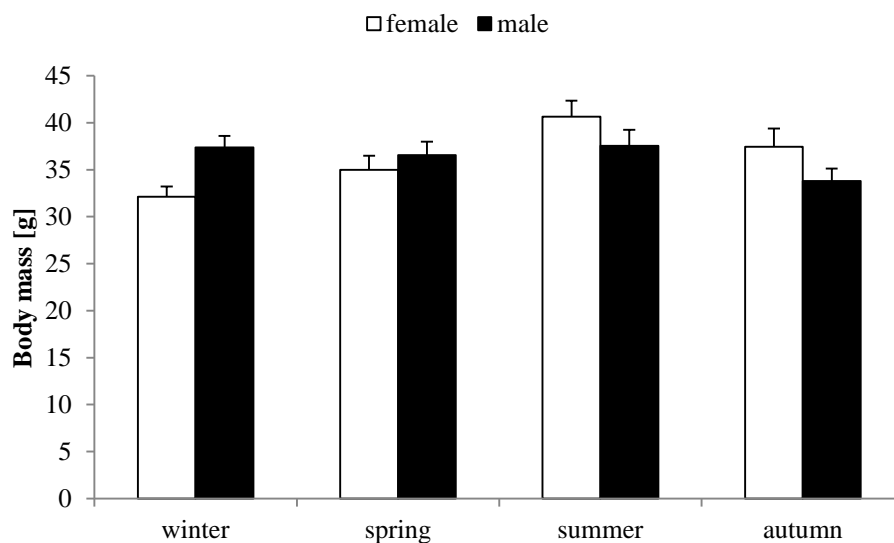
3.3 Males

There were significant differences in relative testes mass between seasons ($\chi^2 =$ 34.536, df = 3, p < 0.0001, Table 2). It was significantly lower in winter compared to summer and autumn (p≤0.002, Fig. 2a). None of the remaining pairwise comparisons was significant (p≥0.151). Relative testes mass increased significantly with temperature ($\chi^2 = 17.412$, df = 1, p < 0.0001, estimate: 0.106 ± 0.025, supplementary Fig. S1) but not rainfall ($\chi^2 = 0.728$, df = 1, p = 0.393, Table 2). However, removal of three extreme values (supplementary material Fig. S1) resulted in rainfall ($\chi^2 = 20.980$, df = 1, p < 0.0001, estimate: 0.019 ± 0.004) instead of temperature ($\chi^2 = 2.794$, df = 1, p = 0.095) significantly affecting testes mass.

Table 2. Overview of the effects of season, rainfall and temperature on the reproductive parameters measured for male and female *A. dimidiatus*. Note that gonads refer to testes and ovaries for males and females, respectively. In addition, hormones are testosterone and progesterone, respectively.

	males			females		
	season	rainfall	temperature	season	rainfall	temperature
Gonad mass	*	ns[a]	*↑[a]	*	ns	ns
Gonad volume	*	*↑	ns	*	*↑	ns
Seminiferous tubules	*	*↑	ns	-	-	-
Hormone levels	*	*↑	ns	*	ns	↑*
Primordials	-	-	-	ns	ns	ns
Primary	-	-	-	*	*↑	ns
Secondary	-	-	-	*	*↑	ns
Tertiary	-	-	-	*	*↑	ns
Graafian	-	-	-	*	*↑	ns
Corpora lutea	-	-	-	*	ns	*↑

-: parameter not measured, ns: factor not significant, *: factor significant, arrows indicate that parameter increased with increasing rainfall or temperature. [a]: results were reversed for rainfall and temperature after the removal of three extreme values.

