

The reproductive biology of the Ethiopian hedgehog, *Paraechinus aethiopicus*, from central Saudi Arabia: The role of rainfall and temperature

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Highlights

- Ethiopian hedgehog is a seasonal breeder.
- Breeding season occurs from spring and to end of summer.
- Female reproduction is activated in spring with the first occurrence of rain.
- Male reproduction is activated in late winter due to changing temperatures.
- Precipitation crucial for onset of female reproduction.

Abstract

We set out to test whether the Ethiopian hedgehog, *Paraechinus aethiopicus* is a seasonal or aseasonal breeder and assess which environmental cues bring about both reproductive recrudescence and its subsequent regression. In this study, the body mass, morphometry of the reproductive tract the histology of the reproductive organs and the hormone concentrations of males and females were studied over 12 consecutive months in a wild population of the Ethiopian hedgehog from central Saudi Arabia. Using these data we investigated the potential proximate environmental cues that may trigger the onset of reproduction.

Temperature is important for the initial activation of the males by bringing about an increase in testosterone concentration. In female hedgehogs the first rains trigger the onset of reproductive activation with ovulation. In turn increased temperature brings about the final activation of the males with increased testes size and seminiferous tubule diameter. Pregnancies were first recorded in early spring and summer coinciding with a rise in plasma progesterone concentration that peaked in spring. The Ethiopian hedgehog was found to exhibit marked seasonal reproduction. This study strengthens the findings that changes in rainfall and temperature in the Middle East are crucial cues for the onset of reproduction in small mammals.

Keywords: Reproduction ; Environmental cues ; Seasonality ; Hormones ; Histology

1. Introduction

Small desert mammals inhabiting these arid regions need to regulate their energy expenditure and water turnover (Schwimmer and Haim, 2009). Deserts are hostile environments with marked daily and seasonal temperatures as well as sporadic rainfall patterns that results in poor primary productivity as a consequence hedgehogs need to cue into times of the year when survival of offspring will be maximal (Schmidt-Nielsen, 1964).

Reproduction in small mammals may be regulated by a number of proximate factors such as photoperiod, temperature, rainfall and in turn availability of food resources (Bronson and Heideman, 1994; Wube et al., 2009). Photoperiod is a common proximate cue utilised by organisms to time reproduction as the changes are constant from year to year (Prendergast et al., 2001; Bronson, 2009). Photoperiod is the cue of choice in organisms inhabiting high latitudes where seasonal variability in climatic conditions and food is predictable. However, in more arid regions different proximate factors may be operational such as rainfall, temperature or food availability (El-Bakry et al., 1998; Shanas and Haim, 2004).

The pattern of rainfall and subsequent availability of water in desert environments has been found to be a very important cue for the onset of reproductive activity (Sicard et al., 1993; Bukovetzky et al., 2012; Khammar and Brudieux, 1987). Reproduction in desert areas is precarious because of the scarcity and unpredictability of rainfall, the extreme temperatures and the associated lack of food resources. Rainfall may be used as an indicator of primary production in arid environments (Nelson and Desjardins, 1987). As a general rule reproduction usually only arises in deserts when food resources and water coincide with favourable temperatures that in turn promote optimal conditions for mating, pregnancy and the subsequent production of young that maximises their survival (Bronson, 1988).

To date there has been relatively few detailed studies investigating reproduction over an entire calendar year in small mammals from Saudi Arabia. Henry and Dubost (2012), studying Cheeseman's gerbil, *Gerbillus cheesmani*, and Sarli et al. (2015, 2016), studying the Baluchistan gerbil, *Gerbillus nanus*, and the Arabian spiny mouse, *Acomys dimidiatus*, from south western Saudi Arabia, found these murids to cue their reproduction to rainfall. In fact, both the males and females synchronise their reproduction to the short spring rains, rapidly producing offspring that can benefit from the subsequent primary productivity.

The Ethiopian hedgehog, *Paraechinus aethiopicus*, is a small terrestrial nocturnal mammal ranging in body mass from between 280 and 500 g with a spiny integument and occurs in the semi-arid and arid deserts of North Africa and the Middle East. In Africa, it is distributed from Mauritania, Western Sahara and Morocco in the west through to Egypt, Sudan, Eritrea and Ethiopia in the east. In the Middle East it has been recorded from Syria, Jordan, Iraq and marginally in Iran, and from most of the Arabian Peninsula except for Hijaz/Asser mountains (Harrison and Bates, 1991; He et al., 2012). The Ethiopian hedgehog, is adapted to the harsh and dry conditions of the desert (Essghaier et al., 2015). It is found in wadis, around oases and sandy plains where there is vegetation, they are even known to frequent the coast (Harrison and Bates, 1991).

The hedgehog feeds on insects and other invertebrates, and may also consume vegetation and vertebrates. Even though they have a wide distribution there is currently a dearth of information pertaining to the reproductive biology of the hedgehog, apart from basic information on time of mating, courtship and numbers of young produced (Al-Musfir and Yamaguchi, 2008; Yamaguchi et al., 2013).

There is limited information on the reproductive biology of the desert hedgehog which is mainly confined to the mating behaviour, gestation period and weaning period from around rubbish dumps in Qatar (Yamaguchi et al., 2013). Yamaguchi et al. (2013) observed mating events to occur in the Ethiopian hedgehog mainly during late winter and into early spring (February and March), following a period of hibernation (December to January), however, they also record a second mating period in early summer (May to July). This implies that females may have the reproductive potential to breed twice a year, whereas males appear to be sexually active throughout the year. Currently, there is no reported histological or hormonal profiles to confirm the reproductive biology of the Ethiopian hedgehog from a natural environment (Yamaguchi et al., 2013).

In this study we investigated the reproductive biology of the Ethiopian hedgehog from central Saudi Arabia by collecting hedgehogs and euthanizing them on a monthly basis with an aim to measuring the testosterone in males and progesterone in females over an entire calendar year. In addition we examined the gross morphology and detailed histology from the post-mortem of the samples. We hypothesized that reproduction in the Ethiopian hedgehog would be highly dependent on rainfall. We predicted that reproductive recrudescence of the gonads and an associated elevation of the reproductive sex hormones would occur following the rains and the increase in ambient temperature, whereas the ovaries and testes would regress and the hormone concentrations would be lower during the cooler and dry months. As a consequence we predicted that the Ethiopian hedgehog would reproduce seasonally following the rains when food availability would be maximal.