Fig. 2 Seasonal variation of (a) relative testes mass, (b) testes volume, (c) seminiferous tubule diameter and (d) plasma testosterone concentration of A. dimidiatus at the National Wildlife Research Centre at Taif, Saudi Arabia. Displayed are means ± SE.
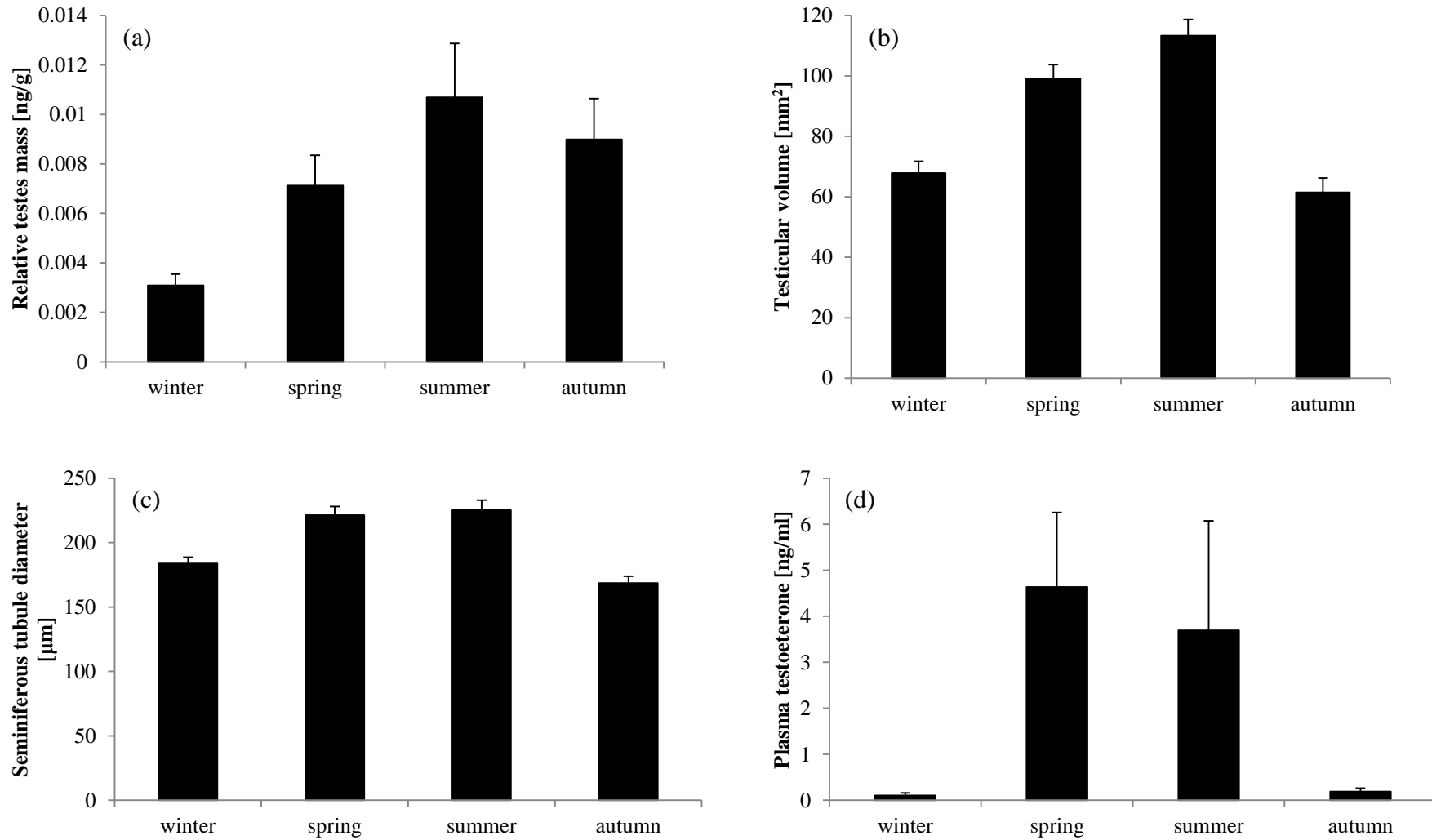
The testes volume differed significantly between seasons (χ^2 = 80.782, df = 1, p < 0.0001, Table 2). It was significantly higher in summer compared to all other seasons (p ≤ 0.043, Fig. 2b). In addition, it was significantly greater in spring compared to winter and autumn (p < 0.0001 for both, Fig. 2b). In contrast, it did not differ significantly between winter and autumn (LSD: p=0.294). The testes volume increased significantly with cumulative rainfall (χ^2 = 18.399, df = 1, p < 0.0001, estimate: 1.094 ± 0.255, Fig. S2a) but not mean temperature (χ^2 = 1.620, df = 1, p = 0.203, Table 2).

The diameter of the seminiferous tubule differed significantly between seasons (χ^2 = 52.360, df = 3, p < 0.0001, Fig. 2c). It was significantly lower in winter compared to all other seasons (p ≤ 0.033). Furthermore, it was significantly lower in autumn compared to spring and summer (p < 0.0001 for both, Fig. 2c). In contrast, the seminiferous tubule diameter did not differ significantly between spring and summer (LSD: p=0.710). It increased significantly with increasing rainfall (χ^2 = 21.291, df = 1, p < 0.0001, estimate: 0.008 ±0.002, Fig. S2b), but not with temperature (χ^2 = 0.084, df = 1, p = 0.771, Table 2).

The plasma testosterone concentration varied significantly with season (χ^2 = 79.768, df = 1, p < 0.0001, Fig. 2d). Post hoc comparisons showed that it was significantly lower in winter and autumn compared to spring and summer (p <0.0001 for all comparisons, Fig. 2d). However, it did not differ significantly between winter and autumn (LSD: p = 0.737) and between spring and summer (LSD: p = 0.481). The concentration of plasma testosterone increased significantly with rainfall (χ^2 = 37.080, df = 1, p < 0.0001, estimate: 0.054 ±0.009, Fig. S2c), but not temperature (χ^2 = 0.283, df = 1, p = 0.595). This was not affected by the removal of three extreme values.

3.4 Females

Pregnant females were caught during all seasons, apart from the winter (Table 1). The relative ovarian mass differed significantly between seasons ($\chi^2 = 9.266$, df = 3, p = 0.026, Fig. 3a). It was significantly lower in winter compared to spring and summer (p ≤ 0.027, Fig. 3a). No other pairwise comparison between seasons was significant (p ≥ 0.248). Neither rainfall ($\chi^2 = 1.474$, df = 1, p = 0.225) nor temperature ($\chi^2 = 2.299$, df = 1, p = 0.129) had a significant effect on relative ovarian mass.

The ovarian volume differed significantly between seasons ($\chi^2 = 39.646$, df = 3, p < 0.0001, Fig. 3b, Table 3). Post hoc tests showed that it was significantly lower during winter compared to all other seasons (p ≤ 0.038, Fig. 3b). In addition, it was significantly higher in summer compared to autumn (LSD: p = 0.007). None of the remaining pairwise comparisons was significant (p ≥ 0.089). Ovarian volume was not affected significantly by temperature ($\chi^2 = 1.664$, df = 1, p = 0.197). In contrast, it increased significantly with both rainfall ($\chi^2 = 11.318$, df = 1, p = 0.001, estimate: 0.012 ± 0.044, Fig S3a) and body mass ($\chi^2 = 20.103$, df = 1, p < 0.0001, estimate: 0.036 ±0.012).

Table 3. Number of primordial, primary, secondary and tertiary follicles per season for *Acomys dimidiatus* at the National Wildlife Research Centre at Taif, Saudi Arabia. Displayed are means ± SD.

	N	Primordial	Primary	Secondary	Tertiary
Winter	23	628.57 (±62.11)	43.52 (±4.34)	19.17 (±3.94)	2.72 (±0.65)
Spring	13	602.42 (±72.97)	60.35 (±5.52)	42.23 (±4.92)	7.35 (±1.14)
Summer	15	607.03 (±77.12)	51.13 (±3.29)	38.07 (±5.45)	7.32 (±0.82)
Autumn	10	502.60 (±58.59)	46.15 (±4.93)	20.10 (±4.02)	4.65 (±0.93)

Fig. 3 Seasonal variation of (a) relative ovarian mass, (b) is ovarian volume, (c) Graafian follicle (open bars) and corpora lutea (solid bars) counts as well as (d) plasma progesterone concentration of A. dimidiatus at the National Wildlife Research Centre at Taif, Saudi Arabia. Displayed are means ± SE.
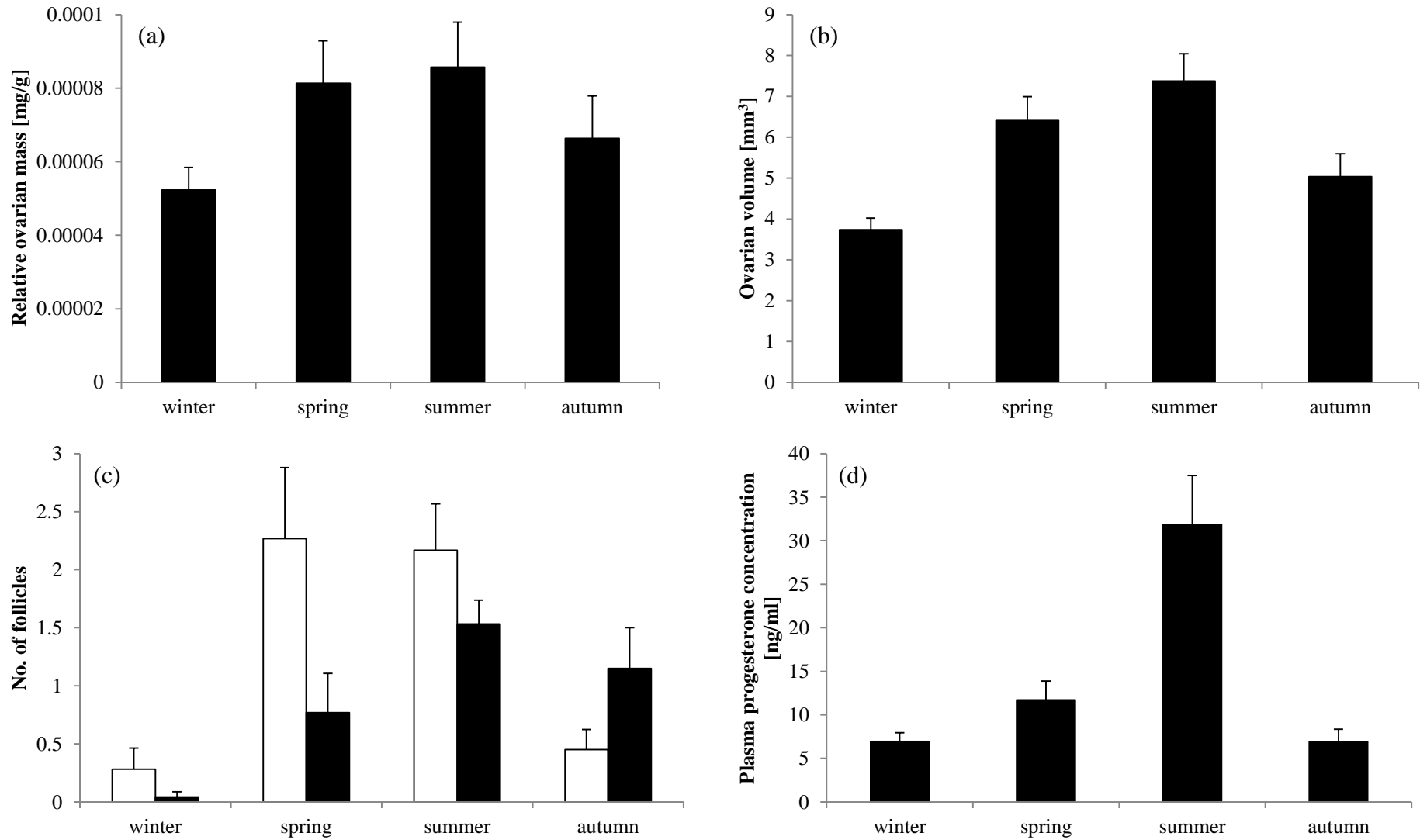
Follicle counts are summed up in Table 2. Primordial follicle counts did not differ significantly between seasons ($\chi^2 = 1.601$, df = 3, p = 0.659, Table 3). Similarly, neither rainfall ($\chi^2 = 0.320$, df = 1, p = 0.572) nor temperature ($\chi^2 = 0.060$, df = 1, p = 0.806) affected the primordial counts significantly (Table 2).