2. Methods and materials

2.1. Study site and animal capture

Sampling took place in central Saudi Arabia, in and around the town of Unizah (26.0840° N, 43.9940° E) (Al Qassim Province). The capture zones were predominantly desert ecosystem comprising annual grasses, small herbaceous plants, small trees, rocky outcrops and small wadis. Hedgehogs were captured on a monthly basis by hand at night, using a beam light while driving around from July 2014 until June 2015 (Table 1).

Table 1. Numbers of the Ethiopian hedgehog captured on a monthly basis in Unizah, Saudi Arabia from July 2014 until June 2015.

Season		Summer			Autumn			Winter			Spring		
Month		Jun.	Jul.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May.
Male		11	4	5	6	5	4	0	9	6	4	5	7
Female		2	6	5	3	7	5	0	1	4	4	4	3

Collection of hedgehogs was authorised by the Saudi Wildlife Authority and the research protocol was approved by the animal ethics committee of the University of Pretoria (Ethics number EC017-16) which follows the South African National Standards for animal welfare and research. The animals were handled and euthanised humanely. The animals were used for a number of other research studies to ensure maximum use of the euthanised animals. The animal experimentation was conducted in accordance with ARRIVE guidelines and carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments.

2.2. Dissection

On the day of capture the animals were weighed using Sartorius U4600P balance 1200 g Capacity, with 01 g readability. The animals were euthanised using an overdose of ether in a euthanizing chamber. Once the animal had expired, whole blood was obtained from the heart using a 3.0 cc syringe with a 25 g x 5/8" needle. Immediately after collection, the blood was transferred into a Vacutainer lithium heparin blood collection tubes and centrifuged at 3000 rpm for 15 min. Plasma was collected and stored at -20 °C for hormonal analysis.

2.3. Histology

Fresh ovaries and testes were weighed using a balance Adam Equipment PW 254 PW Series Analytical Balance, 250 g Capacity, with 0.0001 g readability. The length and width were measured (mm) using a pair of digital callipers (Mitutoyo American Corporation, Aurora, Illinois, USA) once fat and connecting tissue were removed. Testicular and ovarian volume (mm^3) was calculated using gonadal length and width by using the formula for the volume of an ellipsoid ($V = 4/3 \pi ab^2$ where a represents half the maximum length and b half the maximum width (Woodall and Skinner, 1989). The gonads were initially fixed for 24 h in Bouin's fixative and subsequently subjected to a series of histological preparations, involving dehydration and embedding in wax (Drury and Wallington, 1967). Ovaries were cut into 5 μm -thick sections and testes were cut into 8 μm -thick sections with a rotary microtome (820 Spencer, American Optical, Scientific Instrument Division, Buffalo, N.Y., U.S.A.). Sections were mounted in consecutive order on microscope slides with gelatine and dried in an oven at 36 °C for 48 h and thereafter stained with Ehrlich's haemotoxylin and counter-stained with eosin (Drury and Wallington, 1967). Testicular sections were used to measure round seminiferous tubules with a light microscope (Diaplan, Ernst Leitz Wetzlar GmbH, Germany) and photographed at $\times 10$ magnification with a digital camera (Moticam 1000 1.3 M Pixel USB 2.0; Motic China Group, LTD., Xiamen, P.R. China). The programme Motic Images Plus 2.0 ML (Motic China Group, LTD., Xiamen, P.R. China) was used to measure the diameter of 100 seminiferous tubules (μm).

Ovarian sections, taken from both ovaries of each female, were examined serially for stages of follicular growth with a light microscope at $\times 100$, $\times 200$ and $\times 400$ magnifications. Follicular stages were identified and classified according to Bloom and Fawcett (1964). Primordial follicles are generally observed in large numbers on the periphery of the ovary. For all other follicular stages the total number was counted throughout each ovary.

2.4. Hormone analysis

Coat-a-count hormone kits (IBL International GmbH, Hamburg, Germany) were used to measure testosterone and progesterone concentrations in blood plasma of male and female hedgehogs, respectively. This method uses a solid phase radioimmunoassay which is based on a radioactive labelled hormone (I.e., ^{125}I -labelled testosterone) and the hormone in the sample competing for antibody sites (Abraham, 1977). The amount of bound ^{125}I -labelled hormone left in the test tube is counted with a gamma counter. A calibration curve with a known hormone concentrations is generated with the same method and used to obtain the hormone concentration of the sample. All assays were carried out according to the manufacturer's protocol. A serial dilution curve was generated by serially diluting a sample from an individual with high hormone concentrations (I.e., pregnant female). The slopes of the calibration curve and serial dilution curve were tested for parallelism with a General Linear Model (GLM) after log-logit-transformation (Chard, 1987) to validate this method for the blood plasma testosterone and progesterone concentrations in male and female Ethiopian hedgehogs, respectively.

Plasma testosterone concentrations were determined using a commercially available coated tube assay kit (Coat-a-Count MG12191 testosterone kit, IBL International GmbH, Hamburg, Germany). The assay was able to determine plasma testosterone concentrations of 6–1200 ng/dl. Cross reactivity of the Coat-a-Count testosterone antibody was 1.8% with 19-Nortestosterone, 0.31% with dihydrotestosterone and less than 0.01% with other steroids tested. We validated the assay by testing for parallelism between serial dilutions of Ethiopian hedgehog plasma (obtained from an individual with high testosterone concentrations) and the standard curve (Chard, 1987). The curves were parallel and not significantly different from the reference preparation (ANCOVA, $F = 1.8$, $p = 0.2$, $n = 6$). The intra-assay coefficient of variation was 7.6%. The sensitivity of the assay (90% binding) was 5 ng/dl or 0.175 nmols/l.

Plasma progesterone concentrations were determined using a commercially available coated tube assay kit (Coat-a-Count MG12171, IBL International GmbH, Hamburg, Germany). The assay was able to determine plasma progesterone concentrations over the range 6–1200 ng/dl. Cross-reactivity of the antibody was (<0.1%) to other naturally occurring steroids except for 5 α -Pregn-3, 20-dione (3.4%), 17 α -Hydroxyprogesterone (1.5%), 20- β -dihydroprogesterone (3.2%) and 11-Deoxycorticosterone (0.3%). A plasma sample with a high concentration of progesterone was double diluted using the assay buffer as a matrix 1:1 to 1:8 then assayed. The slopes of serial double dilution and standard curve were compared to check for parallelism (ANCOVA: $F = 5.26$, $p = 0.07$, $n = 4$) following a log-logit transformation of the data (Chard, 1987). The intra-assay coefficient of variation for the plasma pool was 6.7% and sensitivity of the assay was 1.35 nmol/L.