Primary follicle counts differed significantly between seasons ($\chi^2 = 8.146$, df = 3, p = 0.043, Table 2-3). They were significantly lower in winter compared to spring (LSD: p = 0.006), but did not differ significantly between any other seasons (p ≥ 0.054). While the number of primary follicles was not significantly affected by temperature ($\chi^2 = 0.401$, df = 1, p = 0.527), it increased significantly with rainfall ($\chi^2 = 6.614$, df = 1, p = 0.010, Table 2).

The numbers of secondary follicles differed significantly between seasons ($\chi^2 = 16.826$, df = 3, p = 0.001, Table 3). It was significantly lower in winter compared to spring and summer (p ≤ 0.010). In addition, it was significantly lower in autumn compared to spring and summer (p ≤ 0.025). None of the remaining pairwise comparisons was significant (p ≥ 0.692). Secondary follicle counts were not significantly affected by temperature ($\chi^2 = 0.136$, df = 1, p = 0.721). In contrast, they increased significantly with rainfall ($\chi^2 = 7.520$, df = 1, p = 0.006, estimate: 0.026 ±0.009, Fig. S3b, Table 2).

The number of tertiary follicles differed significantly between seasons ($\chi^2 = 24.086$, df = 3, p < 0.0001, Table 3). It was significantly lower in winter compared to spring and summer (p = 0.001 for both). None of the remaining pairwise comparisons was significant (p ≥ 0.087). The number of tertiary follicles increased significantly with rainfall ($\chi^2 = 7.163$, df = 1, P = 0.007, estimate: 0.020 ±0.008, Fig. S3c), but not temperature ($\chi^2 = 2.394$, df = 1, p = 0.122, Table 2).

The number of Graafian follicles differed significantly between seasons ($\chi^2 = 49.137$, df = 3, p < 0.0001, Fig. 3c). It was significantly greater in spring and summer compared to both winter and autumn (p < 0.0001 for all comparisons, Fig. 3c), while it did not differ

significantly between summer and spring (LSD: p = 0.864) or between winter and autumn

(LSD: p = 0.521). Graafian follicles counts increased significantly with rainfall (χ^2 = 17.396,

df = 1, p < 0.0001, estimate: 0.028 ±0.007, Fig. S3d), but not temperature (χ^2 = 1.514, df = 1,

p = 0.219, Table 2). This result did not change when one outlier was removed from the data

set.

The number of Corpora lutea differed significantly between seasons (χ^2 = 52.371, df =

3, p < 0.0001, Fig. 3c). It was significantly lower in winter compared to all other seasons (p ≤

0.001, Fig. 3c). In addition, it was significantly greater in summer compared to spring (LSD:

p = 0.019, Fig. 3c). None of the remaining pairwise comparisons was significant (p ≥ 0.255).

Corpora lutea counts increased significantly with temperature (χ^2 = 26.007, df = 1, p <

0.0001, estimate: 0.074 ±0.015, Fig. S4a), but not rainfall (χ^2 = 0.470, df = 1, p = 0.493,

Table 2).

The plasma progesterone concentration differed significantly between seasons (χ^2 =

51.318, df = 3, p < 0.0001, Fig. 3d). It was significantly higher in summer compared to all

other seasons (p ≤ 0.001, Fig. 3d). In addition, it was significantly higher in spring compared

to winter (LSD: p = 0.044, Fig. 3d). None of the remaining pairwise comparisons was

significant (p ≥ 0.064). The plasma progesterone concentration increased significantly with

temperature (χ^2 = 18.063, df = 1, p < 0.0001, estimate: 0.113 ±0.027, Fig. S4b), but not

rainfall (χ^2 = 0.196, df = 1, p = 658). This result was not affected by the removal of three

extreme values.

4. Discussion

The current study found that female body mass was lowest in winter, while it was greater

in winter compared to autumn for males. This could partially be attributed to the recruitment

of a new generation into the population (Medger et al., 2010, 2012a). However, laboratory

studies have shown that food limitation as well as increases in dietary salinity may result in reductions in body mass of arid adapted *A. russatus*, but not mesic adapted *A. cahirinus*, particularly in females (Wube, Fares & Haim, 2008a; Bukovetzky et al., 2012). Consequently, the observed lower body mass during winter may also be a result of the reduced food and water availability during this season. In addition, the presence of pregnant individuals during all seasons but winter may have contributed to the sex difference in this season.

Despite pregnant females being present for most of the year and sperm being found in males during all seasons, the overwhelming majority of the reproductive parameters assessed in the current study exhibited seasonal patterns with a cessation of reproductive activity during winter. Unlike several laboratory studies of *Acomys spp.* we did not differentiate between stages of spermatogenesis (El-Bakry et al., 1998; Wube et al., 2008b, 2009; Bukovetzky et al., 2012; Medger et al., 2012a) and can thus not exclude the possibility that the proportion of mature sperm may have been lower in winter. However, the observed patterns support our hypothesis that the period of reproductive activity of *A. dimidiatus* is shorter in the more arid study site compared to another location in Saudi Arabia as a result of lower rainfall and suggests that *A. dimidiatus* pursues an opportunistic breeding strategy (Al-Khalili and Delany, 1986). Largely maintaining reproductive function throughout the year can ensure quick responses to reproductive opportunities that may arise from unpredictable changes in water and/or food availability as is generally the case in desert environments (Louw and Seely, 1982; Schneider, 2004; Bronson, 2009).

Although the seasonal profiles we observed for gonad (i.e. testes and ovaries, respectively) mass and volume, follicle counts and hormone profiles for both sexes may also be triggered by changes in photoperiod; the strong effect of rainfall we found in our study population suggests that unlike photoperiod is not the main cue regulating reproduction in the

study species. This corresponds with the results from laboratory studies of *Acomys* spp. reporting none or moderate effects of photoperiod on spermatogenic indices or testosterone levels. In addition, photoperiod did not affect the reproductive tract morphometrics or histology in arid adapted *A. russatus* (Wube et al., 2008a,b,). In contrast, mesic adapted *A. cahirinus* and *A. spinosissimus* were photoresponsive and significant seasonal fluctuations in reproductive parameters as well as pregnancies have been reported for a wild population of *A. spinosissimus* (Wube et al., 2008b; Medger et al., 2010, 2012a; Bukovetzky et al., 2012; Ben-Zaken, Haim and Zubidat, 2013). Our data does not allow us to determine whether the observed changes in reproductive activity are a direct result of rainfall or mediated via dietary salinity as has been shown for other *Acomys* spp. (Shanas and Haim, 2004; Wube et al., 2008b, 2009; Bukovetzky et al., 2012). However, our observations corroborate the hypothesis that in the genus *Acomys* populations occurring in relatively mesic habitats predominately use photoperiodic cues to regulate their reproduction, while in arid habitat reproductive function is largely maintained throughout the year and other environmental cues such as rainfall become more important. This appears adaptive for desert environments in light of the observation that desert vegetation flourishes and food availability increases in response to precipitation (Noy-Meier, 1973; Ghazanfar, 1997).

Despite the strong effects of rainfall apparent in the study species, testes mass, the number of corpora lutea as well as the progesterone concentration measured correlated with temperature and not rainfall. However, with regards to testes mass this is likely to be a result of three males with extremely enlarged testes and removal of these data points resulted in a significant relationship with rainfall not temperature for this parameter. Nevertheless, temperature has been implicated as a cue for the regulation of reproduction in small mammals due to the energetic constraints small mammals experience during cold periods when energy expenditure for thermoregulation may constrain the energetic investment into reproduction

(Bronson and Heideman, 1994; Bronson, 2009). As a result, several small mammal species have been reported to cease reproduction during energetically demanding periods (Fietz et al., 2004; Canale, Perret and Henry, 2012) and this may also account for the observed cessation of reproduction in winter in the study species. A similar pattern has also been found in *A. cilicius* (Kivanç et al., 2013). However, given that in females only the corpora lutea and progesterone levels, that are both indicators of pregnancies, were correlated to temperature, it is more likely that this correlation is a result of the time delay between rainfall triggered initiation of reproduction and the resulting pregnancies in the study species.