2.5. Data analyses

Due to the low sample size for some months (Table 1) the data of the 12 months were combined into four seasons: summer (June to August), autumn (September to November), winter (December to February) and spring (March to May). Body mass was found to be normally distributed (Shapiro-Wilk test (S-W test): $D_{110} = 0.990$, $p = 0.640$). The effects of

sex and season on body mass were explored 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 (plasma testosterone level, seminiferous tubule diameter or testicular mass and volume) were normally distributed (S-W test: $p < 0.018$ for all) and log transformations were unsuccessful. Consequently, the effect of season on the reproductive parameters was evaluated employing Kruskal-Wallis (KW) tests. *Post hoc* comparisons were carried out with Mann-Whitney-U (MWU) tests. Spearman Rank correlation test were employed to identify if there was a relationship between testicular mass and testicular volume.

The number of Graafian follicles in the ovaries were found to be normally distributed (S-W test: $D_{44} = 0.966$, $p = 0.219$). The rest of the reproductive parameters measured for females (plasma progesterone level, corpora lutea and ovarian mass and volume) were not normally distributed (S-W test: $p < 0.008$ for all). Ovarian volume and number of corpora lutea once log-transformed were found to be normally distributed (S-W test: $D_{44} = 0.972$, $p = 0.368$, $D_{44} = 0.956$, $p = 0.107$, respectively). The effect of season on log-transformed ovarian volume and number of corpora lutea and number of Graafian follicles parameters was explored using a one-way ANOVA with season as an independent variable. Least significant difference (LSD) post-hoc tests were carried out for significant tests. Consequently, the effect of season on the other parameters was evaluated employing Kruskal-Wallis (KW) tests. *Post hoc* comparisons were carried out with Mann-Whitney-U (MWU) tests. Spearman Rank correlation test was used to investigate the relationship between ovarian mass and ovarian volume.

Weather data were obtained from the weather service Weather underground, <https://www.wunderground.com>, the total precipitation and average temperate per month was gathered for the area around the Prince Nayef Bin Abdulaziz Regional Airport, the regional airport of Al Qassim Province approximately 20 km from the capture sites. To test the effect of rainfall and precipitation, respectively, GLMs were used with total seasonal rainfall and mean seasonal temperature as covariates. Generalized linear models (GZLM) fitted with a Poisson distribution and a log-link function were employed for all non-normal dependent variables. Seasonal precipitation and temperature averages were used as covariates for all models. Body mass was additionally added as a covariate to all models to assess the effect of body mass on the reproductive parameter. Photoperiodic data were obtained from the weather service ClimaTmps, <https://www.ClimaTemps.com>, the number of hours of daylight per day over an entire calendar year was gathered for the area around the Prince Nayef Bin Abdulaziz Regional Airport, the regional airport of Al Qassim Province. To test the effect of photoperiod, GLMs were used with number of hours of daylight per day as the covariate. Generalized linear models (GZLM) fitted with a Poisson distribution and a log-link function were employed for all non-normal dependent variables. The number of hours of daylight per day were used as the covariate for all models. All statistical analyses were implemented using the IBM Statistical Package for the Social Sciences (SPSS) Statistics version 23.0 (2016). The results herein are presented as mean \pm standard error (SE) and were found to be significant at $p < 0.05$.

3. Results

3.1. Climate data

The cumulative precipitation for the study period was a mere 4.31 mm. Precipitation was recorded only during the months of March and April, with the peak precipitation occurring in March (4.06 mm) as presented in Fig. 1. Temperature on average reached above 26 °C (range: 14–36 °C), from May to September being the warmest (34.4 ± 1.14 °C) and February to March being the coolest (16.3 ± 2.08 °C) as seen in Fig. 1. The day length at the study period was greatest during the months of summer and spring, but lowest during winter (Fig. 2).

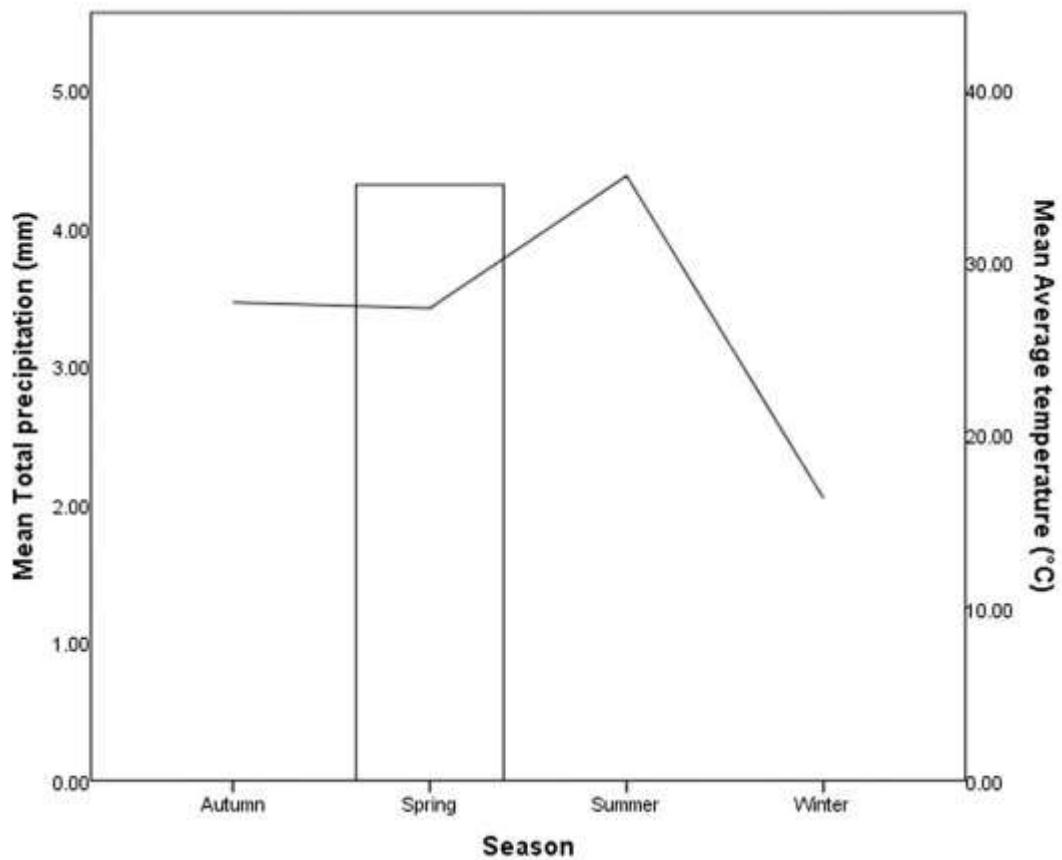


Fig. 1. Climatic data recorded for Prince Nayef Bin Abdulaziz Regional Airport, Al Qassim Province, Saudi Arabia during the 12 month study period.

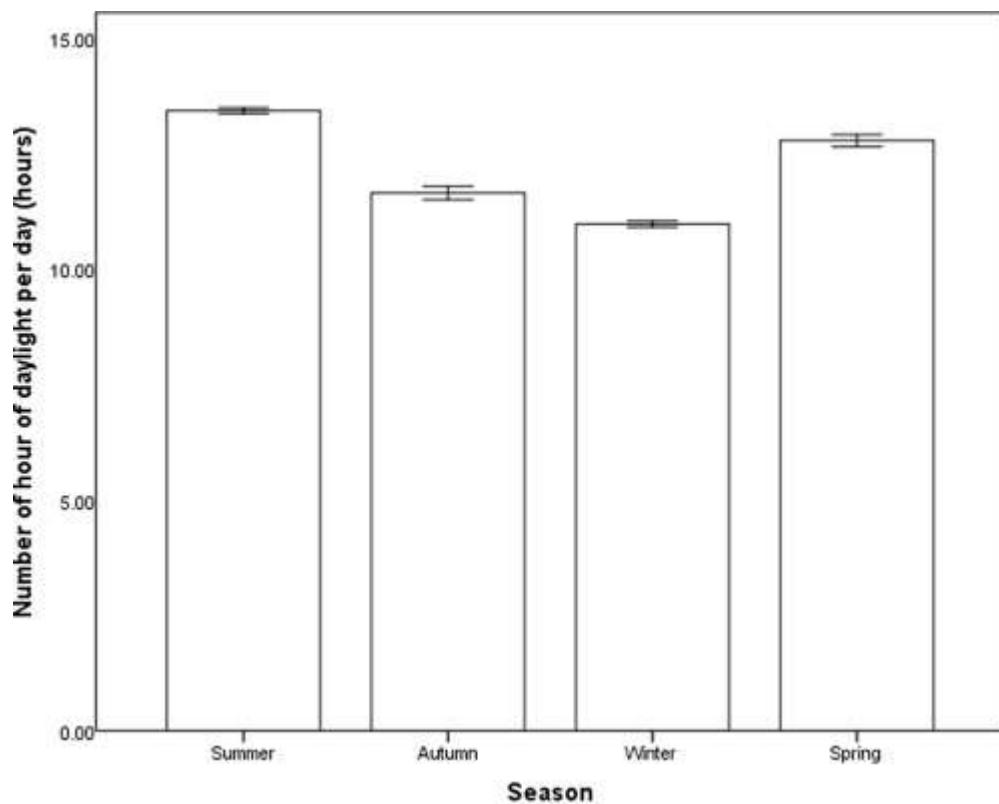


Fig. 2. Photoperiod (Number of hours of daylight per day) Mean day length recorded for the Al Qassim Province, Saudi Arabia during the 12 month study period.

3.2. Body mass

Body mass did not differ significantly between males (347.52 ± 91.92 g) and females (357.89 ± 101.1 g; $F = 0.147$, $df = 1$, $p = 0.702$). Additionally, body mass did not differ between season ($F = 0.459$, $df = 3$, $p = 0.712$) and the interaction between season and sex was not significant ($F = 0.548$, $df = 3$, $p = 0.650$).

3.3. Males

Testicular volume was significantly different between seasons (KW test: $Z = 22.451$, $df = 3$, $p < 0.0001$). The testicular volume in autumn was significantly lower than those of the testicular volumes for winter and spring (MWU: $p < 0.0001$ for both; Fig. 3a).