The reproductive pattern observed for *A. dimidiatus* in the current study strongly contrasts with those of the sympatric Baluchistan gerbil (*Gerbillus nanus*) during the same study period (Sarli et al., 2015). While most male reproductive parameters measured in *G. nanus* showed patterns corresponding to those observed for male *A. dimidiatus* in the current study, there were no seasonal effects on ovarian volume and the counts of primordial, primary, secondary or tertiary follicles and pregnant females were almost exclusively caught in summer (Sarli et al., 2015). In addition, none of the female reproductive parameters were affected by rainfall. Only the number of Graafian follicles and corpora lutea and none of the other parameters were affected by temperature. Temperature also correlated with male testes mass and volume as well as seminiferous tubule diameter while rainfall affected testes volume, seminiferous tubule diameter and testosterone concentrations in *G. nanus* (Sarli et al., 2015). Thus, although experiencing the same climatic conditions, *G. nanus* and *A. dimidiatus* use very different environmental cues to regulate their reproduction. Such divergent reproductive strategies in sympatric desert rodents have previously been reported for rodent communities from North America and Australia (Kenagy et al., 1985; Brown and Zeng, 1989; Predavec, 1994). It has been suggested that differences in body mass may contribute to the observed patterns in the study site (Kenagy et al., 1985). With a mean body

mass of 20.4g (±0.21) *G. nanus* achieves only 56.6% of the body mass of *A. dimidiatus* and is thus likely to be more energetically constrained than *A. dimidiatus* (Schmidt-Nielsen, 1997). Consequently, low temperatures are more likely to be energetically taxing for *G. nanus* and deplete energy resources that could otherwise be invested in reproduction. Similarly, in the Californian desert, the smaller pocket mice (*Perognathus spp.*) exhibited a later onset and shorter breeding period than the larger kangaroo rats (*Dipodomys spp.*) and antelope ground squirrel (*Ammospermophilus leucurus*) (Kenagy et al., 1985). In addition, differences in the diet of *Acomys* spp. and *G. nanus* are likely to contribute to the divergent patterns. While the former rely on an omnivorous diet the latter is granivorous (Harrison and Bates, 1991; Wilson and Reeder, 2005). Including food items such as insects in their diet provides *A. dimidiatus* not only with a higher energetic input, but insects will also have higher water content than the grass seeds consumed by *G. nanus*. Similarly, the extended and predictable breeding season of ground squirrels has been attributed to their omnivorous diet compared to species such as the granivorous Merriam's kangaroo rat (*D. merriami*) with their fluctuating breeding phenology (Kenagy et al., 1985). Consequently, the contributions of body size and diet on the reproductive patterns of desert rodents deserve further attention in future studies.

In conclusion, in an arid region of Saudi Arabia *A. dimidiatus* reproduces seasonally with the cessation of reproduction during winter. Reproduction of *A. dimidiatus* appears to be tightly linked to rainfall, although whether this is regulated directly by rainfall or indirectly via mechanisms such as food salinity remains to be determined. However, the presence of sperm throughout the year suggests that they may retain the potential to reproduce opportunistically should favourable conditions arise. Across the genus *Acomys* the available evidence indicates that reproduction is mainly linked to changes in photoperiod while responding to water-related cues (either direct or indirect) allows arid populations to time their reproduction with the most favourable conditions for successful reproduction.

Acknowledgements

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Supplementary information

Fig. S1 Relationship between relative testes mass and mean temperature for *A. dimidiatus* at