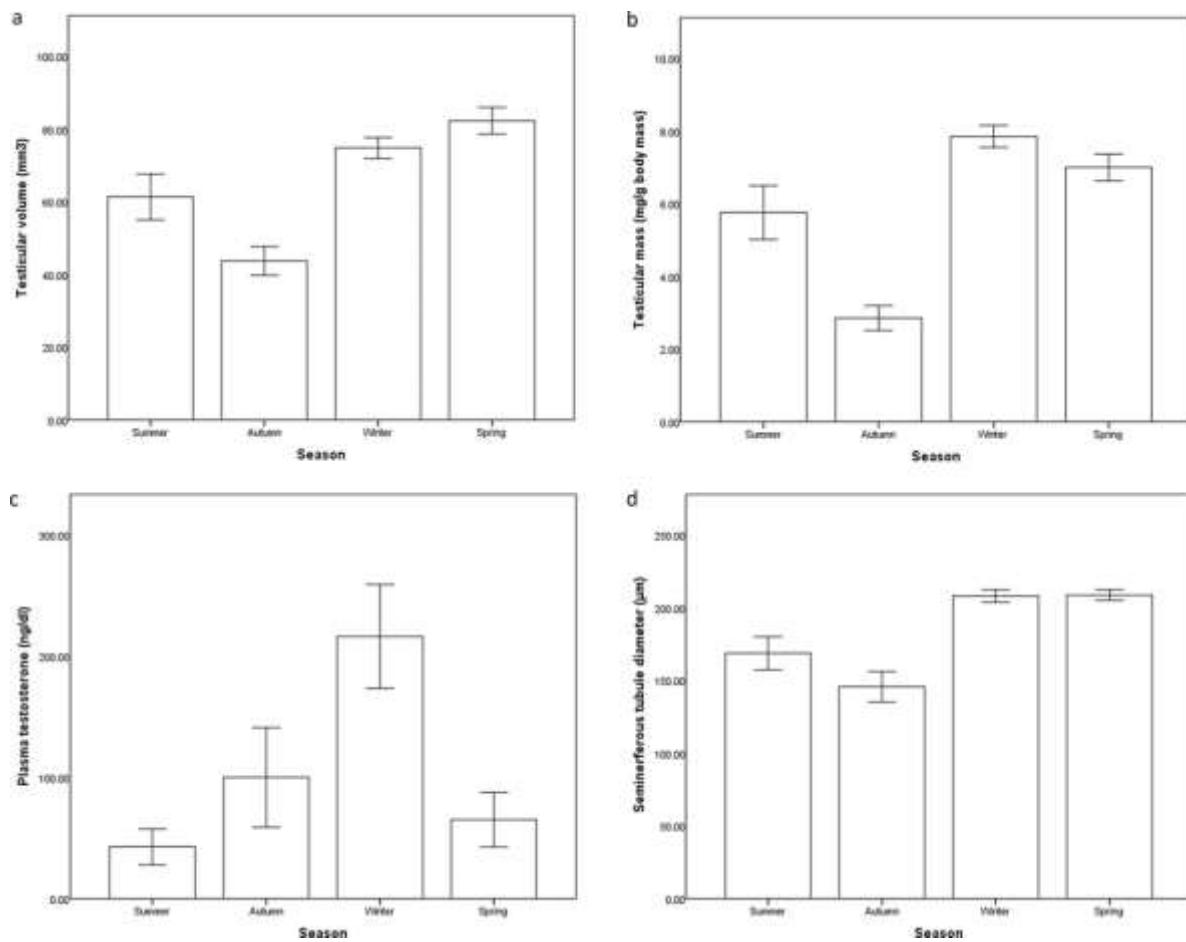


Fig. 3. Seasonal variation of reproductive parameters (mean \pm SE) of *Paraechinus aethiopicus* Showing a, testicular volume (mm^3), b, testicular mass (mg/g body mass), c, plasma testosterone concentration (ng/ml), d, seminiferous tubule length (μm).

Testicular mass was significantly different between seasons (KW test: $Z = 21.385$, $df = 3$, $p < 0.0001$). Testicular mass per gram body mass was observed to be significantly affected by season (KW test: $Z = 31.372$, $df = 3$, $p < 0.0001$, Fig. 3b).

Testicular mass per gram body mass was found to be significantly lower in autumn than the other three seasons (MWU: $p < 0.003$), additionally the testicular mass per gram of body mass in the summer was observed to be significantly lower than in winter (MWU: $p < 0.003$, Fig. 3b). The mean plasma testosterone level was significantly different between seasons (KW test: $Z = 18.832$, $df = 3$, $p < 0.0001$, Fig. 3c).

The mean plasma testosterone concentration of male hedgehogs in winter was significantly higher than plasma testosterone levels in the other three seasons (MWU: $p < 0.0001$). Seminiferous tubule diameter differed significantly with season (KW test: $Z = 24.342$, $df = 3$, $p < 0.0001$, Fig. 3d). The seminiferous tubule diameter in winter was significantly greater than that for autumn and the summer (MWU: $p < 0.0001$ and $p = 0.014$, respectively), however, it was not significantly different to spring (MWU: $p = 0.554$). The seminiferous tubule diameter in the spring was significantly greater than that recorded in the autumn and the summer (MWU: $p < 0.0001$ and $p = 0.022$, respectively). (Fig. 3d). A positive correlation

was found between testicular mass and testicular volume (Spearman rank test: ($r_s = 0.819$, $p < 0.0001$).

Testicular mass and volume increased significantly with precipitation (Wald $\chi^2 = 3.855$, $df = 1$, $p = 0.05$ and Wald $\chi^2 = 9.589$, $df = 1$, $p = 0.002$, respectively). Ambient temperature had no effect on either testicular mass or volume ($p > 0.74$). Testicular mass per g body mass decreased significantly with temperature (Wald $\chi^2 = 4.799$, $df = 1$, $p = 0.028$) and was not affected by precipitation (Wald $\chi^2 = 3.267$, $df = 1$, $p = 0.071$). Testicular mass and volume were significantly affected by body mass (GZLM: testicular mass: Wald $\chi^2 = 15.636$, $df = 1$, $p < 0.0001$ and testicular volume: Wald $\chi^2 = 25.952$, $df = 1$, $p < 0.0001$). The individuals with higher body mass had larger and heavier testes. Plasma testosterone was significantly affected by the ambient temperature, with a decrease in plasma testosterone level with increased ambient temperature (Wald $\chi^2 = 13.618$, $df = 1$, $p < 0.0001$). Rainfall had no effect on plasma testosterone concentration (Wald $\chi^2 = 0.802$, $df = 1$, $p = 0.37$). There was no effect of the body mass on the plasma testosterone concentration or (GLZM: Wald $\chi^2 = 2.507$, $df = 1$, $p = 0.113$). Seminiferous tubule diameter was significantly affected by precipitation, temperature and body mass (Wald $\chi^2 = 8.439$, $df = 1$, $p = 0.004$, Wald $\chi^2 = 13.727$, $df = 1$, $p < 0.0001$, and Wald $\chi^2 = 22.064$, $df = 1$, $p < 0.0001$, respectively). There was a direct relationship between body mass and seminiferous tubules, with larger body mass being related to greater tubular diameter, in turn there was an inverse relationship between temperature and seminiferous tubule diameter, illustrating that as ambient temperature increases, so seminiferous tubule diameter decreases. The seminiferous tubule diameter, however, is proportional to precipitation (i.e. the diameter of seminiferous tubules was greater with precipitation). There was no effect of the photoperiod length on any male reproductive parameters (testicular volume, mass and seminiferous tubule diameter and plasma testosterone) (GLZM: Wald $\chi^2 > 0.051$, $df = 1$, $p > 0.192$).

3.4. Females

Ovarian volume showed significant differences with season ($F = 9.596$, $df = 3$, $p = 0.002$) being in the autumn significantly lower than that in the summer (LSD: $p < 0.0001$) or spring (LSD: $p = 0.003$, Fig. 4a).

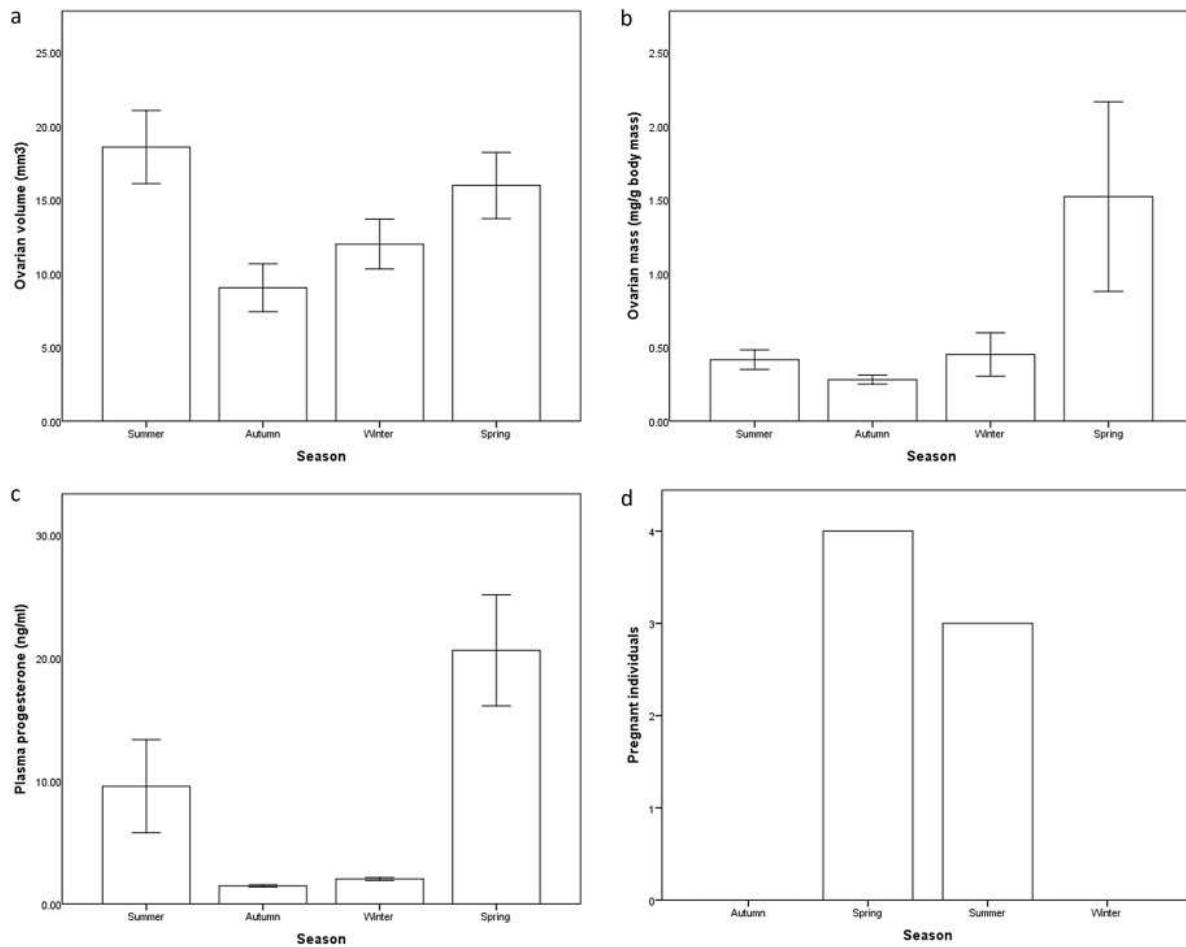


Fig. 4. Seasonal variation of reproductive parameters (mean \pm SE) of female *Paraechinus aethiopicus*. Showing a, ovarian volume (mm³), b, ovarian mass (mg/g body mass), c, progesterone testosterone concentration (ng/ml) and d, Number of pregnancies.

Ovarian mass was significantly different between the seasons (KW test: $Z = 10.26$, $df = 3$, $P = 0.016$). Ovarian mass per g body mass was significantly affected by the season (KW test: $Z = 15.9147$, $df = 3$, $p = 0.001$, Fig. 4b), which in autumn was lower compared to spring and summer (MWU: $p > 0.05$, for both), additionally ovarian mass per g body mass for spring was significantly higher than that of summer (MWU: $0 = 0.011$, Fig. 4b).

Plasma progesterone concentrations were significantly different with season (KW test: $Z = 12.545$, $df = 3$, $p = 0.006$, Fig. 4c). Mean plasma progesterone was significantly lower in the autumn than for the other three seasons (MWU: $p < 0.029$ for all; Fig. 4c). The number of corpora lutea and Graafian follicles in the ovaries of these hedgehogs did not differ between seasons ($p > 0.05$). Seven pregnant females were caught during the spring and summer period (Fig. 4d). A positive correlation was found between ovarian mass and ovarian volume (Spearman rank test: $(r_s = 0.669$, $p < 0.0001$).

Ovarian mass increased significantly with precipitation (Wald $\chi^2 = 21.446$, $df = 1$, $p < 0.0001$), but was not significantly affected by temperature (Wald $\chi^2 = 0.140$, $df = 1$, $p = 0.708$). Ovarian mass per g body mass increased significantly with precipitation (Wald $\chi^2 = 37.556$, $df = 1$, $p < 0.0001$), but it was not significantly affected by ambient temperature (Wald $\chi^2 = 0.172$, $df = 1$, $p = 0.679$). Furthermore, plasma progesterone concentration

significantly increased with precipitation ($\text{Wald } \chi^2 = 26.444$, $\text{df} = 1$, $p < 0.0001$), but significantly decreased with ambient temperature ($\text{Wald } \chi^2 = 16.958$, $\text{df} = 1$, $p < 0.0001$). There was no effect of precipitation or temperature on ovarian volume (GLM: $p > 0.084$ for both). All reproductive parameters (plasma progesterone and ovarian mass, and volume) were significantly affected by body mass (GZLM: plasma progesterone: $\text{Wald } \chi^2 = 4.812$, $\text{df} = 1$, $p = 0.028$ and ovarian mass: $\text{Wald } \chi^2 = 35.707$, $\text{df} = 1$, $p < 0.0001$; GLM: ovarian volume: $F = 9.225$, $\text{df} = 1$, $p = 0.004$). The individuals with greater body mass had larger and heavier ovaries as well as heightened concentrations of progesterone in their plasma. The GLM showed that temperature, body mass and precipitation did not affect number of corpora lutea or Graafian follicles in the ovaries of the hedgehog ($p > 0.05$). There was no effect of photoperiod on the number of corpora lutea, Graafian follicles or ovarian volume (GLM: $F = 0.244$, $\text{df} = 1$, $p = 0.511$). Additionally, there was no effect of the photoperiod on plasma progesterone (GLZM: $\text{Wald } \chi^2 = 0.996$, $\text{df} = 1$, $p = 0.318$). In contrast, ovarian mass and ovarian mass per gram body mass was affected photoperiod length (GLZM: ovarian mass: $\text{Wald } \chi^2 = 11.219$, $\text{df} = 1$, $p = 0.001$ and ovarian mass per gram body mass: $\text{Wald } \chi^2 = 8.801$, $\text{df} = 1$, $p = 0.003$). As the number of hours of daylight increased so did the ovarian mass.