Taif, Saudi Arabia.
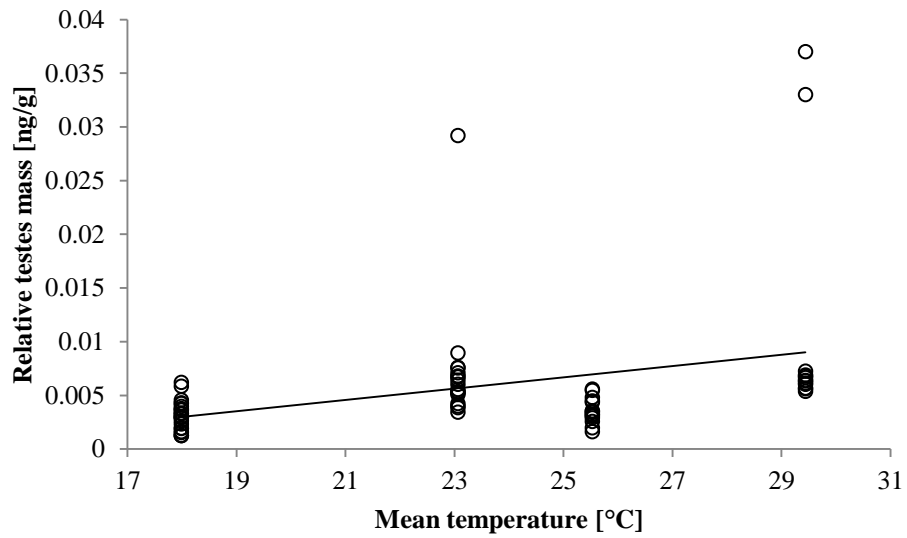
Fig. S2 Relationship between (a) testes volume, (b) seminiferous tubule diameter and cumulative rainfall for male *A. dimidiatus* at Taif, Saudi Arabia.
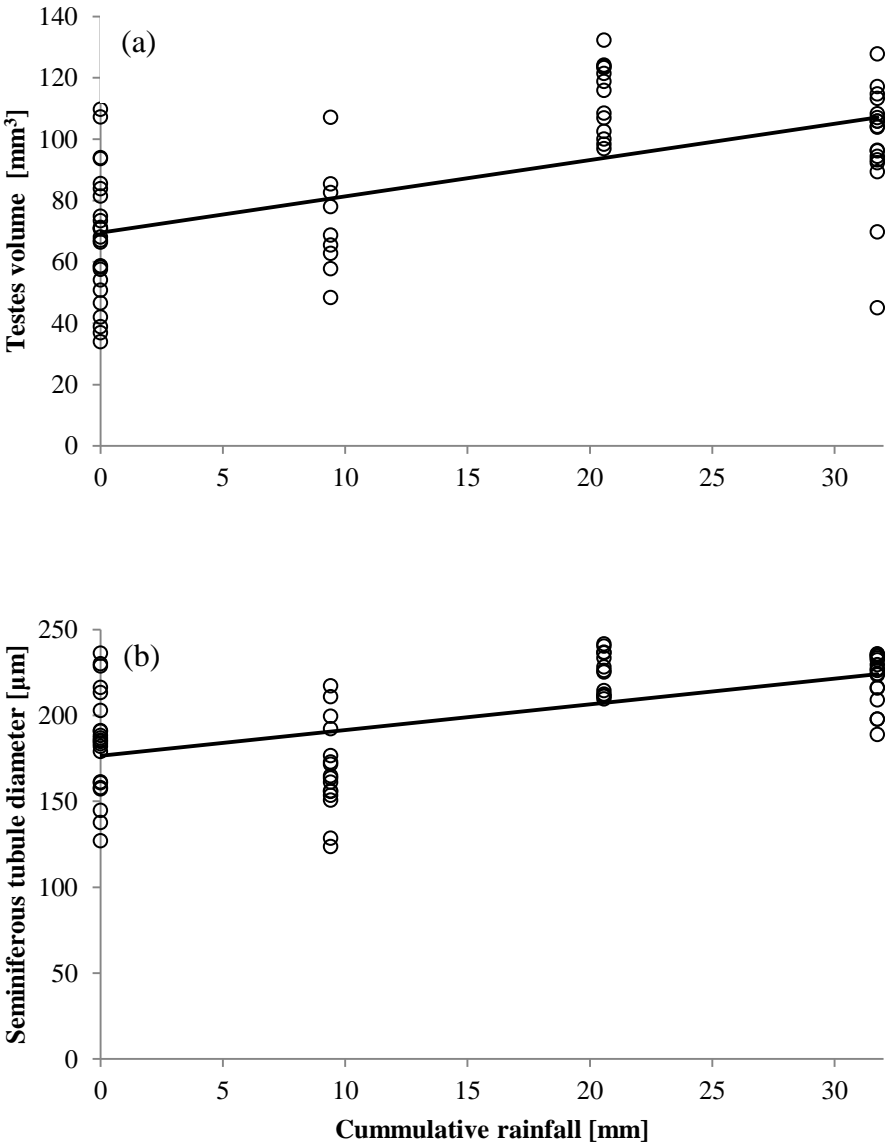
Fig. S3 Relationship between the number of (a) ovarian volume, (b) secondary, (c) tertiary, (d) Graafian follicles and