4. Discussion

Mammals inhabiting arid environments only reproduce when the proximate and ultimate environmental cues promoting procreation are operational. The present study reveals that the Ethiopian hedgehog from the sandy desert of central Saudi Arabia is a seasonal breeder with breeding being initiated in the spring (March to May) and terminating in late summer. The onset of the breeding season in the females was directly associated with the first occurrence of precipitation in spring. Precipitation occurred during March, and following this time vegetation sprouted and insects became abundant enabling reproduction to arise as has been observed in the Baluchistan gerbil and Arabian spiny mouse in south western Saudi Arabia (Sarli et al., 2015, 2016). The results from this study provide additional support of the behavioural observations of Yamaguchi et al. (2013) that courtship and copulation in the hedgehog arises in March, despite the former study taking place around a rubbish dump. Indeed the histological and endocrine data provide important results as to the internal functioning in the organism. Male hedgehogs were observed pursuing females for copulation during the month of March (A.N. Alagaili pers. Obs.). Yamaguchi et al. (2013) reports that successful mating occurred significantly more frequently in the late winter (February and March) than in the summer (May, June and July). In this study, testosterone concentrations were highest in the late winter. Testicular mass, volume and seminiferous tubule diameter increased significantly with the onset of the rains, however, in late summer the males showed signs of reproductive regression with reduced testes size, seminiferous tubule diameter and testosterone concentration.

The rise in plasma testosterone concentration in males during the winter months promotes the development of the testes for the production of spermatozoa, and the subsequent increase in seminiferous tubule diameter. Seasonal changes in temperature are more predictable than patterns of precipitation in these arid regions; it is thus predicted that some species may utilise temperature changes to cue reproductive recrudescence (Henry and Dubost, 2012; Sarli et al., 2015). Temperature has been found to play a role in the onset of reproduction in males of several species of small mammal in deserts (Henry and Dubost,

2012; Sarli et al., 2015). Temperature rises bring about the recrudescence of reproduction in Cheeseman's gerbil and the Baluchistan gerbil, (Henry and Dubost, 2012; Sarli et al., 2015). Towards the end of winter as temperature rises, plasma testosterone in the hedgehogs increases which in turn triggers spermatogenesis in the seminiferous tubules (Henry and Dubost, 2012; Sarli et al., 2015).

The morphological, anatomical and endocrine findings from this study revealed that the female hedgehogs breed seasonally with the onset of reproduction at the first appearance of precipitation in early spring (March) and continuing through to mid-summer (July) (Yamaguchi et al., 2013). In our study, pregnancy was spread evenly through the spring and summer months; however, the plasma progesterone, ovarian mass and volume peaked in the spring. During autumn and winter the ovarian mass, ovarian volume and progesterone concentrations in female hedgehogs were reduced compared to those of spring and summer. Comparable findings have been reported in rodents, where breeding in female Egyptian greater gerbil and Anderson's lesser Egyptian gerbil synchronise procreation with precipitation (Soliman and Mohallal, 2009). It has been reported that the Ethiopian hedgehog has a gestation period of between 30 and 40 days (Alsharham et al., 2008), it is thus entirely possible that hedgehogs could produce two litters per annum if there are good rains, based on the observations of Yamaguchi et al. (2013). Young hedgehogs are weaned after a month (Nowak, 1991). Thus the norm is probably to have a single litter per annum, but if the rainfall is good it is possible that two litters could be produced over the breeding season.

The breeding pattern and months during which reproduction arose in the Ethiopian hedgehogs in Qatar mirrored the pattern observed in hedgehogs from Saudi Arabia (Al-Musfir and Yamaguchi, 2008; Yamaguchi et al., 2013). In Qatar the hedgehogs were captured around well irrigated farms with abundant water and trash dumps with a relatively steady supply of food (Yamaguchi et al., 2013). In the somewhat more optimal conditions of Qatar where food around dumps was readily available, breeding commenced in March (early spring) and continued through until July, a situation similar to that observed in the hedgehog population from a more natural environment in Saudi Arabia. Aggression by males was observed a month prior to breeding in the hedgehogs from Qatar (Al-Musfir and Yamaguchi, 2008; Yamaguchi et al., 2013), this may arise as a result of the heightened testosterone as observed in this study. The hedgehogs caught in Saudi Arabia were not exposed to optimal environmental conditions, however, their period of reproduction paralleled the situation observed in Qatar.

The European hedgehog shares many similarities with the pattern of reproduction with the Ethiopian hedgehog. Females in the two species of hedgehog become fertile in spring, but the European hedgehogs maintain the capacity to reproduce in the late summer and autumn since food supply is more abundant in Europe for longer periods of time in comparison to Saudi Arabia. Furthermore, in the males of both species, testosterone levels increased just after hibernation and just prior to spring, hibernation ends as the food and climatic conditions become optimal (spring) for survival and reproduction, thus resulting in the males beginning to ready themselves for reproduction (Deanesly, 1934; El Omari et al., 1989). The reproductive season of the hedgehog is far longer in Europe compared to that observed in Saudi Arabia, this is a consequence of the disparate rainfall periods which are

more predictable and for a longer period of the year in Europe, which in turn enables the quantity and quality of food to be high enough to sustain reproduction (Deanesly, 1934; El Omari et al., 1989).

As both of these species of hedgehog occur at similar latitudes one could predict that the two species would have very similar breeding patterns if it was cued by photoperiod. Photoperiod length only affected two reproductive parameter (ovarian mass per gram body mass and ovarian mass). As a consequence we conclude that photoperiod plays a minimal role in reproduction, with precipitation being far more important in cueing reproduction in the desert hedgehog.

In deserts, the proximate cues for reproduction usually entail bouts of rainfall and associated sudden flushes of vegetation as well as rising temperature. In this situation we can see how reproduction is triggered by differing proximate cues dependent upon the environment in which these organisms inhabit.

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References

Abraham, G.E., 1977. Handbook of Radioimmunoassay. Marcel Dekker Inc, New York and Basel.

Al-Musfir, H., Yamaguchi, N., 2008. Timings of hibernation and breeding of Ethiopian hedgehogs, *Paraechinus aethiopicus*. In: Qatar. Zool. Middle East, vol. 45, pp. 3-9.

Alsharham, a., Shahlid, S.A., Abdelfattah, M.A., Bottomlay, N., Hamed, S.M., Brown, G., Javed, S., Gardner, A., Cunningham, P.L., Howarth, B., Trafford Gillett, M.P., 2008. Terrestrial Environment of Abu Dhabi Emirate. Environmental Agency Abu Dhabi, United Arab Emirates, p. 440.

Bloom, W.E., Fawcett, D.W., 1964. A Textbook of Histology, eighth ed. W.R. Saunders Company, Philadelphia, London.

Bronson, F.H., Heideman, P.D., 1994. Seasonal regulation of reproduction in mammals. In: Knobil, E., Neill, J.D. (Eds.), The Physiology of Reproduction, second ed. Raven, New York, pp. 541-584.

Bronson, F.H., 1988. Mammalian reproductive strategies: genes, photoperiod and latitude. Reprod. Nutr. Dev. 28, 335-347.

Bronson, F.H., 2009. Climate change and seasonal reproduction in mammals. *Philos. Trans. R. Soc. B* 364, 3331-3340.

Bukovetzky, E., Shwimmer, H., Fares, F., Haim, A., 2012. Photoperiodicity and increasing salinity as environmental cues for reproduction in desert adapted rodents. *Horm. Behav.* 61, 159-163.

Chard, T., 1987. An Introduction to Radioimmunoassay and Related Techniques, 3rd rev. Elsevier, Amsterdam.

Deanesly, R., 1934. The reproductive processes of certain mammals VI. The reproductive cycle of the female hedgehog. *Phil. Trans. R. Soc. B* 223, 239-276.

Drury, R.A.B., Wallington, E.A., 1967. Carleton's Histological Technique. Oxford University Press, Oxford.

El-Bakry, H.A., Zahran, W.M., Bartness, T.J., 1998. Photoperiodic responses of four wild trapped desert rodent species. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 275, 2012-2022.

El Omari, B., Lacroix, A., Saboureau, M., 1989. Daily and seasonal variations in plasma LH and testosterone concentrations in the adult male hedgehog (*Erinaceus europaeus*). *J. Reprod. Fertil.* 86 (1), 145-155.

Essghaier, M.F.A., Taboni, I.M., Etayeb, K.S., 2015. The diversity of wild animals at Fezzan Province (Libya). *Biodivers. J.* 6 (1), 245-252.

Harrison, D., Bates, P., 1991. The Mammals of Saudi Arabia. Harrison Zoological Museum, Sevenoaks, United Kingdom, second ed., pp. 217-222

He, K., Chen, J.H., Gould, G.C., Yamaguchi, N., Ai, H.S., Wang, Y.X., Zhang, Y.P., Jiang, X.L., 2012. An estimation of Erinaceae phylogeny: a combined analysis approach. *PLoS One* 7 (6), 1-14, 39304.

Henry, O., Dubost, G., 2012. Breeding periods of *Gerbillus cheemani* (Rodentia, Muridae) in Saudi Arabia. *Mammalia* 76, 383-387.

Khammar, F., Brudieux, R., 1987. Seasonal changes in testicular contents and plasma concentrations of androgens in the desert gerbil (*Gerbillus gerbillus*). *J. Reprod. Fertil.* 80, 589-594.

Nelson, J.R., Desjardins, C., 1987. Water availability affects reproduction in deer mice. *Biol. Reprod.* 37, 257-260.

Nowak, R.M., 1991. Walker's Mammals of the World. The Johns Hopkins University Press, Baltimore and London.

Prendergast, B.J., Kriegsfeld, L.J., Nelson, R.J., 2001. Photoperiodic polymorphism in rodents: neuroendocrine mechanisms, costs and functions. *Q. Rev. Biol.* 76, 293-325.

Sarli, J., Lutermann, H., Alagaili, A.N., Mohammed, O.B., Bennett, N.C., 2015. Reproductive patterns in the Baluchistan gerbil, *Gerbillus nanus* (Rodentia: Muridae), from western Saudi Arabia: the role of rainfall and temperature. *J. Arid. Environ.* 113, 87-94.

Sarli, J., Lutermann, H., Alagaili, A.N., Mohammed, O.B., Bennett, N.C., 2016. Seasonal reproduction in the Arabian spiny mouse, *Acomys dimidiatus* (Rodentia: Muridae) from Saudi Arabia: the role of rainfall and temperature. *J. Arid. Environ.* 124, 352-359.

Schmidt-Nielsen, K., 1964. *Desert Animals: Physiological Problems of Heat and Water*. Oxford University Press, London. UK.

Schwimmer, H., Haim, A., 2009. Physiological adaptations of small mammals to desert ecosystems. *Integr. Zool.* 4, 357-366.

Shanas, U., Haim, A., 2004. Diet salinity and vasopressin as reproduction modulators in the desert-dwelling golden spiny mouse (*Acomys russatus*). *Physiol. Behav.* 81, 645-650.

Sicard, B., Fuminier, F., Maurel, D., Boissin, J., 1993. Temperature and water conditions mediate the effects of day length on the breeding cycle of the Sahelian rodent. *Arvicanthus Niloticus. Biol. Reprod.* 49, 716-722.

Soliman, S., Mohallal, E.M., 2009. Patterns of reproduction in two sympatric gerbil species in arid Egypt. *Integr. Zool.* 4, 248-253.

Woodall, P.F., Skinner, J.D., 1989. Seasonality of reproduction in male rock elephant shrews. *Elephantulus Myurus. J. Zool.* 217, 203-212.

Wube, T., Haim, A., Fares, F., 2009. Effect of increased dietary salinity on the reproductive status and energy intake of xeric and mesic populations of the spiny mouse. *Acomys. Physiol. Behav.* 96, 122-127.

Yamaguchi, N., Al-Hajri, A., Al-Jabiri, H., 2013. Timing of breeding in free ranging Ethiopian hedgehogs, *Paraechinus aethiopicus*, from Qatar. *J. Arid. Environ.* 99, 1-4, 1Arid Environ. 99.