cumulative rainfall for female *A. dimidiatus* at Taif, Saudi Arabia.
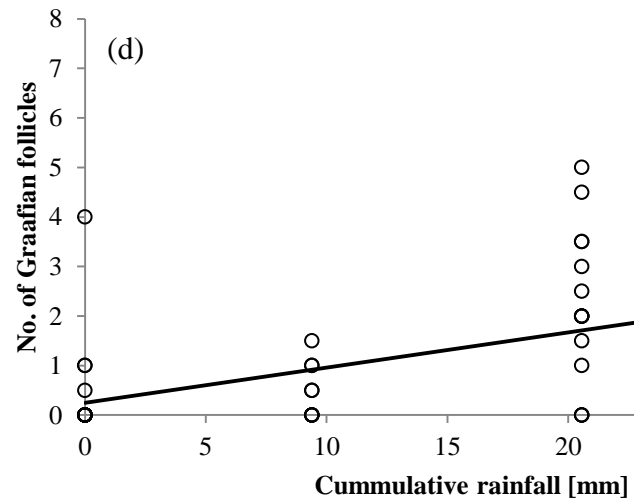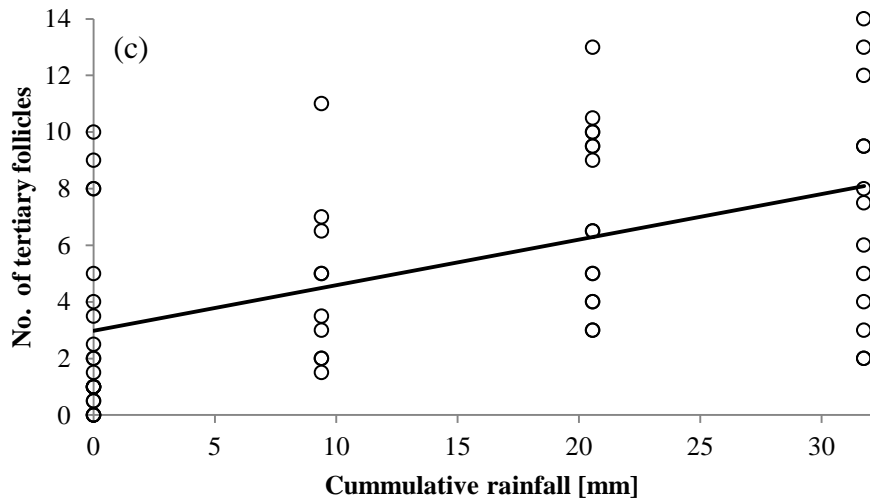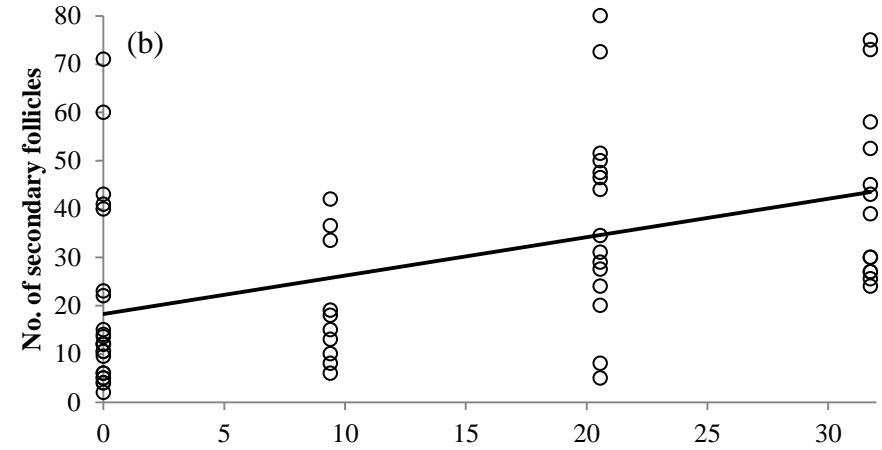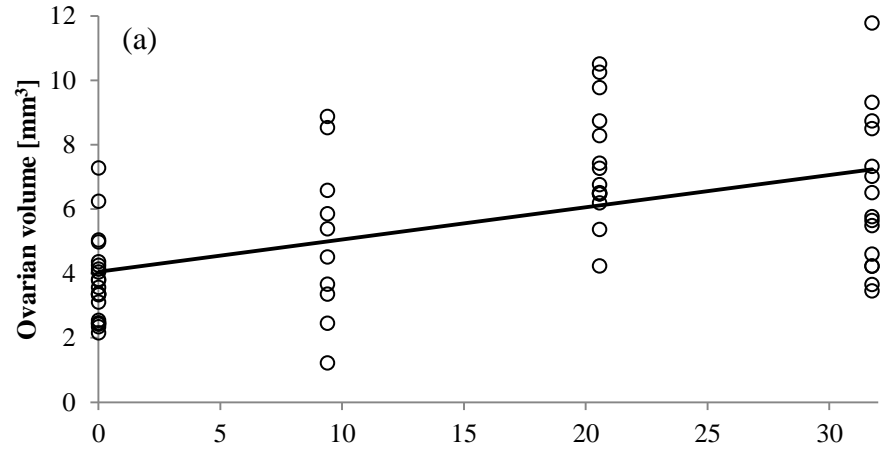
Fig. S4 Relationship between (a) the number of corpora lutea, (b) progesterone levels and temperature for female *A. dimidiatus* at Taif, Saudi Arabia.